



















in the emergency department. Thus, many risk factors for severe exacerbations have been described (Turner, *et al.*, 1998; Westerman, *et al.*, 1979) such as age, smoking history, poor disease control, previous mechanical ventilation, admission to the ICU, history of worsening asthma, use of air conditioning, deterioration in FEV<sub>1</sub>, labile asthma, and others. In comparison, few studies have been published on mild exacerbations in patients with long-term, stable asthma; (Bellia, *et al.*, 1984 ; Ellman, *et al.*, 1999; Pauwels, *et al.*, 1997) Therefore, Information on this aspect is important for determining appropriate future management and may have influence on the quality of life of the patients.

### *Aim and Objectives*

To examine the probability of correlation of mild exacerbation of asthma with eosinophilic inflammation in patients with stable, well-controlled asthma. We aimed to determine the time of exacerbation, and its correlation with eosinophilia in peripheral blood.

## **Material and Method**

### *Subject Demographics and Enrollment*

Retrospective study of symptoms and mild exacerbations in subjects aged 16 to 63 years with at least a 1-year history of bronchial asthma defined as well-controlled asthma was carried out. All the subjects were classified as having moderate asthma and were registered for comparative study and clinical evaluation of efficacy of two Unani coded drugs UNIM 352 and UNIM 353. Subjects were asthmatics who needed regular treatment to remain symptom free and were allergic to house dust mites, as judged by symptoms. They had been receiving treatment regularly with the same dose during the previous year and were free of exacerbations and chest infections in the 3 months preceding selection. A total of 108 patients with exacerbations, out of which 54 patients receiving UNIM 352 and 54 patients receiving UNIM 353 were selected for the analysis. Response and the course of the disease were studied for 1 year or until the period of mild exacerbation occurred. Mild exacerbation was defined as symptoms of asthma lasting > 48 h with a fall in peak expiratory flow > 20%, Complete Blood Count with eosinophil count, blood and sputum examination was done at the first visit and every follow-up.

In GROUP I-35 subjects with mild exacerbation (mainly wheezing and coughing) showed significant eosinophilia in blood and sputum. Remaining 19 Subjects with exacerbations did not show significant blood or sputum eosinophilia Baseline cough, wheezes were occasional and mainly related to exercise.

In GROUP II-40 subjects with mild exacerbation (mainly wheezing and coughing) showed significant eosinophilia in blood and sputum. Remaining 14 Subjects did

not show significant blood or sputum eosinophilia Baseline cough, wheeze were occasional and mainly related to exercise.

### *Study Design*

This was a retrospective longitudinal observational study with blind assessment of outcome measures. Subjects were selected from the cases registered in RRIUM. The end point was defined by the occurrence of mild exacerbation, defined as symptoms of asthma lasting > 48 h and preventing subjects from performing their usual activities, in association with a fall in peak expiratory flow (PEFR)  $\geq$  20% of the mean morning value.

Symptoms including breathlessness, wheezing, cough and sneezing in eligible subjects were studied over a 2-month period. Symptoms and lung function stability were rigorously checked. Subjects were then eligible for the study if their asthma was under control. Specifically whose exacerbations of clinical manifestations were mild and does not warrant emergency measures for controlling the episode.

Retrospective study of the history of Subjects interviewed on 2 consecutive days every 2 months for 1 year was studied. The first day clinical history was obtained, and a physical examination record examined. Records of venous blood and induced sputum samples were obtained and examined.

Laboratory technicians were blinded to the clinical characteristics until the end of the study. In addition, case sheets of the patients recorded the following: PEFRs, Laboratory parameters of complete blood count including eosinophil count and symptom scores.

No changes were made in treatment or follow-up procedures during the study, and the prescribed dose of medication remained the same until mild exacerbation occurred. In the event of exacerbation, subjects were instructed to immediately contact the investigators and come to the hospital within 48 h. The investigators assessed each such event according to preestablished exacerbation criteria, and, in case of mild exacerbation, they increased the dose for a minimum of 4 days or until PEFR returned to normal values.

*Time to Exacerbation:* The primary outcome studied was the time to mild exacerbation, which was defined as the number of days elapsed from the beginning of the study to the onset of the first exacerbation or to the end of the study and the correlation of the eosinophilic count during baseline assessment and during exacerbations.

### *Statistical Analysis*

Sample size was calculated to estimate the probability of mild exacerbation with  $\pm$  10% accuracy assuming an unknown rate of 50%. ANOVA and Students T test

was applied to compare the observations. However, no sample-size assumptions were made about differences between subgroups.

## Observations

Exacerbation of the disease showed male predominance. (Table 1). Out of 108 Group I had 35 and Group II 40 cases of exacerbations during the treatment period along with significant eosinophilia in blood and sputum. Of the remaining 33 subjects, 19 from Group I and 14 from Group II showed no significant blood or sputum eosinophilia.

Mean time to mild exacerbation for the whole group was 293 days (248 to 337 days) (range, 6 to 375 days) and 175 days (105 to 245 days) for those who experienced a mild exacerbation. The cumulative probability of a mild exacerbation was 49% (39 to 59%) independent of treatment.

An absolute number of Eosinophils in blood  $> 0.4 \times 10^9/L$  were associated with a shorter time until an exacerbation occurred. PEFR did not influence mild exacerbation rates significantly. Analysis for the effect of treatment confirmed these results and

**Table-1. Age and Sex distribution of the disease**

Age in years	Sexwise		
	Male	Female	Total
1-10 years	01 (1.7)	—	01
10-20 years	04 (6.7)	10 (20.8)	14
30-30 yrs	09 (15.0)	08 (16.7)	17
30-40 yrs	15 (25.0)	10 (20.8)	25
40-50 yrs	11 (18.3)	09 (18.8)	20
50-60 yrs	10 (16.7)	08 (16.7)	18
60-70 yrs	08 (13.3)	03 (6.2)	11
70-80 yrs	02 (3.3)	—	02
Total	60	48	108

identified blood Eosinophils as the factor with the greatest impact for predicting exacerbations.

## Results and Discussion

More than half of the subjects with well-controlled asthma in our study (according to well-established criteria) had at least one mild exacerbation within 12 months of enrollment, with a cumulative estimated exacerbation rate of 49% and mean time to exacerbation of 175 days for those experiencing a mild exacerbation. In addition, those who showed signs of active eosinophilic inflammation at the beginning also proved to have higher risk of exacerbation.

The aim of current guidelines for asthma treatment is to reach the control of symptoms, airflow limitation, and inflammation, although the last factor is not regularly assessed. Patients with stable asthma are not expected to experience exacerbations when their conditions are well controlled and treatment has been optimized. (Bethesda, 1992) However, our findings suggest the probability of a mild exacerbation means that approximately one half of the subjects followed up for 1 year are likely to suffer at least one exacerbation. At the initial visit, the asthma of our subjects was apparently under control according to guidelines (Hargreave, 1990; Sterk, *et al.*, 1993; Pinet, *et al.*, 1992; and Belda, *et al.*, 1997).

Exacerbation of the disease was higher in patients with average socioeconomic status and in the age group of 30-40 years. (Table 2). Male predominance of the disease as well as the exacerbation was observed during the trial.

The eosinophilic inflammation reflected poorly controlled disease, and we may speculate that uncontrolled inflammation predisposed subjects to experience mild exacerbations. (Koller, *et al.*, 1995) If we interpret the presence of inflammation to reflect under treatment, our findings have clinical relevance, supporting the view that asthma treatment should be guided not only by symptoms and airflow limitation but also by eosinophilic inflammatory markers. (Hargreave, 1999)

We found evidence of higher risk of mild exacerbation in subjects with signs of eosinophilic inflammation over the course of a year, but this effect was independent of the dose of the drug. The effect of these medications on exacerbation is mediated by their capacity to modify inflammation (Pauwels, *et al.*, 1997)

Symptoms, need of treatment, and airflow limitation are well-known markers of asthma severity. (Ogilvie, 1962; Juniper, *et al.*, 1990; and McFadden, 1997)

We did not find a relevant role of these factors in our well-controlled subjects, because in these subjects they may lose their predictive value, and improved monitoring of inflammatory markers is likely to play a more important role in controlling disease.

Similarly, airway responsiveness was not a significant risk factor for mild exacerbation in our study. Recent data suggest a relationship between exacerbation and the

**Table-2. Socioeconomic status of the subjects**

Age in years	Social Status			
	Good	Average	Poor	Total
1-10 years	–	1 (2.0)	–	01
10-20 years	2 (14.3)	8 (16.0)	4 (9.1)	14
30-30 yrs	2 (14.3)	11 (22.0)	4 (9.1)	17
30-40 yrs	4 (28.6)	11 (22.0)	10 (22.7)	25
40-50 yrs	5 (35.7)	7 (14.0)	8 (18.2)	20
50-60 yrs	01 (7.1)	7 (14.0)	10 (22.7)	18
60-70 yrs	–	4 (8.0)	7 (15.9)	11
70-80 yrs	–	1 (2.0)	1 (2.3)	2
Total	14	50	44	108

degree of airway hyper-responsiveness. (Sont, *et al.*, 1999) Based on our current knowledge of asthma pathophysiology, it seems logical that an indirect marker of inflammation like bronchial hyper-responsiveness had less relevance than a direct one, such as the number of eosinophils. The improvement in airway hyper-responsiveness was not clearly paralleled by the improvement in bronchial biopsy findings. (Sont, *et al.*, 1999 and Crimi, *et al.*, 1993)

Moreover, it was also found that nonspecific airway responsiveness was not a predictor of severity of exacerbation. (Crimi, *et al.*, 1993). Therefore, it remains unknown whether or not the reduction in exacerbation rates is because of airway inflammation, which indirectly reduces hyper responsiveness. Subjects with blood eosinophil counts  $> 0.4 \times 10^9/L$  had a 5.4-times higher risk of exacerbation than subjects with eosinophilic count  $< 0.4 \times 10^9/L$ . The relationship between peripheral blood eosinophil and asthma has been known for a long time although insufficient attention was paid to this observation. (Horn, *et al.*, 1975) They showed a good correlation between blood eosinophilia and  $FEV_1$  after an exacerbation. In addition, eosinophilia in blood could be a prognostic factor for exacerbations in asthma. (Crimi, *et al.*, 1993). We suggest that eosinophilia in blood may reflect activation of bone marrow production, as can be inferred from the observations of other authors

expressing a particular predisposition of a subject to suffer exacerbation.(Virchow, *et al.*, 1994) Eosinophilia in induced sputum has been related to exacerbations and is useful in guiding treatment of an exacerbation in severe asthma. (Sont, *et al.*, 1999). Follow up of asthmatic subjects for 1 year, suggested that the degree of airway inflammation can be a risk factor for future exacerbations. (Sont, *et al.*, 1999). In our study, sputum eosinophil count was not significantly related to spontaneous exacerbation in the following year. This lack of association was unexpected.

One reason which might explain this finding is that the systemic effect of eosinophilic airway inflammation. This relates to the fact that the markers of eosinophilic inflammation (mainly in blood) might reflect a more systemic state of eosinophilic activation, resulting in long-term risk of exacerbation. (Hargreave, *et al.*, 1990)

It is also possible that the etiology of an exacerbation plays a determinant role in the importance of sputum eosinophilia. We, like others,(Baigelman, *et al.*, 1983;Virchow, *et al.*, 1994) found most exacerbations to be related to infection, which has been described as non eosinophilic in sputum. (Virchow, *et al.*, 1994). At baseline, the mean eosinophil count was  $0.39 \times 10^9/L$  ( $0.21 \times 10^9/L$ ) in blood. An increased risk of mild exacerbation was associated with blood eosinophil count  $> 0.4 \times 10^9/L$  but was unassociated with other variables. Our data reinforce that high eosinophil levels seem to be related to risk of exacerbations, as several authors have previously described. (Baigelman, *et al.*, 1983)

The overall efficacy of UNIM 352 and UNIM 353 observed during the trial in controlling the exacerbations is shown in Table-3. scoring of the symptoms and signs including breathlessness, cough, wheeze, and grading of auscultatory signs (ronchi) were used or determining the response of the patients to the drug.

Symptoms were scored and patients with 71-100% relief of symptoms were grouped as Relieved, 30-70% relief of symptoms as partially relieved and those with up to

**Table-3. Overall efficacy of UNIM 352 and UNIM 353 observed during the trial.**

Response	UNIM 352	UNIM 353	TOTAL
Relieved	12(24.0%)	19(37.0%)	31
Partially Relieved	40(74.0%)	32(57.4%)	72
Not Relieved	02(2.0%)	03(5.6%)	05
Total	54	54	108

Patients were grouped as Relieved, Partially Relieved and Not Relieved according to the Aas scoring of symptoms with the scoring of 71-100%, 30-70%, Up to 29% respectively.

29% relief of symptoms were grouped as not relieved. UNIM 353 showed better efficacy in completely relieved cases the response of was observed to be better (37.0%) than the response of UNIM 352(24.0%).In partially relieved cases the response of UNIM 352 was good (74.0%) as compared to the response to UNIM 353(57.4%). Eosinophil count observed during enrollment and during exacerbations showed significance with  $p < 0.001$  in group I and  $p < 0.05$  in group II. Table-4.

Effect of exacerbation of bronchial asthma on changes in markers of airway inflammation while on treatment with UNIM 352 and UNIM353 is shown in Table-5. With out the mean eosinophil count was  $0.39 \times 10^9/L$  ( $0.21 \times 10^9/L$ ) in blood. An increased risk of mild exacerbation was associated with blood eosinophil count  $> 0.4 \times 10^9/L$ .Eosinophilia in induced sputum has been related to exacerbations. Significant  $p$  less than 0.01 versus normal subjects by ANOVA.

## Conclusion

Patient with stable, well-controlled asthma are at risk of mild exacerbation during 1 year of follow-up despite regular treatment. Eosinophilic inflammation expressed as eosinophil count is associated with higher risk of mild exacerbation. We conclude that subjects with well-controlled asthma have a high risk of mild exacerbation, which is related to underlying eosinophilic inflammation. Our data indicate that subjects with active eosinophilic inflammation are more likely to experience a mild exacerbation and that, given the great variability in the natural course of the disease, close monitoring of eosinophilic inflammation may be warranted to evaluate the risk of an individual patient. Studies show that Subjects with a sputum ECP level  $> 2$  SDs higher than that reported for healthy subjects were more likely to experience mild exacerbations (68% (14%) vs. 33% (14%)) for subjects with levels within 2 SDs of healthy subject levels.(Belda, *et al.*,1997) ECP (eosinophilic cationic protein) total IgE should be included in the laboratory workup of cases of bronchial asthma for further establishing the correlation of eosinophilic inflammation and exacerbation of disease in cases of bronchial asthma.

**Table-4. Eosinophil count showing increase in subjects with exacerbations**

Groups	Eosinophil count During enrollment	Eosinophil count During exacerbation	Sample size
Group I [UNIM-352]	$3.7 \pm 3.2$	$10.8 \pm 5.2$	40
Group II [UNIM-352]	$4.2 \pm 3.9$	$7.4 \pm 6.2$	35

Group I Mean  $\pm$  SD  $6.8 \pm 4.0$  n= 40  $P < 0.001$

Group II Mean  $\pm$  SD  $5.0 \pm 4.7$  n=35  $P < 0.05$

**Table-5. Effect of exacerbation of bronchial asthma on changes in markers of airway inflammation while on treatment with UNIM-352 and UNIM-353**

Outcome	With exacerbation of asthma	With out exacerbation of asthma	With exacerbation of asthma	With out exacerbation of asthma
Morning PEFR change	-28	-8.9	-27	-8.7
Peak flow Variability change	3.5	14.9	3.2	13.0
FEV <sub>1</sub>	1.1	-27.4	1.6	-25.3
Viability %	65	68	65	*70
Blood eosinophil count	> 0.4 x 10 <sup>9</sup> /L	0.39 x 10 <sup>9</sup> /L	> 0.4 x 10 <sup>9</sup> /L	0.39 x 10 <sup>9</sup> /L (0.21 x 10 <sup>9</sup> /L)
Sputum eosinophil	13.4	0.6	12.2**	0.2

\*p less than 0.05 versus normal subjects by ANOVA.

\*\* p less than 0.01 versus normal subjects by ANOVA.

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# Unani Drugs in the Treatment of Premenstrual Syndrome

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## Abstract

In this study 50 patients were selected from Out Patient Department (OPD) of Ajmal Khan Tibbiya College Hospital, Aligarh Muslim University, Aligarh from January 2005 to August 2006. The study was undertaken to evaluate the clinical efficacy of “Sufoof-e-Mudir” in the patients of Premenstrual Syndrome and to compare the results with a herbal marketed product P Mensa given for the same symptoms. The patients were divided into two groups- Group A and B. Group A patients were administered “Sufoof-e-Mudir” and Group B patients P Mensa. In group A significant relief was observed in physical symptoms and thereby psychological symptoms of Premenstrual Syndrome as compared to group B. The compound “Sufoof-e-Mudir” which is commonly used as diuretic is also reported to possess anti-inflammatory, emmenagogue, resolvent and laxative properties which may be helpful in regulating menstrual cycle and dysmenorrhea.

**Key Words:** Premenstrual Syndrome (PMS), Drug “Sufoof-e-Mudir”. *Raphanus sativus*, *Amomum subulatum*, Potassium Carbonate, Potassium Nitrate.

## Introduction

Premenstrual Syndrome is the cyclic recurrence in the luteal phase of the menstrual cycle of a combination of distress and physical, psychological and behavioural changes of sufficient severity to produce the deterioration of interpersonal relationships and interference with normal activities. Symptoms are relieved with the onset of menses and disappear by day 3 of flow.

The essential character of the syndrome was published in 1931, by an American Gynaecologist, Frank, who described it as a severe syndrome of “indescribable tension and irritability from ten to seven days preceding menstruation which in most instances, continues until the time that the menstrual flow occurs”. But the term PMS was first coined in 1953.

## Incidence

Millions of women experience recurrent emotional and physical symptoms associated with their menstrual cycles. These symptoms constitute PMS when they are isolated to the luteal phase and interfere with daily functioning. Overall, approximately 75% of the general population encounter some kind of premenstrual symptoms. If specific diagnostic criteria for PMS are used, 3 to 8% of women with regular cycles can be diagnosed with PMS. Of those who seek medical treatment approximately 40 to 50% meet these criteria. (www.himalayahealthcare.com)

### *Survey of Unani Literature*

The sign and symptoms for which the term Premenstrual Syndrome” is used since 1953 have been also described by authentic Unani Physicians like Hkm Zakariya Razi, Sheikh Bu Ali Sina and Ali Ibne Abbas Majoosi. According to them the most marked symptoms is edema, which is associated with loss of appetite, pica, nausea, vomiting, dyspepsia, increased thirst and weakness of the mechanism of digestion. Some females also experience breathlessness, cough, headache, giddiness, dysuria, backache and tremers. Sometimes, there is also collection of fluid in the abdominal cavity. The collection of fluid may also be of generalized type leading to a condition called anasarca.

### *Etiology*

Many different etiologies have been proposed for the symptom of PMS; none have been definitely established as a dominant cause. Possible influences include hormonal imbalance, specifically a low progesterone level during the luteal phase of the cycle; abnormal neurotransmitter response to ovarian signaling; disordered aldosterone function leading to sodium and water retention; abnormal hypothalamic-pituitary-adrenal axis function leading to deficient adrenal hormone secretion; nutritional deficiency including magnesium, pyridoxine, carbohydrate intolerance, environmental factors including stress.

Although, more than one mechanism may be concerned in the production of symptoms, an increase in extra-cellular fluid throughout the body is one which is often blamed. This happening explains why some affected women gain 1.8 – 2.3 kg weight just before a menstrual period. The edema is said to be associated with sodium and water retention and this is turn with the high level of estrogen and relative deficiency of progesterone in circulation during the second half of the menstrual cycle.

Many women who suffer from premenstrual tension, however, do not put on weight and controlled observations show that the incidence and intensity of symptoms is not related to weight gain. The tendency, therefore, is to credit the syndrome to a hypothalamic-pituitary disturbance.

### *Clinical Features*

The Premenstrual Syndrome includes a large group of symptoms which appear regularly and predictably about 12 days before the onset of menstruation. It consists of physical and emotional symptoms as well as behavioural changes. Common physical symptoms experienced by women with premenstrual syndrome are breast tenderness, fatigue, joints pain, headache, abdominal distension, abdominal bloating, weight gain. The emotional symptoms most commonly reported include depression, irritability and anxiety. The behavioural changes

often reported by women with premenstrual syndrome are binge eating, the avoidance of social activities, crying episodes and physical or emotional abuse of those around them. Over 150 symptoms have been reported to comprise the premenstrual syndrome.

Herve (1968) defines premenstrual syndrome as mild when the symptoms appear 2-3 days before menstruation, are few in number and modest in severity and disappear the day before the menstrual flow; as medium when it appears 7-8 days before menstruation, with many symptoms of different severity, which disappear sometimes before, sometimes during the first two days of menstrual flow, and as severe when it starts at the time of ovulation or lasts more than 8 days, disappear only after the beginning or even the end of the menstrual flow and shows numerous and severe symptoms.

Typically premenstrual symptoms appear after ovulation, worsen progressively leading up to menstruation, and are relieved at varying rates after onset of menstruation. In some women there is almost immediate relief from psychiatric symptoms with the onset of bleeding while for others the return to normal is more gradual.

The syndrome may be present in varying degrees of intensity and different symptoms may predominate in different women. Some patients complain primarily of the nervous system manifestations, particularly irritability, depression, unreasonable emotional outbursts and episodes of uncontrollable weeping; others chiefly of the swelling and edema of various parts of their bodies, and still others of headache and visual disturbances.

Some behavioural changes are also found to be increased in the patients of PMS such as suicide, child abuse, examination failures, poor performance at work, and alcohol abuse.

### *Diagnosis*

Diagnosis of PMS depends on history and careful questioning. In the present study, a questionnaire was developed based on the symptoms commonly found in patients of PMS as described in literature. These are:

1. Swelling of extremities
2. Weight gain
3. Abdominal bloating
4. Abdominal distension
5. Colonic spasm
6. Fullness and tenderness of breasts

7. Fullness in pelvis
8. Irritability
9. Depression
10. Anxiety
11. Hostility
12. Lassitude
13. Excitability
14. Insomnia
15. Congestive dysmenorrhoea
16. Loss of concentration
17. Fatigue

### *Treatment*

According to their response to the drug the patients were categorized into 3 groups as follows :

1. Completely relieved (C) The patients who showed 60% and above relief in symptoms.
2. Partially relieved (P) – Those who showed 30-60% relief in symptoms.
3. Not relieved (N) – The patients who showed relief in symptoms below 30%.

The compound “Sufoof-e-Mudir” contained four drugs namely Javakhar (Potassium Carbonate), Shora Qalmi (Potassium nitrate), Barg-e-turb (Raphanus sativus), Heel-e-kalan (Amomum subulatum).

Javakhar that is impure potassium carbonate has been known from very ancient times. Its principal source in India is wood ashes because potash is an indispensable element for the growth of most plants. It is prepared by reducing to ashes the 'green spikes of barley, dissolving the ashes in water, straining the solution through thick cloth and evaporating it over fire. Its temperament is hot 3<sup>0</sup> and dry 3<sup>0</sup> having following therapeutic actions: diuretic, anti-inflammatory, resolvent, antacid and stomachic. It is used in urinary diseases, uric acid diathesis leading to gout, rheumatism and uterine irritability.

Shora Qalmi (Potassium nitrate) is commonly found as a surface efflorescence on soils rich in organic matter. The mineral is formed in soils when nitrogenous organic substances decay in contact with potassium salts, the reaction being brought about by nitrifying bacteria. Its temperament is hot 3<sup>0</sup> dry 3<sup>0</sup>. It is an efficient diuretic,

emmenagogue, refrigerant and disphoretic. A mixture of potassium nitrate 2 parts and leaf juice of the radish 1 part is used to relieve scalding and retention of urine, also suppression or scantiness of urine.

Heel Kalan (*Amomum subulatum*) is the fruit 1-1.75 cm long, dark brown in colour. Seeds are also dark brown in colour, round, aromatic and are more numerous held together in each cell by a dark viscid saccharine pulp. Its temperament is hot  $2^0$  and dry  $2^0$ . Its chemical constituent is an essential oil extracted from the seeds. It is an agreeable aromatic, stimulant and is used for flavouring. It acts as a stomachic, used to allay irritation of the stomach produced either by cholera or some other affections. In combination with the seeds of melon it is used as diuretic.

Barg-e-turb are highly nutritious being a good source of vitamins and minerals. The leaves have a high strontium content. Its therapeutic actions are diuretic, laxative lithotriptic and antiscorbutic. The juice of fresh leaves is used as diuretic and laxative.

Hence, the compound Sufoof-e-Mudir contained the drugs which have diuretic, emmenagogue, anti-inflammatory, resolvent, deobstruent, appetizer and laxative properties which helped the patients to overcome physical and thereby psychological symptoms. While P Mensa is a marketed herbal formulation having following ingredients viz. Ashoka (*Saraca indica*), Ailwa (*Aloe vera*), Nirgundi (*Vitex negundo*), Asgand (*Withania somnifera*), Ajwain desi (*Trachyspermum ammi*), Lodh pathani (*Symplocos racemosa*), Devdaru (*Cedrus deodara*). These ingredients possess uterine tonic, spasmolytic, anti-inflammatory, anti-stress, muscle relaxant properties.

#### *Criteria for the Selection of Patients*

1. Cyclic symptoms which occurred only during the luteal phase.
2. Dramatic and complete relief with the onset of full blood flow.
3. Symptom free period of at least 7 days in the first half of the cycle.
4. Severe enough symptoms to require medical advice or treatment.

#### **Material and Method**

In this clinical trial 50 patients of Premenstrual Syndrome were selected as per inclusion criteria. The patients were randomly divided into two groups of 25 patients each. The patients were assessed of their age, duration of illness, family history, socioeconomic status, clinical features etc. Group A or test group received oral "Sufoof-e-Mudir" for 10 days in three consecutive cycles in a dosage of 6 gms twice daily. The drug was administered 7 days before the onset of menses and 3 days during menses for 3 consecutive cycles. Group B

received a marketed herbal product syrup P Mensa two teaspoonful twice daily in the same manner. The effect of each group was analyzed. The results were interpreted at the completion of the study taking into account the relief of symptoms.

There is no objective measure such as biochemical tests available to diagnose the severity of problem. Investigations can help only to exclude other similar disorders of females. Still following investigations were carried out:-

### *1. Routine Investigations*

- (a) Complete Haemogram (Hb%, TLC, DLC, ESR)
- (b) Urine-routine and microscopic
- (c) Stool for ova and cyst.

### *2. Specific Investigations – (When required)*

- (a) Hormone Assay
- (b) U.S.G. of pelvis and adnexae
- (a) Hormone Assay (Serum level of oestradiol and progesterone in luteal phase) was done to rule out any hormonal imbalance. It is advised to those patients in which symptoms related to fluid retention were prominent such as swelling of extremities, feeling of weight gain, abdominal bloating, breast tenderness etc.
- (b) Ultrasound of Pelvis and Adnexae – This investigation was carried out to rule out any uterine and ovarian organic disorders which could account for pelvic pain or abdominal bloating.

Organic causes of uterine pain include leiomyomas of the uterus, adenomyosis, cervical stenosis, infections of the uterus following Dilatation and Curettage or with intrauterine contraceptive devices. Pelvic pain due to endometrial or cervical cancer is usually a late manifestation.

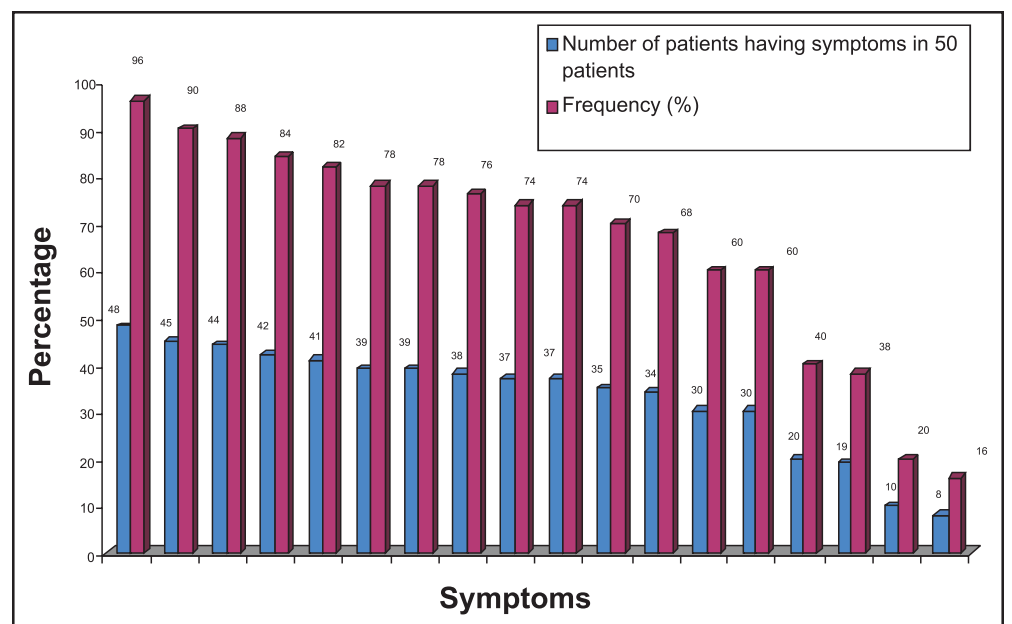
The most common organic cause of adnexal pain is acute salpingo-oophoritis, chlamydial or gonococcal disease with or without a superimposed pyogenic infection. Chronic pelvic inflammatory disease results from either a single episode or multiple episodes of infection and may present as infertility associated with chronic pelvic pain. Endometriosis involving fallopian tubes, ovaries or peritoneum may cause chronic low abdominal pain.

Pregnancy must be considered in the differential diagnosis during the reproductive years.

## Observations and Result

**Table-1. Frequency of Symptoms**

Symptoms	Frequency (%) (In 50 patients)
1 Fatigue	96
2 Lassitude	90
3 Fullness in pelvis	88
4 Abdominal bloating	84
5 Colonic Spasm	82
6 Abdominal distension	78
7 Congestive dysmenorrhoea	78
8 Fullness and tenderness of breasts	76
9 Headache	74
10 Irritability	74
11 Depression	70
12 Anxiety	68
13 Excitability	60
14 Loss of concentration	60
15 Insomnia	40
16 Swelling of extremities	38
17 Weight gain	20
18 Hostility	16

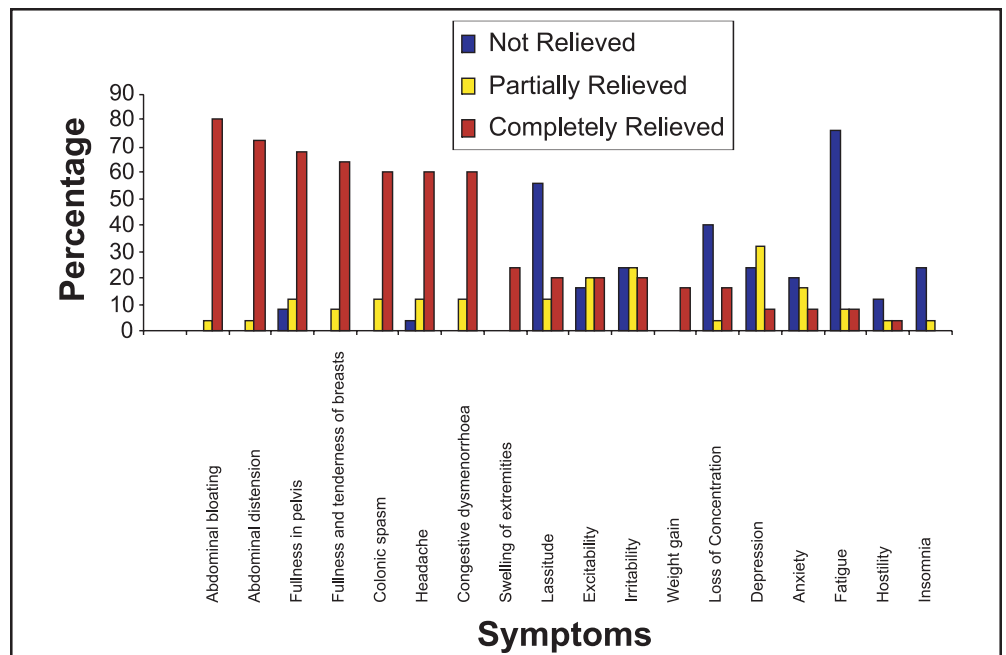


**Fig. 1.** Distribution of symptoms of patients according to the rate of incidence

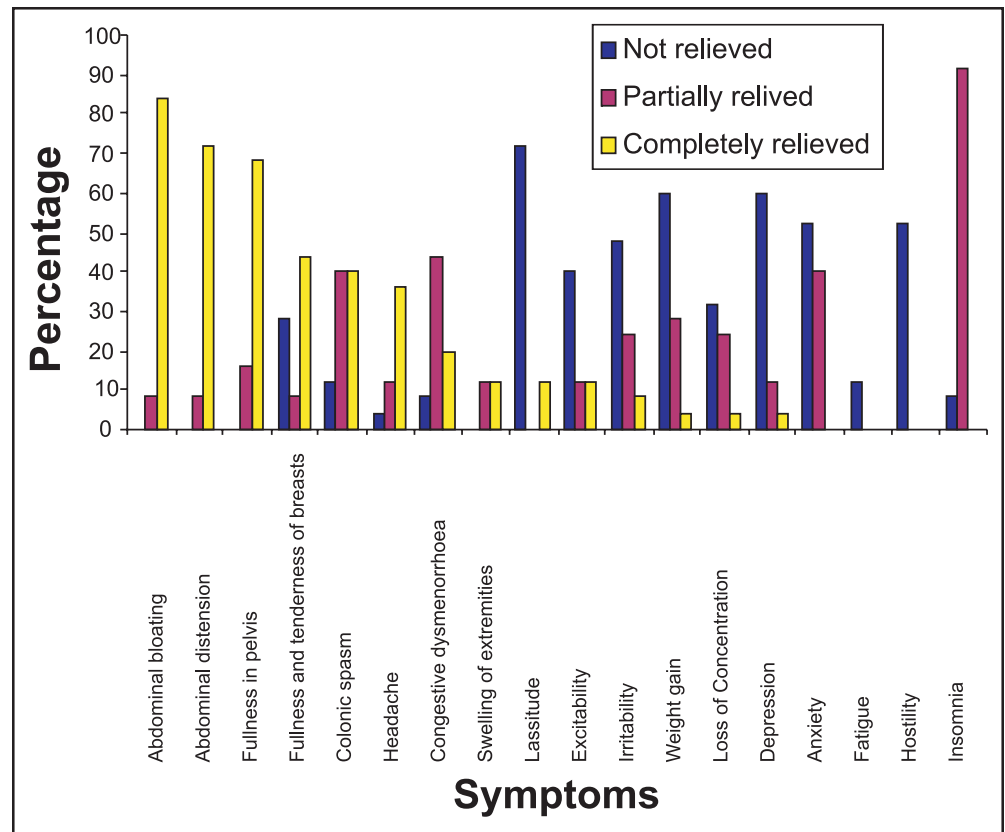


**Table-2. Shows comparison in symptoms relieved by Group A drug and Group B drug**

S.No	Parameter	Response (% Relieved)	
		Group A (Sufoof-e-Mudir)	Group B (P Mensa)
1	Abdominal bloating	80	68
2	Abdominal distension	72	44
3	Fullness in pelvis	68	84
4	Fullness and tenderness of breasts	64	72
5	Colonic spasm	60	4
6	Headache	60	20
7	Congestive dysmenorrhoea	60	12
8	Swelling of extremities	24	36
9	Lassitude	20	40
10	Excitability	20	12
11	Irritability	20	8
12	Weight gain	16	12
13	Loss of concentration	16	4
14	Depression	8	4
15	Anxiety	8	0
16	Fatigue	8	0
17	Hostility	4	0
18	Insomnia	0	0



**Fig. 2a. Response to Sufoof-e-Mudir**



**Fig. 2b.** Response to P Mensa

## Discussion

This study reveals that incidence of this problem is more common in 15-20 yrs (32%) of age, affecting unmarried females most frequently.

