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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *MENTHA PULEGIUM* L.VAR. ERECTA

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ABSTRACT

Mentha pulegium L. var. erecta has remarkable medicinal properties and is recently reported for the first time in Vidarbha region. There is a growing interest of industry to replace synthetic chemicals by natural products with bioactive properties from plant origin. The aim of this study was to validate the therapeutic properties of *Mentha pulegium* L. commonly known as Pennyroyal, by conducting a qualitative and quantitative analysis of phytochemicals, to determine the chemical composition of secondary metabolites. The whole plant has various bioactive components or phytochemical constituents such as alkaloids, flavonoids, phenols, steroids, terpenoids and tannins. The present study was carried out to find the availability of these important phyto-constituents in aqueous, methanol and ethanol crude extracts. Quantitative analysis was also carried out for three metabolites. This species has never been investigated pharmacologically till now, even if its properties were traditionally well-known. In view of this reason, Pennyroyal was selected for phytochemical analysis.

KEYWORDS: Crude extracts, *Mentha pulegium,* Phytochemicals, Secondary metabolites.

INTRODUCTION

Mentha pulegium L.var. erecta (Lamiaceae) is reported for the first time in Vidarbha region (P.S Rao, 2018). During botanical explorations in Bhandara rural area, the author collected a plant specimen from Parsodi village with a 79.538772 E Longitude and 21.146403N Latitude (near Ordnance factory, Jawahar nagar) in Bhandara district of Maharashtra. A critical survey of literature, floral examination and taxanomy expert's opinion, the collected specimen is confirmed as *Mentha pulegium* L., which grown as wild in rice fields. The habit of the previously reported species in J&K and Haryana is prostrate and roots arise in the nodal region (Agnihotri et.al. 2005 and Singh et.al 2001), while in the present report, stem is stout, erect with a height of 30-90 cm and not rooting at the nodes. On the base of critical studies of relevant literature, the present specimen confirmed as *Mentha pulegium* L. var. erecta., this species constitute a new record for Vidarbha, Maharashtra state (Padmavathi Rao, 2018).

In therapeutic applications, this plant and its preparations have been used traditionally for its antispasmodic, carminative, diaphoretic, sedative, stimulant, diuretic, expectorant, antiseptic and digestive effect(Simon, 1980; Zargari, 1990; Mkaddem 2007). It was even used to promote menstruation, cure headaches, treat bronchitis, relieve bites from scorpions and snakes and help against vomiting and kidney disease (Simon et.al. 1980). It also served as a repellent against fleas and other insects due to pungent odour. It was effective in relieving acne and other skin conditions. This plant has been used as a spice and flavoring in various foods, especially desserts, to make herbal teas also as perfume in cosmetics (Zargari, 1990).

Aerial parts of this plant contain a wide diversity of secondary metabolites such as: alkaloids, flavonoids, phenols, tannins, resins, pectin, bitter principles and essential oils (Zargari, 1990). Fresh or dried leaves and flowering tops are commonly used for their healing and culinary properties. The whole plant and its essential oil have a strong and characteristic odour (Simon et.al. 1980).

Pennyroyal is used to make herbal teas, which, although not proven to be dangerous to healthy adults in small doses, is not recommended, due to its known toxicity to the liver. Consumption can be fatal to infants and children. Pennyroyal leaves, both fresh and dried, are especially noted for repelling insects. Pennyroyal essential oil should never be taken internally because it is highly toxic; even in small doses, consumption of the oil

can result in death. Pennyroyal tea has been used for cold relief, fevers, coughs, indigestion, liver and kidney problems and headaches. The fresh or dried leaves of pennyroyal have also been used when treating influenza, abdominal cramps, to induce sweating, as well as in the treatment of diseases such as smallpox and tuberculosis. The pennyroyal plant has also been used as an abortifacient (Franzios et. al., 1997). Chemicals in the pennyroyal plant cause the uterine lining to contract, causing a woman's uterine lining to shed. Women who struggle with regulating their menstrual cycle or suffer from a cystic ovary syndrome may choose to drink pennyroyal tea. In view of this, the present study was carried out to find the phyto-constituents in aqueous, ethanol and methanol extracts. Quantitative analysis was also carried out for the few metabolites in the aerial parts of *Mentha pulegium* crude extract.

MATERIAL AND METHODS

Sample Extraction

The shade dry leaves, stem and inflorescence of *Mentha pulegium* was used for the extraction of bioactive compounds. About 100 gr. of each part of plant material was used to prepare the crude extract. The plant materials were washed thoroughly with distilled water, shade dried and crushed into uniform dry powder. Extracts were prepared using three solvents: methanol (80%), ethanol (80%) and double distilled water. Aqueous, methanol and ethanol crude extracts were isolated by Soxhlet method and subjected to the qualitative chemical tests for the identification of various bioactive phytochemicals.

