WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 12, 1849-1855. Research Article ISSN 2277– 7105

PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF MEDICINAL PLANT *MELIA AZEDARACH*

Hena Khodiar and Sunita Bhatnagar*

Medicinal and Aromatic Plants, Regional Plant Resource Centre, Bhubaneswar. Odisha, India.

Article Received on
15 July 2022,

Revised on 05 August 2022,
Accepted on 25 August 2022,

DOI: 10.20959/wjpr202212-25418

***Corresponding Author**
Sunita Bhatnagar
Medicinal and Aromatic Plants, Regional Plant Resource Centre, Bhubaneswar. Odisha, India.

ABSTRACT

Melia azedarach belongs to the family Meliaceae is used in traditional medicine for curing a number of diseases across the world and the plant is widely distributed across tropical, subtropical and warm temperate regions of the world. In present study the leave extracts of *Melia azedarach* were investigated for their phytochemical and antioxidant properties. The observation for phytochemical analysis revealed the presence of phytochemical constituents such as flavonoids, terpenoids, cardiac glycosides, tannins, saponins and phlobatannin in different extracts (hexane, chloroform, ethyl acetate, methanol) of *Melia azedarach*. Antioxidant activity was carried out qualitatively by DPPH assay, maximum numbers of antioxidant bands were obtained in chloroform extract in BEA solvent.

KEYWORDS: *Melia azedarach*, medicinal plant, phytochemicals, TLC, antioxidant, DPPH.

INTRODUCTION

Melia azedarach is commonly known as Chinaberry tree, bead tree, Cape lilac, Syringa berry tree and Pride of India.[1] belongs to mahogany family, Meliaceae, native to Indo malaya and Australia.[2] Special feature of *Melia azedarach* is that it is unaffected by herbivores and pathogens.[3] Seeds are poisonous but contain an oil rich in linoleic acid and oleic acid.[4]

Medicinally plant has been reported to have anthelmintic, antimalarial, cathartic, emetic and emmenagogue properties.[5] Since prehistoric times medicinal plants have been used in various therapeutic way to eradicate or treat the ailments. As it is cheaply available and there are allopathic medicines which are not as effective as they should be, so medicines derived

from medicinal plant are used by people for their effectiveness and one important thing is that they don't show side effects. As the plant is termite resistant and also not affected by pathogens, it is clear that they might be producing some secondary metabolites which might be active against the organisms. Hence in the present study leaf extracts of the species was explored for the presence of phytochemicals and antioxidant activity if any.

MATERIALS AND METHODS

Collection and processing of plant material

Melia azedarach plant was collected from the medicinal germplasm garden of Regional plant resource Centre (RPRC), Bhubaneswar. The leaves were weighed in a weighing balance for calculating the moisture content of the plant. Samples were then washed with plain water to remove dust and soil particles and were shade dried for about one week. After drying, plant samples were coarsely grinded using electronic mixture grinder. Again, weighed in the weighing balance to calculate the dried weight of plant sample. Powdered plant sample was then used for extraction.

Moisture content of the plant was calculated by using the following formula:

Moisture content (%) = (F_w – D_w/ F_w) x 100

where, F_w = Weight of the fresh plant sample

D_w = Weight of the dried powdered sample

Solvent extraction

Solvent extraction was conducted as per the standard protocols.[6] Powdered plant sample was processed for extraction with four different solvents i.e. hexane, chloroform, ethyl acetate and methanol according to their increasing solvent polarity i.e. 0.1, 4.1, 4.4, 5.1 respectively. Solvent extraction was done by Soxhlet extractor.

The solvent was heated to reflux depending on boiling temperature of each solvent. As the solvent vapours travelled up a distillation arm, condenser ensured that any solvent vapour cools, and drips back into the chamber containing thimble filled with plant sample. The chamber containing the thimble with plant sample slowly filled with warm solvent. When Soxhlet chamber was almost full, the chamber was emptied by the siphon. The solvent was returned to the distillation flask. This cycle allowed repeating many times, over 3-4 hours. During each cycle, a portion of the non-volatile compound dissolved in the solvent. After many cycles the crude extract is accumulated in the distillation flask.

After extraction through different solvents (hexane, chloroform, ethyl acetate, methanol) the four extracted distillation flask was put in the rotary evaporator to remove the solvents from sample i.e. the filtrate extracts were concentrated under vacuum, using Bucchi rotary evaporator R-200 at 45-60^{o}C depending upon the boiling point of the different solvents. Concentrated extracts were stored in screw capped vial. Percentage of yield was calculated of each solvent extract obtained.

Percentage yield = (Extract weight / Powdered weight) x 100

The extract obtained was stored at room temperature for further various analysis.

