



PHYTOCHEMICAL ANALYSIS OF BRIDELIA SCANDENS WILLD A FOLK LORE HERBAL DRUG A REVIEW

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ABSTRACT:

Analytical study deals with the modern pharmaceutical analysis of the leaves of the drug. Here, organoleptic characters, physico-chemical parameters, UV spectrophotometric study, chromatographic study which were carried out for the standardization of both leaf powder and leaf oil samples are documented. The leaf powder sample of the plant yielded moisture, 10.1%, ash, 6.65%, water soluble extractive 16.5% and methanol soluble extractive 22.3%. Whereas the leaf oil sample showed specific gravity 0.9650; refractive index 1.473; acid value 1.17; saponification value 241.4; ester value 240.23 and unsaponifiable matter 0.55%. UV spectrophotometric study of the leaf powder sample showed three absorptions peaks: the absorption being maximum at 218 nm followed by 272 nm and 666 nm. The spectra of the unsaponifiable matter of the leaf oil sample also showed three absorption peaks at 209 nm, 235 nm and 287 nm. Comparison of these two spectra reveals that their pattern is different. The chromatogram of both the samples obtained in day light showed 8 spots in the leaf powder sample and no spots in the other sample. On viewing under short wave UV radiation, the leaf powder sample revealed 3 spots and the other sample 2 spots. Among the spots the spot at Rf 0.66 was present in both the samples. After exposing to Iodine vapour almost all the spots viewed in day light were revealed in the first sample and 5 spots were found in the second sample. On spraying with vanillin sulphuric acid 6 spots were revealed in leaf powder sample and 5 spots in second sample. Among these 2 spots at Rf 0.46 and 0.66 were common in both the samples.

Key Words: *Bridelia scandens*, Folk lore, Analytical study

INTRODUCTION:

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Today, in spite of the exponential development of synthetic pharmaceutical chemistry, 25% of prescribed medicines in industrialised countries contain plant extracts or active principles derived from higher plants. Also, at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs currently in use in one or more countries (Fransworth et al. 1985) Of these 119 drugs, 74% were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine [1,2.]

The isolation of antimalarial drug, quinine, from the bark of *Cinchona* species (e.g. *C. Officinalis*) was reported in 1820 by the French Pharmacists, Caventon and Pilletier. The bark had long been used by indigenous groups in the amazon region for the treatment of fevers, and was first introduced into Europe in the early 17th Century for the treatment of malaria. Quinine formed the basis for the synthesis of the commonly used antimalarial drugs, chloroquine and mefloquine. Another plant long used in the treatment of fevers in traditional Chinese medicine, *Artemisia annua* has yielded the agents, artemisin and its derivatives artemether and arthiter, effective against strains of malaria resistant to quinine and its derivatives (Buss and Waigh, 1995). The analgesic used in ancient Mesopotamia,

morphine, was isolated from the opium poppy (*Papaver somniferum*) in 1816 by the German Pharmacist, Serturmer. This isolation laid the basis for alkaloid chemistry, and the development of a range of highly effective analgesic agents (Buss and Waigh, 1995). In 1785, the English Physician, Withering, published the observations on the use of foxglove, *Digitalis purpurea*, for the treatment of heart disorders; this eventually led to the isolation of the cardiotoxic agent digoxin. The antihypertensive agent isolated from *Rauwolfia serpentina*, reserpine, has been used in Ayurvedic medicine for the treatment of snakebite and other ailments (Kapoor, 1990) [3].

Over the last two decades, there has been a renewed interest in plants; the pharmaceutical industry now fully considers plants as a viable option for the discovery of new leads. Among the estimated 350,000 plant species on the earth, only a small percentage has been phytochemically investigated; the fraction subjected to biological or pharmacological screening is even smaller. Moreover, a plant extract may contain several thousand different secondary metabolites but any phytochemical analysis will reveal only a narrow spectrum of its constituents. The plant kingdom thus represents an enormous reservoir of pharmacologically valuable molecules to be discovered (Hostettman et al., 1998; Hostettman et al., 2000) [4, 5, 6].

