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## Evaluation of antibacterial, antioxidant and GC-MS analysis of ethanolic seed extract of *Myristica dactyloides*

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**Abstract.** In this present investigation we analysed the antimicrobial and antioxidant activities of ethanol extract of *Myristica dactyloides*. The antimicrobial activity of the ethanol extract was evaluated by the agar well diffusion method against of *E. coli*, *Klebsiella pneumonia*, *Streptococcus* sp, and *Staphylococcus aureus* at different concentrations. The antibacterial activity showed the result in a dose-dependent manner. The free radical scavenging was evaluated against DPPH, hydroxyl, and nitric oxide radicals. In DPPH, hydroxyl and nitric oxide scavenging assay showed the IC 50 value of the extract was found to be 20 µg/ml, 48.25 and 30 µg/ml, respectively. The plant can be considered as promising antioxidant properties with high potential value for drug development for various diseases.

### 1. Introduction

Currently, new diseases are evolved in the world which drug resistance capabilities. Diseases are caused by bacteria, fungi, viruses and parasites; they may have multidrug drug resistance. So they cause the major threat to human beings [1]. Medicine from natural products having antimicrobial characters has revived as a result of the existing trouble associated with the exploit of synthetic or chemical antibiotics [2]. The indigenous system of phytomedicine reports a number of natural sources for their antimicrobial efficacy [3-6].

Free radical cause oxidative stress. Antioxidants are playing an important role in preventing oxidative reactions catalyzed by free radicals. The various free radicals include superoxide anions, hydroxyl radical, singlet oxygen, hydrogen peroxide, ferric ion, nitric oxide etc. Free radicals cause such diseases like cancer, inflammation, diabetes, liver cirrhosis, Alzheimer's and aging [7-9]. The chemical drugs of synthetic antioxidants; they cause side effects like carcinogenesis and also low solubility. Hence, these limitations have been replaced by using the naturally available antioxidants. Therefore, emerging natural antioxidant has greatly increased in the recent years of research. Natural antioxidants are derived from medicinal plants in and the phytomedicine is more potent and compatible than synthetic drugs [10].

The plant *Myristica dactyloides* belongs to the family Myristicaceae. It is commonly called as 'kattu jathi'. *Myristica dactyloides* is an evergreen tree that can grow up to 20 meters tall. The tree is commonly harvested from the wild for its fruits. This plant possesses preservative agent, good antioxidant activities and used in some medical treatments due to the presence of phytochemicals [11].



The fruit of this plant has rich phenolic constituents and demonstrated good antioxidant capacity [12]. In this report, the antimicrobial and the free radical scavenging activity of ethanolic extracts of *Myristica dactyloides* seeds was characterized against pathogenic bacteria and DPPH radical scavenging activity, Nitric oxide radical inhibition assay and Hydroxyl radical scavenging assay.

## 2. Materials and methods

### 2.1 Collection of plant material

The medicinal plant *Myristica dactyloides* were collected from Ranipet, Vellore district, Tamilnadu, India.

#### 2.1.1 Preparation of plant extract

Collected seeds of *Myristica dactyloides* were allowed for shade drying and powdered by mixer grinder. Powdered seeds (250 g) were treated with ethanol solvent which was extracted by using Soxhlet apparatus. The solvent was evaporated under vacuum treatment to get the solid mass. The obtained solid mass was weighed and kept in the refrigerator at 4 °C in an airtight container and used further studies.

#### 2.2 Antimicrobial activity of ethanol extract of seed

Antimicrobial activity of ethanol extract of seeds was assessed by agar well diffusion method. 0.1 ml of each fresh culture of *E. coli*, *Klebsiella pneumonia*, *Streptococcus sp.*, and *Staphylococcus aureus* were evenly spread on each Petri dishes containing Muller Hinton agar. Five wells were made by using sterile cork borer with the diameter 6 mm in all plates. Inoculate different concentrations of seed extract was added in all plates. Ampicillin used as a positive control. Plates were incubated in the incubator at 37°C for 24 hours. After incubation, a growth of organisms in Petri plates was examined and the results were recorded as the zone of inhibition around the well.

#### 2.3 Antioxidant activity of ethanolic seed extract

For assessing DPPH scavenging activity, 1 ml of different concentration of seed extract ranging from 20-100µg was taken makeup by using methanol. To this 4 ml of DPPH solution was mixed and the reaction solution was kept 30 min at room temperature. The optical absorbance was measured at 517 nm. 1ml methanol with 4 ml DPPH was considered as control.

