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PHYTOCHEMICAL SCREENING AND RADICAL SCAVENGING ACTIVITY OF MEDICINALLY IMPOTANT PLANT : *MESUA FERREA*

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ABSTRACT : Ethanolic extract of *Mesua ferrea* (Nagkesar) flower was prepared and it's *in vitro* phytochemical composition and radical scavenging activity was carried out of different parameters. Saponin, flavanoid, terpenoid and tannin are found as major phytoconstituents in flower ethanolic extract of *Mesua ferrea* flower. Study also reflected radical scavenging activity in *in vitro* assays. It showed a concentration dependent increase in radical scavenging activity. Outcome of the present study suggested assessing the *Mesua ferrea* at its *in vivo* level to develop it a good phytotherapeutic system.

Key words : *Phytoconstituent, Radical scavenging, Ethanolic extract, Mesua ferrea, In vitro assay.*

INTRODUCTION

Bioactive compounds in plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. Secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes for compounds associated with plant growth and development, and are regarded as products of biochemical "side tracks" in the plant cells and not needed for the daily functioning of the plant. In Ayurveda flowers and their extracts and ashes were used as medicine to cure many ailments related to human physiology like diabetes (Sarkara vikara), Jundis (Pandu roga), Fever (Jwara) and Weakness (Durbalata) etc.

Current day physiological practice becomes modernized in terms of disease on set and treatment. Free radicals can be defined as reactive chemical species having a single unpaired electron in an outer orbit (Riley,1994). The majority of free radicals that damage biological systems are oxygen-free radicals, and these are more generally known as "reactive oxygen species" (ROS). The endogenous sources of ROS include mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation (Inoue *et al.*,2003). Free radical stress leads to tissue injury and can eventually to arthritis, atherosclerosis, diabetes mellitus, neurodegenerative diseases and carcinogenesis. Several studies are ongoing worldwide to find natural antioxidants of plant origin.

The extracts of flowers and roots of *P.venusta* contain significant amounts of phytochemicals with antioxidative properties and could serve as inhibitors or scavengers of free radicals. *P.venusta* could be exploited as a potential source for plant-based pharmaceutical products. These results could form a sound basis for further investigation in the potential discovery of new natural bioactive compounds (Roy *et al.*,2011).

Nagkesar (buds of *Mammea longifolia*) is extensively used in culinary preparations especially in spice blend in India. Previously thirteen compounds were identified from the medium polar fractions of methanol extract of buds of *M.longifolia*. In continuation of the study, the polar fraction of methanol extract exhibited stronger antioxidative and radical scavenging activities (Joshi *et al.*,1969).

A methanol extracts of Nagkesar (buds of *Mammea longifolia*), which showed strong radical scavenging activity, yielded 13 compounds by separations using column chromatography and HPLC.

MATERIAL AND METHODS

Chemicals :

Standard chemicals used for the study were purchased from Himedia labs Mumbai, SDFCL and SRL chem. Mumbai. Spetcrophotometry was carried out in a digital UV-Visible Spectrophotometer manufacturer Systronic international Ltd.

Collection and identification of plant material :

Fresh flowers of Mesua ferrea commonly known as Nagkesar were collected randomly from the area of Indore city, Madhya Pradesh. Fresh flower materials were washed under running tap water, air dried under shed for 3rd days and then homogenized to fine powder and stored in airtight bottles.

Preparation of extracts :

The ethanol extracts were obtained by weighing out a fraction 20 g of the pulverized powdered flowers of the plant and soaking in 100 ml of the 60% ethanol and kept in dark for three days with occasional shaking to take out the extract. The extract was then filtered using Whatman No.1 filter paper. All filtrates were air dried at 28°C for three days to obtain semi dried extracts.

Sample preparations for *in vitro* studies :

A stock solution of concentration of 1000 µg/ml of flower extract was prepared in 10% ethanol while standard Ascorbic

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acid was prepared in 0.1 M phosphate buffer. Then both the solutions were kept on dark narrow mouth bottles and stored at 4°C.

Qualitative determination of the chemical constituents (Shabbir *et al.*,2013) :

Presence of flavonoids, saponins, tannins and terpenoids in the extract and various fractions was confirmed individually by following standard procedures.

