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Comparative analysis of antioxidant activity of leaf and *In vitro* callus of *Mentha spicata* (Lamiaceae)

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Abstract
Our present study was undertaken to identify the new active constituent from *Mentha spicata* for their antioxidant activities to determine novel active to improve the treatment of diseases. In these studies leaf extracts and callus extracts in various solvents (Ethanolic, DMSO). The antioxidant potential of the *Mentha spicata* was investigated by ferric ion reducing antioxidant power (FRAP assay). Among those studied, the ethanolic extract of *M.spicata* leaf showed the highest phenolic content with 0.0110 mg gallic acid /mg of dry weight which was followed by dry weight extract of callus. The total antioxidant activity of ethanolic leaf extract was higher when compared to dry weight extract of callus. The leaves of *Mentha spicata* have excellent antioxidant activities. Hence, it can be employed as a new active ingredient in the antioxidant formulation in plant species. There is no report on the antioxidant activity of *Mentha spicata* L.

Keywords: FRAP, gallic acid, antioxidant activity, DMSO, Dry weight callus

Introduction
From ancient culture to modern era medicinal plants have been playing a vital role in the health remedies of living creatures. Our Indian system of a medicine widely used plant-based drugs or formulations to cure various human diseases. Presently, researchers have been extensive interest in exploiting the biological studies of different ayurvedic formulations for cost-effectiveness and minimum side effects.

These plant species contain many constitutes in which therapeutic value for medicine. *Mentha* is a genus of aromatic perennial herbs belonging to the Lamiaceae family. It is found mainly in the temperate and sub-temperate regions of the world (Ahmed; 2012) [1]. Family Lamiaceae is an ample source of terpenoids and flavonoids. In which includes 264 genera and 6990 species distributed worldwide. Among them, 72 genera and 435 species are represented by Indian subcontinent. Spearmint (*Mentha spicata*) is locally known as "domestic mint or pudina" (Asai *et al*.; 1994) [2]. Mint plant is one of the most exciting research for the antioxidant assay (Benzie; 1996) [3].

Many herbal plants possess antioxidant compounds which protect the cell against the damaging effects of reactive oxygen species. Superoxide anions, hydroxyl radicals and hydrogen peroxide are the ROS (free radicals) which play an essential role in causing various chronic and degenerative diseases such as arthritis, carcinoma, diabetes, cardiovascular and pulmonary diseases, some types of cancer, cataracts, immune autoimmune diseases, inflammation, atherosclerosis and brain dysfunction (Parkinson's, Alzheimer's, Huntington's diseases) as well as aging processes. In the human body, free radicals are produced through aerobic respiration or from exogenous sources (Dani *et al*.; 2010) [7].

In these experiments, determination of antioxidant capacities several assays have been employed viz. 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2- diphenyl-1-picrylhydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP), and the oxygen radical absorption capacity (ORAC). Among these methods; in these experiments performed by using ferric ion reducing antioxidant power (FRAP assay) to estimate the antioxidant capacity; reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. (Fe (II)-TPTZ has an intensive blue color and can be monitored at 593 nm (Farnsworth *et al*.; 1985) [9]. Phenolic compounds exist universally in plants as an essential category of phytochemicals. Being a potential agent for preventing and treating many oxidative stress-related diseases they have attracted increasing attention. At present, various researchers have found considerable interest in determining the total phenolic contents and antioxidant capacities of various plant species (Farooq *et al*.; 2007) [10].

The aim of the present study to systematically evaluate total phenolic contents and antioxidant capacity of *Mentha spicata* L. To understand the comparative antioxidant studies of leaf extract and callus extracts were carried out for the estimate total phenolic content and antioxidant capacities.

Materials and Methods
1. Collection of Plant Material
Fresh leaves of the plant were collected from Botanical Garden, Ganpat University, Mehsana, Gujarat (India)-384012 during summer season (Month of June). Plant materials rinsed with distilled water to remove dust particles followed by removed water by blotting over a filter paper.

2. Sample Preparation
Plant materials were shade dried and powdered.20 gram of powdered plant material was weighed and used for extraction by using ethanol. The cold extraction procedure using ethanol solvent followed by extracted essential oil

using distillation apparatus. After evaporating solvent recovered essential oil. The remaining content of essential oil diluted to 1:20 dilution with ethanol for further assays of antioxidant activity and total phenolic content.