The hydroxyl radical scavenging effect of seed extract was assayed by mixing various concentration of seed extract sample (20-100 µg) with FeCl<sub>3</sub> (0.1 mM), deoxyribose (2.8 mM), EDTA (0.1 mM), H<sub>2</sub>O<sub>2</sub> (1 mM) and final volume of 1.0 ml was made by using phosphate buffer (20 mM, pH 7.4). The reaction mixture was incubated for 1 hr. at 37 °C. After that 1.0 ml of 1%, TBA (w/v) were added to the mixture and heated at 100 °C in a water bath for 20 min. The resulted solution was measured spectrophotometrically at 530 nm.

The nitric oxide radical scavenging effect plant seed extract was assessed by Griess Illosvoy reaction. In this test, various concentration of seed extract (20-100µg), was mixed with 2 ml of 10 mM sodium nitroprusside, 0.5 ml phosphate buffer and incubated for 3 h at 25°C. After incubation, 0.5 ml pf the reaction mixture mixed with 1 ml sulfanilic acid and incubated for 5 min for stop the reaction process. Further, 1 ml naphthyl ethylenediamine dihydrochloride was mixed with the reaction mixture and allowed to stand for 30 min at 25°C and optical density was measured at 540 nm. Vitamin C was used as the standard. The reaction mixture without seed extract was used as the control.

The percentage of antioxidant activity against DPPH, hydroxyl and nitric oxide was calculated by using the following formula

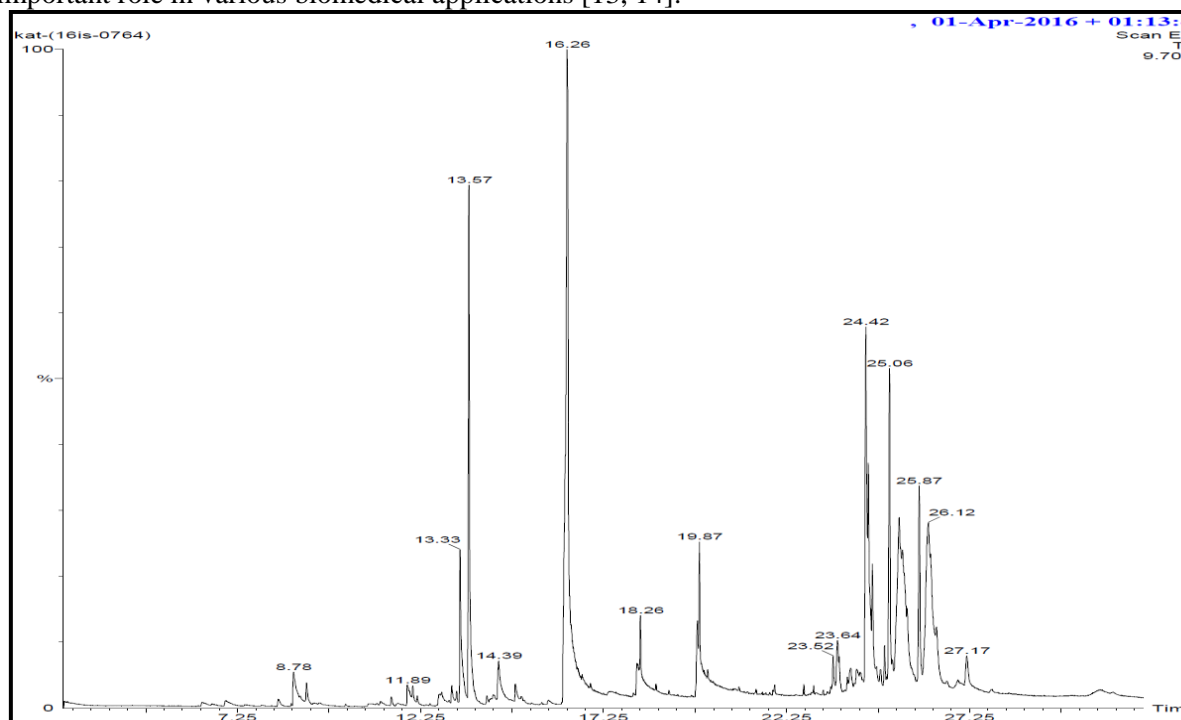
$$\% \text{ antioxidant activity} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}$$

## 3. Results and discussion

### 3.1 GC - MS analysis

The presence of phytochemicals in the ethanol extract of *Myristica dactyloides* seeds was characterized by GC-MS analysis. GC chromatogram shows 11 peaks which are corresponding to 11

phytoconstituents (Figure 1). The plants are the major sources of phytochemicals which play an important role in various biomedical applications [13, 14].



