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Qualitative and quantitative phytochemical evaluation of ethanolic extract of *Mentha pipperita* (Linn.)

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Abstract
The present study was conducted to evaluate the ethanolic extract of *Mentha pipperita* (Linn.) for both qualitative and quantitative phytochemical estimation. The dried leaves were extracted using 95% ethanol in Soxhlet apparatus and the representative sample was used for phytochemical analysis. The results of qualitative estimation revealed the presence of alkaloids, cardiac glycosides, phytosterols, flavonoids and amino acids. Through GC-MS analysis, 24 different peaks were identified and many of which are reported to be pharmacologically active compounds. From the results it was concluded that the ethanolic extract of *Mentha pipperita* (Linn.) possesses remarkable phytochemically active constituents having pharmacological properties which can be used in veterinary therapeutics.

Keywords: *Mentha pipperita*, GC-MS, phytochemistry, peppermint, ethanolic extract

1. Introduction
Among ancient civilization India has been known to be rich repository of medicinal plants as the forests in India has large number of medicinal plants of which about 8000 herbal remedies have been documented in Ayush system of India (Zahid, 2016) [18]. The Lamiaceace family is one of the most important families of medicinal plants and the *Mentha* species is the popular representative of this family. *Mentha piperita* in the dried or fresh form and also their extracts are being used in pharmacology research. The *Mentha piperita* (Linn.) extract is considered as natural source containing many compounds with remarkable antioxidant activity.
Mentha piperita (Peppermint) of Lamiaceae family, is a hybrid form of mint native to Europe and Middle East and is now cultivated throughout the world. It is considered as an oldest herb to be used in medicinal products and especially recognized as a plant source of menthol and menthone. It contains active principles such as terpenoids and flavonoids like eriocitrin, hesperidins and kempferol-7-O-rutinoside. In alternative medicine, mint extract is used to treat common cold, flu, bloat and flatulence and also for rheumatism and arthritis (Antolak *et al*., 2018) [1].
Peppermint can be used in various forms like oil, leaf, leaf extract and leaf water. Its preparations are used in pharmaceutical products as it exerts varieties of therapeutic properties (Herro and Jacob, 2010) [4]. The present study aimed towards providing scientific background for the use of *Mentha piperita* (Linn.) through its detailed phytochemical evaluation.

2. Materials and Methods
2.1 Identification and authentication of plant species
The mature leaves of *Mentha piperita* (Linn.) were collected from the authentic sources. The plant material was duly authenticated and the herbarium sheets (*M. piperita* Linn. herbarium sheet number 10357) were deposited to the department of Botany at Rashtrasant Tukdoji Maharaj University (RTMNU), Nagpur.

2.2 Preparation of ethanolic extract of *Mentha piperita* (Linn.)
Fresh leaves of *Mentha piperita* Linn. were collected from herbal market, washed with clean tap water and dried under the shade for nearly one week. The air dried leaves were milled into fine powder in a commercial blender (Perfect Mix mixer grinder, MX112, manufactured by Omex industries, Solan, H.P.). The 3000 gm powder was subjected to hot extraction in Soxhlet apparatus using 95% ethanol (1:3 w/v) as a solvent for the time required to complete 70-80 cycles of solvent in the assembly.

The extract was collected in a beaker and then filtered through Whatman filter paper number 1, dried over water bath at 60 ^{0}C and then it was collected in a clean, sterilized petridish and stored in an airtight dessicator for further use (Thangapandiyan *et al*., 2013) [16].

The extractability of extract was determined by the following formula:

Eq. (1)

$$\% \text{ Extractability} = \frac{\text{Weight of extract (gm)}}{\text{Weight of dried powder (gm)}} \text{ X } 100$$

2.3 Qualitative phytochemical evaluation

The representative quantity of ethanolic extract of *Mentha pipperita* (Linn.) was subjected to various phytochemical tests for preliminary qualitative estimation to detect presence of active phytoconstituents using a method described by Raaman (2006) [11] and Rosenthaler (1930) [12].