Phytochemical screening

The phytochemical screening of the sample was carried out as described by Nweze et al., (2004) and Senthikumar and Reetha (2009). The samples were screened for alkaloids, flavonoids, steroids, terpenoids, tannins and phenols.

A. Qualitative Phytochemical Analysis

1. Test for Flavonoids

a) To 2 ml of plant extract 1 ml of 1N aqueous NaOH solution was added and observed for the formation of yellow-orange coloration.
b) 2 ml of plant extract was treated with 4 drops of concentrated sulphuric acid and observed for the formation of orange colour.
c) 2 mL of the extract was dissolved in the methanol, to this a small piece of magnesium ribbon was added and 1 mL of concentrated Hydrochloric acid was added from the side of the test tube. A magenta pink colour indicates the presence of flavonoids.

2. Test for Alkaloids

a) To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then 3 drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.
b) 2 ml of each extract was diluted separately to 10 ml with acid alcohol, boiled and filtered. To 5ml of the filtrate 2 mL of dilute ammonia was added. 5 ml of chloroform was added and mixed to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. Few drops of Wagner's solution was added to chloroform solution, reddish brown precipitate indicates the presence of alkaloids.
c). Extract of each plant sample was separately stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents as follows.
d). **Dragendorff's test:** To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added (Potassium bismuth iodide solution). A prominent yellow precipitate indicates the presence of alkaloids.
e) **Mayer's test:** To a few ml of filtrate, added 1 ml of Mayer's reagent (potassium mercuric iodide solution). White or cream-coloured precipitate indicates the presence of alkaloids.
f) **Hager's test:** To a few ml of filtrate, added 2 ml of Hager's reagent (saturated aqueous solution of picric acid); yellow coloured precipitate indicates the presence of alkaloids.
g) **Wagner's test:** To a few ml of filtrate, added 2 ml of Wagner's reagent (iodine in potassium iodide), formation of reddish brown precipitate indicates the presence of alkaloids.

3. Test for Terpenoids

a). 2 mL of extracts was treated with 2 mL of chloroform and concentrated sulphuric acid was carefully added to form a layer. A reddish brown colour formation at the interface confirms the presence of terpenoids.
b). **Test for Triterpenoids (Noller's Test):** Two or three granules of tin metal were added in 2 ml thionyl chloride solution. Then the extract was added into test tube and warmed. The formation of pink colour indicates the presence of triterpenoids.

4. Test for Steroids

a). To 1 ml of plant extract, equal volume of chloroform and 3 drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.
b). 1 ml of extract was taken to which 10 ml of chloroform was added. To this mixture, 10 ml of concentrated sulphuric acid was added along the sides of the test tube. A colour change from violet to blue/green confirms the presence of steroids in the samples.

5. Test for tannin

1 ml of distilled water and 2-3 drops of ferric chloride solution was added to 0.5 ml of crude extract. A black coluration indicated the presence of tannin.

6. Test for phenol
2 ml of alcohol and 2-3 drops of ferric chloride solution was added to 1 ml of crude extract, blue green or black coloration indicated the presence of phenols.

Quantitative Phytochemical Analysis
1. Estimation of Terpenoids
Alkaloid determination by using Harborne (1973) method. One gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH4OH was added by drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

2. Estimation of Flavonoids
One gram of plant sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No 1 filter paper into a pre- weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. (Krishnaiah et. al., 2009).

3. Estimation of Total Phenols
The fat sample was boiled with 50ml of ether for the extraction of the phenolic components for 15 min. Five ml of the extract was pipette out into a 50 ml flask, then 10ml of distilled water was added. Two ml of NH4OH solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was read at 505 nm.

RESULTS AND DISCUSSION

Qualitative Phytochemical analysis
M. pulegium has been well known since ancient times, from Greek, Roman, and Medieval cultures, for its culinary uses and medicinal properties, such as emmenagogue and abortifacient effects (Van De Walle & Riddle in 1994 and Gordon & Khojasteh in 2014), as well as for treatment of gastrointestinal ailments and skin itching, (Nickavar & Jabbareh, in 2018). Nowadays, *M. pulegium* commercialization includes uses as food and drink flavoring, Pennyroyal teas for cold relief, coughs, kidney problems, and headaches (Teixeira et.al. in 2012). Despite these applications, this plant is well-known for its toxicity to humans, especially due to its essential oil (Nadia et.al., 2013). Pennyroyal is not yet described by the Pharmacopoeia 15. Even if its properties were traditionally well-known, this species has never been investigated pharmacologically (Bruneton, 2009).