**Figure 1:** GC chromatogram analysis of ethanol extract of *M. dactyloides* seeds

The compound 1-11 were identified as 7-Oxabicyclo [2.2.1] Heptane, 1-Methyl-4- (1-Methylethyl) - ( $C_{10}H_{18}O$ ), Coniferyl aldehyde, Methyl Ether ( $C_{11}H_{12}O_3$ ), Benzene 1,2,3-Trimethoxy-5 - (2-Propenyl)- ( $C_{12}H_{16}O_3$ ), Phenol, 2,6-Dimethoxy-4- (2-Propenyl) - ( $C_{11}H_{14}O_3$ ), Tetradecanoic acid ( $C_{14}H_{28}O_2$ ), Hexadecanoic acid, Ethyl ester ( $C_{18}H_{36}O_2$ ), Ethyl oleate ( $C_{20}H_{38}O_2$ ), Phenol, 4-[2,3-Dihydro-7-Methoxy-3-Methyl-5-(1-Propenyl)-2-Benzofur ( $C_{20}H_{22}O_4$ ), Isoquinoline, 1,2,3,4-Tetrahydro-8-Amino-2-Methyl-4-Phenyl- ( $C_{10}H_{10}O_4$ ), 5-Methyl-1,10-Phenanthroline ( $C_{13}H_{10}N_2$ ), and 1,2-Bis (Trimethylsilyl)-4-methylbenzene ( $C_{13}H_{24}Si_2$ ). These bioactive components exhibit medicinal property of the plant listed in Table 1. It shows the phytochemicals and its molecular weight based on retention time.

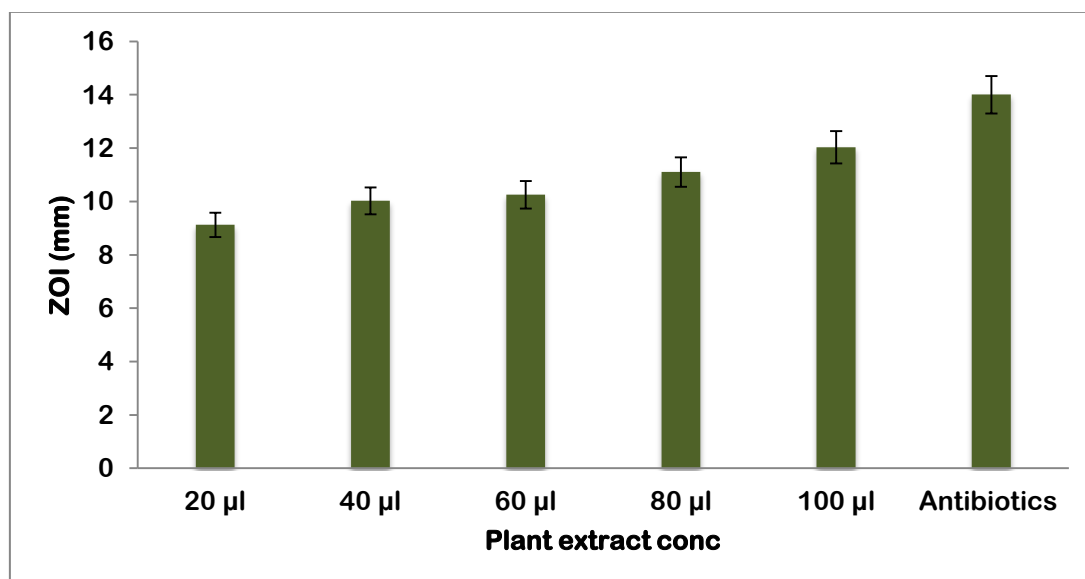
**Table 1:** GC-MS analysis of ethanol extract of seeds of *M. dactyloides*

Rt value	Compound	Molecular weight	Structure
8.78	7-Oxabicyclo [2.2.1] Heptane, 1-Methyl-4-(1-Methylethyl)-	154	$C_{10}H_{18}O$
13.3	Coniferyl aldehyde, Methyl Ether	192	$C_{11}H_{12}O_3$
13.57	Benzene, 1,2,3-Trimethoxy-5-(2-Propenyl)-	208	$C_{12}H_{16}O_3$
14.39	Phenol, 2,6-Dimethoxy-4-(2-Propenyl)-	194	$C_{11}H_{14}O_3$
16.26	Tetradecanoic acid	228	$C_{14}H_{28}O_2$
18.26	Hexadecanoic acid, Ethyl ester	284	$C_{18}H_{36}O_2$