2.3 Quantitative phytochemical evaluation

The GC-MS analysis of ethanolic extract of *Mentha piperita* (Linn.) was carried out using Agilent GC 7890 with triple axis 5975 MS detector. The capillary column was Agilent HP-5MS (30 m x 250 µm x 0.25 µm) composed of 5% phenyl methyl silox. The initial oven temperature was raised at the rate of 40 ºC for 0 min which was raised at the rate of 25 ºC per min up to 160 ºC for 15 min and then at the rate of 2 ºC/min up to 280 ºC for the hold time of 5 min. The injector volume was 4 µl. The gas used as carrier with constant flow rate of 1ml/min with split ratio of 25:1. The MS operating conditions were; source temperature 230 ºC (max-250 ºC), quad temperature 150 ºC (max- 200 ºC), solvent delay time of 4 min. Compounds were identified in terms of Rt values and mass spectra with those obtained from the NIST search library. The obtained compounds were searched for detailed information.

3. Result and Discussion

3.1 Preliminary phytochemical evaluation

The extractability of dried leaves of *Mentha pipperita* (Linn.) was found to be 1.62% (See Table no. 1) when hot extracted using 95% ethanol. This finding was in agreement with that of Thangapandiyan *et al*., 2013 [16]. The alkaloids, cardiac glycosides, phytosterols, flavonoids and amino acids were the major active principles present in the extract (shown in the table no. 2).

Table 1: Details of extract obtained

Sr. No.	Content	Ethanolic extract of *Mentha piperita* (Linn.)
1.	Solvent used	95% ethanol
2.	Quantity of powder used	3000 gm
3.	Colour	Greenish brown
4.	Consistency	Semisolid
5.	Extractabity	1.62%

Table 2: Result of qualitative phytochemical evaluation

Sr. No.	Pharmacologically active compound	Test performed	Result
1.	Alkaloids	i) Mayer's Test	Positive
		ii) Wagner's Test	Positive
		iii) Hager's Test	Positive
		iv) Dragendroff's Test	Positive
2.	Carbohydrates	i) Molish's Test	Negative
		ii) Fehling's Test	Negative
		iii) Barfoad's Test	Negative
		iv) Benedict's Test	Negative
3.	Glycosides	i) Borntrager's Test	Positive
		ii) Legal's Test	Positive
4.	Saponins	Foam Test	Negative
5.	Proteins and Amino Acids	i) Millon's Test	Positive
		ii) Biuret Test	Positive
		iii) Ninhydrin Test	Positive
		iv) Xanthoprotein Test	Negative
6.	Phytosterols	i) Libermann-Burchard Test	Positive
		ii) Salkowski's Test	Positive
7.	Fixed oils and Fats	i) Spot Test	Negative
		ii) Saponification Test	Negative
8.	Phenolic Compounds and Tannins	i) Ferric Chloride Test	Negative
		ii) Lead acetate	Negative
		iii) Alkaline Reagents Test	Negative
		iv) Magnesium and Hydrochloric acid reduction Test	Negative
9.	Gum and Mucilage	Test for gum and mucilage	Negative
10.	Flavonoids	Test for Flavonoids	Positive
11.	Resins	Test for resins	Negative

3.2 GC-MS analysis

The compounds 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (S)- and Phytol was having greatest percent area while Propane, 1, 1, 3-triethoxy-, 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, Methyl 10-methyl-undecanoate, 3-Eicosyne, Hexadecanoic acid, ethyl ester, Phytol, 9, 12-Octadecadienoic acid, ethyl ester, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol- and dl-α-Tocopherol were the compounds showing major peak with peak heights as shown in the fig. 1. The compound Propane, 1, 1, 3-triethoxy- is reported as compound having antinephrotic and antioxidant activity (Palani *et al.*, 2011) [10] while for in-vivo anticancer drug screening with tumor model the compound Propane, 1, 1-diethoxy-(National Center for Biotechnology Information, 2021) [5-7] was reported of being evaluated.

