Journal of Applied Pharmaceutical Science Vol. 4 (12), pp. 123-127, December, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.41221
ISSN 2231-3354

Short Communication

Preliminary phytochemical and physicochemical evaluation of *Carallia brachiata* (Lour.) Merr. leaves

Julfikar Ali Junejo, Kamaruz Zaman*, Mithun Rudrapal, Prodyut Mondal, Khumantham Deepak Singh, Vinod Kumar Verma

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India.

ARTICLE INFO

Article history:
Received on: 06/11/2014
Revised on: 19/11/2014
Accepted on: 05/12/2014
Available online: 29/12/2014

***Key words**: Carallia brachiata*, Physicochemical evaluation, Standardization, Herbal drugs.

ABSTRACT

In the present study, the preliminary phytochemical and physicochemical studies including fluorescence and thin layer chromatographic analyses of the leaves of *Carallia brachiata* (Lour.) Merr. (Rhizophoraceae), an Indian medicinal plant used traditionally in the treatment of a variety of human disorders, were carried out with an objective to standardize the plant/plant part (leaves) to be used either as crude drug or in the form of herbal medicine, considering the quality and safety requirements of herbal drugs as per pharmacopoeial standards and/or WHO guidelines.

INTRODUCTION

Medicinal plants have been used in traditional healing practices for treating various human ailments since time immemorial. Such traditional practices have provided the basis of scientific investigation on medicinal plants which led to the discovery of many potential drug molecules of today's modern medicine. Herbal medicine has therefore become the most reliable form of alternative medicine for treating human disorders around the world. In recent years, there has been a dramatic rise in use of herbal drugs/preparations in the developed countries because of their easy availability and cost effectiveness besides having desired pharmacological effectiveness with high level of safety/low toxicity profile. It is estimated that world's one-fourth population i.e. 1.42 billion people are dependent on traditional herbal medicines for the treatment of various ailments (Kadam *et al*., 2012). However, the lack of documentation and stringent quality control procedures has hindered the easy acceptance of such plant drugs (crude preparations) to be used as herbal medicine. According to the WHO, to ensure reproducible quality of herbal plants (or preparations), physicochemical and phytochemical characterizations are required to be carried out for establishing their identity, purity, and quality standards (Annan *et al*., 2013). Therefore, there is a need for documentation of standardization studies for profiling the quality control parameters of plant-derived crude/herbal drugs. *Carallia brachiata* Merrill (Rhizophoraceae) commonly known as *Karalla* (*Daini jam* in Assamese), is widely distributed throughout India up to an altitude of 1300 meter (Krishnaveni *et al*., 2009b). It is also found in Sri Lanka, Southern China, Thailand and Northern Australia. It occurs as large evergreen trees that reach upto 50 meter tall and 1 meter in circumference. It is often planted as ornamental tree. The fruits and seeds of this plant are edible. The seed oil is also used as a substitute for ghee in several parts of India. The leaves are decussate, simple, elliptical to obovate in shape. The margin is entirely to dentate or serrate and often with black dots beneath. The stipules are interpetiolar and and lance-shape (www.flowersofindia.net, www.biotik.org). In traditional medicine, various parts of *C. brachiata* are used for treating various human disorders.

* *Corresponding Author*
E-mail: z_kamar2003@yahoo.com

A decoction of leaves mixed together with benzion, turmeric and rice dust is used in treatment of sapraemia (Ling *et al*., 2004). The traditional uses of the bark in the treatment of itching, cuts and wounds, oral ulcers, inflammation of the throat and stomatitis are well documented (Nadkarni *et al*., 1995). Some scientific studies have also been performed on this plant. The ethyl acetate and methanol extracts of bark have been reported to possess anti-inflammatory (Krishnaveni *et al*., 2009a), wound healing (Krishnaveni *et al*., 2009b), and antimicrobial activities (Neeharika *et al*., 2010). It has been reported that proanthocyanidins named carallidin, mahuanin and *para*-hydroxy benzoic acid are some compounds isolated from the bark of *C. brachiata*. Proanthocyanidins has been reported to possess free radical scavenging activity (Phuwapraisirisan *et al*., 2006). Other phytoconstituents such as megastigmane diglycoside (3-hydroxy-5,6-epoxy-β-ionol-3-O-βapiofuranosyl (1→6)-β-glucopyranoside), alkaloids (hygroline), tannins, flavonoids and glyceroglycolipids have been reported from the leaves of the plant (Nadkarni *et al*., 1995; Fitzgerald *et al*., 1995). As there is no standardisation work reported so far on the leaves of *C. brachiata*, in our present investigation phytochemical and physicochemical evaluations including fluorescence and thin layer chromatographic analyses were carried out with an objective to standardize the *C. brachiata* leaves.

