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An in vitro analysis on the antioxidant and antigout activity of ethanolic leaf extract of Mentha Piperita Linn.

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Abstract

Introduction:

Mentha piperita is an aromatic herb in the mint family. It is a hybrid mint that is a cross between spearmint and watermint. It can be found naturally in North America and Europe. Herbal extracts are rich in antioxidants which scavenges the free

radicals produced as a result of metabolism in the body. Xanthine oxidase is an enzyme that is involved in purine metabolism, by generating uric acid.

Aim: The aim of the study is to analyse antioxidant and antigout activity of ethanolic leaf extract of Mentha *Piperita Linn.*

Materials and Methods: Methanolic leaf extract for mentha piperita was prepared. Antioxidant and Xanthine oxidase inhibitory potential of the extract was analysed by standard methods.The data were analyzed statistically by a one-way analysis of (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the group. The results with the p< 0.05 level were considered to be statistically significant.

Results: Phytochemical constituents like terpenoids, flavonoids, phenol, alkaloids, saponins and steroids were present in the extract. Antioxidant potential of the ethanolic extract was observed in a dose dependent manner. Xanthine oxidase inhibitory was maximum at 300 and 400 µg/ml of the ethanolic extract.

Conclusion: To conclude the study, ethanolic extract of Mentha piperita possessed potent in vitro antioxidant and xanthine oxidase inhibitory activity.

Keywords: Mentha piperita, Gout, Xanthine oxidase, antioxidant,innovative technology; novel method

INTRODUCTION:

The plant materials play a serious role in primary health care as therapeutics in many developing countries(Shaheen *et al.*, 2019). Various medical plants are used for years in lifestyle to treat diseases globally(Alakbarov, 2001). Mentha piperita is a popular herb that have been used in many forms (i.e. oil, leaf, leaf extract, and leaf water)(Murray, 2013) .Peppermint extracts e.g. ones with ethanol components, have various applications in food industries, cosmeceuticals, hygienic and pharmaceutical products for both its flavouring and fragrance properties(Bährle-Rapp, 2007). Antioxidants are substances that can slow damage or prevent cells caused by unstable molecules, free radicals that the body produces as reaction pressures(Shantanova, 1998). They are sometimes called "free-radical scavengers.The antioxidant plays a crucial role in scavenging oxygen radicals that were released during the metabolism of hypoxanthine to xanthine and acid(Singh, Shrivastava and Kale, 2012).

Gout is a kind of joint pain that causes unexpected joint aggravation typically in a solitary joint(Brzezińska *et al.*, 2021)t. Extreme gout can in some cases influence numerous joints immediately. This is known as polyarticular gout. Gout is brought about by a lot of uric acid in the circulatory system and collection of urate crystals in tissues of the body(Brzezińska *et al.*, 2021). Uric acid crystals in the joints are the reason for aggravation of redness, warmth, and pain in the joints. Xanthine oxidase is a protein that catalyzes purine catabolism, eventually producing uric acid(David Anthony Basil Hugh, 1986). Hence, xanthine oxidase inhibitors are utilised to prevent the conversion of hypoxanthine to xanthine and subsequently in the treatment of gout (Basnet *et al.*, 2020). Allopurinol is one of the synthetic xanthine oxidase inhibitors which has been generally utilised in the remedial and clinical administration of gout(Jeyaruban *et al.*, 2021). Anyway the disadvantage of utilising allopurinol is that it creates free radicals such as superoxide anions. The demerits of using synthetic drugs have resulted in finding natural products as an alternative.

To find a drug which has less side effects than allopurinol, Mentha piperita was chosen. (Wu *et al.*, 2019),(Chen *et al.*, 2019),(Li *et al.*, 2020),(Babu and Jayaraman, 2020),(Malaikolundhan *et al.*, 2020),(Han *et al.*, 2019),(Gothai *et al.*, 2018),(Veeraraghavan, Hussain, *et al.*, 2021),(Sathya *et al.*, 2020),(Yang *et al.*, 2020),(Rajendran *et al.*, 2020),(Barma *et al.*, 2021),(Samuel, 2021),(Samuel *et al.*, 2021),(Tang *et al.*, 2021),(Yin *et al.*, 2021),(Veeraraghavan, Periadurai, *et al.*, 2021),(Mickymaray *et al.*, 2021),(Teja and Ramesh, 2020),(Kadanakuppe and Hiremath, 2016). The aim of this study was to

analyse the phytochemical constituents, in vitro antioxidant and antigout activities of ethanolic leaf extract of Mentha piperita L.