The problem is more common in patients having regular menstrual cycle (78%) as compared to patients having irregular menstrual cycle. (22%). It was observed that patients with moderate menstrual flow (52%) were more prone to premenstrual syndrome and maximum number of married females (20%) with PMS were para two and para three. A significant percentage of patients (75%) were found to have pain in lower abdomen. The problem is commonly found in patients who belong to low socioeconomic group (94%). On analyzing the family history it was found that patients did not have positive family history of similar illness in mother or sister.

After statistical tests, (i.e Correlation, Chi square test, Unpaired T test) significant reduction was observed by test drug in following symptoms: abdominal distension (72%), colonic spasm (60%), Congestive dysmenorrhoea (60%) headache (60%), anxiety (8%).

Symptoms of dysmenorrhoea considerably decreased post treatment for Group A, as the ingredients of “Sufoof-e-Mudir” are reported to possess diuretic, anti-inflammatory and resolvent properties. (“Mamoolat-e-Matab” by Hakeem Abdul Mannan).

## Conclusion

After going through the observation and result of this study, we have arrived to the following conclusions.

The incidence of this problem is common in adolescents. It is more common in average built and in low socio-economic group.

After treatment, there was statistically significant reduction observed by “Sufoof-e-Mudir” in physical symptoms which are abdominal distension, colonic spasm, anxiety, congestive dysmenorrhoea and headache significant relief was observed in other physical symptoms significant such as abdominal bloating, fullness in pelvis, fullness and tenderness of breasts. While physical symptoms showed less response to the treatment by P Mensa and the symptom of lassitude decreased significantly post treatment.

This suggests that the response of “Sufoof-e-Mudir” administered for this problem is better than marketed herbal product P Mensa in physical symptoms. However, psychological symptoms including behavioural changes showed little response to the treatment.

On the basis of above study it is suggested that a long term clinical study must be conducted with more sophisticated parameters for the assessment of effect of these drugs on clinical symptoms of PMS and recurrence of any symptom after treatment. The drug given was tolerated well by the patients and showed no side effects.

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# Role of Unani Drugs in Combating Oxidative Stress Related Diseases

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## Abstract

Oxidative stress (OS) play an important role in the pathogenesis of various disease, which include brain disorders (Alzheimer's disease (AD), Parkinson's disease (PD), Anxiety and CNS Depression, cardiovascular disease (Atherosclerosis, Cerebral Ischaemia and stroke) and Lung disorder (Bronchitis), Metabolic and Aging diseases (Diabetes and Arthritis), Cancer and infectious disease. Although modern medicine have very effective and therapeutic role in the management of different disease, yet its use for a longer time, produce a number of undesirable side effects. On the other hand Unani medicine uses a large number of natural drugs, plant preparations and phytochemicals with no or negligible side effects.

**Key Words:** Oxidative stress, Arthritis, Alzheimer disease, Cancer, Hepatitis

## Introduction

The discovery of superoxide dismutase (SOD) by McCord and Fridovich (1969) provided the first evidence of implicating radicals and oxidative stress as important sources of biological injury. Oxidative Stress (OS) occurs when there is an imbalance in the generation and removal of radical species within an organism. The majority of these radicals involves oxygen and is referred to as reactive oxygen species (ROS) (Sies, 1991). Because of the potential for ROS to damage tissues and cellular compartments such as membranes (Kellogg and Fridovich, 1977), DNA and proteins (Imlay and Linn, 1988; Stadtman and Berlett, 1997). They are involved in causing disease. Therefore, living creatures have evolved a highly complicated defense system with antioxidants, composed of various enzymes which includes Superoxide-dismutase (SOD), Catalase (Cat), Glutathione-peroxidase, (GPx), Glutathione-reductase (GRx), Glucose-6-phosphate-dehydrogenase (G6PDH), Cytochrome- P450 and non-enzymes such as vitamins (Vitamin-A, Vitamin-E and Vitamin-C) and Glutathione (GSH) against oxidative stress in the course of their evolution.

In India, around, 20,000 medicinal plants have been recorded; however traditional communities are using only 7000-7500 plants for curing different diseases (Kamboj, 2000). The medicinal plants are listed in various indigenous systems such as Sidha (600), Ayurveda (700) and Amchi (600) Unani (700) and Allopathic (30) (Rabe and Staden, 1997). The world health organization (WHO) has estimated that most of the world's populations relies on these, "alternative" plants- based medicines as their primary medical intervention (Kroll *et.al.*, 2003).

Till few decades back the traditional systems of medicine were prevalent only in the rural areas of developing countries because of their limitations to afford the high cost of production of modern synthetic drugs. But the increasing resistance of some

disease producing micro-organisms against synthetic drugs has made the policy makers even in developed countries to incorporate traditional herbal medicine in their health care programme. Besides because of increasing apprehensions regarding the toxicity and safety of modern drugs people all over the world are shifting back to traditional medicine. This global resurgence of interest in traditional herbal remedies has become a compelling reason to evolve a mission made approach towards medicinal plants (Butt *et.al.*, 2008). The aim of present review is to understand the role of traditional medicines, extensively used for the treatment of oxidative stress and its related disease like Alzheimer's disease, Parkinson's disease, Atherosclerosis, Stroke, Cancer, HIV/ AIDS, Hepatitis, Bronchitis, Diabetes and Arthritis etc.

## Neurodegenerative Diseases

Aging is the major risk factor for neurodegenerative diseases, including Alzheimer's disease and Parkinson disease. Oxidative stress may cause neuronal damage, ultimately leading to neuronal death by apoptosis or necrosis.

### 1. Alzheimer's Disease

It is progressive neurodegenerative disorder. The people with Alzheimer's often have an acetylcholine deficiency. The synthetic medicines available for treatment and management of Alzheimer's disease have severe side effects and as the drugs are needed to be continued for long period of time. A review of literature indicates that a number of medicinal plants, and their constituents have been traditionally used for the treatment of Alzheimer's disease. These include leaves of Methi (*Trigonella foenum-graecum* Linn.) (Rastogi & Mehrotra, 1993), *Withania somnifera* (Ashwagandha) (Schliebs *et. al.*, 1997; Archana & Namasivayam, 1999), *Bacopa monniera* (Nir-Brahmi) (Singh, H.K & Dhawan, B.N, 1997; Ghosh *et al.*, 2007). Only Asgandh and Nir-Brahmi have antioxidant activity.

### 2. Parkinson's Disease (PD)

PD is neurological syndrome manifested by any combination of tremor at rest, rigidity, bradykinesia and loss of postural reflexes. In PD there is selective degeneration of dopaminergic neurons in the nigrostriatal system. These neurons synthesize and release dopamine (DA), Loss of dopaminergic influence on other structures in the basal ganglia leads to classical Parkinsonian symptoms (Esposito *et.al.*, 2002). Oxidative stress may arise from the metabolism of DA with the production of potentially harmful free radicals.

A combination of high dosage of alpha-tocopherol and ascorbate delayed the emergence of disability by 2.5 years, the time necessary to begin therapy with L-DOPA (Fahn, 1991). Esposito *et.al.*, 2002, suggest that there are many alternative antioxidative approaches that may be considered in future clinical trials, including

free radical scavengers, indigenous antioxidant enzyme boosters, iron chelators and drugs that interfere with oxidative metabolism of DA in Parkinsonism. There is no report about Unani drugs, used for treatment of this disease.

### 3. Psychiatric Disorders

The incidence of anxiety, depression and other psychiatric disorders is increasing day by day in the fast mechanical and materialistic age. As a result, the use of Allopathic psychotropic agents is also increasing, which have further deleterious effects on the brain, nervous system and other organs. The drugs like diazepam, alprazolam may also cause dependence. Their adverse effects include confusion, headache, giddiness, alimentary tract upset, skin rashes, reduced libido etc.

There are many herbs like Sumbul-ut-Teeb, Asgandh (Archana & Namasivayam, 1999), Brahmi (Ghosh *et al.*, 2007) Sankhaholi etc, which are used for the treatment of psychiatric disorders, especially anxiety and stress in Unani Medicine. A review of literature indicates that various plants and their extracts have been used for treatment of various psychiatric disorders. These includes *Sphaeranthus indicus* (Ambavade *et. al.*, 2006) antidepressant like activity of glycyrrhizin (Dhingra & Sharma, 2005; Visavadiya *et al.*, 2009), CNS depressant and anticonvulsant activity of Brahmi Ghrita, a formulation containing *Bacopa monnieri* (Ghosh *et al.*, 2007) *Acorus calamus* (Souza *et al.*, 2007), *Saussurea lappa* and cow's ghee (Achliya *et. al.*, 2005), and Gul-e-surkh (*Rose damascena, mill*) (Husain *et.al.*, 2009). All plants have antioxidant activity except *Saussurea lappa* , Brahmi Ghrita, *Saussurea lappa* and Gul-e-Surkh.

## Cardiovascular Diseases

### 1. Atherosclerosis

Oxidation of low density lipoproteins (LDL) has long been suspected to play a critical role in atherogenesis, in consequence of which antioxidants were expected to have antiatherogenic potential. Such agents were thought to be able to inhibit oxidative modification of LDL that leads to the accumulation of cholesterol in the atherosclerotic lesion. Several plants extract like, *Terminalia arjuna* (Tiwari *et. al.*,1997), garlic (Yeh and Liu, 2001), pomegranate (Aviram *et.al.*, 2002) and ginger (Fuhrman *et.al.*, 2000). contain polyphenols which reduce the capacity of macrophages to oxidatively modify LDL; due to their interaction with LDL to inhibit its oxidation by scavenging reactive oxygen species and reactive nitrogen species and also due to accumulation of polyphenols in arterial macrophages; hence, the inhibition of macrophage lipid peroxidation and the formation of lipid-peroxide-rich macrophages. They are shown to be effective in atherosclerosis.



## 2. Stroke

Stroke is the third leading cause of death and the major cause of disability in USA. Stroke is defined as an abrupt impairment of brain function resulting from occlusion or rupture of intra or extra cranial blood vessels. There are several types of stroke. Cerebral thrombosis and cerebral embolism, also classified as ischaemic stroke and subarachnoid haemorrhage, and intracerebral haemorrhage also classified as haemorrhagic stroke. Calcined gold preparation is used in Unani Medicine as treatments for global and focal ischaemia. Ischaemic brain damage in experimental rats was halted and the animals restored to near normal function following dosing with Kushta Tila Kalan, suggestion that gold preparations have an important therapeutic role (Shah & Vohora, 2002). Shukla *et al.*, 2006 had reported that the ischaemic rats treated with *Acorus calamus* (Ac-002) in middle cerebral artery occlusion (MCAO) also reduced the contra lateral cortical infarct area (19%) as compared to MCAO rats (33%) with antioxidant activity (Souza *et al.*, 2007).

## Cancer

The recent world cancer report released by WHO observes that world cancer rates are set to double by 2020. Cancer is emerging as a major problem globally; both in more developed and in less developed countries. A review of literature indicates that various medicinal plant extract and their constituents particularly polyphenols have been used for the treatment of different types of cancers. These includes *Withania somnifera* (Al-Hindawi *et al.*, 1992; Archana & Namasivayam, 1999), Ginger (*Zingiber officinale*) (Upadhyay, 1997; Lu *et al.*, 2003), Curcumin (Deeb *et al.*, 2003; Wu *et al.*, 2008), Clove (*Eugenia caryophyllata*) (Zheng *et al.*, 1992; Jirovetz *et al.*, 2006), green tea (Stoner and Mukhtar, 1995; Shin *et al.*, 2007), *Aloe vera* gel extract (Shamman *et al.*, 1998; Hu *et al.*, 2003). All the plants have antioxidant activity.

## Infectious Disease

### 1. HIV/AIDS

Acquired Immunodeficiency Syndrome (AIDS), caused by Human Immunodeficiency Virus (HIV), is emerging as one of the most serious health problems of this century and the focus is shifting fast from the developed nations to developing countries and to India due to its vast populations. Presently 40 million (38-46 million) people living with HIV/AIDS and 5 million (4.2-5.8) people newly infected with HIV/AIDS and a total of 3 million (2.5-3.5 million) people died in 2003 because of AIDS. Presently India is estimated to have maximum number of HIV infected individuals (Anonymous, 2005).

A lot of researches have been done in the past and present in the modern medicine and no doubt they are very good Antiretroviral (ART) drugs are found but they have severe side effects. There are many Unani formulations composed of herbs and minerals and particularly gems, which are used as Muqawwi-e-aam (general body tonic) for the purpose of improving functions of vital organs, increasing Hararat-e-ghareezi (Metabolic heat) and Rooh (Pneuma) and for boosting the immune system. Qureshi *et.al.*(2008) have studied that the Unani polypharmaceuticals preparations Safoof Jawahar Mohra improves the immunity and suppresses the viraemia in AIDS patients. A review of literature indicates that a number of medicinal plant extracts, alkaloids, polyphenols, flavonoids etc have been therapeutically used for treatment of different stages of life cycle of HIV. These include: *Ancistrocladus korupensis* (Manfredi *et.al.*, 1991) and *Syzigium claviflorum* (Fujoka, and Kashiwada, 1994) without antioxidant activity.

## 2. Hepatitis

A global estimate indicates that there are about 18000 deaths every year because of the liver cirrhosis mainly caused by hepatitis. As a matter of fact there are no specific drugs in allopathy to deal with hepatitis where as in Unani medicine certain herbal drugs are proved to be highly efficacious in the treatment of hepatitis. However due to tremendous demand and scarcity the commercial samples of certain herbal drugs especially *Swertia chirata* are heavily adulterated with cheaper substituents (Sarin, 1996). Even though certain important formulations like Roghan-e-Kalan, Arq-e-Murakkab, Musaffi-e-khun, Majoon-e-juzam are prepared from *S. chirata*. A review of literature indicates that many plants extracts and phytochemicals such as polyphenols have been traditionally used for the treatment of different types of hepatitis. These includes *Milk thistle* and licorice root (Bean, P., 2002), *Cordyceps sinensis* (Jiang & Gao, 1995), Silybin (Liu *et.al.*, 2003), Dhiman and Chawala (2005) had reported that four commonly used herbal preparations 1. *Phyllanthus* (Karuna *et al.*, 2009) 2. *Silybum marianum* (*Milk thistle*) 3. Glycyrrhizin (licorice root extract) (Visavadiya *et al.*, 2009) 4. Liv-52 (mixture of herbs) (Jeyaprakash & Chinnaswamy, 2005). Liu. *et al.*, (2003) have reported that the combination of matrine (aqueous extract of radix *Sopharae flavescens* and interferon-alpha (IFN-alpha), thymosin or basic treatment showed better effects on viral and liver biochemical responses. Among all these plant and its extracts, only *Phyllanthus*, *Glycyrrhizin* and Liv-52 have antioxidant activity.

## Bronchitis

Chronic bronchitis is a major health problem in this age. For the management of chronic bronchitis there are a large number of antibiotics and bronchodilators with potent efficacy but they have many side effects related to hepatobiliary, digestive and microenzyme system. On the other hand Unani medicine uses a

large number of natural drugs, which not only cure this condition but also prevent recurrence with minimum or negligible unwanted effects. Kazmi *et al.*, (2008) have observed that two formulations Sharbat-e-Akseer-e-Sadar and Qurs-e-Zeequannfas improve chronic bronchitis without any unwanted effects. Kazmi *et.al* (2009) have reported that Unani drug UNIM-352 is effective in treating mild and moderate and severe type of bronchial asthma. These Unani drugs are not reported for its antioxidant activity.

### Diabetes

Diabetes is an important human ailment affecting many from various walks of life in different countries, including India, especially in urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are considered desirable due to lesser side effects and low cost (Madak *et. al.*, 2007). A review of literature indicates that some Unani formulations and a number of medicinal plants extract have been used for the treatment of diabetes. These includes *Gymnema sylvestre* (Mathew, 2007), *Nelumbo nucifera Gaertn* (Rafiullah *et. al.*, 2007; Wu *et al.*, 2003), UNIM-210 (Verma *et al.*, 2009), UNIM-211 (Verma *et al.*, 2009). Among all these drugs only *Nelumbo nucifera Gaertn* has antioxidant activity.

### Arthritis

Rheumatoid arthritis is a type of Wajaul Mafasil (Arthritis) which has become one of the most pressing public problems globally. It is estimated to be the 31<sup>st</sup> leading cause of non fatal burden in the world population (Anonymous, YNM). The prevalence in the general population is 0.5% to 1.0%, and women are two to three times' greater risk for developing the disease (Olsson *et al.*, 2007).

Tibb-e-Unani claims to possess a number of effective and safe drugs against arthritis. Despite the fact that arthritis requires a long term treatment, the drugs have not been found to cause major adverse effects. Many single and compound Unani drugs subjected to experimental and clinical studies have shown very promising results. These includes *Withania somnifera* (Begum *et. al.*, 1988), Suranjan-e-Talkh (*Colchicum luteum*) (Amin and Minhajuddin, 2009; Javed *et. al.*, 2005) and Habb-e-Gul-Aak (Masarrat *et. al.*, 2004).

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# Temperament as a Guiding Force for Treatment of Patients with Bronchial Asthma

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## Abstract

Herbal originated drugs of Unani System of Medicine are being prescribed since ages for the treatment of bronchial asthma. There is also a need of alternative findings with comparatively safe profile. In the present study the role of Temperament of patients on the outcome of treatment was analyzed. Total 576 patients were enrolled for the study during the period of three year, from the OPD of TB & chest diseases. Herbal drugs that are reported as mucolytic, anti-inflammatory, expectorant and bronchodilator property were included. Temperament of all the drugs were hot & dry. Temperament of the patients was assessed as per guide lines of TEN PARAMETERS<sup>-1</sup>. Lung function test was performed before and after the treatment to evaluate the patients. Two respiratory parameters, which were mainly observed, were Peak Expiratory Flow Rate (PEFR) and Forced Expiratory Volume in one second (FEV<sub>1</sub>). Both these parameters after the treatment showed improvement, but it was more marked in Phlegmatic (Balghami) & Sanguineous (damvi) patients which verifies the law of Unani medicine that for treatment drugs opposite to the temperament of patients should be chosen ..

**Key Words:** Unani Medicine, Bronchial asthma; Temperament.

## Introduction

As per earlier notion Bronchial Asthma was regarded as a simple airway obstruction. But after substantial improvement in the understanding of pathogenesis of Asthma it is viewed as an inflammatory illness with bronchial hyperactivity and bronchial spasm as a result. It is close to the concept given by one of the eminent physician of Unani Medicine as "It is narrowing of bronchial tree due to inflammation and accumulation of humour i.e. thick mucoid phlegm" and described its treatment with those drugs that may expectorate the viscid phlegm and relieve the spasm of bronchial tree<sup>2</sup>.

In Unani system of medicine Temperament is one of the central concept. It occupies an important place in defining physiochemical aspect of body, diagnosis and treatment. Human temperament are classified as sanguineous (Damvi), choleric (Safravi), phlegmatic (Balghami), melancholic (Saudavi) and qualities associated with them are hot & moist, hot & dry, cold & moist and cold & dry respectively. Plants and animals like human beings have also particular temperament since numerous plants are used in the formulation of drugs in Tibb they also possess temperament. Temperament of compound drugs is expressed in terms of qualities hot & moist, hot & dry, cold & moist, cold & dry.

According to Unani Concept drugs should be used after considering temperament of patient. The drug of a particular temperament should not be prescribed to a person having similar temperament because it could harm him. The drugs prescribed



should always be opposite the temperament of the diseased person i.e. hot against cold and cold against hot<sup>3</sup>.

So in order to scientifically validate the fact this study was carried out.

## Material and Methods

The present study was carried out in the Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh with the collaboration of Department of Tuberculosis and Chest Diseases during the period of 3 yrs from 2003 to 2006. A total number of 576 patients were enrolled for the study from OPD of T.B. and Chest Diseases. Pulmonary Function Test (PFT) was performed with the help of DT-Spiro Respirometer (Maestros Company, India) before and after drug therapy. Forced expiratory volume in one second (FEV<sub>1</sub>) and Peak expiratory flow rate (PEFR) were taken as important parameters of PFT to assess the severity of asthma. Temperament of patient was assessed according to Performa attached as annexure-1. As per protocol, in the inclusion criteria those patients, who has undergone PFT and showing airway obstruction with more than 15% increase in FEV<sub>1</sub> following administration of  $\beta$  agonist<sup>4</sup> and patients in the age groups of 15-70 yrs of either sex were enrolled. Patients having diabetes mellitus, myocardial infarction, acute asthma and patients of bronchial asthma on oral steroid therapy, pregnancy, severe hepatic or renal failure and aged more than 70 yrs were not included in the study.

### Preparation of Test Drugs

In the present study, 5 drugs of Unani system of medicine with known bronchodilator, anti-inflammatory, antihistaminic, leukotrien inhibitor and expectorant activities was selected (Table 1).

These drugs in a dose of 500 mg were combined in the following proportion. *Piper longum* (65 mg), *Adhatoda vasica* (95mg), *Picrorhiza kuroa* (5mg), *Hyssopus officinalis* (90mg) and *linum usitatissimum* (145mg). The above combination of drugs was manufactured by Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh, India and was ensured that the drugs were of good quality. All tablets were examined and tested for its shape, size and uniformity in weight (500 mg). Following tests were also performed for their stability: tablet dissolution test, tablet disintegration test, tablet friability, tablet hardness and shelf life. When the above tests were found within acceptable limits, these tablets were then used for present clinical trial. All tablets in a dose of 500 mg to 1gm were given orally thrice a day after meals. Spirometry for PFT of every patient was done at 0, 7, 15, 30 and 90th day. The change in FEV<sub>1</sub> and PEFR on 7, 15, 30 and 90 day in comparison to 0 day was considered as improvement in illness. For statistical analysis student's test was applied by using the SPSS software.

**Table-1. Unani drugs selected for clinical trial.**

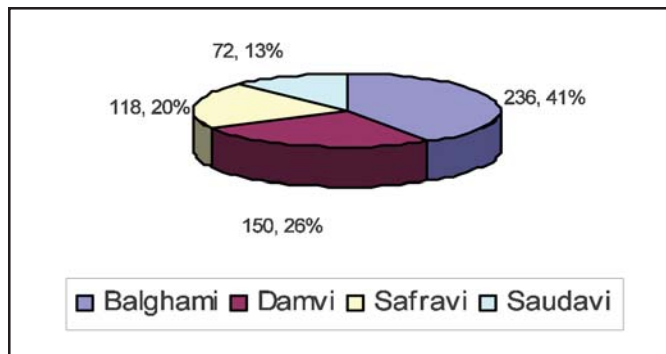
Plants name	Temperament	Pharmacological activities mentioned in modern literature
<i>Adhatoda vasica</i> Linn	Hot & Dry III <sup>o</sup>	Bronchodilator, expectorant, antihistaminic <sup>5-13</sup>
<i>Hyssopus officinalis</i>	Hot & Dry I <sup>o</sup>	Anti-inflammatory, expectorant, diuretic <sup>14-16</sup>
<i>Piper longum</i>	Hot & Dry III <sup>o</sup>	Antihistaminic, antiinflammatory, antitubercular, analgesic <sup>12, 15</sup>
<i>Picrorhiza kurroa</i>	Hot & Dry III <sup>o</sup>	Leukotrien inhibitor, hepatoprotective, antipyretic <sup>14,15-18</sup>
<i>Linum usitatissimum</i>	Hot & Dry I <sup>o</sup>	Anti-inflammatory, antispasmodic, expectorant, demulcent <sup>19</sup>

## Results and Discussion

In the present study 576 patients were enrolled and out of them 150 were of Damvi (Sanguineous) temperament 105 (70%) were male and 45 (30%) were female. 118 patients were of Safravi temperament 75 (64%) patients were male and 43 (36%) were female and 72 patients were of Saudavi temperament. Out of which 56 (77%) were male and 16 (23%) were female. Lastly 236 patients were found to be of Balghami temperament. Out of which 116 (49%) were male and 120 (51%) were female. It indicates that the number of males of all temperament are more prone to morbidity of asthma except in Balghami temperament in which it was equally prevalent in both the sexes. As shown in table 2 & graph-1.

**Table-2.**

Temperament	Total patients	Male	Female
Balghami	236	116(49%)	120(51%)
Damvi	150	105(70%)	45(30%)
Safravi	118	75(64%)	43(36%)
Saudavi	72	56(77%)	17(23%)



**Graph 1:** Showing distribution according to temperament

### *No of Patients Showing Improvement According to Temperament*

In the present study the total number of patients showing improvement and the degree of improvement according to temperament were also noted. Total number of patients which were of Balghami temperament and showed improvement in their respiratory parameters during follow-up on 7<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 90<sup>th</sup> day were counted. It was observed that maximum number of patients showed improvement in nearly all respiratory parameters after 90 days of treatment. The level of improvement was assessed in all respiratory parameters and mean standard deviation at '0' zero day and 90<sup>th</sup> day were calculated with percent improvement and significance level was also assessed as shown in Table 3&4, graph 2.

Similarly the number of patients showing improvement and the level of improvement in all respiratory parameters was also calculated for Safravi temperament as shown in Table 5, Table 6, graph 3 & for Saudavi temperament as shown in Table 7, Table 8 & graph 5 and for Damvi temperament as shown in Table 9, 10 & graph 6.

### **Balghami Patients**

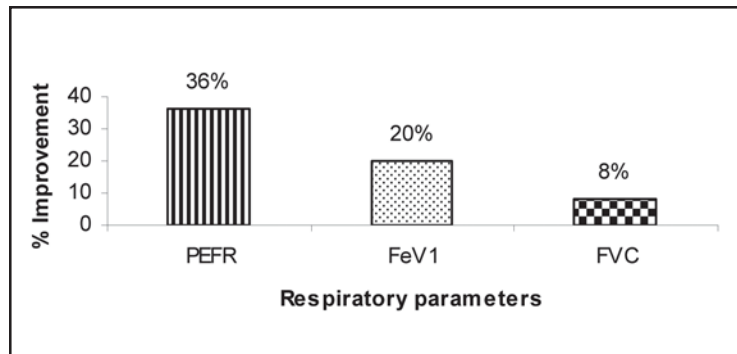
**Table-3. Number of Patients showing Improvement in Respiratory Parameters**

S.No.	Days	Parameters			Total No. of Patients
		PEFR	PEVI	FVC	
1.	7 <sup>th</sup> day	135 (65%)	130 (63%)	126 (61%)	N = 207
2.	15 <sup>th</sup> day	117 (66%)	113 (63%)	117 (53%)	N = 178
3.	30 <sup>th</sup> day	116 (73%)	107 (68%)	109 (68%)	N = 160
4.	90 <sup>th</sup> day	107 (89%)	88 (73%)	86 (72%)	N = 120

Note: Difference in number of patients on days of spirometry was due to fall out.

**Table-4. Showing Percentage Improvement in Respiratory Parameters following administration of asthma-5 for 90 days (n=120, m=58, f=62)**

S. No.	Parameters	Mean $\pm$ SD		% improvement	t-value	p-value	Significance level
		Day-0	Day-90				
1.	PEFR	4.16 $\pm$ 1.17	5.67 $\pm$ 0.83	36	-13.7	0.000	H.S.
2.	FEVI	2.3 $\pm$ 0.75	2.76 $\pm$ 0.87	20	-9	0.000	H.S.
3.	FVC	3.10 $\pm$ 1.8	3.35 $\pm$ 1.02	8	-2	0.048	S



**Graph 2:** Showing percentage improvement in respiratory parameters of Balghami patients after 90 days of treatment

### Damvi Patients

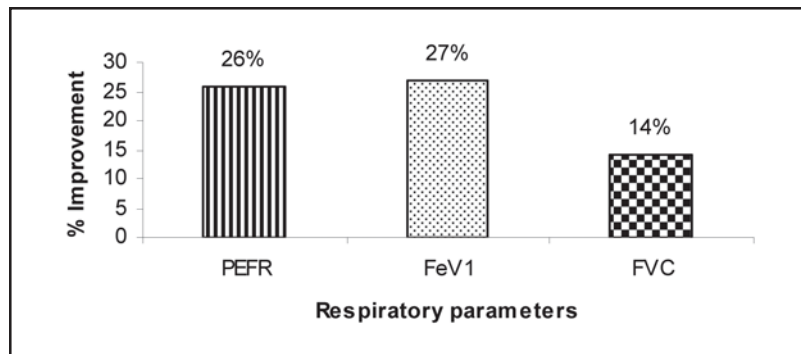
**Table-5. Number of Patients Showing Improvement in Respiratory Parameters**

S.No.	Days	Parameters			Total No. of Patients
		PEFR	PEVI	FVC	
1.	7 <sup>th</sup> day	83 (67%)	78 (63%)	61 (49%)	N = 124
2.	15 <sup>th</sup> day	79 (69%)	74 (65%)	62 (54%)	N = 114
3.	30 <sup>th</sup> day	70 (74%)	63 (67%)	53 (56%)	N = 94
4.	90 <sup>th</sup> day	70 (86%)	59 (73%)	47 (58%)	N = 81

Note: Difference in number of patients on days of spirometry was due to fall out.

**Table-6. Showing Percentage Improvement in Respiratory Parameters following administration of asthma-5 for 90 days (n=81, m=57, f=24)**

S. No.	Parameters	Mean $\pm$ SD		% improvement	t-value	p-value	Significance level
		Day-0	Day-90				
1.	PEFR	5.11 $\pm$ 1.66	6.65 $\pm$ 1.81	26	-12.8	0.000	H.S.
2.	FEV1	2.41 $\pm$ 0.82	3.06 $\pm$ 1.17	27	-4.9	0.000	H.S.
3.	FVC	3.19 $\pm$ 1.29	3.64 $\pm$ 1.23	14	-4.23	0.000	H.S.



**Graph 3:** Showing percent improvement in respiratory parameters of Damvi patients after 90 days of treatment

### Safravi Patients

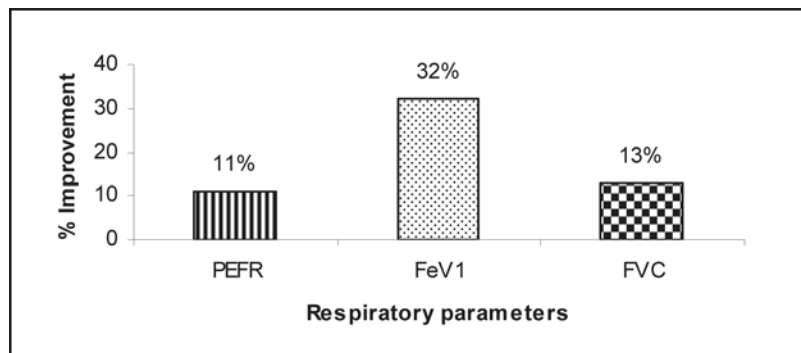
**Table-7. Number of Patients Showing Improvement in Respiratory Parameters**

S.No.	Days	Parameters			Total No. of Patients
		PEFR	PEVI	FVC	
1.	7 <sup>th</sup> day	62 (65%)	58 (61%)	50 (53%)	N = 95
2.	15 <sup>th</sup> day	60 (67%)	59 (66%)	60 (67%)	N = 90
3.	30 <sup>th</sup> day	51 (71%)	48 (67%)	49 (68%)	N = 72
4.	90 <sup>th</sup> day	44 (83%)	40 (75%)	38 (71%)	N = 53

Note: Difference in number of patients on days of spirometry was due to fall out.

