WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 16, 1585-1595. Research Article ISSN 2277– 7105

PHYTOCHEMICAL SCREENING AND ASSESSMENT OF BIOACTIVE COMPOUNDS ON ERYTHROXYLUM MONOGYNUM ROXB. (RED CEDAR)

Dr. A. Logamadevi[1]*, C. Menaka[2], B. Mahalakshmi[3] and T. Poongodi[4]

Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi-642 001, Tamilnadu, India.

Article Received on
13 October 2022,
Revised on 02 Nov. 2022,
Accepted on 23 Nov. 2022
DOI: 10.20959/wjpr202216-26313

***Corresponding Author**
Dr. A. Logamadevi
Department of Botany,
Nallamuthu Gounder
Mahalingam College,
Pollachi-642 001,
Tamilnadu, India.

ABSTRCT

Erythroxylum monogynum is a member of the Erythroxylaceae family. It is commonly found in the southern parts of India. The present study investigated the qualitative and quantitative analysis of the bioactive compounds of *E. monogynum* wood. They were using two distinctive solvents, such as acetone and methanol. The phytochemical screening of the crude extracts of the wood revealed the presence of alkaloids, flavonoids, tannins, glycosides, steroids, reducing sugars, phenols, emodins, phlobatannins, gums, mucilages, and resins. Terpenoids, saponins, proteins, and amino acids are absent in both extracts. As a consequence of qualitative analysis of some phytochemicals, notably alkaloids, flavonoids, phenols were subjected to quantitative analysis. It is the first report on *E. monogynum* wood extracts analysis of qualitative and quantitative phytochemicals. The present study may be useful for the quality and purity of the plant material in future studies.

KEYWORDS: *Erythroxylum monogynum*, extraction, qualitative, quantitative analysis.

INTRODUCTION

Phytochemicals are biologically active and naturally occur in plants' parts. Which provide health benefits for humans beyond those attributed to macronutrients and micronutrients.[1] The qualitative and quantitative determination of bioactive compounds differs from plant to plant and part to part.

Generally, phytochemical constituents are classified into two categories based on their roles in basic metabolic processes, namely primary and secondary metabolites. The primary metabolites are involved in basic life functions. They consist of proteins, carbohydrates, amino acids, hormones, and vitamins. The secondary metabolites are alkaloids, flavonoids, sterols, terpenoids, saponins, tannins, etc.[2] It is crucial in both modern medicine and folk applications for treating a variety of ailments.

Medicinal plants are the richest bio-resources of drugs in traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs.[3] Today, a large sector of the population is relying on medicinal plants for their preventive and curative properties. People from rural areas and semi-urban regions of the world still depend on either raw or refined products from the plant's origin.[4]

E. monogynum belongs to the family Erythroxylaceae, commonly known as Red Cedar. The wood have odor resembling to sandalwood and hence, the called bastard sandal.[5] It is commonly found in the southern parts of India. In India, it is mostly found in Tamil Nadu, Kerala, Andhra Pradesh and Karnataka.[6] It is one of the traditional medicinal plants in the South Asia and each portion of the tree has certain medicinal properties. Since prehistoric times, the plant parts are claimed to have several medicinal benefits. The present study deals with the analysis of phytochemical compounds in wood extracts of *E. monogynum* through qualitative and quantitative analysis.

MATERIALS AND METHODS

Collection of plant material

The plant material was collected and washed thoroughly in tap water. Then the plant parts were shade dried at room temperature for 7-10 days. After drying the plant materials were pulverized into fine powder. The powdered materials were used for the study.

Extraction of plant materials

About 10 g of powder were uniformly packed into a thimble and extracted with 180 ml of different solvents separately. Solvents used methanol and acetone. After the extraction the extracts were taken in Petridis and kept for evaporation. Dried crude extracts stored in the refrigerator. The crude extract was used for qualitative and quantitative phytochemical analysis using standard methods.

