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Phytochemical Analysis and Evaluation of Antimicrobial and Antioxidant Properties of *Mesua ferrea* L.: An Endangered Medicinal Plant

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ABSTRACT

The traditional medicines have been re-validated worldwide by extensive research on different medicinal plant species and their phyto-medicinal properties. The current investigation was carried out on *Mesua ferrea* L., commonly known as Nagakesara, to evaluate phytochemical composition and re-validate its therapeutic applications. The collected leaves and barks were properly shade-dried for 15 days and then powdered by using a kitchen mixer grinder. The phytochemicals were isolated through different extraction techniques using different solvent, out of which Hot extraction method i.e. through Soxhlet apparatus was most appropriate one. The qualitative and quantitative analysis and phytomedicinal properties were analyzed using different protocols. The qualitative analysis revealed the presence of wide rays of phytochemicals like phenol, flavonoids, tannin, steroids, glycosides, alkaloids, quinine, coumarin, saponin, sterol and terpenoids. The quantitative analysis showed the presence of high content of phenolic substances and flavonoids. The presence of high content of phenolic substance and other phytochemicals was responsible for maximum total antioxidant capacity of in the MFMBE (188.97%) followed by MFEBE and least antioxidant activity was found in MFLEAE. Highest antibacterial activity of inhibition zone i.e.16.3 mm was observed in the MFMBE followed by MFELE. The presence of theses phytochemicals was responsible for different phyto-medicinal properties. The high content of phenols/flavonoids makes this plant medicinally important and can be seen as a good source for useful drugs.

Key words: *Mesua ferreae*, phytochemicals, TLC, antioxidant, antibacterial

INTRODUCTION

Medicinal plants are rich in secondary metabolites of different classes like alkaloids, flavonoids, terpenoids, glycosides, etc. Since the beginning of human race, the presence of wide range of secondary metabolites has enhanced the importance of medicinal plants in pharmaceutical applications. Traditional medicines used in folk treatment have commended major attention of the modern day researcher towards plant phytochemistry. Although plant produced a large number of diverse bioactive compounds, some medicinal plants are still neglected, which need to be scientifically evaluated. *Mesua ferrea* is one of the important medicinal plants, commonly known as Nagakesar, indigenous in Asian countries like India, Srilanka, Nepal, Indo-China, Malaysia, Myanmar, etc. *M. ferrea,* belongs to the family Calophyllaceae, is a slow growing evergreen tree of 18-30 m tall and up to 70 cm in diameter. The flowers of the plants are white in colour and have attractive fragrant with a bunch of yellow stamens at the centre, which give the name Nagakesar. The fruits are dark brown in colour, ovoid to conical in shape and 2.5-5 cm long with a woody pericarp, which contain 1-4 seeds. The plant contains a wide range of phytochemicals which include coumarin, triterpenoids, xanthones, alkaloids, steroids and terpenes. The bark contained 4-akylcumarin, Ferone-b in the stamen of flowers, mesuaferone A, mesuaferone B, 1,5-dihydroxyxanthne (II), euxanthone 7-methyl ether and β-sitosterol (Adib *et al.*, 2019). Compounds, namely, isomammeisin, mesuagin and assamene were isolated from the seeds *of M. ferrea*. Friedelin and stigmasterol were later purified from the stem bark. This was followed by the identification of 12, 13-furano-8-hydroxynapthyl-6-0-β-2′,3′, 4′,6′-tetrahydroxy-5′5′ dimethylcyclohexyl ether from the leaves of *M. ferrea.* Similarly, mesuanic acid, α- and β-smyrin, β-sitosterol, 1,5-dihydroxyxanthone and euxanthone-7-