**Table-8. Showing Percentage Improvement in Respiratory Parameters following administration of asthma-5 for 90 days (n=95, m=61, f=34)**

S. No.	Parameters	Mean $\pm$ SD		% improvement	t-value	p-value	Significance level
		Day-0	Day-90				
1.	PEFR	2.97 $\pm$ 0.83	3.31 $\pm$ 0.75	11	-3.8	0.003	S
2.	FEV1	2.17 $\pm$ 0.44	2.87 $\pm$ 0.93	32	-6.98	0.000	H.S.
3.	FVC	3.00 $\pm$ 0.85	3.38 $\pm$ 0.76	13	-3.56	0.001	S



**Graph 4:** Showing percent improvement in respiratory parameters of Safravi patients after 90 days of treatment

### Saudavi Patients

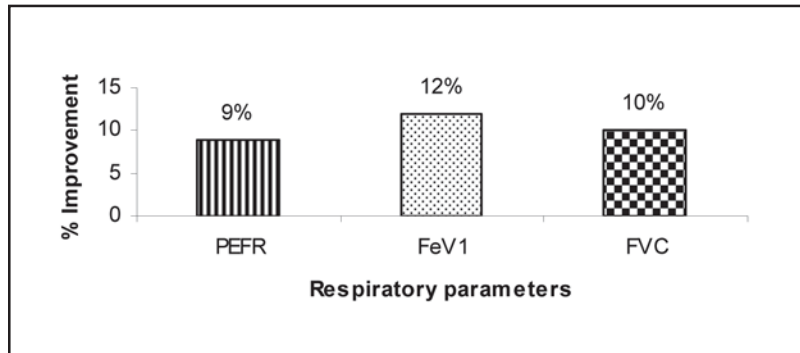
**Table-9. Number of Patients of Saudavi Temperament Showing Improvement in Respiratory Parameters**

S.No.	Days	Parameters			Total No. of Patients
		PEFR	PEVI	FVC	
1.	7 <sup>th</sup> day	38 (66%)	38 (66%)	39 (68%)	N = 58
2.	15 <sup>th</sup> day	26 (68%)	26 (68%)	27 (71%)	N = 38
3.	30 <sup>th</sup> day	22 (73%)	21 (70%)	22 (73%)	N = 30
4.	90 <sup>th</sup> day	17 (77%)	16 (73%)	17 (77%)	N = 22

Note: Difference in number of patients on days of spirometry was due to fall out.

**Table-10. Showing Percentage Improvement in Respiratory Parameters following administration of asthma-5 for 90 days (n=22, m=17, f=5)**

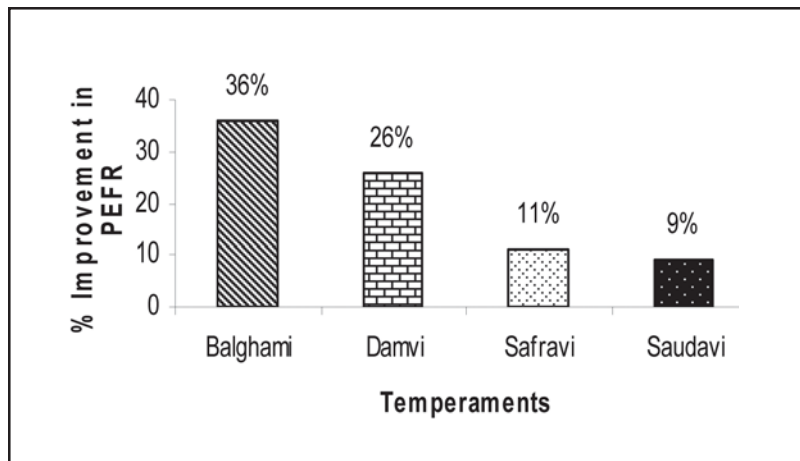
S. No.	Parameters	Mean $\pm$ SD		% improvement	t-value	p-value	Significance level
		Day-0	Day-90				
1.	PEFR	4.31 $\pm$ 1.38	4.68 $\pm$ 1.43	11	-6.68	0.000	H.S.
2.	FEV1	1.73 $\pm$ 0.47	1.94 $\pm$ 0.80	32	-2.6	0.015	S
3.	FVC	2.18 $\pm$ 0.50	2.39 $\pm$ 0.75	13	-3.77	0.001	S



**Graph 5:** Showing percent improvement in respiratory parameters of Saudavi patients after 90 days of treatment

### Improvement

Patients showing overall improvement after 90 days were also calculated according to temperament. And the level of improvement was also calculated as shown in Graph 6 & table 11.



**Graph 6:** Showing improvement according to temperaments

**Table-11. No of patients showing improvement in respiratory parameters according to temperament**

Temperament	Respiratory parameters			No.
	PEFR	FEVI	FVC	
Balghami	107 (89%)	88 (73%)	86 (72%)	N=120
Damvi	70 (86%)	59 (73%)	58 (71%)	N=81
Safravi	44 (83%)	40 (75%)	38 (71%)	N=63
Saudavi	17 (77%)	16 (73%)	17 (77%)	N=22

Note: Difference in number of patients on days of spirometry was due to fall out.

## Conclusion and Discussion

Out of total maximum numbers. of patients were of Balghami temperament (41%) and least were of Saudavi temperament (13%) which indicates that people of Balghami temperament were more susceptible to asthma as compared to other temperaments. It was also observed that prevalence of asthma in male (49%) and female (51%) subjects of balghami temperament were almost equal but in other temperaments males were more prone to morbidity of asthma. In Damvi & Saudavi the numbers. of males were just double than the no. of females suffering from asthma.

After oral administration of drugs for 90 days, the total no.of patients showing improvement were calculated and the level of improvement was also noted. The maximum no. of patients who showed improvement in PEFR & FVC were of Balghami and Damvi temperament (89%) & (86%), respectively. Similarly the level of improvement in all respiratory parameters were maximum in Balghami & Damvi temperament and all the drugs were of hot & dry temperament. They proved beneficial for the patients of Balghami (cold & moist) and which is exactly opposite in temperament.

It verifies the rule the drugs chosen should be exactly opposite to the temperament of person.

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# Traditional Phytoremedies in Health Care Among the Forest Ethnics of Balasore District, Orissa

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## Abstract

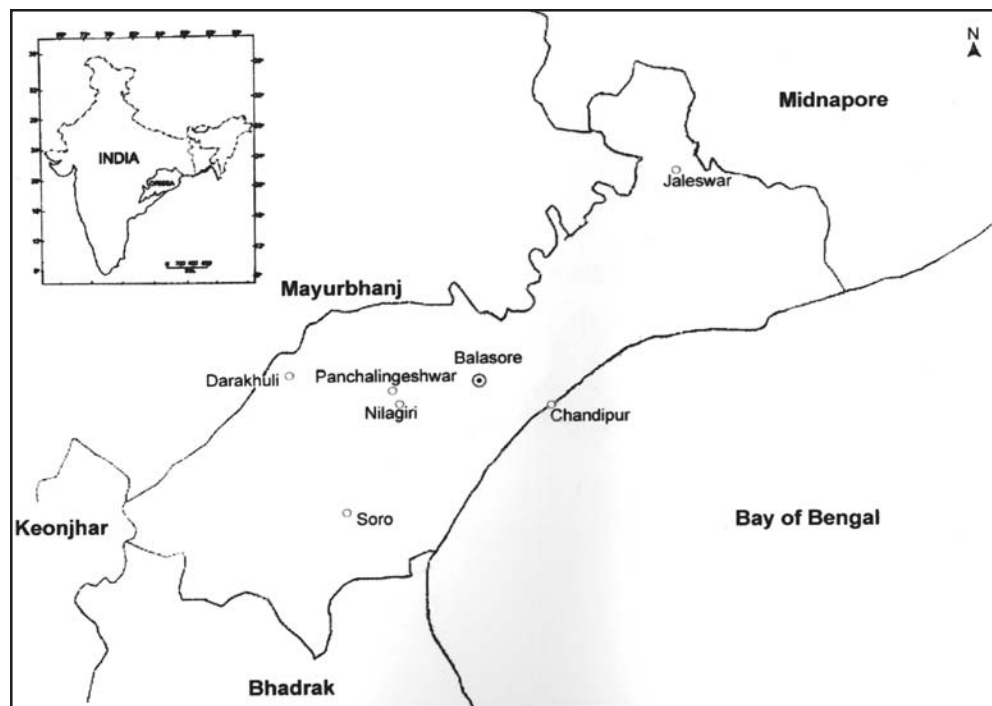
The district of Balasore forms a part of North-east Orissa. It has dense tracts of intact natural forests which are predominantly inhabited by various ethnic and cultural groups. An ethnobotanical survey was undertaken among the rural population of the area. In the course of this survey, drug preparations of herbal origin were found to be commonly employed by the traditional healers in health care. Based on this field study, present report deals with ethnomedicinal uses of 53 plant species belonging to 50 genera and 38 families. Each entry includes the information on correct scientific and prevalent local names, the part used, claimed medicinal use(s) and mode of administration. The study has revealed many unknown and interesting phytotherapeutic uses.

**Key Words:** Ethnobotanical survey, Herbal remedies, Balasore, Orissa.

## Introduction

The state of Orissa has an extensive forest area blessed with richly diverse flora and likewise a large population of tribals. A main fraction of this population remains dependent on ancestral plant knowledge for health care. In the state, much ethnobotanical research work has been done during last few decades and as a result the voluminous literature dealing with folk medicinal uses of various native floras has been published. Some notable contributions to this field have been made by Aminuddin and Girach (1991, 1996); Anonymous (2001); Brahman and Saxena (1990); Choudhury and Mahapatra (2005); Das and Misra (1987); Das *et al.*, (1996); Girach (1992); Girach and Aminuddin (2000); Girach *et al.*, (1994, 1996, 1998); Malik (1996); Mohanty *et al.*, (1996); Mudgal and Pal (1980); Mukherjee and Namhata (1990); Panda *et al.*, (2005); Pattanaik *et al.*, (2005); Saxena and Dutta (1975); Saxena *et al.*, (1981); Sen and Pradhan (1999); Singh and Dhar (1993); Srivastava and Rout (1994); Tribedi *et al.*, (1982), etc. No separate report, however, exists on the plants which are in therapeutic use among the tribal people of Balasore district. Hence, the present paper communicates an account of 53 plant species belonging to 50 genera and 38 families that are traditionally used by local healers of this area as folk drugs for treatment of various diseases and conditions of humans and cattle. The study represents a contribution on our present knowledge on the contemporary herbal pharmacopoeia of the tribals of Orissa.

Balasore district is situated between 21° 03' - 21° 59' N latitude and 86° 20' - 87° 29' E longitude in the northeastern part of Orissa. It is bounded by Midnapur district of West Bengal in north, Bhadrak district in the south, Mayurbhanj and Keonjhar districts in the west and Bay of Bengal in the east (Fig.1). This coastal district is known for its beautiful mountains, famous temples and extraordinary beaches. There are a few hill ranges in the northwestern part of the district which have significant tracts of natural forest and a variety of wildlife. The forests are mainly of



**Fig. 1.** Study area: Balasore district, Orissa, India.

tropical semi-evergreen type. Tribal populations of the district are mostly found in this region. Santhal, Munda, Bhumija, Bathuri, Kolho, Khandait are the predominant tribes in the area. Some specific tribal settlements visited include Ajodhya, Aravand, Asteruki, Bholadangar, Darakhuli, Gobalpala, Hathikot, Jodiwali, Mitrapur, Motgura, Naranpur, Notopara, Sajanagarh, Tenda, Tinkusia, Saganaria and Oupada located in Nilagiri Sub-division of the district.

## Methodology

Fieldwork was carried out in January, 2009. Information on medicinal uses of plants was obtained through interviews with reliable informants who were local healers and knowledgeable village elders. Data on local name of the plant, medicinal use(s), part used, other ingredients added (if any), method of drug preparation and mode of administration were recorded for each claim. Materials were collected and plant species were later identified by the senior author with the help of related floras (Haines, 1921-1925; Mooney, 1950; Saxena and Brahmam, 1994). All voucher herbarium specimens have been deposited in the herbarium of the Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Bhadrak (Orissa), India.

## Observations

In the following enumeration medicinal plants are listed alphabetically by their scientific names. Each entry gives information on correct botanical name, family (in

parenthesis), local name, locality, voucher specimen number and use(s). As far as possible, the probable dosage and duration of these crude drugs are also given.

*Abrus precatorius* L. (Fabaceae), *Runjo*, Sardigh (ZAA8457). Seeds of red variety are given orally to the woman as an abortifacient. The seeds of white variety are burnt to ashes and finely powdered. This is applied in the eyes of cow for excess lacrimation.

*Aerva lanata* (L.) A. Juss. ex Schult. (Amaranthaceae), *Dhora Kaphsa*, Tenda (ZAA8475). The paste of whole plant, obtained by crushing, is given in a dose of 10g three times a day for one month. It is claimed to dissolve and expel small pieces of kidney stones.

*Ailanthus excelsa* Roxb. (Simaroubaceae), *Mahalo*, Mitrapur (ZAA8515). Powdered stem bark is mixed with fodder and given to cattle in general weakness.

*Argemone mexicana* L. (Papaveraceae), *Akranti*, Nilagiri (ZAA8487). The paste of the root in the dose of about 10g is given orally two to three times a day for 10 days to treat eczema.

*Asparagus racemosus* Willd. (Liliaceae), *Gaicheera*, Sardigh (ZAA8409). A root paste (20g), prepared by grinding the fresh root in water, is given to the patient twice daily for two to three week in cases of leucorrhoea and spermatorrhoea.

*Blumea lacera* (Burm. f.) DC. (Asteraceae), Nilagiri, *Poksunga* (ZAA8403). The paste of the fresh leaf is applied to the affected parts in eczema.

*Boerhavia diffusa* L. (Nyctaginaceae), *Khaprasago*, Darakhuli (ZAA8526). Cooked leaves are taken daily for improving eye vision.

*Bombax ceiba* L. (Bombacaceae), *Simli*, Naranpur (ZAA8504). The tap root of the young plant is cut into small pieces and soaked in an earthen pot and left overnight. This infusion is taken once every morning for 15 days consecutively for spermatorrhoea.

*Canavalia gladiata* (Jacq.) DC. (Fabaceae), *Kathsem*, Oupada (ZAA8599). Seeds are cooked and taken for constipation.

*Careya arborea* Roxb. (Barringtoniaceae), *Kumbha*, Saganaria (ZAA8417). Two to three spoons of the juice of freshly crushed stem bark are given twice daily for one month to treat joint pain. In another claim, the paste of stem bark is plastered around the fractured limb after setting the bones right, splints and bandage are used to hold the bone and plaster in position.

*Cassia alata* L. (Caesalpiniaceae), *Dadmari*, Nilagiri (ZAA8532). A freshly made paste of the leaves, obtained by crushing, is applied externally on ringworm and scabies.

*Celastrus paniculatus* Willd. (Celastraceae), *Kujri*, Tinkusia (ZAA8460). The oil, obtained by crushing the dried seeds, is applied locally with light massage to treat

muscular pain. It is also applied on horns of cow for excessive watery secretion from the nose and eyes.

*Cissampelos pareira* L. (Menispermaceae), *Akalbindi*, Motgura (ZAA8516). Leaf paste as poultice is applied on boil.

*Cissus quadrangula* L. (Vitaceae), *Harbhanga*, Tenda (ZAA8472). The paste of fresh stem mixed with little alum is used as plaster for treating bone fracture.

*Clausena excavata* Burm. f. (Rutaceae), *Agnijal*, Raipaal (ZAA8556). The leaf paste is applied to the shoulder of oxen to treat sores caused by yoke.

*Cleistanthus collinus* (Roxb.) Benth. ex Hook. f. (Euphorbiaceae), *Paransi*, Saganaria (ZAA8404). The leaf paste is applied externally to treat dhobie-itch during rainy season.

*Crotalaria pallida* Ait. (Fabaceae), *Nirmishi*, Ajodhya (ZAA8441). Fresh leaf paste is applied around the wound as an antiseptic.

*Croton bonplandianum* Baill. (Euphorbiaceae), *Banmirach*, Kendukhatta (ZAA8449). Latex of the plant is poured on fresh cut. For treating sprain, fresh paste of the stem bark is applied to the affected part using a bandage.

*Cryptolepis buchanani* Roem. & Schult. (Periplocaceae), *Uttardudhi*, Tinkusia (ZAA8466). Root is mixed with *harbhanga* (dried stem of *C. quadrangula*) and turmeric in equal quantities, then ground and used as plaster for bone fracture.

*Diospyros melanoxylum* Roxb. (Ebenaceae), *Kendu*, Saganaria (ZAA8419). Pulp of the ripe fruit is taken as laxative.

*Elephantopus scaber* L. (Asteraceae), *Najarchura*, Hathikot (ZAA8416). About 10g of the root paste are given orally three times a day for paralytic condition.

*Euphorbia hirta* L. (Euphorbiaceae), *Dudhi*, Darakhuli (ZAA8525). Fresh latex of the plant is applied in the eyes for redness.

*Flacourtia indica* (Burm. f.) Merr. (Flacourtiaceae), *Goinchhokoli*, Nilagiri (ZAA8522). Two spoons of the root paste are given with water three times a day for 3 days to treat loose motion.

*Holarrhena pubescens* (Buch.- Ham.) Wall. ex G. Don (Apocynaceae), *Kuring*, Hathikot (ZAA8414). The paste of the seed is administered orally for worm infestation.

*Hygrophila auriculata* (Schum.) Heine (Acanthaceae), *Koilrukha*, Saganaria (ZAA8404). About two spoonful of the soaked seeds are taken daily in the morning for spermatorrhoea.

*Justicia adhatoda* L. (Acanthaceae), *Basang*, Ajodhya (ZAA8438). Leaf juice is given with honey for cough.

*Kalanchoe pinnata* (Lam.) Pers. (Crassulaceae), *Amarpoi*, Kendukhatta (ZAA8445). About one cup aqueous decoction is taken daily for controlling diabetes. It is also given for diarrhoea in children.

*Kirganelia reticulata* (Poir.) Baill. (Euphorbiaceae), *Jandaki*, Tenda (ZAA8490). Tender twig is made into toothbrush and used daily to prevent tooth decay.

*Lygodium flexuosum* (L.) Sw. (Lygodiaceae), *Naginocha*, Saganaria (ZAA8515). Whole plant is crushed and made into a paste. About 20g of this paste are given once daily for two to three week to treat amenorrhea.

*Madhuca longifolia* (Koenig) McBride (Sapotaceae), *Mohulo*, Asteruki (ZAA8413). For treating typhoid fever, the decoction of 25g powder of the stem bark is given twice a day till the cure is obtained.

*Mimusops elengi* L. (Sapotaceae), *Bakoli*, Nilagiri (ZAA8408). Paste of the kernel is given for diarrhoea and dysentery in children.

*Nyctanthes arbor-tristis* L. (Oleaceae), *Singarhar*, Mitrapur (ZAA8518). A decoction of 20-30 leaves mixed with powder of few grains of black pepper is given twice a day for 7 days to treat malarial fever.

*Nymphaea pubescens* Willd. (Nymphaeaceae), *Nalikain*, Hathikot (ZAA8405). The paste of the tuber (20g) is given twice a day for one month for treating leucorrhoea.

*Ocimum basilicum* L. (Lamiaceae), *Tulsa*, Nilagiri (ZAA 8570). Leaf juice is used as an ear drop for earache.

*Oroxylum indicum* (L.) Vent. (Bignoniaceae), *Pharnphora*, Gobalpala (ZAA8541). For treating headache, fresh juice of the stem bark is applied on forehead.

*Pergularia daemia* (Forsk.) Chiov. (Asclepiadaceae), *Utradhi*, Motgura (ZAA8469). One teaspoonful fresh leaf juice is given with honey twice a day for 3 days to treat cough.

*Phyllanthus fraternus* Webster (Euphorbiaceae), *Baarianwla*, Darakhuli (ZAA8527). The paste of the whole plant is given orally two to three times a day for 21 days to treat jaundice.

*Pongamia pinnata* (L.) Pierre (Fabaceae), *Karanjo*, Hathikot (ZAA8432). Seed oil is applied on dry and rough skin. It is also applied externally to keep the body warm, particularly in rainy season.

*Ricinus communis* L. (Euphorbiaceae), *Jada*, Mitrapur (ZAA8449). Seed oil is applied on navel of children for constipation.

*Sansevieria cylindrica* Bojer (Agavaceae), *Mirisanp*, Motgura (ZAA8474). For treating snake bite, root paste is given with water, until the patient develops signs of relief.

*Sansevieria roxburghiana* Schult. f. (Agavaceae), *Gondchitti*, Tenda (ZAA8473). Root paste is given after delivery as a prophylactic drug.



*Sapindus laurifolius* Vahl. (Sapindaceae), *Rithaphal*, Baragudhi (ZAA8506). Fruits mixed with *neembo* (leaf of *Azadirachta indica* A. Juss.) are ground with water. This preparation is applied on scalp to kill lice.

*Semecarpus anacardium* L. f. (Anacardiaceae), *Bhalia*, Hathikot (ZAA 8431). Crushed seeds are boiled in mustard oil and filtered. It is allowed to cool and applied over the hoofs in hoof rots in cases of cow and goat.

*Shorea robusta* Gaertn. f. (Dipterocarpaceae), *Sarjam*, Tinkusia (ZAA8462). Dried oleo-gum resin is powdered and applied on fresh cuts. Tender twig is made into toothbrush and used daily for strengthen gums and teeth.

*Smilax perfoliata* Lour. (Smilacaceae), *Ramnatni*, Saganaria (ZAA8403). Tender twig is used as toothbrush for dental care.

*Smilax wightii* DC. (Smilacaceae), *Ramdatun*, Tinkusia (ZAA8403). Aqueous decoction of the root is given as an aphrodisiac. Tender stem is also used as toothbrush for oral hygiene.

*Streblus asper* Lour. (Moraceae), *Shewra*, Tenda (ZAA8578). Decoction of stem bark is given for common fever. Tender twig is used as toothbrush to treat toothache.

*Strychnos nux-vomica* L. (Loganiaceae), *Kuchila*, Nilagiri (ZAA8401). Paste of the stem bark is applied on abdomen for stomachache.

*Torenia violacea* (Azalo ex Blanco) Pennell (Scrophulariaceae), *Baghua*, Jalesar (ZAA8589). Leaf paste is applied on cuts and wounds.

*Tragia involucrata* L. (Euphorbiaceae), *Bichhati*, Nilagiri (ZAA8488). A paste of the seeds is applied on alopecia.

*Vernonia albicans* DC. (Asteraceae), *Pittaphori*, Tinkusia (ZAA8556). Leaves are ground and made into pills of pea size with solidified sugarcane juice; three to five pills are given with water twice daily for 3 consecutive days to treat worm infestation.

*Vitex negundo* L. (Verbenaceae), *Begunia*, Motgura (ZAA8420). Leaves are used as poultice on inflamed body parts.

*Woodfordia fruticosa* (L.) Kurz (Lythraceae), *Dhatingphulo*, Saganaria (ZAA8408). Dried flowers mixed with *tirphala* (fruits of *Phyllanthus emblica* L., *Terminalia bellirica* (Gaertn.) Roxb. and *T. Chebula* (Gaertn.) Retz.), in equal quantities are ground together. About 20g of this preparation are given with water two times a day for two week in spermatorrhoea.

## Discussion

This paper provides a report on folk medicinal uses of 53 plant species revealed by the tribal people of the Balasore forests. The uses were mostly related to the disorder of digestive, integumentary, musculoskeletal and urino-genital systems. It

was also observed that eczema, scabies, diarrhoea, dysentery and worm infestation were more prevalent complaints, probably due to lack of hygienic care among the villagers. A comparison of the data with relevant literature (Agarwal, 1986; Ambasta, 1986; Anonymous, 2001; Chopra *et al.*, 1956; Jain, 1991; Kirtikar and Basu, 1935; Nadkarni, 1954; Satyavati *et al.*, 1976, 1987) revealed that uses of various plants viz., *Abrus precatorius*, *Bombax ceiba*, *Cassia alata*, *Cissus quadrangula*, *Croton bonplandianum*, *Euphorbia hirta*, *Hygrophila auriculata*, *Justicia adhatoda*, *Kalanchoe pinnata*, *Kirganelia reticulata*, *Nyctanthes arbor-tristis*, *Ocimum basilicum*, *Pergularia daemia*, *Shorea robusta*, *Smilax perfoliata*, *S. zeylanica*, etc. were similar to those already published. But many of these usages were differ either on the part utilized, or on the method of administration or on the type of disease/condition. For other plants, the usages seem to be recorded for the first-time. Majority of medicinal plants are wild, although, a few are cultivated or are weed in waste ground near villages. By and large, importance is given to drugs prepared from the fresh plant material. Generally, an ethnomedicinal preparation consists of a single plant. However, in some cases the final recipe may contain ingredients from more than one species. Moreover, the curative properties of a particular plant may cover more than one disease state or a single disease may be treated with many plant species.

Traditional knowledge of folk medicine is usually hold only by few elderly people who are specialists recognized by the culture as having spiritual power to promote health. These individuals represent a disappearing tradition which is not being passed on to the next generation. Similarly there is a threat to some of the forest species of medicinal importance due to deforestation as a result of expansion of agriculture and dwellings, excessive grazing, forest fire, over exploitation of natural resources, etc. In this situation the forest land in many places has considerably reduced for wild plants to spread naturally. This specialized ethnobotanical knowledge is in danger of being lost permanently. Therefore, intensification of ethnobotanical research work is urgently needed in other ethnobotanically unexplored or under explored regions of the state. This could lead to more medicinal plants and their medicinal uses being revealed and utilized for well being of mankind before these useful plants become extinct or their uses forgotten because of unavailability.

The aim of this study is to highlight information on traditional phytotherapy which may provide ethnobotanical leads in search for new and affordable pharmaceuticals of natural origin. As ethnobotanical leads have led to the discovery of many useful drugs which are in wide modern usages (Jain, 1991; Chadwick and Marsh, 1994).

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# Histo – Pharmacognostic Studies of *Parthenium hysterophorus* Linn. Used in Homoeopathy

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## Abstract

*Parthenium hysterophorus* Linn. (family – Asteraceae), used as a tonic, febrifuge, emmenagogue, analgesic in neuralgia and useful particularly in allergic conditions. It can be characterized by the presence of longitudinal grooves on the stem; irregularly dissected, pubescent leaf; white axillary or terminal flower heads of 5 mm diameter; glandular and non-glandular trichomes on the leaf and stem surfaces; anomocytic stomata, stomatal index 24.5, palisade ratio 4-5, veinlet number 22-28 per sq.mm; vascular bundles encapped by sclerenchyma; roots having secretory canals and funnel shaped medullary rays; presence of alkaloid, lignin, suberin, saponin, carbohydrate & steroid and absence of tannin and flavone. Based on these characters, the study will help in the correct identification of this important homoeopathic drug.

**Key Words:** *Parthenium hysterophorus*, Histo-Pharmacognostic, Anti-allergic, Homoeopathy.

## Introduction

*Parthenium hysterophorus* Linn. is an aggressive annual weed, commonly known as “Congress grass”, and native of southern and central America. In 1956, it was accidentally introduced in India as a contaminate of imported wheat under PL-480 and detected for the first in Poona and adjoining regions but now it has spread and established all over the country.

The plant contains a good number of sesquiterpene lactones. Major one of them is Parthenin (Parthenicin, C<sub>15</sub>H<sub>18</sub>O<sub>4</sub>; m.p. 163-166<sup>0</sup>), second major one is coronopilin. Other sesquiterpene lactones are tetraneurin-A and hysterophorine; others reported in small quantities are ambrosin, gysterin and dihydroisoparthenin (Picman, 1982). Leaves are reported to contain parthenin, hexacosanol, myricyl alcohol, B-sitosterol, campesterol, stigmasterol, betulin, ursolic acid, β-D glucoside of β-sitosterol and a saponin which on hydrolysis yields oleanolic acid and glucose, galactose and 4.8% potassium chloride (Gupta, 1977).

The plant causes allergic contact dermatitis (Sharma & Kaur, 1990). The allergic properties have been attributed to the major and minor sesquiterpene lactones present in this plant. Active principle isolated by fractionating over an ambertile resin was used for preparation of anti-allergic formulation (injection) for hypersensitization of patients suffering from “Congress grass” allergy (Jain, 1975).

The plant is used as a tonic, febrifuge, emmenagogue antiallergic and as an analgesic in neuralgia (W.I., 1976). In Homoeopathic system of medicine it is used as a remedy against allergy, amenorrhoea, dyspepsia, ear affections, toothache, vision disorder, liver & spleen pain, periodic neuralgia. It is also employed in the relief of malarial fever in tropics,. (Blackwood, 1959; Clarke, 1955). A decoction of the root

is given in dysentery. Flowers and leaves are used for inflammation, eczema and skin rashes in Barbados; for harpes and rheumatic pain in Guadeloupe; also used for skin eruption in Guyana; used to prepare a decoction for cold and to make a bath for flees on dog, bush bath and treatment of wounds; as a remedy against headache, ulcerate sores, muscular rheumatism in Mexico; for muscular strains, analgesic, vermifuge and heart troubles in Virgin island of U.S. (Tower, 1977).

Parthenin, the major sesquiterpene lactone of the plant, exhibit significant anti-malarial activity against a multi drug resistant strain of *Plasmodium falciparum* (Hooper, 1990).

## Materials and Methods

The plant was collected locally from various places at Ghaziabad, U.P. Usual hand sections were made for anatomical studies and for anatomical quantification, leaves were bleached with lactic acid. For anatomical characterization, Esau (1960) and Wallis (1967); for powder analysis methods suggested by Jackson & Snowdon (1968) were followed; for chemical analysis methods suggested by Johansen (1940), Youngken (1957), and Trease and Evans (1972) were followed. I.P. (1970) was consulted for determination of physical characters; colours were named by consulting "Indian standard colours for ready mixed paints and enamels" (1984).

## Results and Observations

### Drug Evaluation

#### (i) Macroscopical Evaluation

A much branched herb (fig.1), upto 1m in height; stem longitudinally grooved, diffusely branched. Leaves irregularly dissected, pubescent. Flower heads small, 5mm in diameter, white or very light yellow, in terminal or axillary often leafy inflorescence; ray florets fertile minute, white or light yellow, small, pistillate with bifid stigma; disc florets sterile, tubular with anther at the base of corolla, style of disc florets undivided. Both the florets subtended by innermost series of 2-4 series of broad, dry, herbaceous, involucre bracts. Achene broadly ovoid, dark brown, less than 2mm long.

#### (ii) Microscopical Evaluation

##### (a) Histology

Leaf: Transection shows single layered epidermis with thin cuticle, anomocytic stomata (fig. 4E) and covered with glandular and non-glandular trichomes. Glandular



**Fig. 1.** *Parthenium hysterophorus* Linn. (whole plant).

trichomes are of 3 types- (i) uniseriate, multicellular, (fig. 4B), (ii) bicelled (fig. 4C) and (iii) biseriate, multicellular (fig. 4d); each type having distended, unicellular, thin walled, secretory head. Non-glandular trichomes also are of three types – (i) Thick walled, uniseriate, 2-5 celled, with unicellular base and pointed apex (fig. 4F), (ii) small, uniseriate multicellular with unicellular basal cell and long terminal cell (fig. 4G), (iii) thick walled, uniseriate, multicellular with shriveled intermediate cell and unicellular base (fig. 4H).

The lamina dorsiventral, mesophyll differentiated in to single layer of palisade and 4 to 6 layers of spongy parenchyma (fig. 2). Midrib region prominently bulging towards the lower side, shows single layered epidermis followed by 1-2 layers of collenchyma on the lower side and 2-3 layers below the upper side; palisade discontinuous in this region; meristele consists of three vascular bundles embedded in ground tissue with central bundle larger than the lateral ones, each bundle consisting of xylem towards upper side and phloem towards the lower side and encapped by sclerenchymatous cells. (fig. 3).



