KFRI Research Report No. 296 **ISBN No. 0970-8103**

Phytochemical Characterisation and Evaluation of the Medicinal Plant *Moovila* for Resource Enhancement

N. Sasidharan
Non-Wood Forest Products Discipline
Forest Utilisation Programme Division

KERALA FOREST RESEARCH INSTITUTE
PEECHI, THRISSUR
June 2007

Title: KFRI/405/2003: Phytochemical Characterisation and Evaluation of the Medicinal Plant *Moovila* for Resource Enhancement

Investigator: N. Sasidharan

Objectives:

- Qualitative and quantitative characterization of phytochemicals in *Pseudarthria viscida* and other related species.
- Study the macroscopic, microscopic characters, physical constant values, extractive values, ash values, etc. of the principal drug species and its substitutes for pharmacognostical parameters.
- Prepare the phytochemical profile of *Pseudarthria viscida* and other related species.
- Evaluation of the ingredients with respect to plant parts.

Period: April 2003 - March 2006, extended up to September 2006

Sponsor: KFRI Plan Fund

Research Associate: Dr. T.D. Babu

Technical Assistant: Willy George

CONTENTS

Acknowledgements

The guidance, advice and the moral support and above all, the interest shown by Dr R Gnanaharan, Director and Dr JK Sharma, former Director, Kerala Forest Research Institute are acknowledged with due respect. The financial support for the study by the Kerala State Council for Science, Technology and Environment (KSCSTE) is gratefully acknowledged.

The help received from Dr. Jose Padikkala, Amala Cancer Research Centre, Thrissur and Dr A Augustin, Kerala Agricultural University, Vellanikkara are acknowledged with thanks. Thanks also due to Dr Jose Kallarakkal and Dr M Balasundaran, Kerala Forest Research Institute for the editorial scrutiny of the report.

Finally, I also place on record my sincere thanks to Dr P Sujanapal, Shri Pramod Pathrose, Shri Sreekanth Sreedhar and Shri MM Roy.

Abstract

Pseudarthria viscida is the preferred source of the raw drug, 'salaparni' in the Ayurvedic system of medicine. The plant is known as *Moovila* in Malayalam and is one among the 'Dasamoola' of Ayurveda. Due to scarcity, some other trifoliate leguminous plants, particularly the species of *Desmodium* and *Uraria* are used as substitutes. *Pseudarthria viscida* and its widely substituted species viz. *Desmodium pulchellum* and *Uraria rufescens* were subjected to phytochemical as well as biological property analysis to find out their similarity. The polyphenolic, flavonoid, terpenoid and alkaloid compounds isolated from these plants were separated on TLC with various solvent systems. Profiles obtained for polyphenolic compounds show more or less similar patterns in all the samples used. For flavonoids and terpenoids similar patterns were observed in the samples of *P. viscida* and *U. rufescens*. But different profiles were obtained for *D. pulchellum*. Among the plants studied, the root of *D. pulchellum* only shows the presence of alkaloid. For the biological property analysis the antioxidant hydroxyl, nitric oxide, superoxide and lipid peroxide radical *in vitro* assay systems were performed. Among the plants used, *P. viscida* alone showed significant anti-oxidant properties. Fifty percentage inhibition of hydroxyl radical was found at the concentration of 5.75 and 7.61 μg/ml with methanol and acetone fractions respectively. Nitric oxide generated from sodium nitro-prusside was found inhibited by *P. viscida* extracts. Concentrations needed for 50% inhibition of nitric oxide were 33.34 and 3.48 μg/ml for methanol and acetone fractions respectively. The extracts were also found to inhibit lipid peroxides generated by induction of Fe^{2+}/ascorbate and Fe^{3+}/ADD/ascorbate in rat liver homogenate. Fifty percentage inhibition of lipid peroxidation was observed with 35 and 8.19 μg/ml of methanol and acetone fractions respectively. The results of above assay systems show significant free radical scavenging property of *P. viscida*. Mice treated with acetone extract of *P. viscida* showed significant protection against ethanol induced gastric damage. The acetone extract was found cytotoxic to various transformed cells in *in vitro* assay systems. Among the various concentrations used, 50% cell death was obtained in the concentration of 40 μg/ml. The extract was also found to reduce the tumour volume in solid tumour bearing animals in dose dependent manner. Thus, the study reveals that *Pseudarthria viscida* extracts show only significant free radical scavenging and other biological activities.

Introduction

India is enriched with vast resources of medicinal plants, which have been extensively used in various traditional Indian systems of medicine. Because of more adverse effect of synthetic products in therapy, the interest and usage of medicinal plants have increased tremendously in Asian and Western countries. According to WHO, approximately 80 % of the population in developing countries depends on traditional medicine, mostly plant drugs for their primary health care needs. The All India Ethnobiological Survey (AIES) carried out by the Ministry of Environment and Forests revealed that 7,500 plant species belonging to 386 families are used by 4,635 ethnic communities for health care across the country (Dubey *et al.*, 2004). The study also revealed that India is one of major suppliers of medicinal plants with an annual turnover of herbal products worth Rs.23 billion which includes condiments and food additives, herbal extracts, essential oils, gums, resins, crude drugs, etc. (Patwardhan, 2000).