Qualitative phytochemical analysis

Detection of alkaloids

Mayer's test

A few ml of extract treated with two drops of Mayer's reagent were added along the test tube sides. The formation of creamy yellow creamy precipitate indicates the presence of alkaloids.[7]

Detection of flavonoids

Ferric chloride test

A little extract was dissolved in distilled water. 2 ml of 5% ferric chloride solution was added to this mixture. Formation of blue, green or violet color indicates the presence of flavonoid compounds.[8]

Alkaline reagent test

The extract was treated with a few drops of sodium hydroxide. The presence of flavonoids is indicated by the formation of an intense yellow color that fades when a few drops of diluted sulphuric acid are added.[8]

Detection of tannins

Ferric chloride test

A little extract was dissolved in distilled water. 2 ml of 5% ferric chloride solution was added to this mixture. Formation of blue, green or violet color indicates the presence of tannin compounds.[9]

Detection of Glycosides

Liebermann's test

The extract was mixed with 2 ml of chloroform and 2 ml of acetic acid. Then the concentrated H2SO4 was added. Formation of green color indicates presence of glycosides.[9]

Salkowski's test

The extract was mixed with 2 ml of chloroform and 2 ml of concentrated H2SO4 was added and shaken gently. Appearance of reddish brown color indicates the presence of glycosides.[9]

Detection of Steroids

Acetic anhydride test

2 ml of acetic anhydride was added to 0.5 ml crude plant extract with 2 ml H_2S0_4. The change in coloration from violet to blue or green in extracts indicates the presence of steroids.[8]

Salkowski's test

The 2 ml of extract was mixed with 2 ml of chloroform and concentrated H2SO4 was added along the test tube. Appearance of red in the lower layer of chloroform indicated the presence of steroids.[9]

Detection of terpenoids

To 1ml of the extract add 2 ml of chloroform and then add 3 ml conc. Sulphuric acid. Formation grayish color indicates the presence of terpenoids.[10]

Detection of Saponins

Foam test

Extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Formation of stable foam was taken as an indication for the presence of saponins.[9]

Detection of Proteins and Amino acids

Ninhydrin test

Two drops of ninhydrin solution were added to 2 ml of extract filtrate and heated. The formation of violet color indicates the presence of proteins and amino acids.[9]

Detection of reducing sugars

Benedict's test

To 0.5 ml of extract, 0.5 ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.[9]

Fehling's test

1 ml of extract treated with 1ml of each of fehling solution A and B was added. The mixture was kept in a water bath. Appearance of red precipitate indicates the presence of reducing sugars.[9]

Detection of phenols

Folin ciocalteau's test

To 1 ml of the extract, 2ml of distilled water followed by 0.5 ml of Na_2Co_3 and folin ciocalteau's reagent (0.5ml) added to the extract. Appearance of blue/green color indicates the presence of phenols.[11]

Ferric chloride test

A little extract was dissolved in distilled water. To this, 2 ml of 5% ferric chloride solution was added. The formation of blue, green or violet color indicates the presence of phenolic compounds.[9]

Detection of emodins

The extract was treated with 2 ml of ammonia solution followed by 3 ml of benzene. The formation of a red color indicated the presence of emodins.[10]

Detection of phlobatannins

The extract was mixed with 2 ml of Hydrochloric acid. A boiled water bath was used to keep the mixture warm. A formation of a red precipitate indicates the presence of phlobatannins.[10]

Detection of Gums and mucilages

Alcohol test

To dissolve 50 mg of extract in 5 ml of distilled water, add absolute alcohol (constant stirring). The formation of white or cloudy precipitates indicated the presence of gums and mucilages.[12]

Detection of resins

Acetic anhydride test

Extracts were mixed with 1 ml of acetic anhydride and 1 ml of concentrated H2S04. The change in coloration from orange to yellow in extracts indicates the presence of resins.[13]

Turbidity test

1 ml of extract dissolved in acetone was poured into distilled water. The appearance of turbidity indicated the presence of resins.[14]