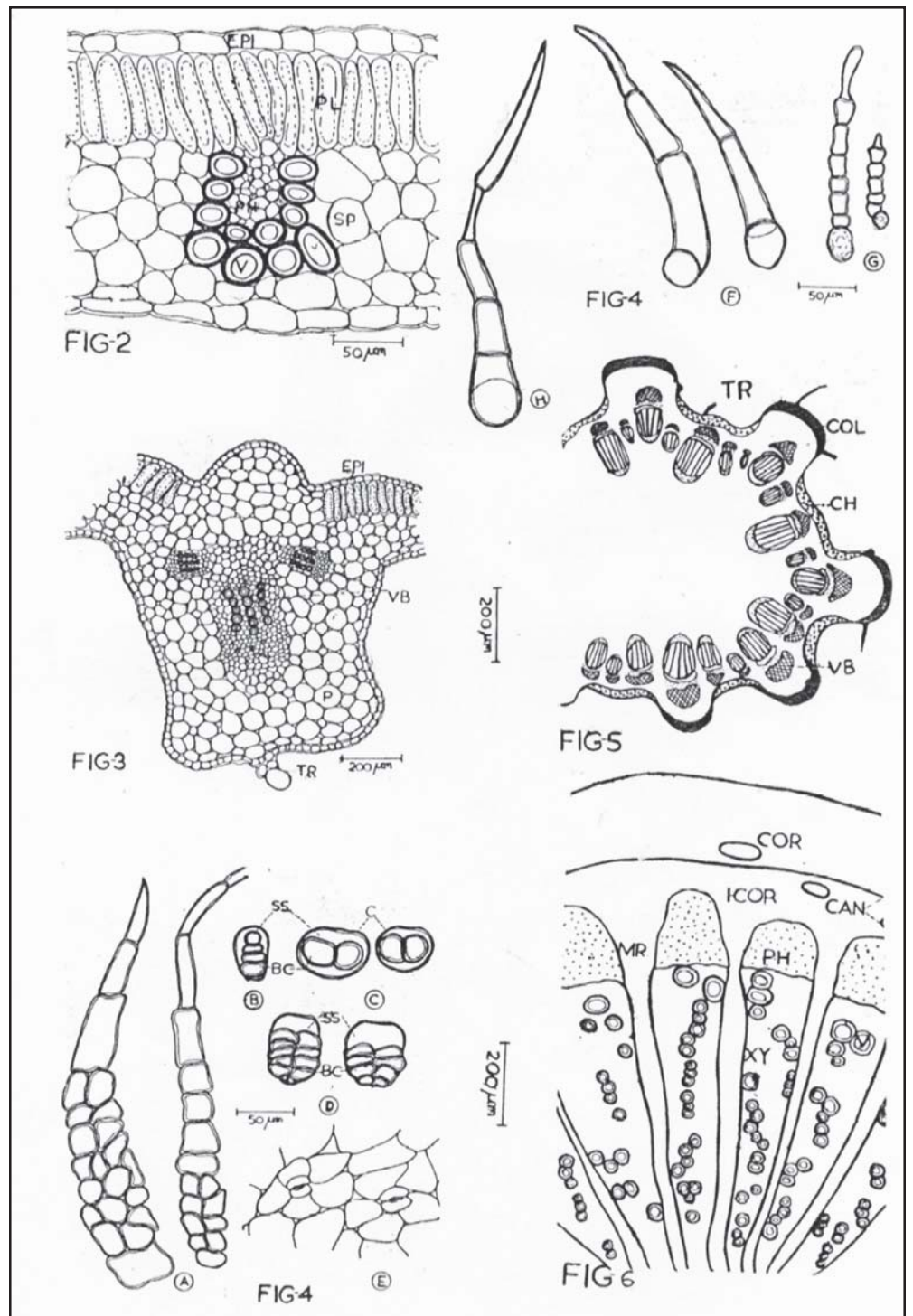


Fig. 2. T.S. of Lamina.

Fig. 3. T.S. of leaf through midrib.

Fig. 4. Trichomes and stomata. A: Non glandular trichomes with multicellular base. B-D: Glandular trichomes. E: Anomocytic stomata in surface view. F-H: Non glandular trichomes.

Fig. 5. T.S. of stem (Diagrammatic representation).

Fig. 6. T.S. of root (Diagrammatic representation).

Palisade ratio- 4 to 5 per epidermal cell.

Vein islet No.- 22 to 28 per sq. mm.

Stomatal Index- 24.5.

**Stem:** In transverse section, almost circular in outline with ridges and furrows (fig. 5), and shows single layer of epidermis consisting of oval, tangentially flattened cells with thin cuticle and trichomes; trichomes long, thick walled, uniseriate, multicellular with pointed apex and biseriate to triseriate, multicellular base (fig. 4,A) along with glandular and non glandular trichomes as has been described in leaf; epidermis followed by collenchyma at ridges and chlorenchyma at furrows; cortex parenchymatous consists of a few layers of thin walled, oval or rounded large cells; vascular bundles are conjoint, collateral, open, and encapped by sclerenchymatous sheath; and are of different sizes and arranged in a ring. Xylem consists of radially arranged vessels. Pith having large polygonal parenchymatous cells and occupying major portion of stem.

**Root:** Epidermis single layered of tangentially elongated cells; cortex having thin walled parenchymatous cells and secretory canals and differentiated into outer and inner cortex by a continuous, well defined layer of parenchyma; stele occupied 2/3<sup>rd</sup> of the space (fig. 6); xylem and phloem in a close cylinder traversed by medullary rays; medullary rays are broad multiseriate, thin walled parenchymatous, funnel shaped and merge with the inner cortex (fig. 6); phloem consists of sieve tubes, companion cells and phloem parenchyma; xylem large, vessels circular or polygonal and show reticulate and scalariform thickenings. Pith absent.

#### (b) Powder analysis

Powder drug is yellowish in colour; odour herbal, not characteristic; taste bitter. Microscopical analysis reveals vessels with reticulate and scalariform thickenings, simple pits, thin-walled parenchymatous cells; leaf fragments with vein islets, epidermal cells, anomocytic stomata, palisade cells; glandular & non-glandular trichomes and a few ducts.

#### (iii) Chemical Analysis

##### (a) Phytochemical Tests

Colour reaction tests suggested the presence of Alkaloids, carbohydrates, Glycosides, Lignin, Suberin, Saponin and Steroids and absence of Tanin and Flavones. (Table-1)

##### (b) TLC (Thin layer chromatography)

TLC observations show the number of chemical compounds. The four different colour spots are found. These are shown in Table-2.



**Table-1. Phytochemical Tests (Preliminary colour Reaction test)**

S.No.	Reagent	Test Performed	Result
1.	Dragendorff's reagent	Alkaloids	+ve
2.	Phloroglucinol + HCl	Lignin	+ve
3.	FeCl <sub>3</sub>	Tannin	-ve
4.	Molish test	Carbohydrates	+ve
5.	Heating with strong KOH + H <sub>2</sub> SO <sub>4</sub>	Suberin	+ve
6.	Molish test after hydrolysis	Glycosides	+ve
7.	Alc. ext. + Acetic anhydride + H <sub>2</sub> SO <sub>4</sub>	Saponin	+ve
8.	Mg powder + Conc. HCl	Flavones	-ve
9.	Liebermann + Conc. HCl	Steroids	+ve
10.	Sudan IV	Oils	+ve

**Table-2. Rf Values (Mobile phase – CHCl<sub>3</sub> : CH<sub>3</sub>OH; 9:3 v/v)**

Colour of spots	Rf values
1. Green	0.48
2. Bluish green	0.57
3. Orange	0.17
4. Orange red	0.15

#### (iv) Physical Analysis

##### (a) Fluorescence behaviour

Various fluorescence behaviour are tabulated in Table-3.

##### (b) Extractive values

The powder of plant was subjected to successive extraction with different solvents of increasing polarity and results obtained are tabulated in Table-4.

(c) Ash value: Total ash determined of powder is 16%.

(d) Total solids : 0.2020 – 0.2090%

(e) Specific gravity: 0.9973 – 0.9976

(f) pH (at 25°C) : 5.2 to 5.4

**Table-3. Flourescence behaviour of plant powder.**

S.No.	Material taken	Colour in day light	Colour in fluorescence light
1.	Powder as such	Yellowish green	Light green
2.	Powder rubbed in filter paper	Light olive green	Pale green
3.	Aqueous extract of powder	Light brown	Apple brown
4.	Alcoholic extract of powder	Olive brown	Light brown green

**Table-4. Extractive values of *Parthenium hysterophorous* Linn.**

S.No.	Reagents	Values (%)
1.	Ethyl Alcohol 30% 50% 70% 90%	34 29 28 16
2.	Acetone	5
3.	Benzene	5
4.	Petroleum Ether	1
5.	Water	25
6.	Chloroform	5

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# Ingredient Identification in Habb-e-Narmushk – Quality Control Strategies

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## Abstract

In the past few decades traditional system of medicine have been gaining interest and acceptance among the masses. The plant materials and herbal drugs derived from them represent a substantial proportion of the current global market. In this scenario, there is a need to ensure the quality of the herbal preparations. The only way to ensure that the herbal drugs and preparations made with them achieve optimum and consistent quality is to create and maintain a comprehensive quality assurance system. Most of the herbal formulations, especially the classical formulations are polyherbal. Ingredient identification of these polyherbal formulations is indispensable not only to ensure the reproducible therapeutic efficacy but also to ensure the safety. Present communication deals with the ingredient identification in Habb-e-Narmushk which is considered as purgative and carminative in unani system of medicine. All the ingredients which are required in the preparation are examined separately (both macroscopically and microscopically) followed by the microscopic examination of the formulation as a whole. This will provide a key of diagnostic histological characters which serves as an important tool in laying down the standards for quality assurance.

**Key Words:** Habb-e-Narmushk, Quality control, Traditional systems of medicine.

## Introduction

Quality of herbal drugs is a burning issue now a days. A number of classical formulation that are used in unani system of medicine are based on the drugs of plant origin. The only way to ensure that herbal drugs and preparations made with them achieve optimum and consistent quality is to create and maintain a comprehensive quality assurance system. Ingredient identification in a polyherbal formulation is the foremost step in this direction which leads in the preparation of quality finished products. Present communication deals in the ingredient identification in Habb-e-Narmushk which is used by unani physicians to cure qulanj (colic pain), meghs (cramps), waj-ul-meda (gastric pain/ stomach ache) since time immemorial (Anonymous, 2007). Identification and authentication of all the ingredients present in this important formulation will help in the preparation of a quality drug with maximum therapeutic potential and hence beneficial for the mankind.

## Methodology

All the ingredients were procured from the local raw drug dealers , New Delhi. Each ingredient was authenticated by examining separately (both macroscopically and microscopically). After detoxifying saqmonia each ingredient was powdered separately and Habb-e-Narmushk was prepared as per formulation composition given in NFUM part II.

### Formulation Composition

S.No.	Ingredients	Botanical name	Part used	Quantity
1.	Zanjabeel	<i>Zingiber officinale</i> Rosc.	Rhizome	250g
2.	Qaranfal	<i>Syzygium aromaticum</i> Merr & L.M. Perry	Floral bud	250g
3.	Filfil daraz	<i>Piper longum</i> Linn.	Berries	250g
4.	Darchini	<i>Cinnamomum zeylanicum</i> Blume	Stem bark	250g
5.	Filfil siyah	<i>Piper nigrum</i> Linn.	Berries	250g
6.	Narmushk	<i>Mesua ferrea</i> Linn.	Floral bud	250g
7.	Mastagi	<i>Pistacia lentiscus</i> Linn.	Resin	250g
8.	Saqmonia	<i>Convolvulus scammonia</i> Linn.	Resin	500g

Further, few pills were broken into powder and mounted in different reagents and cells/ tissues/ cell contents etc. and examined under a microscope according to the methods laid down by Johnsen (1940) and Trease and Evans (1983). The resulting photographs were taken from the microscope with computer attachment.

### Observations

#### Ingredients

##### 1. Zanjabeel (*Zingiber officinale* Rosc.)

Part used : Rhizome

Macroscopy: Rhizome irregularly branched (sympodial), laterally compressed, different sizes, externally pale yellowish-buff, longitudinally striate, ends of branches with depressed stem scars, fracture short, mealy, uneven with projecting fibres, odour agreeably aromatic with characteristic pungent taste.

Microscopy: A cross section of rhizome shows:

Phellem or outer cork few layered, dark brown, irregular parenchyma cells.

Phellogen or inner cork few layered, colourless parenchyma cells, radially arranged in regular rows.

Phelloderm or cortex several layered, thin walled, round- polygonal, parenchyma cells with intercellular spaces containing abundant starch grains which are mostly simple, fairly large, flattened, oblong or subrectangular to oval or sac shaped with terminal beak like projection in which eccentric hilum is situated. Numerous oleo-resin cells and vascular bundles present.

Endodermis single layered with radial walls thickened, starch grains absent.

Stele broad central zone, thin walled, round- polygonal, parenchyma cells with intercellular spaces (same as cortex) just inside the endodermis i.e. to the periphery of the ground tissue a ring or narrow zone of vascular bundle present. Scattered irregularly throughout the remainder of the stele are larger, closed, collateral, fibrovascular bundles.

2. Qaranfal (*Syzygium aromaticum* Merr & L.M. Perry, syn. *Eugenia aromatica* Kuntze.)

Part used: Floral bud

Macroscopy: Flower buds reddish brown, 12-18 mm. long consisting of sub-cylindrical, slightly flattened, four sided hypanthium which exudes oil when pressed. Hypanthium surmounted by four spreading, thick, acute sepals and dome shaped corolla which is formed of four bowl shaped petals; stamens indefinite, free and introse; Ovary inferior, bilocular, situated in the upper part of the stalk, each locule containing many ovules attached to axile placenta; odour strong, spicy and aromatic, taste pungent, aromatic followed by slight tingling of the tongue. (Anonymous, 1985; Nadkarni, A.K. 1986; Kirtikar, K.R. and Basu, B.D. 1988)

Microscopy: T.S. through hypanthium shows:

Epidermis: single layered covered by thick cuticle; square shaped cells with straight walls containing numerous anomocytic type of stomata having 30- 35µ in diameter.

Cortex: several layered, peripheral region containing 2- 3 layers of big ellipsoidal, schizo- lysigenous oil glands embedded in radially elongated parenchyma cells. Middle region composed of 1- 2 rings of bicollateral vascular bundles associated with few pericyclic fibers embedded in thick walled parenchyma cells and an inner region of loosely arranged arrenchyma cells.

Columella: Central cylinder containing thick walled parenchyma cells with a ring of bicollateral vascular bundles towards the periphery.

Numerous cluster crystals scattered throughout the columella and the middle cortical region. Each stamen has a filament with a central vascular strand and oil- glands at intervals beneath the epidermis, the connective has a large oil gland in the apex.

T.S. through the anther wall shows a typical fibrous layer, very small cluster crystals of calcium oxalate present in the filament and along the dehiscence lines of anther lobes. Pollen grains are biconvex with a rounded edge which is triangular in outline and approx. 15- 20 $\mu$  in diameter.

3. Filfil daraz (*Piper longum* Linn)

Part used : Berries

Macroscopy: Fruit black, cylindrical, 2.5- 5 cm. long consisting of minute sessile fruits (approx. 6- 12 fruits) around the central axis, surface rough and composite, odour aromatic; taste pungent producing numbness on the tongue.

Microscopy: T.S. of fruit shows:

Epidermis: Outermost layer covered with thick cuticle; single layered, consisting of irregular cells filled with deep brown content.

Mesocarp: Several layered consisting of irregular shape, usually collapsed, thin walled cells; a number of stone cells, either single or in groups, present.

Endocarp: Several layered, cells filled with starch grains, oval to round measuring 3-8 $\mu$  in diameter.

4. Darchini (*Cinnamomum zeylanicum* Blume)

Part used: Stem bark

Macroscopy: Long, slender, flexible sticks of varying length and width approx. 6mm,; occur either as single or compound double quills; outer surface dull yellowish brown marked with paler, glossy, undulating longitudinal lines with occasional small scars or holes; inner surface darker in colour and finely striated longitudinally; fracture short and splintery; odour fragrant and aromatic, taste sweet, aromatic with sensation of warmth.

Microscopy: T.S. of bark shows patches of cork and underlying parenchyma, cork and cortex are absent. Outermost layer consists of a continuous band of pericyclic lignified parenchyma which is 3-4 cells wide; small groups of pericyclic fibres embedded at intervals, pitted sclereids thickened at the inner and radial walls and contain a few starch grains. Phloem of tangential band of sieve tissue alternating with parenchyma and containing axially elongated secreting cells containing volatile oil or mucilage; phloem fibres with very thick walls, upto 30 $\mu$  in diameter; either isolated or in short tangential rows; medullary rays biseriate, widening slightly as they reach the pericycle, many of these cells contain starch grains approx 6- 10 $\mu$  in diameter and minute acicular crystals of calcium oxalate.

5. Filfil siyah (*Piper nigrum* Linn)

Part used : Berries

Macroscopy: Fruits globular, hard, dark brown to black, 3- 5mm. in diameter with a characteristic coat of deep set wrinkles; odour aromatic, taste pungent.

Microscopy: T.S. of fruit shows :

Epicarp: Single layered epidermis covered by cuticle; epidermal cells polygonal (tabular) containing dark brown-blackish content followed by 2- 3 layers of thin walled parenchyma cells intermingled with thick walled isodiametric to radially elongated lignified stone cells.

Mesocarp: broad zone of tangentially elongated parenchyma cells having larger secretion sacs with suberised walls and oil or resin contents. Cells in the inner mesocarpic region are compressed having few fibro vascular bundles.

Endocarp: single row of beaker shaped stone cells (cells whose radial and inner walls are more strongly lignified than the outer ones)

Testa: single layer of yellow coloured cells.

Perisperm: broad zone of thin walled, radially elongated parenchyma cells filled with abundant starch grains, aleurone grains, oleoresin cells containing oil globules and masses of resin.

6. Narmushk (*Mesua ferrea* Linn.)

Part used : Floral bud

Macroscopy: Stamen consists of anther, connective and filament; copper or golden brown in colour, filament united at the base forming a fleshy ring, stamen 0.9- 2.0 cm in length, anther approx. 0.6 cm in length, linear, basifixed, containing numerous pollen grains; filament 1 m. in length, slender, filiform, more or less twisted, brittle, odour fragrant; taste astringent.

Microscopy: T.S. of anther shows longitudinally dehiscent anther wall of thin walled parenchymatous cells; pollen grains numerous, yellowish, many pollen grains with 1- 3 minute, distinct protuberances on walls, exine thick walled, intine distinct.

*Test Sample (Formulation)*

Microscopic examination of Habb-e-Narmushk shows following components of diagnostic characteristics:-

- Sclereids: abundant sclereids, either single or in groups, show great variation in size and shape; lumen either broad or narrow; with varying thickness on the walls; showing variable striations; with or without pits.



- Fibre: abundant fibres; occur in pieces of variable size, lignified with varying thickness of lumen; with or without pits.
- Pollen grains: numerous pollen grains, present either single or in groups; various shapes.
- Starch grains: abundant starch grains; present either single or in groups; various size.
- Parenchyma cells: various size and shape, some packed with starch grains.

## Results and Conclusion

Habb-e-Narmushk are dark brown pills with the smell of cardamomum. On the basis of above mentioned histological characters, presence of following ingredients were established in Habb-e-Narmushk:-

- Septate fibres with oblique, elongated pits on their walls, parenchyma cells with starch grains measuring 10- 25µin diameter and vessels with spiral thickenings confirms the presence of *Zingiber officinale* Rosc. in Habb-e-narmushk. (Fig. 1-3).



Fig. 1. Septate fibre of *Zingiber officinale* Rosc. x 40



Fig. 2. Spiral vessel of *Zingiber officinale* Rosc. x 40

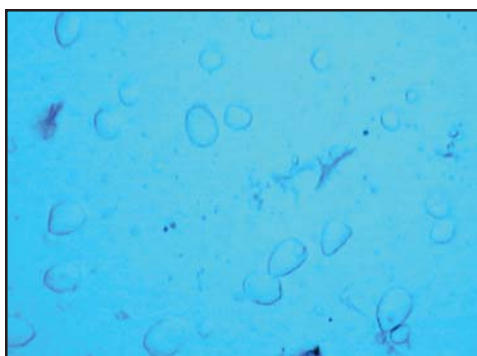


Fig. 3. Starch grains of *Zingiber officinale* Rosc. x 40



Fig. 4. Pollen grain of *Syzygium aromaticum* Merr & L.M. Perry x 100

- Pollen grains biconvex with rounded, triangular outline and smooth exine having diameter approx.  $38\mu$  confirms the presence of *Syzygium aromaticum* Merr & L.M. Perry (Fig. 4).
- Oval elongated stone cells confirms the presence of *Piper longum* Linn. (Fig. 5).
- Moderately thickened sclereids in horse shoe manner thickness with narrow lumen and numerous pits, fibres thick walled, lignified with uneven lumen and slit shaped pits confirm the presence of *Cinnamomum zeylanicum* Blume (Fig. 7 & 8).
- Group of more or less isodiametric or slightly elongated stone cells, beaker shaped stone cells from endocarp confirm the presence of *Piper nigrum* Linn. (Fig. 9 & 10).
- Pollen grains which are spherical, smooth, thick walled, exine and intine distinct having diameter  $65 - 82\mu$  confirms the presence of *Mesua ferrea* Linn. in Habb-e-Narmushk (Fig. 6).



Fig. 5. Stone cells of *Piper longum* Linn. x 40



Fig. 6. Pollen grain of *Mesua ferrea* Linn. x 40



Fig. 7. Fibre of *Cinnamomum zeylanicum* Blume x 40

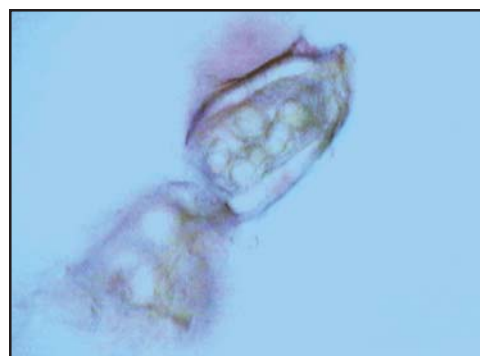


Fig. 8. Sclereids with horse shoe manner thickness of *C. zeylanicum* Blume x 100



Fig. 9. Sclereids of *Piper nigrum*  
Linn. x 40

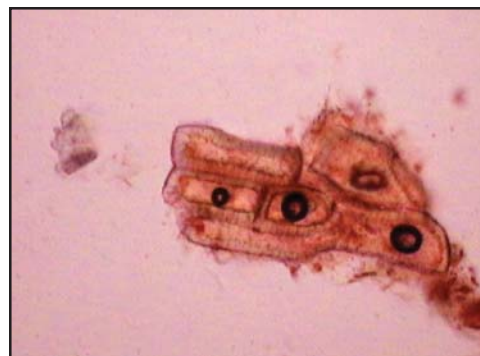


Fig. 10. Sclereids of *Piper nigrum*  
Linn. x 40

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# Biological Contamination Assessment in Commercial Samples of Some Herbal Drugs

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## Abstract

Bacterial, Fungal and insect contaminants have been investigated in commercial samples of herbal drugs viz. *Desmodium gangeticum* DC., *Nardostachys jatamansi* DC., *Picrorhiza kurroa* Royle ex Benth., *Plumbago zeylanica* Linn. and *Withania somnifera* Dunal. Biological contaminants associated with these herbal drugs were isolated and reported. The presences of biological contaminants indicate poor post-harvesting and storage condition of herbal drugs which leads to deterioration of quality of the drugs.

**Key Words:** Biological contamination, Storage, Quality control.

## Introduction

Biological contamination (bacterial, fungal and insect) of herbal drug is a serious concern and threat to the health of consumers. Contamination sets in either at collection stage or during transportation or storage in trade houses and manufacturing units. The trade of herbal drugs has been expended manifold. With this importance and recognition followed by the rapid expansion of the trade, the instances of lack of quality check have increased manifold. This has put a question mark on the quality of herbal drugs. The legislation enacted as 'Drugs & Cosmetic Act & Rules' has previewed this situation by promulgating schedule-T to ensure the quality of drugs. Raw materials used in the preparation of medicine have direct impact on the efficacy of the drug. To ensure the quality of herbal drugs, identity of raw material is prioritized where as freedom from biological contaminants (microbiological, fungal and insect) is restricted to finished products. Presence of biological contaminants in raw material is prime cause of deterioration which shortens the shelf life of a product. Post-harvest and storage practices play major role on this aspect. The existing work is carried on herbal drugs of root and rhizome origin resourced from *Desmodium gangeticum* DC., *Nardostachys jatamansi* DC., *Picrorhiza kurroa* Royle ex Benth., *Plumbago zeylanica* Linn. and *Withania somnifera* Dunal. All these herbal drugs are attributed for different therapeutic activities and important medicinal plants used in a number of formulations of Ayurvedic and Unani System of Medicine (Anonymous, 1940; 1948-1976; 1978, Chopra *et. al*, 1956).

## Materials & Methods

The commercial samples of following herbal drugs were drawn from different drug dealers and pharmacies as per schedule recommended in USP XX (Anonymous, 1979) (Table 1).

**Table-1. Herbal Drugs taken for study.**

Sl. No.	Botanical Species	Herbal Drugs	Morphological part
1.	<i>Desmodium gangeticum</i> DC.	Shalparni	Root
2.	<i>Nardostachys jatamansi</i> DC.	Jatamansi	Rhizome
3.	<i>Picrorhiza kurroa</i> Royle ex Benth.	Katuki	Rhizome
4.	<i>Plumbago zeylanica</i> Linn.	Chitraka	Root
5.	<i>Withania somnifera</i> Dunal.	Ashvagandha	Root

### *Determination of Biological Contaminants*

The determination of biological contamination was established for fungi, bacteria and insects in the commercial drug samples of above plant species. The methodology applied for the studies are prescribed protocols (Anonymous, 1980a, 1980b) and methodology adopted by Sharma (2009).

### *Sampling*

The commercial samples of the drugs were kept in sterile container and brought to laboratory avoiding further contamination. The contamination studies were carried immediately after the sampling employing following steps for fungal, bacterial and insect contamination.

### **Observations**

Based on the studies carried out on commercial samples of herbal drugs viz. *Desmodium gangeticum* DC., *Nardostachys jatamansi* DC., *Picrorhiza Kurroa* Royle ex Benth., *Plumbago zeylanica* Linn. and *Withania somnifera* Dunal. following biological contaminants were recorded and are elaborated in Table-2.

### **Discussion**

The contamination of the drug material by biological contaminations viz. microbes, fungi and insects does not cause the destruction only but ultimately leads to health hazards to consumers. Sometimes, drug materials are so badly infested with biological contaminants that the possibility of the production of toxins can not be ignored. Among all the different toxins known so far, aflatoxins activity leading to strong hepatocarcinogenic activity, frequently occur in high concentrations in stored drug materials. Considerable interest therefore lies in the investigations pertaining

Table-2. Components of Biological Contaminants and their Distribution in commercial samples

Sl. No.		Contaminants and their components	DG				NJ				PK				PZ				WS			
			A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D				
Bacteria (Pathogenic)																						
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-		
Fungi																						
		-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	+	-	-		
		-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-		
A. candidus		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
A. flavus		+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+	-		
		-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-		
		+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		
A. fumigatus		-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-		
		+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		
A. nidulans		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+		
		+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+		
		-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		
A. sulphureus		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-		
A. terricola		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-		
Botrytus cinerea		-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-		
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

Table-2. (Contd.)

Sl. No.	Contaminants and their components	DG				NJ				PK				PZ				WS			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	<i>Circinella simplex</i>	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Curvularia lunata</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>C. pallaescens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	<i>Fusarium nivale</i>	+	+	+	-	-	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-
	<i>F. oxysporum</i>	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+	-	+
	<i>Fusarium</i> sp.	+	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-
	<i>Gliocladium penicillioides</i>	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
	<i>Monilia</i> sp.	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Mucor fragilis</i>	+	-	+	+	-	-	-	-	+	-	+	+	-	-	+	-	+	+	-	-
	<i>M. hiemalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	<i>M.mucedo</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	+
	<i>M. pusillus</i>	+	+	-	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-	-	-
	<i>Mucor</i> sp.	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
	<i>Penicillium chermesinum</i>	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	+	+
	<i>P. chrysogenum</i>	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
	<i>P. decumbens</i>	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-
	<i>P. javanicum</i>	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+
	<i>P. lanosum</i>	-	-	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-	-	+

Table-2. (Contd.)

Sl. No.	Contaminants and their components	DG				NJ				PK				PZ				WS			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	<i>P. pallidum</i>	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-
	<i>P. purpureogenum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	<i>P. spinulosum</i>	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-
	<i>Penicillium</i> sp.	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Periconia byssoides</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	<i>Rhizopus arrhizus</i>	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-	-
	<i>R. nigricans</i>	+	+	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-
	<i>R. oryzae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	<i>R. stolonifer</i>	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-
	<i>Rhizopus</i> sp.	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-
	Sterile mycelium, black-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
	Sterile mycelium white	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
	<b>Insect</b>																				
	<i>Lyctus africanus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

**Abbreviations:** DG – *Desmodium gangeticum* DC.; NJ – *Nardostachys jatamansi* DC.; PK – *Picrorhiza kurroa* Royle ex Benth.; PZ – *Plumbago zeylanica* Linn. and WS – *Withania somnifera* Dunal.

'A' & 'B' – Samples drawn from drug houses of Dehradun and Hardwar

'C' & 'D' – Samples drawn from pharmacies of Hardwar

Indications: '-' Absence and '+' Presence



to the biological contaminants associated with the drug samples. The results of the present study are summarized in Table 2.

The result elucidates the various components of different contaminants associated with all the commercial samples of drugs studied. The identification of bacterial contaminants was only made regarding *Escherichia coli* and *Salmonella* species. Both these bacteria are pathogenic and are responsible for a number of human infections. The presence of *Salmonella* species has been found in a few samples of drugs viz. *Plumbago zeylanica* Linn. and *Withania somnifera*. Dunal. This pathogenic bacteria is quite common in soils where faecal matters are frequently available. In open and waste lands animal and human excreta is not uncommon. The contaminated samples of drugs are obtained from such plants which grow in open and waste land habitats. The occurrence of these pathogenic bacteria can be attributed to the above.

In the present study forty-one fungal species were isolated from the drug samples, and their respective distribution in each sample has been indicated in Table 1. The dominant genera are *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. *Aspergillus fonscaeus*, *Curvalaria lunata*, *C. pallaescens*, *Mucor fragilis* and *Periconia byssoides* were found to be of restricted occurrence. A few species on the other hand viz. *Aspergillus clavatus*, *A. flavos*, *A. niger*, *Fusarium nivale*, *Mucor pusillus*, *Rhizopus nigricans*, black and white sterile colonies flourished well on the drug samples probably due to the availability of the suitable substratum in the environment. The preponderance of deuteromycetes especially *Aspergilli* might be due to their wide range of distribution and greater tolerance to various limiting factors.

The insect contamination was not found in any of the commercial sample of studied herbal drug. The incidence of biological contaminants in commercial drug samples is self-explanatory as all the samples of subjected drugs have been found to be infested with microbial and fungal contaminants. While all the drug samples were found free from attack by the insects. The major cause of the contamination is that most of the drug material is collected regardless of materials being mutilated and affected by foreign organisms. This negligence causes deterioration of valuable drug material and also proves media for the dissemination of contamination to healthy material. Besides this collectors are also least concerned about the proper processing of drug material. They try to transport out the material to destination for the sale to drug dealers and pharmaceutical units without proper processing. The stock of the drug material are generally packed in gunny bags and placed in open or closed shades. Some times, closed godowns are damp and humid. These practices of storage and transit prove an aid in profuse growth and development of contaminants rendering them unfit to therapeutic utility. The survival of the contaminants on drug material depends upon their nutrition through the decomposition of organic material available from host. It can be thus concluded that the association of these contaminants with the drug material is because of various negligences

during collection, processing, storage and transit. Besides these, field storage devices and storage conditions also provide ample chance of contamination.

### Acknowledgement

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# Evaluation of In-vitro Antioxidant Activity of the Rhizome of *Alpinia officinarum* Hance

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## Abstract

The *In-vitro* antioxidant activity of the rhizome of *Alpinia officinarum* Hance investigated by Ferric Thiocyanate (FTC) and Thio Barbituric Acid (TBA) methods was measured colorimetrically at 500 and 532 nm. The ethanol extract of the rhizome of *A. officinarum* possesses significant antioxidant activity. The antioxidant activity of the extract is close and identical in magnitude and comparable to that of standard antioxidant compounds used.

**Key Words:** *Alpinia officinarum* Hance, Antioxidant activity.

## Introduction

Khulanjaan in Unani, Kulanjan in Ayurveda and Chitrarttai or lesser galangal commerce is botanically equated to *Alpinia officinarum* Hance., Zingiberaceae. The rhizome is reddish brown, about 2cm (3/4in) in diameter (Fig. 1). They are more pungent than the greater and are similarly ringed (Khare, 2007 and Anonymous, 1948). The rhizome of *A. officinarum* is being used as a traditional medicine in China for relieving stomach-ache, cold, invigorating the circulatory system and reduces the swelling. It is especially useful in flatulence, dyspepsia, vomiting, sickness of stomach and recommended as a remedy for sea-sickness. It tones up the tissues and sometimes prescribed in fever (Nadkarni, 1976, Anonymous, 2005). In Unani system of medicine, the *A. officinarum* is considered sea-sickness. It tones up the tissues and sometimes prescribed in fever (Nadkarni, 1976, Anonymous, 2005). In Unani system of medicine, the *A. officinarum* is considered as one of the constituent for compound drug "Jawarish Jalinoos" used in diuretic, antimicrobial and gastro-intestinal disorders (Asolkar *et al.*, 1992). The chemical constituents of *A. officinarum* rhizome contains galangin, eugenol, quercetin, kaempferol, quercetin-3- methylether, isorhamnetin, galangin-3-methylether, kaempferol-7-methyl ether, 7-hydroxy-3,5-dimethoxyflavone, alpinin, 7-(4"-hydroxyphenyl)-1-phenyl-4-hepten-3-one, 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-3-heptanone, 5-methoxy-7-(4"-hydroxyphenyl)-1-phenyl-3-heptanone, 5-hydroxy-7-(4"-hydroxy-3"-methoxyphenyl)-1-phenyl-3-heptanone, (3R,5R)-1-(4-hydroxy-phenyl)-7-phenylheptane-3,5-diol, octahydrocurcumin, 17-diphenylhept-4-en-3-one, 7-(4"-hydroxy-3"-methoxyphenyl)-1-phenylhept-4-en-3-one, 7-(4"-hydroxy-3"-methoxyphenyl)-1-phenyl-3,5-heptadione, 5-hydroxy-7-(4"-hydroxyphenyl)-1-phenyl-3-heptanone, 3,4- dihydroxybenzoic acid,  $\beta$ -sitosterol, stigmasterol, campesterol, 6-zingerol, benzylacetone and eualpinol (Chopra *et al.*, 1956).