India has not been able to capitalize its herbal wealth by promoting its use. The reasons are that many Indian products are not available in standardized form due to improper identification, harvestation and processing, lack of standardization and quality control checking, research and development of herbal drugs, clinical trials, etc. In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines (WHO, 2000).

Non-wood forest products contribute to food security, income and employment opportunities, particularly to the tribal communities. Most of the NWFPs are medicinal and are used in the Indian systems of medicines. Over the years, due to the increased demand and over-exploitation, the much needed medicinal plants have become rare or even depleted from their natural habitats. Medicinal plants are valued for their active ingredients and are best represented when growing in their natural habitats. In the Indian systems of medicines, the botanical source of a raw drug is often attributed to one species. The continuous extraction of a particular species will lead to its rarity as well as the depletion from the

habitats. In the absence of the preferred species, the demand is met with substitutes consisting of related or unrelated species (Rajshekharan, 2002).

The increasing commercialization trends in plant based drug preparations leads to overexploitation and destruction of medicinal plant populations. Because of the unavailability of the actual plant materials, a variety of approaches have been developed to increase productivity of plants/plant products. Cultivation is an important strategy to overcome the scarcity of medicinal plants. The other important criteria is to increase the production of plant ingredients by generating modified plants through biotechnology tools like plant tissue culture, culture of genetically modified plants and induction of secondary metabolite production by applying hormones/chemicals or fungal elicitors. The increased application of synthetic fertilizers and pesticides may cause the accumulation of toxic chemicals in the plant parts (Patwardhan *et al.*, 2004).

The other general trend is the usage of related species as substitutes often without proper evaluation. Hence, the identification of plants and its chemical constituent analysis in phytopharmaceutical industry are very important criteria. Some of the laboratories have already initiated analytical parameter studies significantly to determine the concentrations of active ingredients, contamination by pesticides, fertilizers, hormones, mycotoxins and general efficacy of medicinal plants.

Generally the plants possess the medicinal properties due to the presence of biologically active compounds like polyphenolics, terpenes, alkaloids, flavonoids etc. (Fransworth, 1984 and Cragg *et al.*, 1997). The isolation and characterization of these compounds from plant tissue is an important task in phytochemistry. It is often tedious and time consuming especially when the mixture to be separated is complex. Over the past 25 years, several new technologies with highly sophisticated instruments have emerged to accelerate the complicated purification and separation steps. Although, there is no universal technique to solve all the separation problems, best results may be obtained by using the

combination of two or more different methods with the use of more sophisticated instruments (Grably and Thiericke, 2000). According to the recent study conducted by United Nations Industrial Development Organisation (UNIDO), the inefficient processing techniques, poor quality control measures, lack of trained personnel and equipments are leading to low yields and poor quality products. The understanding of active ingredients along with their mechanisms of action may help to maintain or assure the quality of drugs. The study thus advocates an integrated approach to promote the export of medicinal plants/drug by assuring the quality.

According to modern medicine, most of the diseases are caused by the over production of free radicals. These free radicals damage the biological macromolecules including oxidation of sulfhydril containing enzymes, modification of amino acids, loss of function and fragmentation of proteins, damage of polysaccharide and excessive DNA strand break (Muller and Walin, 1998). To protect the action of highly reactive oxygen, the body system has several 'safe guard' mechanisms. They include enzymes such as superoxide dismutase, glutathion peroxidase, catalase, etc. Many antioxidants in the body such as vitamin E and ascorbic acid also inhibit the generation of oxygen free radicals. Sometimes these protective mechanisms were not found to be sufficient to the insult produced by excess stress. In these circumstances supplementation of non-toxic antioxidants may have a chemoprotective role in the body (Logani and Davis, 1979). Most of the plant related compounds also have the ability to activate free radical scavenging enzymes. Hence, in the present study we have analyzed the compounds profile and free radical scavenging properties of *P. viscida, U. rufescens* and *D. pulchellum*.

Species profile of '*Moovila*' and its substitutes

Pseudarthria viscida, commonly known as *Moovila* is an important plant in the Indian systems of medicine. The root is one of the *Dasamoola* in Ayurveda and is known by the Sanskrit name *Salaparni*. It is used to treat ulcer, hepatic disorders, bronchitis, helminthiasis, piles, strangury,

asthma, cardiovascular diseases, nerve disorders, snake bite etc., in the Indian systems of medicine (Nambiar *et al.*, 1985; Warrier *et. al.*, 1995; Kirthikar and Basu, 2000). The annual consumption of the root by the Ayurvedic medicine industry in Kerala is 140 tones (Sasidharan and Muraleedharan, 2000). The medicinal preparations containing *Moovila* roots are:

