ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 1(F), pp. 23242-23245, January, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

PHYTOCHEMICAL SCREENING, THIN LAYER CHROMATOGRAPHY AND QUANTITATIVE ESTIMATION OF BIOACTIVE CONSTITUENTS IN AQUEOUS EXTRACT OF *MANILKARA HEXANDRA* (ROXB.) DUBARD

Sisodiya, D[1]* and Shrivastava, P[2]

[1]Department of Zoology, AISECT University, Bhopal, M.P
[2]Department of Zoology, AISECT University, Bhopal, state- Madhya Pradesh, India

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1427

ARTICLE INFO

Article History:
Received 7th October, 2017
Received in revised form 21st November, 2017
Accepted 6th December, 2017
Published online 28th January, 2018

Key Words:

Manilkara hexandra, Phytochemicals, Total Phenolic Content, Total Flavonoid Content

ABSTRACT

The present study was aimed to investigate the qualitative and quantitative analysis of the major bioactive constituents of medicinally important plant *Manilkara hexandra* (Roxb.) in its aqueous extract of leaves of the plant. Studies were carried out in terms of aqueous extraction, total extractive values, qualitative and quantitative estimation of phytochemicals. The percentage value of yield extraction was found to be 5.15%. The preliminary phytochemical analysis confirmed the presence of phenols, flavonoids, saponins, carbohydrates, alkaloids and glycosides. The total phenolic content ranged from 31.857 mg/100mg of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 25.097 mg/100mg, expressed as quercetin equivalents. It signifies that results revealed the presence of various bioactive compounds which could be further exploited and isolated for their potential applications for medicinal purposes.

INTRODUCTION

Plants are universally recognized as vital component of the world's bio-diversity and very essential resources for the planet. The art of healing has its origin in the ancient past of human civilization. The medicinal value of the plant lies in some of its chemical substances that produce a definite physiological action on human body.

Manilkara hexandra (Roxb.) Dubard belongs to family Sapotaceae. It is commonly called as Rayan/ Khirni. The plant is an evergreen tree, native of Central India and Deccan peninsular. It is also found growing at Sri Lanka, Thailand, Indochina and Hainan (Almeida, 2001). The fruit is edible; the seed is used as cooking oil by the natives. The Koli tribe uses the decoction of the bark in diarrhea for children. The stem bark is also recommended for fever, jaundice, helminthiasis, flatulence, stomach disorder etc (Kirtikar and Basu, 2001). Ethnopharmacological studies show that *Manilkara hexandra* is used to cure a large number of diseases in many parts of India. Mostly the western and the central parts of India especially in Madhya Pradesh, Rajasthan, Maharashtra, Tamil Nadu and Andra Pradesh have a long history of traditional medicinal use of *Manilkara hexandra*.

The current scenario reveals the demand for herbal drugs throughout the world due to their valuable phytochemicals. There are thousands of medicinal plants known to have a long history of usage for their curative properties against various diseases and ailments. However, screening of medicinal plants for their useful activities is very essential and requires an urgent attention to know the value of the plant. The rich knowledge base of countries like India in medicinal plants and healthcare has lead the pharmaceutical companies to take keen interest in order to use this traditional knowledge as a good resource for research and development in the pursuit of discovering novel drugs. However, several plants are used for various aspects in India in the crude form without scientific evidence of efficacy. At this juncture it is of interest to determine the scientific basis for the traditional use of these plants (Singh *et al*, 2011; Shah and Gilani, 2010). With this background an attempt has been made to establish the phytochemical constituents like phenols, flavonoids, glycosides, alkaloids, saponins, etc. present in the aqueous extract of *Manilkara hexandra* through qualitative, quantitative analysis and TLC techniques. These studies will be helpful for the scientific verification of folklore claim related to the usefulness of this plant.

Corresponding author:* **Sisodiya, D
Department of Zoology, AISECT University, Bhopal, M.P

MATERIALS AND METHODS

Reagents

The reagents and solvents used for the extraction, phytochemical analyses and TLC profiling were analytical grade reagents.