Phytochemical analysis

Phytochemical analysis of plant extract of *Melia azedarach* was done by standard phytochemical methods.[7] Plant samples were tested for presence of different phytochemicals like Alkaloids, Flavonoids, Anthraquinones, Saponins, Terpenoids, Cardiac glycosides, Tannins. Protocols of the phytochemicals are as follows:

Tests for alkaloids: Alkaloid tests were done by using three different reagents.

Dragendorff's test- To 1ml of leaf extract 2ml of 1% HCl was added and then boiled for a few minutes. After boiling two to three drops of Dragendorff's reagent was added and sample was observed for reddish-brown precipitate.

Wagner's test- 1ml of leaf extract 1 ml of 1% H_2SO_4 was added followed by few drops of Wagner's reagent. Formation of precipitate detects the presence of alkaloids.

Mayer's test- 1ml of leaf extract 2ml of 1% HCl and Mayer's reagent was added dropwise and was observed for the formation of precipitate.

Test for flavonoid: 2.5 mL of leaf extract, 1ml of 10% NaOH was added. From the side of the test tube drops of concentrated HCl was added, yellow colour turns to colourless which indicates presence of flavonoids.

Test for Anthraquinone: 1 ml of leaf extract was taken to which 2ml of 5% of KOH was added and was observed for pink coloration.

Test for Saponin: To 1ml of leaf extract 2ml of $NaHCO_3$ was added and on shaking it forms lather.

Test for Terpenoids: To 1ml of extract 400 micro litre of chloroform and four to five drops of concentrated H_2SO_4 was added from the walls of the test tube. Positive results form reddish-brown ring.

Test for Cardiac glycoside: To 2.5 ml of extract 2 ml of glacial acetic acid, few drops of $FeCl_3$ and concentrated H_2SO_4 was added from the walls of the test tube. Presence of cardiac glycoside is determined by a reddish-brown ring.

Test for Tannin: It can be observed by two methods:

Method A - 1 ml of extract was boiled and few drops of $FeCl_3$ were added to it. The sample was observed for blue, black, green colour.

Method B - To 1 ml of extract 500 micro litre of lead acetate was added which gives yellow colour.

Test for starch: To 1 ml of extract 500 micro litre of iodine was added, which results in blue colouration. **Test for Phlobatannin:** To 1 ml of extract 1% HCL was added and boiled, formation of Precipitate occurs on positive test.

Antioxidant activity

TLC is one of the most widely used and potent technique to resolve mixture of plant compounds. TLC sheet (Silica gel 60 F_{254}, Merck company, Germany) was used as stationary phase and solvents (BEA, CEF, EMW) was used as mobile phase. DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as a stable free radical molecule. The developed TLC plate was sprayed with 0.2% DPPH in methanol as an indicator.

Three types of solvent were prepared for TLC chromatography technique.

1. **BEA -**Benzene: Ethanol: Ammonium hydroxide (90:10:1) [Non polar/basic]
2. **CEF -**Chloroform: Ethyl acetate: Formic acid (5:4:1) [Intermediate polarity/Acidic]
3. **EMW -** Ethyl acetate: Methanol: water (40:4.5:4) [Polar/neutral]

Qualitative screening of the constituents in each of the plant extract of *Melia azedarach* for antioxidant activity was done by TLC analysis. The pre-coated TLC sheets were activated at 100^{o}C for 2 minutes. The samples were then spotted with the help of microtips leaving 2cm from the bottom of the sheet. All the above-mentioned solvents were used for TLC chromatography separation of solvent extracts. After drying of the sheet's DPPH solution was sprayed. Yellow/Orange bands in purple background represent antioxidant bands of extract.[8]

R_f values of all the antioxidant bands were calculated using the following formula:

$$\textbf{Retardationfactor } (R_f) = \frac{\textbf{Distance travelled by the compound}}{\textbf{Total distance travelled by the solvent}}$$

RESULT AND DISCUSSION

The whole plant of *Melia azedarach* was collected and the moisture content was estimated. For calculating moisture content fresh plant weight and dried powder weight of plant were recorded as follows:

Weight of the fresh plant sample = 538g

Weight of the dried powdered sample = 203g

Moisture content (%) = ($F_w - D_w / F_w$) x 100

= (538-203/538) x 100

= 62.26%

It was found that moisture content of the whole plant was 62.26%.

Yield of methanol was highest amongst the solvent's extracts followed by hexane, chloroform and ethyl acetate. Thus polar molecules were more in number in comparison to non polar.

