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Gas Chromatographic analysis of potentially bioactive compounds in leaf and root extracts of *Muntingia calabura* and their expected antibacterial activities

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ABSTRACT: The study aimed to identify bioactive compounds in *Muntingia calabura* leaf and root methanolic extracts. The Gas Chromatography and Mass Spectroscopy (GC-MS) technique were used to identify bioactive compounds. GC-MS analysis revealed 38 compounds in the leaf and 15 compounds in the root methanolic extracts of *M. calabura*. The prime potent compound found in leaf extract is 2-{3-[(E)-2-(1H-indol-3-yl)ethenyl]-1,2,4-oxadiazol-5-yl}phenol with 5.78% peak area and cholest-4-en-6-on-3-ol is found in root extracts, has the highest 63.7% peak area and another potent compound Lupeol has 7.3% peak area. The bioactive compounds identified in *M. calabura* have antibacterial activity against various bacterial strains such as gram-positive and gram-negative bacteria, which showed the efficacy of *in vivo* plant extracts. These findings validate the therapeutic potentiality of *M. calabura* leaf and root samples. Furthermore, these screened potential bioactive compounds can be used effectively for biomedical and therapeutic applications.

1. INTRODUCTION

Medicinal plants are an essential source of several bioactive compounds, which have been used to treat several diseases caused in human beings. The World Health Organization (WHO) estimates that 80% of developing countries rely on therapeutic drugs (Vital & Rivera, 2009). Their low cost, easy accessibility and the search on medicinal plants have led to the discovery of novel therapeutic drugs against diseases.

Gas chromatography is an exciting tool used nowadays to determine new bioactive compounds from critical medicinal plants, which interest is discovering novel drugs. The identified compounds are used in cosmetics, drugs, pharmaceuticals, the food industry and forensic applications (Uma et al., 2009). Identifying bioactive compounds from different plant parts leads to an additional investigation of pharmacological and biological studies (Farid et al., 2015; Momin et al., 2014). Different potent active compounds in the various plant extracts can treat various human diseases (Konappa et al., 2020). Most of the world's population depends on plant-derived medicines, which has drawn the interest of researchers to invent new drugs. (Iwu et al., 1999). The infections caused by microbes threaten a major health hazard that is around 25% of all the deaths among the countries (Priyanka et al., 2015). Natural products have been mainly a rich source of anti-infective agents. Many researchers have screened the *in vitro* crude extracts from plants with a history of use in folk medicine have been screened for antibacterial activity by many researchers (Cushnie & Lamb, 2005).

According to these reports, several pharmacological studies have been demonstrated using various plant parts of *M. calabura*. The *M. calabura* is a beautiful flowering shrub that belongs to the Muntingaceae family. The secondary metabolites of plants such as alkaloids, flavonoids, phenols, tannins and saponins were reported in the leaf extracts of *M. calabura*. (Buhian et al., 2016). These active compounds are essential in the food and research industry (Leema & Prakash, 2019). The leaf and root parts of *M. calabura* have high anti-inflammatory, antiulcer, antibacterial, antipyretic, and antioxidant activities (Krishnaveni et al., 2015; Sufian et al., 2013; Zakaria et al., 2007, 2011, 2014).

Thus, the present study evaluates the presence of bioactive compounds present in methanolic extracts of *M. calabura* leaf and root parts using GC-MS analysis and evaluations of their antibacterial activity by using the agar well diffusion method.

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2. MATERIALS AND METHOD

2.1. Plant material collection and sample preparation

Fresh *M. calabura* leaves and roots were collected from the greenhouse at (18°01'35.1"N 79°33'31.7"E). The collected plant materials were thoroughly washed under running tap water to remove impurities. These were dried for 12 days in the shade at room temperature (27 ± 2 °C) and ground into a fine powder. Five gram of plant materials were mixed with 50 mL of methanol and incubated in an orbital shaker for 48 hours at 130 rpm. Whatman No. 1 filter paper was used to filter the supernatant. The supernatants were evaporated at room temperature to obtain a crude extract, then subjected to GC-MS analysis and anti-microbial activity testing.

