Pharmacognostical and physio-chemical standardization of *Jasminum auriculatum* Vahl leaves

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ABSTRACT

Jasminum auriculatum commonly known as Jhui; an evergreen shrub belonging to family Oleaceae, is traditionally used in the treatment of urolithiasis, various skin diseases and wounds. Until date no scientific evaluation has been reported on its leaves. Therefore, the present study is aimed to evaluate the pharmacognostic characters of an important medicinal plant Jasminum auriculatum Vahl. Micro and macroscopic characters of fresh and dried leaf samples were analysed. Physciochemical studies and preliminary phytochemical investigation were performed using WHO guidelines. The characteristic microscopic features of leaves were observed as multicellular trichomes, xylem cells, phloem cells, collenchymas, spongy parenchyma and palisade cells. Physicochemical parameters such as extractive values, ash values, foreign matter, loss on drying, volatile oil content, swelling index, foaming index, crude fibre content, fluorescent behaviour, microbial contamination, aflatoxin content, heavy metal profile, pH values of drug solution were also determined. Preliminary phytochemical screening showed the presence of carbohydrate, terpenoids, steroids, saponins, flavonoids, tannins and phenolics compounds. This is the first report on the pharmacognostic studies of J. auriculatum and the data thus generated may be used as an analytical feature to ascertain the authenticity and quality of the crude drug.

Keywords: *Jasminum auriculatum,* Pharmacognostic character, Fluorescence analysis, Aflatoxin content, Microbial contamination.
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INTRODUCTION

Standardisation of herbal medicines is the process of laying down a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that hold an assurance of quality, safety, efficacy and reproducibility. It is the procedure of developing and agreeing upon technical standards. Specific standards are laid by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by a particular herbal medicine. Hence standardisation is a tool for the quality control process [1].

Jasmine is a genus of shrubs and vines in the olive family Oleaceae with about 200 species throughout the world, out of which around 40 species are reported to be growing in India [2]. Irrespective of the species, extracts from different parts such as leaves, stem, bark and roots of the *Jasminum* plant have been used in ethno-medicines for a long time. It is a large scrambling, sub erect, twining, evergreen shrub; native to Deccan Peninsula, Circars and Carnatic extending south wards to Travancore. It is commercially cultivated for its fragrant flowers mainly in Ghazipur, Jaunpur, Farrukhabad and Kanauj districts of U.P, Bihar and Bengal. Its various parts have been reported

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to possess beneficial effects as aphrodisiac, antiseptic, anthelmintic, aromatherapy, cardiotonic, corns, deobstruant, diuretic, emollient, hyperdipsia, leprosy, nephrolithiasis, odontalgic, ophthalmopathy, stomatopathy, strangury, suppurative, skin diseases, thermogenic, urolithiasis, ulcers and wounds [3].

Besides its uses in traditional system of medicine; there is a lack of information available in the literature regarding pharmacognostical evaluation and standardisation of leaves of *Jasminum auriculatum* Vahl.. Therefore the present study has been conceived with aim to establish standardisation parameters for the selected plant parts and it comprises of macroscopy, microscopy, determination of extractive values, ash values, loss on drying, crude fibre content, volatile oil content, bitterness value, foaming index, swelling index, heavy metal analysis, total microbial count and aflatoxin content.

MATERIALS AND METHODS

Plant collection and authentication
Leaves were collected from healthy plants of *Jasminum auriculatum* Vahl. from the medicinal garden of Sri Venkateswara University, Tirupati (Andhra Pradesh). Herbarium so prepared was authenticated by Dr. H. B. Singh (Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi) under voucher specimen no. NISCAIR/RHMD/Consult/-2011-12/1763/63 dated June 24; 2011 and a specimen was deposited in the department. The plant material was dried under shade and then coarsely powdered.

Macroscopy
Untreated sample was examined under diffused day light and the colour of sample was recorded. The powder was rubbed slowly between fingers and odour was examined. Taste of the powder was also checked. Surface material was touched to determine whether it was soft or hard [4].

Microscopy
Thin transverse free hand sections of fresh leaves were made with the help of sharp blade and cleared with chloral hydrate solution. The sections were stained with phloroglucinol and conc. hydrochloric acid and mounted in glycerine-water mixture. These were observed under compound microscope and photographed [5-8].

