ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 1, 2016

ISSN - 0974-2441

Research Article

PHYTOCHEMICAL, PHYSICOCHEMICAL, AND FLUORESCENCE ANALYSIS OF LEAF EXTRACT OF *SYZYGIUM CALOPHYLLIFOLIUM* WALP.

SUMITHRA NAGULAN*, SEVANAN RAJESH KUMAR

Department of Botany, Government Arts College, Udhagamandalam, Affiliated to Bharathiar University, Coimbatore, Tamil Nadu, India.
Email: sumithranagulan@gmail.com

Received: 12 September 2015, Revised and Accepted: 10 October 2015

ABSTRACT

Objective: The aim of the study is to investigate the phytochemical, physicochemical, and fluorescence analysis of *Syzygium calophyllifolium* Walp. leaf extract.

Methods: The plant powder was extracted with different solvents such as petroleum ether, benzene, ethyl acetate, methanol, ethanol, and water. The different extracts were tested qualitatively for the identification of various phytochemical constituents. The plant powder was subjected to fluorescence analysis in daylight and in ultraviolet-light (254 nm and 365 nm).

Results: Water soluble extractive value was found to be higher than ethanol, methanol, acetone, petroleum ether, benzene, and chloroform. The total ash value was observed 10.84±0.13. The result showed the presence of secondary metabolites such as alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside, xanthoprotein, and fixed oil.

Conclusion: It can be concluded from the present study the leaves of *S. calophyllifolium* contain various phytochemical constituents which may be used as phyto medicines.

Keywords*: Syzygium calophyllifolium*, *Eugenia calophyllifolia*, Myrtaceae, Phytochemical screening, Physicochemical constant, Flourescence analysis.

INTRODUCTION

Plant-based therapy has been used as a vital component in the traditional medicine systems and the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The therapeutic potential, including the antioxidant, antimicrobial, and anticarcinogenic properties of higher plants, is due to the presence of secondary metabolites [1,2] which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, and phenols. Drug discovery led to the isolation of early drugs from medicinal plants such as cocaine, codeine, digitoxin, quinine, and morphine, of which some are still in use [3-5]. Plant secondary metabolites consist of low-molecular weight compounds that are regarded as not essential for sustaining life, but as crucial for the survival of the producing organism [6].

The World Health Organization supports the use of traditional medicines as it is believed that herbal medicines are naturally superior to synthetic drugs and they are proven to be efficacious and safe [7]. The growing attractiveness of herbal medicines is that many people accept them, as true and innocuous, in contrast to pharmaceutical drugs, and the idea that what is natural can only be good [8]. In spite of the numerous studies reported on Myrtaceae family, the preliminary phytoconstituents of the *Syzygium calophyllifolium* have not been reported so far. The present investigation is the first attempt to analyze the secondary metabolites and the physicochemical constituents of the leaf extract of *S. calophyllifolium* (*Eugenia calophyllifolia* (Walp.)Wt.) commonly called Neerodam (Malayalam) belongs to the family Myrtaceae.

METHODS

Plant material

Healthy and disease free leaves of the plants were collected from Doddabetta hills, The Nilgiri District, Tamil Nadu and was identified as *S. calophyllifolium* Walp. at the Department of Botany, Government Arts College, Udhagamandalam, Tamil Nadu, India. The leaves were shade dried, blended into coarse powder in a blender to a constant weight and stored in an airtight plastic container at room temperature.

Physicochemical constant

Determination of total ash

Three grams of the powdered drug was accurately weighed in a silica crucible, which was previously ignited and weighed. The powdered drug was spread like a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get the constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Determination of water soluble ash

The ash obtained in the determination of total ash was boiled for 5 minutes with 25 mL of water. The insoluble matter was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a pre-weighed silica crucible and ignited for 15 minutes at a temperature not exceeding 450°C. The procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried powder.