Agasthyarasayanam
Ajamamsarasayanam
Amrithaprasam
Amrutharishtam
Amruthathi Thailam
Anu Thailam
Badradarvadhi Kashayam
Balajeerakadhi Kashayam
Bhrahathyadhi Kashayam
Chyavanaprasam
Danthyarishtam
Dasamoolaharithaki
Dasamoolakaduthrayam
Dasamoolakadutryamka
Dasamoolarasayanam
Dasamoolarishtam
Dhanwandhararishtam
Dhanwanthara Ghritham
Dhanwantharam Kashayam
Dhanwantharam Kuzhambu
Dhanwantharam Thailam
Elakanadhi Kashayam
Gandha Thailam
Indukantha Kashayam
Indukantha Ghritham
Kallianaka Ghritham
Mahakalyanaka Ghritham
Mahalpanchagavya Ghritham
Mahamasha Thailam
Mahapaisachika Ghritham
Manasamithravadakam
Narayana Thailam
Rajannyadi Choornam
Rasnadasamoola Ghritham
Rasnadi Thailam
Sahacharadi Kuzhambu
Sahacharadi Thailam
Sivagulika
Sudarsana Choornam
Sukumara Ghritham
Sukumara Kashayam
Sukumara Rasayanam
Vidaryadhi Lehyam
Vidaryadhi Kashayam
Vidaryadhi Ghritham

Pseudarthria viscida belonging to the family Fabaceae (Leguminosae) is the preferred source of *Moovila*. It is observed that, a few other 3-leaved shrubby or subshruby leguminous plants, particularly *Uraria rufescens* and *Desmodium pulchellum* belonging to the family Fabaceae are also collected and marketed as *Moovila*. Brief botanical profile of the plants used as *Moovila* are provided below.

Pseudarthria viscida (L.) Wight & Arn., Prodr. 209. 1834.

Hedysarum viscidum L., Sp. Pl. 747. 1753.

Description: Annual or perennial diffuse subshrubs, branches slender, stem and branches with greyish-white hairs. Leaves 3-foliolate, leaflets 7-10 x 5-7 cm, broadly ovate, apex acute, base rounded, densely hairy; stipellate; petiole to 7 cm long; stipule lanceolate. Flowers small, in terminal or axillary racemes or panicles; calyx 2-lipped, 2 mm long, lobes lanceolate; petals red or pink, standard 6 mm diam, orbicular, retuse; wings 3 mm long, oblong; keel glabrous; stamens 9 + 1; ovary sessile, many-ovuled; style inflexed. Pods 2-3 x 0.4-5 cm, oblong, compressed, viscid hairy; seeds 3-5, subreniform, brownish-black.

*Habitat:*Moist deciduous forests, scrub jungles, forest plantations and plains

Flowering & Fruiting: October-February

World distribution: Peninsular India and Sri Lanka

In Kerala: Throughout the State

Part used: Root

Average dry weight (per 100gms): Small/Young (root from 15-25 plants), Medium (root from 8-20 plants), Mature/Large (root from 5-10 plants).

Uraria rufescens (DC.) Schind., Feddes Repert. 21: 14. 1925

Desmodium rufescens DC., Ann. Sci. Nat. (Paris) 4: 101. 1825.

Doodia hamosa Roxb., Fl. Ind. 3: 367. 1832.

Uraria hamosa (Roxb.) Wall. ex Wight & Arn., Prodr. 222.1834.

Description: Subshrubs to 1 m tall, viscid pubescent. Leaves 3-foliolate; leaflets 6-8.5 x 3-4.5 cm, elliptic, acute or obtuse at both ends, hispid below, lateral ones smaller than the terminal; stipules 12 mm long, acuminate. Flowers distant, in terminal cylindric racemose

panicles or racemes ; bracts 7 mm long, hispid; calyx 3 mm long, glabrous; petals bluish-pink, standard 6 x 8 mm, orbicular, cuneate at base; wings 5 x 3 mm, oblique, auricled at base; keel 6 x 3 mm. Joints of pods 6-8, each 2 x 2 mm, hispid, black.

Habit: Scandent subshrubs

Habitat: Moist deciduous forests and Forest plantations; occasional in the plains

Flowering & Fruiting: November-February

World distribution: Indo-Malesia

Kerala: Throughout the State, particularly in Central Kerala

Part used: Root

Average dry weight (per 100gms): Small/Young (root from 15-25 plants), Medium (root from 10-20 plants), Mature/Large (root from 5-10 plants).

Desmodium pulchellum (L.) Benth., Fl. Hongk. 83. 1861; 1876.

Hedysarum pulchellum L., Sp. Pl. 747. 1753.

Phyllodium pulchellum (L.) Desv., J. Bot. ser. 2, 1: 124.

Description: Shrubs, to 1.5 m tall, stem angular. Leves 3-foliolate, sometimes i-foliate; leaflets 7-10 x 4-6 cm, obovate, apex obtuse, base acute, thinly hairy above, densely below, lateral leaflets smaller than the terminal one. Racemes panicled, terminal or subterminal, floral leaves bifarious, to 12 mm across, orbicular, hispid, with one filiform appendage at base. Flowers 1-3 together; bracts and bracteoles minute; calyx puberulous, teeth lanceolate, shorter than the tube; standard petal 4-5 mm long, pale blue, glabrous. Pods 2-jointed, 7 mm long, joints orbicular, pubescent.