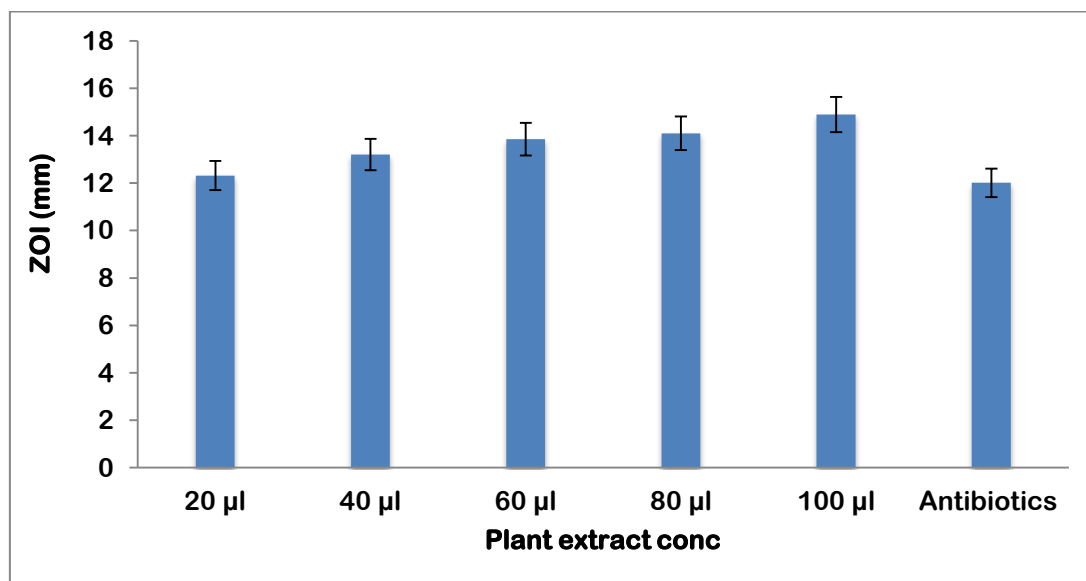
<b>19.87</b>	Ethyl oleate	310	$C_{20}H_{38}O_2$
<b>24.42</b>	Phenol, 4- [2,3-Dihydro-7-Methoxy-3-Methyl-5- (1-Propenyl) -2-Benzofur	326	$C_{20}H_{22}O_4$
<b>25.06</b>	Isoquinoline,1,2,3,4-Tetrahydro-8-Amino-2-Methyl-4-Phenyl-	194	$C_{10}H_{10}O_4$
<b>26.12</b>	5-Methyl-1,10-Phenanthroline	194	$C_{13}H_{10}N_2$
<b>27.17</b>	1,2-Bis(Trimethylsilyl)-4-methylbenzene	236	$C_{13}H_{24}Si_2$

### 3.2 Antimicrobial activity of Plant leaves

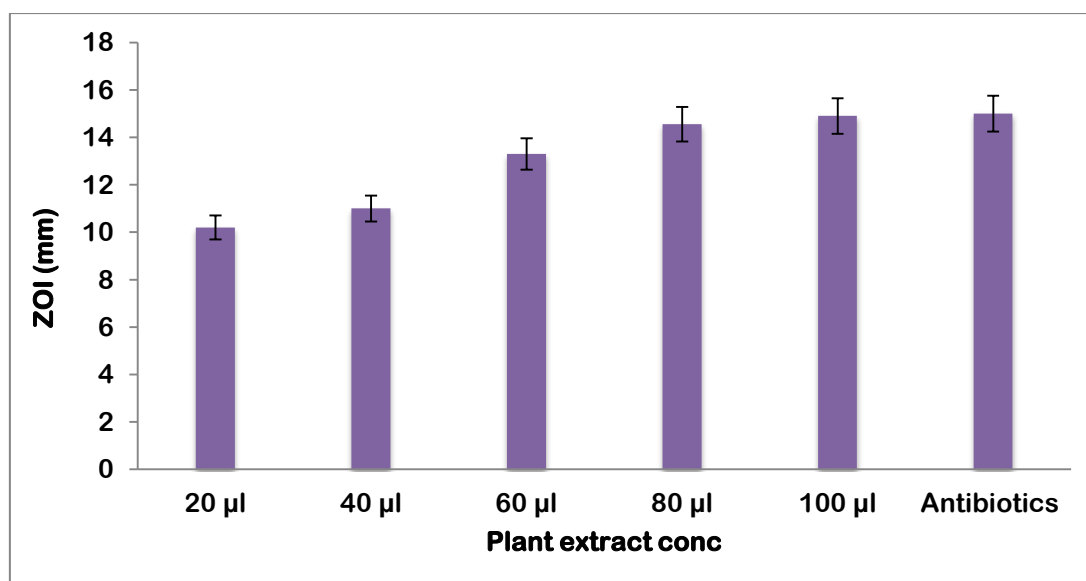
The antibacterial activity of ethanol extract of seeds of *Myristica dactyloides* was analyzed by agar well diffusion method. This assay was performed by measuring the diameter of the zone of inhibition of bacterial growth around the well. The antibacterial activity of ethanolic *Myristica dactyloides* seeds extracts shown in the Figures 2-5. The plants are playing a major role in the antibacterial activity against pathogenic microbes isolated from different types of clinical samples [15, 16,17].