Collection and identification of plant

Manilkara hexandra plant material was collected from 'Santosh nursery' Shujalpur located in Shajapur district, Madhya pradesh, during the month of December, 2016. Further plant material was identified and voucher specimen was submitted in 'Herbarium', Department of Botany, Dr. Hari Singh Gour University, Sagar, M.p. The registration number allotted to *Manilkara hexandra* P2 (bot/BG/201199).

The plant material (leaves) were shade dried at room temperature for about 15 days. The dried plant sample was powdered by mechanical grinder and sieved to give particle size 40-100 mm. The powdered material was stored in a polythene bag at room temperature before extraction.

Preparation of extract

Manilkara hexandra dried and powdered plant material (50 g) was extracted with hot continuous percolation method (Soxhlet extraction). The temperature during extraction was maintained at 70°C. The extraction process was carried out by using water as solvent. The extract was filtered through a paper filter (Whatman, No.1) and then evaporated to dryness under the reduced pressure developed by the rotary evaporator. The obtained crude extract was stored in dark glass bottles for further processing. Yield of extract obtained was calculated by using formula as mentioned below:

Extractive yield value = Weight of concentrated extract/Weight of plant dried powder × 100

Qualitative phytochemical analysis of plant extract

The extract was examined for the presence of various phytoconstituents such as carbohydrate, alkaloids, glycosides, saponins, phenolic compound, tannins and flavonoids. All tests were done as per the procedure given in the standard book (Kokate, 2004).

TLC (Thin Layer Chromatography) profile

For the separation of different phytochemical compounds in the aqueous extract of *Manilkara hexandra* (leaves), the extract was spotted manually using a capillary tube on precoated silica gel G TLC plates (15X5 cm with 3 mm thickness). The spotted plates were put into a solvent system to detect the suitable mobile phase as per the method of Wagner *et al*., (1984 and 1996). After the separation of phytochemical constituents, the spraying reagents such as Dragendorff reagent, 10% ethanolic sulphuric acid, 10% sulphuric acid, 5% ferric chloride, Kedde reagent, vanillin sulphuric acid reagent and vanillin phosphoric acid reagent were used in order to identify the respective compounds. The colour of the spots were noted and Rf values were calculated by using the following formula: Retention factor (Rf) = Distance travelled by the solute/Distance travelled by the solvent

Quantitative phytochemical analysis

Total phenols determination

The amount of total phenolic contents of extract of *Manilkara hexandra* was determined by the spectrophotometric method of Kim *et al*., (2003) with slight modification. A diluted plant extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml Folin-Ciocalteu's phenol reagent was then added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample. The solution was diluted to 25 ml distilled water and mixed thoroughly. This mixture was kept in dark for 90 min. at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of phenolic compounds was carried out in triplicate. The Total phenolic content was expressed as milligrams of Gallic acid equivalents (GAE) per 100 mg of dried sample.

Total flavonoids determination

The total flavonoids assay was conducted according to Katasani Damodar (2011). Total flavonoids content was determined by using Aluminium chloride colorimetric method. Aqueous extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It was remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 0 to 150 µg/ml in methanol. The total flavonoids content was expressed as milligrams of Quercetin equivalents per gram of dried sample.

RESULTS

The crude extracts obtained after soxhlet extraction process was then further concentrated on the water bath evaporation completely to obtain the actual yield of extraction. The yield of extract was obtained from water as solvents and results are depicted in the table;

Table 1 Percentage yield of plant material

S. No.	Extract	% Yield (W/W)
1.	*Manilkara hexandra* (aqueous extract)	5.15%

A small portion of the extract was subjected to the phytochemical test for alkaloids, glycosides, tannins, flavonoids, saponins, amino acids, carbohydrates and diterpenes. Small amount of the extract was resuspended into sterile distilled water to make the concentration of 1 mg per ml. The findings of the results are described in the table;

Table 2 Results of phytochemical screening

S. No.	Chemical Tests	*Manilkara hexandra*	Observation
		Alkaloids	
1.	*Mayer's reagent*	-	Yellow coloured precipitate
	Hager's reagent	+	Yellow coloured precipitate
	Wagner's reagent	-	Brown/reddish coloured precipitate
	Dragendorff's reagent	-	reddish coloured precipitate
2.		Glycosides	
	Legal's test	+	Pink to blood red colour

