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INVESTIGATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY OF *MESUA FERREA* L. SEED OIL

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ABSTRACT

The paper describes the *in-vitro* antioxidant activity of seed oil extracted from seeds of *Mesua ferrea* L. The plant extract has been found to possess various pharmacological activities which have been proved scientifically based on the ethnomedical data. The *in-vitro* antioxidant activity of *M. ferrea* seed oil (MFSO) was evaluated. The EC_{50} values of free radical scavenging activity (10.25 µg/ml), $ABTS^{+}$ (652.5025 µg/ml) discoloration assay and nitric oxide scavenging activities (13.17 25 µg/ml) and determination of total phenolic content (121.5 µg GAE/ml), total antioxidant content 241.25 µg GAE/ml and total flavonoids content (66 µg QE/ml) in MFSO indicated its potential antioxidant activity.

INTRODUCTION: Physicians generally prefer to take food rich in phenolic compounds because of their ability to prevent the incidence of cardiovascular diseases, neurodegenerative diseases like Alzheimer and Parkinson diseases and even cancer [1]. The phenolic components such as flavanoids, phenolic acids and phenolic diterpenes shows strong antioxidant properties due to their redox properties [2], which can play important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides [3].

The most commonly used antioxidants at present are butylated hydroxyanisole (BHA), butylatedhydroxy toluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) [4]. However they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals [5]. Therefore the development and utilization of most potent antioxidants of natural origin are required, therefore the food containing phenolics from herbal origin are increasingly of interest in food industry as they prevent oxidative degeneration of lipids and improve the nutritional value of food.

Mesua ferrea (Nagakesar) is traditionally being used as a carminative, expectorant, cardiotonic, diuretic, antipyretic, antimicrobial, wound healing and and as a treatment for colds and asthma. It has been scientifically evaluated for hepatoprotective and antivenom, anticancer, antiulcer, anti-inflammatory, antiasthmatic and antirheumatic activity. The fixed oil is used for cutaneous infection, wounds, rheumatism and mesuol isolated from seed oil was proved for analgesic and anti-inflammatory and *in-vivo* antioxidant and immunomodulatory activity by our research group [6].

In the present study, the investigation was made to investigate the *in-vitro* antioxidant property of *Meusea ferrea* seed oil (MFSO).

MATERIALS AND METHODS:

Plant material: The seeds of *Mesua ferrea* were collected in August 2010 from Shimoga, Karnataka, India and authenticated by Prof. K. Siddappa, Department of Botany, Sree Siddaganga Boy's College, Tumkur (Karnataka), India. A voucher specimen is preserved in college herbarium (SSCP11PC0010).

Chemicals: 1, 1-diphenyl1-2-picryl-hydrazil (DPPH), Methanol, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonicacid) diammoniumsalt ($ABTS^{+}$), Sodium Nitroprusside, Potassium persulphate, Sulfanilamide, Phosphoric acid, N-(1-naphthyl) ethylenediamine dihydrochloride, Aluminium chloride, Potassium acetate, Folin-Ciocalteu reagent, Sodium carbonate, Sulphuric acid, Sodium phosphate, Ammonium molybdate, Gallic acid, Quercetin. All the chemicals used were of analytical grade.

Extraction of seed oil: The sun dried seeds were coarsely powdered by pulverization method, 100 g seeds were extracted with 500 ml of petroleum ether by soxhlet apparatus for 6 h, the residue was removed by filtration and concentrated. The concentrated extract was transferred to china dishes and allowed to dry. The final percentage yield of the extract was 70%. The extract was liquid, brownish in color. The preliminary phytochemical screening confirmed the presence of flavanoids and triterpenoides.

IN-VITRO ANTIOXIDANT ACTIVITIES:

1. **Free Radical Scavenging Activity:** The antioxidant activity of MFSO and the standard were assessed on the basis of the radical scavenging effect on the stable DPPH free radical. Briefly, 0.1mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 3 ml of solution of different concentration of MFSO (0.515-16.5 µl) in methanol at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The DPPH radical concentrations were calculated using the following equation [7]:

$$\text{DPPH Scavenging Effect} = 100 - [(A_0 - A_1/A_0) \times 100]$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2. **$ABTS^{\cdot+}$ Radical Cation Discoloration Assay:** The $ABTS^{\cdot+}$ cation radical was produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulphate, stored in the dark at room temperature for 12h. Before usage, the $ABTS^{\cdot+}$ solution was diluted to get an absorbance of 0.700 ± 0.025 at 734 nm with phosphate buffer (0.1M, pH 7.4). Then, 1ml of $ABTS^{\cdot+}$ solution was added to 3 ml of solution of different concentration of MFSO (133.92-1071.4 µl) in methanol at different concentrations. After 30 min, the percentage inhibition at 734 nm was calculated for each concentration relative to blank absorbance (methanol). The scavenging capability of $ABTS^{\cdot+}$ radical was calculated using the following equation [8]:

$$ABTS^{\cdot+} \text{ Scavenging Effect} = 100 - [(A_0 - A_1/A_0) \times 100]$$

3. **Nitric Oxide Radical Scavenging Activity:** Sodium nitroprusside (10 mM) and phosphate-buffered saline were mixed with different concentrations of MFSO (1.04- 33.33 µl) incubated at 37°C for 150 min. After the incubation, 0.5 ml of Griess reagent was added. The absorbance of the chromophore which was formed in the reaction was measured at 546 nm against blank [9].