Table 1: **Percentage of Yield.**

Solvent Extracts	Percentage of Yield
Hexane	3.185 %
Chloroform	1.315 %
Ethyl Acetate	1.255 %
Methanol	12.815 %

Phytochemical analysis of solvent extracts of Melia azedarach

The phytochemical tests were carried out to test the presence of secondary metabolites in the different extract (hexane, chloroform, ethyl acetate, methanol). As can be observed from Table 2. out of nine phytochemicals screened, six were present in various solvent extracts. These were flavonoid, saponin, terpenoid, cardiac glycoside, tannin and phlobatannin. Alkaloid, anthraquinone and starch were absent in all samples. Hexane showed 4 numbers of secondary metabolites i.e. flavonoid, terpenoid, cardiac glycoside, tannin. Chloroform contained 5 numbers of secondary metabolites i.e. flavonoid, terpenoid, cardiac glycoside, tannin, phlobatannin. Ethyl acetate showed 4 numbers of secondary metabolites i.e.

flavonoid, terpenoid, cardiac glycoside, tannin. Methanol contained 5 number of secondary metabolites i.e. flavonoid, saponin, terpenoids, cardiac glycosides, tannin. Thus, almost all extracts showed the presence of medicinally important class of compounds.

Table 2: Phytochemical Analysis of *Melia azedarach.*

Phytochemicals	Hexane	Chloroform	Ethyl acetate	Methanol
Dragendorff's test	-	-	-	-
Wagner's test	-	-	-	-
Mayer's test	-	-	-	-
Flavonoid	+	+	+	+
Anthraquinone	-	-	-	-
Saponin	-	-	-	+
Terpenoids	+	+	+	+
Cardiac Glycosides	+	+	+	+
Tannin A	+	+	+	+
Tannin B	+	+	+	+
Starch	-	-	-	-
Phlobatannin	-	+	-	-

TLC based Qualitative Antioxidant Assay

Qualitative antioxidant activity was conducted using TLC based DPPH assay. The presence of antioxidant compounds was detected by yellow spots against a purple background on TLC plate sprayed with 0.2% DPPH in methanol. As can be observed in Table 3, Maximum number of antioxidant bands were obtained in BEA solvent which is a nonpolar basic solvent suggesting that large number of antioxidant molecules are nonpolar but with of alkaline nature. Amongst the solvent extracts choloroform extract was richest in antioxidant molecules with 10 bands followed by hexane with 9 antioxidant bands. From the above results, it can be concluded that methanol and chloroform extracts carry medicinal properties and need further exploration.

Table 3: Qualitative TLCanalysis of solvent extracts of *Melia azedarach.*

Solvent extract	Solvent	Number of bands	Rf values
Hexane	BEA	9	0.775,0.625,0.55,0.487,0.35,0.325,0.162,0.087,0.05
	CEF	0	-
	EMW	0	-
Chloroform	BEA	10	0.612,0.537,0.475,0.4,0.362,0.325,0.275,0.162,0.112,0.07
	CEF	1	0.837
	EMW	3	0.775,0.625,0.325
Ethyl acetate	BEA	7	0.625,0.387,0.337,0.262,0.175,0.15,0.087
	CEF	3	0.9,0.875,0.775
	EMW	3	0.912,0.875,0.837

Methanol	BEA	1	0.1
	CEF	1	0.9
	EMW	2	0.9,0.85

ACKNOWLEDGEMENTS

Authors acknowledge the Forest, Environment and Climate change Department, Government of Odisha for providing funding for the smooth conduct of entire work and also acknowledge Chief Executive, Regional Plant Resource Center, Bhubaneswar, Odisha for providing lab facilities.

REFERENCES

1. Kannahi, M., Sangeetha, K., Madhavan, S., Umamaheshwari, N. Antimicrobial activity of *Azardirachta indica* A juss and *Melia azedarach*L against wound causing pathogenic microorganisms. *Advances in Plant Sciences,* 2009; 22(2): 605-606.
2. Jabeen, K., Javaid, A., Ahmad, E., Athar, M. Antifungal compounds from *Melia azedarach* leaves for management of Ascochyta rabiei, The cause of chickpea blight. *Nat. Prod. Res.*, 2011; 25(3): 264-276.
3. Ahmed, S., Idris, S. *Melia azedarach*. In: Hanum IF, Maesen LJB van der, eds. Plant Resources of South-East Asia. No. 11 Auxiliary plants. Indonesia. Leiden, Holland, Backhuys Publishers, 1997; 187-190.
4. Carpinella, C., Ferrayolli, C., Valladares, G., Defago, M., Palacios, S. Potent limonoid insect antifeedant from *Melia azedarach*. *Biosci. Biotech. Bioch*, 2002; 66(8): 1731- 1736.
5. Cropley, TG., Haseqawa, GR. *Melia azedarach*: New potential for an old medicinal plant. *J. Am. Acad. Derma*, 2007; 52(2): 366-367.
6. Nostro A., Germano, MP, Angelo, DV., Marino, A. and Cannatelli, MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol*, 2000; 30(5): 379-390.
7. Harborne, JB. Phytochemical methods: A guide to modern techniques of plant analysis. *Harborne JB (Ed).* Chapman and hall, London, UK., 1984.
8. Eloff, JN., Katerere, DR., Mcgaw, LJ. The biological activity and chemistry of the southern African Combretaceae. *J. Ethnopharmacol*, 2008; 119(3): 686-699.