The powder microscopy was carried out after passing the powdered drug through #60. The powder so obtained was treated with chloral hydrate solution and stained with phloroglucinol and conc. hydrochloric acid and mounted in glycerine-water mixture. This was observed under compound microscope and photographed [5-8].

Qualitative parameters
Extractive values and successive extractive values of *Jasminum auriculatum* Vahl. leaves powder were determined according to standard procedures using petroleum ether (60-80°C), chloroform, ethanol and water. Total ash, water soluble ash and acid insoluble ash values were studied according to standard procedures [8-13].

Preliminary phytochemical analysis of successive extract of leaves extracts was performed according to standard procedure [7, 12, 13].

Fluorescence analysis was conducted according to standard procedure [14-15].

Quantitative studies
Loss on drying, foaming index, swelling index, volatile oil content, crude fibre content, aflatoxin content, microbial contamination, heavy metal analysis of powdered leaves and pH values of 1% w/w and 10% w/w powder in water were determined as per WHO guidelines [8-13].

RESULTS

Macroscopy
Leaves are trifoliate and ovate in shape as depicted in Figure 1. The terminal leaflet is bigger having 2-3cm length and 1.5-2cm width. The basal lateral leaflets are two having 0.5-1cm length and 0.4-0.5cm width. The apex is acute, margin entire, base symmetrical. The lateral veins are 3-5 and not prominent on the upper surface. Taste bitter, odour faint and aromatic.

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Figure 1: Leaflets of *Jasminum auriculatum* Vahl

Microscopy

T.S of the leaflet passing through midrib shows the following structure as shown in Figure 2(a).

The leaflet is dorsiventral with upper layer of epidermis having barrel shaped cells. It is covered with very thin cuticle and contains no stomata but the lower epidermis has paracytic stomata. Both the epidermis has glandular and covering trichomes. Glandular trichomes have 1-2 celled stalks with 4-8 celled head. The covering trichomes are warty, multicellular, uniseriate type.

Below the upper epidermis the laminar region has 1-2 layers of palisade cells. These cells have microrosette crystal of calcium oxalate. Palisade is followed by 2-3 layers of spongy parenchyma; some of which have simple starch grains.

In the midrib region upper epidermis is followed by 2-4 layers of collenchyma. The vascular tissue is present in the centre. It is shaped like horse shoe and is surrounded by phloem cells; below which are present 3-4 layers of collenchymas cells followed by lower epidermis.
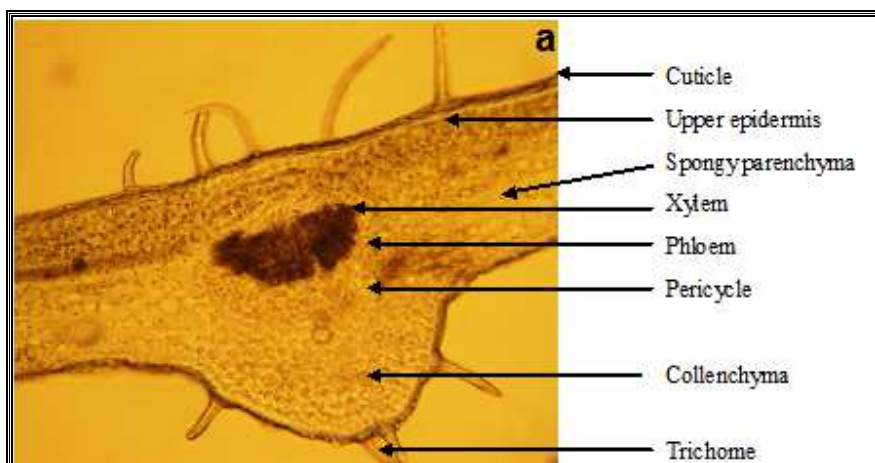

Figure 2: Transverse section of *Jasminum auriculatum* Vahl. leaf

Powder Microscopy

The powder microscopy of the leaf shows following structures as shown in Figure 3(a, b, c, d). Powder of leaf shows numerous simple thick walled warty, uniseriate, multicellular covering trichomes. Many of them were