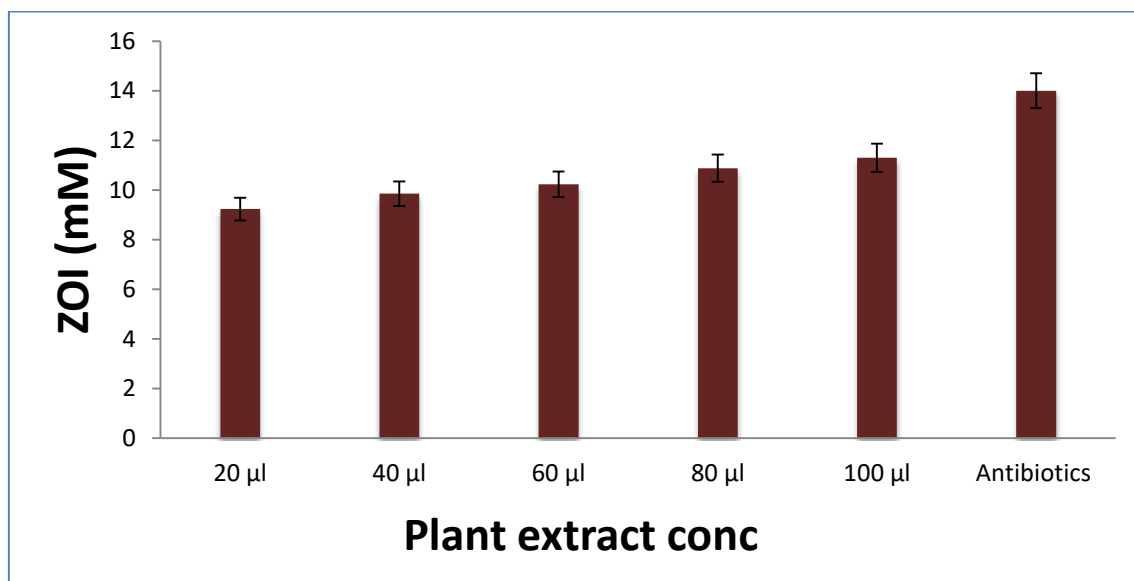
**Figure 2:** Antibacterial activity of ethanolic *Myristica dactyloides* seeds extract against *Streptococcus sp.*



**Figure 3:** Antibacterial activity of ethanolic *Myristica dactyloides* seeds extract against *E. coli*



**Figure 4:** Antibacterial activity of ethanolic *Myristica dactyloides* seeds extract against *Klebsiella pneumoniae*.



**Figure 5:** Antibacterial activity of ethanolic *Myristica dactyloides* seeds extract against *Staphylococcus aureus*.

There are five different concentrations of ethanolic seed extract 20 µl, 40 µl, 60 µl, 80 µl and 100 µl were tested against the bacterium like *Streptococcus sp*, *E. coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus*. Ethanolic extract of *Myristica dactyloides* seeds was very strong active against all the microorganisms. Herein, increased inhibition activity was noted with the increasing of concentration of seed extract. The zone of inhibition was shown figures against *Streptococcus sp*, *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* are 17, 18, 17.5, and 16.5 mm in diameter, respectively. It clearly indicates the antibacterial capability of the medicinal plant *Myristica dactyloides* seeds.

