DOI:10.26524/krj.2022.7

Kongunadu Research Journal 9 (1): 47-52, 2022
Publisher: Kongunadu Arts and Science College, Coimbatore.

https://krjournal.com/index.php/krj/article/view/536

RESEARCH ARTICLE

SCREENING OF PHYTOCHEMICAL CONSTITUENTS AND QUANTITATIVE ESTIMATION OF TOTAL FLAVONOIDS AND PHENOLIC COMPOUNDS OF LEAFEXTRACTS OF *MITRACARPUS HIRTUS* (RUBIACEAE)

Binoodha Remina, C., Vimal Priya, S. and Karthika, K.*
Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641029, Tamil Nadu, India

ABSTRACT

Plants have the ability to synthesize mixtures of structurally diverse bio-active compound, with multiple and mutually potential therapeutic effects. The objective of the study is to cover the preliminary phytochemical screening of traditional medicinal plant *Mitracarpus hirtus* belonging to the family Rubiaceae. The preliminary screening revealed the presence of alkaloids, flavonoids, steroids, tannins, phenolics, glycosides, carbohydrates, proteins and amino acids. In quantitative estimation, among all the extracts, acetone extract exhibited the maximum amount of phenolics (40.26 mg GAE/g extract), and it depicted the maximum quantity of flavonoids (84.43 mg RE /g extract) which explains that the plant must have valuable medicinal properties and so it can be explored.

Keywords: *Mitracarpus hirtus,* Phytochemical analysis, Phenols, Rubiaceae.

1. INTRODUCTION

Origin of medicinal herbs. They have been using them for curative purposes successfully. The records are available in ancient texts. In India itself, there are more than 20000 medicinal plants grown all over the wild forests. Of these, some 60 genus are used immensely in medicinal preparation. Despite their demands today, they are not grown in controlled manner. Rather tribes use them as their livelihood in some belts where they are grown in the wild. Unlike India, in china, the spurts in demand for traditional medicine have made government to allow growth of these plants for further research and development. About 100 units have nearly 600 plant type, grown for their medicinal value. Herbal medicines are used in Ayurveda, Naturopathy and Homeopathy, tradition and Native American medicine (Rangari, 2002). The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, the medicinal plant plays a major role and constitutes the backbone of traditional medicine (WHO, 1978). A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants, which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, contain minerals and vitamins, possess medicinal properties (Adnan, 2002).

Mitracarpus hirtus is a generally erect, annual plant that can be simple or sometimes much branched. The relatively stout stems can be 30 - 60cm tall. The plant is sometimes gathered from

*Correspondence: Karthika, K., Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641029, Tamil Nadu, India. E.mail: karthikak_bo@kongunaducollege.ac.in

the wild for local medicinal use. The dried leaves are said to heal old ulcers rapidly. The plant is an antidote for arrow poison (Ken Fern, 2021). Considering the medicinal activity of *M. hirtus* based on the aforesaid traditional information, the present study was focused on the phytochemical screening and quantitative estimation of secondary metabolites of the selected plant sample to add scientific conclusion to the traditional claims.

2. PLANT DESCRIPTION

Systematic Position

Kingdom: Plantae
Class: Dicotyledonae
Subclass: Gamopetale
Order: Rubiales
Family: Rubiaceae
Genus: Mitracarpus
Species: hirtus
Synonym: Mitracarpus villosus

Vernacularnames

Common name: Tropical girdle pod, Odia; Gothia gobi, Malayalam; Thaval

Fig. 1. Habit of *Mitracarpus villosus*

3. MATERIALS AND METHODS

3.1. Study area

The selected plant *Mitracarpus hirtus* was collected from Kozhikode District in the Northern part of Kerala. Keezhariyur is a small village situated in Kozhikode district, Kerala state, India. It comes under Keezhariyur Panchayath.

It is located 35 Km from District headquarters Kozhikode. The average annual rainfall of the state ranges from 101.6 to 362cm. The highest temperature recorded was 39.4°C in March and the lowest was 14◦C on December. The District has a generally humid climate with a very hot season extending from March to May. The rainy season is during the South West Monsoon, which sets in the first week of June and extend up to September.