Searching for new drugs in plants implies screening of the extracts for the presence of novel compounds and an investigation of their biological activities. Suspected novel or bioactive compounds

are generally isolated in order to elucidate the structure and to perform further biological and toxicological testing. The path that leads from an intact plant to its pure constituents is long. It involves work that might last from weeks to years and includes the following steps [7,8,9].

- Collection of the plant material with precautions to avoid artefact formation.
- Identification of the species by a botanist.
- Extraction using different solvents, followed by the analysis of these extracts by different chromatographic methods.
- Fractionation and isolation steps using different preparation chromatographic techniques.
- Structure elucidation of the constituents by combination of various spectroscopic and chemical methods.
- Pharmacological and toxicological testing.
- Synthesis or semi synthesis of the natural product.
- Synthesis of analogues with the aims of establishing structure activity relationships.

Before starting phytochemical and pharmacological investigations, chemotaxonomic information can be obtained by literature or data bank search; it is therefore possible to gain access to all the previous research which has been performed on the plant selected for study.

Selection of plants based on data from traditional medicine can also lead to promising new molecules. Plants from tropical and subtropical regions represent an enormous reservoir of new

molecules with potential therapeutic activity waiting to be discovered.

How can the information gathered from traditional healers be evaluated? For example, the efficacy of plants used as laxative or externally to treat sores or wounds are of great interest because their healing effect is easy to verify. However, diagnosis of healing effects on internal problems is much more difficult to rely on. The treatment is very often symptomatic and moreover, traditional healers often call upon supernatural forces. The placebo effect is also often involved. Therefore, an evaluation of the obtained information is required before selecting plants for further investigation [10].

Here, in the present study, a sincere attempt is made to analyze the test drug "*Bridelia scandens* Willd" physically as well as chemically within the limitations of the facilities available.

AIMS AND OBJECTIVES:

- To evaluate the organoleptic characters of the test drugs;
- To assess the physicochemical parameters of the test drugs;
- To analyse the test drug U.V. spectrophotometrically;
- To evolve suitable thin layer chromatography (TLC) pattern of the drug.

MATERIALS AND METHODS:

The pharmacognostically authenticated drug sample was finely powdered and used in the present study. However, the oil prepared from the leaves of the same drug, with the classical method, in gingelly oil base was also studied. The samples were analysed by using various parameters,

quantitative and qualitative analysis of phytochemicals, U.V. spectrophotometric analysis and Thin Layer Chromatography (TLC).

(A) Organoleptic characters:

The organoleptic characters of Ayurvedic drugs are of utmost importance and will provide a general idea regarding the proper identification of the sample. This also corresponds to *Pancajnanendriya Pariksa* of Ayurveda. The characters like colour, odour, taste, touch and consistency of both the samples were noted .

(B) Physicochemical Parameters

(1) Leaf powder

The sample number one, leaf powder of "*Bridelia scandens* Willd ", was analysed for the following parameters:

- (i) Loss on drying,
- (ii) Ash value,
- (iii) Water soluble extractive, and [11,12]
- (iv) Methanol soluble extractive. [12]

(i) Loss on drying [13]

The loss on drying of the leaf powder was determined by taking 2 g. accurately weighed powder in a dish, previously weighed, kept in an oven at 110°C for drying till constant weight. The weight after drying was noted and the loss on drying was calculated and presented in percentage w/w.

(ii) Ash value [14]:

About 2 g of the leaf powder was accurately weighed and taken in a silica crucible, which is weighed previously. It was incinerated in a muffle furnace at a temperature not exceeding 450°C for about four hours, cooled and weighed. From the

weight of the residue obtained, the ash value was calculated in percentage.

(iii) Determination of water soluble extractive [15]:

The extractive material in different solvent is related to amounts of different types of constituents in a given amount of medicinal plant material.

For the determination of water soluble extractive 5g accurately weighed dried powder sample was taken in a conical flask, to it 100 ml of water was added, shaken well, closed tightly and allowed to stand for 24 hours with occasional shaking, and filtered. 20 ml of filtrate was taken in a previously weighed porcelain evaporating dish; evaporated to dryness on a hot water bath and dried to constant weight in an oven.

