

The quantification of phytochemical constituents and *in-vitro* antioxidant activity of the crude extracts of *Mesua ferrea* L. (seeds)

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Abstract

Objective: To investigate the quantification of total phenolic, flavenoid content and *in-vitro* antioxidant activity of the hexane and ethanol extracts of *Mesua ferrea* (Seeds).

Methods: The quantification of the total phenolic and flavonoid contents were estimated by taking gallic acid and rutin as a standard; *In-vitro* antioxidant activity was evaluated for extracts by using DPPH assay.

Results: The hexane extract of *M. ferrea* (Seeds) have more phenolic and flavonoid content than other extracts. The selected plant extracts were produced concentration dependent percentage inhibition of different free radicals and produced maximum activity at a concentration of 700 µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations.

Conclusion: In the present study we found that the extracts of *M. Ferrea* (Seeds) showed good antioxidant activity. Among the two extracts Hexane extract showed better activity than the other extract.

Keywords: *Mesua ferrea* L. (Seeds), Phenolic content, flavenoid content, *In-vitro* antioxidant activity

1. Introduction

Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent against several disease, no side effects and economic viability. Several compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, antiinflammatory, anti-carcinogenic etc[1-4]. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids which prevent free radical damage, reducing risk of chronic diseases. Living cells may generate free radicals and other reactive oxygen species byproducts as a results of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans [5]. *Mesua ferrea* (*M. ferrea*) (Ceylon ironwood, Indian rose chestnut is a species in the family Calophyllaceae, many parts having medicinal properties. It enhances the complexion. It leads to fragility transparency to the skin. The flowers are acrid, anodyne, digestive, constipating, and stomachache. They are useful in conditions like asthma, leprosy, cough, fever, vomiting and impotency. The seed oil is considered to be very useful in conditions like vata and skin diseases.

The goals of the present study on the crude extracts of *M. Ferrea* L. (seeds) were the following: (i) to determine the total phenolic and flavonoid content, and (ii) to estimate the antioxidant activity by DPPH radical scavenging assay.

2. Materials and Methods

2.1. Chemicals

The organic solvents used in the experiments were of analytical grade and purchased from Qualigen Chemicals, India. The other chemicals used were of analytical grade and obtained from Merck, India.

2.2. Plant sample

Air-dried seeds of *M. Ferrea* L. were collected from the local spice market of District Ujjain, India. Plant samples were duly authenticated by department of Botany, Vikram University, Ujjain, India.

2.3. Extraction procedure

The plant samples were washed several times with tap water and finally with distilled water to remove dust. The samples were dried under shade at room temperature. The seeds were separated from dried pods by crumbling and then screening. The shade dried seeds were further ground by means of a mechanical blender (Bajaj GX10, India) to fine powder. One hundred grams of the seed powder was sequentially extracted for 3 days with each solvent hexane (500 mL ×3) and ethanol (500 mL×3) using a Soxhlet apparatus over a water bath. The extracts obtained were filtered through Whatman No. 1 filter paper and then evaporated to dryness by using a rotary evaporator (Buchi, Switzerland). The final crude extracts were collected in an airtight container and then refrigerated at $4 \pm 2^\circ\text{C}$ until further use.

2.3. Quantification of the total phenolic contents (TPC)

The concentration of the phenolics in the plant extracts was determined using the Folin Ciocalteu assay [6]. In brief, 1 mL of extracts (1 mg/mL in methanol) or a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to a 25-mL volumetric flask containing 9 mL of distilled water. One millilitre of Folin Ciocalteu reagent was added to the mixture and then shaken. After 5 min, 10 mL of a 7% Na_2CO_3 solution was added to the mixture. The final volume was brought up to 25 mL by adding double distilled water and then mixed. After 90 min of incubation at room temperature ($23 \pm 2^\circ\text{C}$), the absorbance was determined against a blank at 750 nm (UV-2550, Shimadzu spectrophotometer). The total phenolic content was calculated using a calibration curve for gallic acid ($R^2 = 0.981$). The results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate.

2.4. Quantification of the total flavonoid contents (TFC)

The total flavonoid content of the crude extracts was determined using the aluminium chloride colorimetric method [7]. In brief, 3 mL of the extract (1 mg/mL in methanol) or a standard solution of rutin (20, 40, 60, 80 and 100 mg/L) were mixed with 5 mL of 2% AlCl_3 in methanol. After a 60-min incubation at room temperature ($23 \pm 2^\circ\text{C}$), the absorbance against blank was determined at 510 nm (UV-2550, Shimadzu spectrophotometer). The total flavonoid content was calculated using a calibration curve for rutin ($R^2 = 0.985$). The results were expressed as the rutin equivalent per gram of dry weight of extract (mg of RU/g of extract). All samples were analyzed in triplicate.