Habitat: Moist deciduous forests, occasional in semi-evergreen forests and forest plantations

Flowering & Fruiting: October-January

World distribution: Tropical Asia and Australia

In Kerala: Throughout the state, but not very common

Part used: Root

Average dry weight (per 100gms): Small/Young (root from 10-20 plants), Medium (root from 5-10 plants), Mature/Large (root from 2-6 plants).

Materials and methods

Pseudarthria viscida, Uraria rufescens and *Desmodium pulchellum* (Fig. 1) were collected from Peechi-Vazhani Wildlife Sanctuary and also from Tholpetty, Wyanad Wildlife Sanctuary. Root, stem and leaves of each sample were separated and dried at 50^0C and powdered. The powder of each sample was extracted with soxhlet extraction system using various solvents. The solvent of each extract was evaporated with rotary evaporating system (Buchi, Switzerland) and subjected to chromatographic and antioxidant studies.

TLC analysis

The powder of each sample was extracted with methanol and acetone, fractionated through Thin Layer Chromatography (TLC) with various solvent systems and analyzed for polyphenolics, flavonoids, terpenoids and alkaloids.

Solvent systems used for TLC analysis

1. Acetone : methanol : water (4:3:3)
2. Benzene : acetone : methanol (3:3:4)
3. Petroleum benzene : acetone : methanol : water (2:4:3:1)
4. Petroleum benzene : acetone : methanol (4:4:2)
5. Acetone : methanol (1:1)
6. Chloroform : acetic acid (9:1)

Comparative analysis of protein profile obtained through SDS-PAGE analysis

Proteins were extracted with 0.2 M tris buffer pH 7.5 using mortar and pestle and electrophoresed on 10% SDS PAGE (Biorad, USA) with 20 mA current for 2 hr. The gel was stained with silver nitrate to visualize the banding profile of protein.

Superoxide scavenging activity

Superoxide scavenging activity was determined by light induced superoxide generation by riboflavin and subsequent reduction of

nitrobluetetrazolium into coloured diformazan as described by McCord and Fridovich (1969). The reaction mixture contained 6 μm EDTA, 3 μm NaCN, 2 μm riboflavin, 50 μm NBT, test material and phosphate buffer in a final volume at 3 ml. The reaction mixture was uniformly illuminated with incandescent lamp for 15 min and the optical density was measured at 530 nm after illumination. The percentage inhibition was evaluated by comparing the absorbance value of the control and experimental tubes.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the test material was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substance (Elizabeth and Rao, 1990). The reaction mixture containing 2.8 mM deoxyribose, 0.1 mM ferric chloride, 0.1 mM EDTA, 0.1 mM ascorbate, 1 mM H_2O_2, 20 mM KH_2PO_4-KOH pH 7 and test material in a volume of 1 ml was incubated for 1h at 37^0C. Deoxyribose degradation was measured as thiobarbituric acid reactive substances by the method of Ohkawa *et al.* (1979). The inhibition produced by different concentrations of the sample as well as the concentration required for 50% inhibition were calculated.

Nitric oxide radical inhibition activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which were measured by Griess reaction (Green *et al.*, 1982 and Marcocci *et al.*, 1994). The reaction mixture containing 10 mM sodium nitroprusside in phosphate buffered saline (PBS) and the test material was incubated at 25^0C for 150 min. After incubation, 0.5 ml of the reaction mixture was added to 0.5 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethyne diamine dihydrochloride). The absorbance of the chromophore formed was determined at 546 nm.

Lipid peroxidation assay

Lipid peroxidation was induced in rat liver homogenate by the method described by Bishayee and Balasubramonian (1971) in the presence of different concentrations of test material and estimated by thiobarbituric acid reactive substance by the method of Ohkawa *et al.* (1979). Different concentrations of the test material were incubated with 0.1 ml rat liver homogenate (25%) containing 30 mM KCL, 0.04 mM tris buffer pH 4, 0.06 mM ascorbic acid and 0.16 mM ferrous ion in total volume of 0.5 ml for 1hr. At the end of the incubation period, 0.4 ml of the reaction mixture was treated with 0.2 ml SDS, 1.5 ml thiobarbituric acid (0.8%) and 1.5 ml acetic acid (20%) pH 3.5. The total volume was made up to 4 ml by adding distilled water and kept in water bath at 95^0C for 1hr. After cooling, 1 ml distilled water and 5 ml butanol pyridine mixture (15:1v/v) was added and shake vigorously, the tubes were centrifuged and the upper layer containing the chromophore was read at 532 nm. The percentage inhibition and the concentration required for 50% inhibition were calculated.

$$\% \text{ Inhibition} = \frac{(\text{Control OD} - \text{Treated OD}) \times 100}{\text{Control OD}}$$

Gastroprotective activity

The animals were divided into 5 groups containing 6 animals in each group. Group I was kept as standard without any treatment and all other groups received 1 ml of 80% ethanol/kg b. wt. Group II animals received 1 ml 80% ethanol alone. Group III was kept as positive control by treating Ranitidin (5 mg/kg b. wt.) along with ethanol. Group IV, V, and VI were treated with *P. visida*, *U. rufescens* and *D. Pulchellum* acetone extract (50 mg/kg b. wt) respectively one hour prior to the administration of 80% ethanol. The animals were sacrificed after 4 hour of the administration of ethanol.