From the weight of the residue obtained, the extractive values, in percentage w/w was calculated with reference to the air-dried sample.

(iv) Determination of Methanol soluble extractive [16]:

5 g accurately weighed leaf powder sample was taken in a conical flask, to it 100 ml of methanol was added, shaken well, closed tightly and allowed to stand for 24 hours. Rest of the procedure is similar to that of water-soluble extractive. From the weight of the residue obtained, the methanol soluble extractive was calculated.

2. Leaf oil

The sample number 2, the leaf oil of *Bridelia scandens* Willd was analysed for the physical parameters like refractive index, specific gravity and loss on drying and chemical parameters like acid

value, saponification value, ester value and unsaponifiable matter by following the method prescribed in Indian Pharmacopoeia, 1985. 9Pno 144-145

(i) Specific gravity [17]

Definition:

Specific gravity of a liquid is the weight of a given volume of the liquid at a specific temperature compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air.

Significance:

The presence of dissolved substances in oil is expected to change its specific gravity. So, it is considered to be an important parameter for analysing medicated oils.

Methods:

A clean and dry 25-ml capacity pycnometer was taken and its weight was noted. It was filled with the sample, cleaned properly from outside and the weight was taken at 40°C. Then it was cleaned, rinsed and filled with distilled water, dried from outside and the weight was noted at 40°C. The weight of sample and the distilled water was calculated. Then the specific gravity was determined by dividing the weight of the sample by the weight of the water.

(ii) Refractive index [18,19]

Definition:

The refractive index of a substance is the ratio of the sine of the angle of incidence to the sine of the angle of refraction. In otherwise, it is the ratio of the velocity of the light in vacuum to the velocity in the substance or a chosen media.

Significance:

The consistency of the media and solutes present in the media bring the difference in the refractive index. So, it is an important parameter for differentiating the oils.

Method:

Refractive index of a substance varies with temperature. Hence, temperature is to be noted while determining R.I. The R.I. of the sample was measured in Abbe's Refractometer at 40°C. The temperature was maintained at 40°C by circulating warm water.

(iii) Acid value [20]:

Definition:

Acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acid present in 1 gm of the sample.

Method:

Acid value is determined by taking about 10 gm accurately weighed sample in a 250 ml flask, dissolving it in a mixture of equal volume (50 ml) of alcohol (95%) and solvent ether and titrating with N/10 potassium hydroxide using Alkali Blue - 6B as indicator. The appearance of pink colour from blue was the end point. Acid value was calculated with following formulae:

No. of ml of N/10 Alkali used

Acid value = -----

Weight of sample in gram

(iv) Saponification value [21]:

Definition:

Saponification value is the number of mg of potassium hydroxide required to neutralize fatty

acids resulting from the complete hydrolysis of 1 gm of the substance.

Method:

Weigh accurately about 2 g of the sample into a conical flask and add exactly 25 ml of the alcoholic potassium hydroxide solution. A reflux condenser was attached, and heated in boiling water for one hour, shaking frequently. It was titrated, while hot, using alkali blue 6B as indicator. A blank was also performed.

$$(b-a) \times 0.02805 \times 1000$$

Saponification value = -----

Wt. in gm. of the sample

Where "a" is titer value of sample and "b" is titer value of blank.

(v) Ester value [22]:

Definition:

Ester value of a substance is the number of mg of potassium hydroxide required to neutralize the acids resulting from the complete hydrolysis of 1 gm. of fat and not the fatty acids present in it.

Method:

The value is obtained by subtracting the acid value from saponification value.

Ester value = Saponification value - Acid value

(vi) Unsaponifiable matter [23]:

Definition:

The unsaponifiable matter consists of substances present in oils and fats which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent after saponification.