Fig. 1: Leaves of *Carallia brachiata.*

MATERIALS AND METHODS

Collection and extraction of plant material

Fresh leaves of *Carallia brachiata* (Lour.) Merr. were collected in the month of November 2013 from the Dibrugarh forest, Dibrugarh district, Assam, India. The plant species was identified and authenticated by Botanical Survey of India, Eastern Regional Centre, Shillong, India, and a voucher specimen (BSI/ERC/2014/Plant identification/360) was deposited. The collected plant leaves were shade dried, coarsely powdered (Sieve no. 40), and stored in a well closed airtight container. The fresh leaves were used for organoleptic evaluation and the dried powdered sample of leaves was employed for physicochemical characterization. The powdered leaves were subjected to successive solvent extraction as follows: 50 gm of the powdered leaves was extracted successively by Soxhlation with different solvents such as petroleum ether (60- 80^{o}C), chloroform, ethyl acetate, methanol and water. Each time before extracting with the next solvent of higher polarity the extracted leaves (marc) was dried in a hot air oven below 50^{0}C for 10 minutes. The extracts were concentrated by distilling off the solvent, and were subsequently evaporated to dryness and the dry residues were then used for physicochemical analysis. The percentage yield (w/w) of each extract was calculated in terms of the weight of initial air dried plant material.

Reagents and Chemicals

All chemicals used in the study were procured from Rankem, Mumbai and Himedia Labs., Mumbai. All other chemicals and reagents used were of analytical grade.

Organoleptic evaluation

The fresh leaves of *C. brachiata* were visually examined and different organoleptic features such as colour, odour and taste were characterized.

Physicochemical analysis

Powder samples were subjected to determination of various physicochemical parameters such as moisture content (%LOD), total ash, acid insoluble ash, and water soluble ash according to the methods specified in the Indian Pharmacopoeia. Water and alcohol soluble extractive values were determined by cold maceration method as per WHO guidelines. Each study was performed in triplicate; mean values with standard error of mean (SEM) were calculated.

Phytochemical screening

Preliminary phytochemical screening of the extracts were carried out for detection of the presence of different phytoconstitutents such as alkaloids, glycosides, flavonoids, phenolic compounds, saponins, tannins etc. present in *C. brachiata* leaves. The qualitative chemical tests were performed according to the standard procedures (Khandelwal *et al*., 2005; Kokate, 1994).

Fluorescence analysis

The fluorescence characteristics of the leaf powders (40 mesh) was examined both in daylight and UV light (254 nm and 365 nm), and after treatment with different reagents like ferric chloride, glacial acetic acid, hydrochloric acid, iodine, nitric acid and sodium hydroxide etc. (Gupta *et al*., 2006; Kokashi *et al*., 1995).

Thin layer chromatographic (TLC) analysis

The chemical fingerprint of the extracts was determined by thin layer chromatography using aluminium precoated silica gel 60 F254 (0.25 mm thick) HPTLC plates (Merck, Germany). The plates were developed using hexane:chloroform (3:1) and toluene:ethyl acetate:formic acid (4:5:1) as mobile phase. One dimensional ascending method was used for the development of plates as per standard protocol (Indian Pharmacopoeia 1996). The TLC plate was air-dried and spots were visualized under ultraviolet light (254 & 365 nm). The R_f values of the spots were also recorded.

RESULTS AND DISCUSSION

Organoleptic evaluation

The organoleptic study reveals that fresh leaves are deep green in colour, with characteristics odour and nasty taste. They are large and palmate shaped.

Organoleptic evaluation is based on the study of morphological and sensory profiles of whole drugs (Kokate *et al*., 2007). It is therefore considered as a primary screen in the qualitative assessment of crude drugs. The parameters such as the structure of leaves, the hairy surface of leaves, the typical tongue sensation and the odour are some important diagnostic as well as qualitative organoleptic indicators of leaf drugs. For example, the characteristic aroma of leaves (or any other plant parts) is a true indicator of the presence of volatile active principles.