2.2. GC-MS instrumentation

M. calabura leaf and root methanol extracts were GC-MS analysed using a Perkin-Elmer GC system coupled with a silica capillary column 30m x 0.25mm ID x 0.25m df equipped with Elite-5MS (5 % diphenyl and 95 % dimethyl polysiloxane). Helium was used as a gaseous carrier in a 1:1 mg/minute ratio, with an injector volume of 2μl and a temperature of 250 °C and ionization at 70-eV. The oven temperature began at 110 °C and ended at 280 °C. The extraction time was 29 minutes for *M. calabura* leaf extracts and 13 minutes for root methanolic extracts. The total peak area of *M. calabura* was used to calculate the percentage of each bioactive compound in leaf and root extract. The Sophisticated Analytical Instrument Facility, IIT Bombay, performed the GC-MS analysis.

2.3. Microbial cultures and culture conditions

Muntingia calabura methanolic leaf and root extracts were tested against gram-negative and gram-positive bacteria. *Escherichia coli*, *Proteus vulgaris*, *Bacillus sphericus*, and *Pseudomonas fluroscens* were used as test organisms. Preparation of 24 hr old bacterial strains: inoculate nutrient broth with original cultures and incubate overnight at 37 °C. The antibacterial activity was tested using the agar well diffusion method with streptomycin (10 μg/mL) as the standard. The media was prepared and poured 15 mL into a petri dish for 5 minutes to solidify. The inoculums were swabbed uniformly over the media and dried. The standard (10 μg/mL) and three different leaf and root extract concentrations (45, 60, and 75 μg/mL) were incubated overnight at 37 °C in each well. After incubation, zones emerged, and the proportion of inhibition was scaled in millimetres.

3. RESULTS AND DISCUSSION

M. calabura phytochemical components were detected in methanolic leaf and root extracts. The percentage of the compounds was estimated using the retention time (RT) and peak area.

3.1. GC-MS analysis of leaf methanolic extract

The bioactive molecules found in methanolic leaf extracts were thirty-eight compounds. Among the thirty-eight compounds, the first compound identified with less retention time (4.85 min) was 1,3-dihydroxypropan-2-one and the peak area (0.24%), whereas the last compound with the longest retention time (33.68 min) was 3-Hydropregn-5-en-20-one hydrazone and the peak area (0.33%). Many of these compounds possess various pharmacological activities. The remaining phytochemical compounds are as follows: 1-(6-oxabicyclo[3.1.0]hexan-1-yl)ethan-1-one (or) 6-oxabicyclo(3.1.0)hexan-3-one, a phytoconstituent of methanolic leaf and root extracts of pili and safedshatavar (*Asparagus racemosus*), known to possess antioxidant activity (Banakar & Jayaraj, 2017). The 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one reported antimicrobial, automatic nerve, and antioxidant properties in ethanolic stem extracts of an important medicinal plant *Waltheria indica* L (Ashwathanarayana & Naika, 2018). 2,3-dihydro-1-benzofuran, a bioactive compound, possesses anti-inflammatory activity in crude extracts of leaf and flower of *Pavetta crassicaulis* Bremek (Correa et al., 2017). Decan-3-yl acetate, reported to possess activities like asthma, anticancer, anti-inflammatory activities and Histamine H3 and H4 receptors, have been discovered drugs for Parkinson's and Alzheimer's disease (Tatipamula et al., 2019). The 1,2,3-benzenetriol (Pyrogallol), identified as a phytochemical compound that possesses anticancer activity in ethanolic extract of *Clathria baltica* (Sukprasert et al., 2020). The compound (1R,2S,3R,4S)-cyclopentane1,2,3,4-tetrol, known to possess antiviral activity in *Lysiphyllum strychnifolium* plant extracts that treat against influenza virus (Rubaye et al., 2018). The compound, 4-[(1E)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenol have multiple biological properties like anti-hyperglycemic, hepatoprotective, anti-obesity and antibiotic activities in root extracts of Eclipta alba (L) (Naik et al., 2019). More recently, it was reported that the compound (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol has antifungal and antibacterial activity in leaf extracts *Rhizophora apiculata* (Lakshmanan et al., 2019).

