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Preliminary phytochemical screening and quantitative analysis of secondary metabolites of *Mentha arvensis* and *Azadirachta indica*

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Abstract
This study includes the Preliminary Phytochemical screening and quantitative analysis of secondary metabolites of leaves and stems extract of *Mentha arvensis* and *Azadirachta indica*. In this study we were checked the presence of various active metabolites like-alkaloid, tannins, terpenoid, steroid, saponin, anthraquinones, glycosides, flavanoids and phlobatannins. The result of preliminary Phytochemical screening of *Mentha arvensis* (leaves and stem) showed the presence of various secondary metabolites like alkaloid, tannins, terpenoid, steroid, saponin, anthraquinones, terpenoid, glycosides and flavanoids. *Azadirachta indica* (leaves and stem) also showed the presence of various secondary metabolites like alkaloid, tannins, terpenoid, steroid, saponin, anthraquinones, saponin, anthraquinones and glycosides. In quantitative analysis the leaves of *Mentha arvensis* showed the maximum presence of phenolic content (260.995±0.004710645) and flavonoid content was (86.282±0.001).

Keywords: preliminary phytochemical screening, quantitative analysis, *Mentha arvensis* and *Azadirachta indica*

1. Introduction
Plants are endowed with various Phytochemical substances such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites. Phytochemical are those bioactive components that originate from plants and they are called as secondary metabolites. These active components are synthesized by the plants and plants may have little need for these components. They are naturally synthesized in all parts of the plant body like- stem, leaves, bark, root, flower, seeds and fruits etc. and any part of the plant body may contain active components (Tiwai *et al*., 2011). Phytochemicals are known to possess antioxidant (Wong *et al*., 2009) [24], antibacterial (Nair *et al*., 2005) [14], antifungal (Khan and Wassilew, 1987) [11], antidiabetic (Singh and Gupta, 2007; Kumar *et al*., 2008a) [19, 13], anti-inflammatory (Kumar *et al*., 2008b) [12] and radio-protective activity (Jagetia *et al*., 2005) [5], and due to these properties they are immensely used for medicinal purpose. *Mentha arvensis* commonly known as "Pudina" in Hindi, "Corn mint" or "Field mint" in English and in Sanskrit it is known as "Rochani". Wild mint is an herbaceous perennial plant generally growing to 10–60 cm (3.9–23.6 in) and rarely up to 100 cm (39 in) tall. *Mentha arvensis* is a species of mint with a circumboreal distribution. It is native to the temperate region of Europe, Western and Central Asia, east to the Himalaya, Eastern Siberia and North America. *Mentha arvensis* leaf and oil contain acetaldehyde, amyl alcohol, methyl esters, limonene, β-pinene, β-phellandrene, cadinene, dimethyl sulphide, traces of α- pinene, sabinene, terpinoline, trans-ocimene, g-terpinene, fenchene, α-thujone, β- thujone, citronellol and lutiolin- 7-o-rutioside. It is also include menthol (35-70%), menthone (15-30%), menthyl acetate (4-14%) and pulegone (1-4%). *Azadirachta indica* commonly known as "Neem" belongs to family Meliaceae. Neem contains various antibacterial, antifungal and antiviral activities against many diseases like- vaccine, chikungunya, measles etc. (Biswas *et al*., 2002) [3]. Since ancient times the use of each part of *A. indica* is known for its medicinal value and has been used as herbal medicine to treat different diseases. It is an evergreen tree indigenous to South Asia and most part of Indian sub-continent (Govindachari *et al*., 1993) [6]. *A. indica* is a perennial, fast-growing tree that can reach a height of 15–20 meters (49–66 ft.) and rarely 35–40 meters (115–131 ft.). Active constituents of Neem leaf extract include isomeldenin, nimbin, nimbinene, 6-desacetyllnimbinene, nimbandiol, immobile, nimocinol, quercetin and beta-sitosterol (Siddiqui *et al*., 2004; Tiwary,1985) [18] Two additional tetracyclic triterpenoids zafaral [24,25,26,27-tetranorapotirucalla-(apoeupha)-6alpha-methoxy-7alpha-acetoxy-1,14-dien-3,16-dione-21-al and meliacinanhydride (24,25,26,27-tetranorapotirucalla-(apoeupha)-6alpha-hydroxy,11alpha-methoxy-7alpha, 12alpha diacetoxy,1,14, 20(22)-trien-3-one have been isolated from the methanolic extract of Neem leaves (Siddiqui *et al*., 2004) [18]. The main aim of present investigation was to screen the active components through the process of preliminary Phytochemical analysis and also quantified the secondary metabolites present in *Mentha arvensis* and *Azadirachta indica*.

