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Assessment of Anti-Inflammatory Potential of *Myristica fragrans* Houtt upon Membrane Stabilization

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Abstract: Inflammation is complex biological response of vascular tissues to harmful stimuli. It is also a protective attempt by the organism to remove the injurious stimuli and initiate the healing process. With the onset of inflammation, the cells undergo activation and release inflammatory mediators. Upon screening for membrane stabilization assay using aqueous extract of the plant *Myristica fragrans*, it was clearly observed that the aqueous extract possessed 30.93% membrane stability. The optimum concentration of 250 mg/ml extract of *Myristica fragrans* showed the maximum stabilization activity of 82.73%. Phytochemical screening of aqueous extract of *Myristica fragrans* Houtt indicated the presence of various primary and secondary metabolites such as steroid, phenol terpenoids, flavonoids, tannins, coumarins alkaloids and saponin. From the GCMS data it was evident that bioactive fragment composed of 17 compounds. From the study it is concluded that the aqueous extract of *Myristica fragrans* has good membrane stability, hence is a good anti-inflammatory agent. Further laboratory study and chemical isolation of this plant can confirm an effective drug molecule in pharmacological aspects effectively.

Keywords: Anti-inflammatory potential, *Myristica fragrans* Houtt, Phytochemical screening, GCMS

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Introduction

Inflammation is a highly dynamic process, which can be characterized as the first protective response of body's immune system against pathogens, irritants or damaged cells. It can be classified as either acute or chronic, and involves a cascade of biochemical events comprising the local vascular system, the immune system, and different cell types found in the injured tissue. A wide variety of molecules are involved in the induction and maintenance of the inflammatory response. Along with the pivotal cytokines such as interleukin -1, interleukin -6 and tumor necrosis factor-α (TNF-α), prostaglandins and nitric oxide are important chemical mediators of inflammation

(Jin *et al*., 2010). Inflammatory mediators also include histamine, serotonin, prostaglandins and some plasma enzymes systems such as complement system, fibrinolytic system and clotting system (Anosike *et al*., 2012).

Recently, many medicinal plants are found useful for reducing pain and in exhibiting anti-inflammatory activity. *Myristica fragrans*, commonly known as nutmeg is commonly served in powder form or essential oil in the food industry worldwide. It has been reported that nutmeg oil, commercially obtained by steam distillation from kernels of nutmeg, usually contains more than 50 chemical components such as linalool, terpineol, eugenol, myristicin, camphene, di pentene, and pinene (Zhang *et al*., 2016).

Here an attempt is made to prepare aqueous extract of *Myristica fragrans* Houtt., using Soxhlet method to evaluate its *in vitro* membrane stability, to optimize biological action of *Myristica fragrans* Houtt., to characterize the bioactive fraction by the employment of GC-MS and to determine the phytochemical constituents present in the aqueous extract of *Myristica fragrans* Houtt.

Materials and Methods

Myristica fragrans Houtt leaves were washed thoroughly using distilled water. The leaves were then dried in hot air oven at 60°C. It was powdered using electric blender and used. From this, 5 g of the powdered sample was weighed and boiled with 100 ml of distilled water in heating mantle at 80°C.The plant extract was filtered and collected separately. For membrane stability assay, human blood was collected in EDTA coated vials to prevent coagulation. It was mixed and kept in incubator and used for the experiment within 24 h of collection. Preparation of erythrocyte suspension was done by using standard protocols (Sakib *et al*., 2021). Serially diluted concentrations of plant extract were used to evaluate the effect of hemolysis on human's red blood cells (RBC). The absorbance was read at 540 nm. The percentage inhibition of hemolysis was calculated using the following equation:

Percentage of membrane stability =100 – (O.D. of Test/O. D. of Control) *100

Different factors like concentration, pH and temperature were measured to know the effect in maximizing the membrane stability activity of *Myristica fragrance* Houtt aqueous extract. pH was maintained by three different buffers; acetate (pH 3.6 to 5.6), phosphate (pH 5.8 to 7.4), and bicarbonate (pH 9.2 to 10.6). The effect of temperature was understood by incubating the reaction mixture in various temperature (3 to 60°C). Denaturation of proteins is the main cause of inflammation. Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi (1968) and Sakat *et al*. (2010) with slight modification. Heat induced human red blood stabilization was done by using standard protocols (Ezzat *et al*., 2018).

The bioactive constitutes in leaf extract was determined by Gas Chromatography (Agilent 6890 series) equipped with HP-5MS column mass spectrometer operated at initial column temperature of 30°C and heated up to 300°C at 10°C/5 min. Chromatographic conditions were: flow of 1 ml/min of high purity helium as carrier gas in split mode. The identification of the compounds in spectra was done based on retention time and integral area of peaks. The similarity of compounds matched with >70% are listed based on NIST library search. Phytochemical screening was carried in de-ionized water extracts of *Myristica fragrance*.

