

## Qualitative Phytochemical Screening of *Mentha arvensis* L.

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### ABSTRACT:

Phytochemicals are non-nutritive plant chemicals. Plant metabolites represent enormous chemical diversity with largely unexplored biological activities. Our extensive knowledge on the chemistry and pharmacology of some secondary metabolites has led to their use in a range of medical applications. Thin Layer Chromatography is a common analytical technique widely used for the analysis of phyto-constituents in plant extracts. In the present study was to investigate the presence of various phytochemical from different solvents (methanol, ethanol, ethyl acetate, petroleum ether, hexane and aqueous) extracts of *Mentha arvensis* L. Among them methanol, ethanol solvent extracts were found with rich secondary metabolites (flavonoids, glycosides, phenols, terpenoids, alkaloids) due to highest number of various metabolites compounds.

**KEYWORDS:** *Mentha arvensis* L, In Vitro Regeneration, Phytochemicals, Solvents, Extraction, TLC

### I. INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostroet al., 2000). Phytochemicals are fascinating chemical molecules, very useful and of great importance in nature, as well as highly diversified in structures, properties, uses, chemistry etc. *Mentha arvensis* L. is well known important medicinal and aromatic plant widely used in several indigenous systems of medicine as various therapeutic powers viz. analgesic, anesthetic, antiseptic, astringent, carminative, decongestant, expectorant, nervier, stimulant, inflammatory disease, ulcer and stomach problems (Campbell et al., 1973; Blumenthal, 1998; Jamal et al., 2006). In India, mint is used to tone the stomach, stimulate the mind and body, rid the intestines of gas, and

relieve muscle spasms (Pandey, 2003). The Commission E approved internal use of mint oil for flatulence, functional gastrointestinal and gallbladder disorders, catarrhs of the upper respiratory tract, and external use for myalgia and neuralgic ailments. Menthol crystals are used in different pharmaceutical products and cosmetics as antiseptic, stimulant and inhibitor. It gives minty flavour to various food products. It is also used in oral products e.g. tooth paste and mouth fresheners due to its physiological cooling effect. Extensively used as fragrance component in soaps, detergents, cosmetics and perfumes, toothpastes, and industrial fragrances (Alviet al., 2001).

### II. MATERIALS AND METHODS

In vitro plants were removed from MS supplemented with 2.0 mg/l BAP + 0.4 mg/l NAA standardized cultured medium (Bariya and Pandya, 2014). Whole plant material was washed under the running tap water. 10 gm of plant material (shade dried powder) was extracted separately with 100 ml of each solvents i. e. water, ethanol, methanol, ethyl acetate, petroleum ether and hexane and allowed to stand for 24 hours soaked in air tight Erlenmeyer flask. Later it filtered through a whatman filter paper no. 1 (Souriet al, 2008; Khan and Nasreen, 2010). The filtrate was evaporated for drying to yield a dark-residue and % yield of extracts were calculated. Each sample was then transferred to glass vials and kept in refrigerator at 4°C for their future use in phytochemical analysis.

**Distillation of essential oil-**An air-dried powdered sample (100 gm) was subjected to hydro distillation for 3 h using a Clevenger-type apparatus to produce the essential oil. The obtained essential oil was dried over anhydrous sodium sulphate and stored at 4 °C for further experiments. Hydrosols are the condensate water coproduced during the Hydro distillation of plant material.

### Phytochemical Screening

The qualitative phytochemical screening of the plant extracts using carried out by testing of different class of compounds using standard methods (Harborne, 2005; Raman, 2006). In TLC screening Chromatography plates were prepared by spreading slurry of Silica Gel G in distilled water (1:2) uniformly over clean glass plates (20 cm.) The layer (thickness 2 mm) was allowed to dry and activated by heating in an oven at 110 °C for one hour. Samples were applied to adsorbent surface at 10 mm edge using a capillary tube and developed in glass chamber preciously saturated with the vapours of standardized solvent system. after the development plates left to dry for about 10 minutes, then viewed under UV fluorescence light at 254 and 366 nm wavelength and finally sprayed with the required detection reagent (dragendorf reagent-alkaloids, vanillin sulphuric acid-terpenoids, saponins, Ferric ferrocynide- phenols, ammonium vapour-flavanoids after dry the plate heated at 110° C for 5-10 min. Then plate was evaluated for chromatographic measurement to determine the compounds present.