Quantitative phytochemical tests

Quantitative determination of phytochemicals was quantified using the gravimetric method.[15, 16]

Determination of Alkaloids

3 g of plant sample was dissolved in 100 ml of 10 % acetic acid. The solution was covered and allowed to stand for 4 hours. It was filtered using whatman filter paper (No. 42) transferred to a water bath to evaporate one quarter of the original volume. The concentrated ammonia solution was added to the filtrate drop by drop until precipitate formed. The extract was filtered again and washed with 1 % of ammonia solution. Filter paper containing an alkaloid precipitate, which was dried using an oven. After drying and allowed to cool for a few minutes and weighted.

Determination of flavonoids

10 g of sample was repeatedly extracted with 100 ml of 80% aqueous methanol. The extract was filtered through a whatman (No. 42) filter paper in a pre-weighed 250 ml beaker. The filtered extract was transferred to an oven and allowed to evaporate to dryness and was weighed.

Determination of Phenolic content

Determination of total phenolic content was determined using the Folin-ciocalteu method. A calibration curve was prepared by mixing 1 ml of gallic acid (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml) and 1 ml of distilled water with 0.5 ml Folin-ciocalteu reagent was added test tubes. After 3 minutes, 2 ml of 2% Sodium carbonate (Na2CO3) was added, and the mixture was allowed to stand for 30 min at room temperature. 1 ml of extract was mixed with the above used reagents. The mixture was kept at room temperature for 30 minutes. The absorbance was measured at 765 nm using a spectrophotometer to determine total phenolic content of the extract. From the calibration curve, the amount of phenolic compounds was determined and expressed as milligrams of gallic acid equivalent (GAE)/g of the dried extract.

RESULTS

Qualitative phytochemical screening

Phytochemical screening of acetone and methanolic extracts of *E. monogynum* wood were presented in Table 1. Methanolic extract of *E. monogynum* wood revealed that presence of

bioactive compounds such as alkaloids, flavonoids, tannins, glycosides, steroids, reducing sugars, phenols, emodins, phlobatannins, gums, mucilages, and resins.

Table 1: Qualitative phytochemical analysis of wood extract of *E. monogynum*.

S. No	Phytochemical tests	Acetone extract	Methanol extract
1.	**Alkaloids**		
	a) Mayer's test	-	+
2.	**Flavonoids**		
	a)Ferric chloride	+	+
	b) Alkaline test	-	+
3.	**Tannins**		
	Ferric chloride test	+	+
4.	**Glycosides**		
	a) Liebermann's test	-	-
	b) Salkowski test	+	+
5.	**Steroid**		
	a) Acetic anhydride test	-	-
	b) Salkowski test	+	+
6.	**Terpenoids test**	-	-
7.	**Saponins**		
	Foam test	-	-
8.	**Proteins and Amino acids**		
	Ninhydrin test	-	-
9.	**Reducing sugars**		
	a) Benedict test	+	+
	b) Fehling' s test	+	+
10.	**Phenols**		
	a)Folin-ciocalteau's test	+	+
	b) Ferric chloride test	+	+
11.	**Emodins**	+	+
12.	**Phlobatannins**	+	+
13.	**Gums and Mucilage**		
	Alcohol test	-	+
14.	**Resins**		
	a) Acetic anhydride test	-	-
	b) Turbidity test	+	-

The result obtained from the extracts revealed the absence of terpenoids, saponins, proteins, and amino acids in acetone and methanolic extracts. However, alkaloids, gums and mucilage were absent in acetone extract. Resins were absent in methanolic extract.

Quantitative determination of phytochemicals

A quantitative determination of phytochemicals is presented in table 2. The alkaloids content of *E. monogynum* wood extract was found to be (14.94%), followed by the phenolic content (0.32%), and then the flavonoids content (3.3%).

Table 2: Quantitative determination of phytochemicals analysis of *E. monogynum*.