Results and Discussion

Optimization of variables such as concentration, temperature and pH were analyzed to maximize the membrane stabilization activity of *Myristica fragrans* extract. From the optimization studies it was evident that administration condition has immense role in influencing the membrane stabilization activity. The maximum activity was observed in the concentration of (250 mg/ml) plant extract. Similarly, in order to check whether

Table 1: Effect of concentration in membrane stabilization activity

Concentration (mg/ml)	Percentage stability (%)
100	30.93%
150	53.9%
200	94.9%
250	82.73%
300	95.68%

Table 2: Effect of pH in membrane stabilization activity

Hydrogen ion concentration (pH)	Percentage stability (%)
4	96.4%
7	94.96%
10	95.68%

Table 3: Effect of temperature in membrane stabilization activity

Temperature (°C)	Percentage stability (%)
3	69.7%
37	66.18%
60	58.27%

Table 4: OD value and percentage of heat induced human red blood stabilization

S. No.	Total sample concentration (mg/ml)	O.D. value	Percentage %
1	100	0.58	72.18
2	150	0.39	82.84
3	200	0.34	83.43
4	250	0.30	85.79
5	300	0.28	88.16

at which pH the maximum activity was in 250mg/ml (Table 1) the reaction mixture was incubated at different pH varying from 4 to 10 (Table 2). The desired hydrogen ion environment was maintained using three different buffers (acetate, phosphate, carbonate buffers). From the analysis it was observed that activity was independent of pH as all concentration showed similar percentage. The temperature was found to be independent as all the percentages were approximately similar (Table 3). The aqueous extract of the plant, *Myristica fragrans*, upon screening for membrane stabilization assay, clearly showed that the aqueous extract possessed 30.93% stabilization activity. As the concentration of *Myristicafragrans* extract increased it provided more protection to human erythrocyte against heat induced lysis (Table 4).

From GCMS data it was evident that the bioactive fragment was composed of 17 compounds (Table 5). The different compounds in the aqueous extract of *Myristica fragrans* Houtt. includes Dodecane**,** Pentadecane, 2-cyclobuten-1-one, Diethyl Phthalate, n-phenylpropanamide, Dihydro-beta-ionone, N-(trifluoro acetyl)-N, O,O', O''-tetrakis(trimethylsilyl)norepinephrine, 1,2-benzenedicarboxylic acid, 1,2,4-dithiazolidine-3,5-dione, Bis(2-ethylhexyl) ester, Ethanone, Stannane, Phthalic acid, [3,3'-bifuran]-2,5-dicarboxylic acid, Terephthalic acid, octahydro-3,5a-dimethyl, p-acetyl benzyl tert-butyl ether.

Some of the compounds have antifungal, anti-microbial and anti-inflammatory activity.

The above-mentioned identified compounds are known to possess several biological activities and industrial applications such as Stannane and Phthalic acid was known to have anti-inflammatory, and anti-microbial activities; Ethanone have anti-fungal activity; 2-cyclobuten-1-one act as an antioxidant, Terephthalic acid has anti-bacterial properties, Bis(2-ethylhexyl) ester is an apoptosis inhibitor as well as plasticizer and n-phenylpropanamide also posseses anti-inflammatory activity. Overall, these phytoconstituents have been shown to possess different biological activities and industrial applications such as anti-inflammation, anti-oxidant, anti-microbial, etc. So, the present study is expected to help in identifying the anti-inflammatory activity of aqueous extract of *Myristica fragrans* Houtt.

GC-MS analysis of the aqueous extract of leaves of *Myristica fragrans* Houtt. revealed the presence of different acids, heterocyclic compounds, esters among others (Figs. 1-18). This confirms the results on presence of the various