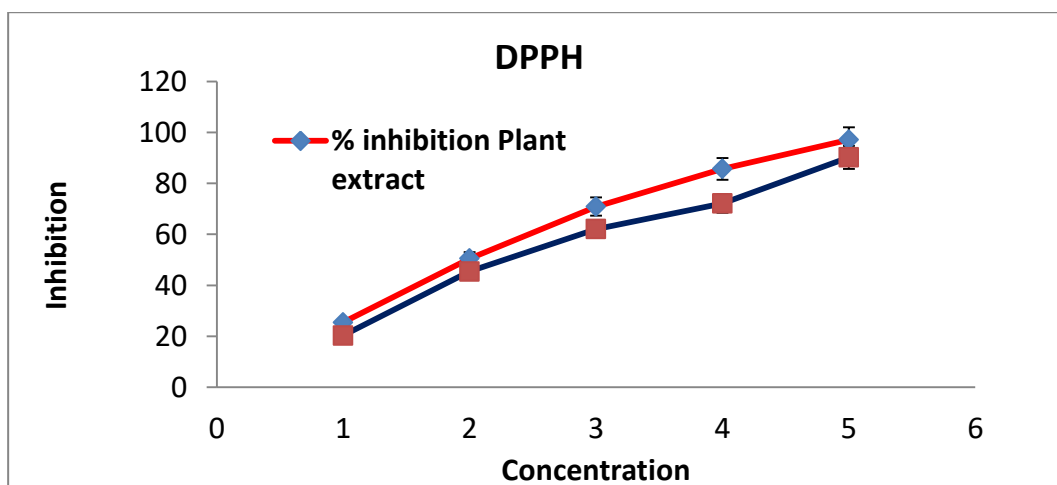
### 3.3 DPPH radical scavenging activity

In this activity of ethanolic *Myristica dactyloides* seeds extract is shown in Figure 6. Scavenging effect of the seed extract exhibited increased activity in a significant dose-dependent approach. The results were noticed in different concentrations of seed extract from 10 to 50 µg/ml. Vitamin C was used as a standard antioxidant agent which showed 50% of inhibition at 25.65 µg/ml. The results of DPPH scavenging activity by ethanolic extract of seeds of *Myristica dactyloides* seeds showed IC<sub>50</sub> values at 20 µg/ml of the extract.

**Table 2:** DPPH Scavenging activity using ethanolic *Myristica dactyloides* seeds extract

Concentration (µg/ml)	% inhibition	
	Plant extract	Standard
10	26.33 ±0.12	20.35±1.25
20	50.25±0.24	45.45±0.85
30	70.85±0.65	62.12±0.98
40	80.35±0.84	72.13±1.65
50	95.05±1.05	90.25±1.24

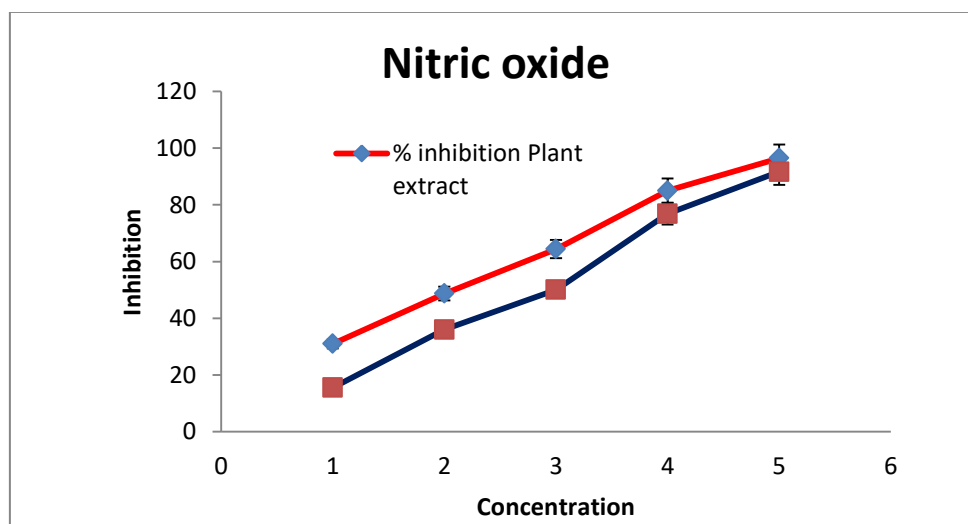
± Standard deviation



**Figure 6:** Effect of ethanolic *Myristica dactyloides* seeds extract and Standard Vitamin C on scavenging of DPPH radical Results

### 3.4 Nitric oxide radical inhibition assay

Figure 7 represents that the scavenging capability of the ethanol extract of *M. dactyloides*, which was compared with standard antioxidant agent vitamin C. The scavenging activity of the extract was increased with the concentration of the test sample. The 50% of the scavenging effects of *M. dactyloides* seed extract was observed at 21.95  $\mu\text{g/ml}$ . Standard showed the 50% (IC<sub>50</sub>) of inhibition concentration at 30  $\mu\text{g/ml}$ . However, increasing the extract concentration range from 10 to 50  $\mu\text{g/ml}$ , the scavenging activity also increased in the dose-dependent manner (Table 3).



