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RESEARCH **P**APER

Effect of thermal processing on total phenolic content and antioxidant activity of *Mentha* leaves

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Mint is a promising health promoting herb, which is not only used for flavour and aroma, but also has many potential health benefits. Effect of refrigeration as well as thermal processing methods (blanching and boiling) on potential health benefits of mint was studied by determination of antioxidant activity and content of total phenolic substances in ethanolic extracts of mint leaves. The leaves were subjected to blanching (80°C), boiling (100°) as well as storage at refrigerated temperature (4°C). A qualitative phytochemical screening was performed. The ethanolic extracts were analyzed for total phenolic content using Folin-Ciocalteau assay and free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Thermal treatment caused significant decrease in antioxidant activity as well as total phenolic content of mint leaves. Total phenolic content in fresh sample was 115.81 mg GAE/g, which decreased to 3.59 mg GAE/g when leaves were subjected to 100°C. Antioxidant activity reduced from 77.9 per cent in fresh leaves to 48.7 per cent in boiled leaves. The study indicated that polyphenols and phenolic acids, responsible for antioxidant action of mint, were degraded by heat, thereby reducing the medicinal value of herb. The study thus, suggests the consumption of fresh mint leaves to obtain the maximum health benefits.

Key words : *Mentha*, Bioactive components, Phytochemical screening, Antioxidant potential, Total phenolic content

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INTRODUCTION

Mint is a common food adjunct cultivated widely in India, having nutritional as well as medicinal benefits. Its discovery dates back to seventeenth century in England (Raghavan, 2006). *Mentha* (mint) is a genus of flowering plants in the mint family Lamiaceae (Harley *et al*., 2004). The genus includes 18 species and 11 named hybrids, placed in four sections (Lawrence, 2006). Mints are aromatic, almost exclusively perennial, rarely annual, herbs. Some of the common varieties are the Apple mint, Curly mint, Pennyroyal, Peppermint, Pineapple mint, Spearmint and Water mint. The plant is widely grown for its leaves and essential oil. Mint leaves and essential oil, both are used for culinary and medicinal purposes. When placed on the tongue, it produces a hot, tingly sensation, which fades into a cooling feel. The cooling is due to the menthol, which is the major component of the oil (Chawla and Thakur, 2013). Other active components include p-menthane, menthone, limonene and carvone, which are primarily responsible for flavour and aroma. Mints contain minerals like calcium, potassium, sodium, magnesium, phosphorus and iron, as well as vitamin A, C, K, folic acid, thiamine, riboflavin and niacin (Raghavan, 2006). Mint was originally used as a medicinal herb to treat stomach ache and chest pains. It is considered to be an astringent, antiseptic, carminative, antispasmodic, anti-inflammatory, anti-tussive, analgesic, and anti-carcinogenic (Chawla and Thakur, 2013; Gardiner, 2000).

Mentha piperita has numerous pharmacological, cosmetic and alimental applications due to its ability to produce terpene and terpenoid compounds. It produces oils rich in menthol and flavonoids, making it economically very important (Barbalho *et al*., 2011). The phenolic components present in the leaves include rosmarinic acid and several flavonoids, primarily eriocitrin, luteolin and hesperidin (McKay and Blumberg, 2006). These polyphenols are responsible for strong antioxidant action of *Mentha*. These bioactive components can protect the human body from free radicals and could retard the process of many chronic diseases

such as cancer, cardiovascular disease and diabetes; they can also reduce lipid oxidative rancidity in foods (Regnault-Roger *et al*., 2004; Arts and Hollman, 2005; Williamson and Manach, 2005). The antioxidant activity of phenolic acids is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons or chelate metal cations (Tahira *et al*., 2011). Over the years, researchers have developed great interest in antioxidant activities of phenolic compounds due to their abundance in diet as well as their probable role in prevention of cardiovascular and neurodegenerative diseases (Nambiar *et al*., 2010; Chawla and Thakur, 2013).

The paper demonstrates the effect of refrigeration and different cooking methods such as blanching and boiling on total phenolic content and antioxidant activity of ethanolic extracts of mint leaves as well as correlation between total phenolic content and antioxidant activity of mint leaves. The plant extracts were also subjected to qualitative phytochemical screening.

RESEARCH METHODOLOGY

Chemicals :

Ethanol, methanol, sodium carbonate, gallic acid, Folin-Ciocalteu reagent, and DPPH (1, 1-diphenyl-2-picrylhydrazyl) were used. All the chemicals and reagents used were of analytical grade.

Collection of plant material and sample preparation :

Fresh leaves of *Mentha* were purchased from a local vendor in Delhi, India and washed with tap water. 200 g of leaves were taken and divided into 4 equal parts (50 g each). One portion was retained fresh; others were given different thermal treatments, as given below :

- *Blanching:* Mint leaves (50 g) were blanched at 80°C for 2 min. The sample was drained off and cooled rapidly with cold water.
- *Boiling:* Leaves (50 g) were boiled for 20 min, drained off and cooled rapidly.
- *Refrigeration:* Leaves were kept at 4°C in refrigerator for 5 days.