methyl ether were isolated from the stamens of the plant. Furanoxanthone, mesuaferrin-A and macluraxanthone were identified in the root bark, acting as strong anti-cancer agent. The presence of mesuaferrin B, caloxanthone C1, 8-dihydro-3-methoxy-6-methylantraquinone, and β-sitosterol was reported from the root bark of *M. ferrea*. The plant was extensively used in traditional Ayurvedic medicine against various diseases (Chaitanya *et al.*, 2015). The whole plant is consumed in tropical Asia and India. The dried flowers were used against fever, astringent, anti-inflammation, dysentery and Typhoid (De Filipps *et al.*, 2018). Stem bark, leaves and seeds are used as antioxidant, antimicrobial, analgesic, anti-venom, immune-modulator and anti-arthritic medicine. In siddha system of medicine, flower bud was used as carminative and astringent (Rajalakshmi *et al.*, 2019). Anti-diarrheal activity has been reported in the aqueous flowers extract of *M. ferrea* using Balb/C mice induced by *E. coli* (Puspitarini *et al.*, 2020). Mazumder *et al.* (2019) reported anti-diarrheal and anti-nociceptive activity of methanolic unripe fruit peels extract of *M. ferrea* on mice models. *M. ferrea* flowers methanolic extract (MFME, 200 mg/kg bw) showed anti diabetic activity and underlying mechanisms for its activity. In diabetic rats, MFME treatment significantly restored body weight and blood glucose levels to normal (Balekari and Veeresham, 2015). The plant is extensively used in folk medicine for various pharmacological actions and constitutes a part of the number of the of Ayurvedic medicine preparation such as Nagakesher yoga, Nagaeshar-adi-churna, eladichurna, lavangadichurna and dasamoolarishta. The search for new classes of antimicrobial compounds is required since there are still occurrences of resistance to all major classes of antibiotics. Plant is a very useful source to discover a new potential antimicrobial agent since it naturally produces secondary metabolites with highly diversified and unique chemical structures and mode of action in killing or controlling the growth of pathogenic bacteria. In the protection against numbers of diseases and delaying the ageing process antioxidants have played a crucial role. Antioxidants have the power to scavenge the free radicals, which are responsible for cell damage in human. The advantages of antioxidants from plants are probably because of their protective effects against the radicals which are reactive oxygen species (ROS). Though this plant is used as constituents of different medicine only a few reports were available on its phytochemical analysis and phytomedicinal properties. On the basis of above fact, the present study was carried out to evaluate its phytochemical composition in different extract and analysis of antibacterial and antioxidant properties.

MATERIALS AND METHODS

The aerial parts of plant samples (leaves and barks) were collected from the plants grown in the Athagada Reserve Forest of Ganjam district, Odisha and authenticated by Dr. P. C. Panda and were cross verified with the digital Herbarium (Accession No. 505) of Regional Plant Resource Centre, Bhubaneswar, Odisha. The leaves and barks collected were properly shade- dried for 15 days and then powdered by using a kitchen mixer grinder. The extract was prepared in different solvents like water, methanol, ethanol, chloroform and ethyl acetate by taking 20 g of powdered sample using Soxhlet apparatus. The apparatus was run about 6-8 h. The extracts were concentrated to dryness using rotary evaporator maintaining a temperature of 50°C and stored for future use (Fig. 1).

Both the qualitative and quantitative analysis of different extracts was carried out to find out the diversity of phytochemicals and their quantitative estimation. The qualitative phytochemical analysis of plant extract for different bioactive compounds was made following Chaitanya *et al.* (2015).

One ml of plant extract was taken in a test tube. Then 2 ml of double distilled water was added to the test solution followed by addition of few drops of 10% $FeCl_3$. The colour of the test solution changed to blue indicating the presence of phenols. Two ml of 2% NaOH solution was added to the extract. Formation of intense yellow colour as well as colourless after addition of dilute acid, indicated the presence of flavonoids. Ferric chloride ($FeCl_3$) solution was added drop by drop to the extract. Bluish black precipitate was found in the test tube indicating presence of tannins. Concentrated H_2SO_4 and chloroform were added to the extract and shaken well.

