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ANALYSIS OF PHYTOCONSTITUENTS PRESENT IN *MENTHA PIPERITA*

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ABSTRACT

The present investigation aims to assess the phytochemical content of the methanolic,acetonic, hot and cold leaf extract of locally available *Mentha piperita* , the mint plant. *Mentha piperita* is the best medicinal plant. Different tests are performed to find the phytochemicals that are present so as to subject the plant for further medicinal uses. These chemical constituents are mainly responsible for various biological activities .From the phytochemical studies conducted it was found that there is the presence of phenols, flavonoids, carbohydrates ,glycosides ,reducing sugar and tannins in the plant sample taken from the medicinal garden of the Institute of Biotechnology (Patwadangar), Nainital during the month of june. After the collection of leaves, they were dried and crushed into a powdered form. Finally an extract was prepared using different solvents. After this the phytochemical analysis was performed to find out the above mentioned chemical constituents in *Mentha piperita* from patwadangar.

Keywords- Phytochemicals , extraction methods , medicinal plant

INTRODUCTION

The genus of ***mentha piperita*** has a subcosmopolitan distribution across Europe, Africa, Asia, Australia and North America. Mints are aromatic, almost exclusively perennial, rarely annual, herbs. Mentha piperita L. (Peppermint) is a perennial glabrous and strongly scented herb belonging to family Lamiaceae. The plant is aromatic, stimulant and used for allaying nausea, headache and vomiting. Its oil is one of the most popular widely used essential oils in food products, cosmetics, pharmaceuticals, dental preparations, mouthwashes, soaps and alcoholic liquors. *Mentha* species are used for their flavouring and medicinal properties widely throughout different countries of the world. ***Mentha piperita*** Linn. emend. Huds. is currently one of the most economically important aromatic and medicinal crops. It is commonly known as ***peppermint***, ***Brandy mint*** or ***Paparaminta***. It is native to Europe but today, it is cultivated worldwide and also occurs in the wild, especially in the damp and temperate regions of the globe. 75% of the peppermint produced in the world is from the USA.

The world producton of peppermint oil is about 8000 tonnes per year (Eccles,1994). It is a popular medicinal plant in several traditional systems of medicine. In *Ayurveda*, this is an important ingredient of several compound formulations used in management of gastro-intestinal and skin disorders. It is thought to be a natural hybrid between spearmint (M. spicata Linn. emend. Nathh.) and water mint (M. aquatica Linn), the latter itself being a hybrid of M. rotundifolia (Linn.) Huds. and

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M.longifolia (Linn.) Huds., so M. piperita is a triple hybrid (Fleming, 1998; Wealth of India-Raw Materials, 1962).

The scientific name for peppermint (*Mentha* x *piperita*) is derived from the name Mintha, a Greek mythological nymph who transformed herself into the plant, and from the Latin piper meaning "pepper".

MATERIALS AND METHODS

Phytochemical Analysis:"Phytochemistry is study of phytochemical foundin plants describing the extraction, isolation, purification, identification and structural elucidation of various plant secondary metabolites"

Extraction of phytochemicals

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powderedextracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. This product contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides,terpenoids, flavonoids and lignans. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfleurage (cold fat extraction) may be employed.

Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase microextraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation.

Extract preparation:

First step is the collection of leaves after which the leaves are first washed in running water for 15 minutes. After washing let the leaves dry on the paper sheets(3-4 days). After the leaves are dried, grind the leaves in grinder and filter the leaf extract by cloth and store the filtered part of the leaf sample.

Methods of extraction:

- Aqueous extraction
  - Cold water extraction
  - Hot water extraction
- Solvent extraction by soxhlet apparatus:
  - Alcoholic extraction (by methanol)
  - Extraction by acetone

Cold water extraction:

1. Weight the plant sample(5g).
2. Add 150 ml cold water i.e. at room temprature.
3. Shake the sample for 30 minutes.
4. Filter the sample with muslin cloth and then whatmann filter paper no. 1.
5. Collect the transparent decant and evaporate in an oven to get a dry extract.

Hot water extraction:

1. Weight the plant sample (5g).
2. Add 150 ml hot water(65°C-75°C).
3. Shake the sample for 30 minutes.
4. Filter the sample with muslin cloth and after that Whatmann filter paper no. 1.
5. Collect the transparent decant and evaporate the content inside the oven so as to get the dry extract.

Solvent extraction by soxhlet method:

1. Weight the leaves (5g).
2. Put the content in thumble and set up the soxhlet apparatus.
3. Add 150 ml of the solvent.
4. After the completion of 10-12 cycles take the solvent.
5. Evaporate in oven so as to get dry extract.