In the present investigation, qualitative analysis is used to reveal the chemical constituents or the secondary metabolites of the different parts of the plant. The preliminary phytochemical screening for dry stem, leaves and inflorescence in Aqueous, Methanol and Ethanol extract of *M. pulegium* revealed the presence of alkaloids, flavonoids, steroids, phenols, terpenoids and tannin (Table 1). Similar results were observed in both Ethanol and Methanol extracts. Therefore only Ethanol extract results were not represented in the table. Alkaloids were present in the aqueous extract in leaf and inflorescence, absent in methanol extracts of stem. Tannins were present in inflorescence of both aqueous and methanol extracts. Steroids were revealed in both aqueous and methanol extracts in leaves as well as in inflorescence, absent in methanol extract in stem. Whereas flavonoid, terpenoids and phenols are present in all parts of both aqueous and methanol extracts. On the other hand, number of various environmental factors and physiology of the plant has a major role for the quality and quantity of the herbal ingredients present in a particular species. In the present investigation, the plant material was collected in afternoon around 1.00 PM and the plant was fully blooming stage. These conditions may produce major variations in the bioactive compounds present in the plants (Kokate C.K. 1991). It is explained that the polarity level and species nature are playing major role in extracting the secondary metabolites (Paulsamy Set. al. 2011). Qualitative phytochemical screening will help to understand variety of chemical compounds produced from different parts of the plants. The secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses.

The positive test for methanol and water extracts of all the parts showed that methanol extract registered higher percentage of yield. It may be due to high polarity of methanolic solvent which can draw high variety of plant constituents than the water extract (Vishnu R et.al. 2013).

Quantitative Phytochemical Analysis
Presence of Terpenoids, flavonoids, essential oils and phenolic compounds are the basic characterstic of any aromatic plant. *Mentha pulegium*, the whole plant exhibits pungent aromatic smell due to the presence of large quantities of these secondary metabolites. Terpenoids are important ecological mediators and play a role in plant defense against herbivory, disease resistance, attract pollinators, as well as potentially plant-plant communication *(Martin,2003 & Pichersky, 2006).* They appear to play roles as insect repellent (Davis et.al, 2000). On the other hand both flavonoids and many other phenolic components have been reported on their effective antioxidants, anticancer, antibacteria, cardioprotective agents, anti-inflammation, immune system promoting, skin protection from UV radiation, and interesting candidate for pharmaceutical and medical application Kumar S., Pandey, 2013, Chen X et.al., 2014, Andreu et.al. & Meng et.al., in 2018. In view of this, quantitative analysis for terpenoids, flavonoids and phenols also have been estimated and observed in large quantities in almost all parts of the plant.

Fig:1. Habitat of *Mentha Pulegium* L. Var. erecta (Phenology: March-May)

Table 1: Phytochemical analysis of *Mentha pulegium* L.

A. Qualitative Phytochemical analysis.

Sr.No	Name of the Plant part	Aqueous						Methanol					
		A	S	F	Te	Ta	P	A	S	F	Te	Ta	P
1.	*Leaves*	+	+	+	+	-	+	+	+	+	+	+	+
2.	*Stem*	-	-	+	+	-	+	-	+	+	+	-	+
3.	*Inflorescence*	+	+	+	+	+	+	+	+	+	+	+	+

'+': Present, '-': Absent, A-Alkaloid, S-Steroid, F-Flavonoid, Te –Terpenoid, Ta-Tannin, P-Phenol

B. Quantitative Phytochemical Analysis.

Sr.No	Name of the Plant part	Terpenoids	Flavonoids	Phenols
1.	*Stem*	8.80 mg/gdw	7.00 mg/gdw	8.32 mg/gdw
2.	*Leaves*	10.04mg/gdw	8..90mg/gdw	10.56mg/gdw
3.	*Inflorescence*	12.42mg/gdw	14.42mg/gdw	13.56mg/gdw

mg- milligram, gdw-gram dry weight

CONCLUSION

Different secondary metabolites were identified from various parts of the plant; Flavonoids, Steroids, Tannins, Terpenoids, Alkaloids and Phenols. Therefore, pennyroyal can be seen as a potential source of useful drugs. This work aims to provide an overview of the biocomponents as the potential sources of pharmaceutical and cosmetic applications and also some interesting directions for future researches on this plant.

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