Free radicals have been implicated in causation for various ailments. Free radicals are continuously produced by the body for normal use of oxygen such as respiration and some cell mediated immune functions. There is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench or scavenge them and protect the body against their deleterious effects. The cause of majority diseases like alzheimer's, parkinsonism, cancer, diabetes mellitus and inflammatory



**Fig. 1.** Rhizome of *A. officinarum* Hance.

conditions are being considered to be primarily due to the imbalance between pro-oxidant and antioxidant homeostasis. Antioxidant principles from natural sources possess multifacetedness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance. Hence, there is no doubt that phytochemicals deserve a proper position in the therapeutic armamentarium.

The literature survey revealed that no systematic study has been carried out on *in vitro* antioxidant activity on rhizome of *A. officinarum*. Hence, in the present study, an effort was made to establish its anti-oxidant property.

## **Materials and Methods**

### *Collection of Plant Materials*

Rhizome of the *A. officinarum* were collected from local market Chennai, identified and authenticated by botanist at Regional Research Institute of Unani Medicine (CCRUM), Chennai-13. The dried rhizome of the plant was used for the study.

### *Preparation of the ethanolic extract of A. officinarum Hance.*

Rhizomes of *A. officinarum* were air dried, powdered and stored in an air tight container at 27°C. Then 10 g of dried powder of *A. officinarum* was accurately weighed and exhaustively extracted by ethanol (absolute 99%) using Soxhlet apparatus. The extract was filtered, evaporated to dryness under vacuum and used for the assay.

### *Instrument*

UV spectrophotometer used was Perkin Elmer, Lambda EZ20.

#### *Ferric Thiocyanate Method (Inhibition of Lipid Peroxide Formation)*

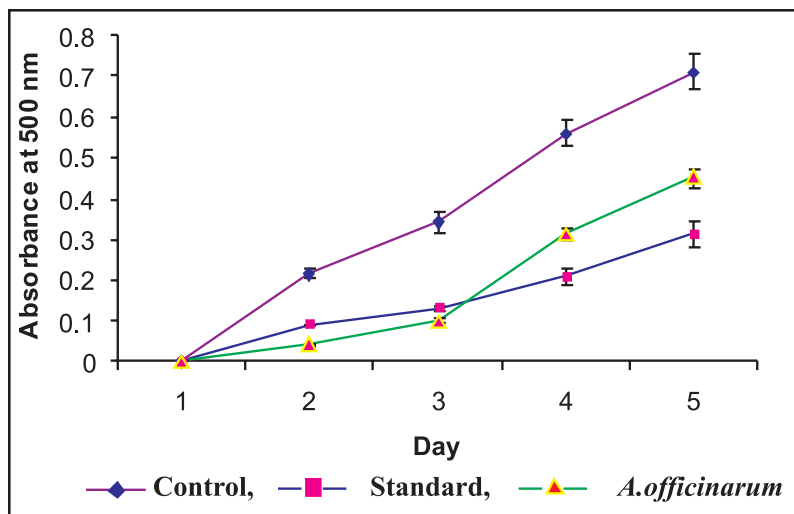
This method is based on the determination of peroxide (lipid) at the primary stage of linoleic acid peroxidation. The peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment which was measured at 500 nm. The standard method as described by Kikuzaki and Nakatani was used. A mixture of 4 mg of sample in 4 ml of 99.5% ethanol (final concentration, 0.02%), 4.1 ml of 2.52% linoleic acid in 99% ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with a screw cap and placed in oven at 40° C in the dark. To 0.1 ml of this mixture, 9.7 ml of 75% ethanol (v/v) and 0.1 ml of 30% ammonium thiocyanate was added. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% Hydrochloric acid was added to reaction mixture; the absorbance of red colour was measured at 500 nm by UV spectrophotometer for every 24 hrs until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was used and for the standard, 4 mg of the sample was replaced by 4 mg of Vitamin E (Kikuzaki and Nakatani, 1993).

#### *Thiobarbituric Acid (TBA) method*

The method of Ottolenghi was used to determine the TBA value of the samples. The formation of malonaldehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation. At low pH, and high temperature (100° C), malonaldehyde binds TBA to form a red complex that can be measured at 532 nm. TBA method is used to measure the carbonyl compounds obtained by linoleic acid oxidation and at the later stage of linoleic acid (lipid) peroxidation. 2 ml of 20 % trichloro acetic acid and 2 ml of 0.67 % TBA solution were added to 1 ml of the mixture containing the sample prepared in the FTC method. This mixture was kept on a water bath (100° C) for 10 min and after cooling to room temperature it was centrifuged at 3000 rpm for 20min and the absorbance of the supernatant was measured at 532 nm. Antioxidant activity was carried out based on the absorbance on the final day of the assay (Ottolenghi, 1959).

### **Results and Discussion**

The antioxidant activity of ethanolic extract of the rhizome of *A.officinarum* was assessed by both FTC and TBA methods at the concentration of 0.02% and compared with Vitamin E. The ethanolic extract was carried out by FTC method showed the low absorbance values, which indicates high levels of antioxidant activity shown in Fig.2. In general, rhizome of *A.officinarum* and Vitamin E markedly inhibited the oxidation of linoleic acid for a period of 5 days when compared to control. Vitamin E showed the least increase in absorbance values, followed by *A.officinarum*. The control increased steadily reached maximum level on day 5 and finally dropped on day 6.

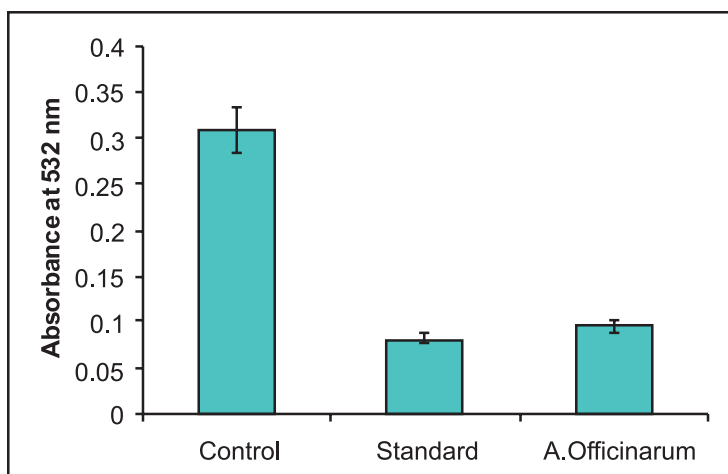


**Fig. 2.** Antioxidant property of *A.officinarum* determined with FTC method  
Each legend represents mean  $\pm$  SD of six determinations

Fig. 3 gives the absorbance values from TBA method that showed total peroxide values produced by the oxidation of linoleic acid. Higher absorbance values indicate lower level of antioxidant activity. The control has the highest absorbance value (0.31) followed by *A.officinarum* (0.095) and Vitamin E (0.081). Based on the present result, *A.officinarum* found to possess antioxidant activity which is comparable to the standard. However, the literature survey showed the presence phytochemicals like flavonoids and phenols which might be responsible for the In-vitro antioxidant activity of this drug.

## Conclusion

Thus the results of the present study support the view that some traditionally used Indian medicinal plants are promising source of potential antioxidants.



**Fig. 3.** Antioxidant activity of *A.officinarum* determined by the TBA method

## Acknowledgement

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# Ethnomedicinal Studies in Chittoor Forest Division (East & West) of Andhra Pradesh

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## Abstract

Based on an ethnopharmacological survey of Chittoor Forest Division (East & West) in Chittoor District of Andhra Pradesh conducted during January – February 2009, the paper presents some 40 contemporary folk recipes comprising 40 taxa of folk medicinal plants by various tribes eg. Koyas, Valmiki and Telaga agriculturists etc. for the treatment of various common ailments. Botanical name, family, local name, unani name, field book number, part(s) used, name of the disease(s) against which used and mode of administration is given for each recipe discussed. The information provided will help to discover new drugs of natural origin for many of the diseases, thus far, incurable in modern medicine.

**Key Words:** Ethnopharmacological Survey, Tribal Medicine, Chittoor Forest

## Introduction

During an ethnopharmacological survey of Chittoor forest division (East & West) in Chittoor District of Andhra Pradesh undertaken from 25<sup>th</sup> January to 8<sup>th</sup> February 2009 first hand information on folk medicinal uses of plants for treatment of various diseases and conditions were recorded. The area from which data were derived is situated in North latitude 12°37' to 14°81' and between East Longitudes of 78°15' and 79°. The area explored include Madanpally, Horsley Hills, Kotapet, Punganur, Palmner, Palleru, Bhakrapet, Talakona, Tirupathi, Tirumalla Hills and Karvetinagar. In Chittoor East Division of the District, the forest area is mostly hilly with wide valley such as Mamandur, Somala and Talakona. In Chittoor West Division, the Western Ghats, traverse this division from South West to North East standing from Kangeundi in South West and running in a North and North Eastern direction across Palmaner, Punganur, Madanpally and Voyalpad Taluka Horsley Hills is the prominent hill in this division.

The area has not been investigated exhaustively earlier in this direction except for some sporadic reports on medicinal uses of plants (Kumar *et al.*, 1991; Chetty & Rao, 1989; Hemadari, 1987, 1988, 1991; Kumar and Pullaiah, 1998; Nagaraju & Rao, 1990; Balaji Road *et al.*, 1995; Gupta *et al.*, 1997; 2005; 2008; 2009; Surya Narayana, 1996; Kapoor and Kapoor, 1993; Jain 1991 and Pullaiah & Yasoda, 1989). The study presents 40 folk medicinal species used by the tribal and other ethnic groups for various ailments among local population in the areas surveyed.

## Methodology

An ethnopharmacological survey of Chittoor Forest Divisions of Chittoor District was conducted during January – February 2009 with a view to study the medicinal herbs of the area and also to record the folk-wisdom of tribals known as Koyas and valmiki and Telaga agriculturists who have since long settled in the river side

villages. The data on folk medicinal uses of plants were collected from the well reputed herbalists (medicine men) through their direct field interviews who also accompanied the survey team in the field to help identify the folk plants and also from the tribals who have long been prescribing the folk medicine to locals for treatment of various common and chronic diseases. Information about the efficacy of the herbs was also recorded.

Botanical specimens of all folk drugs were collected, identified and voucher specimens prepared and deposited in the herbarium of Survey of Medicinal Plants Units, Central Research Institute of Unani Medicine, Hyderabad for future reference and study. Ingredients and adjuvant drugs in a particular recipe have been recorded by their local names in field and scientifically identified at the Institute.

### *Enumeration of Folk Medicinal Species*

Adverting shortly to the scheme of presentation of data, the medicinal plants used as folk medicine in the study area are arranged in alphabetical order. Each entry gives the information. Plant's scientific name with family (in bracket), Field Book No., Local Name (s), Unani Name (Wherever available), part(s) used disease and condition and method of usage, in sequence.

*Acacia chundra* Willd. (Mimosaceae), CRI. 8826; Sundra; Lalkhair; Stembark; Cough & Cold; Decoction of the stem bark is given for cough and cold.

*Aerva lanata* (Linn.) Juss. (Amaranthaceae); CRI 8798; Magavira; Patharphodi; Whole plant; Kidney stones; Decoction of the whole plant is given for kidney stones.

*Alpinia galanga* Willd. (Zingiberaceae); CRI 8802; Pedda-dumparashtam; Kulanjan; Roots; Joint pains; Root's powder is given for Joint pains.

*Anacyclus pyrethrum* DC. (Asteraceae); CRI 8824; Akkalakara; Akarkara; Roots; Cleaning of teeth. Root's powder is used as toothpowder for cleaning teeth & tooth pain also.

*Anogeissus latifolia* (Roxb. Ex.DC.) Wall. (Combretaceae); CRI 8820; Chirumanu; Dhawa; Gum; tonic for good health; gum is used as a tonic for good health.

*Boswellia serrata* Roxb. ex. Coleb. (Burseraceae); CRI 8828; Madi; Luban; Gum; Diarrhoea & Dysentery; Gum powder is used for diarrhea and dysentery.

*Buchanania lanzan* Spreng. (Anacardiaceae); CRI 8830; Sara; Chironji; Gum; Diarrhoea & Dysentery; Gum powder is used for Diarrhoea.

*Capparis zeylanica* Linn. (Capparidaceae); CRI 8788; Kariramu; Kabra; Stem bark; Rheumatic pains; stem bark's powder is used for rheumatic pains.

*Cardiospermum helicacabum* Linn. (Sapindaceae); CRI 8800; Buddakakara; Habul Qil-Qil; Whole plant; Diabetes; Decoction of the whole plant is used for diabetes.

*Centella asiatica* (L.) Urban. (Apiaceae); CRI 8792; Saraswatiaku; Brahmi; Leaves; Epilepsy; Leaves powder is used for epilepsy.

*Dalbergia latifolia* Roxb. (Fabaceae); CRI 8832; Cittegi; Shisham; Stem bark; Dyspepsia; Decoction of the stem bark is used for dyspepsia.

*Dioscorea pentaphylla* Linn. (Dioscoreaceae); CRI 8810; Dukapenda leaves; Kantalu; Rhizomes; Swellings; Rhizome's powder is used for swellings.

*Diospyros melanoxylon* Roxb. (Ebenaceae); CRI 8835; Tumki; Tendu; Leaves; Diuretic; Decoction of the leaves is used as diuretic and strengthen to the kidney.

*Dregea volubilis* (Linn.f.) Benth. (Asclepiadaceae); CRI 8885; Dudhipaala; Nakchikni; Leaves; Boils & Wounds; Paste of the grinded leaves is applied to boils and wounds.

*Emblica officinalis* Gaertn. (Euphorbiaceae); CRI 8838; Usirikai; Amla; Fruits; Cooling & Stomachic; Fruit's powder is used as cooling and stomachic.

*Gardenia gummifera* Linn. (Rubiaceae); CRI 8840; Tella-Manga; Dikamali; Gum; nervine tonic; Gum powder is used as nervine tonic.

*Gloriosa superba* Linn. (Liliaceae); CRI 8818; Adavi-nabhi; Karihari; Tubers; Joint pains; Tuber's powder is used for joint pains.

*Grewia tiliaefolia* Vahl. (Tiliaceae); CRI 8845; Dhamni; Pharsa; Stembark; Dysentery; Decoction of the stem bark is used in dysentery.

*Helicteres isora* Linn. (Sterculiaceae); CRI 8812; Kavanchi; Marorphali; fruits; stomach pain; fruit's powder is used for stomach pain of children.

*Holarrhena antidysenterica* (Roth.) DC. (Apocynaceae); CRI 8790; Kodaga; Inderjao-Talkh; Leaves; Diabetes; Leaves powder is used for diabetes.

*Lagerstroemia parviflora* Roxb. (Lythraceae); CRI 8848; Chinangi; Dhaura; Leaves; Diabetes; decoction of the leaves is used for diabetes.

*Manilkara hexandra* (Roxb.) Dubard. (Sapotaceae); CRI 8850; Manjipala; Khirni; stembark; fevers; decoction of the stem bark is used for fevers.

*Mimosa pudica* Linn. (Mimosaceae); CRI 8795; Attapatti; Sharmili; Whole plant; Nervine tonic; Decoction of the whole plant is used for kidney stones.

*Morinda citrifolia* Linn. (Rubiaceae); CRI 8852; Maddi; Surangi; leaves; gout and joint pains; paste of the grinded leaves is applied for gout and joint pains.

*Piper longum* Linn. (Piperaceae); CRI 8808; Pippuloo; pipulamul; roots; asthma; root's powder is used for asthma.

*Plumeria alba* Linn. (Apocynaceae); CRI 8815; veyvivarahaalu; Gulchin; roots; ulcers; root's powder is used for ulcers.

*Premna tomentosa* Willd. (Verbenaceae); CRI 8855; Nagaru; Chambara; roots; stomach pain; decoction of the root's powder is used for stomach pain.

*Prosopis cineraria* (L.) Druce. (Mimosaceae); CRI 8858; Vilayati-kikkar; Vilayati-babul; pods; food; pods are used as a food by the tribals.

*Pterocarpus marsupium* Roxb. (Fabaceae); CRI 8865; Yegisi; Bijasar; Stemwood; Diabetes; Keep water in the wooden glass of Bijasar for the whole night and take in the morning for diabetic patients.

*Pterocarpus santalinus* Linn.f. (Fabaceae); CRI 8860; Rakta-gandhamu; Sandal surkh; Stem bark; inflammations; paste of the stem bark is applied for area of inflammation.

*Rauvolfia tetraphylla* Linn. (Apocynaceae); CRI 8805; Sarapagandhi (Susstitute); Barachandrica; Roots; Reducing blood pressure; Root's powder is used for reducing blood pressure.

*Salvadora persica* Linn. (Salvadoraceae); CRI 8868; Varagogu; Jhak; Leaves; Asthma; decoction of the leaves is used for asthma.

*Solanum surattense* Burm. (Solanaceae); CRI 8775; Pinnamulaka; Katai-kurd; Leaves and roots; fever; leaves & root's paste is given with honey for fever by the tribals.

*Sterculia urens* Roxb. (Sterculiaceae); CRI 8875; Thapasi; Karyagum; gum; good for digestion and keeping stomach cool; soak little gum in the water at night and take in the morning with sugar keeps stomach cool and good for digestion.

*Strychnos nux-vomica* Linn. (Loganiaceae); CRI 8785; Mushti; Kuchla; seeds; nervous disorders; seed's powder is used for nervous disorders.

*Strychnos potatorum* Linn.f. (Loganiaceae); CRI 8880; Chillaginjal; Nirmali; Seeds; diabetes; seed's powder is given two times to the diabetic patient.

*Tinospora cordifolia* (Willd.) Miers. (Menispermaceae); CRI 8782; tippateege; Gulbel; stem and whole plant; fevers; decoction of the stem and whole plant is used for fevers.

*Wedelia chinensis* Merrill. (Asteraceae); CRI 8778; Bhringaraja; Pila-Bhangra; Leaves; blackening of the hairs; leave's paste is used for blackening of the hairs.

*Wrightia tinctoria* R.Br. (Apocynaceae); CRI 8780; Amkuda; Indrajau-Sharien; Stem bark; biliousness; decoction of the stem bark is used for biliousness.

*Xeromphis spinosa* Keay. (Rubiaceae); CRI 8862; Manga; Mainphal; Seeds; improves digestion and appetite; seed's powder is given for digestion.

## Discussion

Traditional phyto-therapy is an art practiced mainly by few older people whose empirical knowledge is respected by everyone in the villages and tribal settlements.

The great potential of ethno-botanical knowledge as a key resource for developing new kinds of pharmaceuticals and other chemicals of Industrial use has been increasingly realized. In the present study some traditional therapeutic methods employed by the natives of Chittoor Forest Division of Chittoor District have been discussed. Out of 100 taxa of medicinal plants collected and identified from the study area 40 are used locally in folk medicines by the local tribals and other ethnic people viz. Valmikies; Koyas and Telaga agriculturists etc. for the treatment of various common ailments; including cough and cold, fever, diarrhea & dysentery, ulcers, skin diseases, cardiac troubles and rheumatic arthritis.

The usual methods of application of folk medicines are as decoctions paste, powder; juice and pills. These are taken internally or applied externally. Most of the recipes include only one plant species, however in some preparations are the combination of several herbs. Moreover the some plant is used for more than one disease and single diseases may be treated by many plant species.

It is, therefore, difficult to say which plant is actually effective in curing; laboratory investigations and clinical trials are suggested to establish therapeutic properties of these herbal preparations for their effective and safe use.

The data on folk medicinal uses have been compared with recent available literature (Anonymous, 1948-1976; Hussain *et al.*, 1992; Anonymous 1987, 1992, 1997; Jain *et al.*, 1991; Rastogi and Mehratra 1990-1998; Chetty & Rao, 1989; Hemadari, 1987, 1988, 1991; Vijay Kumar & Pullaiah, 1998; Nagaraju & Rao, 1990; Balaji Rao *et al.*, 1995; Gupta *et al.*, 1997, 2005, 2007, 2008 & 2009; Surya Narayana, 1996; Shaik Imam *et al.*, 2007; Hussain *et al.*, 2000 and Vedavathy, 1986) and found that most of the folk medicinal plants are duly reported in the literature, however, their mode of application, ingredients and parts used are different. Therefore, the present study represents contemporary folk uses of medicinal plants of the area investigated. It would be worthwhile to subject all these folk drugs to scientific testing in the context of claims reported herein

It was, therefore, considered important that this valuable knowledge regarding folk medicinal uses of plants be recorded before these time tested use of herbal drugs are lost forever because of even dwindling number of tribal men belonging to various caste and ethnic groups. Therefore, there is an urgent need to protect and conserve medicinal plants; some of which could be listed because of over exploitation by traders and folk medicine men.

Through such investigations many more new plant drugs can be revealed from the unique folk-lore lying hidden among the traditional communities of other ethno-pharmacologically unexplored area of India and elsewhere, which may be utilized to the well being of human health. However, experimental and clinical evidence are needed to demonstrate the effectiveness and safety of these folk drugs before they can be accepted by the modern health care system.

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# A Medico-Ethnobotanical Study Against Gynaecological Diseases of Nilgiri Tehsil and Adjacent Areas of District Balasore, Orissa, India

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## Abstract

The present paper enumerates 21 medicoethnobotanical plant species belonging to 16 families used by the tribals of Nilgiri Tehsil and adjacent areas of Balasore district of Orissa for the cure of gynaecological diseases. Tribals generally collect these plants from the nearby forests and prepare the medicine under the guidance of Kaviraj (Vaidya) or "village medicine man" in a traditional way. These medicinal plants are becoming extinct day by day because of their heavy use and transport to urban areas for commercial purposes. These plants help the pharmaceutical industries for manufacturing herbal medicines.

**Key Words:** Medico-ethnobotany, Tribals, Gynaecological diseases, Nilgiri Tehsil, Balasore.

## Introduction

India is predominantly a village-based country. The use of common plants around us is of holistic nature which took into the account of all aspects of human health and diseases, the philosophy of the treatment enunciated by great Rishi/ Muni. They bear testimony to our tradition in the health care of our people. The major part of population lives with poor socio-economic status, inadequate transport and communication facilities and poor hygienic conditions and the first-hand and timely provision of treatment becomes impracticable.

So the people of rural India, by and large, are still dependent on traditional medicine for their health care and treatment of diseases. These medicines have been developed through the experience of many generations assimilating the knowledge, in course of time, from fragments of Unani, Ayurveda, Yoga and Naturopathy, Siddha as well as traditional system of medicine. This paper deals with the native plants and their uses for the cure of gynaecological disorders practiced by the tribals of Nilgiri tehsil and adjacent areas of Balasore district of Orissa.

The district of Balasore is one of the coastal district of Orissa, lies between 21°03'- 21°59' North latitude and 86°20'- 86°29' East longitudes in the northeastern part of the state. Its boundaries extend in the north up to Midnapur district in west Bengal, in the south of Bhadrak district, in the West to Keonjhar and Mayurbhanj district and in the east to the Bay of Bengal. It has a massive coast line of 81 kms. Climate of the district is generally hot with high humidity. The district has a total area of 3634 km<sup>2</sup>. The Nilgiri tehsil of Balasore district is mostly terrain and vegetated with tropical semi-evergreen forests. The hills of Nilgiri have the highest peak of 543 meter above the Sea level.

## Methodology

Ethnobotanical surveys were conducted in the tribal villages (randomly selected) of namely Nilgiri, Badapokhari, Jaleswar, Sajangarh, Kendukhunta, Saganaria, Jodowali, Mitrapur, Tenda, Naranpur and Darakholi of Balasore District of Orissa. Out of 6 million tribal about 62 notified tribes are seen in Orissa (Mohapatra, 1993). Balasore is dominated by tribal like Santhals / Majhi, Kolhos, Mundas, Bhumij, Naik, Khandait etc. First hand information regarding the therapeutic properties of wild plants was recorded from these areas. Frequent visits were made to collect plants from the forest. The ethno-medico-botanical information was collected on the basis of interviews and cross examination of the inhabitants and village medicine man commonly known as Local Vaidya or Kabiraj during field trips. Information was collected in respect of 33 ethno-medicinal plants belonging to 24 families used by the tribals of Nilgiri Tehsil of Balasore district of Orissa to cure various gynaecological diseases. Voucher specimens were collected and preserved as herbarium specimen and deposited in the Survey of Medicinal Plant Unit, Regional Research Institute of Unani Medicine, Bhadrak. Identification of plants was done by using Haines (1921-25) and Saxena and Brahman (1994-96).

## Enumeration

The plant species are arranged in alphabetical order. The description follows botanical name, family name in bracket, Vernacular Names (Unani, local, Hindi and Sanskrit name), locality and voucher specimen (VS) number. The details such as the part(s) used single or in combination with other plants, methods of preparation, dosage and mode of administration are as under.

Botanical Name : *Abutilon indicum* (L.) Sweet. (Malvaceae)

Vernacular Names : Jhumka (O), Kanghi (U), Kakaiya (H), Atibala (S).

Locality with V.S. No. : Nilgiri-8573

Ethnomedicinal Uses : The bread prepared from the mixture of leaf powder (10 g) and wheat flour (200 g) is taken daily during night for about one month for the cure of uterus displacement.

Botanical Name : *Alstonia scholaris* (L.) R.Br. (Apocynaceae)

Vernacular Names : Chhatani (O), Satoona (U), Chatiwan (H), Saptaparni(S)

Locality with V.S. No. : Badapokhari-8698

Ethnomedicinal Uses : The powder of the bark (one teaspoon) with equal amount of sunthi powder is taken along with cow's milk (one cup) twice a day to cure post-pregnancy fever.

- Botanical Name : *Amaranthus spinosus* L. (Amaranthaceae)
- Vernacular Names : Kanta marisha (O) Choulai (H) Meghanad (S)
- Locality with V.S. No. : Jaleshwar -8660.
- Ethnomedicinal Uses : The paste of the plant's root (10 g) along with rice washed water is taken twice daily for three to four days to cure menorrhagia.
- 
- Botanical Name : *Bombax ceiba* L. (Bombaceae)
- Vernacular Names : Simil (O), Saimbhal (U), Semal (H), Mocharas (S)
- Locality with V.S. No. : Nilgiri- 8574
- Ethnomedicinal Uses : The petals of the flower fried in pure-ghee, is taken with black salt for one week to cure menorrhagia.
- 
- Botanical Name : *Butea monosperma* (Lam.) Taub. (Fabaceae)
- Vernacular Names : Palasa (O), Dhak (H/U), Palash (S)
- Locality with V.S. No. : Sajanagarh- 8553
- Ethnomedicinal Uses : The powder of the dried leaves (one teaspoon) is taken twice daily for one month to cure white discharge (Leucorrhoea) and menorrhagia.
- 
- Botanical Name : *Ficus hispida* L.f.. (Moraceae)
- Vernacular Names : Dumer (O), Anjeer-e-Dasti (U), Gular (H) Udumbar (S)
- Locality with V.S. No. : Nilgiri- 8572
- Ethnomedicinal Uses : The paste of the bark (10gm.) with water is taken twice daily to cure white discharge (Leucorrhoea) & menorrhagia.
- 
- Botanical Name : *Ficus religiosa* L. (Moraceae)
- Vernacular Names : Usto (O), Pippal (H / U), Aswatatha(S)
- Locality with V.S. No. : Nilgiri- 8562
- Ethnomedicinal Uses : The paste of the bark (10 g) is taken with water (one glass) twice daily for one month to cure white discharge (Leucorrhoea).

- Botanical Name : *Hibiscus rosa-sinensis* L. (Malvaceae)
- Vernacular Names : Mandar (O), Jabakasam (U), Gudahal (H), Japapuspa (S)
- Locality with V.S. No. : Kendukhunta- 8692
- Ethnomedicinal Uses : a. White flowers of the plant (five nos.) are taken in the morning on empty stomach for about two to three months to cure white discharge (Leucorrhoea).  
b. Red flowers of the plant (five nos.) fried with pure-ghee are taken in the morning daily to cure irregular periods (metrorrhagia).
- Botanical Name : *Holarrhena pubescens* (Buch-Ham.) Wall.ex.G.Don (Apocynaceae)
- Vernacular Names : Kuro (O), Inderjo Talkh (U), Kuda (H), Kutaj(S)
- Locality with V.S. No. : Saganaria- 8484
- Ethnomedicinal Uses : The powder of the bark (one teaspoon) along with rice washed water (one cup) is taken twice daily for a week to cure menorrhagia.
- Botanical Name : *Holoptelea integrifolia* (Roxb.) Planch. (Ulmaceae)
- Vernacular Names : Dharanj (O), Chilbi (H), Chirabilwa (S)
- Locality with V.S. No. : Jodiwali- 8630
- Ethnomedicinal Uses : The paste of the bark (5 g) with water (one glass) is taken in the morning in the empty stomach to cure post-pregnancy fever.
- Botanical Name : *Mangifera indica* L. (Anacardiaceae)
- Vernacular Names : Amba (O), Aam (H), Amra (S)
- Locality with V.S. No. : Jaleshwar- 8655
- Ethnomedicinal Uses : The skin of unripe mango fried in desi-ghee is taken daily to cure menorrhoea.
- Botanical Name : *Mimosa pudica* L. (Mimosaceae)
- Vernacular Names : Lajkoli (O), Lajwanti (H/U), Lajjawati(S)
- Locality with V.S. No. : Kendukhunta- 8517

Ethnomedicinal Uses : The powder of seeds (5 g) is taken daily on empty stomach for one month to cure menorrhagia.

Botanical Name : *Mimusops elengi* L. (Sapotaceae)

Vernacular Names : Boula (O), Maulsree (H/U), Bakula (S)

Locality with V.S. No. : Nilgiri- 8474

Ethnomedicinal Uses : The powder of the bark (one teaspoon) is taken twice daily on empty stomach for four to five days to cure uterus problems.

Botanical Name : *Phyllanthus fraternus* Webster. (Euphorbiaceae)

Vernacular Names : Badionla (O), Bhuinamla (U), Jharamla (H), Bhuamalika (S)

Locality with V.S. No. : Darakholi- 8597

Ethnomedicinal Uses : The paste of whole plant or fruit (5 g) along with rice washed water is taken twice daily in empty stomach for three-four days to cure menorrhoea.

Botanical Name : *Ricinus communis* L. (Euphorbiaceae)

Vernacular Names : Gaba (O), Baid Anjir (U), Arandi (H), Aranda (S)

Locality with V.S. No. : Mitrapur- 8514

Ethnomedicinal Uses : The stem of the plant is cut into pieces and is sun dried and then burnt. The ash along with equal amount of amla powder is taken (one teaspoon) twice daily on empty stomach to cure white discharge (Leucorrhoea) and menorrhagia.

Botanical Name : *Saraca asoca*(Roxb.) de.Wilde (Caesalpiniaceae)

Vernacular Names : Oshoko (O), Ashok (H / U), Ashoka(S)

Locality with V.S. No. : Sajanagarh- 8503

Ethnomedicinal Uses : The bark of the plant (120 g) and black rasi (120 g) is boiled in one glass of milk and three glasses of water for sometime. The rest part (milk) is taken thrice daily on empty stomach to cure white discharge (Leucorrhoea), menorrhagia and irregular periods (menorrhagia).



- Botanical Name : *Streblus asper* Lour. (Moraceae)
- Vernacular Names : Sahada (O) Sahoda (H), Sakhotak (S)
- Locality with V.S. No. : Tenda- 8548
- Ethnomedicinal Uses : The bark of the root (5 g) along with rice washed water is taken twice daily to cure white discharge (Leucorrhoea).
- 
- Botanical Name : *Syzygium cuminii*(L.) Skeels. (Myrtaceae)
- Vernacular Names : Jamkoli (O), Jamun (H/ U), Jambu(S)
- Locality with V.S. No. : Naranpur-8575
- Ethnomedicinal Uses : The soft leaf or the bark juice (10ml.) along with rice washed water is taken twice daily on empty stomach to cure menorrhagia.
- 
- Botanical Name : *Tamarindus indica* L. (Caesalpinaceae)
- Vernacular Names : Tamar Hindi (U), Tentel (O), Imli(H), Tentula (S)
- Locality with V.S. No. : Tenda- 8546
- Ethnomedicinal Uses : The seeds are soaked overnight, then the paste of those soaked seeds (two teaspoons) along with milk (one glass) is taken twice daily to cure white discharge (Leucorrhoea).
- 
- Botanical Name : *Tinospora cordifolia*(L.) Merr. ( Menispermaceae)
- Vernacular Names : Gilu (U), Gilochi (O), Giloy (H), Amrata (S)
- Locality with V.S. No. : Nilgiri-8583
- Ethnomedicinal Uses : The stem of the plant (50 g) first crushed and then soaked in two glasses of water overnight and then filtered, the filtrate is taken daily for one month to cure white discharge (Leucorrhoea).
- 
- Botanical Name : *Woodfordia fruticosa*(L.) Kurz. (Lythraceae)
- Vernacular Name : Dhatuki (O), Dhaya (H), Dhataki (S)
- Locality with V.S. No. : Saganaria- 8478
- Ethnomedicinal Uses : The powder of flowers (10 g) along with honey is taken thrice daily on empty stomach to cure white discharge (Leucorrhoea) and menorrhagia.