Physicochemical analysis

The results of the physicochemical determinations are presented in table 1. The moisture content (%LOD) of the powdered leaf drug was found to be 25.26±00.49 (%w/w) which indicates that the drug was properly dried and stored. The determination of moisture content is important for the plant drugs because insufficient drying may lead to possible enzymatic deterioration of active principles (Kokate *et al*., 2007). This parameter is therefore essentially used to control the quality of crude drugs and/or herbal drugs/drug products. The purity of crude drugs could also be evaluated by the determination of ash values which represent the content of foreign matter such as inorganic salts or silica present as a form of adulterant in the drug sample. An analytical result for total ash was found to be 17.83±0.20 (%w/w). The total ash includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. The amount of acid insoluble and water soluble ash were found to be 8.26±0.23 and 7.3±0.28 (%w/w), respectively. Acid insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash (Kokate *et al*., 2007; Evans, 2002). From results, it is clear that the amount of water soluble ash is less than that of acid insoluble ash, whereas the amount of total ash was almost double the quantity of water soluble ash. The ash content gives an idea about the inorganic content of powdered leaves under investigation and thus the quality of the drugs can be assessed. On the other hand, the water soluble extractive value of the drug was found to be 11.63 ±0.60 (%w/w) which indicates the presence of water soluble components such as sugar, acids and inorganic compounds etc.; and the alcohol soluble extractive value was found to be 3.96 ±0.12 (%w/w) which indicates the presence of polar constituents like phenols alkaloids steroids glycosides flavonoids. The results of physicochemical analyses lie within the acceptable limit which in turn ascertains the quality as well as purity of leaf drugs. These parameters are therefore some useful quality standards included in the standardization of *C. brachiata* leaf drugs.

Table 1: Physicochemical parameters.

Parameter	% w/w±SEM*
Moisture content (%LOD)	25.26 ±0.49
Ash values	
Total ash	17.83±0.20
Acid insoluble	08.26±0.23
Water soluble	07.3±0.28
Extractive values	
Water soluble	11.63±0.60
Alcohol soluble	03.96±0.12

*Values are expressed as mean ± SEM of three replicates

Phytochemical screening

The results of the phytochemical screening are depicted in the table 2. The results reveal the presence of alkaloids, glycosides, polyphenols, tannins, flavonoids, saponins, carbohydrates, saponins, proteins, lipids, gums and lignins in the leaves of *Carallia brachiata*. The ethyl acetate, methanol and aqueous extracts contain alkaloids and glycosides, and carbohydrates and phenolic compounds are present in all the solvent extracts except petroleum ether extract. The saponins are present in methanol and aqueous extracts only. All the extracts contain flavonoids except petroleum extract. The percentage yields of the extracts were also recorded and are given in the table 3.

Table 2: Phytochemical screening of various solvent extracts of *Carallia brachiata* leaves

Constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Alkaloids	- -	- -	+ +	++	++
Glycosides	- -		+ +	+ +	++
Phenolic compounds and tannins	- -	+ +	+ +	++	++
Flavonoids	- -	+ +	+ +	++	++
Carbohydrates	- -	+ +	+ +	+ +	++
Proteins	- -		++	++	--
Fats and oils	++	- -	- -	- -	--
Saponins	- -	- -	- -	++	++
Gum	- -	- -	- -	- -	++
Lignins	- -	- -	- -	- -	- -

'+ +' indicates presence '- -' indicates absence

Table 3: Extractive values of solvent extracts

Extract	Extractive values (% w/w)	Color of extract
Pet. Ether extract	1.68	Black
Chloroform extract	2.48	Light black
Ethyl acetate extract	2.6	Green
Methanol extract	3.8	Dark brown
Aqueous extract	4.2	brown

Fluorescence analysis

The fluorescence properties of powder drug sample of *Carallia brachiata* obtained after treatment with different reagents are exhibited in the table 4. The crude drugs are often qualitatively assessed by their fluorescent characteristics which indicate the presence of certain chemical constituents under experimental conditions. It is therefore an important qualitative parameter for the identification of marker components, and hence considered as a useful analytical tool for the standardization of crude drugs.

Some plant constituents show fluorescence in the visible range of day light. Under ultra violet light many natural products (e.g., alkaloids like berberine) produces fluorescence. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by the application of different reagents (Gupta *et al.*, 2006; Ansari *et al*., 2006)]. However, in this study as the powdered sample of *Carallia brachiata* leaves possess fluorescent properties which may therefore be used to standardize the crude drugs in terms of its fluorescent marker profile.