Determination of cytotoxicity assay

Cytotoxicity was assessed by incubating $1x10^6$ transformed cells in 1.0 ml Phosphate Buffer Saline (PBS) containing various concentrations of the test materials at 37^0C for 3 hours. The viable cells were counted with a haemocytometer using trypan blue exclusion method (Babu *et al.*, 1995).

Anti-tumour studies with mouse ascites tumour model

The animals (Swiss albino mice) were injected with $1x10^6$ DLA cells subcutaneously into the right hind limb for the solid tumour development. The animals were administered the extract orally. Tumour diameter was measured every third day with vernier calipers and tumour volume was calculated using the formula.

Tumour volume = $(4/3)\ \Pi\ r_1^2 \times r_2^2$

where r_1 and r_2 are the two independent measurements of tumour radius

Results and discussion

Comparison of Optical Density (O.D) of methanolic extracts

The maximum absorbance (O.D) between the ranges of 200 nm to 700 nm was analyzed by spectrophotometer (Spectronic 20, USA). The absorbance profile of methanolic extract was between 271-328 in root, 271-328 in stem and 271- 277 in leaf samples (Fig. 2).

Comparison of polyphenolic compounds

The extracts of root, stem and leaf of each plant in methanol and acetone were subjected to thin layer chromatography using various solvent systems. The plates were sprayed with Folin Ciocaltive reagent for phenolic compounds. By spraying, 9 bands were observed for polyphenolic compounds (Fig. 3) in acetone extract with the solvent system of petroleum benzene: acetone: methanol (4:4:2). Six bands were separated for the same extract using chloroform: acetic acid (9:1) solvent system (Fig. 4). Profiles obtained for polyphenolics show more or less similar patterns in all the samples used. When methanolic extract was used, separation and intensity of bands were less.

Spectrophotometric analysis of bands separated on TLC

The absorbance of the most intensive bands with Rf. 0.95 present in root, stem and leaf of all plants were 283λ. The other less intensive bands with Rf. 0.2, 0.4 and 0.6 present in all samples gave the maximum absorbance at 277λ, 262λ, and 340λ respectively. The results obtained showed that the absorbance profile of polyphenolic compounds of *P. viscida, U. rufescens* and *D. pulchellum* were more or less similar.

Comparison of flavonoids

The methanolic and acetone extracts of root, stem and leaf of each plant were subjected to thin layer chromatography using solvent systems as

described. The plates were sprayed with 10% lead acetate solution. By spraying, 6 bands were observed for flavonoids in acetone extract with both solvent systems of chloroform: acetic acid (9:1) (Fig. 5) and petroleum benzene: acetone: methanol (Fig. 6). Similar patterns were observed in all the samples of *P. viscida* and *U. rufescens*. But different profiles were obtained for *D. pulchellum* with additional bands of Rf. 0.4 and 0.15.

Comparison of terpenoides

The extracts of root, stem and leaf of each plant in methanol and acetone were subjected to thin layer chromatography using solvent systems as described. The plates were sprayed with Liebermann–Burcard reagent.

By spraying, 10 bands were observed for terpenoids in acetone extract with the solvent system of chloroform: acetic acid (9:1) [Fig. 7] and petroleum benzene: acetone: methanol (4:4:2) [Fig. 8]. Similar patterns were observed in all the samples of *P. viscida* and *U. rufescens*. But different profiles were obtained for *D. pulchellum* with an additional band of Rf. 0.95 in chloroform: acetic acid solvent system and Rf. 0.06 in petroleum benzene: acetone: methanol (4:4:2).

Comparison of alkaloids

The methanolic and acetone extracts of root, stem and leaf of each plant were subjected to thin layer chromatography using solvent systems as described. The plates were sprayed with Dragondorff reagent. By spraying, root samples of *D. puchellum* only showed the bands for alkaloids (Fig. 9).

SDS PAGE Analysis

Total protein from leaves were fractionated through SDS-PAGE and prepared a comparative account between *Pseudarthria viscida* and its substitute plants. Results (Fig. 10) showed that the maximum similarity between *P. viscida* and *U. rufescens* (56.6%). The similarity between *P. viscida* and *Desmodium pulchellum* is 30.3%.

Fig. 10: SDS-PAGE analysis of total protein from leaves
Lane 1 and 2: *P. viscida;* 3 and 4: *U. rufescens,* 5 and 6: *D. pulchellum*

Analysis of antioxidant properties

For the preliminary study, superoxide radical scavenging assay was carried out by various solvent extractions of *Pseudarthria viscida*, *Uraria rufescens* and *Desmodium pulchellum*. Significant scavenging activity was observed in *Pseudarthria viscida* with the fractions obtained in acetone and methanol. Fifty percentage inhibition of superoxide radical was found at concentrations of 98.0 and 16.0 μg/ml with methanol and acetone fraction respectively (Table 1). But, in the case of *Uraria rufescens* and *Desmodium pulchellum*, no significant antioxidant activity was observed.