3.	*Ferric chloride*	Phenols/Tannins +	Bluish black coloured
4.	*Lead acetate test*	Flavonoids +	Yellow coloured precipitate
	Alkaline reagent test	+	Colourless
5.	*Foam test*	Saponins +	Layer of foam
6.	*Fehling's solution test*	Carbohydrates +	Red coloured
7.	*Xantoprotein Test*	Amino acids -	Yellow coloured
8.	Diterpenes	-	Emerald green

The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.0028X- 0.0257, R^2= 0.98, where X is the absorbance and Y is the gallic acid equivalent (GAE).

Table 3 Preparation of calibration curve of gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	25	0.049
2	50	0.093
3	75	0.155
4	100	0.255
5	125	0.315
6	150	0.421

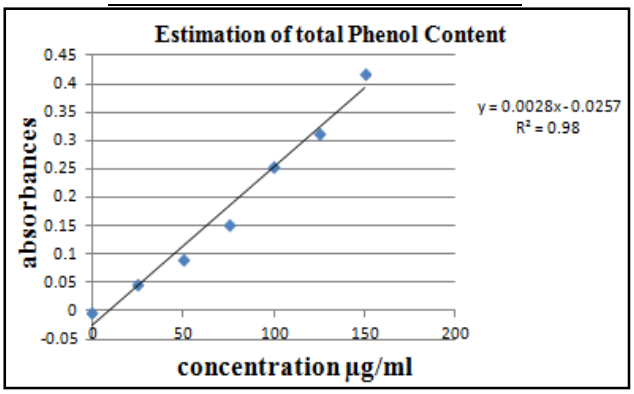

Figure 1 Graph of estimation of total phenolic content

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.0041 X - 0.0012, R^2=0.9962, where X is the absorbance and Y is the quercetin equivalent (QE).

Table 4 Preparation of calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	25	0.119
2	50	0.195
3	75	0.297
4	100	0.387
5	125	0.517
6	150	0.626

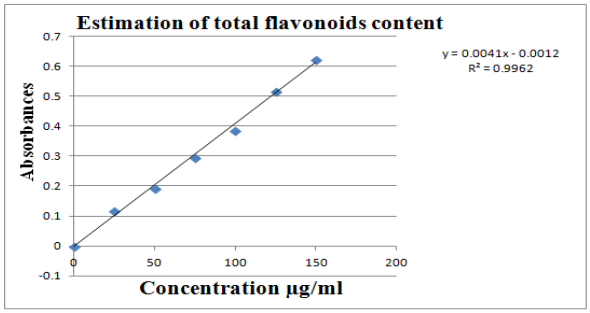

Figure 2 Graph of estimation of total flavonoid content

Table 5 Estimation of total phenolics and total flavonoid content

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100mg of dried extract)
1	*Manilkara hexandra*	31.857	25.097

Table 6 Results of thin layer chromatography

Compound	Mobile phase	R_f value
Quercetin	Toluene: ethyl acetate: Formic acid (7:5:1 v/v/v)	0.65
Gallic acid	Toluene: ethyl acetate: Formic acid (5:4:1 v/v/v)	0.23

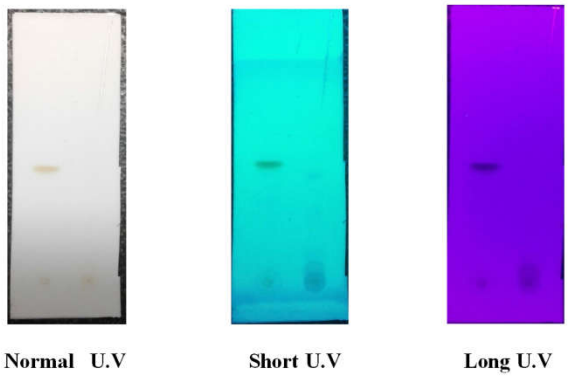

Figure 3 Results of TLC (Quercetin)