Test for saponins :

The criterion of oil emulsion formation of saponins was used for the screening of saponins. Briefly, extract and various fractions (20 mg) suspended in 20 ml of distilled water and boiled for 5 min. In 10 ml of the above filtrate 5 ml of distilled water was added and mixed well to develop the froth. Development of emulsion after mixing the froth with olive oil confirmed the existence of saponins.

Test for terpenoids :

Briefly, 2 ml of chloroform was mixed with 5 ml (1 mg/ml) of each sample in a test tube then 3 ml of concentrated H_2SO_4 was added to develop the color. Exhibition of reddish brown coloration at the interface confirmed the presence of terpenoids.

Test for flavonoids :

The ethanolic extract of Nagkesar flower was prepared by adding 50 mg of each sample to 100 ml of distilled water and filtered. An aliquot of 5 ml of dilute ammonia solution was mixed with 10 ml of the filtrate. Appearance of yellow coloration by addition of few drops of concentrated sulfuric acid indicated the presence of flavonoid

Test for tannins :

A mixture was prepared by mixing 50 mg of Ethanol extract in 20 ml of distilled water and boiled. Appearance of brownish green or blue-black coloration after mixing few drops of 0.1% $FeCl_3$ confirmed the existence of tannins.

DPPH radical scavenging activity :

The free radical scavenging activity of flower extracts was measured by using DPPH assay. The quantitative estimation of radical scavenging activity was determined according to the methods described by Sridharan *et al.*,2011 and Puranik *et al.*,2014 with slight modifications. Two ml of 0.004% DPPH radical solution was added to one ml plant extract solutions ranging from 25-500 µg/ml. The mixtures were vortex-mixed and kept at room temperature under dark conditions for 30 min. The optical density (OD) was measured at 517 nm. Methanol was used as a blank, the methanol and DPPH solution as a baseline control (A0) and Ascorbic acid as standard. The DPPH radical concentration was calculated using the following equation :

$$\% \text{ Inhibition} = \frac{\text{O.D. control - O.D. sample}}{\text{O.D. control}} \times 100$$

RESULTS AND DISCUSSION

Test for phytochemical screening reveals the presence of saponins, flavanoids, terepenoids and tannins in the flower ethanolic extract of *Mesua ferra* (Table.1).

Table. 1 Qualitative determination of phytochemicals in flower ethanolic extracts of *M.ferra.* Results expressed as mean±S.D.

S.	Test performed	*Mesua ferra*
1	Saponins	+
2	Flavanoids	+++
3	Terepenoids	+++
4	Tannins	+++
5	Alkaloids	++

Radical scavenging potential of *Mesua ferra* flower ethanolic extract was assessed by the method already described using DPPH model system. *Mesua ferra* flower ethanol extract at a highest concentration 400 ug/ml showed percent inhibition of 98.47±0.003 followed by standard ascorbic acid. The analyte showed concentration dependent increase in inhibition of DPPH radical (Table.2).

Table. 2 Inhibition of DPPH radical (% inhibition) at various conc. (100, 200 and 400 ug/ml) of Flower ethanol extracts of *M.ferra* compared to standard Ascorbic acid. Results expressed as mean ± S.D.

S.	Conc. Ug/ml	Ascorbic acid	*Mesua ferra*
1	100	75.01±0.004	91.1±0.005
2	200	78.41±0.002	93.48±0.002
3	400	82.41±0.003	98.47±0.003

Free radical induced oxidative damage in DNA is the serious issue in cell metabolism it directly induces either dysmetabolism by corrupting normal process of DNA replication or gene expression or causes severe chromosomal or gene mutations. Plants with medicinal importance reported to protect *in vitro* DNA damage inhibition. However, *Mesua ferra* showed the very good ability to scavenge oxidative DNA damage at *in vitro* level.

The literature mentioned in ancient Ayurveda and folk medicine, the use of plants and their extracts are accounted beneficial effects in treatment of many diseases The Data of present study reflected enormous potential of radical reduction mechanism. So the Study concluded as an example to study more on flowers and medicinal plants and their use in natural, herbal and folk medicine to develop them as good therapeutics.

It is also suggested that the phyto extracts may be processed as nano particles to develop their pharmacological potentials.

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