Table 5: Showing the bioactive fragment of 17 compounds

	RT	COMPOUND NAME	NATURE	ACTIVITY
1	6.845	Dodecane	Volatile	Plant metabolite
2	9.170	Pentadecane	Volatile	Antimicrobial activity
3	17.520	2-cyclobuten-1-one	Organic compound	antioxidant
4	19.815	Diethyl Phthalate	Phthalate ester	Antimicrobial activity
5	20.155	n-phenylpropanamide	Chemical inhibitor	Anti- inflammatory activity
6	20.270	Dihydro-beta-ionone	Diethyl ester	Antibacterial and anti fungal activity
7	20.755	N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine	Natural product	Anti-inflammatory activity
8	26.480	1,2-benzenedicarboxylic acid	Phthalic acid	Anti-fungal activity
9	28.205	1,2,4-dithiazolidine-3,5-dione	Natural product	Antihelmithic and anti inflammatory activity
10	34.835	Bis(2-ethylhexyl)ester	Phthalate ester	Apoptosis inhibitor and plasticizer
11	35.990	Ethanone	Volatile, Flammable	Antifungal activity
12	36.385	Stannane	Inorganic compound	Anti-microbial activity
13	36.670	Phthalic acid	and anti-inflammatory activity	
14	37.785	[3,3'-bifuran]-2,5-dicarboxylic acid	Aromatic dicarboxylic acid	Anti-inflammatory activity and antimicrobial activity
15	38.005	Terephthalic acid	Polyester	Antibacterial activity
16	38.115	octahydro-3,5a-dimethyl	Organic compound	Antibacterial activity
17	38.290	p-acetylbenzyltert-butyl ether	Natural product	Anti-inflammatory activity

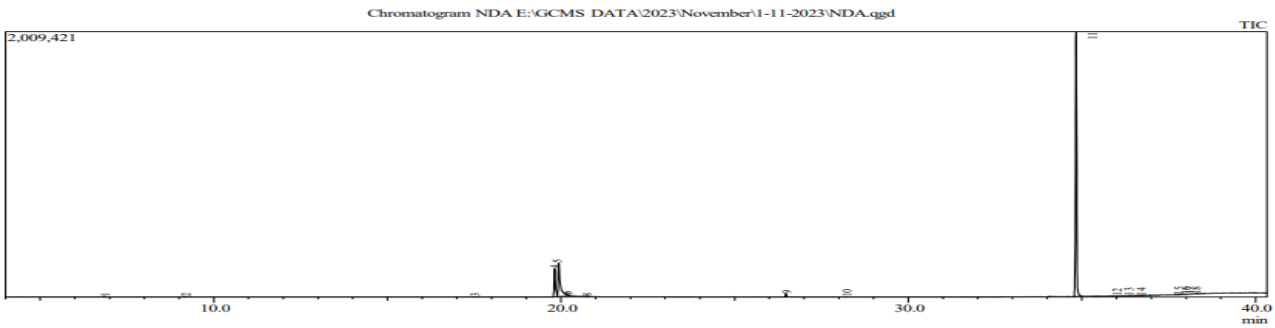

Fig. 1: GCMS analysis of *Myristica fragrans.*

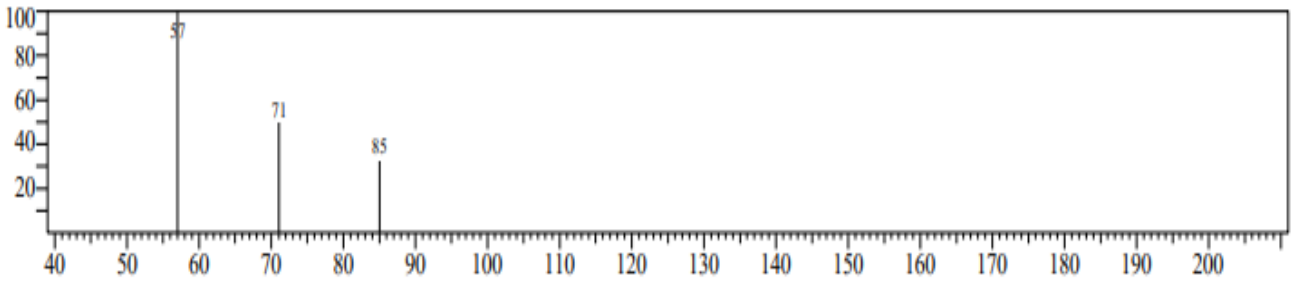

Fig. 2: GCMS analysis of compound Dodecane.

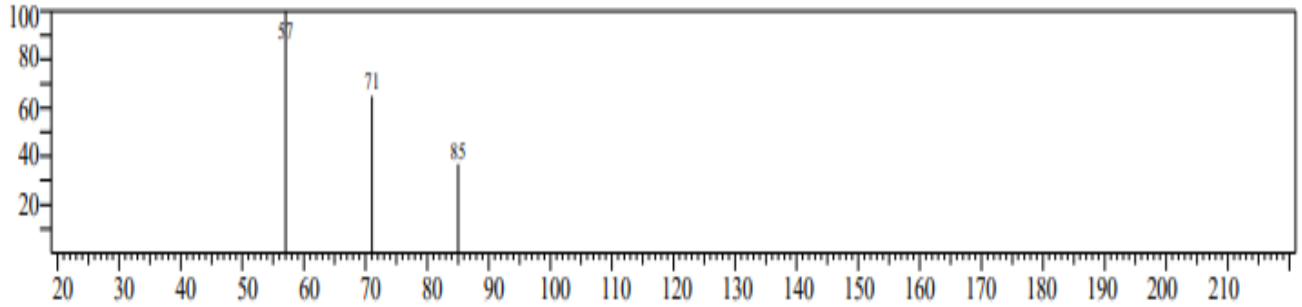

Fig. 3: GCMS analysis of compound Pentadecane.

