



# Comparative Phytochemical Evaluation of *Mentha piperita* Varieties (citrate and perpeta) Leaves and Stem Extracts

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**Abstract:** The present research work was carried out to compare the phytochemical analysis of two menthe *piperita* varieties (citrate and perpeta) stem and leaves extracts. The methanol and ethanol leaves extracts showed large quantities of the investigated phytochemical in *Mentha piperita* citrate. *n*-Hexane extracts were found the less phytochemical contents as compared to the other extracts.

**Keywords:** *Mentha*, Alkaloids, Phenols, Flavonoids, Tannins, saponins, Amino Acids

## INTRODUCTION

*Mentha piperita* is found in different parts of the world both as wild and cultivated. *Mentha piperita* is utilized internally as extract, oil, tincture and tea, while externally applied as liniment or rub. Plant experts consider it as an anti-aging, stimulant, emmenagogue, rubefacient, antimicrobial, astringent, antiscatarrhal, antipyretic, antispasmodic and antiseptic properties (Ali *et al.*, 2002).

The conventional medicinal methods, particularly the exercise of therapeutic herbs still keep a main position in the budding countries. The history to utilize herbal drug might be as old as the mankind history. Nowadays 80% of the world population utilize plants to cure different diseases (Srinivas & Arun, 2012). Hence, the objective of the study was to assess the phytochemical properties of the locally grown mint plant leaves and stem.

## Materials and Methods

### Plant Collection and preparation

Healthy plants (*Mentha piperita* L.) were collected from Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar Khyber Pakhtunkhwa-Pakistan, washed thoroughly in tap water and shade dried for two weeks.

### Preparation of leaves and Stem Solvent Extracts

Fifty grams powder of *Mentha piperita* (leaves and stem) were extracted in 500 ml of aqueous ethanol, acetone, methanol, chloroform and *n*-hexane for 48 hrs. These extracts were filtered through Whatman filter paper (repeated three times). The extracts were concentrated in a rotary evaporator and then transferred into a sterilized beaker for heating in a water bath (50°C) to get a dehydrated filtrate. The resultant crude extracts were transferred into sealed bottles and reserved at 4°C until used.

### Phytochemical Analysis

#### Preliminary phytochemicals screening:

One gram of the aqueous, methanol, ethanol, acetone, chloroform, and *n*-hexane extracts of *Mentha piperita* citrate and *Mentha piperita* perperita stem and leaves were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening following the methodology of Harborne (1998) and Kokate (2001).

#### Screening procedure

**Terpenes:** 02 ml of each extract, 2 ml acetic anhydride, 5 ml chloroform and H<sub>2</sub>SO<sub>4</sub> (concentrated) were mixed gently till formation of layer. At the result brown reddish color formed indicated the terpenes (Harborne, 1973).

**Test for steroids:** The extracts 01 mL was mixed in chloroform (10 ml) and added equivalent amount of H<sub>2</sub>SO<sub>4</sub> (concentrated) by the test tubes sides. At the results, H<sub>2</sub>SO<sub>4</sub> layer revealed yellow with green appearance and top side went red. This observation showed the steroids presence.

**Test for glycosides:** Each tests portion was hydrolyzed with hydro chloric acid on water bath for some hours. The resultant hydrolysate, added 1ml of pyridine and some drops of sodium troprusside solutions were mixed and after that it was formed basic with the help of NaOH solution. Pink to red colour indication confirm the glycosides existence.

**Test for alkaloids:** 2 mL of HCl was taken and 5 mL of extract was added, then Dragendroff's reagent (1 mL) was added to this acidic mixture. Red or orange precipitate formed instantly verified the existence of alkaloids.

**Phenolics:** To 2 ml of test portion (extract), 1 ml of ferric chloride solution (1%) was added. Green or blue color indicated phenols (Martinez & Valencia, 2003).

**Test for flavonoids:** A few drops of NaOH (dilute) were added to 1 mL of the extract. A strong yellow color was formed in the extract, which was converted to colorless solution when some drops of HCl (dilute) were added. This reaction showed that tested extract has flavonoids.

**Test for tannins:** To 5 mL of the test portion, little drops of lead acetate (1%) was added. The formation of yellow precipitate was a sign of tannins.

**Test for saponins:** To 20 mL of distilled water, extract was added to become diluted and for 15, it was rotated in a graduated cylinder. The development of foam layer (1cm) indicates the existence of saponins.

**Test for amino acids:** A few drops Ninhydrin reagents were added to 1 mL of the extract. Purple colour formation indicated the presence of amino acid presence.

### Results

The qualitative analysis of the major natural constituents of the medicinally important plants was conducted in *M. piperita* citrate and *M. piperita* perperita. The qualitative phytochemical screening was carried out in the aqueous, methanol, ethanol, acetone, chloroform, and *n*-hexane leaves extract of both plants and results are

shown in Table 1. Terpenoids, steroids, glycosides, phenol, tannins, saponins and amino acid were present in more amounts in methanol and ethanol extract of *M. piperita citrate* whereas, the leaves extract of *M. piperita* perpeta was found bioactive in average quantity. The tannins was absent in the chloroform and n-hexane extracts of both variety of *M. piperita*. Flavonoid was found more quantity in aqueous, methanol and ethanol extracts while absent in the acetone, chloroform and n-hexane extracts of *M. piperita* perpeta.