### III. RESULTS AND DISCUSSION

Phytochemical results revealed the presence of various bioactive secondary metabolites in the different solvent extracts (Table-1). The maximum numbers of secondary metabolites were observed in ethanolic and methanolic extracts which were rich in flavonoids, phenols, glycosides, alkaloids and terpenoids. Ethyl acetate extract shown the presence of high amounts of phenols and flavonoids. Petroleum ether extract shown highest amount of terpenoids, very less of alkaloids and absence of glycosides. Hydrosol sample (aroma) shown high content of flavonoids, terpenoids, saponins and glycosides. The similar findings were also reported by John et al., (2012), Suresh (2012) and Rachel and Meera Bai (2011). In addition Singh et al. (2011), Naidu et al. (2012) who observed in essential oil contain most of the phytoconstituents including flavonoids, saponins, cardiac glycosides, reducing sugars and steroids, alkaloids.

**TABLE-1 QUALITATIVE PHYTOCHEMICAL SCREENING**

| Phyto constituents        | Aqueous extract | Methanol extract | Ethanol extract | Ethyl acetate extract | Petroleum ether extract | Hexane extract | Essential oil | Hydrosol |
|---------------------------|-----------------|------------------|-----------------|-----------------------|-------------------------|----------------|---------------|----------|
| <b>Alkaloids</b>          |                 |                  |                 |                       |                         |                |               |          |
| 1. Mayer's test           | +               | ++               | +++             | +                     | +                       | -              | +             | +        |
| 2. Dragendorff's test     | +               | +                | +               | -                     | +                       | +              | -             | +        |
| 3. Wagner's test          |                 |                  |                 |                       |                         |                |               |          |
| <b>Flavonoids</b>         |                 |                  |                 |                       |                         |                |               |          |
| 1. Alkaline reagent       | ++              | +++              | +++             | ++                    | ++                      | ++             | ++            | ++       |
| 2. FeCl <sub>3</sub> test | +               | ++               | ++              | ++                    | +                       | +              | +             | ++       |
| <b>Phenolic compounds</b> |                 |                  |                 |                       |                         |                |               |          |
| 1. Lead acetate test      | +               | ++               | +++             | +++                   | ++                      | +              | ++            | +        |
| 2. FeCl <sub>3</sub> test | ++              | ++               | ++              | ++                    | ++                      | +              | ++            | +        |
| <b>Terpenoids</b>         |                 |                  |                 |                       |                         |                |               |          |

|  |   |    |    |   |     |     |    |    |
|--|---|----|----|---|-----|-----|----|----|
| 1. Libermann test                              | - | ++ | ++ | - | +++ | +++ | ++ | ++ |
| 2. Salkowas test                               | - | +  | +  | + | ++  | ++  | ++ | ++ |
| <b>Glycosides</b>                              |   |    |    |   |     |     |    |    |
| 1. Kellerkilliani test                         | + | +  | +  | + | -   | -   | +  | ++ |
| 2. Borntrager's test (athraquinone glycosides) | + | +  | +  | - | -   | -   | +  | +  |
| <b>Saponins</b>                                |   |    |    |   |     |     |    |    |
| 1. Froth test                                  | + | ++ | ++ | + | +   | +   | ++ | ++ |

**TABLE-2 FLAVANOID SOLVENT SYSTEM**

| S. No. | Solvent system (Flavanoids)   | Treatment with ammonia vapour | No. of Bands     |                 |                       |                         |                |                 |
|--------|---|-------------------------------|------------------|-----------------|-----------------------|-------------------------|----------------|-----------------|
|        |   |                               | Methanol Extract | Ethanol Extract | Ethyl acetate Extract | Petroleum ether Extract | Hexane Extract | Aqueous Extract |
| 1.     | ethyl acetate: formic acid : glacial acetic acid : water (100:11:11:20) |                               | 3                | 3               | 2                     | 2                       | 2              | 1               |
| 2.     | con. HCL: acetic acid: water (3:30:10)                                  |                               | 2                | 2               | 1                     | 1                       | 1              | 2               |
| 3.     | butanol: acetic acid: water (4:1:5)                                     |                               | 3                | 3               | 2                     | 1                       | 2              | 3               |
| 4.     | Phenol: water (3:1)   |                               | 3                | 4               | 3                     | 3                       | 2              | 3               |