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attached to the epidermal cells. The upper and lower epidermis was seen in their surface view. The lower epidermis shows the presence of paracytic stomata. The leaf fragments showing palisade cells in surface view were also visible. Fragments of vascular tissue were also present.
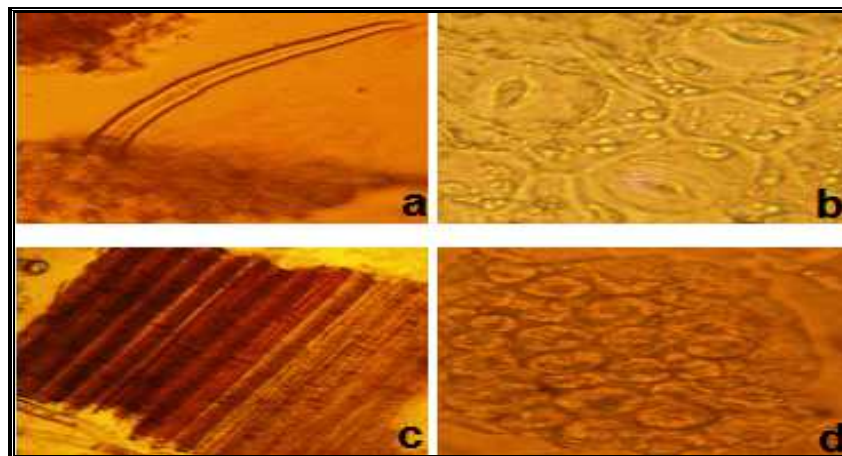
Figure 3: a) Covering trichome at 100x; b) Paracytic stomata at 100x;
c) Vascular bundle at 40x; d) Palisade cells at 40x (surface view).

Qualitative Analysis

The ethanol soluble and water soluble extractive values were found to be 13.13% and15.33% w/w respectively. The leaves yielded successive extractive values of 1.13%, 2.53%, 12.43% and 13.38% w/w with petroleum ether (60-80°C), chloroform, ethanol and water respectively. The total ash value of the crude drug was found to be 8.28% w/w while water soluble ash and acid- insoluble ash values were determined as 2.14% and 1.37% w/w, respectively. The preliminary phyto-chemical examination showed the presence of Steroids, Saponins, Flavonoids, Tannins & phenolics compounds. The fluorescence behaviour of the powder of leaves, moistened with solvents and chemical reagents; under UV (long and short) and normal day light is given in Table 1.

Table 1: Fluorescence analysis of Leaves of *J. auriculatum*

Treatment of Powder with	Observation under		
	Visible light	UV light	
		254 nm	356 nm
As such	Green	Black	Green
1N HCl	Light brown	Black	Dark green
1N H_2SO_4	Light brown	Greenish black	Dark green
1N HNO_3	Brown	Greenish black	Greenish black
5% $FeCl_3$(Alc.)	Black	Greenish dark blue	Dark blue
5% $FeCl_3$(Aq.)	Greenish black	Bluish black	Greenish black
1N NaOH(Alc.)	Blackish brown	Black	Greenish black
1N NaOH(Aq.)	Yellowish brown	Greenish black	Yellowish green
1% nitrocellulose in amyl acetate	Dark green	Greenish black	Dark green
1N NaOH(Alc.) +1% nitrocellulose in amyl acetate	Brownish green	Greenish blue	Dark green
1N NaOH(Aq.) +1% nitrocellulose in amyl acetate	Greenish brown	Black	Dark green
1N HCl +1% nitrocellulose in amyl acetate	Light brown	Cherry brown	Light green

Quantitative Studies

Loss on drying content and crude fibre content was determined to be 6.20% and 6.27% w/w respectively. The drug was devoid of volatile oil content and foaming index was found to be less than 100. The swelling index of crude drug was also found to be nil. Aflatoxin content and microbial contamination of leaves powder were confirmed to be within limits as shown in Table 2. Heavy metal analysis revealed that each element was present within specified limits as per Ayurvedic Pharmacopoeia of India as shown in Table 3. The pH values of 1% and 10% w/w drug solutions were found to be 7.2 and 6.5, respectively.

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Table 2: Aflatoxin and Microbial Contamination Test

Parameter	Value	Specified limit
Total bacterial count	56×10^3 c.f.u/g	1×10^5 c.f.u./g
Total yeast/mould count	Nil	1×10^3 c.f.u/g
E. coli	Nil	Nil
Salmonella sp.	Nil	Nil
S. aureus	Nil	Nil
P. aeruginosa	Nil	Nil
Aflatoxin B_1	Absent	0.5 ppm
Aflatoxin B_2	Absent	0.1 ppm
Aflatoxin G_1	Absent	0.5 ppm
Aflatoxin G_2	Absent	0.1 ppm

Table 3: Heavy Metal Content

Heavy metal	Result (ppm)	Specified limit (ppm)
Arsenic	Nil	3.00
Cadmium	0.67	0.30
Lead	Nil	10.0
Mercury	Nil	1.00

DISCUSSION

Albeit the accessibility of contemporary analytical techniques, identification and evaluation of plant drugs by pharmacognostical and physico-chemical parameter study is still more reliable, accurate and inexpensive. According to W.H.O. the macroscopic and microscopic determination of the plant is the primary stride in the direction of establishing its identity and purity and should be conceded out before any tests are under taken [16].