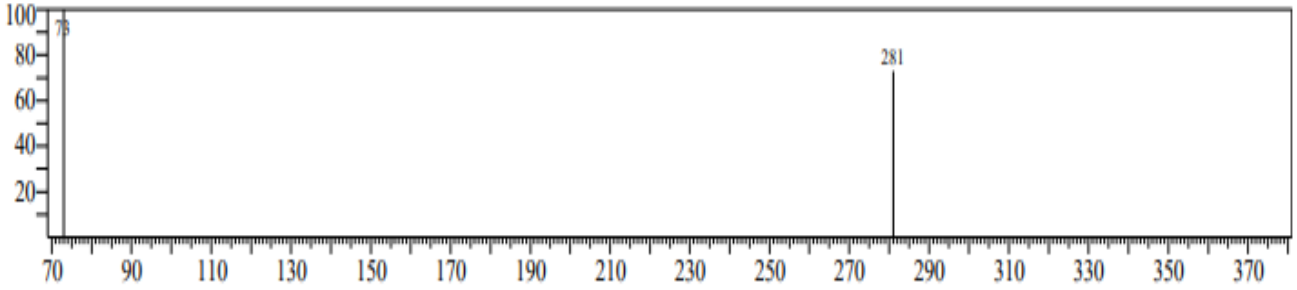

Fig. 4: GCMS analysis of 2-cyclobuten-1-one.

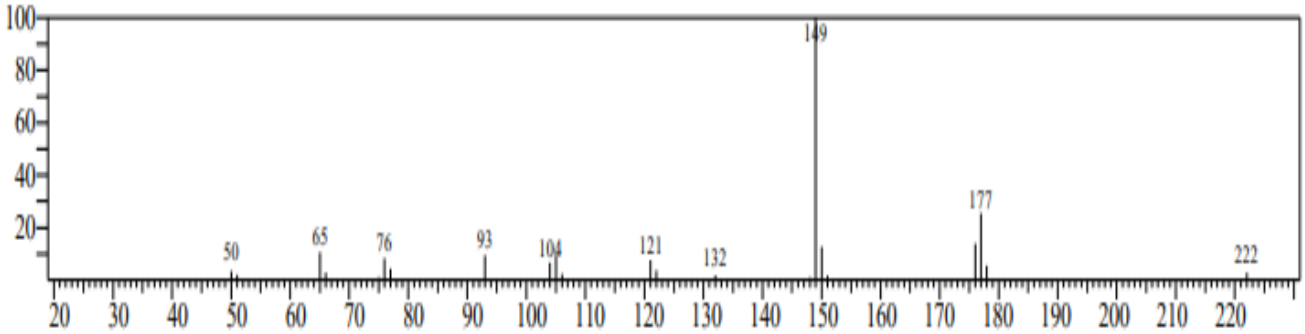

Fig. 5: GCMS analysis of compound Diethyl Phthalate.

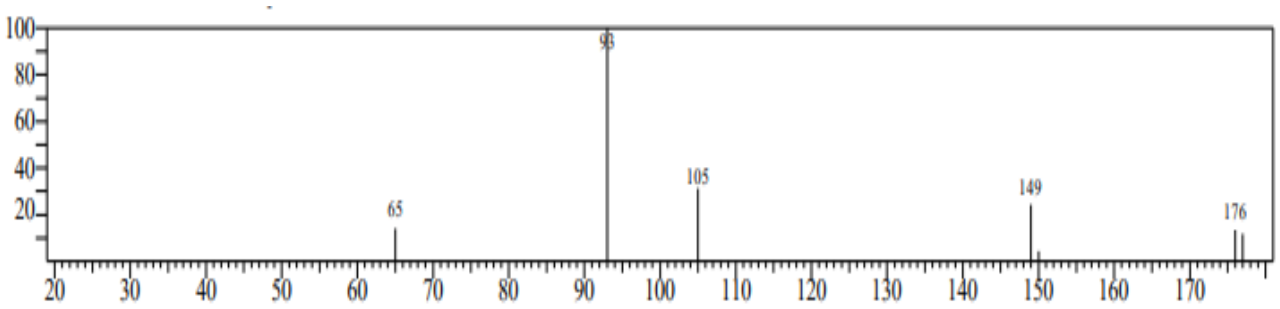

Fig. 6: GCMS analysis of compound N-phenylpropanamide.