**TABLE-3 ALKALOID SOLVENT SYSTEM**

| S. No. | Solvent system (Alkaloids)                    |   | No. of Bands     |                 |                       |                         |                |                 |
|--------|---|---|------------------|-----------------|-----------------------|-------------------------|----------------|-----------------|
|        |   |   | Methanol Extract | Ethanol Extract | Ethyl acetate Extract | Petroleum ether Extract | Hexane Extract | Aqueous Extract |
| 1.     | cyclohexane: ethanol: diethylamine (80:10:10) | Before Spray  | 5                | 5               | 2                     | 4                       | 3              | 4               |
|        |   | After Spray (Drag. + H <sub>2</sub> SO <sub>4</sub> ) | 7                | 8               | 3                     | 7                       | 5              | 8               |
|        |   | H <sub>2</sub> SO <sub>4</sub>                        | 11               | 10              | 6                     | 9                       | 8              | 13              |

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| 2 | toluene: ethyl acetate: diethyl amine(70:20:10) | After Spray(Drag.+ H <sub>2</sub> SO <sub>4</sub> ) | 4 | 5 | - | 3 | - | 6 |
| 3 | butanol: glacial acetic acid: water (40:40:10)  | Before Spray  | 2 | 2 | - | 1 | 1 | 3 |
|   |   | After Spray(Drag.+ H <sub>2</sub> SO <sub>4</sub> ) | 3 | 3 | 1 | 1 | 1 | 5 |

(Drag.=Dragendoeff's reagent)

**TABLE-4GLYCOSIDES SOLVENT SYSTEM**

| S. No | Solvent system (Glycosides)                        | After Spray(Anisaldehyde sulphuric acid reagent) | No. of Bands     |                 |                       |                         |                |                 |
|-------|--|--|------------------|-----------------|-----------------------|-------------------------|----------------|-----------------|
|       |  |  | Methanol Extract | Ethanol Extract | Ethyl acetate Extract | Petroleum ether Extract | Hexane Extract | Aqueous Extract |
| 1.    | ethyl acetate: methanol: ethanol: water(81:11:4:8) |  | 8                | 9               | 3                     | 2                       | 2              | 4               |

**TABLE-5PHENOLS SOLVENT SYSTEM**

| S. No. | Solvent system (Phenols)       | Ferric ferrocynide reagent | No. of Bands     |                 |                       |                         |                |                 |
|--------|--------------------------------|----------------------------|------------------|-----------------|-----------------------|-------------------------|----------------|-----------------|
|        |                                |                            | Methanol Extract | Ethanol Extract | Ethyl acetate Extract | Petroleum ether Extract | Hexane Extract | Aqueous Extract |
| 1.     | benzene : ethyl acetate (11:9) |                            | 9                | 9               | 4                     | 5                       | 2              | 6               |
| 2      | chloroform: acetic acid (9:1)  |                            | 7                | 8               | 6                     | 6                       | 7              | 8               |

**TABLE-6 TERPENOID SOLVENT SYSTEM**

| S. No. | Solvent system (terpenoids)        | Vanillin sulphuric acid reagent | No. of Bands     |                 |                       |                         |                |                 |
|--------|------------------------------------|---------------------------------|------------------|-----------------|-----------------------|-------------------------|----------------|-----------------|
|        |                                    |                                 | Methanol Extract | Ethanol Extract | Ethyl acetate Extract | Petroleum ether Extract | Hexane Extract | Aqueous Extract |
| 1.     | Dichloromethane                    |                                 | 9                | 10              | 9                     | 8                       | 7              | 5               |
| 2      | toluene: chloroform (1:1)          |                                 | 10               | 12              | 8                     | 6                       | 4              | 5               |
| 3      | toluene: ethyl acetate (97:3)      |                                 | 8                | 10              | 8                     | 5                       | 6              | 3               |
| 4      | chloroform: benzene (1:1)          |                                 | 6                | 9               | 4                     | 6                       | 8              | 3               |
| 5      | chloroform: ethyl acetate: ammonia |                                 | 15               | 16              | 7                     | 15                      | 15             | 1               |

|   |                                 |  |    |    |    |    |    |   |
|---|---------------------------------|--|----|----|----|----|----|---|
|   | (97:2.5:05)                     |  |    |    |    |    |    |   |
| 6 | chloroform:<br>methanol (90:10) |  | 12 | 14 | 13 | 15 | 13 | 4 |

The results of TLC screening of ethanolic and methanolic extracts exposed with maximum number of bands in all the solvent systems. Ethyl acetate extract revealed maximum number of bands presence in phenols and flavonoid solvent system followed by petroleum ether extracts detected 15 bands in terpenoid solvent system, aqueous extracts found with highest number of bands in phenol and alkaloid solvent system.