## Discussion and Conclusion

The ethnomedicobotanical survey of the area revealed that the people of the area are possessing good knowledge of herbal drugs but, as the people of the societies are in progressive exposure to modernization; their knowledge of traditional uses of plants may be lost in due course. So it is important to study and record the uses of the plant by different tribes and sub-tribes for future study. Such studies may also provide some information to phyto-chemists and pharmacologists in screening of individual species in rapid accessing of phyto constituents.

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# Characterization of Herbals and Herbal Drugs

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## Abstract

The assessment of herbals as drug is directly linked with the ability to accurately determine the marker constituents which are also finger prints of plant material. Over the past several decades the herb industry has developed sophisticated and complex chemical assays to identify specific marker compounds in plant materials to ensure identity and qualitative aspects for the proposed botanical preparation. The increasing acceptance to herbal drugs resulted to large number preparations on market. These products face challenges from regulatory and safety authorities because plants contain a wide variety of potentially active constituents, even same species exhibit a broad range of chemical profiles. It has been noted that same species is being used for different remedies in different traditional system, with history of successful and widely accepted treatment. Established specification, quality control, quality assurance and use of validated process are the parameter to over-come from such problems.

**Key Words:** Herbals, Active constituents, Process validation, Finger prints, Regulatory and safety aspects.

## Introduction

The use of herbal products for therapeutic properties is as ancient as human civilization. With advancement of pharmaceutical science, they have been made into more acceptable and in easy ingestible form such as decoctions, syrup, tinctures, herbal teas, tablets, confections, steam distillate etc. The success of herbal drug is based as much on its purity and uniformity of the constituents, so their physico-chemical, pharmacological and pharmacognostic properties are studies along with quantitative characteristics. (Anonymous, 2001; Anonymous, 2003; Saraswathy *et al.*, 2005). The pharmacological spectrum of a drug is ascertained through experimental trials on laboratory animals under standard laboratory conditions for estimation of dosage, evaluation of efficacy, safety and mode of action. (Barrava *et al.*, 2005). Recent industrial attempts for regulating the accepted standard criteria for chemical evaluation and authentication of the claims made for the product have global impact on trade and success of herbal industry. (Anonymous, 1999). Botanicals are identified using macroscopic and microscopic techniques along the place of origin. (Mukherjee *et al.*, 2002). Every step of manufacturing process is deciding for the quality of the product. Chemical and chromatographic testing to generate the supporting evidence of principal constituents, are documented. (Asper *et al.*, 2005). Binders and fillers in the final products are specified with stability and expiry parameters before supplying to consumers. (Anonymous, 2001; 2003).

### *Herbals: Chemistry and Quality Control*

Herbals and herbal drugs are not merely mixtures/-extracts of medicinal plants, but are integrated, chemically modified and suitably formulated combination of standardized moieties derived from plant materials. The role of each ingredient may be different, even not be directly contributing to the array of active molecules, as it can be precursors, artifacts, neo-molecules, emulsifying agents, alkaline or acidic components contributing for the pH for chemical changes. The chemistry connected with the active principles and their precursors reveals the potential of the drug. The detailed studies have shown that most of the therapeutically active compounds are secondary metabolites, which can be grouped in classes as below: (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

#### *Alkaloids*

These are nitrogenous organic compounds usually of plant origin, having a complex structure, which are made up of carbon, hydrogen, usually one or sometimes more than one nitrogen atom and generally oxygen, which possesses significant pharmacological activity. Alkaloids are product of plant metabolism and are biosynthesized, usually from amino acids. Depending on the types of raw material, the method of extraction may vary, but in general the plant material is basified using diethyl amine or ammonia and plant tissue extracted with organic solvent. Alkaline medium ensures that the alkaloids are in their base or unionized state, and can be extracted with chloroform, dichloromethane or diethyl ether, even alcohol can be used. The second approach is to treat the plant material with aqueous acid, when alkaloid form salt, soluble in water which can be recovered as free base by basifying the aqueous phase, except a few (berberine, colchicine, hyoscyne and hyoscyamine) other alkaloids are insoluble in water in free form. (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

#### *Carotenoids*

The red, orange and yellow pigments observed in the plants usually are tetraterpenoid derivatives and divided into hydrocarbon and their oxygen derivatives, known as xanthophylls. For extraction of these compounds petroleum ether is used for hydrocarbons while xanthophylls being more polar due to alcoholic or ketonic or aldehydic or acidic or epoxide nature, are extracted with alcohol or mixture of alcohol and chloroform. (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

#### *Flavonoids*

It is a largest group of naturally occurring phenol, contain fifteen carbon atoms in their basic nucleus. The best solvents for extraction are acetone with water or

alcohol with water in certain ratio. (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

### *Glycosides*

Compounds formed by the condensation of sugar (glycone) with organic compounds (aglycone) are characterized by their property of being hydrolysed in presence of enzymes, dilute acids or alkalis. Mostly glycosides are extracted with polar solvent such as acetone, ethanol, methanol, water and mixture of water with these. Cardiac glycosides (e.g. Digoxin) contain bulky steroidal aglycones, so soluble in chloroform. When extracting with water enzymatic breakdown is possible due to 'glycosidase enzyme', so water above 50°C temp. or with significant proportion of alcohol or ammonium sulphate are used for extraction. Glycoside may have link via -O,-N or -S with aglycone moiety, so are called -O-glycoside,-N-glycoside or -S-glycoside, which can be easily hydrolysed in water with 10% H<sub>2</sub>SO<sub>4</sub> at certain temperature. (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

### *Phenolic Compounds*

These are of high biological value compounds, may be characterized having at least one aromatic ring substituted by at least one hydroxyl group. They are weak acid and can be extracted in hydro-alcoholic medium. Phenolic compounds undergo extensive polymerization reaction by the action of 'phenol oxidase'. The reaction is responsible for brown coloration of damaged plant material, when exposed to air. Phenolics are the compounds that inhibit oxidation. Higher phenolics in food tend to generate higher anti-oxidants level. (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

### *Saponins*

Saponins are glycosides, which forms colloidal solution with water, on shaking produces a foam or froth. Structurally saponins can be grouped as; steroidal saponins (tetra cyclic) and triterpenoid saponins(pentacyclic).The aqueous extraction of saponins is not recommended as it promotes hydrolysis, so hydro- alcoholic extraction is preferred. Purification of saponins can be achieved by exchanging the aqueous syrup with water insoluble alcohol. (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

### *Tannins*

Tannins are poly phenolic compounds, wide- spread in nature and probably present in every plant which can be categorized as hydrolysable tannins and condensed tannins. Tannins are generally extracted with water and acetone, not with methanol

as it causes methanolysis. (Anonymous, 1999; Mukherjee *et al.*, 2002; Asper *et al.*, 2005; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002).

### *Volatile Oil/Essential oil*

These are complex and highly volatile mixtures containing terpenoids (monoterpenes and sesquiterpenes), mainly mono and sesquiterpenoids and are phenolic in nature. Which are relatively non polar and can be extracted into light petroleum. However slightly more polar solvents e.g. chloroform, dichloromethane can be used. Steam distillation is popular method for selective extraction of volatile oils while supercritical fluid extraction is going to be latest option to extract the volatile oils.

### *Phytochemicals and their suitable derivatives*

The naturally occurring constituent may not be ideal due to side effects or poor activity. Therefore once the structure and therapeutic behavior of the constituent is known, research is directed to develop derivatives/analogues, which are more potent, less toxic and find together new application e.g. colchicine to thiocolchicine and colchicoside to thiocolchicoside, 10-deactylbacattin to taxol, curcuminoids to tetrahydrocurcuminoids (THC) are the steps in this direction. (Dubey *et al.*, 2004; Chakkaravarti *et al.*, 2005).

### *Validation of manufacturing process*

The process of validation is linked with actual development and implementation of manufacturing parameters because the developer does not know whether the method conditions are acceptable until validation studies performed. The results of validation of a manufacturing and analysis method adopted indicate that either any change in the procedure is necessary, which may then require revalidation. During each validation study, key method parameters are determined and then used for all subsequent validation steps. To minimize repetitious studies and ensure that the validation data are generated under conditions equivalent to the final procedure, studies on the following sequence may reduce the labor. Proper raw material selection is an essential requirement to generate the quality product, adopted for selection of good raw materials. The manufacturing process with established process chemistry for product to have consistency in term of yield of finished goods and economy of the product is the validation of manufacturing process. The deviation / modification in the process, is matter of re-validation of the process. Proper documentation with cleaning validation during process scale-up is a matter of great concern, for generation of finished product specification. (Anonymous, 2001; 2003; Mukherjee *et al.*, 2002; Dubey *et al.*, 2004).

### *Analytical validation & experimental design*

The evidence for intended purity is determined by validated analytical method, which includes studies on specificity, linearity, accuracy, precision, range, detection limit, ruggedness and robustness of the method. In pharmaceutical industries, where methods are submitted to regulatory agencies and further changes may require formal approval before implementation. The best way to minimize administrative problems is to perform adequate validation experiments during development. The first step in the method development and validation cycle is to set minimum requirements, which are specifications for the method. A complete list of criteria is prepared as agreed by the developer and the end users before the method is developed so that expectations are clear. During actual studies and in the final validation report, these criteria allow clear judgment about the acceptability of the analytical method. The statistics generated for making comparisons are similar to what analysts generate later in the routine use of the method and therefore will serve as a tool for evaluating later questionable data. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Specificity of the method*

Specificity of a method is the ability to accurately measure the analyte response in presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components is compared with the response of a solution containing only the analyte. Other potential sample components are generated by exposing the analyte to stress conditions {as heat, light, acid (0.1 N-HCl), base (0.1 N-NaOH), and oxidant (3% H<sub>2</sub>O<sub>2</sub>)} sufficient to degrade it to 80-90% purity. For formulated products, heat, light, and humidity (85%) are often used. The resulting mixtures are then analyzed and the response is evaluated for purity with standard and resolution from the nearest eluting peak. Once acceptable resolution is obtained for the analyte and potential sample components, the chromatographic parameters, such as column type, mobile-phase composition, flow rate, and detection mode are considered to be set. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Accuracy of the method*

The accuracy of a method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined in one of four ways. First, accuracy can be assessed by analyzing a sample of known concentration and comparing the measured value to the true value. The second approach is to compare test results from the new method with results from an existing alternate method that is known to be accurate. The third and fourth approaches are based on the recovery of known amounts of analyte spiked into sample matrix. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).



### *Precision of the method*

The precision of an analytical method is the amount of scatter in the results obtained from multiple analyses of a homogeneous sample. The first type of precision study is instrument precision or injection repeatability. A minimum of 10 injections of one sample solution is made to test the performance of the chromatographic instrument. The second type is repeatability or intra-assay precision. Intra-assay precision data are obtained by repeatedly analyzing, in one laboratory on one day, aliquots of a homogeneous sample, each of which has been independently prepared according to the method procedure. From these precision studies, the sample preparation procedure, the numbers of replicate samples are prepared, and the number of injections required for each sample in the final method procedure will be set. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Linearity of the method*

A linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration. This study is generally performed by preparing standard solutions at five concentration levels, from 50 to 150% of the target analyte concentration. The standards are evaluated using the conditions determined during the specificity studies, which is analyzed a minimum of three times. The 50 to 150% range for this study is wider than what is required by the FDA guidelines, so final method procedure, a tighter range of three standards is generally used, such as 80, 100, and 120% of target. Validating over a wider range provides confidence that the routine standard levels are well removed from nonlinear response concentrations, that the method covers a wide enough range to incorporate the limits of content uniformity testing, and that it allows quantification of crude samples in support of process development. At the completion of linearity studies, the appropriate concentration range for the standards and the injection volume are set for all subsequent studies. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Range of the method*

The range of an analytical method is the concentration interval over which acceptable accuracy, linearity, and precision are obtained. In practice, the range is determined using data from the linearity and accuracy studies. These precision data are generated from the triplicate analyses of spiked samples in the accuracy study. Higher variability is expected as the analyte levels approach the detection limit for the method. The decision of analyst about concentration becomes valuable for the intended use of the method. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Detection limit of the method*

The detection limit of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically, three times the noise level. The detection limit needs to be determined only for impurity methods in which chromatographic peaks near the detection limit will be observed. The detection limit is to be estimated early in the method development-validation process and is repeated using the specific wording of the final procedure for any changes. It is important to test the method detection limit on different instruments, such as those used in the different laboratories to which the method will be transferred. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Quantification limit of method*

The quantification limit is the lowest level of analyte that can be accurately and precisely measured. This limit is required only for impurity detection and is determined by reducing the analyte concentration until a level is reached where the precision of the method is unacceptable. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Robustness of the method*

The ability to remain unaffected by small changes in parameters such as variation in mobile phase composition, pH of the mobile phase, buffer concentration, temperature, and injection volume. These method parameters are evaluated one factor at a time or simultaneously as part of a factorial experiment as different columns (different lots/-suppliers). The consequence results as data for system suitability parameters to ensure the validity of analytical procedure to be included in the final method procedure as pharmacopoeial or non-pharmacopoeial. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

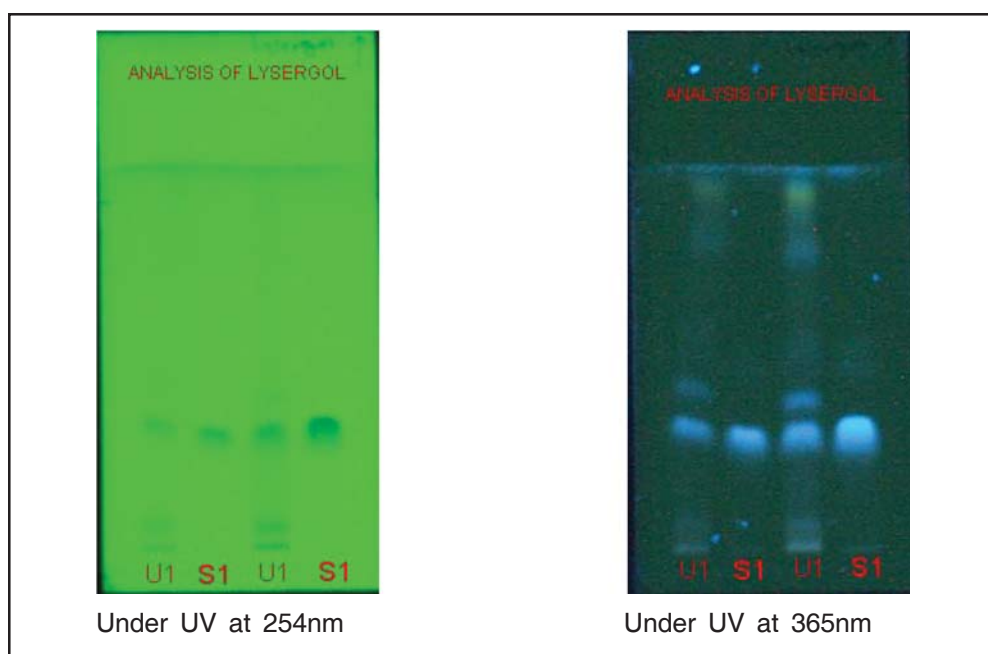
### *Stability of the method*

The stability of reagents used during validation studies (mobile phases, standards and sample preparation) is documented as solutions to be stable enough to allow for delays such as instrument breakdowns or overnight analyses using auto-samplers. At this point, the limit of stability is tested. Samples and standards are studied over at least a 48-hours period, and quantification of components is determined by comparison to freshly prepared standards. If the solutions are not stable over 48 hours, storage conditions or additives are identified that can improve stability. (Anonymous, 1999; 2001; 2003; Mukherjee *et al.*, 2002).

### *Finger prints documentation*

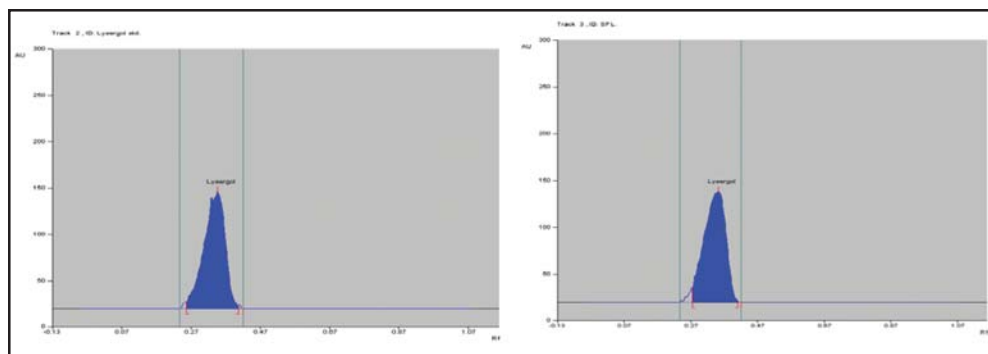
Finger printing in herbal industries has been used to highlight impurity profiles, thereby giving indication of its purity. Chromatographic techniques, most frequently

used are TLC, HPLC, HPLC, GLC and some times UV-Vis., I.R. Spectroscopy for finger prints is followed. The choice depends on the nature of the constituents that are present in plant material or on customer's specification. The chemical profile (fingerprint) of the raw material, or of intermediate product (specific extracts) or of the finished products against reference standard, (Fig.1 & Fig. 2) defines claims made on certificate of analysis, which are documented with batch no./ Lot no., process stage, date, signature of operator and supervisor in batch manufacturing record (BMR). When HPLC techniques are used for analytical purpose to quantify constituent(s) of the finished product, chromatograms are used as finger prints. (Mukherjee *et al.*, 2002; Hostettmann *et al.*, 2001; Psascual *et al.*, 2002; Chakkaravarti *et al.*, 2005).



**Fig. 1.** TLC Finger Prints <sup>12</sup> Where S= Standard of Lysergol, U= Kaladana extract (Mobile phase:  $\text{CHCl}_3$ : MeOH:  $\text{NH}_4\text{OH}$ ::94:5.5: 0.5, Silica gel60F<sub>254</sub>)

**HPTLC finger prints**<sup>12</sup> Evaluation of Lysergol (scanning @410nm after derivatisation)



**Fig. 2.** Kaladana Extract for Lysergol Lysergol Standard

## Conclusion

The concept to provide adequate information to the consumers about the safety and credibility, with evidence has accelerated the consumption and prescription of herbals and herbal drugs. (Balachandran *et al.*, 2005; Chidambara *et al.*, 2003). For characterizations of these products a team of Botanists, Ethnobotanists, Medical Botanists, Pharmacognosists, Natural Products Chemists, Biologists, Health Professionals (e.g., Naturopaths, Acupuncturists and frequently Chiropractors) requires, to diffuse the misleading claims by reliable validated data and standard test procedures (STPs) for the contents, claimed in the product. At present the herbal and natural products industries are at the places where scientific discipline combine with business. (Kala *et al.*, 2006; Chakkaravarti *et al.*, 2005). Now, with impending and increasing government regulations that define good manufacturing practices (GMPs) for herbals and herbal drugs, the application of these disciplines joined with good research practices reflects as standard operating procedures (SOPs) for product development and manufacturing activities. (Kala *et al.*, 2006; Chakkaravarti *et al.*, 2005). The present paper aims at increasing awareness on these issues and facilitating a critical analysis of the present market situation. It is intended to lead to a stronger partnership and collaboration among all the stakeholders who have a role in this important sector.

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# Hepatic Safety of the Unani Anti-Arthritic Drug Bisfaij (*Polypodium vulgare*)

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## Abstract

Bisfaij (*Polypodium vulgare*), a less used Unani anti-arthritic drug was shown by us to have good anti-arthritic activity in Freund's Adjuvant Arthritis in rats which is considered to be a good model of Rheumatoid Arthritis. Unani texts recommend it for Jaundice also. However, as the anti-arthritic activity demonstrated by us could be due to NSAIDs-like effect which may have deleterious effect on liver function so it was considered worthwhile to test the drug for effect on liver function. Therefore, two doses of the 50% Ethanolic extract of Bisfaij, the lower corresponding to the Unani clinical dose were studied for effect on biochemical markers of liver function in rats. The study showed Bisfaij to have no effect on serum Bilirubin but it produced a small, significant increase in serum levels of ALT, AST and Alkaline Phosphatase, which was significantly lesser than the increase produced by Diclofenac and within normal limits. Thus, the study does not challenge the hepatic safety of Bisfaij as an Anti-Arthritic Agent but it suggests that the Test Drug should be studied for effect on compromised liver function before being cleared for use in Jaundice and other conditions with hepatic insufficiency.

**Key Words:** Bisfaij, *Polypodium vulgare*, Unani Medicine, Anti-Arthritic, Liver Function.

## Introduction

One basis of the great importance and potential of Tib-e-Unani (Unani Medicine) is the presence of effective and safe drugs for the serious and common disease group of Rheumatological disorders such as Rheumatoid Arthritis, Osteoarthritis etc for which Western Medicine has no effective treatment. But the actualization of this potential is somewhat compromised by the absence of scientific studies of these drugs. Such studies would not only widen the confidence in Unani anti-arthritic drugs but also improve the efficacy and safety of their clinical application. Secondly, it could be of benefit to main-stream healthcare by providing more efficacious and safe anti-arthritic drugs to Western Medicine which it currently lacks.

Thus, we have subjected many Unani anti-arthritic drugs to experimental pharmacological study including the most commonly used Unani anti-arthritic drug, namely, Suranjan (*Colchicum luteum*) (Amin & Minhajuddin, 2009) as well as Bisfaij (Amin, 2007), which though not commonly used for this purpose, is described as an anti-inflammatory (Muhallil-e-Awram) (Ibn Baitar) agent since the earliest times, the finding mentioned by the first Unani Pharmacologist, Dioscorides (Dymock, 1891). It is of special interest as it is described to purge not only Balgham (Phlegm) (Ibn Sina, 1038) but also Sauda (Black Bile) (Ibn Baitar), and being Mulattif (Demulcent) to Mutasallab Maddah (Hardened Pathological Matter) (Hakeem, 1343 H), hence, particularly suitable for chronic forms of arthritis such as Rheumatoid

Arthritis, which are likely to involve both Sauda and Tasallub (Hardening). We, showed Bisfaij to significantly suppress Freund's Adjuvant Arthritis (Amin *et al.*), which is considered to be a good model of Rheumatoid Arthritis. This could be due to NSAIDs-like activity which usually possess Analgesic Activity. The NSAIDs may have deleterious effect on Liver Function but Unani texts report Bisfaij to be useful in Yarqan (Jaundice) (Ibn Baitar, Pub.1871). In view of this possible discrepancy it was considered worthwhile to study Bisfaij for effect on Liver Function in rats with Diclofenac sodium as the standard NSAID for comparison and for the validation of the methodology of the Test.

## Material and Methods

### *Preparation of Extract*

Bisfaij (*Polypodium vulgare*) was obtained from the Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh. The drug was identified at the Pharmacognosy Section of Department of Ilmul Advia, AMU, Aligarh. It was dried in Hot air oven at 45°C for 6 hours and powdered in Haawan Dasta (Iron Mortar) as a coarse powder for extraction and kept in an airtight container. The powdered drug was separately extracted in 50% alcohol with Soxhlet's apparatus for 6 hours. The extracts were filtered and dried by evaporation on water bath. The yield percentage was calculated with reference to crude drug and was found to be 31.95%. Fresh suspension of the extract was prepared in distilled water with 1% methylcellulose, which was administered orally with the help of oral feeding cannula. The dose for the animal was calculated by multiplying the Unani clinical dose of test drugs by conversion factor of 7 for rat (Frierich *et al.*, 1996). The two different doses selected for the study of Bisfaij (*Polypodium vulgare*) were 560 mg/kg and 700 mg/kg. The standard drug Diclofenac sodium was administered in the dose of 5 mg/kg.

### *Liver Function Test (Biochemical)*

The test was carried out in albino rats of either sex of 150-200 g body weight. The animals were divided into 4 groups of 6 animals each. Animals in Group I served as control and were administered with 20 ml/kg of distilled water. The standard drug Diclofenac sodium was given to animals in Group II, in a dose of 5mg/kg orally. The animals in Group III and IV were administered with 560 mg/kg and 700 mg/kg of Bisfaij (*Polypodium vulgare*) extract, respectively, once a day for 6 days after overnight fasting. On the seventh day all animals were sacrificed and blood was collected for the estimation of bio-chemical markers of liver function, namely, S Bilirubin by the method of Malloy and Evelyn (1937), AST (SGOT) and ALT (SGPT) by the method of Reitman & Frankel (1957) and S Alkaline Phosphatase by the method of Kind and Kings (1954), using Reagent kits supplied by SPAN Diagnostic Ltd (Code No. 25920).



## Observations and Result

S. Bilirubin was found to be  $0.98 \pm 0.07$  mg/100ml in Control Group while, it was significantly increase to  $1.51 \pm 0.15$  ( $P < 0.01$ ) mg/100ml in Diclofenac Group and  $1.13 \pm 0.10$  mg/100ml and  $1.13 \pm 0.13$  mg/100ml with the 560 mg/kg and 700 mg/kg of *P. vulgare* extract, respectively. The concentration of S. Bilirubin with both doses of *P. vulgare* was not significantly different in comparison to Control Group. The concentration of S. Bilirubin with the lower dose of *P. vulgare* was significantly lesser in comparison to Standard drug Diclofenac sodium ( $P < 0.05$ ). However, it was not significantly different with the higher dose of *P. vulgare*.

The concentration of ALT/SGPT was found to be  $21 \pm 1.34$  IU/L in the Control Group while, it was significantly increased to  $54.33 \pm 2.03$  IU/L ( $P < 0.001$ ) in the Diclofenac Group and  $37 \pm 2.81$  IU/L ( $P < 0.001$ ) and  $44.66 \pm 2.29$  IU/L ( $P < 0.001$ ) with 560 mg/kg and 700 mg/kg of *P. vulgare* extract, respectively. The concentration of SGPT with the lower dose of *P. vulgare* was significantly lesser than the higher dose ( $P < 0.05$ ). The concentration of SGPT with both the doses of *P. vulgare* was significantly lesser than with the standard drug Diclofenac sodium ( $P < 0.01$ ).

The concentration of AST/SGOT was found to be  $25.33 \pm 1.76$  IU/L in the Control Group while, it was significantly increased to  $77.66 \pm 3.19$  IU/L ( $p < 0.001$ ) in the Diclofenac Group and  $36.33 \pm 2.50$  IU/L ( $P < 0.001$ ) and  $51.33 \pm 1.61$  IU/L ( $p < 0.001$ ) with the 560 mg/kg and 700 mg/kg of *P. vulgare* extract, respectively. The concentration of SGOT of with lower dose of *P. vulgare* was significantly lesser than the higher dose ( $P < 0.01$ ). The concentration of SGOT of both the doses of *P. vulgare* was significantly lesser in comparison to the Standard drug Diclofenac sodium ( $P < 0.001$ ). However, the effect of the higher dose was not significantly different from the Diclofenac sodium.

The concentration of S. Alkaline phosphatase was found to be  $9.4 \pm 0.67$  KAU/100ml in the Control Group while, it was significantly increased to  $15.6 \pm 0.96$  KAU/100ml ( $P < 0.001$ ) in the Diclofenac Group and  $12.9 \pm 0.69$  KAU/100ml ( $P < 0.01$ ) and  $14.4 \pm 1.06$  KAU/100ml ( $P < 0.001$ ) with the 560 mg/kg and 700 mg/kg of *P. vulgare* extract, respectively. The concentration of S. Alk. Phosphatase with both the doses of *P. vulgare* was not significantly different. The concentration of S. Alk. Phosphatase with the lower dose of *P. vulgare* was significantly lesser in comparison to the Standard drug Diclofenac sodium. However, with the higher dose of *P. vulgare* it was not significantly different in comparison to Standard drug. The results are presented in Table 1.

## Discussion

The findings of the test for effect on biochemical parameters of liver function shows that Bisfaij has no effect on S Bilirubin both at the low as well as high dose. However, both doses produce a small but significant increase in ALT/SGPT, AST/



**Table-1. Effect of Test Drug on Biochemical parameters of Liver Function with Diclofenac as the Standard Anti-inflammatory agent**

Group	S. Bilirubin mg/100ml	SGPT/ALT IU/L	SGOT/AST IU/L	S. Alk. Phos. KAU/100ml
Control	0.98±0.07	21.0±1.34	25.33±1.76	9.40±0.67
Diclofenac (5mg/kg)	1.51±0.15 x <sup>2</sup>	54.33±2.03 x <sup>3</sup>	77.66±3.19 x <sup>3</sup>	15.60±0.96 x <sup>3</sup>
<i>P. vulgare</i> (560mg/kg)	1.13±0.10 y <sup>1</sup>	37.0±2.81 x <sup>3</sup> y <sup>3</sup> a <sup>1</sup>	36.33±2.50 x <sup>1</sup> y <sup>3</sup> b <sup>3</sup> a <sup>2</sup>	12.90±0.69 x <sup>2</sup> y <sup>1</sup>
<i>P. vulgare</i> (700mg/kg)	1.13±0.13	44.66±2.29 x <sup>3</sup> y <sup>2</sup>	51.33±1.61 x <sup>3</sup> y <sup>3</sup> b <sup>2</sup>	14.40±1.06 x <sup>3</sup>
F- value	2.27	35.54	42.51	6.90

**n = 6**

x = Against Control

1 = p < 0.05

y = Against Diclofenac Sodium

2 = p < 0.01

**a = Against *P. vulgare* (700mg/kg)**

**3 = p < 0.001**

SGOT and S Alkaline Phosphatase. But this increase is significantly lesser than the increase caused by Diclofenac sodium, which also significantly increases S Bilirubin. Thus, Bisfaij is shown to have a mild to negligible deleterious effect on liver function which is significantly lesser than the effect of Diclofenac sodium. However, the increase in biochemical parameters of liver function by both Diclofenac sodium and Bisfaij is by and large within normal limits. Thus, the study does not challenge the safety of Bisfaij for the Liver as an Anti-Arthritic Drug, however, it does put a question mark on it. However, it does indicate that the use of Bisfaij in Jaundice, as reported in Unani texts, and in other conditions where liver function is compromised should be put on hold till it is shown to be safe to hypofunctioning Liver.

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# Therapeutic Uses of Earthworm – A Review

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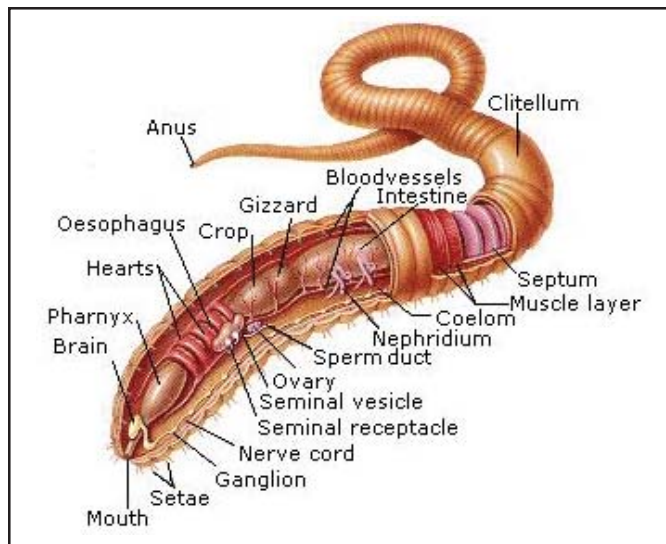
## Abstract

Based on review of literature on Unani Medicine and contemporary scientific investigations, the study demonstrates enormous potential of common Indian earthworm to combat many human ailments. The information on clinical, pharmacological and chemical studies carried out on earthworms between 1930 and 2009 in India and abroad have been reviewed in an effort to substantiate their medical efficacy. In this paper, the methods of collection and processing of earthworms, their pharmaceutical composition, preliminary pharmacological and clinical effects on (i) nervous system; (ii) blood circulatory system; (iii) cardiovascular system; (iv) respiratory system; (v) uterus smooth muscle function; and (vi) anticancer properties are reviewed. Therapeutic efficacy of earthworms has been shown for *tracheitis* and *bronchial asthma*, *epilepsy*, *high blood pressure*, *schizophrenia*, *leg ulcers*, *mumps*, *eczema*, *urticaria* and *anaphylaxis diseases*, *burns* and *scald fractures*, *erysipelas*, *sequel of encephalitis*, *chronic lumbago*, *skin crevices*, *blood-deficiency apoplexy*, *acute injury of soft tissues*, *vertigo*, *hematemesis* and *hematuria*, *digestive ulcer*, *vesicle calculus* and *cancer*. Taking lead from the present reports detailed scientific investigations are needed to discover new therapeutic agents of zoological origin which might be very specific to treat certain diseases and conditions, thus far, incurable in modern medicine.