Hexadecanoic acid, a phytochemical compound, has properties like strong antimicrobial, anti-inflammatory activities and acts against arthritis in medicinal plants like *Albizia adianthifolia*, *Pterocarpus angolensis* and in some plants against food-borne pathogens (Abubakar & Majinda, 2016; Preethi et al., 2010). In Cycas revolute, a medicinal plant is known to have antioxidant properties in the 1–hentetracontanol bioactive molecule (Olabisi & Olubunmi, 2019). The (9Z,12E)-octadeca-9,12-dienoic acid and (9Z)-octadec-9-enoic acid have phytochemical properties like hypercholesterolemia, anti-acne, anti-cancer, anti-bacterial, and diabetes mellitus in methanolic leaf extract *Crateva adansonii* DC and root extract of *Jatropha pelargoniifolia* Courb (Christiana et al., 2014). Another bioactive molecule, 3-[(4-ter-butylcyclohexyl)oxy]-3-phenyl-2-benzofuran-1(3H)-one is identified to synthesize green synthetic particles using ferrous and nickel nanoparticles by leaf extract of eucalyptus (Weng et al., 2017).

Table 1
GC-MS of bioactive compounds present in the methanolic extracts ofleaves derived from *in vivo* grownplants of *Muntingia calabura* L

S No	RT	Compound name	Formulae	Mwt	Area	Biological activity
1	4.85	1,3-dihydroxypropan-2-one	$C_3H_6O_3$	90	0.24%	Ant oxidative (Leema & Prakash, 2019)
2	8.49	1-(6-oxabicyclo[3.1.0]hexan-1- yl)ethan-1-one (or) 6-oxabicyclo(3.1.0)hexan-3-one	$C_7H_{10}O_2$	126	0.25%	Antimicrobial, automatic nerve activity and antioxidant (Banakar & Jayaraj, 2017)
3	9.68	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	144	0.16%	Anti-inflammatory (Ashwathanarayana & Naika, 2018)
4	11.20	2,3-dihydro-1-benzofuran	C_8H_8O	120	0.3%	Asthma, Anticancer, Parkinson's and Alzheimer's disease (Correa et al., 2017)
5	11.56	decan-3-yl acetate	$C_{14}H_28O_2$	228	0.07%	Anticancer (Tatipamula et al., 2019)
6	14.20	benzene-1,2,3-triol (Pyrogallol)	$C_6H_6O_3$	126	0.17%	Antiviral (Sukprasert et al., 2020)
7	15.22	-D-Glucopyranosyl-(1->3)-β-Dfructofuranosyl β-Dglucopyranoside (or) D-(+)-Melezitose	$C_{18}H_{32}O_{16}$	504	0.03%	Nutritional Anemia (Wei et al., 2020)
8	15.81	(1R,2S,3R,4S)-cyclopentane1,2,3,4-tetrol (or))-cyclopentane1,2,3,4-tetrola,4b,3b,2a(1	$C_5H_{10}O_4$	134	0.44%	Antibacterial and antifungal (Rubaye et al., 2018)
9	18.02	4-(ethoxymethyl)-2-methoxyphenol	$C_{10}H_{14}O_3$	183	0.10%	No activity
10	19.30	4-[(1 E)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenol	$C_{10}H_{12}O_3$	180	0.29%	Antihyperglycemic, hepatoprotective, Immunodulatory, Antiviral, Antifungal, Analgesic (Naik et al., 2019)
11	21.14	(2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.13%	Antibacterial and antifungal (Lakshmanan et al., 2019)
12	22.35	hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.35%	Anti-inflammatory and Arthritis (Abubakar & Majinda, 2016)
13	24.35	1 –hentetracontanol	$C_{41}H_{84}O$	592	0.07%	Antioxidant (Olabisi & Olubunmi, 2019)
14	24.75	(9Z,12E)-octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$	280	0.74%	Hypocholesterolemic, Anti-acne (Christiana et al., 2019)
15	24.97	(9Z)-octadec-9-enoic acid Or Oleic acid	$C_{18}H_{34}O_2$	282	0.29%	Diabetes mellitus (Aati et al., 2019)
16	26.77	N'-(4-tert-butylcyclohexylidene)-4-methylbenzene-1-sulfonohydrazide	$C_{17}H_{26}N_2O_2S$	322	0.08%	No activity