Table 4: Fluorescence analysis of powdered leaves of *Carallia brachiata*

Powdered drug/Treatment	Visible/Day light	Short UV (254 nm)	Long UV (365 nm)
Powder drug	Light yellow	Brown	Yellowish brown
Powder + Methanol	Light yellow	Yellowish brown	Brownish black
Powder + 1% Glacial acetic acid	Brown	Dark brown	Blackish brown
Powder +10% NaOH	Yellowish brown	Dark yellowish brown	Bluish brown
Powder + Dil. NH_3	brown	Light brown	Brown
Powder + Conc. HNO_3	Brown	Blackish brown	Dark brown
Powder + Dil. NH_3 + Conc. HNO_3	Yellowish brown	Light brown	Blackish brown
Powder +1M H_2SO_4	Light brown	brown	Yellowish brown
Powder +1M HCl	Light yellow	Light Brown	Dark brown
Powder + 10% $FeCl_3$	Reddish brown	Light brown	Brownish yellow
Powder +Acetone + Methanol	Light brown	Brown	Black
Powder +10% Iodine	Brown	Dark brown	Blackish brown

Thin layer chromatographic analysis

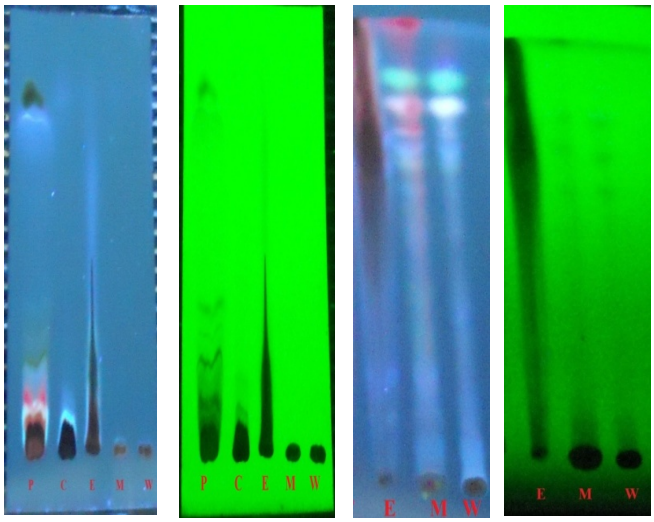
Table 5 reveal the TLC fingerprint profile of different extracts which depicts the number of spots obtained with their relative R_f values. TLC chromatograms depicted in figure 2 show certain distinct spots with their relative intensities. The colors of the spots were recorded as yellow, yellowish green, redish and violet.

Table 5: Thin layer chromatographic analysis

Extract	No of spots (Plate A)	R_f values (Plate A)	No of spots (Plate X)	R_f values (Plate X)
Pet ether extract	6	0 .07, 0.15, 0.23 0.31, 0.79, 0.85	- -	
Chloroform extract	2	0 .07, 0.15	- -	
Ethyl acetate	2	0.23, 0.78	3	0.06, 0.18, 0.70
Ethanol	- -		8	0.06, 0.18,0.62, 0.67, 0.70, 0.78 0.82, 0.85
Water	- -		8	0.06, 0.18,0.62, 0.67, 0.70, 0.78, 0.82, 0.85

'- -' indicates absence

The chromatograms (TLC) showing characteristic spots with their relative R_f values can be used for the identification of marker components present in the leaves of *C. brachiata*. Eight spots having respective R_f values are found in the TLC plate X (methanolic & water extract) which may indicate the presence of various phytoconstituents like alkaloids, flavonoids, saponins, and tannins. Therefore, with the help of TLC fingerprinting the plant *C. brachiata* can be identified as well as standardized. Analytical TLC is sometimes also useful for ascertaining the purity of crude drugs by detecting the presence or absence of adulterants and substitutents (Ravichandra *et al*., 2011).



A B X Y

Fig. 2: TLC Finger print profile of *Carallia brachiata* under 254 nm (A & X) and 366 nm (B & Y)
(P is Pet. Ether extract, **C** is Chloroform extract, **E** is Ethyl extract, **M** is Methanol Extract, **W** is Water extract)
TLC Plate **A** was Developed with Hexane: Chloroform (3:1) and **X** was Developed with Toluene :Ethyl Acetate : Formic Acid (4:5:1)

CONCLUSION

Standardization is an essential analytical aspect for the study of identity, purity and quality of crude drug sample of plant origin. Chemical and physiochemical analyses reveal useful information which is of utmost importance for the quality control of *C. brachiata* leaves to be used as crude drugs. The documentation of standardized parameters therefore are an indispensable element in the development of herbal drugs from raw plant drugs (crude preparations), considering their desired therapeutic and safety profile.