In vitro grown callus dried at room temperature were shaded and wholly dried callus subsequently homogenized in a mortar pestle and make powdered of callus. Cold extraction procedure using DMSO solvent followed by essential oil throughdistillation apparatus. The mixture filtered by using Whatman filter paper for removing cell debris. The remaining content of essential oil diluted to 1:20 dilution with ethanol for further assays of antioxidant activity and total Phenolic content. Dried extract stored in the refrigerator for their future use in antioxidant activities and phytochemical screening.

3. Phytochemical Screening

The dry extract dissolved in particular solvent, i.e., ethanol and DMSO for further determination of constitute of phytochemical screening were performed for establishing a phytochemical profile of ethanolic and DMSO extracts obtained from cold extractions. Chemical tests were carried out using both extracts to detect various phytoconstituents present in them. (Trease and Evans; 1989) and (Sofowara; 1993).

4. Antioxidant activity and Phenolic content extraction method

a. Ferric Ion Reducing Antioxidant Power Assay

By Benzie and Strain; (1996) [3]; Ferric Reducing antioxidant power assay followed by the determination of total antioxidant activity. In this method, aliquots of 100µl different sample viz. Ethanolic extract (B) and Callus extract(C) were taken, and each sample was mixed with 3ml of FRAP reagent, and the mixture was incubated at 37^0 C for 10min and absorbance of the reaction mixture was measured at 593nm. Using five different concentration of FeSO4.7H2O (1000,750, 500,250 and 125µmol/L) calibration curve was prepared. The values were expressed as the concentration of antioxidant having a ferric reducing ability equivalent to 1mmol/L FeSO4.

b. Total phenolic compound analysis

The total amount of phenolic contents in the extracts measured by using Folin- Ciocalteu (FC) agent. 100 µl of undiluted (FC) reagent mixed with 20 µl of Ethanolic extract and Callus extract followed by incubating at 40 °C in the dark for 30 min. After incubation color development determined at 765 nm. By using the standard curve of gallic acid, phenolic contents of the samples calculated and expressed as Gallic acid equivalents (GAE)/mg the plant materials.

Results & Discussion

Our result indicated that production of plant secondary metabolites is a part of climatic and seasonal factors. It allows flexible production cycles of secondary metabolites. Additionally, the *In vitro* production overcomes the asymmetrical distribution of secondary metabolites in the whole plants (Dani *et al*.; 2010) [7]. However, media composition has to be optimized for an absolute biomass increase and the accumulation of the desired metabolites, for efficient spearmint callus production. In this study, results indicated that medium supplemented with growth regulators 2,4-D (2,4-Dichlorophenoxyacetic acid) and BAP (6-benzylamino purine) in a combination of (3mg/L + 2mg/L) under monochromatic blue light is favorable to induce maximum callus for *M. spicata* L. as shown in figure no. 1.

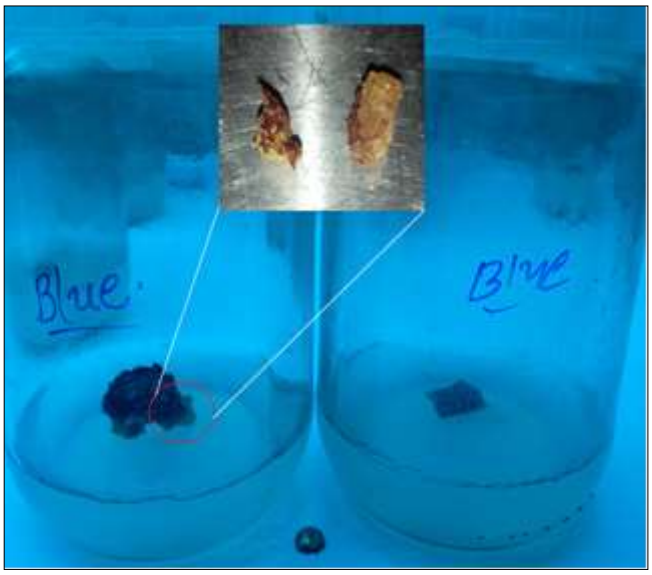

Fig 1: A maximum growth of friable yellowish dark callus obtained from blue light with the combination of growth regulators 2,4-D + BAP (3mg/L + 2mg/L).