4. **Total Antioxidant Activity:** The total antioxidant capacity of the seed oil was determined with phosphomolybdenum using gallic acid as the standard. An aliquot of 0.1 ml of solution of different concentration of MFSO (0.52- 16.66 µl) in methanol was combined with 1.0 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min.

   After cooling, the absorbance was measured at 695 nm against the blank using an UV-Visible spectrophotometer. The total antioxidant capacity was expressed as µg equivalents of gallic acid by standard gallic acid curve. The standard curve was prepared using 50, 75, 100, 125, 150, 175, 200, 225 and 250 µg/ml solutions of gallic acid in methanol [10].

5. **Total Flavanol Determination:** Aluminum chloride colorimetric method was used for flavonol determination. The solution of different concentration of MFSO (0.52- 16.66 µl) was mixed in methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The prepared solution was left to stay for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer. Total flavonoid values were expressed as quercetin equivalents (mg/g of dry mass), which is a common reference compound for flavonoids and standard curve was prepared using 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml solutions of qurcetin in methanol [11].

6. **Phenolic Content Determination:** The total phenolic content was determinated by Folin-Ciocalteu's colorimetric method. The solution of different concentration of MFSO (0.52- 16.66 µl) or gallic acid was mixed with 450 µl of distilled water and then 2.5 ml of Folin-Ciocalteu's reagent 0.2 N was added. The mixtures were allowed to stand for 5 min, and then 2 ml of aqueous Na_2CO_3 (75 g/L) was added. After incubation of the resulting reaction mixtures (90 min/30° C) the total phenols were determined by colorimetry at 765 nm. Total phenol values were expressed as gallic acid equivalents (µg/ml) [2].

RESULTS AND DISCUSSION: The results of antioxidant activity of MFSO by various methods are shown in **table 1**.

TABLE 1: ANTIOXIDANT ACTIVITY OF MFSO BY DIFFERENT METHODS

Method	DPPH (EC_{50})	ABTS (EC_{50})	NO (EC_{50})	Total antioxidant	Total flavanoids	Total Phenols
Value	10.25 µg/ml	652.5 µg/ml	13.17 µg/ml	241.25 GAE	66 QE	121.25 GAE

GAE – Gallic acid equivalents; QE – Quercetin equivalents.

Free Radical Scavenging Activity: With this method it is possible to determine the anti-radical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm, resulting a colour change from purple to yellow. The $DPPH^{\cdot}$ was scavenged when the absorbance decreased by an antioxidant through donation of hydrogen ion and form a stable $DPPH^{\cdot}$ molecule. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. MFSO is effective antioxidant with EC_{50} value 10.25 µg/ml but less than compared to BHT and ascorbic acid which are termed as positive controls.

$ABTS^{\cdot+}$ Radical Cation Discoloration Assay: The basis of this method is to monitor the decay of the radical cation $ABTS^{\cdot+}$ produced by the oxidation of 2, 2' azinobis (3-ethyl-benzothiazoline-6-sulphonate) caused by the addition of antioxidants [12]. MFSO showed very high EC_{50} value of 652.5 µg/ml when compared with DPPH. Usually the antioxidant activities against $ABTS^{+}$ were correlated with concentration, chemical structures and polymerization degree of organ antioxidants [13].

It is known that some compounds which have $ABTS^{+}$ scavenging activity did not show DPPH scavenging activity. In this study, the MFSO showed strong scavenging activity against DPPH and ABTS radicals. This further showed the capability of the MFSO to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical related pathological damage.

Nitric Oxide Radical Scavenging Activity: The scavenging of nitric oxide by MFSO was increased in a dose-dependent manner. The nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The MFSO inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide.[14] MFSO showed EC_{50} value of 13.17 µg/ml.

Total Antioxidant Activity: The antioxidant activity of MFSO was expressed as the number of gallic acid equivalents. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm.

The study revealed that the total antioxidant activity of MFSO was 241.25 GAE. This antioxidant activity of MFSO might be attributed to the presence of phyotochemicals such as phenolic compounds [15].

Total Flavanoids Determination: Total flavanoid content of MFSO is 66 QE. The capacity of flavanoids to act as antioxidants depends upon their molecular structure. Flavonoids show antioxidant effect as they contain hydroxyl functional groups. The position of hydroxyl groups and other features in the chemical structure of flavanoids are important for their antioxidants and free radical scavenging [16].

Total Phenolic Content Determination: Phenolics are the most important constituents in MFSO. Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals [17]. Total phenolic content of MFSO was found out to 121.25 GAE.

CONCLUSION: Therefore, it could be concluded that the phenolic compounds were highly involved in the antioxidant activity found in MFSO and also able to enhance or complement their activity.

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