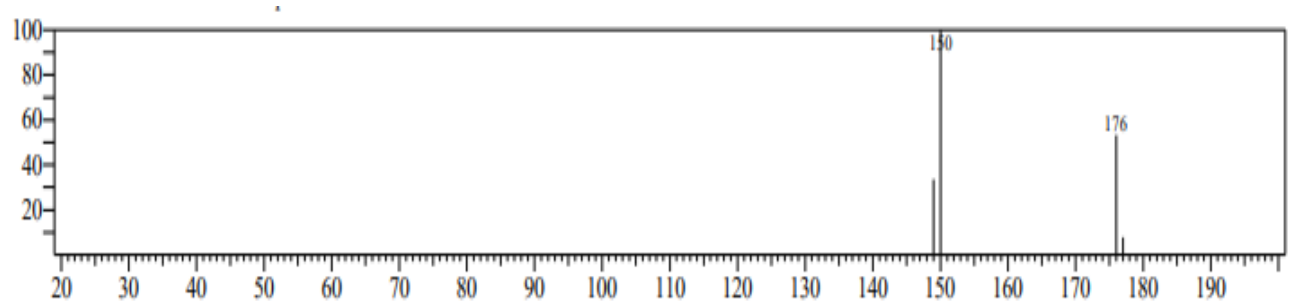

Fig. 7: GCMS analysis of compound Dihydro beta-ionone.

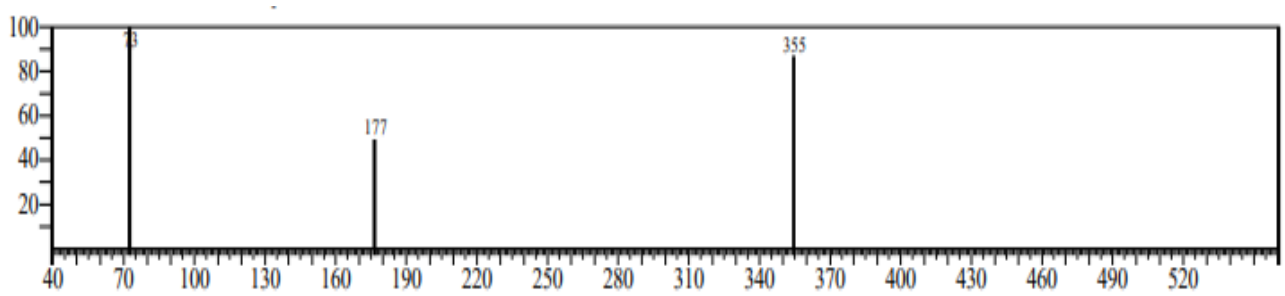

Fig. 8: GCMS analysis of compound N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine.

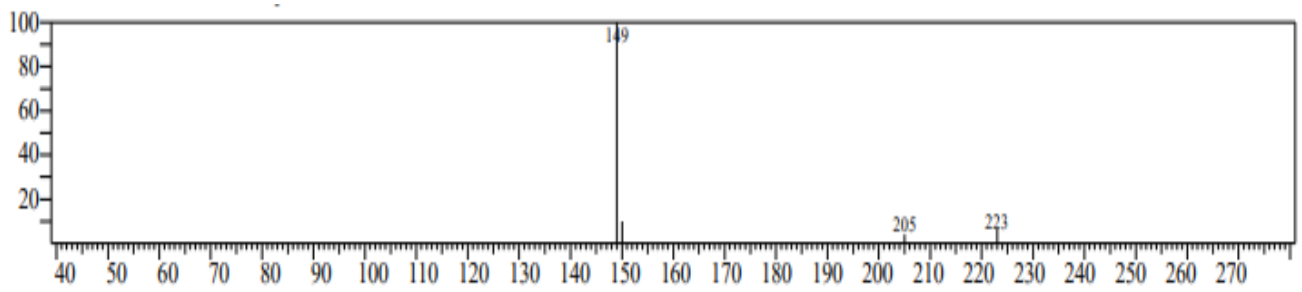

Fig. 9: GCMS analysis of compound 1,2-Benzenedicarboxylic acid.