Preparation of extracts :

Leaves were extracted with ethanol at room temperature prior to removal of the solvent. Mint leaves were soaked in 500 ml of 99.9 per cent ethanol for 2-3 days separately. The soaked material was filtered and the extracts were collected. This process was repeated thrice and filtrates were collected. The filtrates obtained were concentrated under vacuum on a rotary evaporator (Buchi Rotary Evaporator, Model R-124) and stored at 4°C for further use (Song *et al.*, 2010).

Phytochemical screening :

Ethanolic extracts of fresh mint leaves were used for qualitative screening of phytochemicals as per standard biochemical procedures. The tests were performed to confirm the presence of alkaloids, carbohydrates, proteins and amino acids, glycosides, flavonoids, tannins, phenolics, terpenoids and steroids (Tiwari *et al*., 2011).

Estimation of total phenolic content :

The total phenolic content in ethanolic extracts of mint leaves was estimated by Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965). Gallic acid stock solution (1000 µg/ml) was prepared by dissolving 100 mg of gallic acid in 100 ml ethanol. Various dilutions of standard gallic acid were prepared from this stock solution. Calibration curve was plotted by mixing 1 ml aliquots of 1.0, 2.5, 5.0, 10, 25, 50 and 100 µg/ml of gallic acid solution with 5.0 ml of Folin-Ciocalteu reagent (diluted ten-fold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured after 30 min at 20°C at 765 nm. 1 ml of ethanol extract was mixed separately with the same reagents and absorbance was measured at 765 nm after 1 hour. The total phenolic compound in all the extracts was determined using the formula :

$$C = C_1 \times \frac{V}{m}$$

where,

C= Total content of phenolic compounds in mg/g in GAE (Gallic acid equivalent); C_1= The concentration of gallic acid established from the standard curve in mg/ml; V=The volume of extract in ml, M=Weight of plant extract in grams.

Determination of free-radical scavenging activity using DPPH method :

The free radical scavenging activity of test samples was measured by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) (Kaur and Arora, 2011). A 0.1 mM solution of DPPH in methanol was made and 1.5 ml of this solution was added to 0.5 ml of extract solution in methanol at different concentrations (100-500 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature in dark for 30 min. The absorbance was then measured at 517 nm using a spectrophotometer. A blank without DPPH was used to remove the influence of the colour of samples. A methanolic solution of DPPH was used as negative control. The DPPH radical scavenging activity was calculated using the following equation :

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_S}{A_0} \times 100$$

where,

A_o is the absorbance of negative control and A_S is the absorbance of sample .

RESEARCH FINDINGS AND ANALYSIS

The results of qualitative phytochemical analysis of ethanolic extracts of fresh mint leaves revealed the presence of proteins and amino acids, carbohydrates, phenols, terpenoids, sterols, flavonoids and tannins (Table 1).

Table 1 : Qualitative phytochemical screening of ethanol extracts of mint

Sr. No.	Phytochemical	Mint
1.	Alkaloids	-
2.	Proteins and amino acids	+
3.	Carbohydrates	+
4.	Phenols	+
5.	Terpenoids	+
6.	Sterols	+
7.	Saponins	-
8.	Glycosides	-
9.	Flavonoids	+
10.	Tannins	+

Total phenolic content was determined using Folin-Ciocalteu reagent. Gallic acid was used as the standard compound. The standard curve of gallic acid concentrations and absorbance is shown in Fig 1. Standard curve equation found was :

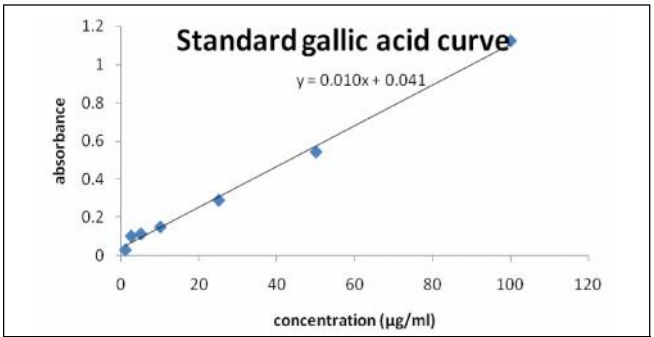

Fig. 1 : Gallic acid standard curve

Y = 0.0106x + 0.041

R^2 = 0.996

The total phenolic content of ethanol extracts of mint is given in Table 2 (Fig. 2). Data expressed as mean ± standard error of three samples analyzed separately.

