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Preliminary phytochemical analyses of hydromethanolic leaf extract of *Melia azedarach* L

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Abstract

Melia azedarach L. is considered as an important medicinal plant of India which is traditionally used for curing malaria, diabetes, skin diseases, etc. Medicinal value of plants lies in their natural bioactive phytochemical constituents. In the present study fifty percent hydromethanolic extract of leaves of *Melia azedarach* (MAE) was prepared and analyzed for presence of various phytoconstituents in MAE employing different qualitative and quantitative biochemical analyses. The extraction yield of MAE was found to be 6.72%. Biochemical analyses revealed presence of various phytoconstituents in MAE viz., resins, tannins, Saponins, flavonoids, alkaloids, glycosides, etc. The total phenolics content of MAE was estimated to be 69.77mg/g while total flavonoids content of MAE was found to be 18.57 mg/g. Thus it could be inferred that MAE displayed presence of various phytoconstituents which could be responsible for its antioxidative potential and medicinal value.

Keywords: *Melia azedarach*, phytochemical analysis, flavonoids, phenolics

1. Introduction

Ever Floral biodiversity is the major source of herbal medicine. Human beings are relying on the herbal medicines that are used since ancient ages in traditional health care system. Therapeutic potential of plants lies in their natural bioactive phytochemical constituents which are the basis of numerous modern drugs. These phytoconstituents work with nutrients and fibres to form an integrated part of human defense system against various disease and stress condition. Phytochemicals are basically divided into two groups i.e. primary and secondary constituents according to their function in the plant metabolism. Primary constituents comprise common sugars, amino acids, proteins, chlorophyll, etc. while secondary constituents consist of alkaloids, flavonoids, saponin, phenolics and so on [1].

Melia azedarach L. (Bakain) belong to family *Meliaceae* which is native to tropical Asia and is almost found everywhere in the India. Different parts of the plants i.e. leaf, flower, seed, fruit, young branches has been used for treatment of malaria, diabetes, cough, skin diseases, strangury, amenorrhoea, bronchitis, leprosy, eczema, asthma and antipyretic, etc [2, 3]. *Melia* also have the antioxidant, antimicrobial, anti-inflammatory, cardio protective, analgesic, anticancer, antiulcer properties which are proven by experimental and clinical studies [4-8]. A variety of chemical constituents has been detected in *M. azedarach* leaf including kampherol, quercetin, stigmasterol, β -sitosterol, campesterol, phytol, beta-carotene, tocopherol and squalene, 1-eicosanol, etc [9]. The compound 2, 3- Dihydrobenzofuran (0.22%) present in bark of *Melia azedarach* is an essential oil used in the treatment of diabetic retinopathy and arthritis and 5-hydroxypipicolic acid (0.52%-imino acid) showed platelet aggregation inhibition. Pyrazol-5(2H)-one (0.26%- flavonoids) possessed several biological activities, such as anti-inflammatory, antipyretic, analgesic [8, 10, 11]. In the present study, fifty percent hydromethanolic extract was prepared from leaves and it was subjected to various qualitative and quantitative phytochemical analyses.

2. Materials and Methods

2.1 Plant Material

The authentic plant material i.e., leaves of *Melia azedarach* were obtained from Medicinal Plant Research and Development Centre (MRDC), Pantnagar, Uttarakhand, India.

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2.2 Preparation of Extract of *Melia azedarach* (MAE)

The method reported by Raghavan and Kumari ^[12] was used for preparation of *Melia azedarach* leaf extract. The plant materials were washed thoroughly with running tap water and finally with distilled water. It was then shade dried and on complete drying it was grinded to make a fine powder in a grinder. 50 gram of this shade dried powder was added to 500 ml of 50% methanol (v/v) for 48 hours under continuous agitation in a shaking incubator. Afterwards it was filtered through muslin cloth and then through Whatmann filter paper no 1. The methanol water (1:1) extract was rotary evaporated at 45°C to evaporate the organic solvent and then subjected to freeze drying. Finally the extracts were obtained after lyophilisation, weighed and stored at -20°C deep freezer till further use.