Table 1: Phytochemical analysis of leaf and callus extract of *Mentha spicata*

Chemical constitutents	Ethanol	Callus
Alkaloid	Present	Present
Tannins	Present	Present
Saponins	Absent	Absent
Phlobatannins	Absent	Absent
Flavanoid	Present	Present
Terpenoid	Present	Present
Glycosides	Absent	Absent
Steroid	Absent	Absent

Fig 2: Extraction of oil from sample

Table 2: Total antioxidant activity and total phenol content

Sample	Total antioxidant activity (mMF^{II}/g Dry weight)	Total phenolic content (g GA/g sample)
Oil (1:20 dilution)	0.550	110
Callus	0.370	15

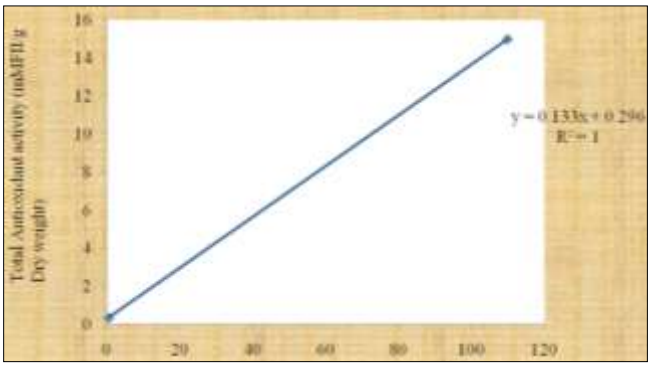

Fig 3: Correlation between Fe II equivalent antioxidant activity and total phenolic content in oil extract from leaf and callus extract

As per result is shown in Table-2 Antioxidant activity found 0.550 mMFII/g Dry weight in essential oil extracted from leaves and 0.370 mMFII/g Dry weight *In- vitro* grown callus. Phenolic content was found 110 g GA/g in essential oil and 15 g GA/g in *In-vitro* grown callus. Correlation analyses of antioxidant activity versus the total phenolic content was carried out using the correlation and regression program of SPPS16.0, and the results are shown in figure 3. Light irradiation has a remarkable effect on plant cell and tissue growth and secondary metabolite biosynthesis. The action of light on higher plants occurs in mainly two aspects. First, light provides the energy source required by the plant through photosynthesis. Second, light is a signal received by photoreceptor to regulate the growth and differentiation and metabolism (Ahmed *et al.*, 2012) [1].
Almost similar total antioxidant activities found in both leaf and callus extracts. Antioxidant activity of essential oil of leaf and callus extract of *Mentha spicata* 0.550 and 0.37 mMFII/g respectively. The phenolic content found higher in essential oil, i.e., 110g GA/g sample than that of callus i.e.15 g GA/g sample. The antioxidant effects of essential oil and callus were estimated using in vitro ferric ion reducing antioxidant power assay to identifying samples possessing high antioxidant power for further studies. The study also attempted to quantify the total phenolic content present in essential oil and Callus. Mentha is rich in compounds such as catechins, flavonoids, theaflavins, thearubigins, and phenolic acids that could potentially have health-promoting properties. Our studies have confirmed that callus possesses significant antioxidant capacity. Antioxidants are known to protect tissues against damage caused by oxygen free radicals and lipid peroxidation; It is proposed that the protective effect of callus against cardiovascular disease may be attributed to its antioxidant components.

Conclusion

Our Present study indicates that, for spearmint regeneration, maximum *In vitro* growth of callus obtained by using 2,4-D (2,4-Dichlorophenoxyacetic acid) + BAP (6-benzylamino purine) in combination with (3mg/L + 2mg/L) under the blue monochromatic light. Variation of medium composition could lead to enhanced spearmint regeneration efficiency with a lower cost (due to a lower concentration of medium growth regulators). At the same time, the essential oil extracted from ethanolic extracted leaves constitutes more antioxidant activity, and total phenolic content than *In vitro* grown callus of *Mentha spicata* L. Our research suggests positive relation between antioxidant activity and total phenol content. The present research work is carried out for comparative estimation of antioxidant capacity of ethanolic extract and *In-vitro* grown callus. It indicated significant antioxidant power compared to phenolic content. It used as a new antioxidant source of the plant.

Acknowledgement (Conflict of Interest)

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