ACKNOWLEDGEMENT

Authors are thankful to the University Grants Commission (UGC), New Delhi for providing Junior Research Fellowship (MANF Fellowship) to first author. The authors also sincerely thank Prof. Dipak Chetia, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh for his needful help in carrying out the research work.

REFERENCES

Ansari SH. 2006. Essentials of Pharmacognosy. New Delhi, India: Birla Publications Pvt. Ltd.

Annan K, Dickson RA, Amponsah IK, Jato J, Nooni IK. Pharmacognostic evaluation and physicochemical analysis of *Paullinia pinnata* L. (Sapindaceae). *J Pharmacogn Phytochem*, 2013; 2(2): 203-208 2013.

Available at: http://www.flowersofindia.net/catalog/slides/Freshwater%20Mangrove.html [08 October 2013].

Carallia brachiata (Lour.) Merrill RHIZOPHORACEAE. Available at: http://www.biotik.org/india/species/c/carabrac/carabrac_en.html [Accessed 12 December 2013].

Evans WC. 2002. Trease and Evans Pharmacognosy. Edinburgh, USA: W. B. Saunders Publication.

Fitzgerald JS. (+)-Hygroline, the major alkaloid of Carallia brachiata (Rhizophoraceae*). Aust J Chem*, 1965; 18:589-90.

Gupta MK, Sharma PK, Ansari SH, Lagarkha R. Pharmacognostical evaluation of *Grewia asiatica* fruits. *Int J Plant Sci*, 2006; 1 (2):249-251.

Kadam PV, Deoda RS, Shivatare RS, Yadav KN, Patil MJ. Pharmacognostic, phytochemical and physiochemical studies of *Mimusops Elengi* Linn stem bark (Sapotaceae). *Der Pharm Lettr*, 2012; 4 (2):607-613.

Krishnaveni B, Neeharika V, Srikanth AV, Reddy MB. Antiinflammatory activity of *Carallia brachiata* bark. *Int J Pharm Sci Nanotech*, 2009; 1: 375-78.

Krishnaveni B., Neeharika V, Venkatesh S, Padmavathy R, Reddy MB. Wound healing activity of *Carallia brachiata* bark. *Indian J Pharm Sci*, 2009; 71:576-78.

Khandelwal KR. 2005. Practical Pharmacognosy. Techniques and Experiments. Pune, India: Nirali Prakashan.

Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J Am Pharm Assoc*, 1958; 47:715-717.

Kokate CK. 1994. Practical Pharmacognosy. New Delhi, India: Vallabh Prakashan.

Kokate CK, Purohit AP, Gokhale SB. 2007. Pharmacognosy. Pune, India: Nirali Prakashan.

Ling SK, Takashima T, Tanaka T, Fujioka T, Mihashi K, Kouno I. New diglycoside megastigmane from *Carallia brachiata*. *Fitoterapia*, 2004; 75:785-6.

Nadkarni KM, Nadkarni AK. 1995. Indian Materia Medica (Vol. 1). Mumbai, India: Popular Prakashan Pvt. Ltd.

Neeharika V, Krishnaveni B, Swetha T, Lakshmi PK, Reddy MB. Antimicrobial activity of *Carallia brachiata*. *Pharma Science Monitor*, 2010; 1 (2):1-5.

Phuwapraisirisan P, Sowanthip P, Miles DH, Tip-pyang S. Reactive radical scavenging and xanthine oxidase inhibition of proanthocyanidins from *Carallia brachiata*. *Phytother Res*, 2006; 20:458-61.

Ravichandra VD, Padmaa MP. Pharmacognostic and phytochemical investigation on leaves of Ficus hispida. *Int J Pharm Pharm Sci*, 2011; 131-134.

How to cite this article:

Julfikar Ali Junejo, Kamaruz Zaman, Mithun Rudrapal, Prodyut Mondal, Khumantham Deepak Singh, Vinod Kumar Verma. Preliminary phytochemical and physicochemical evaluation of *Carallia brachiata* (Lour.) Merr. leaves. J App Pharm Sci, 2014; 4 (12): 123-127.