3.2. Collection of Plant Material

The plant leaf of M.hirtus were collected during the month of December, 2021, Keezhariyur, Kozhikode district, Kerala. The authenticity of the selected plant materials were duly identified and confirmed by Botanical Survey of India, Coimbatore. Fresh and healthy plant leaf of *M. hirtus* was harvested shade dried and coarsely powdered for extraction.

3.3. Preparation of Plant Extracts

The powdered plant samples (40 g/200 ml) were extracted successively with hexane, acetone and ethanol using Soxhlet apparatus at 55-86℃ for 9-10 hr in order to extract the polar and nonpolar compounds. The powder was air dried and packed then used for each solvent extraction. The solvent of respective extracts were reduced under room temperature and stored at 5℃ for further use.

3.4. Qualitative screening of Phytochemical Compounds:

3.4.1. Extractive yield

The air dried leaves were exhaustively extracted with successive solvent extraction using soxhlet apparatus viz., petroleum ether, benzene, chloroform, ethyl acetate, ethanol and hot water was which indicates the presence of earthy materials in the sample. The water soluble ash is used to estimate the amount of inorganic compounds present in drugs (Thomas et al., 2008; Vaghasiya et al., 2008; Dave et al., 2010) Table 2. Physico-chemical analysis of Kedrostis foetidissima leaves performed as per Indian Pharmacopoeia

(Peach and Tracey, 1955). The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE 300; Yamato, Japan), Lyophilized (4KBTXL – 75; Vir Tis Benchtopk, New York, USA) to remove traces of water molecules and their extractive yield percentage wascalculated.

3.4.2. Qualitative phytochemical evaluation

Phytochemical screening for crude solvent extracts were carried out and their bioactive compounds were determined using standard methods (Brain and Turner, 1975; Trease and Evans, 1983; Harborne, 1984).

3.5. Quantitative phytochemical analysis

3.5.1. Determination of total flavonoids

The total flavonoid content of samples was determined by following the modified colorimetric method of Zhishen et al. (1999). 0.5 ml extract was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% NaNO2 solution. After 6 min, 0.15 mL of 10% AlCl3 solution was added and allowed to stand for 6 min, then 2 mL of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was recorded at 510 nm versus prepared water blank. Rutin was used as a standard compound for the quantification of total flavonoid. All the values were expressed as milligram of rutin equivalent (RE) per gram of extract.

3.5.2 Determination of total phenolics

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). Aliquots of each extract were taken in test tubes and made up to the volume of 1 mL with distilled water. Then 0.5 mL of folinciocalteu phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents (GAE).

4. RESULT AND DISCUSSION

4.1. Extractive Yield Percentage

The yield of sequential extracts (%) is shown in Figure 2.

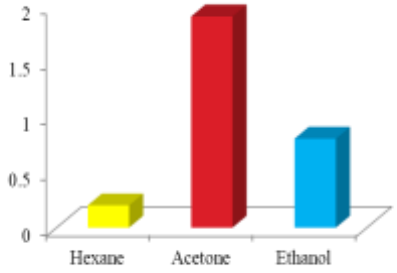

Fig. 2. Extractive Yield Percentage

4.2 Qualitative Phytochemical analysis

Phytochemical screening of the sequential extract of *M. hirtus* revealed the presence of various bioactive components of which phenolics, saponins, alkaloids, tannin, Glycosides, Proteins, Carbohydrates, and Amino acids are the most prominent components and the result of phytochemical test given in the Table 1.

Among these phytochemical tests, Alkaloids, were present in all solvent extracts. Whereas most of the active compound are alkaloid, flavonoid, Glycosides, Tannin, Phenols are present in the acetone extract of plant material.

Table 1. Qualitative phytochemical analysis of the different extracts of *Mitracarpus hirtus* leaf

S. No	Phytocons-tituents	Hex	Ace	EtOH
1	Carbohydrates	++	+	+
2	Proteins	_	_	_
3	Alkaloids	+	+	++
4	Glycosides	_	++	+
5	Saponin	-	+	+
6	Phenol	+	++	+
7	Flavonoids	+	++	+

(+++ abundant; ++ moderately present: +weakly present; -----absent,) Hex, Hexane; Ace, Acetone; EtOH, Ethanol

4.5. Quantitative Phytochemical analysis

Total flavonoids and phenolic content of leaves of *Mitracarpus hirtus* in rutin and gallic acid equivalents are presented in Table 2. The highest phenolic and flavonoid content was noted in the acetone leaf extract, 84.43 mgRE/g extract, and 40.26 mgGAE/g extract flavonoids and phenols were present in leaves respectively. The flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996.). They are also effective antioxidants and show strong anticancer activities (Salah *et al.,* 1995; Del-Rio *et al.,* 1997; Okwu, 2004). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.,* 2007). They possesses biological properties such as anti-apoptosis, antiaging,anticarcinogen,antiinflammation,antiath erosclerosisand cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.,* 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001).