MATERIALS AND METHODS:

1. **Phytochemical Screening test**

Test for phlobatannin
1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

Test for Carbohydrates
Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids
Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids
2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids
2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test for proteins
One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins
Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.
Test for steroids
One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2. DPPH free radical scavenging activity of ethanolic leaf extract of *Mentha piperita* L.

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0.

5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

3. In Vitro Xanthine Oxidase Inhibitory Activity of ethanolic leaf extract of *Mentha piperita* L.

In vitro Xanthine oxidase inhibitory of the extract was assessed as per the method of (Nguyen et al, 2004; Umamaheswari et al., 2007). Briefly, the assay mixture consisted of 1 ml of the fraction (0.1 to 0.5g/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25◦C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25◦C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5mg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25◦C. XOI activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage of inhibition as follows.

Inhibitory activity (%) = $(1 - A_s/A_c) \times 100$ Where,
As – absorbance in presence of test substance, Ac – absorbance of control

STATISTICAL ANALYSIS

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

RESULTS

Antioxidant potential of the ethanolic extract was observed in a dose dependent manner. Xanthine oxidase inhibitory was maximum at 300 and 400 μg/ml of the ethanolic extract. From this study, we can observe the presence of phytochemicals such as Flavonoids, Alkaloids, Terpenoids, Steroids and Saponins. DPPH radical scavenging activity (Figure 1) and xanthine oxidase inhibitory potential (Figure 2) of the ethanolic extract was observed.

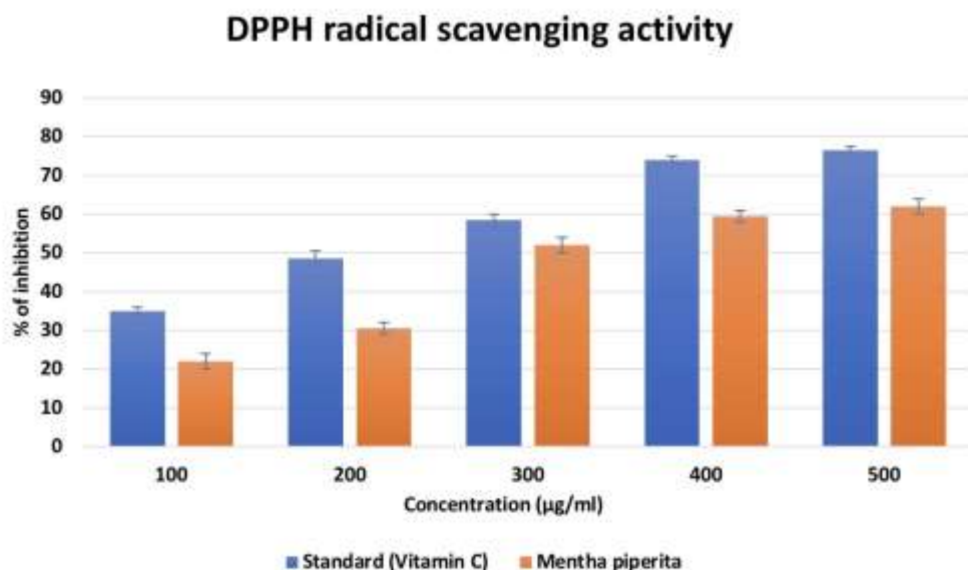

Figure 1: DPPH RADICAL SCAVENGING ACTIVITY

The bar graph depicts the In vitro antioxidant activity of *Mentha piperita L*. The X axis represents the different concentrations of *Mentha piperita L* and the Y axis represents the percentage of inhibition. The blue colour denotes the concentration of standard drug vitamin C and the orange colour denotes the *Mentha piperita L*. The difference was statistically significant. Each bar Represents Mean ± SEM of 3 independent observations. Significance of $p \leq 0.05$.