Method:

About 5 gm. of the sample, accurately weighed, was taken into a 250-ml flask fitted with a reflux condenser. A solution of 2 gm. of potassium hydroxide was added in 40 ml of alcohol and heated on water bath for one hour shaking frequently. The contents of the flask was transferred to a separating funnel with the aid of 100 ml of hot water and while the liquid is still slightly warm, extracted very carefully with three quantities, each of 100 ml, of solvent ether. Combined ether extracts were taken in a second separating funnel containing 40 ml of water, swirled gently for a few minutes, allowed to separate and the lower layer is rejected, the ether extract is washed with two quantities, each of 40 ml, of a 3 percent w/v solution of potassium hydroxide, each treatment being followed by a washing with 40 ml of water. Finally, the ether layer was washed with successive quantities, each of 40 ml of water until the aqueous layer is no longer alkaline to phenolphthalein. The ether layer is transferred to a weighed flask, washing out the separating funnel with solvent ether. The ether is distilled off and added to the residue 6 ml of acetone. The solvent was completely removed from the flask with the aid of a gentle current of air. It was dried at 100-105°C for 30 minutes, cooled in a desiccator and the residue is weighed. The unsaponifiable matter was calculated as a percentage (w/w). The residue was dissolved in 20 ml of alcohol previously neutralized to phenolphthalein solution, and titrated with 0.1 N alcoholic sodium hydroxide. If the volume of 0.1 N alcoholic sodium hydroxide exceeds 0.2 ml, the

amount weighed cannot be taken as unsaponifiable matter and the test must be repeated.

C) UV VISIBLE SPECTROPHOTOMETRIC ANALYSIS:

Principle:

Different chemicals when subjected for photometry in white light (including UV) have specific affinity to absorb or to transmit a particular range of wavelength, which is related to that compound.

Spectrophotometric analysis involves the measurement of the ability of the dissolved substance to absorb electromagnetic radiation of definite and narrow wavelength ranges. These absorptions are measured at wavelength that are generally a characteristic of the chemical composition of a dissolved absorbing substance. Radiant energy waves range from 200 nm to about 400 nm in the UV region and from 400 nm to about 750 nm in the visible region. The UV or visible spectrum of a molecule is the result of change in energy of a molecule as a whole or rather than of a particular band. The UV and visible spectrum of a substance generally do not have a high degree of specificity but they are suitable for quantitative assays for many substance and useful as additional means of identification. Hence, the UV spectrum of the drug was selected as one of the parameter.

The UV spectra was recorded in a Shimadzu double beam UV visible recording spectrophotometer (Model UV - 160A)

Methanol extract of the leaf powder and the unsaponifiable portion (in ether), after suitable dilution, was used as sample for scanning and the

details of the U.V. visible spectra obtained was recorded.

(D) THIN LAYER CHROMATOGRAPHIC STUDY (TLC):

TLC and gas chromatography are widely used for the analysis of different samples. In the present study TLC has been adopted as a separation technique. Thin layer chromatography is a technique where a solute distributes between two phases.

(i) Stationary phase (adsorbent layer): In the form of a thin layer of adsorbent on a glass plate or aluminium plate

(ii) Mobile phase (solvent system): In the form of a liquid (pure solvent or mixtures)

By this we can separate individual compound from a mixture. By observing the intensity and R_f value of separated spots we can identify different compounds present in it.

In 1938, Izmailov and Schraiber introduced this technique for the first time at the Ukrainian institute for experimental pharmacy. But it was not accepted until late 1950, when Stahl publicized the method, developed a kit of basic equipment and made then available. Since then, the TLC has become an important tool for both qualitative and quantitative analysis.

Thin layer chromatographic study of the two samples was carried out by using the following conditions:

Adsorbent layer: Silica gel GF 254

Solvent system: Toluene : Ethylacetate (85:15)

Detection

i) Day light,

ii) Exposure to U.V. light,

- iii) Exposure to Iodine vapour, and
- iv) Spraying with Vanillin sulphuric acid spray reagent followed by heating the plate at 110o C for 10 minutes.

The methanol extracts of both the leaf powder and the unsaponifiable matter of leaf oil (in ether) of *Bridelia scandens* Willd were spotted on a precoated TLC plate in two separate bands and the chromatogram was developed by using Toulene – Ethylacetate (85 : 15) as the solvent system. The chromatograms obtained after using each detection system were observed carefully, and the details like number and colour of the spots and their Rf values were recorded.