Degradation of deoxyribose by hydroxyl radical generated by Fe^{3+}/ascorbate/ EDTA/H_2O_2 system. Fifty percentage inhibition of

hydroxyl radical was found at concentrations of 5.75 and 7.61μg/ml with methanol and acetone fraction respectively (Table 2).

Table 1: Superoxide radical scavenging activity of *Pseudarthria viscida*, *Uraria rufescens* and *Desmodium pulchellum* extracts in various solvents with different polarity

Extracts	Concentration needed for 50% inhibition (μg/ml)		
	P. viscida	*U. rufescens*	*D. pulchellum*
Petroleum benzene	----	----	----
Chloroform	----	----	----
Ethyl acetate	----	----	----
Acetone	16.0	200	283
Methanol	98.0	----	----
Water	----	----	----

Table 2: Free radical scavenging activity of *P. viscida* extracts

Free radical	Concentration needed for 50% inhibition (μg/ml)	
	Methanol fraction	Acetone fraction
Hydroxyl radical	5.75	7.61
Nitric oxide	33.34	3.48
Lipid peroxidation	35.00	8.19

Nitric oxide generated from sodium nitroprusside was found inhibited by *P. viscida* extracts. Concentration needed for 50 % inhibition was 33.34 and 3.48 μg/ml for methanol and acetone fractions respectively (Table 2).

The extracts were found to inhibit lipid peroxides generated by induction of Fe^{2+}/ascorbate and Fe^{3+}/ADD/ascorbate in rat liver homogenate. Fifty percentage inhibition of lipid peroxidation was observed at 35 and 8.19 μg/ml for methanol and acetone fractions respectively (Table 2).

But, in the case of *Uraria rufescens* and *Desmodium pulchellum*, no significant hydroxyl radical, nitric oxide and lipid peroxides activity were observed as shown in the Tables 3 and 4.

Table 3: Free radical scavenging activity of *U. rufescens* extracts

Free radical	Concentration needed for 50% inhibition (μg/ml)	
	Methanol fraction	Acetone fraction
Hydroxyl radical	12.66	14.00
Nitric oxide	84.56	79.34
Lipid peroxidation	204.76	198.45

Table 4: Free radical scavenging activity of *D. pulchellum* extracts

Free radical	Concentration needed for 50% inhibition (μg/ml)	
	Methanol fraction	Acetone fraction
Hydroxyl radical	650.00	482.78
Nitric oxide	11.45	48.76
Lipid peroxidation	610.56	257.00

Cytotoxicity assay

In the cytotoxicity assay, about 80μg/ml of *P. viscida* acetone extract showed 100% death in Daltons Lymphoma Ascites Tumour (DLA) cells. The extract with other solvents methanol, petroleum benzene, chloroform and water didn't produce any significant toxicity (Table 5). Thus, preliminary study suggests that *Pseudarthria viscida* possess cytotoxic effect.

Table 5. Cytotoxicity of *P. viscida* acetone fraction on Daltons Lymphoma Ascites Tumor cells

Group	Concentration of drug in μg	% Cell death
1	0	3
2	5	8
3	10	15
4	20	28
5	40	50
6	80	100
7	100	100

Anti-tumour study

Administration of the acetone extract of *P. viscida* significantly reduced the development of murine solid tumour in dose dependent manner as compared to the control group (Fig. 11). On day 25, the tumour volume was 3.2 cm^3 in case of the control animals while it was 2.1 and 1.6 cm^3 in 0.5 and 1.0 mg receptively.

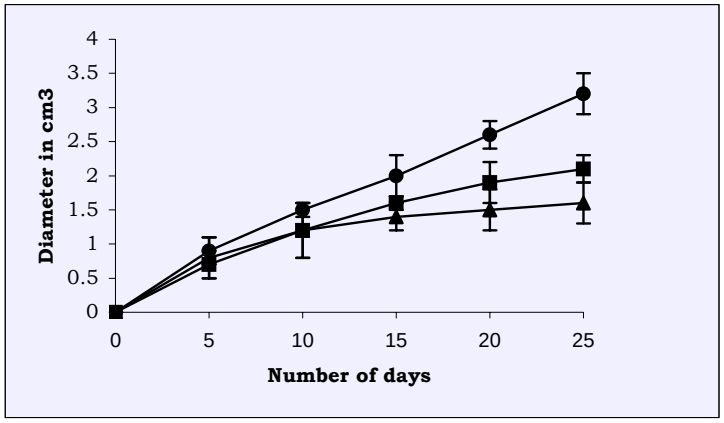

Fig. 11. Effect of *P. viscida* acetone extract on mouse solid tumour model.

Values are the mean ± S.E.M. of six animals in each group. (●) Saline treated control group, (■) 0.5 mg and (▲) 1mg/gm b. wt. treated animals

Gastroprotective effect

Multiple hemorrhagic red bands or patches of different sizes along the longitudinal axis of glandular stomach are characterized as lesions. On examination, a marked gross of mucosal lesions in stomach were observed in animals that received ethanol alone (Fig. 12 and 13). Animals treated with acetone extract of *P. viscida* and Ranitidine showed significant protection against ethanol induced gastric damage. The inhibition is 49.18% in Ranitidine treated group and 68.85% in *P. viscida* treated group (Table 6).