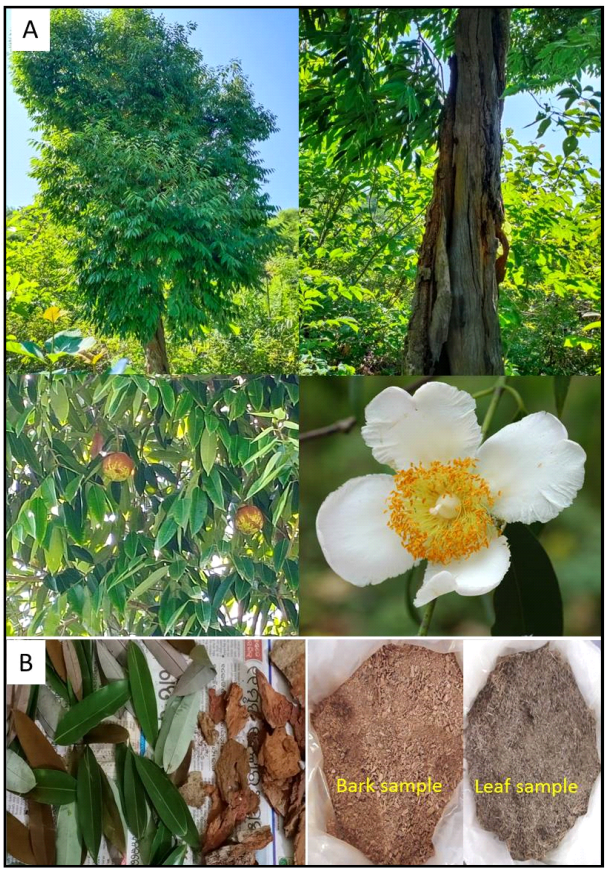

Fig. 1. *M. ferrae* plant (A) growing in natural habitat showing leaves, fruits and flowers (B).

Appearance of reddish brown colour in the lower layer indicated steroids. Similarly, the extract was boiled after addition of a few drops of acetic anhydride then the mixture was cooled. After the mixture was cooled, concentrated sulphuric acid was added slowly to the sides of test tube. A brown ring was formed at the junction of the two layers indicating the presence of steroids. Equal amount of chloroform and acetic acid was added to the 5 ml extract. The mixture was cooled then H_2SO_4 was carefully added, the colour changed from violet blue to green indicating presence of glycosides. To each 2 ml of filtrate a few drops of Hager's reagent were added and the observation of yellow precipitation indicated the presence of alkaloids. To each 2 ml of filtrate a few drops of Wagner reagent (solution of potassium iodide) were added, the formation of reddish brown precipitate revealed the presence of alkaloids. Five ml of extract was treated with concentrated HCl. Formation of yellow precipitate indicated the presence of quinones. Similarly, Five ml of extract was treated with concentrated H_2SO_4 for the formation of red colour indicating quinines 10% NaOH followed by chloroform was added to the extract for the formation of yellow colour indicating the presence of coumarins. Five ml of extract was shaken vigorously with water and warmed, formation of stable foam indicated saponins. Few drops of conc. H_2SO_4 were added to the test solution. Shaked well and allowed to stand, the lower layer turned red indicating the presence of sterols. Similarly, 5 ml of test solution were taken in a test tube, then few drops of acetic anhydride were added and the solution was mixed. Then conc. H_2SO_4 was added to the test solution from the side of the test tube. Formation of brown ring at the junction of the two layers and the upper layer turning green indicated the presence of sterol. Five ml of 1 mg extract were mixed with 2 ml of chloroform. Then 3 ml of concentrated H_2SO_4 were added carefully to form a layer, the formation of reddish brown interface implied the presence of terpenoids.

Folin Ciocalteu reagent was used as oxidising agent and galic acid was used as a standard in determination of total phenolic content in the plant extracts. Plant extracts were solubilized with 2% DMSO (Di methyl sulfoxide). Briefly 0.5 ml of plant extracts (2 mg/ml) were mixed with 1.5 ml (1:10 V/V diluted with distilled water) of Folin Ciocalteu's reagent and allowed to stand for 5 min at room temperature. Then 2 ml of sodium carbonate (Na_2CO_3, 7.5% W/V) was added and the mixture was kept steady for another 90 min, and kept in dark with intermittent shaking. The absorbance of blue colour developed was measured at 760 nm using UV spectrophotometer. The experiment was carried out in triplicates. Galic acid was used for constructing the standard curve (50-500 µg/ml) and the total phenolic concentrations in the plant extracts were expressed as milligrams of galic acid equivalent per gram of dry plant weight (mg of GAE/g).