2.3 Phytochemical analyses of MAE

MAE was subjected to various qualitative and quantitative analyses for phytochemical characterization of important bioactive molecules. Chemicals used for biochemical analysis were procured from Himedia, India and Merck, India.

2.3.1 Qualitative tests for phytochemicals

Qualitative tests for phytochemicals were carried out to detect the presence of phenolics, flavonoids, tannins, polysterols, alkaloids, saponins and carbohydrates as per the methods described by Mir *et al.* ^[13], Singh and Bag ^[14] and Kodangala *et al.* ^[15].

- **Ferric Chloride test for Phenolics** - The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.
- **Ammonium test for flavonoids** - A few drop of 1% ammonia solution is added to the hydromethanolic extract of each plant sample in a test tube. A yellow coloration was indicative of presence of flavonoids compounds in the sample.
- **Lead acetate test for tannins** - Small quantity of the extract was taken separately in water and tests for the presence of tannins was carried out by adding 10% lead acetate solution. Formation of white precipitate indicates presence of tanins.
- **Salkowski's test for polysterols** - Small quantity of the

extract was dissolved in 5ml of chloroform separately. Then this chloroform solution was subjected to the Salkowski test. Salkowski test: To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

- **Dragendorff's test for Alkoids** - Small quantity of extract was separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the test with Dragendroffs reagent. The appearance of orange brown precipitate indicates the presence of alkaloids.
- **Foam test for Saponins** - Small quantity of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.
- **Benedict's test for Carbohydrates** - Extract was dissolve in 5ml of distilled water and filtered. Filtrate was treated with benedict's reagents and heated gently. Orange red precipitate indicates the presence of reducing sugars.

2.3.2 Quantitative tests for phytochemicals

Estimation of total phenolic and flavonoid content in MAE was carried out as per the methods detailed out here under.

Estimation of total phenolic content in MAE

The total phenolic content in MAE was determined according to the method described by Boubaker *et al.* ^[16] with slight modification. Stock solutions of the plant extract and gallic acid were prepared by dissolving 1 mg of extract or Gallic acid in 1 ml of methanol:water mixture (50:50 v/v). 50µl of different concentrations of extract ranging from 20µg to 100µg were taken in a series of test tubes. 250µl of 50% Folin- Ciocalteu reagent was added to each tube and mixed properly. The mixture was allowed to stand for 10 min followed by addition of 500µl 20% sodium carbonate. The mixture was vortexed and final volume was made up to 5ml using autoclaved distilled water. After 30 min of incubation the absorbance of the blue colored complex was measured at 765 nm wavelength in spectrophotometer. Result was calculated from the standard curve of Gallic acid ($y= 0.0029x$) as shown in Figure 1 and expressed as mg Gallic acid equivalents (GAE)/g of extract.