In the present work the macroscopic and microscopic study of *J. auriculatum* leaves was carried out. The results of macroscopic study might be valuable for distinguishing it from its substitutes and adulterants.

Microscopic evaluation allows more meticulous examination of crude drug and enables to identify the organized structural features such as epidermis, trichomes, parenchymatous cells.

The physico-chemical parameters are helpful in judging the purity and quality of the drugs. The foreign matter was present in negligible amount in leaves. This may be due to first hand collection of plant material from non polluted area [17].

Loss on drying for *J. auriculatum* leaves was nearly 6%. It signifies the considerable amount of moisture in leaves. The percentage of active chemical constituents in the crude drugs is usually mentioned on air-dried basis. Hence, the moisture content of a drug should be determined and controlled to make the solution of definite strength. The moisture content of a drug should be minimized in order to prevent decomposition of crude drug either due to chemical change(s) or due to microbial contamination.

The extractive values in different solvents give an idea about the chemical nature of the active constituents present in the drugs. The result suggests that the drug has high water soluble extractive value compared to ethanol soluble extractive value. The water soluble extractives indicate the presence of water soluble constituents such as alkaloids, amino acids, carbohydrates, mucilage and flavonoids. These organic ligands possess promising biological activities and can be utilized to develop prospective therapeutic agents [18].

Ash values were used to detect the presence of any siliceous contamination and water soluble salts. These values are important quantitative standards as it is useful in determining authenticity and purity of drugs [19]. Lower value of total ash in the result signifies low level of carbonates, phosphates, silicates and silica. The total ash value for a crude drug is not always reliable, since there is possibility of presence of non-physiological substances. So, authentication of acid insoluble ash was also performed which showed low content of acid insoluble ash in leaves.

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The result of fluorescence analysis of leaf powder showed their characteristic fluorescent color in different organic and inorganic solvents. The fluorescence behaviour of powdered drugs plays a vital role in the determination of quality and purity of the drug material. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range of daylight. The ultra violet light produces fluorescence in many natural product (e.g. alkaloids like berberine), which is not visible in day light. If the substances themselves are not fluorescent, these may often be converted into fluorescent derivatives or decomposition products by treating with different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [20].

The outcome of preliminary phytochemical analysis showed the presence of various phytochemical compounds in the leaves which are known to have various salutary value in medical sciences. For instance saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have wound healing and anti-inflammatory effects. Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities [21-22]. Saponins possess hypocholesterolemic and antidiabetic properties [23]. The terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids and terpenoids showed the analgesic properties [24]. The steroids and saponins are responsible for central nervous activities [25].

Medicinal plants are usually contaminated with microbes. This blemish can arise during cultivation, harvesting, processing and storage. Hence the total microbial load represents the care taken during these procedures.

Microbes are mainly represented by bacteria and fungi. The materials of vegetable origin usually have higher level of microbial contamination compare to synthetic products. The fungi can produce aflatoxin; some of which can be potentially dangerous. Even the bacteria can lead to exo or endo toxins. The presence of microorganism coupled with moisture can lead to enhanced enzymatic activity hence transforming some of the active constituents to other metabolites which may be less or non potent. All this necessitate the control of microbial contamination with in prescribed limits of pharmacopeia [26].

Heavy metals like lead, cadmimum, mercury and arsenic are natural constituents of environment (soil, water and air). The medicinal plants accumulate these metals from their environment. The heavy metals are health perilous for humans and animals. Hence their content in medicinal plant is controlled with in prescribed limits of pharmacopeia [27].

So the leaves under study can be utilized as a potential source of useful therapeutics and the data arrived at will be beneficial for quantitative and qualitative standardization of herbal preparation containing *J.auriculatum* leaves. Further studies are in progress on these leaves in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with exploration of their pharmacological activity.

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