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Table 1 continued

17	27.22	4,8,12,16, tetramethylheptadecan-4-olide or 5-Methyl-5-(4,8,12-trimethyltridecyl)dihydro2(3H)-furanone	$C_{21}H_{40}O_2$	324	0.16%	No activity
18	27.45	2H-1-Benzopyran-7 Ol,3,4-dihydro-5-methoxy-6-methyl 2-phenyl	$C_{17}H_{18}O_3$	270	0.15%	No activity
19	27.61	3-[(4-ter-butylcyclohexyl)oxy]-3-phenyl-2-benzofuran-1(3H)-one	$C_{24}H_{28}O_3$	364	0.06%	Green synthetic particles (Weng et al., 2017)
20	27.92	5- hydroxy 2 - phenyl - 4 – chromanone	$C_{15}H_{12}O_3$	240	1.24%	Helicobacter pylori and Gastric antisecretory activity (Das et al., 2010)
21	28.46	(2Z)-2-{[5-(methoxycarbonyl)-3,4-dimethyl-1H-pyrrol-2-yl]methylidene}-3,4,5-trimethyl2H-pyrrol-1-iumbromid	$C_{17}H_{23}BrN_2O_2$	366	0.47%	No activity
22	28.64	1-tetradecene,2–decyl	$C_{24}H_{48}$	336	0.02%	Anti-inflammatory, Arthritis and antioxidant (Fernandes & Krishnan, 2019)
23	28.92	4H-1-benzopyran-4-one,2,3-dihydro-5,7-dihydroxy-2-phenyl(3)	$C_{15}H_{12}O_4$	256	2.11%	No activity
24	29.12	1,2-bis-[2-[4-cyclohepta-2,4,6-trienyl-phenoxy]-ethoxy]-ethane	$C_{32}H_{34}O_4$	482	1.20%	No activity
25	29.24	(2E)-1,3-diphenylprop-2-en-1-one	$C_{15}H_{12}O_3$	240	2.48%	Anti-proliferative (Landim et al., 2019)
26	29.56	2-{3 -[(E) - 2 -(1H -indol - 3 -yl)ethenyl]-1,2,4-oxadiazol-5-yl}phenol -	$C_{18}H_{13}N_3O_2$	303	5.78%	Antioxidant and Antitumor (Kopoytkoba & Caxho, 2019).
27	29.92	5 -hydroxy - 7 -methoxy - 2 -phenyl - 4H-1-benzopyran-4-one	$C_{16}H_{12}O_4$	268	0.63%	Anticancer, anti-prostate cancer and anti-inflammatory activities (Wang et al., 2019).
28	30.01	(2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4H-1-benzopyran-4-one	$C_{15}H_{12}O_5$	272	2.08%	Anti-tuberculosis, Anticancer (Sahu et al., 2020).
29	30.32	7-hydroxy-3-methoxy-2-(4-methoxyphenyl)-4 H-1-benzopyran-4-one	$C_{17}H_{14}O_5$	298	2.57%	Antioxidant (Suttiarporn et al., 2016)
30	30.44	4(1,1-Dimethylaaly)9-methoxy7H-furo (3,2-g)(1) benzopyrn-7-one	$C_{17}H_{16}O_4$	284	0.52%	No activity
31	30.83	2,2' -((1E,1'E) -(1,4 -phenylenebis(azanylylidene))bis(methanylylidene))diphenol	$C_{20}H_{16}N_2O_2$	316	1.10%	Anti-tumour and Cancer phototherapy (Kopoytkoba & Caxho, 2019)

Continued on next page

Table 1 continued

32	31.29	5,7-dihydroxy - 2 -(4 - hydroxyphenyl)-6-methoxy-4Hchromen-4-one	$C_{16}H_{12}O_6$	300	2.46%	Antioxidant (Karker et al., 2019; Mu et al., 2009; Yausheva et al., 2019)
33	31.67	2,3,7,8-tetramethoxy-5,11-dihydro10H-dibenzo[a,d][7]annulen-10-one	$C_{19}H_{20}O_5$	328	1.08%	Arthritis, Anti-inflammatory (Fernandes & Krishnan, 2019)
34	32.17	1-(4-methylphenyl)anthracene - 9,10-dione	$C_{21}H_{14}O_2$	298	2.15%	Anticancer (Chu et al., 2019)
35	32.78	8,9-dihydro-aflatoxin B1	$C_{17}H_{14}O_6$	314	0.66%	Antimycotoxin (Pok et al., 2020)
36	33.15	1-Docosene	$C_{22}H_{44}$	308	0.74%	Anticancer (Swantara et al., 2019)
37	33.45	3,6,8-trimethoxy-5,7-dimethyl-2-phenyl-4H-1-benzopyran-4-one	$C_{18}H_{16}O_7$	344	0.09%	No activity
38	33.68	3-Hydropregn-5-en-20-one hydrazone	$C_{21}H_{34}N_2O$	330	0.33%	No activity

5-hydroxy2-phenyl-4–chromanone is one compound that possesses *Helicobacter pylori* and Gastric antisecretory activity (Das et al., 2010). 1-tetradecene,2–decyl reported to have phytochemical properties like an anti-inflammatory in leaf extracts of *Azima tetracantha* Lam (Jose & Panneerselvam, 2019) and antioxidant activity in leaf and stem of *Strobilanthes* species (Fernandes & Krishnan, 2019). Recently, the anti-inflammatory activity of (2E)-1,3-diphenylprop-2-en-1-one has been reported by Landim et al. (2019) in *Lonchocarpus cultratus*.