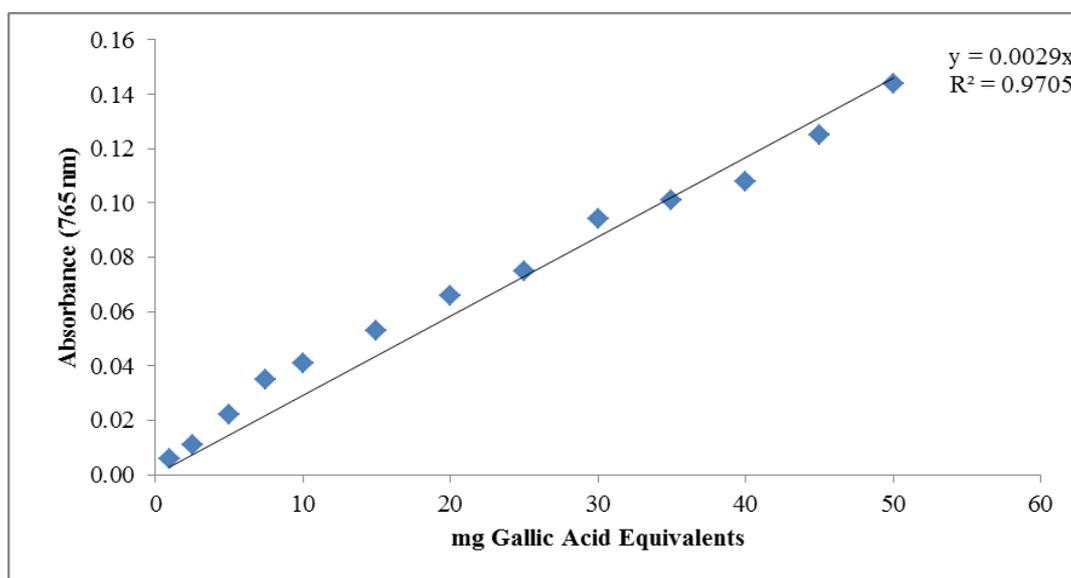


Fig 1: Standard curve of gallic acid for estimation of total phenolic content using Folin ciocalteu's method

Estimation of total flavonoid content in MAE

The total flavonoid content in MAE was measured through Aluminum chloride colorimetric assay as described by Moneim^[17] with slight modification. 1mg/ml of stock solutions were prepared in distilled water for rutin and MAE. Different dilutions of standard solution of rutin and MAE (10-100µg/ml) were taken in a series of test tubes and the volume was made up to 5 ml with distilled water. To the above mixture, 0.3ml of 5% NaNO₂ was added. Then after 5 min

0.3ml of 10% AlCl₃ was added which gave yellow colour. After incubation of 6 min at room temperature, 2ml of 1M NaOH was added and the total volume was made up to 10ml with the distilled water. The solution was mixed well and the absorbance was measured against a reagent blank devoid of the extract at 510 nm wavelength in spectrophotometer. Total flavonoid content was calculated from the standard curve of rutin ($y = 0.0072x$) as shown in Figure 2 and was expressed as rutin equivalents (RE) in mg per g of extract.

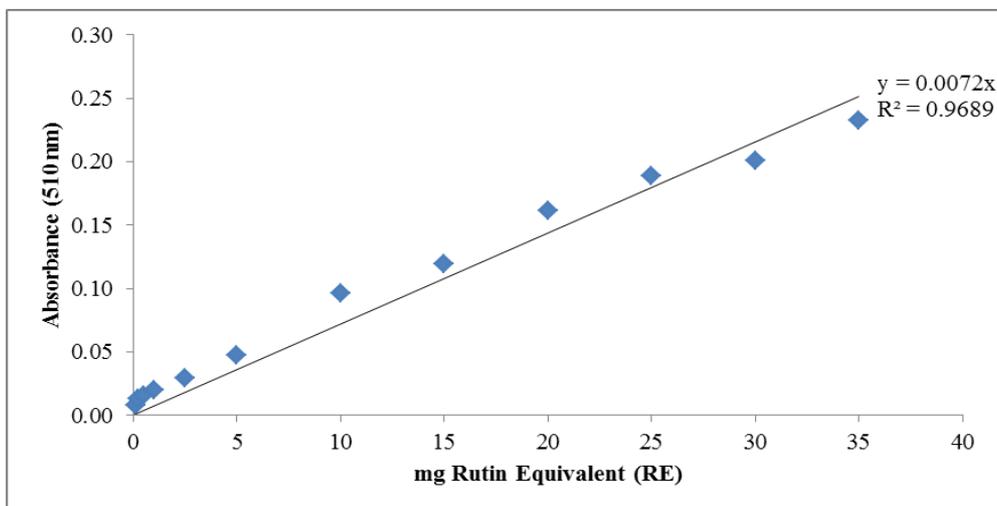


Fig 2: Standard curve of rutin for estimation of total flavonoid content using Aluminium chloride method

3. Results

3.1. Percent Yield of MAE

6.72 gram of the hydromethanolic extract was prepared from 100 gram of leaves of *Melia Azedarach* with Percent yield of 6.72%.

3.2. Phytochemicals analyses of MAE

Various biochemical tests for detection of different phytochemicals in MAE were conducted. Estimation of total phenolic and flavonoid content in MAE was also carried out.