In study of different solvent systems, chloroform: ethyl acetate: ammonia showing maximum number of bands followed by cyclohexane: ethanol: diethylamine (terpenoids), benzene: ethyl acetate (phenol), Ethyl acetate: methanol: ethanol: water (glycosides) and Phenol: water (flavonoids). When a new drug is to be discovered, qualitative phytochemical analysis is a very important step as it gives information about the presence of secondary metabolite in plant extracts which having a clinical significance. TLC screening of all extracts gives a remarkable result that directing towards the presence of number of phytochemicals. Various phytochemicals have different  $R_f$  values in different solvent system. This variation in  $R_f$  values provide a very important clue in understanding of polarity of compound.

#### IV. CONCLUSION

In present investigation, methanol and ethanol extracts of *Mentha arvensis* L. showed the high and positive response than other solvent extracts. Qualitative phytochemical screening is necessary for determination of metabolites occurring in plant. For the further study of metabolites, secondary screening was done by Thin layer chromatography technique using different solvent system. TLC provides a very important clue in understanding of compounds polarity and also helps in selection of appropriate solvent system for separation of pure compounds.

#### REFERENCES

- [1]. Alvi A, Afrasiab H, Saeed M and Iqbal J (2004) An in vitro study of regeneration and micro-propagation of *Mentha arvensis*. *Int. J. Biol. Biotechn.*, 1(4): 519-528.
- [2]. Bariya R R and Pandya H A (2014) Evaluation and establishment of promising large-scale In vitro production of corn mint. *International Journal of Recent Scientific Research* 5(2) 509-512.
- [3]. Blumenthal, M. (1998) *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*. American Botanical Council. Austin TX.
- [4]. Campbell H, Cline W, Evans M, Lloyd J, Peck AW (1973) Comparison of the effects of dexamphetamine and 1-benzylpiperazine in former addicts', *Eur. J. Clin. Pharm.*, 6:70-176.
- [5]. Harborne JB (2005) *Phytochemical Methods*. New Delhi: Springer (India) Pvt. Ltd, 17.
- [6]. Jamal A, Siddiqui A, Tajuddin A, Jafri M A (2006) A review on gastric ulcer remedies used in Unani System of medicine. *Natural Product Radiance*, 5:153-159.
- [7]. John DB, Sebastian SR and Sujin MR (2012) Antimicrobial activity of selected species of Lamiaceae against human pathogens. *Indian journal of natural products and resources*, 3(3):334- 342.
- [8]. Khan ZS and Nasreen S (2010) Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens. *Journal of Agricultural Technology*, 6: 793- 805.
- [9]. Naidu JR, Ismail RB, Yeng C, Sasidharan S and Kumar P (2012) Chemical Composition and Antioxidant Activity of the Crude Methanolic Extracts of *Mentha spicata*. *Journal of Phytology*, 4(1): 13-18
- [10]. Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl Microbiol* 30: 379-384.
- [11]. Pandey A K, Rai M K and Acharya D (2003) Chemical composition and antimycotic activity of the essential oils of corn mint (*Mentha arvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi. *Pharm. Biol.*, 41, 421-425



- [12]. Suresh SN, Rathishkumar S, Rajeshwari V, Sagadevan P, Gayathri S And VithyaEswari D (2012) Studies On Phytochemical Composition And Antibacterial Potential Of Methanolic Leaf Extract of MenthaArvensis .Linn. International Journal of Pharmaceutical Research & Development, 4(08): 001 – 004.
- [13]. Rachel MSB and MeeraBai G (2011) Antimicrobial activity of Menthaarvensis L. Lamiaceae.J Advanced Laboratory Research in Biology., 2(1): 1-4.
- [14]. Raman N (2006) Phytochemical Technique. New Indian Publishing Agencies: New Delhi, 19.
- [15]. Sethi PD (1996) HPTLC – High Performance Thin Layer Chromatography.1st Ed. CBS Publication.3-71.
- [16]. Singh D, Singh B and Goel RK (2011) Traditional uses, phytochemistry and pharmacology of Ficusreligiosa: A review. Journal of Ethnopharmacology., 134: 565-583.
- [17]. Souri E, Amin G, Farsam H and Barazandeh TM (2008) Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. DARU., 16: 83- 87.