S. No	Secondary metabolites	% in *E. monogynum* wood extract
1.	Alkaloids	14.94
2.	Flavonoids	3.3
3.	Phenols	0.32

DISCUSSION

In the present study, qualitative phytochemical analysis of acetone and methanolic extracts of *E. monogynum* revealed the presence of alkaloids, flavonoids, tannins, glycosides, steroids, reducing sugars, phenols, emodins, phlobatannins, gums, mucilages, and resins.

Furthermore, the quantitative determination of the phytochemicals is also being studied. Among the compounds quantified, the alkaloids (14.94%) were present in a large amount compared to the other compounds like flavonoids (3.3%) and phenols (0.32%). Alkaloids show quite diverse medicinal properties. More than 3000 of alkaloids are known in over 4000 different plant species.[17] These alkaloids possibly possess interesting properties for medical, pharmaceutical, synthetic and many other useful therapeutics.

Flavonoids are the largest group of naturally occurring phenols. They have possessed a number of medicinal benefits, including antioxidant, anticancer, anti-inflammatory and antiviral properties. They also have neuroprotective and cardio-protective effects. So far, over 10,000 flavonoid compounds have been isolated and identified. Most of them are widely accepted as therapeutic agents.[18,19] Phenols are the largest group of the plant's secondary metabolites. Many of the phenolic compounds are also effective antioxidant and free radical scavengers.[20]

The wood contains alkaloids, diterpenes, monogynol such as erythoxydiol, erthroxytriol, hydrocarbons, primaradiene, isoatisirens, artisirene, devadorene.[21]

E. monogynum possesses several medicinal properties. It is scientifically proved to be effective against bacterial infections and its antibacterial properties;[22] also helpful for prevent damage to liver and treat diabetes.[23, 24] The infusion of wood and bark is used as a

diuretic, stomach ache, diaphoretic, stimulant and also used in mild cases of dyspepsia.[25] The wood shows medicinal applications for rheumatoid arthritis and urticaria and rashes.[26] The wood oil contains sandalwood aroma and is used as a perfume.[27]

CONCLUSION

The wood's phytochemicals have therapeutic effects against antiviral, hepatoprotective, diabetic, rheumatoid arthritis, and skin diseases etc. This plant contains a great number of phytochemicals with important disease-curing properties. However, only a few bioactive compounds are now being discovered in this plant. Further studies recommended for the isolation and characterization of the specific bioactive compounds may be responsible for curing a variety of diseases.

ACKNOWLEDGEMENT

We would like to thank the Head of the Department and all the staff members, Research scholars of the Department of Botany at NGM College, Pollachi.

REFERENCES

1. Hasler CM and Blumberg JB. Phytochemicals: Biochemistry and physiology. Introduction. J Nutr, 1999; 129: 756S-757S.
2. Motaleb MA. Selected medicinal plants of Chittagong hill tracts. IUCN, Bangladesh, 2011.
3. Velavan S. Phytochemical Techniques - A Review. W J Sci Res, 2015; 1(2): 80-91.
4. Chukwuebuka Egbuna, Toske L. Kryeziu, Minakshi Mukherjee, Narasimha Rao GM, Jonathan C. Ifemeje, Hameed Shah, Laurence John Francis J. Gido, Habibu Tijjani. Introduction to Phytochemistry, Vol. 1 Fundamentals, modern techniques and applications, 2018; 1-29.
5. Nair NC and Henry AN. Flora of Tamil Nadu, India, Vol. I, Botanical Survey of India, Southern Circle, Coimbatore, India, 1983; 184.
6. Dhanunjaya C, Anitha S, Varalakshmi and Dowlathabad Muralidhara Rao. Evaluation of potential phytochemicals and phyto pharmacological activities of *Erythroxylum monogynum* Roxb. Biosci Biotech Res Asia, 2019; 16(2): 441-449.
7. Evans WC. "Trease and Evans Pharmacognosy", Harcourt Brace and company. Asia private Limited Singapore, 1997.
8. Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG (2014). Comparative preliminary phytochemical analysis of ethanolic extracts of leaves

of *Olea dioica* Roxb., infected with the rust fungus Zaghouania oleae (E.J. Butler) Cummins and non-infected plants. J Pharm Phytochem, 2014; 3(4): 69-72.