The basic parameters influencing the quality of an extract are:

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

Effect of extracted plant phytochemicals depends on

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon:

1. Type of extraction
2. Time of extraction
3. Temperature
4. Nature of solvent
5. Solvent concentration
6. Polarity

Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants.

The choice of solvent is influenced by what is intended with the extract. Since the end product will containtraces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted.

The various solvents that are used in the extraction procedures are:

1. Water

Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound.

2. Acetone

Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol. Both acetone and methanol were found to extract saponins which have antimicrobial activity.

3. Alcohol

The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms ascompared to ethanol. The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results.

4. Chloroform:

Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tanninsand terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents.

5. Ether

Ether is commonly used selectively for the extraction of coumarins and fatty acids.

Dichloromethanol: It is another solvent used for carrying out the extraction procedures. It is specially used for the selective extraction of only terpenoids.

Phytochemical Screening

Qualitative determination

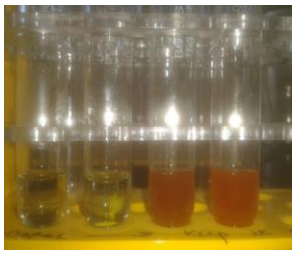
Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug as per the standard methods.

Procedure: Few mg of plant extract was taken in different-different test tube and required amount of solven added in each test tube. Then it was subjected to different-different chemical reagents and tests and observation was noted.

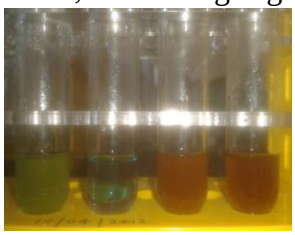
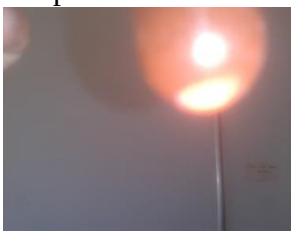
1. Test for Carbohydrate:

a. **Molisch's test:** Treated the extract with few drop of alcoholic α-naphthol, added 0.2 ml of concentrated sulfuric acid slowly through side surface of test tube, purple to violet color ring appears at the junction.
(α-napthol : 10g of α-napthol in 100ml of 95% alcohol).

b. Benedict's test: Treat the extract with few drop of Benedict reagent (alkaline solution containing cupric citrate complex) and boil on water bath, reddish brown ppt forms if reducing suger is present.




c. Fehling's test: Equal volume of fehling A (copper sulfate in distilled water) and fehling B (potassium tartarate and sodium hydroxide in distilled water) reagent are mixed along with few amount of extract, boil on water bath, brick red ppt of cuprous oxide forms, if reducing sugar are present.

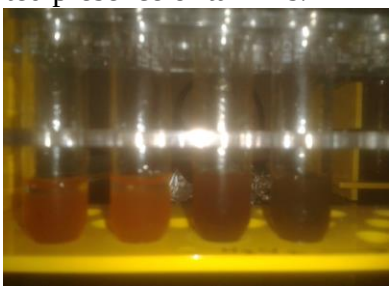



2. Test for Glycoside

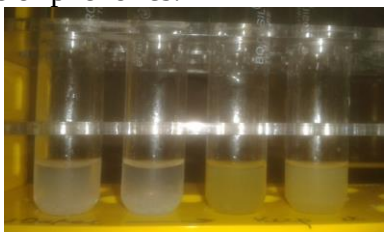
a. **Legal test:**Concentrated extract was made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside solution was added to the solution. Presence of blue coloration indicated the presence of glycosides in the extract.

3. Test for Tannin and phenols:

a. Ferric chloride test: Extract give blue-green color with 5% ferric chloride solution indicated presence of tannins.

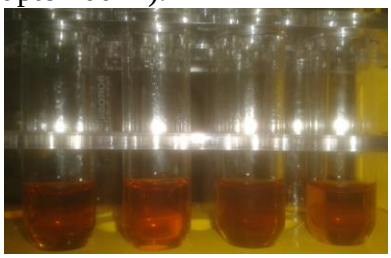


b. Lead acetate test : Lead acetate added to 2ml of the extract a black precipitate indicated presence of phenolics.

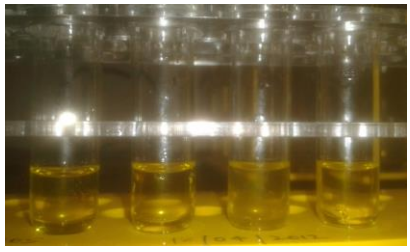


4. Test for Alkaloids:

a. Wagner's test (Solution of iodine in potassium iodide): Alkaloids give reddish brown precipitate with Wagner's reagent.(mix 1.27g iodine with 2g potassium iodide and make it upto 100ml).