The carvotenacetone common name of compound 2-Cyclohexen-1-one, 2-methyl-5- (1-methylethenyl)-, (S)- was shown to having cytotoxic activity against hepatocellular carcinoma cell clines HEPG-2 using MTT assay (Shahat *et al*., 2017) [14]. The Methyl tetradecanoate was used in qHTS assay for small molecule agonists of vitamin D receptor signaling as per pubchem bioassay records (National Center for Biotechnology Information, 2021) [5-7]. In the molecular studies, the compound 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol was found to be important for transcription in cells via factor PPAR-alpha & retinoid X receptor (RXR) and also shown to be effective in conditions like autoimmune encephalomyelitis and oxidative stress responses (National Center for Biotechnology Information, 2021 and Blum *et al*., 2018) [5-7].

The compound Hexadecanoic acid, methyl ester was reported to having cytoprotective potential and also for exerting anti-inflammatory activity (El-Demerdash, 2011 and Saeed *et al*., 2012) [3, 13]. The compound phytol was reported to be effective against polynephritis infection (Srinivasan *et al*., 2017) [15] and its derivatives for having potential antihyperglycemic property (Upadhyay *et al*., 2020) [17]. The 9, 12-Octadecadienoic acid, ethyl ester is being used as a base compound for manufacturing of various veterinary drugs (National Center for Biotechnology Information, 2021) [5-7]. dl-alpha-Tocopherol is a synthetic form of vitamin E, a fat-soluble vitamin with potent antioxidant properties (National Center for Biotechnology Information, 2021) [5-7].

Table 3: Result of GC-MS analysis

Sr. No.	Compound identified	Molecular formula	Peak height	RT (Min.)	Percent area	Molecular weight (g/mol)
1.	Propane, 1, 1, 3-triethoxy-	$C_9H_{20}O_3$	138094	5.85	1.492	176.25
2.	Propane, 1, 1-diethoxy-	$C_7H_{16}O_2$	60098	6.53	0.619	132.2
3.	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (S)-	$C_{10}H_{16}O$	658501	10.19	22.13	152.23
4.	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{16}O$	110897	10.91	1.786	152.23
5.	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{16}O$	1128571	11.72	19.432	152.23
6.	4- (2, 2-Dimethyl-6-methylenecyclohexyl)butanal	$C_{13}H_{22}O$	44371	15.75	0.638	194.31
7.	Methyl 10-methyl-undecanoate	$C_{13}H_{26}O_2$	312412	19.75	4.098	214.34
8.	Methyl tetradecanoate	$C_{15}H_{30}O_2$	245837	24.53	2.913	242.39
9.	3-Eicosyne	$C_{20}H_{38}$	187146	27.25	3.277	278.51
10.	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	54797	28.56	1.275	296.53
11.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	95246	30.29	2.177	270.45
12.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	142696	33.33	3.732	284.47
13.	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	96551	37.68	1.552	296.48
14.	Phytol	$C_{20}H_{40}O$	972646	37.94	15.843	296.53
15.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	47839	38.39	0.487	298.50
16.	9, 12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	132199	39.05	1.302	308.49
17.	Ethyl 9, 12, 15-octadecatrienoate	$C_{20}H_{34}O_2$	514187	39.16	5.744	306.48
18.	Methyl 17-methyl-octadecanoate	$C_{20}H_{40}O_2$	71902	39.73	0.657	312.53
19.	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol-	$C_{20}H_{40}O$	114288	42.65	1.312	296.53
20.	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol-	$C_{20}H_{40}O$	120721	46.46	1.959	296.53
21.	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol-	$C_{20}H_{40}O$	125068	52.21	2.183	296.53
22.	2, 2, 4-Trimethyl-3- (3, 8, 12, 16-tetramethyl-heptadeca-3, 7, 11, 15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	39790	53.22	0.579	428.70
23.	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	91427	58.13	1.523	324.62
24.	dl-α-Tocopherol	$C_{29}H_{50}O_2$	176449	58.42	3.287	430.70