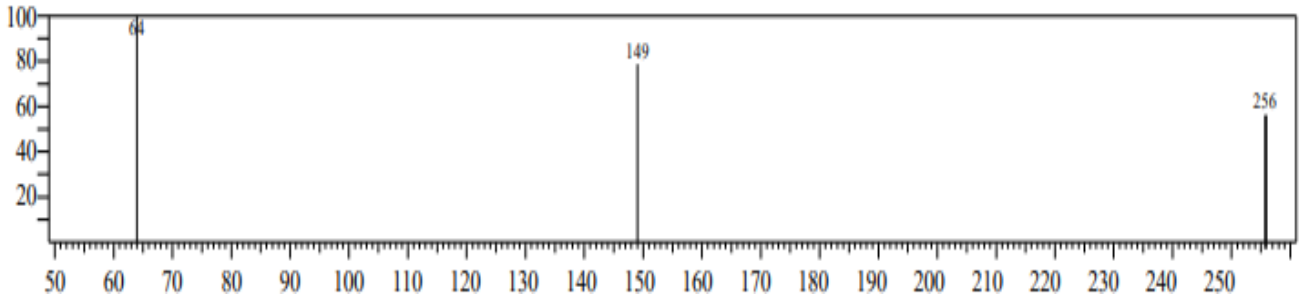

Fig. 10: GCMS analysis of 1,2,4-dithiazolidine-3,5-dion**.**

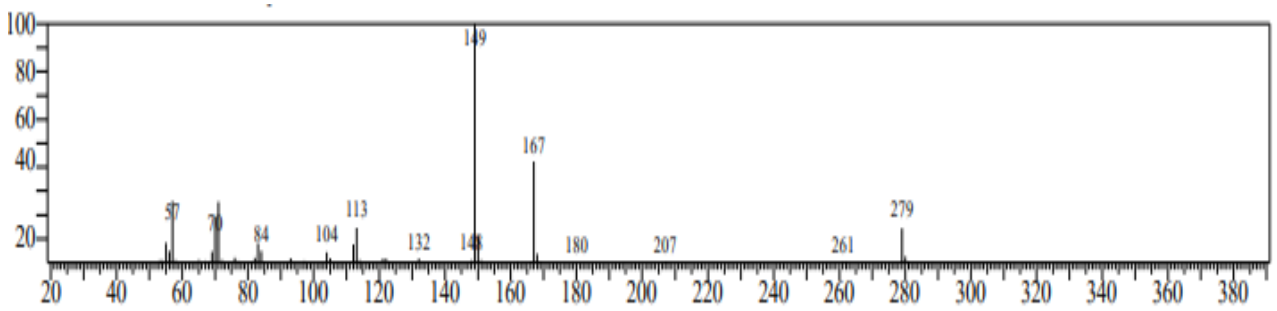

Fig. 11: GCMS analysis of compound Bis(2-ethylhexyl)ester.

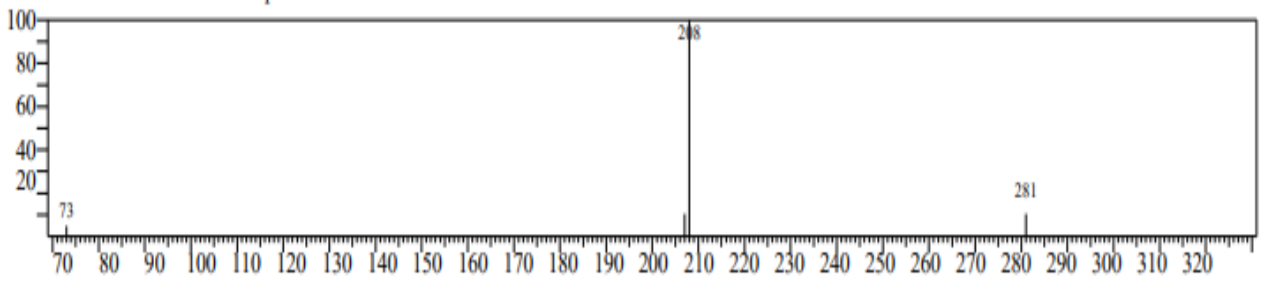

Fig. 12: GCMS analysis of compound Ethanone.

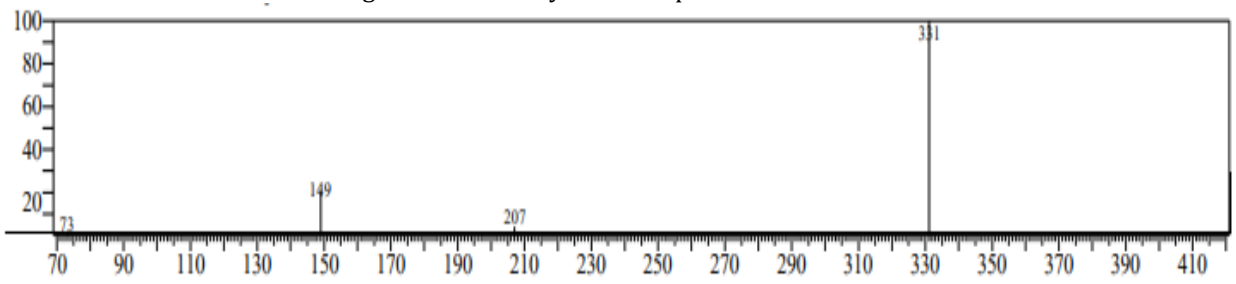

Fig. 13: GCMS analysis of compound Stannane.