3.2.1. Qualitative tests for phytochemicals

As per the biochemical tests conducted, MAE showed presence of almost all the tested phytochemicals, viz. tannins, flavonoids, alkaloids, steroids, phenols, etc (Table 1).

Table 1: Qualitative analyses of phytochemicals present in MAE

S. No.	Phytoconstituents	MAE
1	Phenolics	+
2	Flavonoids	+
3	Tannins	+
4	Alkaloids	+
5	Polysteroids	+
6	Saponins	+
7	Carbohydrate	-

3.2.2 Quantitative tests for phytochemicals

Estimation of total phenolic content in MAE

Total phenolic content was reported as mg / gm of extract gallic acid equivalent (GAE) as presented in Table 2. The total phenolic content of the MAE was estimated to be 69.77 mg/g.

Table 2: Total phenolics content in MAE

S. No.	Plant extract	Volume taken (µl) from 1mg/ml of extract	Total phenolics content (µg/µg extract)	Total phenolics content (mg/g extract)
1.	<i>Melia azedarach</i> (MAE)	5	0.0758	69.77 ± 0.006
		20	0.0637	

• Estimation of total flavonoid content in MAE

The total flavonoids content was represented as mg rutin equivalent (RE) per g of extract with reference to standard

curve as presented in Table 3. Total flavonoids content of the *Melia azedarach* extract was found to be 18.57 mg/g.

Table 3: Total flavanoids content in MAE

S. No	Plant extract	Volume taken (µl) from 1mg/ml of extract	Total flavanoids content (µg/µg extract)	Total flavanoids content (mg/g extract)
1.	<i>Melia azedarach</i> (MAE)	5	0.0186	18.57 ± 0.00
		20	0.0185	

4. Discussion

Knowledge regarding phyto- constituents is desirable because information will help to understand therapeutic potential of

the plant extract. The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been

found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases [13]. In the present study MAE revealed presence of various phytochemicals such as phenols, flavonoids, tannins, polysterol and alkaloids. Phenolic compounds having biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-oxidant, hepatoprotective, anti-inflammations, anti-atherosclerosis, cardiovascular protections and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [18-20].

The presence of flavonoids, which are beneficial for human health due to a large range of biological activities such as anti-mutagenic, immunestimulating, anti-inflammatory, arteriosclerosis inhibiting effects, anti-oxidant or free radical scavengers [21]. Flavonoids like baicalin possess antipyretic effect by suppressing TNF α [22]. One of the compounds pyrozol-5 (2H)-one (0.26%-flavonoids) isolated from *Melia azedarach* leaves displayed wide spectrum of biological activities such as anti-inflammatory, antipyretic and analgesic [23]. Tannins are polyphenolic compounds which considered as primary anti-oxidant or free radical scavengers and have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals [24].

Aoudia *et al.* [25] studied the antioxidative potential of *Melia azedarach* leaves and found the presence of flavonoids and phenolic compounds in the *Melia azedarach* hydromethanolic extract. Moneim [17] evaluated the phytochemical and antioxidant activity of *Melia azedarach* and reported the phenolics component of the ethanolic extract to be 492 mgGAE/g whereas in case of petroleum ether extract phenolics content was found to be 412mg/g. Nahak *et al.* [26] showed that the extract of *Melia azedarach* contain higher amount of phenolic compounds and exhibited greater anti-oxidant activity in comparison to *Azadirachta indica*. Kaneria *et al.* [27] reported the total flavonoid content in *Melia azedarach* leaves to be 21.90 mg/g. Munir *et al.* [28] also studied the total flavonoid content of hydromethanolic extract of leaves and stem bark of the *Melia azedarach* and found that Stem bark having 23.45mg/g and leaves having 16.99mg/g of flavonoid. In present study, the total phenol content is more than the total flavonoids in hydromethanolic leaves extract of the *Melia azedarach*. Phenols are responsible for the variation in the antioxidant activity of the plant. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [29]. Thus it could be inferred from the present finding that various phytochemicals are present in MAE that may be responsible for medicinal properties of *Melia azedarach*. However, there is need for methodical and detailed characterization of phytoconstituents to exactly pin point their specific role for therapeutic activity of *Melia azedarach*.

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