2. Material and Methods
Collection of Plant material
Different parts of plants like leaves and stem were collected from Jiwaji University campus, Gwalior (M.P.), India during November 2015 to January 2016.

Preparation of Plant Extract

Fresh leaves and stem of *M. arvensis* and *A. indica* were washed 2-3 times with tap water and subjected to shade drying at room temperature. The dried plant material was powdered using a clean mixer grinder and filled in air tight container and store in a dry place on room temperature until analysis (Harborne, 1979) [7].

Methanol Extraction

The shade dried, 15 gm of powder materials were extracted with soxhlet apparatus using methanol as solvent. The extraction was done for 48 hours duration and up to 8 cycles of extraction of each plant the crude extract were evaporated at 40^0 C with the help of Hot plate stirrer. After that extract obtain with solvent is weighed. Its percentage was calculated with the compare of initial weight of plant materials. The extracts were collected and stored at 4^0 C in sterile air tight containers for further analysis (Harborne, 1979) [7].

Calculation of Extraction Yield (% Yield)

The yield (%, w/w) from dried extract was calculated as:

Yield (%) = $(W_1 \times 100) / W_2$

W_1 is the weight of the extract after lyophilization of solvent and W_2 is the weight of the powdered material.

Preliminary Phytochemical analysis

Qualitative Phytochemical analysis for the identification of secondary metabolites was carried out for both extracts. The plant parts (leaves and stem) were shade dried in laboratory and grind into homogenized powder and stored in airtight bottles. Those plant parts were subjected to preliminary or qualitative chemical screening for the identification of various classes of active chemical constituents using standard prescribed methods (Harborne, 1984; Adebayo and Ishola, 2009; Sofowora, 1993; Trease and Evans, 1989) [8, 1, 17, 22].

For certain compounds several tests were carried out. Positive result of any one test was considered as an indicative of the presence of that compound. The reason of this is that certain tests are possibly more sensitive than others. Positive tests was denoted as (+) and absent was (-).

The following active compounds observed in plants are as follow:

1. Alkaloids.
2. Tannins.
3. Steroids.
4. Flavanoid.
5. Anthraquinones.
6. Phlobatannins.
7. Glycosides.
8. Saponins.
9. Terpenoids.

Alkaloids

Powdered extract was warmed with 1% aqueous hydrochloric acid for two minutes. The mixtures were filtered and few drops of Dragendroff's reagent were added. A reddish- brown color and turbidity with the reagent indicated the presence of Alkaloids.

Flavonoid

Small quantities of the extracts were dissolving in 10% of sodium hydroxide (NaOH) and Hydrochloric acid (HCl). A yellow solution that turned colorless on addition of HCl indicated the presence of flavonoids.

Anthraquinones

Powdered extracts was shaken with 10 mL of benzene. The solution was filtered and 5 mL of 10 % NH_4OH solution was added to the filtrate. A pink, red or violet color in the ammonical (lower) phase indicated the presence of anthraquinones.

Glycosides: (Borntrger's test)

Crude extract was mixed with 2 mL of dilute sulphuric acid and 2 mL of 5 % aqueous ferric chloride solution, boiled for 5 minutes which lead to oxidation to anthraquinones, indicating the presence of glycosides.

Tannins

Powdered extract was stirred with 10 mL of hot distilled water, filtered and ferric chloride was added to the filtrate and observed for blue- black, blue-green or green precipitate.

Steroids: (Salkowski test)

Crude extract was mixed with chloroform and a few drops of conc. H_2SO_4, shaked well and allowed to stand for some time. Red color appeared at the lower layer indicated the presence of Steroids.