Table 2. Total phenolic and flavonoid content of *M.hirtus* leaf extract

Extracts	Total phenolics (mg GAE/g extract)	Total flavanoids (mg RE/g extract
Hexane	12.31±0.3	10.008±0.036
Acetone	84.43±0.1	40.26±0.06
Ethanol	41.20±0.11	20.10±.03

(GAE - Gallic acid equivalent, RE – Rutin equivalent. Values are expressed as mean±SD (n=6).)

5. CONCLUSION

The results revealed the presence of medicinally important constituents such as flavonoids and phenol in the study plant. Further the plant could be considered for antioxidant, anticancer, immunomodulatory activities.

REFERENCES

1. Adnan, S.M., 2002. Feasibility of Community Involvement in the Sustainable Use of Medicinal Plants in Roringar valley of Swat, Ethnobotany Project, WWF-Pakistan.
2. Agbor, A.G., L. Talla and J.Y. Ngogang, (2004). The Antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. Phytother Res. 18(11):873-876.
3. Alarcon, S.R., L.S.N. Ocampos, L.V. Flores Galleuillo, A. Pacciaroni and V.E. Sosa, (2007). phytochemistry and phytotoxicity activity of *Lagascea mollis* (Asteraceae). The journal of tye Argentine Chem Soc. 95(1-2):25-31.
4. Aqil, F., I. Ahmed and Z. Mehmood, (2006). Antioxidant and free radical scavenging properties of twelve traditionally used.
5. Indian medicinal plants. Turk J Biol. 30: 177-183.
6. Ayodele, S.Q. (2003). The effects of herbal remedies. Paper presented at the 12th Annual Conference of Botanical Society of Nigeria, University of Logos, South Africa.
7. Bohlmann, F., C. Arndt, H. Bornowski, H. Jastrow and K.M. Kleine, (1962). Chem Ber. 95:1320.
8. Brain, K.R. and T.D. Turner, 1975. The Practical Evaluation of Phytopharmaceuticals. Wright Scientechica, Bristol pp.57-58.
9. Brain KR and Turner TD. 1975. The practical evaluation of phytopharmaceuticals, Wright Scientechnia. 6: 81.
10. Brown, J.E. and C.A. Rice-Evans, (1998). Luteolin rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radical Res. 29: 247-255.