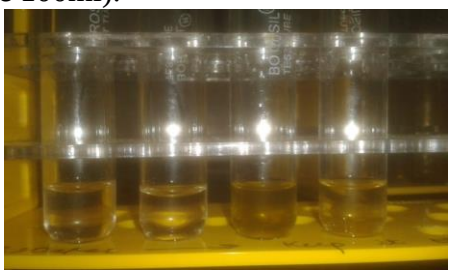


b.**Hager's test (Saturated solution of picric acid):** Alkaloids give yellow color precipitate with Hager's reagent.
(add 1g picric acid in 100ml distilled water).



c. Mayer's test (potassium mercuric iodide): Alkaloids give yellow colour precipitate with Mayer's reagent.

(1.36g mercuric chloride in 60 ml distilled water and add a solution of 5g potassium iodide in 20ml distilled water and make volume 100ml).



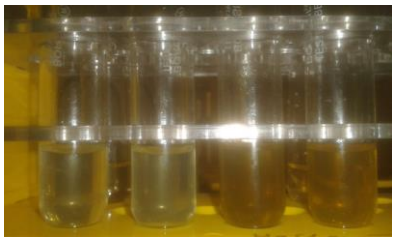
5. Test for Sterols and Terpenoids:

a. .Salkowski test: 2ml of concentrated sulfuric acid was added to the extract, a yellow ring was formed at the junction, which turned red after one minute.

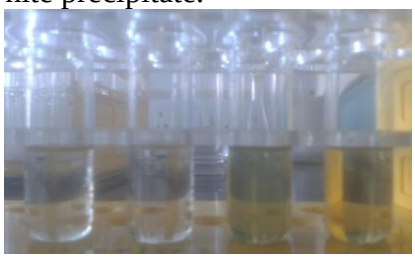
6. Test for Protein and Amino acid:

a. Biuret test : to 3ml test solution add 4% NaOH and few drops of 1% $CuSO_4$ solution. Presence of red/violet colouration indicates the presence of proteins and free amino acids.

b.

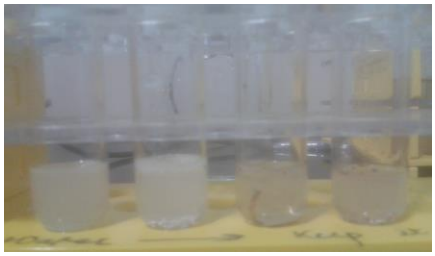


c. Xanthoprotein test: 2ml of the test solution with 1 ml concentrated H_2SO_4 will form white precipitate.

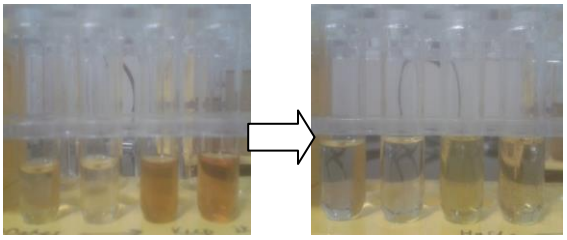


7. Test for Flavonoids:

a. Shinoda test (Magnesium hydrochloride ribbon test): To the extract add few fragments of magnesium ribbon and add concentrated hydrochloric acid dropwise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.



b. **Alkaline reagent test:** To the extract add few drop of sodium hydroxide solution, formation of an intense yellow color which turns to colorless on addition of few drops of dilute acetic acid indicate the presence of flavonoid.



8. Detection of phenols:

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

9. Detection of phytosterols

a. Salkowski's Test Extracts: were treated with chloroform and filtered. The filtrates were treated with few drops of Conc.Sulphuric acid, shaken and allowed to stand.Appearance of golden yellow colour indicates the presence of triterpenes.

Table: The result of the phytochemical analysis of the plant *Mentha piperita* is as follows:

TEST	HOT EXTRACT	COLD EXTRACT	METHANOL EXTRACT	ACETONE EXTRACT
Molish's test	-	-	-	-
Benedict test	-	-	+	+
Fehling test	-	-	+	+
Legal test	-	-	-	-
Ferric chloride test	-	-	+	+
Lead acetate test	-	-	-	-
Wagner	-	-	-	-
Hager	-	-	-	-
Mayer	-	-	-	-
Biuret test	-	-	-	-
Xanthoprotein test	-	-	-	-
Shinoda test	-	-	-	-
Alkaline reagent test	-	-	+	+
Salkowaski test	-	-	-	-

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