Saponins: (Frothing test and Emulsion test)

Small quantity of powdered extract was boiled in 10 mL of distilled water for 5 minute and decanted while still hot. The filtrate was used for the following test.

(a) Frothing test: 1 mL of filtrate was diluted with 4 mL of distilled water and mixture was shaken vigorously and observed for persistent foam which lasted for at least 15 minutes.

(b) Emulsion test: This was performed by adding 2 drops of olive oil to the frothing solution and shaken vigorously. Formation of an emulsion indicated a positive test.

Phlobatannins

Deposition of a red precipitate when an aqueous extract was boiled with 1% aqueous hydrochloric acid indicated the presence of phlobatannins.

Terpenoids

5 mL of aqueous extract of each plant sample is mixed with 2 mL of $CHCl_3$ in a test tube 3 mL of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Quantitative Phytochemical analysis

Total phenolic content

Capacity of total phenolic contents was determined using method with slight modification. Total phenolic content of

isolated crude was determined by the method described by (Singleton *et al*., 1965) [16]. 1.0 ml of sample was mixed with 1.0 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1.0 ml of saturated Na_2CO_3 (~35 %) was added and the mixture made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, observed under UV-Vis spectrophotometer at 510 nm absorbance. Gallic acid was used as a standard with varied concentration from 20 ppm to 100 ppm. A calibration curve was constructed with different concentrations of gallic acid (0.01- 0.1 mM) as standard. The results were expressed as mg of gallic acid equivalents/g of extract and same procedure was done with extract of plants.

Total Flavonoid content

Flavonoids content of isolated crud (leaf powder were determined this method) (Jia *et al*., 1999) [9]. Take a clean test tube and add 0.5 ml of the sample (Extract) containing 1.25 ml of distilled water. Then added 0.075 ml of 5 % sodium nitrite solution and allowed to stand for 5 min. Added 0.15 ml of 10 % aluminium chloride, after 6 min 0.5 ml of 1.0 M sodium hydroxide were added and the mixture were diluted with another 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. The flavonoid content was expressed as mg quercitin equivalents /g sample and same procedure were done with extract of plants.

3. Result and Discussion

In the present study we determine the Preliminary Phytochemical analysis of methanolic extract of medicinal plant *Mentha arvensis* and *Azadirachta indica*. The extraction yields of both methanolic plant extracts were shown in Table 1 and 2 respectively. The methanolic leaf and stem extract yield of *Mentha arvensis* were 8% and 7.79% respectively and the leaf extract yield of *Azadirachta indica* was 7.06% and stem extract yield was 7.1%. The yield of extracts depending on the solvent and plant material used (Dellavalle *et al*., 2011) [4]. The study of the bioactive constituent of the medicinal plants has acquired a lot of significance all over the world. The plants were collected, shade dried and powdered and were subjected to Phytochemical screening. The dried powdered leaves and stem of *Mentha arvensis* and *Azadirachta indica* were subjected to extraction with methanol. The investigation showed that the methanolic extract of *Mentha arvensis* contains alkaloids, flavonoid, glycosides, saponins, tannins, steroids and terpenoids and the methanolic extract of *A. indica* contains alkaloids, steroids, saponin, tannin, triterpenopids, and glycosides. The results were shown in Table 3 and Table 4 respectively. In this study we also quantified the secondary metaboloites. The amount of Phytochemicals which are found in methanolic extract of *M. arvensis* and *A. indica* was quantitatively determined by standard procedures. Both of plant extracts showed different amount of Phytochemicals. Leaf extract of *M. arvensis* showed highest amount of phenolic content (260.995±0.004710645) and the stem extract of *M. arvensis* showed highest amount of flavanoid content (86.282±0.001) and the quantity of leaf extract of *M. arvensis* is 81.234±0.001527525 and the quantity of total phenolic content is (164.461±0.001044663). Leaf extract of *A. indica* also showed excellent amount of phenolic content i.e. 241.642±0.000797874 and the quantity of stem extract of *A. indica* is 178.218±0.000870552. Total flavanoid content present in stem and leaf extract of *A. indica* is 85.386±0.001 and 82.781±0.00057735 respectively which is showed in Table No. 5.