2.5. In-vitro antioxidant activity

The ability of the plant extracts to scavenge DPPH free radicals was assessed using the standard method [8]. Aliquots (2 mL) of various concentrations (62.5–1000 g/mL) of the plant samples were added to 2 mL of a 0.004% methanolic solution of DPPH. After an incubation period of 30 min in darkness at room temperature ($23 \pm 2^\circ\text{C}$), the absorbance was recorded against a blank at 517 nm (UV-2550, Shimadzu spectrophotometer). Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the control. where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. BHT (butylated hydroxyl toluene) was used as a positive control. Samples were analyzed in triplicate.

$$\% \text{ DPPH inhibition activity} = [A_0 - A_1/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. BHT was used as a positive control. Samples were analyzed in triplicate.

3. Results and Discussion

3.1. Percentage yield and total phenolic content and total flavonoid content

Comparing both of the extracts of *M. Ferrea* (seeds), the extraction yield was almost double with hexane than with ethanol (Table 1). The amount of the total phenolic content and total flavonoid content ranged 10.10–40.14 gallic acid equivalents (GAE mg/g) and 12.91–42.64 rutin equivalents (RU mg/g) of dry weight of extract, respectively (Table 1). The efficiency of the solvents to extract phenolic and flavonoid compounds was in the order of hexane > ethanol.

Table 1: Percentage yield, total phenolic content (TPC) and total flavonoid content(TFC) of crude extracts of *M. Ferrea* L. (Seeds)

Extract	% yield	TPC (GAE mg/g) of dry weight of extract	TFC (RU mg/g) of dry weight of extract
Hexane	14.2	40.14±0.72	42.64±0.26
Ethanol	7.4	10.10±0.45	12.91±0.10

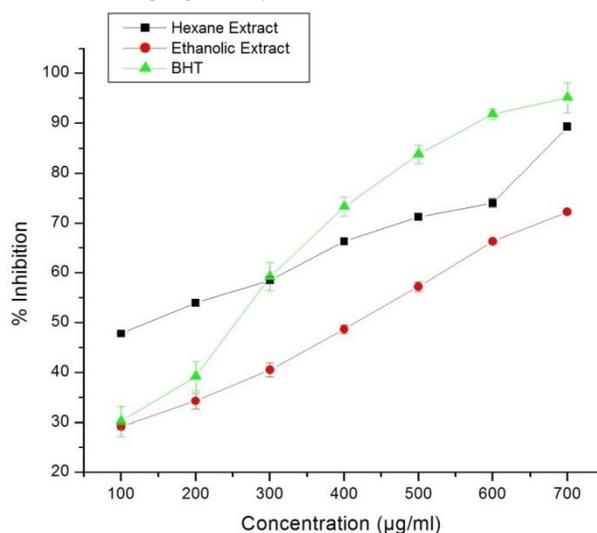
TPC and TFC values are mean± standard deviation of three separate experiments (P <0.05).

Phenolic compounds are secondary metabolites that can act as antioxidants due to their ability to donate hydrogen, quench singlet oxygen and act as metal chelators [10]. Flavonoids consist of a large group of polyphenolic compounds, have a benzopyrone structure, and provide benefits in multiple ways to the plant producing them [9]. Flavonoids are found to be very useful as an antimicrobial agent, a mitochondrial adhesion inhibitor, an antiulcer agent, an antiarthritic agent, an antiangiogenic agent, and an anticancer agent [11]. It has been confirmed that consumption of phenolic rich foods or beverages prevent diseases, such as cancer, heart disease, inflammation, arthritis, immune related diseases, neurodegenerative diseases and diabetes [12]. In the present study, the presence of phenolic and flavonoid compounds in *M. ferrea* seeds confirm the health benefits associated with it.

3.2. Antioxidant activity by DPPH assay

The hexane extract exhibited 89% DPPH radical scavenging activity at a concentration of 1 mg/mL, while for the ethanol extract at the same concentration, 70% DPPH radical scavenging activity was observed (Figure 1).

Figure 1: DPPH radical scavenging ability of the crude extracts of *Mesua ferrea* L. (Seeds).



The DPPH assay is a very common spectro-photometric method to determine the activity of any antioxidant. The advantage of this method is that the antioxidant activity is measured at ambient temperature, and thus, the risk of thermal degradation of the molecule tested is eliminated [13]. Free radical scavenging activity is one of the known mechanism by which antioxidants inhibit lipid oxidation [14].

The result of scavenging activity assay in this study indicates that the plant was potently active. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.

The plant extracts were capable of scavenging DPPH in a concentration dependent manner. The data clearly indicated that the extracts, hexane and ethanol of *M. Ferrea L.* (Seeds) showed good antioxidant activity. Among the two the hexane extract showed better activity.

Acknowledgements

The authors thank department of biochemistry university of Kashmir, (India) for performing the DPPH assay and also thank Dr. B.K mehta, Head, Department of chemistry, vikram university Ujjain , M.P (India) for providing the laboratory facilities.

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