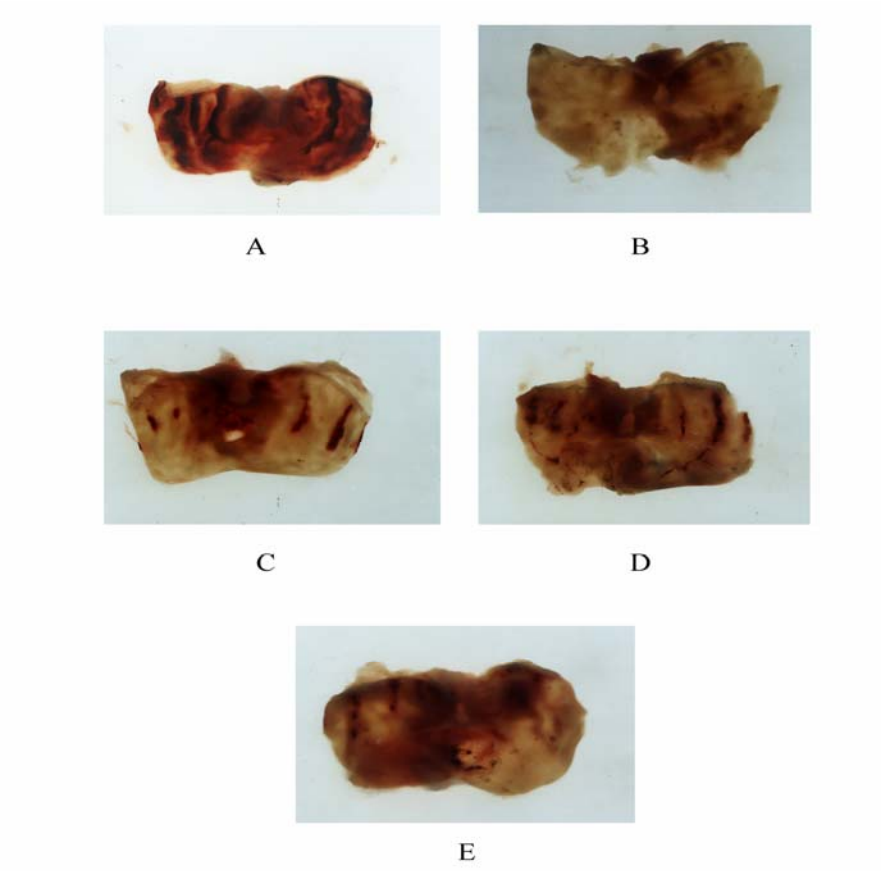

Fig. 12. Morphology of stomach of treated mice. A. Normal; B. Ethanol 80% (1 ml/kg b. wt.); C. Ethanol 80% (1 ml)+Ranitidine 50 mg/kg b. wt; D. Ethanol 80% (1 ml) + Extract 50 mg/kg b. wt; E. Ethanol 80% (1 ml)+Extract 100 mg/kg b. wt

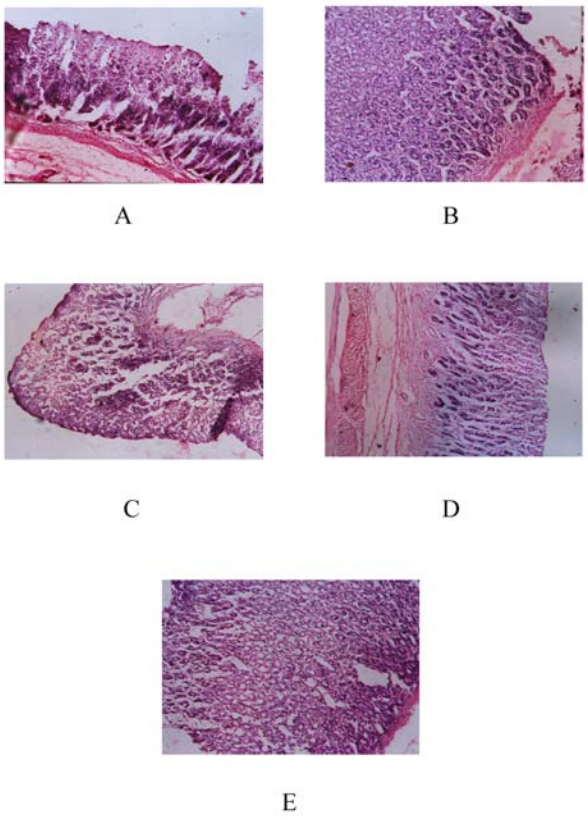

Fig. 13. Cytology of stomach of treated mice. A. Normal; B. Ethanol 80% (1 ml/kg b. wt.); C. Ethanol 80% (1 ml)+Ranitidine 50 mg/kg b. wt; E. Ethanol 80% (1 ml) + Extract 50 mg/kg b. wt; F. Ethanol 80 % (1 ml) +Extract 100 mg/kg b. wt