RESULTS AND DISCUSSION

The results of the analysis of the samples of ‘*Bridelia scandens*, Willd’ carried out by adopting various parameters and methods, are presented here under:

(A) Organoleptic characters

The organoleptic characters of the leaf powder sample and oil are being presented below in the Table No. 1 and 2 respectively.

Table No 1: Organoleptic characters of *Bridelia scandens* Willd leaf powder.

Sr No	Colour	Light greyish green
1	Odour	Like tea powder
2	Taste	Astringent
3	Touch	Coarse

Table No 2: Organoleptic characters of *Bridelia scandens* Willd leaf oil.

Sr No	Colour	Dark green
1	Odour	Like tea powder with odour of heated oil
2	Taste	Astringent and acrid
3	Touch	Unctous

(B) Physico-chemical parameters:

The analytical data pertaining to the two samples i.e. *Bridelia scandens* leaf powder and leaf oil is presented in table no 3 and 4 respectively.

Table No.3: Analytical data of *Bridelia scandens* Willd leaf powder.

Sr. No.	Parameter	Value
1.	Loss on drying at 110°C	10.1% w/w
2.	Ash value	6.65% w/w
3.	Water soluble extractive	16.5% w/w
4.	Methanol soluble extractive	22.3% w/w

It can be observed from the above table that the loss on drying of the leaf powder sample at 110 °C is 10.1% w/w and the ash value is 6.65% w/w. The water soluble extractive is 16.5% w/w and methanol soluble extractive is 22.3% w/w.

Table No. 4: Analytical data of *Bridelia scandens* Willd leaf oil

Sr. No.	Parameter	Value
1.	Specific gravity at room temperature (21°C)	0.9150
2.	Refractive index at 40°C	1.4730
3.	Acid value	1.17

4.	Saponification value	241.4
5.	Ester value	240.23
6.	Unsaponifiable matter	0.55% w/w

It can be observed from the above table that the specific gravity of *Bridelia scandens* Willd leaf oil is 0.9150 at room temperature (21°C), refractive index at 40°C is 1.4730; acid value is 1.17, saponification value is 241.4; ester value is 240.23 and unsaponifiable matter is 0.55% w/w.

(C) U.V. SPECTROPHOTOMETRIC ANALYSIS :

The U.V. visible spectra of the methanol extract of *Bridelia scandens* Willd leaf powder and unsaponifiable matter of leaf oil taken in ether, after suitable dilution, was recorded and presented in Fig. 1, 2 and 3. As could be seen from the figure. 1, the methanol extract of leaf powder showed three absorption peaks; the absorption being maximum at 218nm followed by 272 nm and 666 nm. The visible spectra of the unsaponifiable matter of leaf oil are presented in Fig. 2, show 3 absorption peaks at 209 nm 235 nm and 287 nm. Comparison of these two spectra reveals that their pattern is different.

(D) THIN LAYER CHROMATOGRAPHY (TLC):

Thin layer chromatographic study of both the samples i.e. the methanol extract of leaf powder and the unsaponifiable matter of leaf oil taken in ether was carried out using the following conditions:

Adsorbant layer-Silica gel GF254 pre-coated plates (Merck)

Solvent system Toluene: Ethylacetate (85:15)

Detection

i) Day light,

ii) Exposure to U.V. light,

iii) Exposure to Iodine vapour, and

iv) Spraying with Vanillin sulphuric acid spray reagent followed by heating the plate at 110°C for 10 minutes.

The chromatogram obtained in daylight has been presented in Fig. 1, which showed 8 spots at Rf. 0.11 (light green), 0.15 (orange yellow), 0.20 (dark green), 0.31 (light green), 0.43 (very light green), 0.47 (very light green), 0.64 (green), and 0.99 (yellow) in leaf powder and showed no spot in the unsaponifiable matter of the leaf.

The chromatogram obtained when viewed under short wave U.V. radiation revealed 3 spots at Rf. 0.16, 0.49 and 0.66 in leaf powder sample and 2 spots at Rf. 0.56 and 0.66 in the unsaponifiable matter of the leaf oil sample (Fig. 2). Among the spots the spot at Rf0.66 was present in both the samples.