Table 2 : Total phenolic content in ethanolic extracts of mint

Sr. No.	Sample	Absorbance at 765 nm (Mean ± SEM)	Total phenolic content (mg gallic acid equivalents per gram)
1.	Fresh leaves	0.906 ± 0.0073	115.81 ± 0.98
2.	Refrigerated leaves	0.501 ± 0.0017	61.64 ± 0.23
3.	Blanched leaves	0.446 ± 0.0028	54.38 ± 0.39
4.	Leaves at 100° C	0.066 ± 0.0023	3.59 ± 0.31

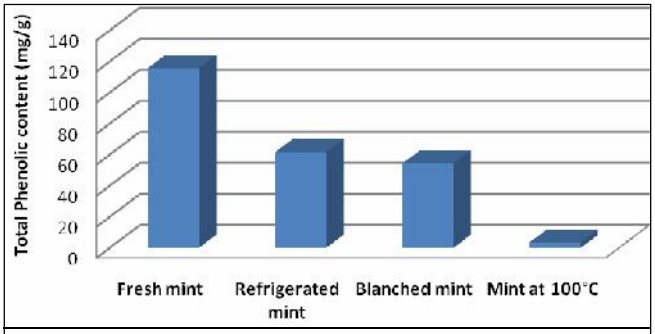

Fig. 2 : Effect of thermal processing on total phenolic content of mint leaf extracts

The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm induced by the antioxidants Ascorbic acid was used as the standard. DPPH reacts with the antioxidants and gets converted into 1, 1-diphenyl-2-picrylhydrazine by accepting a hydrogen atom and thus, reducing the absorbance. The methanolic extracts of mint showed a concentration-dependent DPPH radical scavenging activity. Results are presented in Table 3 (Fig. 3).

Table 3 : Per cent scavenging activity in mint extracts at different concentrations

Concentration (µg/ml)	% Scavenging activity				
	Ascorbic acid	Fresh leaves	Refrigerated leaves	Blanched leaves	Boiled leaves
100	51.5	62.11	57.4	30.3	32.6
200	61.1	64.4	57.9	49.9	38.0
300	77.3	66.9	64.5	57.4	39.9
400	78.6	75.2	73.4	63.5	40.6
500	88.46	77.9	75.5	64.5	48.7

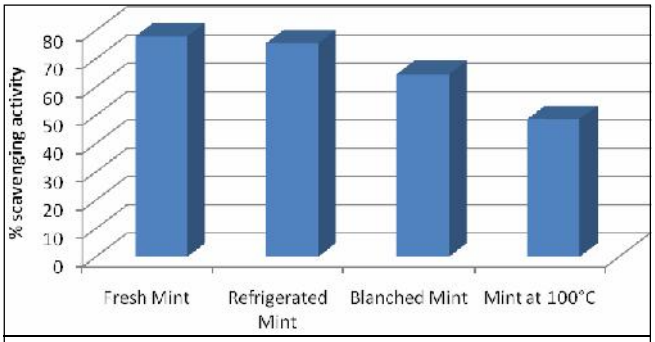

Fig. 3 : Per cent scavenging activity in mint leaf extracts at 500µg/ml

The concentration of total phenolic substances was highest in fresh mint leaves (115.81 ± 0.98 mg GAE/g) and thus, fresh mint leaves had the highest antioxidant activity (77.9% at 500 µg/ml concentration) (Fig. 4). Thermal treatment significantly decreased the total phenolic content as well as antioxidant potential of mint leaf extracts. Blanching caused a significant decrease (53.04%) in total

phenolic content of leaves and this change was drastic in case of boiling (96.9%). Refrigeration also affected the total phenolic content but to a lesser extent (46.77%). Hence, the total phenolic content followed the order :

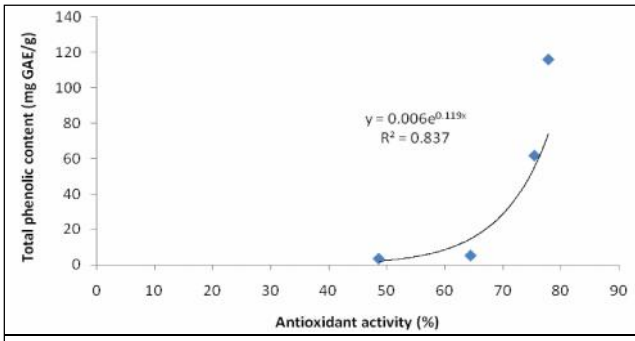

Fig. 4 : Correlation between antioxidant activity and total phenolic content of fresh and treated mint samples

Fresh leaves > Refrigerated leaves > Blanched leaves > Boiled leaves

The same trend was observed in case of antioxidant activity, suggesting that antioxidant activity diminished due to the inactivation of bioactive compounds caused by different chemical reactions accelerated by the effect of heat.

Conclusion :

Qualitative phytochemical screening of leaf extracts of *Mentha* revealed the presence of phenolic compounds and other bioactive components, which are responsible for strong antioxidant and antimicrobial action of mint. However, these compounds get destructed when the herb is subjected to cooking methods such as blanching and boiling as well as stored in refrigerator for longer periods, thus reducing the antioxidant potential of the herb, making it less beneficial for consumption. The research suggests the use of fresh mint leaves in order to get the maximum benefit from the herb.

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