9. Vikram Kumar Sharma and Rashmi Mishra. Phytochemical analysis of floral extract of some medicinal plants used by local people of Ranchi District, Jharkhand (India) used to cure skin diseases. J Emerg Tech Inno Res, 2020; 7(11).
10. Abdur Rauf, Muhammad Oaisar, Ghias Uddin, Samina Akhtar and Naveed Muhammad. Preliminary phytochemical screening and antioxidant profile of *Euphorbia prostrata*. Middle-Last J Med Plants Res, 2012; 1(1): 09-13.
11. Kalpana Devi Rajesh, Subramani Vasantha, Nakulan Valsala Rajesh, Annamalai Panneerselvam. Qualitative and quantitative phytochemical analysis in four Pteridophytes. Inter J Pharm Sci Rev Res, 2014; 27(2): 408-412.
12. Raaman N. Phytochemical Techniques, New India Publishing agency, New Delhi, 2006; 226: 19-24.
13. Kumar R, Venkateshwar C, Samuel G, Rao SG. Phytochemical screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emerginata* (Grah.) used by Gondu tribes at Adilabad District, Anthrapredesh, India. Inter J Eng Sci Inven, 2013; 2(8): 65-70.
14. Pooja S, Vidyasagar GM. Phytochemical screening for secondary metabolites of *Opuntia dillenii* Haw. J Med Plants Studies, 2016; 4(5): 39-43.
15. Krishnaiah DT, Devi A, Bono and Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J Med Plants Res, 2009; 3(2): 67-72.
16. Harbone JB. Phytochemical methods. London. Chapman and Hall, Ltd., 1973; 49-188.
17. Chisholm H. Encyclopedia Brittanica: A Dictionary of Arts, Sciences, Literature and General Information. Vol. 22. New York: Sagwan Press, 2015.
18. Thilakarathna S, Rupasinghe H. Flavonoid Bioavailability and Attempts for Bioavailability Enhancement. Nutrients, 2013; 5: 3367-3387.
19. Aleksandra Kozlowska, Dorota Szostak-Wegierek. Flavonoids-food sources and health benefits. Rocz Panstw Zakl Hig, 2014; 65(2): 79-85.
20. Golawska S, Sprawka I, Lukasik I, Golawski A. Are naringenin and quercetin useful chemicals in pest-management strategies? J Pest Sci, 2014; 87(1): 173-180.
21. Rastogi and Mehrotra. Compendium of Indian Medicinal Plant, Vol. I., 1972; 112-114.
22. Srinivasan D, Sangeetha N, Suresh T, Perumalsamy PL. Antimicrobial activity of certain Indian medicinal plants used in folklore medicine. J Ethnopharm, 2001, 74(3): 217-220.

23. Sabeena SH, Namdeo AG. Hepatoprotective effects of leaves of *Erythroxylum monogynum* Roxb. On paracetamol induced toxicity. Asian Paci J Tropi Biomed, 2013; 3(11): 877-81.
24. Rupesh SK, Ravindra KR, Jayveera KN. Evaluation of Anti-diabetic potential of *Erythroxylon monogynum* in streptozotocin induced diabetic rats. Inter J Adv Res, 2014; 2: 550-560.
25. Kirtikar KR, Basu BD. Indian Medicinal plant. Second edtion, Vol I. Dehradun: International Book Distributors, 1987; 415.
26. Muthamizh S, Ramachandran VS. Medicinal plants of Dharmapuri District of Tamil Nadu used in primary healthcare system. Inter J Bot Stud, 2018; 3(1): 109-11.
27. Uphof, Johannes Cornelis Theodorus. Dictionary of Economic plants, Weinheim, 1959; 400.