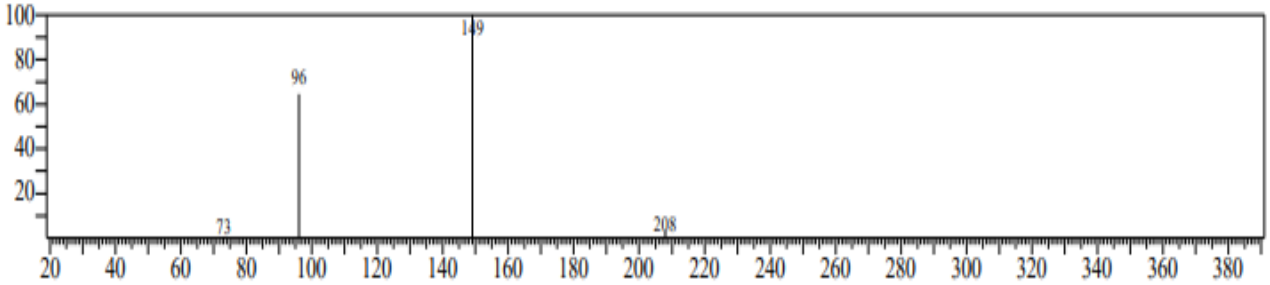

Fig. 14: GCMS analysis of compound phthalic acid.

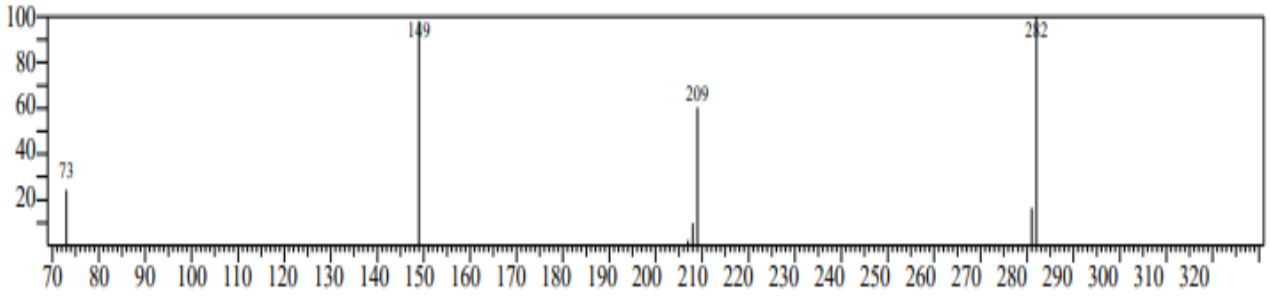

Fig. 15: GCMS analysis of compound [3,3'-bifuran]-2,5-dicarboxylic acid.

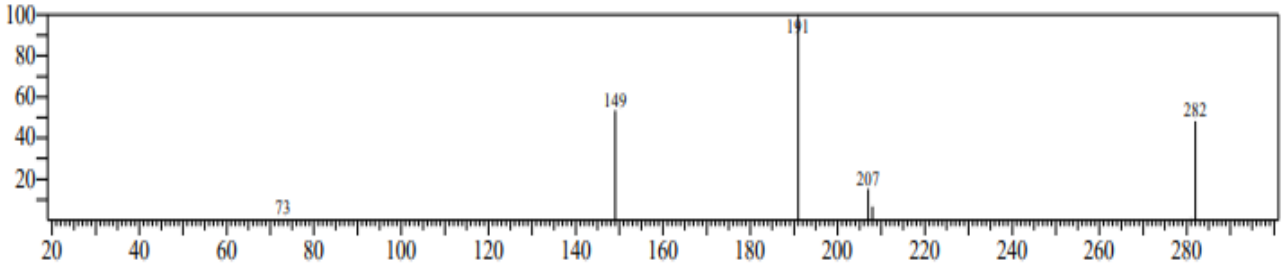

Fig. 16: GCMS analysis of compound Terephthalic acid.