After exposing to Iodine vapour the leaf powder sample showed almost all the spots as seen in daylight while there were five spots at Rf. 0.37, 0.44, 0.55, 0.66 and 0.... In unsaponifiable matter sample (Fig. 3). The spot at Rf. 0.66 was present in both the samples.

The chromatogram obtained after spraying with Vanillin sulphuric acid spray reagent followed by heating the plate at 110°C for 10 minutes has been presented in Fig. 4.

The chromatogram revealed 6 spots at Rf. 0.16 (green), 0.37 (light blue), 0.46 (light green), 0.52 (light blue), 0.66 (dark blue), and 0.99 (dark gray) in leaf powder sample and 5 spots at Rf. 0.38 (light blue), 0.46 (blue), 0.53 (light blue), 0.57 (yellow),

and 0.66 (pink). It can be seen that two spots at Rf. 0.46 and 0.66 are common in both the samples.

Observation and comparison of the chromatograms revealed only 1 or 2 common spots present on both the samples and other spots are different. It indicates that some common compounds are present in both the samples. The unsaponifiable matter of the oil sample may contain mainly sterols, which is supported by the fact that it

gave positive L.B. test. Sterols will be extracted even in methanol. Possibly due to this reason some common spots have been observed in the T.L.C. of the two samples.

The data obtained in the present study will not only help in routine analysis and standardisation of *Bridelia scandens* Willd leaf samples but also the pharmaceutical preparations manufactured by using *Bridelia scandens* Willd leaf.



Fig 1

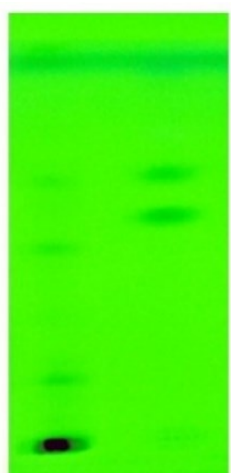


Fig. 2



Fig. 3

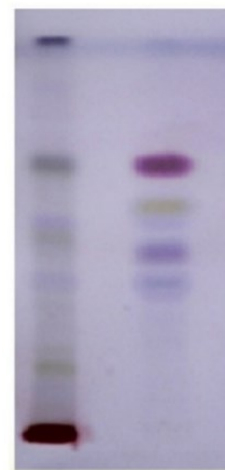


Fig. 4

DISCUSSION AND CONCLUSION:

Bridelia scandens Willd. is a large, woody, evergreen, scandent shrub with pendant branches large deflexed spines abundantly seen in western ghats. Plant when subjected for physicochemical analysis powder had astringent taste and tea powder odour 21,22,24,25. Whereas oil was astringent and acrid with odour of heated oil. Powder showed permissible limit of moisture as well as ash value. It was observed drug had more of methanol soluble extractive (22.3%) than water

soluble extractive (16.5%). Leaf oil which is traditionally used in various diseases had Specific gravity at room temperature (21°C) was 0.9150. Refractive index at 40°C was 1.4730 Acid value was 1.17 Saponification value was 241.4 and Ester value was 240.23. TLC

Comparison of UV Spectra of leaf powder and leaf oil reveals that their pattern is different indicating changed drug activity in two different forms.

TLC study of the drug revealed different Rf in different exposures. Common to all being at 0.66

which could be marker compound helping for the identification of the drug. Thus basic analysis of the drug of *Bridelia scandens* Willd a folk lore drug may help in identity of it as well be a benchmark for further analysis of the drug. In order to discover new bioactive compounds from plant sources, which could become new leads or new drugs extracts should be simultaneously evaluated by chemical screening and by various biological or pharmacological targets. Chemical screening using hyphenated techniques such as LC/ UV and LC/MS and more recently LC (NMR, quickly provide ample structural information, leading in many cases to the identification of compounds. This allows researchers to distinguish between known compounds and new molecules directly from crude plant extracts. Thus, the tedious isolation of known compounds can be avoided, and a targeted isolation of constituents presenting novel or unusual spectroscopic feature can be undertaken 24.

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