11. Bruneton, J. (1993). Plants medicinales: Phytochimie, Pharmacognosie. Zema. New York. lavoisier: P.914, (Fre).
12. Chourasia, O.P. and J.T. Rao, (1987). Antibacterial Efficacy of Some Indian Essential Oils, Perfumery and Cosmetic. Perfume and Cosmetic 68(9): 564-66.
13. Ciulci, I. (1994). Methodology for the analysis of vegetable drugs. Chemical industries Branch, Division of Industrial Operations. UNIDO, Romania 24: 26 - 67.
14. Dave R, Nagani K, Chanda S. 2010. Pharmacognostic studies and physicochemical properties of the *Polyalthia longifolia* var. pendula leaf. Pharmacognosy Journal. 2: 572-576.
15. Del-Rio, A., B.G. Obdululio, J. Casfillo, F.G. Main and A. Ortuno, (1997). Uses and properties of citrus flavonoids. J. Agric. Food Chem. 45: 4505-4515.
16. ElNagar, S.F. and R.W. Doskotch, (1979). Patulitrin and Acetyl Patulitrin, Flavonol Glycosides From *Lagascea mollis* . J. Nat Prod. 42(2):126-28.
17. Giday, M. (2001). An ethnobotanical study of medicinal plant used by the Zay people in Ethiopia – CBM:s skriftserie 3: 81-99.
18. Gokhale, S.B., C.K. Kokate and A.P. Purohit, (2008). A text book of phrmacognosy, Nirali Prakashan, Maharashtra, India pp: 889.
19. Han, X., T. Shen, and H. Lou (2007). Dietry polyphenols and their biological significance. Int. J. Mol. Sci. 950-988.
20. Harborne, J.B. (1973). Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York.
21. Harborne, J.B. (1984). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, UK.
22. Heinrich, M. (2000). Ethnobotany and its role in drug development, Phytother Res, 14: 479.
23. Jackson, B.P. and D.W. Snowdon. (1968). Powdered Vegetable Drugs. Thames, London.
24. Jagtap, A.P. and N.P. Singh, (1999). Fascicles of Flora of India, Fascicle 24, Botanical Survey of India, Calcutta, India.
25. Karthikeyan, S., M. Sanjappa and M. Kothari 2009. Flowering Plants of India: Dicotyledons. Vol. 1. (Acanthaceae Avicenniaceae). Botancial Survey of India, Kolkatta.
26. Karuppusamy, S. (2007). Medicinal plants used by Paliyan tribes of Sirumalai hills of southern India. Natural Product Radiance. 6(5) 436-442.
27. Ken Fern (2021, july 30). *Mitracarpus hirtus* information collected from Tropical Plants Database, Ken Fern. tropical.theferns.info. 2022-06-14. <tropical.theferns.info/viewtropical.php?id=*Mitracarpus*+*hirtus*>
28. Kokate CK., Purohit AP. and Gokhale SB. 1995. Pharmacognosy, 3rd edition, Nirali Prakashan, Pune.
29. Kokoshi, J., R. Kokoski and F.J Slama, (1958). Fluorescence analysis of powered vegetable drugs under ultraviolet radiation. Journal of the American Pharmacists Association. 47: 75-77.
30. Kokwaro and O. John (2009). Medicinal plants of East Africa, 3rd Edition – Nairobi: University of Nairobi Press, pp 111.
31. Krishnamoorthy Karthika,, & Vimal priya Subramanian. (2021). A phyto pharmacological review of medicinaly important plant *Solena amplexcaulis* (cucurbitaceae). *Kongunadu Research Journal*, *8*(2): 44-48.
32. Peach and M.V. Tracey, (1955). Modern Methods of Plant Analysis, Vol. III, Springer and Verlag, Berlin. 321-322.
33. Pullaiah, T. (2006). Biodiversity of India, Regency publications, New Delhi, India. pp 235.
34. Rangari, D.V., Pharmacognosy and Phytochemistry, First Edition, Part-March-2002; pp.10.
35. Sethiya, N.K., A. Trivedi, M.B. Patel and S.H. Mishra, (2010). Comparative pharmacognostical investigation on four

ethnobotanicals traditionally used as Shankhpushpi in India. Journal of Advanced Pharmaceutical Technology and Research. 1: 388.
36. Thomas, S., D.A. Patil, A.G. Patil and N. Chandra, (2008). Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. Journal of Herbal Medicine and Toxicology. 2: 51-54.
37. Trease, G.E. and W.C. Evans, (1983). Pharmacognosy 12th Ed. Bailliere Tindall, London. pp 622.
38. Trease, K. And W.C. Evans, (1985). Textbook of pharmacognosy. 12th Ed, Balliere Tindall Publication, London. 537-541.
39. Vaghasiya, Y., R. Nair and S. Chanda, (2008). Antibacterial and preliminary phytochemical and physico-chemical analysis of *Eucalyptus citriodora* Hk leaf. Natural Product Research. 22: 754-762.
40. Vimal priya, S., R. Shyam Praveen, K. Karthika (2022). Phytochemical analysis and TLC profile of *Tylophora subramanii* A. N. Henry (Asclepidaceae)-an endemic medicinall plant species of southern India. *International journal of Scientific development and Research* (IJSDR), 7(3): 168-174.
41. WHO. (2002). Quality Control Methods for Medicinal Plant Materials. (An authorized publication of World health organization, Geneva). A.I.T.B.S. Publishers & Distributors, New Delhi.
42. World Health Organization, (1978). The Promotion and Development of Traditional Medicine Technical Report Series 622, Geneva: World Health Organization.

About The License