The flavonoids content was determined according to aluminium chloride reducing method as described by Akhtar *et al.* (2017). The sample was prepared by taking 0.2 ml of plant extract in 0.6 ml methanol, 40 µl aluminium chloride (10%), 40 µl 1 M potassium acetate and the final volume was made up to 2 ml with distilled water. The sample mixture was incubated at room temperature for 30 min. Then the absorbance was taken at 420 nm. Quercetin was used as the standard.

The isolated phytochemicals were fractioned through column chromatography using silica gel as the matrix and different solvents and different fractions were collected. The isolated fractions were further used for identification of phytochemicals through thin layer chromatography using standard protocol. The TLC was performed on precoated 20×20 cm and 0.25 mm thick plates. The TLC plates were prepared by using silica gel G and left overnight for air drying. These plates were activated by hot air oven at 100°C for 1 h. Fractions of the extracts were spotted on TLC plates using a capillary tube. The plates were dried and run in the suitable solvents and dried at room temperature. Derivatisation of TLC plates was done by UV trans-illuminator. Different bands were observed and corresponding Rf values were determined for each spot by the formula:

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

The assay was made through DPPH free radical scavenging assay. This assay was based on the reduction of DPPH to non-radical form of DPPH in presence of hydrogen donating antioxidant constituents of plant extracts. The reduction of DPPH was associated with a colour change from purple to yellow. An aliquot of 1 ml, 0.3 mM DPPH ethanolic solution was added to 2.5 ml of plant extract and incubated at room temperature in dark condition. The absorbency was taken at 517 nm. Ethanol was used as the blank and DPPH along with ethanol used as the control. Ascorbic acid (100 µg/ml) was used as standard.

% of DPPH reduction = 100 x [(Absorbance of control–Absorbance of test sample)/(Absorbance of control)]

Antibacterial activity of various extracts was studied through agar-well diffusion method. The bacterial strains i.e. Bacillus collected from the Department of Microbiology, OUAT, Bhubaneswar was used to study the antibacterial properties of different plant extracts. The bacterial culture 106 cfu/ml each strain was spread on nutrient agar plate with a sterile glass spreader. Subsequently, wells of 8.0 mm were punched into the agar medium using a sterile borer and the wells were filled with 200 µl of plant extract and incubated at 37°C for 24 h. Same volume of solvents was used as –ve control. Standard antibiotics disc was used as the +ve control. After incubation, the diameter of the growth inhibition zones was measured in mm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth micro-dilution assay.

RESULTS AND DISCUSSION

Plants and herbs are an integral part of Ayurveda and Unani medicine. The plants have been used effectively and efficiently against severe disease due to presence of different phytochemicals. *M. ferrea* is the only species from the genus of *Mesua*, which has been chemically studied. The qualitative phytochemical analysis of plant extract isolated from the bark and leaf sample in five different solvents of various polarities was carried out to assess the presence of different bioactive compounds. The screening of phytochemicals in different solvent extracts showed the presence (+) and absence (-) of different compounds (Table 1). Tannins were found to be absent in ethyl acetate and ethanol extract, whereas coumarin was not found in aqueous, methanol and chloroform extract. Whereas all other extracts showed presence of phenol, flavonoids, tannin, steroids, glycosides, alkaloids, quinine, coumarin, saponin, sterol and terpenoids.

The quantitative phytochemical analysis of plant extract isolated from the bark and leaf sample in eight different solvents of various polarities was carried out to quantify different active compounds through spectroscopy.

Quantification was made using gallic acid as the standard. The phenolic content was expressed as mg of gallic acid equivalent (GAE) per g of sample. The results showed that the phenolic content was high in *M. ferrea* methanolic bark extract (27.24) followed by ethanolic bark extract and minimum quantity i.e. 09.27 was obtained from the ethyl acetate leaf extract (Fig. 2A).