Similar trend was also found in stem extract of two varieties of *Mentha piperita*. The same natural compound as mentioned above in leaves was present in large amount in extract methanol and ethanol. The saponins and amino acid were noted in average quantity in *M. piperita* citrate. The glycoside, alkaloids, flavonoid, tannins and saponins were not detected in the extract of chloroform and n-hexane of *M. piperita* perpeta (Table 2).

## Discussion

Different types of disease are raise globally particularly in the developing countries, and their treatment by synthetic drugs are always associated with many side effects. The use of phytochemical could be a safe way to solve these problems. Phytochemicals commonly produce their antimicrobial activities through special principles which may be different from synthetic drugs (Scalbert, 1991). The results of the phytochemical analysis of different extracts of *Mentha piperita* showed that each of the plant part tested were rich in at least one of alkaloids, flavonoids, phenols, tannins and steroids (Para *et al.*, 2013).

The tannins containing plants as their main component are astringent in nature and are used for the treatment of stomach disorders such as diarrhea and dysentery (Dharmananda, 2003). Flavonoids are considered as the main phytochemicals present in plant, and are used for the treatment of various types of microbial infections (Xiao, 1980). Extract from leaves of mint, contained 1.24 mgGAE/100 ml of total phenolic compounds and 34.21% antioxidant activity (Fahad & Kashif 2011). Flavonoids are known to be synthesized by plants in response to microbial infection and should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Para *et al.*, 2013).

## Conclusion

Herbal medicine development research is in progress throughout the world, so the present report will be helpful for the isolation and production of new drugs, medicine and health care product. At the end, it can be concluded that the active biological compound present in in the *Mentha piperita* would definitely find place to cure different bacterial and fungal infections and this aromatic plant should be further studied extensively for exploration of its ability to cure different ailments.

## Competing interests

The author declares that they have no competing interests.

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**Table 1. Phytochemical analysis of *Mentha piperita* leaves extracts.**

Constituents	Varieties	Aqueous	Methanol	Ethanol	Acetone	Chloroform	n-hexane
Terpenoids	<i>M. piperita</i> citrate	++	+++	+++	+++	+++	++
	<i>M. piperita</i> perpeta	+	++	++	++	++	++
Steroids	<i>M. piperita</i> citrate	++	+++	+++	++	++	+++
	<i>M. piperita</i> perpeta	+	++	++	+	+	++
Glycosides	<i>M. piperita</i> citrate	++	+++	+++	++	++	+++
	<i>M. piperita</i> perpeta	++	++	++	+	+	++
Alkaloids	<i>M. piperita</i> citrate	++	+++	+++	++	+	+
	<i>M. piperita</i> perpeta	+	++	++	+	ND	ND
Phenols	<i>M. piperita</i> citrate	+++	+++	+++	++	++	+
	<i>M. piperita</i> perpeta	++	++	++	+	+	ND
Flavonoids	<i>M. piperita</i> citrate	++	++	++	+	+	ND
	<i>M. piperita</i> perpeta	+	+	+	ND	ND	ND
Tannins	<i>M. piperita</i> citrate	+++	+++	+++	++	ND	ND
	<i>M. piperita</i> perpeta	++	++	++	+	ND	ND
Saponins	<i>M. piperita</i> citrate	++	+++	+++	++	++	+++
	<i>M. piperita</i> perpeta	++	++	++	+	ND	++
Amino	<i>M. piperita</i> citrate	++	+++	+++	++	++	+++

Acids	<i>M. piperita</i> perpeta	++	++	++	+	ND	++
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Note: + sign shows detection level of the phytochemicals present in extracts, + = Small quantity, ++ = Average quantity, +++ = Large quantity, ND = Not detected.

**Table 2. Phytochemical Analysis of *Mentha piperita* Stem extracts.**

Constituents	Varieties	Aqueous	Methanol	Ethanol	Acetone	Chloroform	n-hexane
Terpenoids	<i>M. piperita</i> citrate	++	+++	+++	++	++	+++
	<i>M. piperita</i> perpeta	+	++	++	+	+	++
Steroids	<i>M. piperita</i> citrate	++	+++	+++	++	++	+++
	<i>M. piperita</i> perpeta	+	++	++	+	+	++
Glycosides	<i>M. piperita</i> citrate	++	+++	+++	++	+	+
	<i>M. piperita</i> perpeta	+	++	++	+	ND	ND
Alkaloids	<i>M. piperita</i> citrate	++	+++	+++	++	+	+
	<i>M. piperita</i> perpeta	+	++	++	+	ND	ND
Phenols	<i>M. piperita</i> citrate	++	+++	+++	++	++	++
	<i>M. piperita</i> perpeta	+	++	++	+	+	+
Flavonoids	<i>M. piperita</i> citrate	++	+++	+++	++	+	+
	<i>M. piperita</i> perpeta	+	++	++	+	ND	ND
Tannins	<i>M. piperita</i> citrate	++	+++	+++	++	+	+
	<i>M. piperita</i> perpeta	+	++	++	+	ND	ND
Saponins	<i>M. piperita</i> citrate	++	++	++	++	+	+
	<i>M. piperita</i> perpeta	+	+	+	+	ND	ND
Amino Acids	<i>M. piperita</i> citrate	++	+++	++	++	++	+
	<i>M. piperita</i> perpeta	+	++	+	+	+	ND

Note: + sign shows detection level of the phytochemicals present in extracts. + = Small quantity, ++ = Average quantity, +++ = Large quantity, ND = Not detected.