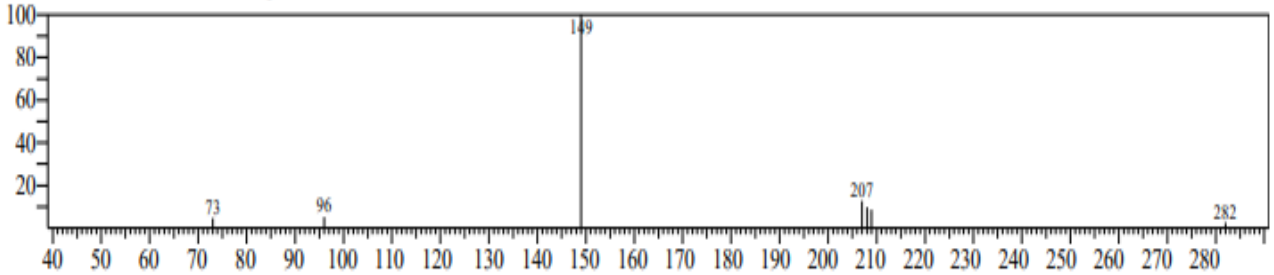

Fig. 17: GCMS analysis of compound octahydro-3,5a-dimethyl.

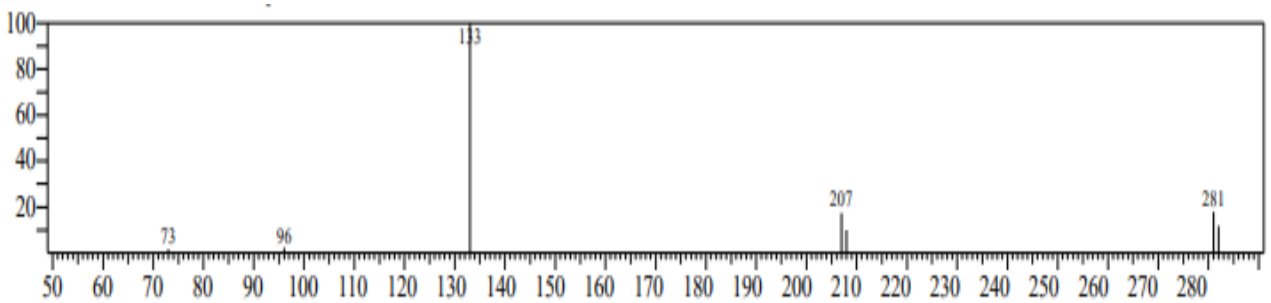

Fig. 18: GCMS analysis of compound p-acetyl benzyl tert-butyl ether.

Table 6: Showing the Primary metabolites identified from *Myristica fragrans*

Test	Result
Test for carbohydrate	+
Test for Aldehyde	+
Test for Sugar	-
Test for ketose	-
Test for amino acid	+
Test for protein	+
Test for starch	-

Table 7: Showing the Secondary metabolites identified from *Myristica fragrans*

Test	Result
Test for alkaloids	+
Test for steroids	-
Test for terpenoids	+
Test for flavonoids	+
Test for phenols	+
Test for tannins	+
Test for saponins	+
Test for cardiac glycoside	-
Test for phlobetannin	-
Test for coumarins	-
Test for tannins	+
Test for anthraquinones	-
Test for acids	-
Test for resin	-

secondary metabolite compounds detected by the qualitative procedures. These mass spectra are fingerprint of the compound which can be identified from the data library. Hence, the identified phyto-components using GC-MS can be used as a pharmacognostical tool for the identification of adulterants. The current pioneering study suggests that the extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity, as well as the unidentified compounds (Seca *et al*., 2014). Phytochemical analysis of aqueous extract plant were analysed and given in the Tables 6 and 7. Phytochemical screening for aqueous extract of *Myristica fragrans* indicated the presence of various primary and secondary metabolites such as carbohydrate, aldehyde, amino acid, protein, alkaloids, terpenoids, flavonoids, phenols and tannins.

Conclusion

It is concluded that the optimum concentration of 250mg/ml of aqueous extract of *Myristica fragrans* showed the maximum stabilization activity of 82.73%. The optimization of pH suggested that all pH showed similar membrane stabilization. Temperature was also optimized as it also showed similar result. Phytochemical screening for aqueous extract of *Myristica fragrans* indicated presence of various primary and secondary metabolites such as steroid, phenol terpenoids, flavonoids, tannins, coumarins alkaloids and saponin. From the GCMS data it was evident that bioactive fragment composed of 17 compounds. From the study it may be concluded that the aqueous extract of *Myristica fragrans* has good membrane stability, hence is a good anti-inflammatory agent. Further laboratory study and chemical isolation of this plant can confirm pharmacological aspects of this plant.

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Conflict of Interest

The authors declare no conflicts of interest.

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