**Key Words:** Earthworm, Unani Medicine, Materia Medica, Indian Systems of Medicine.

## Introduction

Earthworms (Fig. 1) have been used in traditional medicine in India for at least 2,300 years (Puri, 1970). Results from the studies of Zhang *et al.*, (1988), Alumets *et al.*, (1979) and Reynolds and Reynolds (1972) show that some nitrogenous substances extracted from the earthworm can dilate bronchi and can be used as an anti-histamine to treat asthma. In Unani texts, such as *Khawasul Advia* (1911), *Unani Chikitsa Sagar* (Shukla, 1950), *Village Physician* (1959), *Havial Mutradat Wa Jamial Mustalehal* (Hussain, 1901) and '*Makhzanal-Advia*' (Hussain, 1771), it is recorded that '*Kharteem*' (Unani name for earthworm) was used as an antipyretic and anesthetic, for detoxification, treatment of hypertension and hastening parturition, as well as in the treatment of many common ailments, such as arthritis, itching, burns, carbuncles, erysipelas, and inflammation. With the development of modern science, some active compounds from earthworm, such as linmbritin and terrestrolumbralisim, have been isolated. Recently, an enzyme has been extracted from earthworm that can dissolve blood thrombi in experimental conditions. This will probably be made into an oral medicine by the pharmaceutical industry for use in the prevention of cardio-vascular disease (Zhenjun, 2007). Based on earlier studies the present paper highlights the enormous medicinal potential of earthworm to



**Fig. 1.** *Drawida grandis* – An important medicinal earthworm

combat many of the present day diseases and conditions. Further, investigations comprising advanced Pharmacological, Phytochemical and Clinical studies are needed to develop new drugs of zoological origin.

### *Chemical Composition*

Earthworms contain lumbrofebrine, terrestrolumbrylsin, lumbritin, hypoxanthine and other purines, pyrimidines, choline and guanidine. The fat of earthworm is composed of octade acids, palmitic acids, high-chain unsaturated fatty acids, linear and carbon fatty acids, branched fatty acids, phosphate, cholesterol etc. The yellow chloragenous cells and organs of *Lumbricus terrestris* contain large amount of carbohydrates, lipid, protein, pigments and some alkaline amino acids. The yellow pigments perhaps consist of riboflavine or its analogues (Anonymous, 1985).

The tissues of *Pheretima* species contain large amount of microelements-Zn 59.1 µg/g, Ca 25.4 µg/g, Fe 1735.5 µg/g, Cr 10.93 µg/g, Mo 0.25 µg/g, Ca 1019.2 µg/g and Mn 1143 µg/g (Zhang, 1988). Those of *Allolobophora caliginosa* contain crude protein 57.96%, crude fat 6.53%, crude ash 21.09%, crude fibre 0.36%, N extract 14.06%. Those of *Eisenia foetida* contain crude protein 64.61%, crude fat 12.29%, crude ash 10.16%, crude fibre 0.27%, N extract 12.67%. Those of *E. rosea* contain crude protein 63.71%, crude fat 12.29%, crude ash 10.66% crude fibre 0.21%, N extract 12.67% (Zhang, 1987).

The blood and body fluids of *Lumbricus terrestris* contain small concentration of glucose (0.01-0.05 µg/ml), considerable lipids, including 35.14% neutral fat, 41.74% glucolipid, and 23.12% phosphatide.

The neutral fat consists mainly of lauric acid, oleate, myristic acid and decanoic acid. The fatty acids of the glucolipids are decanoic acid and some short chain fatty

acids. The acids of phosphatide are mainly oleate, decanoic, linoleate and behenic acids. The proportion of unsaturated fatty acids is higher than that of neutral fatty acids and saccharides (Hu, 1980). A peptide substance exists in gut wall of *Lumbricus terrestris* (Kaloustain, 1986).

The dormant species of *Allolobophora caliginosa* contain a protein which can hydrolyze collagen (Kaloustain, 1986). Scientists from Japan, China and Korea isolated the enzymes from earthworm gut and body fluids which can dissolve fibrin. These enzymes have been developed as innovative medicines to treat cerebral thrombosis and myocardial infarction (Cheng, 1985). Sun (1989) reported a kind of acid antibacterial peptide, a tetradecapeptide, which has produced a disease resistant, nutrient earthworm preparation and which can be used in plant and animal production. There is also an enzyme in the earthworm body tissue, which can dissolve the earthworms after death under certain conditions (Sun, 1997).

Some active enzymes occur in the yellow chloragenous cells and organs of *Lumbricus terrestris* in high concentrations. These include catalase, peroxidase, dismutase,  $\alpha$ -D-glucosyl-enzyme, alkaline phosphatase and porphyrin synthetase. The body fluids of *Eisenia* species contains at least 18 proteins with molecular weights between 1000 and 95,000 Da. (Cheng, 1985).

## Pharmacological and Clinical Effects

### 1. Effects on the Nervous System

Zhang (1984) first reported that earthworm can reduce blood pressure (Zhang, 1984). Xu *et al.*, (1963) observed the phenomenon of significant blood pressure decrease of anesthetized dogs, that were injected with macerated earthworm extracts in hot water and ethanol solution significantly. Kaloustain, (1986) reported that an 100% extract of earthworm tissue can improve the metabolism of dopamine (DA) and 5-hydroxy tryptamine (5-HT), monoamine nerve medium of the central nervous system, so it can have a protective function against blood-deficiency brain death.

### 2. Effects on Blood

Rao (1986) reported that the enzymes in earthworm body fluids can dissolve fibrin thrombosis. Hu (1980) studied extracts from earthworms to restrain the formation of blood thrombi, by comparing six indices of thrombosis including viscosity angle, development time of prothrombosis, formation time of a characteristic thrombus, dissolving time of the fibrin thrombus, length of the thrombus and dry weight. Bharati and Shweta (2009) reported the effect of different extracts of earthworm on the rates of decomposition of experimental thrombus of rabbits, with whole blood coagulum, blood plasma with platelets and with pure fibrin coagulum of albino mice.

### *3. Effects on Cardiovascular System*

Shukla (1950) reported that earthworm injections (0.5g/ml) could act against arrhythmia of various experimental models involving chloroform-adrenaline, ectisine and barium chloride. It can also cause short blockade conduction between the atrium and ventricle, which is not due to  $K^+$  in the agent.

### *4. Effects on Respiratory System*

An effective asthma-preventing component from earthworms was separated early in the 1930s. This component was used in experiments with rabbit lungs and it was reported that the component produced broncho-dilation, which could be used to resist asthma caused by histamine and pilocarpine. This component was injected intra-venously in experimental animals.

### *5. Effects on Uterine Smooth Muscle*

Xu (1964) separated a kind of substance, which can contract the uterus. Experimental results show that this substance significantly increased the tension of the pregnant or non-pregnant uterus. Xu (1964) reported earthworm injections increased contraction of the mouse uterus more than the standard solution of pituitrin (0.01 mg/ml).

### *6. Anticancer effect*

Earthworm extracts have been used successfully to cure transplanted cancer, in S-180 cells of rats (Wang, 1986) and it suppressed the cancer significantly after treatment for 88 days' with 5mg/ml of extract as enema without any adverse side effects (Wang, 1988). Han (1991) isolated some components by a dialysis method and observed their effects on MGc 803 gastric cancer in participation of 3H-TdR. The results showed that some earthworm components could inhibit 3H-TdR participation of MGc 803 gastric carcinoma ( $p < 0.01$ ), and still had an inhibitory function, even when the component was heated up to  $56^{\circ}\text{C}$  for half an hour ( $p < 0.05$ ). This means that the dialysis components of earthworm have a strong heat-resistance on a limited scale (Han, 1991). Sun (1989) compared the cancer killing ability of four treatments, including cancer cell suspension, earthworm extract-blood porphyrin derivative-laser, blood porphyrin derivative-laser, and earthworms extracts. The depression rate on cancer cells was highest in a treatment with an earthworm extract-blood porphyrin derivative-laser. With a chemical-luminous method, Sun (1989) concluded that the mechanism by which the earthworm extract increased the cancer-killing capability of blood porphyrin derivative-laser is by increasing active oxygen.

## 7. *Sperm-killing effect of Earthworm Extracts*

Succinic acids and hyaluronic acids in earthworm tissue can agglutinate and kill sperm (Zhang, 1987 & 1988). The results showed that earthworms contain upto 200 ppm of arsenic. The arsenic toxicity can decrease by washing and was comparatively low in experiments with rabbit, rats and dogs that were administered with earthworm extract as enema or intravenously. Zhang (1990) suggested the use of sperm-killing function for birth control in China.

## Clinical Applications of Earthworms

### 1. *Treatment of Tracheitis and Bronchial Asthma*

Fried hot earthworm powder was taken orally 3-4 times per day. With a dose of 3-4 g each time, for the treatment of bronchial asthma (Cheng, 1985). An earthworm preparation, "Chuan-shu-ning pill" was used for treatment of bronchial asthma patients, and 84.09% of the patients responded favorably to the treatment. This method is characterized by lasting and moderate anti-asthma (Ling, 1961). A single earthworm injection was used to treat 275 cases of bronchial asthma and 78% of patients recovered fully; especially for children, the therapeutic effects were better than for adults (Shanghai Huashan Hospital, 1971). According to a report by Huang Wenda, a 30% earthworm injection was used to treat children's asthma and adult stubborn asthma with a single dose of 0.1-2ml for children and 2ml for adults, once a day after the asthma occurred. After 10 to 30 minutes' treatment, the breathing became smoother. Wheezing etc, of asthma eased and phlegm was expelled easily. With two to four treatments, the symptoms of asthma disappeared entirely (Hu, 1980). Usually a 1 ml earthworm extract preparation (equal to 1g earthworm) for adults was used as intra muscular injections on the first day and 2ml per day for a second dose if no side effects appeared on the first day. Ten days was regarded as complete course of treatment (Anonymous, 1985). Some reports said that mixtures from several earthworm species were better than single species earthworm preparations, in curing 101 cases of asthma. A dose of 2ml intramuscular injection of earthworm extract per day, every other days, resulted in 88.1% of the patients responding to the treatment in 1 to 2 weeks (Shanghai Cooperative Group for asthma treatment, 1982). The results of germ culture and bacterial checks showed that earthworm tissue components were effective in controlling tracheitis inflammation and repairing mucosa membranes. Earthworm powder was used in treatment of 100 cases of children with asthma, and the therapeutic effects were very good, especially for active asthma (Liang, 1984).

### 2. *Treatment of Epilepsy*

An earthworm pill, a secret recipe handed down from generation to generation, which was composed mainly of earthworms (*A. caliginosa*) had therapeutic effects



against epilepsy. Xu (1963) concocted a mixture of earthworm 3 to 5 g, used once a day, to treat 20 cases of partial epilepsy, 16 cases recovered fully, 3 cases were improved and in one case there was no effect. Zhang used another earthworm extract to treat 12 cases of epilepsy, dosed once a day for 10 to 20 days. In four cases there were no epilepsy attacks for one year, in five cases there were hardly any epilepsy attacks for half a year, and there were obvious decrease in attack times in other patients (Zhang, 1984).

### *3. Treatment of High Blood Pressure*

An earthworm extract tincture, applied twice a day at 20ml per dose was used to cure 34 cases of hypertension in patients who were treated with other medicines without effect. High blood pressure was usually reduced within 4 to 10 days by this earthworm treatment (Hu, 1980). An earthworm extract (named "Earth dragon B1") was used 3 times per day and 2ml once, to treat 11 cases of hypertension, the results showed an effective ratio of hypertension suspension of 90.9% without any obvious side-effects. An earthworm mixture extract was also used to treat 17 cases of hypertension with very good therapeutic results (Hu, 1980). An K factor extracted from earthworm was injected intramuscularly to lessen high blood pressure, and 86.6% improvement occurred in 30 cases, and were better than most chemical treatments to control high blood pressure (Zhang, 1984).

### *4. Treatment of Schizophrenia*

Zhenjun (2007) reported that earthworms were used to treat 110 cases of schizophrenia which were divided into two groups; 60 cases in the first group with treatment of earthworm powder and 50 cases in the second group with treatment with an earthworm extract injection. During a 60 day treatment course, 18 patients were improved in the first group and 11 in the second group. Materials from fresh earthworms were reported more effective than dried ones.

### *5. Treatment of Leg Ulcers*

An earthworm ointment for external uses, "Xin-fu-Shuang" in Chinese was used to treat 50 cases of leg ulcers; 17 cases recovered fully and 37 cases improved. It functioned to lessen pain, to dispose of rotten tissues, remove pus and improve the growth of muscle buds. A syrup, made from earthworms and powdered sugar, was put on the ulcers with good results (Anonymous, 1960). Earthworms were also used orally to cure leg ulcers. The method was to rinse earthworms with cold water and soya-bean milk. This was taken before dinner. A patient took 300 earthworms and recovered fully (Wang, 1963).

## 6. *Treatment of Mumps*

Earthworms were rinsed and put into container, and the same quantity of sugar added to the container, and the earthworms were submerged. The earthworms gradually secreted yellow-white mucus and this mucus was put on to affected parts covering them with gauze. The mucus was changed once every 2 to 3 hours. Observation of 20 cases showed that this was a better method of treating mumps, because of its fast detumescence and antifever effects. In another report 170 cases of mumps were cured fully within 1 to 3 days using this method (Li, 1988).

## 7. *Treatment of Eczema, Urticaria and Anaphylaxis diseases*

Earthworm tissue extracts were used to treat 35 cases of eczema with injections at acupoints and results indicated that 14 patients recovered fully, 13 improved, 5 responded to the treatment and in 3 patients there were no effects. A sample of 60g earthworms was mixed with 30g sugar and the patients recovered after application of the mixture. It was put on the affected part, 4 to 5 times daily. This method was used to treat skin chronic itches and repeated eczema attacks (Hu, 1980).

Earthworm injections were used to treat 100 cases of urticaria, treated once a day with 2ml dose of treatment resulted in an 84% of cure rate. With this 15 patients were recovered, 24 improved and 9 responded to the treatment and in 2 there were no effect (Anonymous, 1980).

## 8. *Treatment of Burns and Scald*

A sample of 15 earthworms were put into a sugar solution and soaked for 10 hour to produce an infusion. Using this earthworm infusion on the wounded surface, 50 cases of burns and scalds (10 to 110) recovered fully in one week (Zhang, 1990). Li (1988) reported application of 5011 cases of burns and scalds (first and second degree) showed that 98.7% of wounds recovered fully. In 32 cases of serious burns and scalds, 23 were completely cured.

## 9. *Healing of Fractures*

Using earthworm extracts to treat 63 cases of femur fracture, the pain stopped within one hour of treatment, tumescence disappeared within 24 hours and the bone grew well over an average of 38.7 days. Li (1988) observed 264 cases of fractures of femur stem (within 7 days) and found that the healing time of fractures was 3.6 days earlier after earthworm treatments, compared to that of other treatments.

## 10. *Treatment of Erysipelas*

A mixture of fresh earthworm tissues and red sugar was applied to parts affected with erysipelas and some 11 patients were cured in 3 to 5 days (Vohora and Khan, 1978).

#### 11. *Treatment of Sequelae of Encephalitis B*

Fresh earthworms were stewed into an extract which was taken orally to treat 10 cases of sequelae of encephalitis B, for 30 days, as a treatment and satisfactory therapeutic results were obtained (Gates, 1982).

#### 12. *Treatment of Blood Deficiency Apoplexy*

Han (1991) reported that when extracts from fresh earthworms were used to treat 381 cases of blood deficiency apoplexy, there was an average effective cure rate of 79%.

#### 13. *Treatment of vertigo*

An earthworm tissue extract was used to treat vertigo. Of 32 treatments, 20 cases were cured fully, 7 proved, 2 responded to the treatment and 3 had no effects (Zhang, 1988).

#### 14. *Treatment of Hematemesis*

A batch of 50 fresh earthworms was mixed with 250 g red sugars. A yellow secretion emerged from the body pores of earthworms. Patients took this secretion orally at a dose of 20 ml per treatment which stopped *Hematemesis* within 2 hours. The disease healed fully when a dose of 100 ml of the secretion was taken (Wu, 1985; Li, 1988).

#### 15. *Treatment of Digestive Ulcer*

A dried earthworm powder was taken orally to treat 40 cases of digestive ulcers at the rate of, 2g per dose, 3 to 4 times a day, with this 34 cases were cured fully and 6 cases improved (Shweta and Singh, 2006).

#### 16. *Treatment of Vesical Calculus*

Chen (1985) reported that earthworms were applied to treat 5 cases of vesical calculus of cystolith with significant therapeutic effects.

### **Summary**

Indian systems of medicine have understood the importance of the drugs of animal origin right from the ancient times. The Indian *Materia medica*, which includes drugs of Ayurveda, the Indian system of medicine, and Unani the Greco-Arabian system of medicine, at present has about 2000 drugs, out of these about 200 are obtained

#### Some common medicinal giant earthworm species found in India

Sl.No.	Species	Length (mm)	Distribution
1.	<i>Drawida grandis</i> Bourne	520	Tamil Nadu
2.	<i>Drawida naduvatamensis</i> Bourne	500	Kerala
3.	<i>Drawida nilamburensis</i> Bourne	1000	Tamil Nadu
4.	<i>Megascolex imperatrix</i> Bourne	650	Karnataka
5.	<i>Megascolex konkanensis</i> Fedarb	415	Annamalai Hills
6.	<i>Megascolex Konkanensis longus</i> Stephenson	570	Tamil Nadu
7.	<i>Eutyphoeus gammiei</i> Beddard	405	E. Himalaya
8.	<i>Perionyx macintoshi</i> Beddard	375	E. Himalaya

from animals (Zhenjun, 2007). The present review provides various therapeutic uses of earthworm to treat many human ailments. Earthworms have been used in traditional medicine in India for at least 2,300 years (Puri, 1970). In ancient India, *Kharteem* (Unani name of earthworm) has been used as antipyretic and anaesthetic, for detoxification, treatment of hypertension and hastening parturition, as well as in the treatment of many common ailments, such as *arthritis*, *itching*, *burns*, *carbuncles*, *erysipelas* and *inflammation*. With the development of science, some active compounds of the earthworm, such as linmbritin and terrestrolumbrolysin, have been isolated. In 1986 a Japanese scientist extracted an enzyme from earthworms that can dissolve thrombi in laboratory experimental conditions. This enzyme preparation has been made into an oral medicine by the pharmaceutical industry for use in the prevention of cardiovascular disease in Hong Kong, Japan, Korea and China, (Han, 1991). In view of the enormous potential of earthworm to treat many diseases and pathological conditions as indicated in preliminary clinical and pharmacological investigations, detailed scientific studies are suggested to develop new therapeutics of animal origin.

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# Development of Water-Hyacinth (*Eichhornia crassipes* (Mart) Solms based Vermicompost and Its Application in Ashwagandha (*Withania somnifera* (L) Dunal): An Important Medicinal Crop

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## Abstract

Organic cultivation of medicinal crops is receiving world-wide attention. In this context, efforts have been directed to utilize various waste substrates for production of organic manure. Water hyacinth is one such waste weed. The aim of this work is to investigate the potential of water-hyacinth (WH) based reactor into vermicompost and their effect on cultivation of Ashwagandha. Two vermireactors WH and WH spiked with cowdung (1:1) were run under laboratory conditions for 180 days. The maximum worm growth and vermicast were recorded in WH spiked with cowdung. The significant decrease in TK and TOC was observed at the end. Vermicompost obtained from this reactor was used in the cultivation of Ashwagandha and there was significant increase in the yield. No adverse effect of this vermicompost was observed.

**Key Words:** Water-hyacinth (*Eichhornia crassipes*), Ashwagandha (*Withania somnifera*), Vermicompost, Vermireactors.

## Introduction

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms) family: Pontederiaceae) (Fig.1) has been listed as most troublesome weed in aquatic system. It is a severe environmental and economical problem in many tropical and sub tropical parts of the world. The weed forms dense mats that prevent river traffic, block irrigation canals, interfere with hydel power projects and destroy rice fields. As water-hyacinth decays, there is a sharp increase in nutrient levels in water body, which ultimately creates the problem of eutrophication in aquatic system. Chemical control of water-hyacinth with herbicides like 2, 4-D, dalapan, diquat and glyphosate was considered most effective but it resulted in water pollution (Singh and Gill, 1997). The stringent and rigid standards for pesticide use in water bodies and public consciousness also call for some alternate technology for aquatic weed management. Abbasi and Ramaswamy (2001) have reported that water hyacinth has successfully resisted chemical, physical, biological or hybrid means used to eradicate, it. The only accepted use of water hyacinth is in treating the biodegradable waste waters (Tchnobanoglous and Burton, 1991). The final disposal of water hyacinth used in wastewater treatment is still an unsolved problem (Gajalakshmi *et al.*, 2002). Therefore, a novel technology with ecological sound and economically viable is urgently required to solve the problem of aquatic weed disposal and management. The present attempt is an endeavor in this direction.

It has been well established that epigeic forms of earthworms hasten the composting process to a significant extent with production of a better quality of composts as compared with those prepared through traditional composting methods (Ndegwa and Thompson, 2001). Use of earthworms for waste management, organic matter stabilization, soil detoxification and vermicompost production have been well





**Fig. 1.** Water-hyacinth (*Eichhornia crassipes* (Mart) Solms)

documented (Bansal and Kapoor, 2000; Kaushik and Garg, 2003; Garg and Kaushik, 2005; Suthar, 2006). The effect of water hyacinth on life cycle of different earthworms has been documented by other workers (Gajalakshmi *et al.*, 2001, 2002).

Ashwagandha (*Withania somnifera* (L.) Dunal) is medicinal herb cultivated on field scale. The ethnomedical studies (Asthana and Raina 1989) have shown that the populations of this plant have become low and scattered in the wild, which may be attributed to its intensive harvesting for traditional medicine coupled with its low potential for natural regeneration. A large variation may occur in the crude drug available in the market as plant materials are coming from various sources. There is, therefore, a necessity for standardizing the quality parameters for the crude drug production. Now organic production of the raw plant drugs is mandatory as per WHO-guidelines. Hence, the study was carried out to explore the potential of water-hyacinth vermireactor as value added biofertilizer to answer their impact on quality of Ashwagandha.

## Methods

Water-hyacinth (WH), Cow dung (CD) and vermi seed (*Eisenia foetida*): Fresh water hyacinth plants were collected from a natural wet land infested with water hyacinth. The soil particles/mud adhered with the root and leaves of the plants were washed with running water. The plants were cut into pieces of 2-3 cm for the present study before mixing with CD. The main physico-chemical characteristics of WH were moisture (%)  $92.8 \pm 1.30$ ; ash content ( $\text{gKg}^{-1}$ ),  $417 \pm 3.6$ ; pH (1:10 ratio),  $8.1 \pm 0.06$ ; TOC ( $\text{g Kg}^{-1}$ ),  $338 \pm 2.1$ ; TKN ( $\text{gKg}^{-1}$ ),  $9.5 \pm 0.3$ ; C:N ratio,  $36.0 \pm 1.63$ ; TKN ( $\text{gkg}^{-1}$ ),  $9.7 \pm 0.7$ ; TAP ( $\text{g Kg}^{-1}$ ),  $5.4 \pm 0.5$ ; Total-Fe ( $\text{mg kg}^{-1}$ ),  $1640 \pm 59$ ; total Cu ( $\text{mg Kg}^{-1}$ ),  $312 \pm 28$  and total-Zn ( $\text{mg Kg}^{-1}$ ),  $640 \pm 33$ .

Fresh CD was procured from an intensively live stock farm at Aligarh, India. The main physico-chemical characteristics of CD were-moisture (%),  $79.4 \pm 7.35$ ; ash content ( $\text{mg Kg}^{-1}$ ),  $195 \pm 10.4$ ; pH (1:10 ratio),  $8.2 \pm 0.065$ ; TOC ( $\text{gKg}^{-1}$ ),  $467 \pm 6.0$ ; TKN( $\text{gKg}^{-1}$ )  $7.7 \pm 0.3$ ; C:N ratio,  $60.6 \pm 2.66$ ; TK ( $\text{gKg}^{-1}$ ),  $4.8 \pm 0.1$ ; TAP ( $\text{g Kg}^{-1}$ )  $3.3 \pm 0.3$ ; total-Fe ( $\text{mgKg}^{-1}$ ),  $282 \pm 366$  and total-Zn ( $\text{mg Kg}^{-1}$ ),  $317 \pm 47$ .

Eathworms used in experiment were picked from stock culture maintained in our laboratory.

### *Experimental design*

In bench-scale vermireactors (vol. 10L, diameter 40cm, depth 12cm), shredded WH was mixed with CD in 1:1 ratio. One Kg of feed mixture (on dry weight basis) was put in each circular plastic vermireactor. All the CD and WH quantities were used on dry weight basis that were obtained by drying the known quantities of material at  $110^{\circ}\text{C}$  to constant mass in hot air oven.

These mixtures were turned manually every 24h for 21 days in order to eliminate volatile gases potentially toxic to earthworms. After 21 days, 20 adult individuals of *Eisenia foetida* (weighing between 250 and 400 mg) were introduced in each vermireactor. The moisture content was maintained at  $70 \pm 10\%$  of water of holding capacity by periodic sprinkling of adequate quantity of distilled water. All the containers were kept in the dark under identical ambient conditions (room temperature  $25 \pm 3^{\circ}\text{C}$ , relative humidity 60-80%). The experiments were replicated thrice for each feed mixture. At the end of experiment (after 147 days), the substrate material in each vermireactor was turned out. The earthworms, hatchlings and cocoons were separated from the feed by hand sorting, after which they were counted and weighed after washing with water and dried by paper towels. The worms were weighed with full gut. No correction has been applied for gut content. A sample of final compost was ground in a stainless steel blender. Stored in airtight plastic vials for further chemical analysis.

### *Chemical analysis*

The samples were used on dry weight basis for chemical analysis that was obtained by oven drying the known quantities of material at  $110^{\circ}\text{C}$ . The pH was determined using a double distilled water suspension of each vermicompost in the ratio of 1:10 (w/V). Total organic carbon (TOC) was measured using the method of Nelson and Sommers (1982). Total kjeldhal nitrogen (TKN) was determined by following Bremner and Mulvaney (1982) procedure. Total available phosphorus (TAP) was analyzed using colorimetric method (Bansal and Kapoor, 2000). Total potassium (TK) was determined after digesting the sample in diacid mixture [conc.  $\text{HNO}_3$ ; conc.  $\text{HClO}_4$ , (4:1v/v)] (Bansal and Kapoor, 2000) by flame photometer. Total Fe, Cu and Zn were determined by atomic absorption spectrophotometer (AAS) (Bansal and Kapoor, 2000).

### *Field experiment*

The field experiment was carried out during 2007-2008 at Vermiculture Research Station, D.S. College, Aligarh, Uttar Pradesh, India. The experimental site lies between 27°54'50"N latitude and 78°4' 26" E longitude representing a dry climate. The cold weather lasts longer and extends from middle of October to end of the March. The mean annual rainfall recorded was 1641 mm, and the mean number of rainy days were 48 per annum. The maximum and minimum temperature were 30.8°C and 18.1°C, respectively. The mean relative humidity often was 66.93 percent.

### *Cropping history of experimental site*

Field where no experiments were conducted during the three previous seasons was selected to conduct field trials (table 6). The planting material was collected from Rajasthan Agro Corporation Ltd., Sonamukhi Nagar, District Jodhpur, Rajasthan. Framyard manure and chemical fertilizers was purchased locally and vermicompost was prepared from water hyacinth and cow dung.

The plot size was uniform for the field experiments, 9.45 m<sup>2</sup> (6.3m x 1.5m) grossplot and 7.2 m<sup>2</sup> (6m x 1.2m) net plot.

### *Statistical analysis*

One-way ANOVA was used to analyze the significant difference between different reactors of observed parameters. Turkey's t-test was also performed to identify the hours genus type of reactors for their different chemical properties and earthworms growth parameters *i.e.* individual weight, earthworm weight gain, individual growth ratio, total cocoon numbers, juveniles etc. The probability levels used for statistical significance were  $P < 0.05$  for the tests.

## **Results and Discussion**

### *Growth and reproduction of E. foetida in different reactors*

No mortality was observed in any feed mixture during the study period. In our experiments, all the wastes were precomposted for 21 days and during this period all the toxic gases produced might have been eliminated. It is established that precomposting is essential to avoid the earthworm mortality (Kaushik and Garg, 2003).

Table 1 and 2 shows the values obtained for different growth and reproduction parameters in *E. foetida* over the experimental period. The highest biomass production was in the vermireactor at 60 days of vermicomposting. The maximum no of juveniles were also reported in vermireactor. However, the higher cocoon production was recorded in vermireactor 2. Average vermicast production per unit

**Table-1. Total no. of hatchlings, residual cocoons and biomass produced in CD+WH fed vermireactor 1 (1 : 1 ratio).**

Days	Temp	No. of worm	No. of cocoon	No. of jurenile
25	26.66±1.62	22.32±1.50	2.22±0.37	1.33±0.56
40	26.00±2.65	28.23±0.85	10.44±0.70	3.66±0.79
60	26.10±1.81	32.11±1.54	21.55±1.30	5.90±0.98
Average	26.25±2.02	27.55±1.29	11.40±0.79	3.63±0.77

**Table-2. Total no. of hatchlings, residual cocoons and biomass produced in WH fed vermireactor 2.**

Days	Temp	No. of worm	No. of cocoon	No. of jurenile
25	25.33±0.66	17.66±0.72	2.10±0.26	0.33±0.22
40	26.10±0.72	22.89±1.09	9.88±1.05	2.66±1.09
60	26.13±1.42	25.22±1.07	26.33±1.00	4.66±0.44
Average	25.85±0.93	21.92±0.96	12.77±0.77	2.55±0.55

**Table-3. Vermicast ( $\text{mg l}^{-1} \text{ d}^{-1}$ ) production in vermireactor 1.**

Days	Vermicast output ( $\text{mg l}^{-1} \text{ d}^{-1}$ )	
	Average	$\text{mg l}^{-1} \text{ d}^{-1}$
30	1069±1.29	35633
60	1097.7±0.88	36590
90	1150.8±2.32	38360
120	1275.2±1.96	42506
150	1363.3±1.31	45445
180	1418.7±1.23	47290
Average	1229.1±1.52	409706

of the feed mixture was significantly higher in vermireactor 1. The difference between biomass and cocoon production in different vermireactors could be related to the biochemical quality of feed, which was one of the important factors in determining the onset of cocoon production (Flack and Hartenskin, 1984). Recently, Garg and Kaushik, (2006) summarized that except for the chemical properties of waste, the microbial biomass and decomposition activities during vermicomposting were also important. Micro-flora played an important role in earthworm nutrition and growth.

**Table-4. Vermicast (mg1-d-1) production in vermireactor 2.**

Days	Vermicast output (mg1 <sup>-1</sup> d <sup>-1</sup> )	
	Average	mg1 <sup>-1</sup> d <sup>-1</sup>
30	552.9±1.82	18430
60	576.3±0.92	19210
90	615.9±2.37	20530
120	623.4±1.96	20781
150	657.8±1.59	21728
180	647.7±1.83	21591
Average	611.3±1.76	20378.3

**Table-5. Physico-chemical analysis of initial feed mixture and vermicompost obtained from different vermireactors.**

Nutrient	WH	WH:CD	CD
TOC	28.50±0.23	15.6±0.23	7.10±0.82
TKN(%)	1.84±0.36	1.78±0.49	1.07±0.22
TAP (%)	0.456±0.03	0.461±0.39	0.421±0.23
TAK (%)	1.89±0.06	0.741±0.40	0.730±0.52
PH	7.3±1.18	7.2±1.26	7.3±1.02

Better growth and cocoon production on beddings containing plant origin wastes could be due to great microbial biomass and activities and also due to more availability of nutrients.

On the basis of present investigations, it was concluded that feed mixture containing 50% WH could be a suitable growth medium for *E. foetida* production (Table 1). Exclusive application of WH in vermireactors significantly reduced the biomass production and number of juveniles produced. This might be due to the fact that higher proportion of WH in the feed mixture made it harder and more tensile, which could not be easily utilized by the earthworms (Gajalakshmi *et al.*, 2002). The feed with higher proportion of WH might not have sufficient amount of easily metabolizable organic matter and non-assimilated carbohydrates which are essential for the growth and reproduction of earthworm (Edwards, 1988).

Gajalakshmi *et al.*, (2005) showed that 100% water hyacinth as feed was not preferred by *Endrilus eugeniae*, whereas addition of cow dung (≈14% CD) had a positive input on biomass gain and hatchling production. Manna *et al.*, (2003) also reported the addition of farmyard manure in 1:1 ratio in the leaf litter of *Tectona*

**Table-6. Characteristics of soil used in experiment.**

Parameter	Soil Characteristics
Texture	Clay loam
pH	6.00±0.64
Available N (P Pm)	148.40±0.84
Available P (P Pm)	2.96±1.20
Available K (P Pm)	66.00±1.87
Available Ca (P Pm)	170.65±0.95
Available mg (P Pm)	18.09±1.10
Available S (P Pm)	5.20±1.10
Available Fe (P Pm)	19.87±0.62
Available Zn (P Pm)	1.63±0.24
Available Cu (P Pm)	3.79±0.28

**Table-7. Effect of water-hyacinth vermireactor on growth & yield of *Withania somnifera*.**

Parameter	Control	Plant growth		
		Vermireactor 1	Vermireactor 2	FYM
Height (cm)	45.79±1.23	46.28±1.09	48.63±1.23	46.00±1.86
Leaves (no.)	110.98±2.12	112.96±2.93	117.60±0.32	112.10±1.81
Root length (cm)	18.94±2.20	20.84±2.36	22.66±0.32	19.23±1.28
Total yield (t/ha)	1.76±0.06	1.86±0.58	1.92±0.31	1.82±0.52

*grandis*, *Madhuca indica* and *Butea monosperma* during vermicomposting employing different earthworm species viz. *E. foetida*, *Perionyx excavatus* and *Dicogaster bolani*. Suthar (2006) studied the vermicomposting of crop residues and farm yard manure mixed with some animal dung under laboratory conditions. Different growth and reproduction patterns of *P. excavatus* in vermibeds were possibly related to the concentrations of polyphenols and related substances present in plant-derived waste materials.