Determination of sulfated ash

1 g of sample was weighed in a crucible and ignited was moistened adding 1 mL of sulfuric acid and heated until the white fumes are no longer evolved and ignited at 800±25°C until all black particles have disappeared. Allow the crucible to cool and add a few drops of sulfuric acid and heat. Ignited as before, allowed to cool and weighed. The

operation was repeated until two successive weighing does not differ by more than 0.5 mg.

Determination of acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25 mL of 2 N HCL acid for 5 minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred to pre-weighed silica crucible. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Extractive values

The extractive values were determined as per the standard procedures [9].

Preliminary phytochemical screening

Preparation of extracts

The coarse powder (100 g) was extracted successively with different solvents such as petroleum ether, benzene, ethyl acetate, methanol, ethanol, and water, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper and subjected to qualitative tests for the identification of various phytochemical constituents.

Qualitative phytochemical analysis of different extracts

The chemical tests for various phytoconstituents in the extracts were carried out as per standard procedures [10-12].

Fluorescence analysis

The drug powder was treated with acids such as 1 N HCL and 50% H_2SO_4; and alkaline solutions such as aqueous sodium hydroxide, alcoholic sodium hydroxide; and other solvents such as nitric acid, picric acid, acetic acid, ferric chloride, and nitric acid with ammonia. They were subjected to fluorescence analysis in daylight and in the ultraviolet (UV)-light (254 nm and 365 nm) [13].

RESULTS

In this present investigation, the ash values of the dried powdered leaves of *S. calophyllifolium* were evaluated (Table 1). In this evaluation, it was found that sulfated ash was higher (11.26±0.11) than that of water soluble ash (4.81±0.04) and acid insoluble ash (2.11±0.01). The total ash value of the powder was found to be 10.84±0.13.

The dried leaf powder was extracted using different solvents and the extractive values were tabulated (Table 2). The result showed the water soluble extractive is higher (9.40±0.14) than the other solvents followed by ethanol (8.78±0.05) and methanol (8.54±0.11). The extractive values of acetone (7.75±0.04), petroleum ether (5.54±0.05), benzene (4.16±0.01), and chloroform (3.78±0.03) were recorded.

Table 1: Ash values of *S. calophyllifolium* leaf extract

Serial number	Type of ash	Ash value (%)[a]
1	Total ash value of powder	10.84±0.13
2	Water soluble ash	4.81±0.04
3	Acid insoluble ash	2.11±0.01
4	Sulfated ash	11.26±0.11

[a]All values are mean of triplicate determinations. *S. calophyllifolium: Syzygium calophyllifolium*

Table 2: Extractive values of *S. calophyllifolium* leaf extract

Serial number	Name of extract	Extractive value (%)[a]
1	Petroleum ether	5.54±0.05
2	Benzene	4.16±0.01
3	Chloroform	3.78±0.03
4	Acetone	7.75±0.04
5	Methanol	8.54±0.11
6	Ethanol	8.78±0.05
7	Water	9.40±0.14

[a]All values are mean of triplicate determinations. ±standard error.
S. calophyllifolium: Syzygium calophyllifolium

In the present study, the qualitative phytochemical tests on the leaf extract of *S. calophyllifolium* for secondary metabolites revealed the presence of alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinine, saponin, steroid, tannin, terpenoid, sugar, glycoside, xanthoprotein, and fixed oil (Table 3). The qualitative phytochemical analysis of various solvent extracts of *S. calophyllifolium* for secondary metabolites revealed the presence of alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside, xanthoprotein, and fixed oil. From this analysis, methanol and ethanol extracts of leaves were found to contain more phytochemical constituents compared to petroleum ether, benzene, and ethyl acetate. Alkaloid, coumarin, flavonoid, phenol, steroid, sugar, glycoside, xanthoprotein, and fixed oil were present in all the five extracts. Saponin was found in benzene, ethyl acetate, methanol, and ethanol but absent in petroleum ether. Catechin and quinone were present in ethyl acetate, methanol, and ethanol but absent in petroleum ether and benzene. Anthraquinone and tannin were present in methanol and ethanol but absent in petroleum ether, benzene, and ethyl acetate. Terpenoid was found in petroleum ether, methanol, and ethanol but absent in benzene and ethyl acetate.