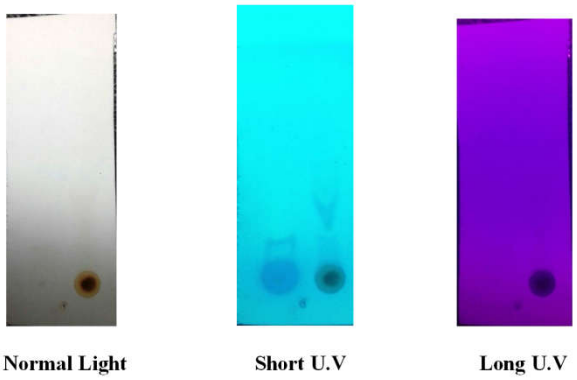

Figure 4 Results of TLC (Gallic acid)

DISCUSSION

The plant screened for important phytochemical compounds appeared to have the potential to be used as a source of drugs effective in improving the health status of the people due to the presence of various constituents necessary for good health. *Manilkara hexandra* revealed the presence of phytochemical constituents such as alkaloids, phenols, flavonoids, saponins, carbohydrates and glycosides whereas diterpenes and amino acids were absent in the aqueous extracts of *Manilkara hexandra* leaves (Table 2). The total phenolic content found in the aqueous extract of *Manilkara hexandra* was 31.857 mg/100mg and the total flavonoid content was 25.097 mg/100 mg in the aqueous extract (Table 5). Phenols and flavonoids seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers. TLC analysis revealed the presence of different types of phytochemicals based on the number of spots. The developed TLC methods will help the manufacturer for quality control and standardization of herbal formulations, such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally valuable plant

species in the Pharma industries as well as plant systematic studies. The data presented here could be helpful in standardizing extracts of this plant. The results of the present study also supplement the folkloric usage of the selected plant which possesses several known and unknown bioactive compounds with bio-activity. By isolating and identifying the bioactive constituents new drugs can be formulated to treat various diseases and disorders. The compounds present in the mixture are to be screened and purified for qualitative estimation.

CONCLUSION

Manilkara hexandra is a well-known medicinal and commercially important tree species which is widely used as herbal remedies for several kinds of diseases and as a source of livelihood support by local tribal population. The authenticity of the crude drug could be equally judged by these physicochemical constants providing a reliable aid for detecting the adulteration. Histochemical and preliminary phytochemical analyses are useful in determining the biochemical constituents present in the said plant drug. This report confirmed the presence of the rich variety of bioactive compounds in the species, *Manilkara hexandra* and it could lead for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. For further study, with the help of developing analytical method pure active chemical compound should be isolated and identified on the basis of reference standards.

References

1. Almeida MR, (2001). Flora of Maharashtra, Vol. III A, Orient Press, Mumbai, pp.168.
2. Kirtikar KR and Basu BD (2001). Indian Medicinal Plants, Vol. VII, Oriental enterprises, pp. 2058-2061.
3. Singh R, Sharma PK, Malviya R (2011). Pharmacological properties and Ayurvedic value of Indian buch plant (Acorus calamus): A short review. *Adv Biol Res*, 5: 145-154.
4. Shah AJ, Gilani AH (2010). Bronchodilatory effect of Acorus calamus(Linn.) is mediated through multiple pathways. J Ethnopharmacol, 131: 471-477.
5. Kokate CK (2004). Practical Pharmacognosy. Published by Nirali Prakashan New Delhi, p. 29-107.
6. Wagner H, Bladt S and Zgainski EM (1984). Plant drug analysis - A Thin Layer Chromatography Atlas. Springer - Verlag, Berlin.
7. Wagner H, Baldt S and Zgainski EM (1996). Plant drug analysis. New York, Berlin, Springer.
8. Kim DO, Jeong SW and Lee CY (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem*, 81:321-326.
9. Katasani D (2011). Phytochemical screening, quantitative estimation of total phenolic, flavanoids and antimicrobial evaluation of Trachyspermum ammi. *J Atoms and Molecules*. 1:1-8.

How to cite this article:

Sisodiya, D and Shrivastava, P.2018, Phytochemical Screening, Thin Layer Chromatography and Quantitative Estimation of Bioactive Constituents in Aqueous Extract of Manilkara Hexandra (Roxb.) Dubard. *Int J Recent Sci Res.* 9(1), pp. 23242-23245. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1427

****