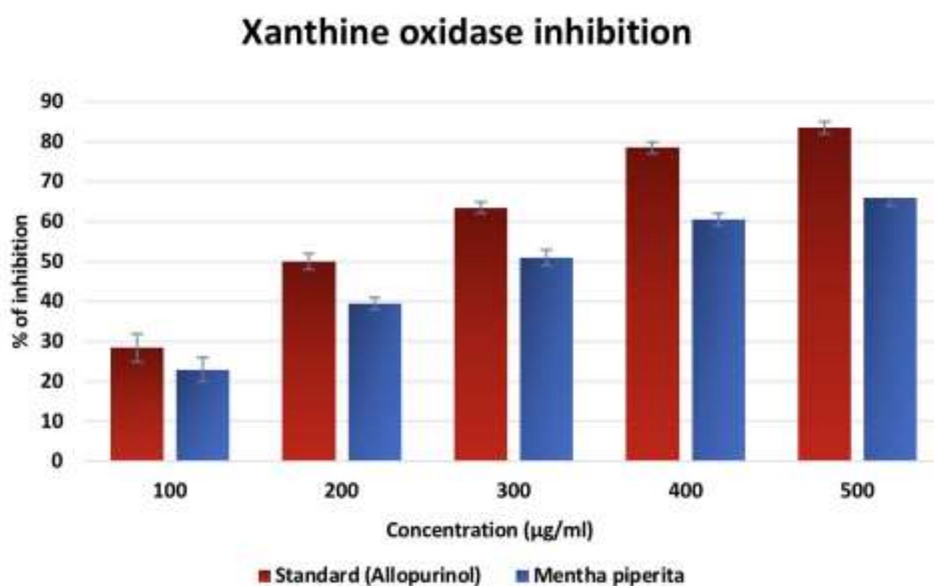

Figure 2: XANTHINE OXIDASE INHIBITORY

The bar graph depicts In vitro xanthine oxidase inhibitory activity of Mentha piperita L. The X axis represents the different concentrations of *Mentha piperita L* and the Y axis represents the percentage of inhibition.The red colour denotes the concentration of standard drug allopurinol and the dark blue colour denotes the *Mentha piperita L*. The difference was statistically significant. Each bar represents Mean ± SEM of 3 independent observations. Significance of $p \leq 0.05$.

Table 1 : Phytochemical screening of Mentha piperita L.

Protein	-
Amino acid	-
Flavonoids	+
Alkaloids	+
Terpenoids	+
Saponins	+
Steroids	+

DISCUSSION:

Phytochemical screening showed that the ethanolic leaf of mentha piperita is rich in alkaloids, saponins, steroids, terpenoids and flavonoids. Hence the results indicate the presence of phytochemicals in the extract might have contributed to its beneficial activities. The results of the study indicated that Mentha piperita extract showed a dose dependent increase in the in vitro antioxidant activity. The results also revealed that the plant extract possessed xanthine oxidase inhibitory activity in a concentration dependent manner, although activity is less when compared to the standard drug allopurinol. The presence of secondary metabolites in the plant might have contributed to the biological activity of the plant. Hence our finding showed a potent in vitro antioxidant which was evident in the DPPH scavenging assay.

Xanthine oxidase may be a versatile flavoprotein, ubiquitous among species. The enzyme catalyses the oxidative hydroxylation of purines like adenine and guanine to xanthine and uric acid. There is subsequent reduction of oxygen at the flavin center with generation of free radicals, either molecular oxygen, superoxide radical or peroxide radical (Fried and Fried, 1974). Xanthine oxidase inhibitors prevent the production of uric acid and subsequently help in the treatment of gout(Wyngaarden, 1965) . The results of *in vitro* screening provided useful information for the future invivo studies of the ethanolic extract in animal models.

Antioxidant activity is the delay in oxidation of lipid, protein, DNA that occurs by blocking the initial stage in oxidative chain reactions. Primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction(Huang and Wong, 2013). It is evident from the present study that the ethanolic extract of Mentha piperita L. has significant antioxidant activity. Keeping in view its high antioxidant property, this ethanolic extract can also be used to protect the body from deleterious effects of free radicals.

The study can be extended in animal models to develop the potential herbal remedies for clinical use for the treatment for gout.

CONCLUSION

To conclude the study, ethanolic extract of Mentha piperita L. possessed potent antioxidant and xanthine oxidase activity. In this study, in vitro antioxidant assay and antigout assay indicate that the plant extract showed increased antioxidant activity, flavonoid contents and xanthine oxidase inhibitory potential, which could be a significant source of natural alternative drug. This might be helpful in preventing the progress of various oxidative stresses and helps in the treatment of gout. Further research may be required to study the active compounds of Mentha piperita L. that confers the antioxidant and antigout activity.

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STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the present study.

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