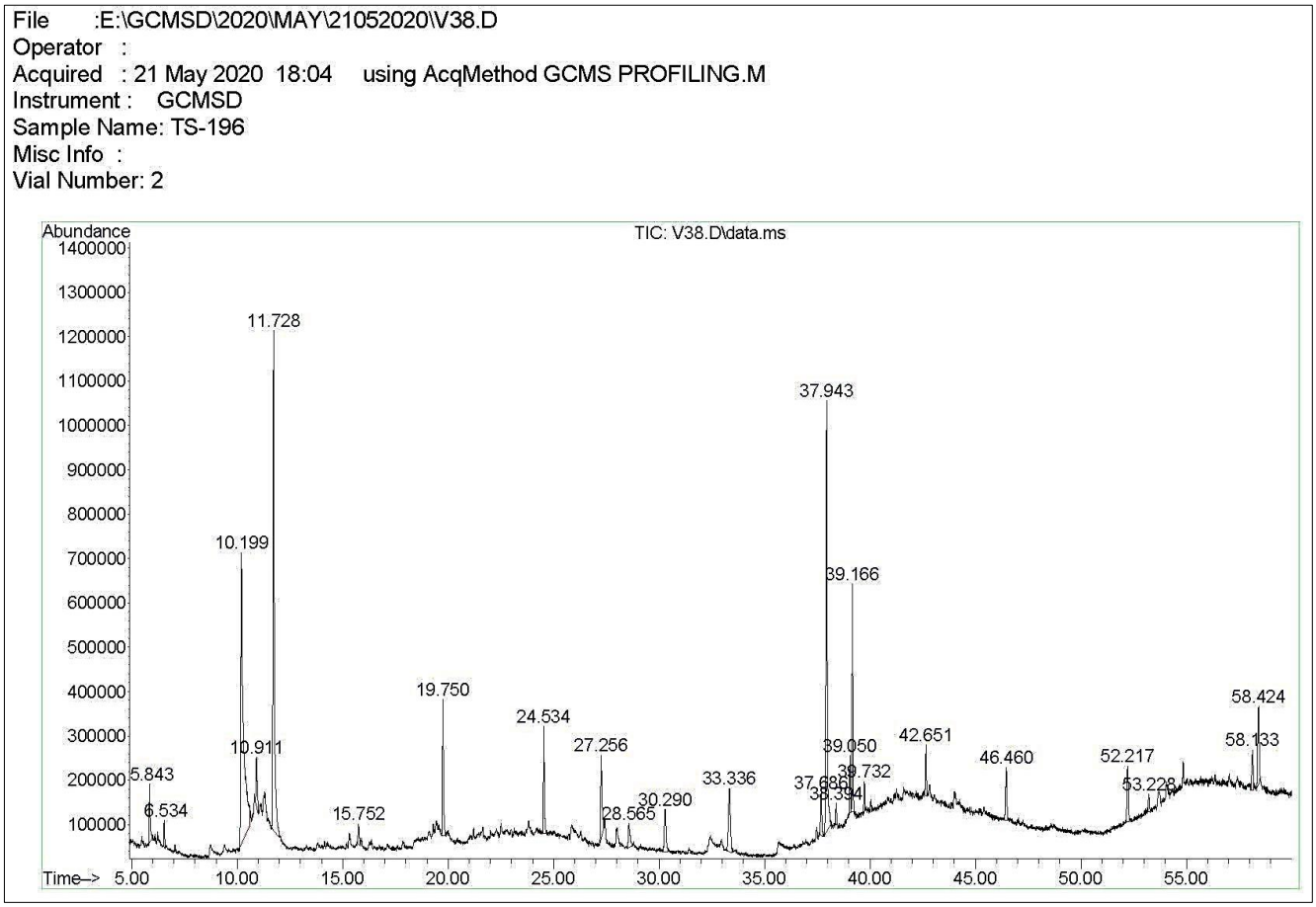

Fig 1: Image showing GC-MS spectra of ethanolic extract of *Mentha piperita* (Linn.)

4. Conclusion

The present study revealed that ethanolic extract of *Mentha pipperita* (Linn.) contains various active principles and bioactive compounds that are having pharmacological importance which can be used in different metabolic disorders of animals and humans. For more exploratory insights the extract can be standardized further followed by separation and identification of individual molecule that will help better understanding of medicinal chemistry of this indigenous medicinal plant.

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