The result of fluorescence analysis was summarized (Table 4). The powder from the leaf fluoresced green under daylight and short UV-light (254 nm), dark green under long UV-light (365 nm). The leaf of *S. calophyllifolium* showed the characteristic fluorescent green treated with 50% H_2SO_4, Con. HNO_3, 50% HNO_3, 40% NaOH+10% lead acetate, NH_3, acetone, ethanol under short UV-light (254 nm).

DISCUSSION

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus, ash value is a validity parameter describe and to assess the degree of purity of a drug [14]. Determination of ash value plays a major role in evaluating crude drugs. The presence of adulterants and the method of processing during drying or storage can be evaluated. The water soluble extractive value shows that the constituents of the drug are more extracted and soluble in water. Many biomass ashes are rich in metals, e.g. potassium, phosphorous, calcium, nitrogen, etc., which are important plant nutrient. Thus, ashes can be used as fertilizer to increase the soil fertility. Many such ashes may also contain heavy metal contaminants such as cadmium, which is a pollutant. So, before considering ashes as fertilizers, the metals concentration should be initially checked.

The leaves of *Syzygium cumini* (L.) (Myrtaceae) showed the highest water soluble ash than the acid insoluble ash [15]. In this present investigation, the ash values of the dried powdered leaves of *S. calophyllifolium* were evaluated, and it was found that sulfated ash was higher followed by water soluble ash and acid insoluble ash.

The solvent extracts are giving clear evidence that the active compound is an alcohol soluble component. As it is more soluble in alcohol, the purity of the compound can be ensured and can be easily extracted from the mixture. The shelf life of the drug can be increased as the alcohol soluble components will carry a very less moisture and escape from humidity. The various phytoconstituents can be extracted depending on the polarity and solubility of the solvent which is used for extraction [16]. In this present investigation, it was found that the extractive value of water was higher followed by ethanol and methanol.

The previous study revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides, and tannins as the chemical class present in the leaf extracts of *S. cumini* (L.) (Myrtaceae) [17]. This result is showing that the Myrtaceae family is sharing a very common phytochemistry which may be unique to this family. There are chances for a drastic difference in

Table 3: Preliminary phytochemical screening of *S. calophyllifolium* leaf extract

Serial number	Test	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
1	Alkaloid	+	+	+	+	+
2	Anthraquinone	-	-	-	+	+
3	Catechin	-	-	+	+	+
4	Coumarin	+	+	+	+	+
5	Flavonoid	+	+	+	+	+
6	Phenol	+	+	+	+	+
7	Quinone	-	-	+	+	+
8	Saponin	-	+	+	+	+
9	Steroid	+	+	+	+	+
10	Tannin	-	-	-	+	+
11	Terpenoid	+	-	-	+	+
12	Sugar	+	+	+	+	+
13	Glycoside	+	+	+	+	+
14	Xanthoprotein	+	+	+	+	+
15	Fixed oil	+	+	+	+	+

+: Present, -: Absent, *S. calophyllifolium: Syzygium calophyllifolium*

Table 4: Fluorescence analysis of powdered leaf of *S. calophyllifolium*

Serial number	Experiments	Visible/ daylight	UV-light	
			254 nm	365 nm
1	Powder as such	Green	Green	Dark green
2	Powder+1 N Aqueous NaOH	Yellowish green	Greenish yellow	Dark blue
3	Powder+1 N Alcoholic NaOH	Yellowish green	Dark green	Dark brown
4	Powder+1 N HCl	Green	Greenish yellow	Dark brown
5	Powder+Con. H_2SO_4	Light brown	Light green	Brown
6	Powder+50% H_2SO_4	Brown	Fluorescent green	Dark green
7	Powder+Con. HNO_3	Light green	Fluorescent green	Dark green
8	Powder+Con. HCl	Green	Light green	Dark green
9	Powder+50% HNO_3	Greenish yellow	Fluorescent green	Violet
10	Powder+40% NaOH+10% lead acetate	Pale green	Fluorescent green	Dark green
11	Powder+Acetic acid	Yellowish green	Light green	Dark green
12	Powder+Ferric chloride	Brown	Dark green	Dark blue
13	Powder+HNO_3+NH_3	Pale green	Dark green	Brown
14	Powder+NH_3	Green	Fluorescent green	Bluish green
15	Powder+Benzene	Dark green	Yellowish green	Pink
16	Powder+Petroleum ether	Green	Yellowish green	Dark blue
17	Powder+Acetone	Yellowish green	Fluorescent green	Dark brown
18	Powder+Chloroform	Dark green	Pale yellow	Blue
19	Powder+Methanol	Yellowish green	Yellowish green	Brown
20	Powder+Ethanol	Yellowish green	Fluorescent green	Dark brown