The compound, 5-hydroxy-7-methxy-2-phenyl-4H-1-benzopyran-4-one, possesses anti-cancer, anti-prostate and anti-inflammatory activities (Marques et al., 2019; Wang et al., 2019). Similarly, a compound is known as (Naringenin) (2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4H-1-benzopyran-4-one is observed to have two biological properties like anti-tuberculosis and anti-cancer activities (Sahu et al., 2020). The major compound identified in the *M. calabura* leaf extract was found to be 2-{3-[(E)-2-(1H-indol-3-yl)ethenyl]-1,2,4-oxadiazol-5-yl}phenol 5.78% peak area, and have antioxidant and antitumor activity (Figure 1). These bioactive compounds are synthetic aromatic C-nucleoside derivatives developed by Sadek et al. (2014). 2,2'-((1E,1'E)-(1,4-phenylenebis(azanylylidene))bis(methanylylidene))diphenol (Nitrilo methyl dyne) has been reported to have an anti-tumor activity (Kopoytkoba & Caxho, 2019).The compound 7-hydroxy-3-methoxy-2-(4- methoxyphenyl)-4H-1-benzopyran-4-one that is identified to possess antioxidant activity from the bran of Thai black rice (Suttiarporn et al., 2016) and 5,7-dihydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-chromen-4-one reported to have strong antioxidant activity (Karker et al., 2019; Mu et al., 2009; Yausheva et al., 2019). 2,3,7,8-tetramethoxy-5,11-dihydro10H-dibenzo[a,d][7]annulen-10-one has anti-inflammatory activity (Fernandes & Krishnan, 2019). The compound, Anthraquinone, 1-(4-methylphenyl)anthracene-9,10-dione is identified to act against the cancer cell and has antibacterial activities (Chu et al., 2019). 8,9-dihydro-aflatoxin B1 has the mycotoxin activity (Aparna et al., 2012; Pok et al., 2020), and 1-Docosene has the anticancer activity of *Xestospongia testudinaria*. Some compounds have been identified, but their biological activity has not yet been discovered (Table 1).

3.2. GC-MS analysis of root methanolic extract

Of all the bioactive compounds exhibited, cholest-4-en-6-on-3-ol has the highest 63.7% retention time (24.58 min) and has anti-obesity activity (Shaheed et al., 2019). At the same time, another compound, Lupeol, was observed during prolonged retention (35.9 min), and the maximum area percentage was 7.36% (Figure 2). Similarly, a compound is known, 17-octadecynoic acid has phytochemical properties like Hypocholesterolaemia, anti-inflammatory, anti-acne, and anti-coronary activities from various plant extracts (Benzidia et al., 2019; Mishra & Patnaik, 2020; Revathi & Dhanaraj, 2019). (2R)-2,5,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-1-benzopyran-6-ol (β-Tocopherol) is one of

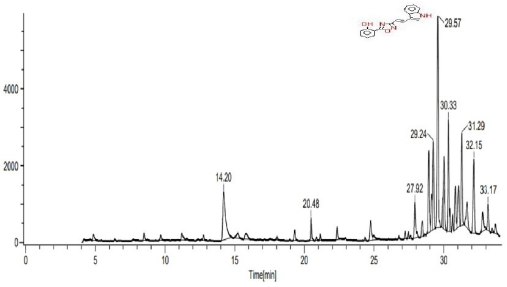

Figure 1. GC-MS chromatogram of leaf methanolic extracts of *Muntingia calabura* and potent bioactive compound 2-{3-[(E)-2-(1H-indol-3-yl)ethenyl]-1,2,4-oxadiazol-5-yl}phenol (antioxidant and antitumor 5.78%).

the compounds that have anti-spasmodic activity (Lanuzza et al., 2017). The compounds like cholestan-3-one-4,4-dimethyl-(5α); 9-octadecenoic acid(Z)-phenyl methyl ester; (9Z)-octadec-9-enoic acid (Oleic acid); androst1-en-3-one,4,4-dimethyl-(5α) identified to possess anti-cancer, anti-inflammatory, antioxidant, antiulcer genic, antipyretic activities in various plant extracts of medicinally important plants (Safwat et al., 2018; Shelke & Bhot, 2019; Yamuna et al., 2017; Yue et al., 2020).