#### *Physico-chemical changes in different vermireactors*

There were very little changes in the pH of feeds (Table 5). Other workers have also reported similar observations (Mitchell, 1997; Gunadi and Edwards, 2003;

Ndegwa *et al.*, 2009; Atiyeh *et al.*, 2000). The pH shift towards acidic conditions was attributed to mineralization of the nitrogen and phosphorus into nitrates/nitrites and orthophosphates; bioconversion of the organic material into intermediate species of organic acids (Ndegwa *et al.*, 2000). It has also been reported that different substrates could result in the production of different intermediate species and different wastes showed a different behavior in pH shift. Haimi and Hutha (1986) postulated that lower pH in the final vermicomposts might have been due to the production of CO<sub>2</sub> and organic acids by the microbial activity during the process of bioconversion of different substrates in the feed given to earthworms. TOC reduction in vermireactor 2 was significantly higher than vermireactor 1. TOC was directly related to the water hyacinth content in the vermireactor. The findings are similar to those of Kaviraj and sharma (2003), who reported 45% loss of carbon during vermicomposting of municipality, or industrial wastes. Elvira *et al.*, (1996) have attributed this loss to the presence of earthworms in the feed mixtures. Suthor (2006) reported that earthworms promoted such microclimatic conditions in the vermireactors that increased the loss of TOC from substrates through microbial respiration.

TKN content of WH vermireactor was higher than spiked with CD (Table 5). The final TKN content in vermicompost is dependent on the initial nitrogen present in the feed material and the degree of decomposition (Crawford, 1983). According to Viel *et al.*, (1987) losses in organic carbon might be responsible for nitrogen addition. Addition of nitrogen in the form of mucus, nitrogenous excretory substances, growth stimulating hormones and enzymes from earthworms has been reported (Tripathi and Bhardwaj, 2004; Suthar *et al.*, 2005). These nitrogen rich substances were not originally present in feed and might have contributed additional nitrogen content. No significant change in phosphorous was reported. TK was significantly higher in vermireactor 2 Suthar (2006) suggested that the earthworm processed waste material contains high concentration of exchangeable K due to enhanced microbial activity during the vermicomposting process, which consequently enhance the rate of mineralization. In contrast Orozco *et al.*, (1996) reported a decrease in TK in coffee pulp waste after vermicomposting. These differences in the results can be attributed to the difference in the chemical nature of the initial feed mixture (Garg, *et al.*, 2006).

#### *Effect of Vermireactors on the Growth & Yield of Withania Somnifera*

Highest vegetative growth were recorded in vermireactor 2 and maximum plant length were recorded in vermireactor 1.

#### **Conclusions**

From the results it is concluded that if WH was mixed with up to 50% in CD, the vermicompost quality was not effected; but a higher percentage of WH in the feed mixture retarded the growth and fecundity of the worms used and also affected the



nutritional quality of vermicompost. The findings confirmed the general hypothesis that growth patterns of composting species showed close relation with quality of the feed stock used as substrate and higher yield of *Withania somnifera*.

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# Antimicrobial activity of *Abutilon indicum* (Linn) SW. (Kanghi Booti) Extracts

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## Abstract

The aqueous and alcoholic extract of fresh plants of *Abutilon indicum* (Linn) SW. commonly known as 'Kanghi' was compared for its antibacterial activity by the Agar well method at different concentrations gradient. The plant of *Abutilon indicum* Linn. has been widely used for the treatment of respiratory disorders, bronchiectasis, fever and used as anti-inflammatory, diuretic and in urinary tract infection. Seeds of "Kanghi" are aphrodisiac and astringent. Alcoholic extracts showed a wide range of antibacterial activity against Gram Positive and Gram Negative bacteria, while the aqueous extract does not show any significant result. The zone of Inhibition was taken as the measuring criteria for its antibacterial activity and was compared with standard drugs (Ampicillin for Gram Positive bacteria and Gentamicin for Gram Negative bacteria). Among the Gram Positive bacteria tested, the antibacterial activity was limited to *Bacillus cereus* (MTCC 430); *Staphylococcus aureus*; *Streptococcus mutans* (clinical isolates) with the best activity at 500 µg/ml. Among the Gram Negative bacteria tested, the antibacterial activity was limited to *Klebsiella pneumoniae*. (MTCC 109) *Pseudomonas aeruginosa* (MTCC 424), *Proteus vulgaris* (MTCC 426) and *Escherichia coli* (clinical isolate).

Chemical screening reveals the presence of alkaloids, glycosides, flavonoids, tannin, oleonic acid based saponins, amino acids, essential oil, steroids/terpenes, and resins as major compounds, and the activity may be attributed to any of these compounds. The ethno botanical studies were also carried out and the four districts of western Uttar Pradesh surveyed. The phenological data and the medicinal uses, as communicated by villagers, were recorded.

**Key Words:** *Abutilon indicum*; *Staphylococcus aureus*; *Streptococcus mutans*; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; *Proteus vulgaris*; *Escherichia coli* (clinical isolate); Ethnobotany.

## Introduction

Out of the several varieties of *Abutilon indicum* (Linn) SW. Family: Malvaceae, is the most remarkable, being tomentose, hoary variety, which produces the 'Balbij'. The other with purple stems called 'Kali kanghi' in Hindi and 'Koran-tutti' in Tamil. The leaves and seeds have been used for various purposes among the 'Hindus' on account of their mucilaginous and diuretic properties. Under the name of "Masht-el-ghoul" and "Deishar", short notices of the plant *Abutilon Indicum* (Linn) SW. in Arabic and Persian books are available. Avicenna mentioned a drug "Abútílún" which was applied to wounds, but as he mentioned that it bears Pumpkin size fruit it must have been a different plant, unless his meaning is that the fruit resembles a mini pumpkin in shape; in this case *Abutilon avicenna* Gäertn., may have been the plant (Dymock *et al.*, 1890). The literature revealed that the root of *Abutilon indicum* Linn. contains Fixed oil and gallic acid; Leaves contains Gossypetin-8 and

7-glucoside; cyaniding-3-rutinoside; alantolactone; isoalantolactone; amino acids; glucose; fructose and galactose and Essential oil, that contain  $\beta$ -pinene; caryophyllene; caryophyllene oxide; cineole; geraniol; geranyl acetate; elemene; eudismol; farnisol and borneol (Rastogi and Mehbooba, 1995).

In the present study the ethnobotanical information of the plant and its antimicrobial activity of alcoholic and aqueous extract of different dilutions have been presented.

## Material and Method

A survey of four districts (Aligarh, Mathura, Agra and Ghaziabad) of Western Uttar Pardesh was made during the three season and the plants were collected and deposited in the Museum of the Department of Ilmul Avia (Voucher No. SC 0115/09). The herb in bulk was collected locally from AMU Campus, Aligarh and dried for the antimicrobial activity. The villagers and local healers of different districts were consulted for its medicinal or economic uses, and their statements were cross checked and recorded. The visits in the fields were made at different intervals to record the phenological data. The dried drug was subjected to physico-chemical standardization i.e. ash values, successive extraction in different solvents, moisture content, loss on drying. The plants were also studied for quantitative analysis of active compounds (Afaq *et al.*, 1994) and the quantitative estimation of alkaloids (IP, 1966), carbohydrates (Paech & Tracey, 1955), Phenols (King, 1951, Paech & Tracey, 1955). Water and Alcoholic extracts were made separately and subjected to antimicrobial studies. As expected antimicrobial activity has been attributed to alkaloids and/or Phenols.

For antimicrobial studies 50 gm of crude drug powder was extracted with 250 ml of 95% alcohol and DDW (Double distilled water) separately by refluxing for six hours. The extract was subjected to dryness using Water bath at 40-45°C.

Different concentrations of alcoholic and aqueous extracts were prepared separately from the dried extract using their respective solvent. viz., 50  $\mu$ g, 125  $\mu$ g; 250  $\mu$ g and 500  $\mu$ g per ml, and then dried and suspended in an inert and sterilized solvent (DDW).

Clinical isolates of Gram Positive and Gram Negative bacteria were obtained from the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital and Department of Biotechnology, Interdisciplinary Unit, Aligarh Muslim University, Aligarh. The clinical bacterial species used were *Staphylococcus aureus*, *Streptococcus mutans*, *Bacillus cereus* and *Escherichia coli*. Bacterial control strains were procured from the Institute of Microbial Technology, Chandigarh, India. Bacterial control strains used were *Bacillus cereus* (MTCC 430); *Klebsiella pneumoniae*. (MTCC 109) *Pseudomonas aeruginosa* (MTCC 424) and *Proteus vulgaris* (MTCC 426). Bacterial strains were grown on nutrient broth or MacConkey agar (M0085) plates at 37°C and maintained on nutrient agar slants at 37°C.

Antibacterial tests were performed as per National Committee for Clinical laboratory Standards (NCCLS; now CLSI) (2000). Corkborer was used to make well of equivalent size and than 20µl of extract of each dilution was poured into the separate wells for susceptibility testing. For different bacteria different plates were used. An inoculums size of 10<sup>6</sup> cfu/ml of bacteria was used for inoculating the susceptibility plates. Mueller Hinton Agar No. 2 (M1084 Hi media, India 084 Hi media, India) was used for Antibacterial susceptibility testing. All the plates were incubated at 37°C overnight for bacterial strains .Ampicillin discs (SD007) 30 mcg was used as standard drug for Gram positive bacterial strains while Gentamicin (SD170) 30 mcg for Gram negative bacterial strains. All experiments were performed in triplicate.

## Results and Discussion

*Abutilon indicum* (Linn.) Sw. (syn. *Sida indica* Linn.) (Fig.1) is an erect herb attaining the height of 1.0-2.5 m with the stem and branches that are stellate, pubescent and mixed with long spreading hairs. Leaves are simple, alternate, ovate, cordate, irregularly and coarsely toothed. 4.5-9.5x7.5 cm. Flowers are solitary, axillary on 2.5-5.0 cm long pedicels, yellow or orange yellow, 2.5 cm across. Sepals are 5, connate, calyx lobe ovate, acute, silky edges, 5-6 mm long. Petals are 5, slightly connate at base, 1.50-1.75 long, prominently veined. Staminal tube is divided into anther bearing filament at the apex. Carpel 15-20, exceeding the calyx tube truncate or with short spreading beak, stellately tomentose that are approximately 1 cm long. Seeds are dark brown compressed, reniform 3-4 mm across, stellately hair

Flowering: September to November; February to April

Fruiting: December to January; April to May.

Locality: Common plant, abundantly found among the hedges.

Part used: Whole plant, root and seed

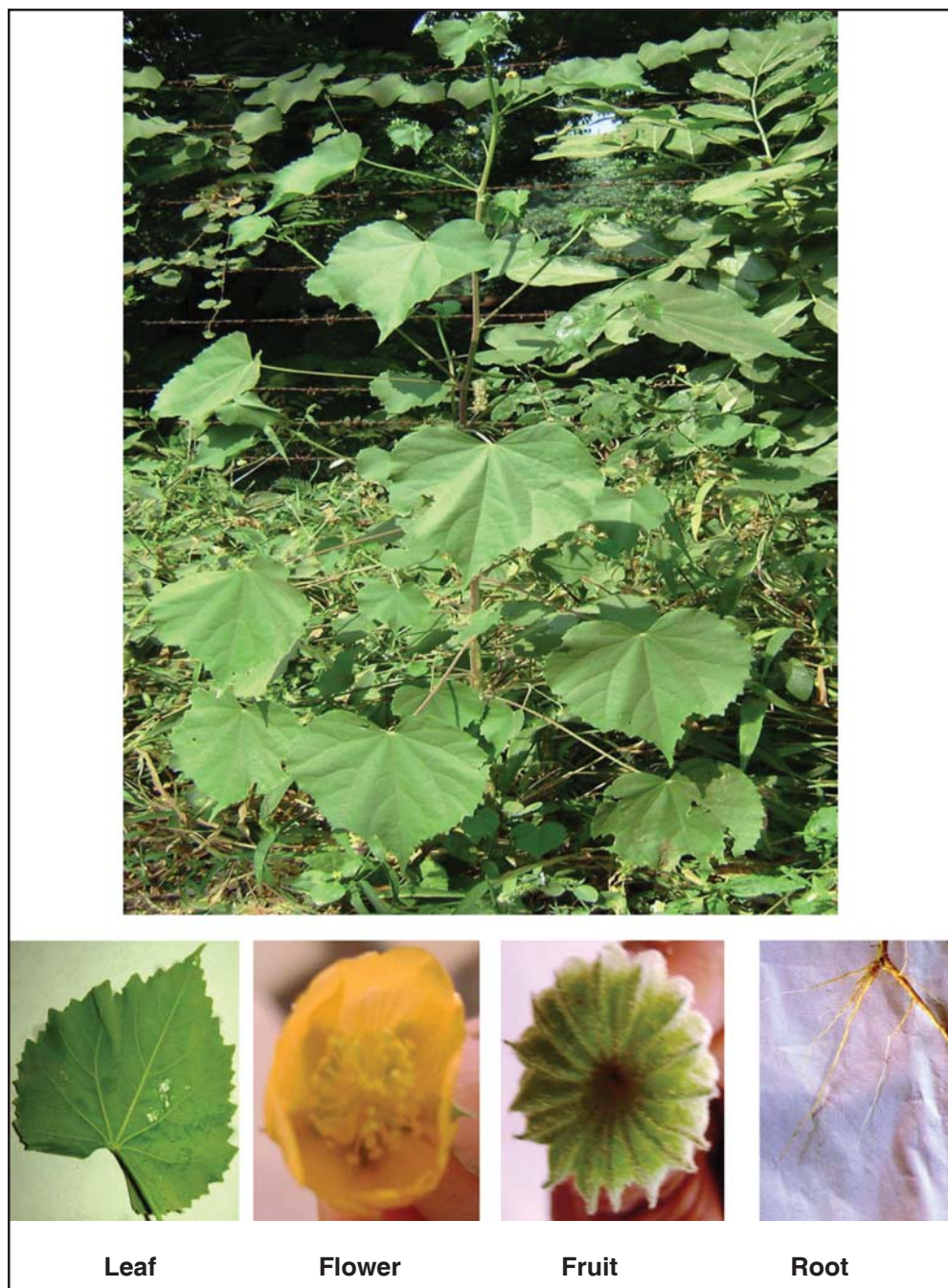
Uses: The plants are used as demulcent, aphrodisiac, laxative, diuretic and sedative. The seeds are useful in piles, gonorrhoea and chronic cystitis. The root is said to be useful in relieving hematuria and leprosy.

Alcoholic and Aqueous extracts has more extractive values as compared to Petroleum ether (60-80°C), Diethyl ether, Chloroform, Benzene .The chemical analysis of the whole herb of *Abutilon indicum* showed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, tannins/phenols, saponins, steroids/terpenes and resins. The total alkaloid percentage is 1.67 in whole plant. The other physico-chemical standard is depicted in Table 1.

### *Antibacterial activity*

All the alcoholic extracts showed a wide range of antibacterial activity against Gram Positive and Gram Negative bacteria; best given by extracts at the concentration



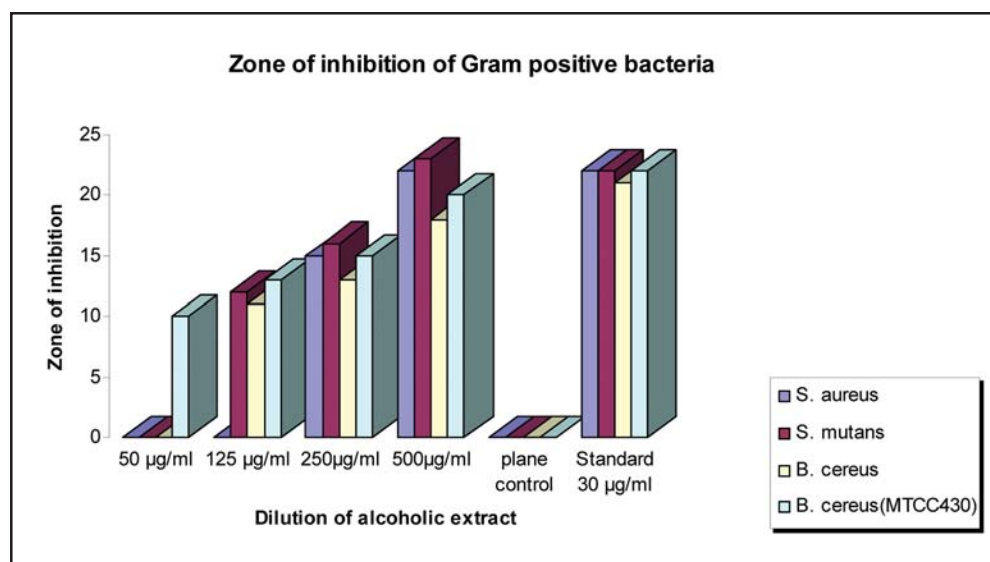


**Fig. 1.** *Abutilon indicum* (Linn) S.W.

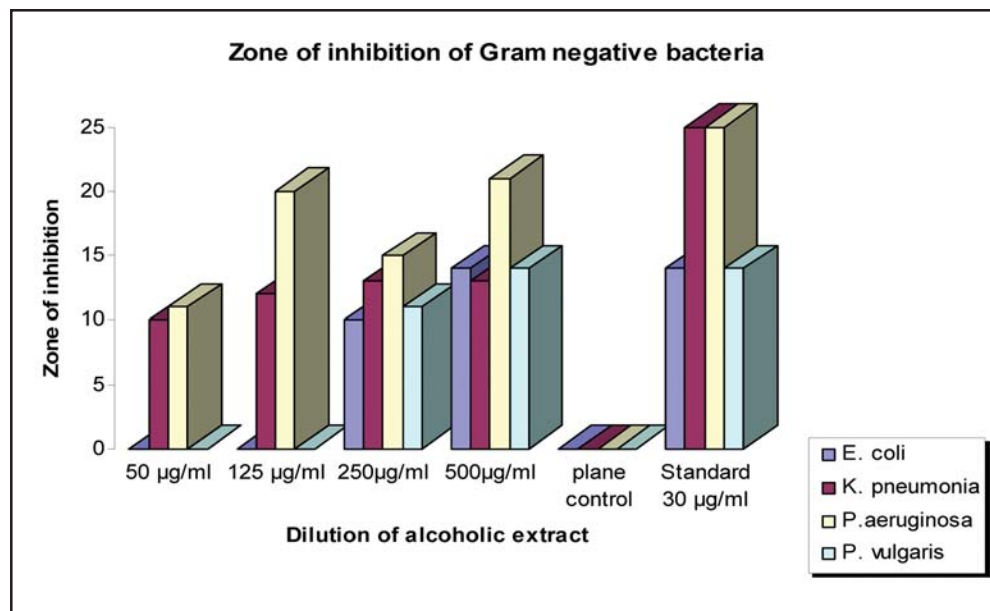
of 500 µg/ml (Figure 2 and 3). Best results were shown against the *Bacillus cereus* among Gram Positive bacteria while among the Gram Negative bacteria the antibacterial activity was comparable to the Standard drug used against *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebseilla pneumoniae* (Table 2 and 3). The aqueous extract fails to give any response at any concentration.

**Table-1. Quantitative Analysis of *Abutilon indicum* (Linn.) SW.**

S.No.	Chemical Constituent	Percentage
1	Total Ash	12.20± 0.25
2	Acid insoluble ash	1.90± 0.18
3	Water soluble ash	6.30± 0.33
4	Soluble Part	
	Pet. Ether	1.47± 0.05
	D. Ether	1.26± 0.12
	Chloroform	2± 0.05
	Alcohol	17.33± 0.44
	Aqueous	23.33± 0.49
5	Successive Extraction	
	Pet. Ether	1.59± 0.23
	D. Ether	3.18± 0.20
	Chloroform	1.17± 0.05
	Benzene	0.22± 0.05
	Alcohol	8.33± 0.23
	Aqueous	14.27± 0.52
6	Moisture content	7.05± 0.05
7	Loss on drying	7.19± 0.05
8	Alkaloid	1.67± 0.05



**Fig. 2.** Effect of different dilutions of alcoholic extract on gram positive bacteria



**Fig. 3.** Effect of different dilutions of alcoholic extract on gram negative bacteria

## Conclusion

As a whole the alcoholic extract of entire herb of *Abutilon indicum* (Linn) S.W. at 500µg/ml dilution have remarkable antibacterial activity, potential against clinical and standards strains and thus could be used to derive antimicrobial agents to fight against the number of infectious life threatening diseases mainly against *Staphylococcus aureus*, *Streptococcus mutans*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebseilla pneumoniae*.

**Table-2. Effect of alcoholic extract on Gram positive Bacteria (Zone of inhibition)**

S.No.	Bacteria tested	Dilution of Alcoholic extract					
		50µg/ ml	125µg/ ml	250µg/ ml	500µg/ ml	Plane control	Standard drug (30µg)
1	<i>Staphylococcus aureus</i>	–	–	15mm	22mm	–	22mm
2	<i>Streptococcus mutans</i>	–	12mm	16mm	23mm	–	22mm
3	<i>Bacillus cereus</i>	–	11mm	13mm	18mm	–	21mm
4	<i>Bacillus cereus</i> (MTCC 430)	10mm	13mm	15mm	20mm	–	22mm

**Table-3. Effect of alcoholic extract on Gram negative Bacteria (Zone of inhibition)**

S.No.	Bacteria tested	Dilution of Alcoholic extract					
		50µg/ ml	125µg/ ml	250µg/ ml	500µg/ ml	Plane control	Standard drug (30µg)
1	<i>Escherichia coli</i>	–	–	10mm	14mm	–	14mm
2	<i>Klebsiella pneumoniae</i> (MTCC 109)	10mm	12mm	13mm	13mm	–	25mm
3	<i>Pseudomonas aeruginosa</i> (MTCC 424)	11	20mm	15mm	21mm	–	25mm
4	<i>Proteus vulgaris</i> (MTCC 426)	–	–	11	14mm	–	14mm

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# Plants Used for the Treatment of Urinary Disorders Among Tribal and Rural Folks of Orissa

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## Abstract

The present paper deals with the selected folk medicines practiced among the tribal and rural population of Orissa for the treatment of urinary disorders. The information presented in this paper were recorded during 1980-1984 as a result of interviews with tribal medicinal practitioners and other inhabitants during the course of ethnobotanical explorations in tribal dominated areas of the state. The paper includes ethnopharmacological information on seven plants species which are less known or unreported so far. Available phytochemical reports and pharmacological actions of the drugs have been incorporated from the published literature to support or contradict the claims. It is suggested that the folk medicines reported here for the treatment of urinary disorders may be screened for their detailed phytochemical and pharmacological study to determine the therapeutic potential of the folk drugs and validation of the folk claims for their use in medicine.

**Key Words:** Ethnopharmacology, Urinary disorders, Orissa.

## Introduction

Ethnopharmacological studies are invaluable for the discovery of novel active compounds from natural sources, particularly from plants. Since new diseases as well as drug resistant strains of known pathogens continue to emerge, the search for newer compounds is ongoing. Traditional knowledge on medicinal plants and indigenous use of plant material have provided the basis for many potential pharmaceuticals used today. There are still many important pharmaceutical compounds yet to be discovered. However, this traditional base of medicinal plants is losing this precious resource as many indigenous cultures as well as medicinal plants themselves are facing threats with extinction. It is therefore, prime need to utilize the accumulated traditional knowledge about medicinal plants as basis for discovering new lead compounds for pharmaceuticals. Based on this rationale, ethnopharmacological field studies were undertaken in the state of Orissa between 1980 and 1994 to record basic data for further investigations in the search of new drugs of plants origin.

The forests of Orissa are rich in vegetation (Haines, 1912-1925) and inhabited by 62 different ethnic groups. Some of these are: Binjhals, Bondos, Bhuiyans, Gouds, Korwas, Kondhs, Kharias, Kols, Majhis, Oraons, Paharias, Saoras and others. Living close to nature, they have unique knowledge about the use of wild flora and fauna, most of which is not known to the outside world. Though Orissa state is ethnically important but only few references are met on ethnomedicinal studies. Some important contributions are those of (Pal, 1980; Saxena & Dutta, 1975; Mudgal & Pal, 1980; Saxena *et al.*, 1981; Brahman and Saxena, 1990).

During a series field studies in different forest areas, first hand information on ethnopharmacological uses of plants for a variety of ailments were recorded by

survey team stationed at Bhadrak in Orissa state. Some of the data recorded has already been published (Aminuddin and Girach, 1991; 1993; 1996; Girach 1992, 2001; Girach & Aminuddin, 2000). The information on plants used in the treatment of urinary disorders was extracted out of the data recorded during ethnobotanical survey tours. As a result, information on 7 plants species that are used in urinary related disorders like burning micturation and haemeturia, have been presented. Uses of these plants as recorded in the literature on Indian system of medicine have been included through literature survey. Available phytochemical and pharmacological data has also been given with a view to support or contradict the claims recorded from the field.

### Methodology

The survey team of Regional Research Institute of Unani Medicine (RRIUM), Bhadrak conducted extensive ethnobotanical survey tours in tribal pockets in the state of Orissa during 1980-94. The tribal inhabitants and other knowledgeable persons were interviewed to collect information on the local utility of plant resources for various purposes including medicinal usage. The information thus collected was further confirmed and cross-checked form other inhabitants of the areas surveyed. Voucher specimens collected are deposited in the Herbarium of Regional Research Institute of Unani Medicine, Bhadrak (Orissa).

### Observations

The plant species colleted from the areas surveyed for the treatment of urinary disorders are presented in alphabetical order by botanical name, family in parentheses, voucher specimen number, local name, source of information disease/condition part used, method of usage followed by therapeutic use in traditional system of medicine and phytochemical and pharmacological reports wherever available in published literature.

*Aegle marmelos* Corr. (Rutaceae), 3657, Belo

Bansjor, Rural folk, Burning micturation,

Pounded leaves (5-10 gm.) with sugar candy water is given 2-3 times a day.

Traditional Uses:

Expectorant, febrifuge, useful in dropsy, bronchial asthma. Leaf Juice applied in abscess ash used as wormicide (Chatterjee and Pakrashi, 1994).

Phytochemical Reports:

Essential oil,  $\beta$ -sitosterol, amino acids, alkaloids-aegeline, aegelenine, anthocynins, marmesinin, leucoanthocyanins, flavone glycosides and lupeol (Chatterjee and Pakrashi, 1994).

#### Pharmacological Actions:

Essential oil from leaves exhibit antifungal activity (Asolkar *et al.*, 1992). Aqueous extract exhibits cardiac stimulant, smooth muscle relaxant and uterine stimulant properties. Alcoholic extracts showed cardiac depressant, smooth muscle relaxant and uterine relaxant properties (Rastogi and Mehrotra, 1995).

*Gardenia turgida* Roxb. (Rubiaceae), 3847, Karhar

Bhandar, Majhi, Haemeturia

Fruit with Satawar (*Asparagus racemosus*) root in equal quantity pounded and given (10 gm.) twice a day.

#### Traditional Uses:

Affections of mammary glanda (Anonymous, 1986). Pulp pounded and applied to forehead in fever (Asolkar *et al.*, 1992).

#### Pharmacological Actions:

Ethanollic extract exhibit antiviral, hypotensive and anticancer activity (Asolkar *et al.*, 1992).

*Ichnocarpus frutescens* R. Br. (Apocynaceae), 1886, Suanloi

Hanumanthpur, Majhi, Haemeturia

Powdered root (5 gm.) is given with water twice a day.

#### Traditional Uses:

Demulcent, alterative, tonic, diaphoretic, diuretic; powdered root used in diabetes and to remove bladder stone (Anonymous, 1986).

#### Phytochemical Reports:

$\beta$ -sitosterol, alkaloids and flavonoids (Satyavati *et al.*, 1987).

#### Pharmacological Actions:

The 50% ethanolic extract (whole plant) showed antiviral activity (Satyavati *et al.*, 1987).

*Kirganelia reticulata* (Poir.) Baill. (Euphorbiaceae), 242, Jhojangi

Pharangia, Kondh, Haemeturia

Leaf decoction (two tea spoonsful) is given twice a day.

#### Traditional Uses:

Diuretic and cooling. Leaf juice with camphor and Cubebs (*Piper cubeba*) is useful in bleeding gums and also for infantile diarrhoea (Satyavati *et al.*, 1987)

Phytochemical Reports:

Friedelin, glochidonol and  $\beta$ -sitosterol (Asolkar *et al.*, 1992).

Pharmacological Actions:

Ethanolic extract (50%) of aerial parts exhibit antiprotozoal, antiviral, spasmolytic and hypotensive activity (Satavati *et al.*, 1987; Asolkar *et al.*, 1992).

*Lawsonia inermis* Linn. (Lythraceae), 3729, Manjuati

Chhotanpalli, Rural folk, Burning micturation

Powdered root (5 gm.) is given daily for 5 days

Traditional Uses:

Root bark alterative, astringent, sedative. Infusion given in calculous affections, jaundice enlargements of liver and spleen, (Nadkarni, 1954; Chatterjee & Pakrashi, 1994).

Phytochemical Reports:

Betulin, betulinic acid, lupeol,  $\beta$ -sitosterol (Chatterjee & Pakrashi, 1994).

*Mimosa pudica* Linn. (Mimosaceae), 4332, Lajwanti

Santhiya, Rural folk, Burning micturation

Leaf juice (one tea spoonful) is given twice a day for one week

Traditional Uses:

Leaf juice in sinus, sores, piles and fistula; paste applied to glandular swellings (satyavati *et al.*, 1987; Chatterjee & Pakrashi, 1994).

Phytochemical Reports:

Leaves contain alkaloids (Satyavati *et al.*, 1987).

Pharmacological Actions:

Leaf decoction showed moderate diuretic activity in experimental models (Satyavati *et al.*, 1987).

*Ventilago denticulata* Willd. (Rhamnaceae), 1799, Aratiki

Malavarum, Kondh, Haemeturia

Powdered stem bark (5 gm.) twice a day is given.

Traditional Uses:

Powdered stem bark mixed with gingelly oil is used as an application for skin diseases, itch and sprains (Chopra *et al.*, 1992; Chaterjee & Pakrashi, 1994).

Phytochemical Reports:

Friedelin (Chatterjee & Pakrashi, 1994).

## Results and Discussion

The present study has yielded first-hand information on native phytotherapy for urinary disorders i.e. burning micturation and haemeturia from various tribal dominated areas of Orissa state. The information presented here is based on oral interviews of local tribal medicinal practitioners and/or other inhabitants who have been using herbal material available in their vicinity since long for the treatment of various health disorders.

Data presented here on seven plants species when compared with published literature on ethnomedicine (Jain, 1991; 1996), it was observed that use of the plant resources for the treatment of urinary problems was new or less known. The screening of literature on traditional medicine revealed that root of *Ichnocarpus frutescens* is diuretic and useful to remove bladder stone, and use of *Lawsonia inermis* in calculus affections (Anonymous, 1986; Nadkarni, 1954). But pharmacological action of these drugs is yet to be worked out in this context.

Use of leaves of *Aegle marmelos* and *Mimosa pudica* and root of *Lawsonia inermis* in burning micturation have been reported from the area surveyed. And, root, stem bark, leaf and fruit of *Ichnocarpus frutescens*, *Ventilago denticulata*, *Kirganelia reticulata* and *Gardenia turgida* respectively were reported to be used in haemeturia.

In the present case four species *Gardenia turgida*, *Ichnocarpus frutescens*, *Kirganelia reticulata* and *Ventilago denticulata* have been recorded to be used again Haemeturia. Whereas three species, namely, *Aegle marmelos*, *Lawsonia inermis* and *Mimosa pudica* have been recorded to be used to treat burning micturation.

Efficacy of essential oils from leaves of *Aegle marmelos* as antifungal and aqueous extract having muscle relaxant activity have been reported (Asolkar *et al.*, 1992; Rastogi and Mehrotra, 1995). Similarly ethnolic extracts of *Gardenia turgida* and *Ichnocarpus frutescens* exhibit antiviral activity. Ethnolic extract of *kirganelia reticulata* (aerial parts) exhibit antiprotozoal, antiviral, spasmolytic and hypotensive action (Satyavati *et al.*, 1987, Asolkar *et al.*, 1992). These pharmacological action and chemical compounds present in the plant parts like alkaloids,  $\alpha$  sitosterol, flavonoids, essential oils etc. may have some role in combating the problems like haemeturia and micturation.

The diuretic activity in leaf decoction of *Mimosa pudica* (Satyavati *et al.*, 1987) might be having a role to overcome urinary disorders.

In view of the above it may be concluded that the ethnopharmacological data on urinary disorders reported from the tribal and other rural population in the state of Orissa may provide basic information for drug development. Therefore, further screening of these folk drug plants as claimed by the informants may be taken up to determine the therapeutic potential of such drugs.

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