Total flavonoids content was quantified using quercetin as the standard and the total flavonoid was expressed mg equivalents of quercetin per g of the crude plant extract. Maximum total flavonoid content (188.97) was found in the methanolic extract and a

Table 1. Qualitative phytochemical analysis of leaf and bark extract of M. ferrea. The extracts were prepared in different solvents through Soxhlet extraction method

S. No.	Phytochemical groups	Aqueous		Methanol		Ethyl acetate		Ethanol		Chloroform	
		L	B	L	B	L	B	L	B	L	B
1.	Phenol	+	+	+	+	+	+	+	+	+	+
2.	Flavonoids	+	+	+	+	-	-	-	-	+	+
3.	Tanin	+	+	+	+	+	+	+	+	+	+
4.	Steroids	+	+	+	+	+	+	+	+	+	+
5.	Glycosides	+	+	+	+	+	+	+	+	+	+
6.	Alkaloids	+	+	+	+	+	+	+	+	+	+
7.	Quinine	+	+	+	+	+	+	+	+	+	+
8.	Coumarin	-	-	-	-	+	+	+	+	-	-
9.	Saponin	+	+	+	+	+	+	+	+	+	+
10.	Sterol	+	+	+	+	+	+	+	+	+	+
11.	Terpinoid	+	+	+	+	+	+	+	+	+	+

+ indicated presence and - indicated absence of phytochemical group. L was leaf, and B was bark.

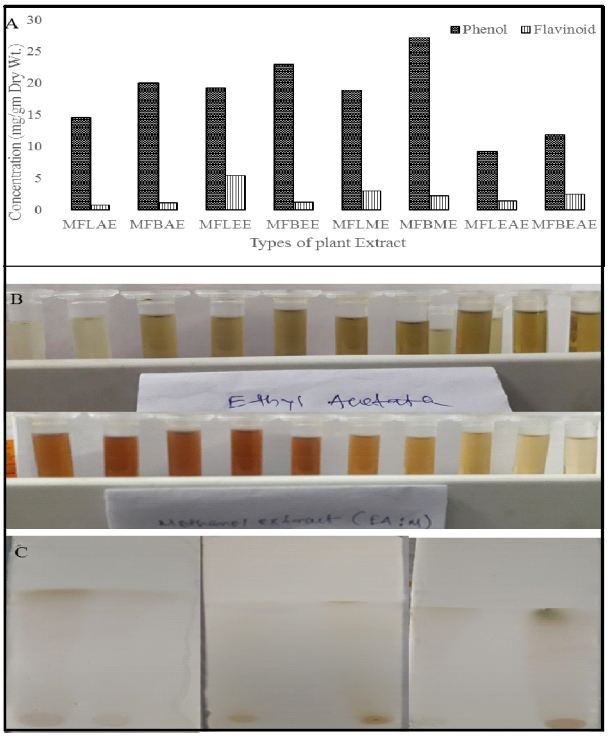

Fig. 2. Qualitative analysis of phenol and flavonoids contents in plant extract of *M. ferrea* using different solvents (A); representative photographs of column chromatography of different plant extracts using (B); and representative photograph of separation of different fractions through TLC (C).

minimum quantity was observed in ethyl acetate leaf extract.

Plant extracts isolated from leaf and bark samples in different solvents were fractionated through column chromatography using different mobile phase. Out of different mobile phase tested, the solvent of Iso-butanol : glacial acetic acid : water (4:1:1) was found more suitable for separation of phyto-constituent in different fractions (Fig. 2B). Each fraction was spotted over the TLC plate and the pigments were separated through TLC, then the R_f value was calculated and it was observed that the fraction contained mostly carotenoids and phenolic compounds (Fig. 2C).

A wide range of antioxidant activities was found in plants due to the presence of wide rays of secondary metabolites like phenolic compounds, flavonoids, terpenoids, etc. *M. ferrare* was found to be a good source of phenolic compounds and flavonoids for which it was also showed high total antioxidant activity. Maximum antioxidant activity was found in the MFMBE (188.97) followed by MFEBE and least antioxidant activity was found in MFLEAE (Fig. 3A).

The clear zones of inhibition of bacterial growth were observed on the plates after the incubation period. The bacterial growth inhibition was due to antibacterial compounds present in the tested sample. The MIC value for different extracts ranged from 1.3-0.313 mg/ml and the MBC value was around 2.5 mg/ml. Maximum size of inhibition zone i.e. 16.3 mm was observed in the MFMBE followed by MFELE (Fig. 2). Minimum growth inhibition zone of bacteria was found in the MFALE i.e. 8.2 mm (Fig. 3B).