Table 1: Percentage Yield of *Mentha arvensis* (Leaf and Stem) using methanol as solvent.

Plant part used	Extract	Extract Yield (%)
Leaf	Methanol	8%
Stem	Methanol	7.79%

Table 2: Percentage Yield of *Azadirachta indica* (Leaf and Stem) using methanol as solvent

Plant part used	Extract	Extract Yield (%)
Leaf	Methanol	7.06%
Stem	Methanol	7.1%

Table 3: Preliminary Phytochemical analysis of methanol extracts of *Mentha arvensis* for the presence of various phytocomponds

Plant part used	Solvent type	Alkaloids	Flavonoids	Steroids	Tannins	Glycosides	Saponin	Phlobatannins	Anthraquinones	Terpenoid
Leaf	Methanol	+	+	+	+	+	+	_	_	+
Stem	Methanol	+	+	+	+	_	+	_	_	+

Table 4: Preliminary Phytochemical analysis of methanol extract of *Azadirachta indica* for the presence of various phytocomponds

Plant part used	Solvent type	Alkaloids	Flavonoids	Steroids	Tannins	Glycosides	Saponin	Phlobatannins	Anthraquinones	Terpenoids
Leaf	Methanol	+	_	+	+	+	+	_	_	+
Stem	Methanol	+	_	_	+	_	+	_	_	+

Table 5: Total Phenolic contents of Methanol extract samples of leaf and stem extract of *Mentha arvensis* and *Azadirachta indica*

S.No.	Sample ID	Quantity
01	M-1	260.995 ± 0.004710645
02	M-2	164.461±0.001044663
03	M-3	178.218±0.000870552
04	M-4	241.642±0.000797874

Where,
M-1= Leaf extract of *Mentha arvensis*
M-2= Stem extract of *Mentha arvensis*
M-3= Leaf extract of *Azadirachta indica*
M-4= Stem extract of *Azadirachta indica*

Table 5: Total Flavanoid contents of Methanol extract samples of leaf and Stem extract of *Mentha arvensis* and *Azadirachta indica*

S. No.	Sample ID	Quantity
01	M-1	81.234±0.001527525
02	M-2	86.282±0.001
03	M-3	82.781±0.00057735
04	M-4	85.386±0.001

Herbal medicine contain some organic substances which provide definite physiological action on the human body as well as their physiological activities due to the presence of secondary metabolites include tannins, alkaloid, flavanoid, glycosides, phlobatannins, anthraquinons, saponin, steroids (Edoga *et al*., 2005). Asha *et al*., 2015 [2] worked on Phytochemical constitution, antioxidant activity and flavonoid profiling of methanolic extracts of aerial parts of four important herbs of Lamiaceae family *Ocimum basilicum* L.., *Mentha arvensis* L., *Hyptis suaveolens* L. (Poit.) and *Coleus aromaticus* Benth. Sagadevan *et al*., 2014 [20] also worked on Studies on Phytochemical csomposition, antibacterial and antioxidant potential of methanolic leaf extract of *Mentha arvensis*. Very little work has been done in the field of quantitative analysis of *Mentha arvensis*. Prasanth *et al*., 2014 also worked on chemical composition of Neem (*Azadirachta indica*) and found the presence of alkaloids, reducing sugars, saponins etc. Therefore, the comparison of *Mentha arvensis* and *Azadirachta indica*, *Mentha arvensis* gave excellent result.

4. Conclusion

In this study we can conclude that the selected leaf and stem extracts of *Mentha arvensis* and *Azadirachta indica* were showing many secondary metabolites. Phytochemical analysis of methanolic extract of *Mentha arvensis* and *Azadirachta indica* leaves and stem extracts were obtained by soxhlet method. The screening of Phytochemical constituents of both plants indicated the presence of many secondary metabolites like alkaloids, glycosides, tannins, steroids, saponins, terpenoids and flavonoids and excellent amount of phenol and flavanoid content. These plants contain maximum metabolites and *Mentha arvensis* have excellent amount of secondary metabolites. This study may be useful to explore the pharmacological and biosynthetic activity of *Mentha arvensis* plant further.

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