S. calophyllifolium: Syzygium calophyllifolium

phytochemical constituents between the plants belonging to Myrtaceae family collected from different places. Those differences might be due to the change in climatic condition and other soil conditions. So, there is a need to explore this family in intense.

Thus, it is clearly evident that the active compound of this selected plant *S. calophyllifolium* is a secondary metabolite and shows bioactivities (results not included).

CONCLUSION

It is evident that plants having therapeutic values usually contain diverse groups of secondary metabolites and *S. calophyllifolium* proved no exception. In conclusion, the leaves of *S. calophyllifolium* possess the secondary metabolites which are an important source to produce the plant-based medicines.

REFERENCES

1. Canigueral S, Tschopp R, Ambrosetti L, Vignutelli A, Scaglione F, Petrini O. The development of herbal medicinal products: Quality, safety and efficacy as key factors. Pharm Med 2008;22(2):107-18.
2. Kaur R, Arora S. Chemical constituents and biological activities of *Chukrasia tabularis* A. Juss: A review. J Med Plant Res 2009;3(4):196-216.
3. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat Prod Rep 2000;17(3):215-34.
4. Butler MS. The role of natural product chemistry in drug discovery. J Nat Prod 2004;67(12):2141-53.
5. Samuelsson G. Drugs of Natural Origin: A Textbook of Pharmacognosy. 5th. Stockholm: Swedish Pharmaceutical Press; 2004.
6. Hadacek F. Secondary metabolites as plant traits: Current assessment and future perspectives. Crit Rev Plant Sci 2002;21:273-322.
7. World Health Organization (WHO). ???. Chronicle 1985;39:51.
8. Abugassa IO, Bashir AT, Doubali K, Etwir RH, Abu-Enawel M, Abugassa SO. Characterization of trace elements in medicinal herbs by Instrumental neutron activation analysis. J Radioanal Nucl Chem 2008;278(3):559-63.
9. Anonymous. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. Vol. I & II. Delhi: The Controller of Publications, Civil Lines; 1996.
10. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies on merugan kizhangu. Bull Med Ethnobot Res 1981;3:84-96.
11. Anonymous. Phytochemical Investigation of Certain Medicinal Plants Used in Ayurveda. New Delhi: Central Council for Research in Ayurveda and Siddha; 1990.
12. Lala PK. Lab Manuals of Pharmacognosy. Kolkata: CSI Publishers and Distributers; 1993.
13. Chase CR Jr, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc Am Pharm Assoc 1949;38(6):324-31.

14. Kokate CK, Purohit AP, Gokhale SB. Analytical pharmacognosy. In: Pharmacognosy. 42nd ed. Pune: Nirali Prakashan; 2008. p. 6.19-20.
15. Soni H, Nayak G, Patel SS, Mishra K, Singhai AK. Pharmacognostic Studies of the leaves of *Syzygium cumini* Linn. Int J Res Pharm Biomed Sci 2011;2(2):507-9.
16. Venkatesh KV, Girish KK, Pradeepa K, Kumar SR. Antibacterial activity of ethanol extract of *Musa paradisiaca* CV. Puttable and *Musa acuminate* CV. Grand naine. Asian J Pharm Clin Res 2013;6(2):169-72.
17. Gowri SS, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J PharmTech Res 2010;2(2):1569-73.