**Figure 7:** Effect of ethanolic *Myristica dactyloides* seeds extract on Nitric oxide radical inhibition assay



**Table 3:** Nitric oxide Scavenging activity using ethanolic *Myristica dactyloides* seeds extract

Concentration ( $\mu\text{g/ml}$ )	% inhibition	
	Plant extract	Standard
20	19.87 $\pm$ 0.86	16.32 $\pm$ 0.22
40	39.21 $\pm$ 0.42	32.98 $\pm$ 0.84
60	58.02 $\pm$ 0.37	55.28 $\pm$ 0.96
80	81.77 $\pm$ 0.29	70.63 $\pm$ 0.64
100	91.25 $\pm$ 0.57	85.45 $\pm$ 1.21

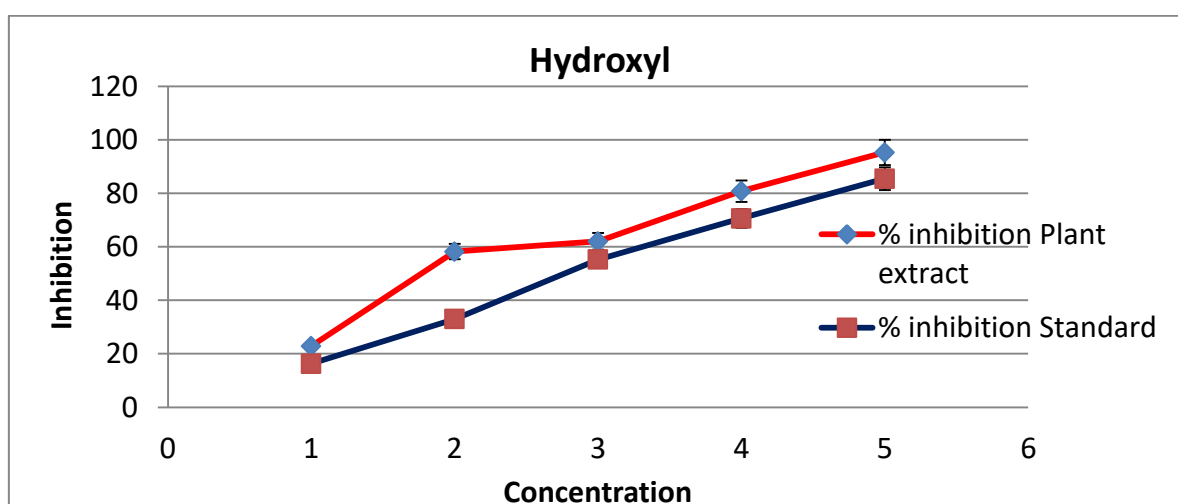
### 3.5 Hydroxyl radical scavenging assay

In hydroxyl radical scavenging assay, ethanol extracts of seeds of *M. dactyloides* show higher inhibition activity compared to standard (Figure 8). The IC<sub>50</sub> of 48.25 and 56.05  $\mu\text{g/ml}$  were observed for ethanol extract of seeds and standard vitamin C, respectively (Table 4). From these results, seed extract may protect from free radicals toxicities by scavenging reactive biomolecules and reducing them to less reactive molecules.

**Table 4:** Hydroxyl radical scavenging activity of ethanolic *Myristica dactyloides* seeds extract

Concentration ( $\mu\text{g/ml}$ )	% inhibition	
	Plant extract	Standard
10	30.93 $\pm$ 0.42	15.48 $\pm$ 0.63
20	48.68 $\pm$ 2.25	35.95 $\pm$ 0.78
30	64.36 $\pm$ 0.63	50.02 $\pm$ 0.69
40	85.02 $\pm$ 0.69	76.87 $\pm$ 2.25
50	96.45 $\pm$ 1.24	91.65 $\pm$ 1.34

$\pm$  Standard deviation

**Figure 8:** Hydroxyl radical scavenging assay of ethanolic *Myristica dactyloides* seeds extract

#### 4. Conclusion

The result of phytochemical analysis of ethanol extract of *Myristica dactyloides* seeds was characterized by GC-MS assay. It showed the presence of 11 biochemical constituents which they may responsible for biological activity. The extract showed the highest antibacterial activity compared to commercial antibiotic disc. In the case of antioxidant activity also showed the highest activity than standard vitamin C. So, it is concluded that ethanol extract of seeds has the highest antimicrobial property and antioxidant activity. As a result, it can be suggested for drug development in the pharmaceutical industry.