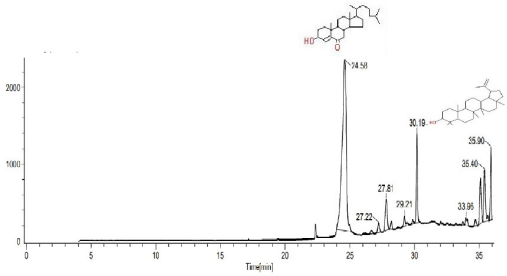

Figure 2. GC-MS chromatogram of root methanolic extracts of *Muntingia calabura*, potent anti-obesity compound cholest-4-en-6-on-3-ol(63.7%) and anti-cancerous compound Lupeol (7.3%).

Cholest-4-en-6-on-3-ol and Lupeol are the two identified compounds with highest percent of compounds among all the compounds in root methanolic extracts of *M. calabura* and reported to possess anticancer, antioxidant, anti-prostate, anti-inflammatory activities in different plant extracts of medicinal plants (Geetha & Varalakshmi, 2001; Shahaby et al., 2019; Shaheed et al., 2019; Yang et al., 2020). There are a few identified compounds, 1,12-Dibromododecane; 5-(2-bromopropan-2-yl)-2-methylcyclohexan-1-ol; 2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimethyl(-2-4,8,12-trimethyl tridecyl); A'-neogammacer-22(29)-en-3-one; 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6 yet to be

Table 2
GC-MS of bioactive compounds present in the methanolic extracts of root-derived from *in vivo* grown plants of *Muntingia calabura* L

S No	RT	Compound name	Formulae	MWt	Area	Biological activity
1	22.36	hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.15%	Anti-inflammatory and arthritis (Abubakar & Majinda, 2016)
2	24.58	cholest-4-en-6-on-3-ol	$C_{27}H_{46}O_2$	400	63.7%	Anti-obesity activity (Shaheed et al., 2019).
3	26.22	1,12-Dibromododecane	$C_{12}H_{24}Br_2$	326	0.40%	No activity
4	26.66	17-octadecynoic acid	$C_{18}H_{32}O_2$	280	0.66%	Hypocholesterolemic, Anti-inflammatory, Anti-acne, Anti-coronary (Revathi & Dhanaraj, 2019)
5	27.22	(2R)-2,5,8-Trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-1-benzopyran-6-ol	$C_{28}H_{48}O_2$	416	1.34%	Anti-spasmodic (Lanuzza et al., 2017)
6	28.2	5-(2-bromopropan-2-yl)-2-methylcyclohexan-1-ol	$C_{16}H_{32}O_2$	256	0.94%	No activity
7	28.71	cholestan-3-one-4,4-dimethyl-(5α)	$C_{29}H_{50}O$	414	0.25%	Anti-cancer and anti-inflammatory (Shaheed et al., 2019)
8	29.41	9-octadecenoic acid(Z)-phenyl methyl ester	$C_{25}H_{40}O_2$	372	0.48%	Anti-ulcer genic, anti-androgenic, anti-inflammatory, anti-cancer (Shelke & Bhot, 2019).
9	29.87	(9Z)-octadec-9-enoic acid (Or)Oleic acid	$C_{18}H_{34}O_2$	282	0.48%	Anti-pyretic, anti-nociceptive, anti-inflammatory (Aati et al., 2019)
10	30.19	(2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol (or) Vitamin E	$C_{29}H_{50}O_2$	430	8.26%	Anti-cancer and anti-oxidant (Shahaby et al., 2019)
11	33.73	2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimethyl(-2-4,8,12-trimethyl tridecyl)	$C_{27}H_{46}O_2$	402	0.34%	No activity
12	34.7	androst 1-en-3-one,4,4-dimethyl-(5α)	$C_{21}H_{32}O$	300	0.87%	Breast and cancer cell activity (Yue et al., 2020)
13	35.1	A'-neogammacer-22(29)-en-3-one	$C_{30}H_{48}O$	424	5.82%	No activity
14	35.4	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6	$C_{30}H_{48}O$	424	6.78%	No activity
15	35.9	1,2,5,14,18,18-hexamethyl-8-(prop-1-en-2-yl)pentacyclo[11.8.0.0^{2},^{10}.0^{5},^{9}.0^{1},^{19}]henicosan-17-ol (or) Lupeol	$C_{30}H_{50}O$	426	7.3%	Anti-prostate and anti-cancer (Yang et al., 2020).

identified (Figure 2 and Table 2).