In the current investigation, the plant extracts of *M. ferrea* prepared in different solvents and extracted through different methods has shown the presence of a wide range of phytochemicals like phenols, flavonoids, glycosides, saponins steroids, terpenoids and alkaloids in the qualitative phytochemical analysis. Similar results were reported by earlier researchers like Chaitany *et al.* (2015). The production of such a huge range of

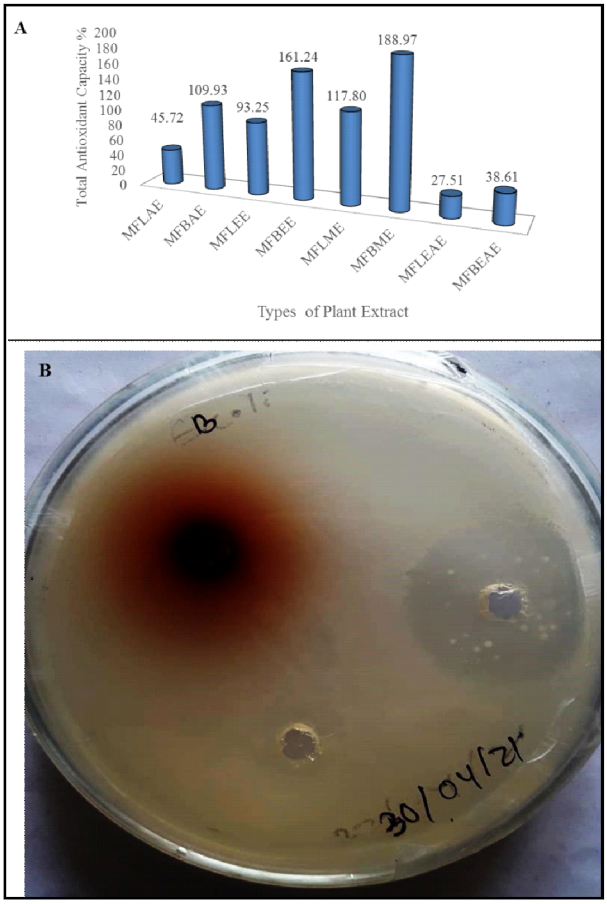

Fig. 3. Total antioxidant capacity of plant extract of *M. ferrae* isolated in different solvents (A) and representative photograph showing antibacterial activity of methanolic plant extracts of *M. ferrae* against Baccilus strain (B).

secondary metabolites by this plant was due to the environmental stress faced by plants in their natural habitat. The presence of theses phytochemicals is responsible for different phyto-medicinal properties. The quantitative phytochemical analysis revealed that the extract contained more phenolic compounds. The phenolic compounds such as flavonoids and phenolic acids, etc. were responsible for antioxidant activities of the medicinal plants. In the current study, maximum antioxidant activity was found in the MFMBE (188.97) followed by MFEBE and least antioxidant activity was found in MFLEAE. This was due to the maximum concentration of phenolic substances in MFMBE than that of the other extracts. In response to the microbial infection the hydroxylated phenolic substances known to be flavonoids synthesis was increased in plants. The increase in concentration of flavonoids had shown an antimicrobial property against a wide range of microorganisms. The results showed that MFMBE followed by MFELE and MFALE showed antibacterial properties against the Bacillus bacteria; this may be due to the binding with the extracellular soluble proteins and to complex with bacterial cell wall. Antibacterial activity was also reported in the aqueous extract of bark and seed (Bhatt *et al.*, 2022). The current results suggested that the identified phytochemical compounds might be the bioactive constituents and these plants proved to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Similar results were also reported by Patangia *et al.* (2023) i.e. pharmacological actions of Nagkesar were revealed due to the presence of secondary metabolites like coumarins and their derivatives and xanthones with their derivates.

CONCLUSION

Presence of different medicinally important constituents in *M. ferrea* contributed towards medicinal as well as physiological properties of the plants. The high content of phenols/ flavonoids made this plant medicinally important as reported earlier also. Therefore, this plant could be seen as a good source for useful drugs. The traditional medicine practice recommended for these plants as well as suggested that further work should be carried out to isolate, purify and characterize the active constituents responsible for the activity of these plants. In order to elucidate the possible mechanism of action of these extracts, further research should be encouraged.

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