#### References

1. Okeke IN, Laxminarayan R, Bhutta ZA Antimicrobial resistance in developing countries. Part 1: recent trends and current status. *Lancet Infect Dis* 2005; **5**:481-93.
2. Abu-Shanab B, Adwan G, Abu-Safiya D Antibacterial activities of some plant extracts used in Palestine in popular medicine. *Turk J Biol* 2004; **28**: 99-102.
3. Nostro A, Germano MP, D'Angelo V, Marino A, Cannaelli MA Extraction methods and bioautography for evolution of medicinal plant antimicrobial activity. *Letters in Applied Microbiology* 2000; **30** (5):379.
4. Bhavani R, Kotteeswaran R, and Rajeshkumar S Hepatoprotective effect of Brassica oleracea vegetable and its leaves in Paracetamol induced liver damage in albino rats, *Int.J. ChemTech Res* 2014; **6(7)**: 3705-3712.
5. Bhavani R, Geetha G, Santhoshkumar J and S Rajeshkumar Evaluation of Antibacterial action and Hepatoprotective efficiency of *Solanum nigrum* leaves extract on acetaminophen induced hepatotoxicity. *Research J. Pharm. and Tech.* 2015; **8(7)**:893-900
6. Rajeshkumar S, Kayalvizhi D Antioxidant and hepatoprotective effect of aqueous and ethanolic extracts of important medicinal plant *Pongamia pinnata* (family: Leguminosae) *Asian J Pharm Clin Res* 2015; **8(5)**: 67-70
7. Gutteridge J Free radicals in disease processes: a compilation of cause and consequence. *Free Radical Res* 1993; **19**:141-58.
8. Knight JA Diseases related to oxygen derived free radicals. *Ann Clin Laboratory Sci* 1995; **25(2)**:111-21.
9. Rechner AR, Kuhnle G, Bremmer P, Hubbard GP, Moore KP, Rice Evans CA The metabolic fate of dietary polyphenols in humans. *Free Radical Biol Med* 2002; **33**: 220-35.
10. Ajish AD, Vagdevi HM, Asha K, Jayanna ND Potential antimicrobial, anthelmintic and antioxidant activities of *Myristica dactyloides* gaetrn bark, *Int J Pharm Pharm Sci* 2015; **7(5)**: 117-122.
11. Bamidele O, Akinnuga AM, Alagbonsi IA, Ojo OA, Olorunfemi JO, Akuyoma MA Effects of ethanolic extract of *Myristica fragrans* Houtt. (nutmeg) on some heamatological in albino rats. *Int J Med Med Sci* 2011; **3(6)**:215-8.
12. Tan KP, Khoo HE, Azrina A Comparison of antioxidant components and antioxidant capacity in different parts of nutmeg (*Myristica fragrans*). *Int Food Res J* 2013; **20(3)**:1049-52.
13. Bhavani R, Yamuna U and S Rajeshkumar Hepatoprotective activity of aqueous extracts of *chrysanthemum indicum* flowers on paracetamol induced liver injury in albino rats *Asian J Pharm Clin Res* 2017; **9(3)**: 246-249

14. Asha S., Asha A., S. Rajeshkumar Evaluation of Phytochemical constituents and Antimicrobial Activity of silver nanoparticle synthesized Ipomoea nil against selected Pathogens. *Asian Journal of Pharmaceutical and Clinical Research Asian J Pharm Clin Res* 2017; **10(3)**: 1-5.
15. Sabah Al Muhtaseb, Najah Al-Muhtaseb, Mahmoud Al Masri, Elham Al Kaissi, Ibrahim Al Adham, Amjad Abu Sirhan. Chemical characterization and antimicrobial activity of jordanian propolis and *Nigella sativa* seed oil against clinically isolated microorganisms. *Int J Pharm Pharm Sci* 2017; **9(6)**:97-102.
16. Henok Gulilat Azul work, Sahabjada, Asif Jafri, Md Arshad, Tabarak Malik. The phytochemical investigation, GC-MS profile and antimicrobial activity of a medicinal plant *ruta graveolens* L. from ethiopia. *Int J Pharm Pharm Sci* 2017; **9(6)**:29-34.
17. Fatima Yahiaoui, Qada Benameur, Meriem Hind Ben-Mahdi. Antibacterial activity of *Mentha pulegium* essential oil against avian isolated ESBL producing bacteria and its synergistic potential with antibiotics. *Int J Pharm Pharm Sci* 2017; **9(6)**:35-41.