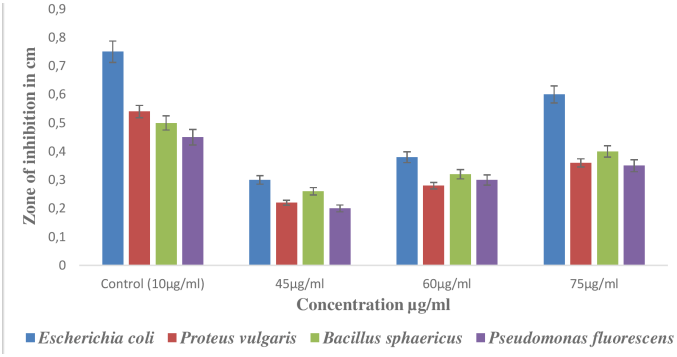

Figure 3. Effect of *Muntingia calabura* leaf methanolic extract on various bacterial cultures.

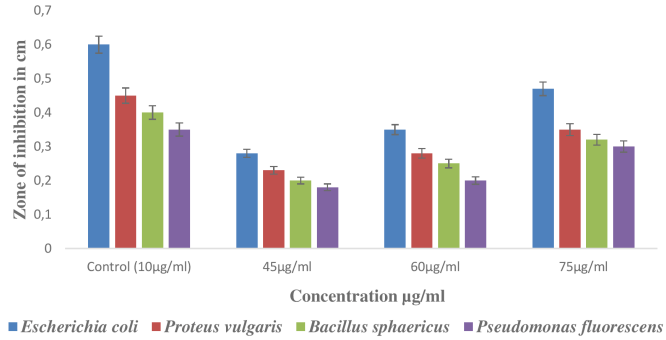

Figure 4. Effect of *Muntingia calabura* root methanolic extract on various bacterial cultures.

3.3. Antibacterial activity for leaf and root methanolic extracts

The antibacterial activity of leaf methanolic extracts (45μg/mL, 60 μg/mL, 75 μg/mL) and the control Streptomycin (10 μg/mL) was evaluated. The maximum

antibacterial activity was shown towards E. coli at 75μg/mL concentration, followed by *B. sphericus*, *P. vulgaris* and *P. fluorescens*. As the concentration of the plant extract increased, the inhibition zone was also found to increase. (Figure 3 and 4). The leaf methanolic extract of *M. calabura* was considered the most effective extract with high anti-bacterial activity (Zakaria et al., 2010). In *M. calabura*, the ethanolic leaf extract has shown strong antibacterial activity against *E. coli* (Gurning et al., 2021). The root extracts of *Carica papaya* have shown the highest antibacterial activity against *Salmonella typhi* among tested gram-negative and gram-positive bacterial strains (Doughari et al., 2007).

4. CONCLUSION

This study revealed the presence of various bioactive molecules in the leaf and root methanolic extracts of *M. calabura*. Leaf methanolic extracts yielded thirty-eight compounds, while root extracts yielded a total of fifteen compounds based on their molecular weight, retention time, and peak area. The leaves of *M. calabura* contain the highest concentration of the antioxidant and antitumor compound is 2-{3-[(E)-2-(1H-indol-3-yl)ethenyl]-1,2,4-oxadiazol-5- yl}phenol, followed by roots of cholest-4-en-6-on-3-ol and Lupeol. These bioactive substances have been shown to have antibacterial effects against gram-positive and gram-negative bacteria. According to this study, the abundance of phytochemicals and bioactive substances in *M. calabura* makes it a potential source of medicines.

CONFLICTS OF INTEREST

Conflict of interest the authors declare that there are no conflicts of interest in this study.

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AUTHOR CONTRIBUTIONS

SV conducted the experimental work, writing the article, PC analyzed the data and designed the manuscript helped in experimental work, and SV designing Chromatogram figures and interpretation the data. ST extended overall guidance and finalized the manuscript.

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