Table 6. Gastroprotective effect of plant extracts (acetone)

Groups	Dose	Ulcer	%Inhibition
Group I	Without treatment	----	----
Group II	1 ml 80% ethanol/kg b wt.	3.05	0.0
Group III	1 ml 80% ethanol50mg/kg b wt. Ranitidine	1.55	49.18
Group IV	50mg/kg b wt. *P. viscida*	0.95	68.85
Group V	50mg/kg b wt. *U. rufescens*	3.00	1.64
Group VI	50mg/kg b wt. *D. pulchellum*	2.80	8.20

Secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicine. Thus, phytochemicals with adequate free radical scavenging efficacy would be used as antioxidants. Various reports suggest that the phenolics and flavonoids are effective groups of compounds for free radical scavenging among the plant derived metabolites. In the present study, fractions obtained with acetone gave comparatively good separation profile with intensive bands for polyphenolic compounds and flavonoids. Also acetone fraction showed better antioxidant activities. This may be due to the extraction of more compounds with acetone solvent, which has low polarity than methanol. The phytochemical profile was estimated by TLC, confirmed that all the three contain high level of polyphenolics, flavonoids and terpenoids except alkaloids. The significant free radical scavenging activities of *Pseudarthria viscida* with acetone extract indicate its antioxidant efficacy. It scavenges various oxygen and nitrogen free radicals of biological importance. These properties may be playing an important role in the therapeutic efficacy of various medicine prepared with *P. viscida*.

Conclusion

Among the plants studied, *P. viscida* alone shows significant antioxidant and other biological properties. Even though the same chemical profiles were obtained in *P. viscida* and *U. rufescens*, only *P. viscida* exhibited significant antioxidant and curative properties in animal trials. The plant *D. pulchellum* produce comparatively different chemical profile.

References

Babu T D, Kuttan G and Padikkala J (1995). Cytotoxic and anti-tumor properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L) Urban. *Journal of Ethanopharmacology* 48:53-57.

Bishayee S and Balasubramanian A S (1971). Assay of lipid peroxide formation. *Journal of Neurochemistry* 18: 909-920.

Cragg G M, Newman D J and Snader K M (1997). Natural products in drug discovery and development. *Journal of National Proceedings* 60: 52-60.

Dubey N K, Rajesh K and Pramila T (2004). Global promotion of herbal medicine: India's opportunity. *Current Science* 86: 37-41

Elizabeth K and Rao M N A (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics* 58: 237-240.

Fransworth N R, Akerde O, Bingel A S, Soejarto D D and Guo Z (1985). Medicinal plants in therapy. *Bulletin of WHO* 63: 965.

Grably S and Thiericke R (1999). Bioactive agents from natural sources: trends in discovery and application. *Advanced Biochemical Engineering and Biotechnology* 64:101-154.

Green L C, Wagner D A, Glogwski J, Slapper P L, Wishnok J S and Tannenbaum SR (1982). Analysis of nitrate, nitrite and ^{15}N in biological fluids. *Analytical Biochemistry* 126, 131-136.

Kirthikar K R and Basu B D (2000). *Punica ganatum*, In: *Indian Medicinal Plants*, Sri Satguru Publications 5: 1508-1513.

Logani M K and Davis R E (1979). Lipid peroxidation in biologic effects and antioxidants. *A Review Lipids* 15: 485-493.

Marcocci L, Maguire J J, Droy-Lefuix M T and Packer L (1994). The nitric oxide scavenging property of *Ginkgo biloba* extract EGb 766. *Biochemistry and Biophysics Research Communication* 201:748-755.

McCord J M and Fridovich T (1969). Super oxide dismutase and enzymatic function for erythrocuprein. *Journal of Biological Chemistry* 244: 6049-6055.

Muller P and Walin H (1998). Adduct formation, mutagenisis and nucleotide excision, repair of DNA damage produced by reactive oxygen species and lipid peroxidation product. *Mutation Research* 410: 271-290.

Nambiar, V P K., N Sasidharan, C Renuka and M Balagopalan (1985). Studies on the Medicinal Plants of Kerala Forests. KFRI Research Report No. 42.

Okhawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbicuric acid reaction. *Analytical Biochemistry* 95: 351-358.

Patwardhan B, Ashok D B V and Mukund C (2004). Ayurveda and natural product discovery. *Current Science* 86: 789-798.

Patwardhan B (2000). Ayurveda: The designer medicine. *Indian Drugs* 37: 213-227.

Rajshekharan P E (2002). Herbal Medicine. In World of Science, *Employment News* 21-27, 3.

Sasidharan N and Muraleedharan PK (2000). Survey on the commercial exploitation and consumption of medicinal plants by the drug industry in Northern Kerala. *KFRI Research Report No.* 193.

Warrier P K, Nambiar V P K and Ramankutty C (1995). *Indian Medicinal Plants* Ed. 4: 366-370.

WHO (2000). General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization, Geneva.