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HR-LCMS based Metabolites Profiling of *Madhuca Longifolia* (J.Konig) J. F. Macbr. Flower Extract and its Bioactivity

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Abstract: **Madhuca longifolia,** ***commonly known as mahua is a very important medicinal plant belongs to family sapotaceae. It is large size tree frequently occurred in India and Indian sub continent. Their flowers are used as food, fermented and non fermented products among the tribal people. It is also used as medicine to cure various ailments, due to presence of secondary metabolites [14]. So in this present study, we investigate its methanolic extracts of flowers for bioactive compounds by HR-LCMS and antibacterial properties by using disc diffusion method against plant pathogenic bacteria, such as Xanthomonus axonopodis and Pseudomonas syringae. Plant extract were showed antibacterial activity against both plant pathogenic bacteria. The maximum growth of inhibition were recorded against X. axonopodis (16 mm) followed by P. syringae (15 mm). Due to the presence of some important bioactive compounds such as Mebeverine metabolite, Quercitrin, Dihydrodeoxystreptomycin, Leucine, Dihydromyricetin, Protorifamycin-I, Chlortetracycline, Racepinephrine, asparatic acid, 4-Trimethylammoniobutanal, arginine, Glutamic acid, Salicyl acyl glucuronide, Petunidin, Betaxolol (hydroxylation), 16-hydroxy-5-hexadecenoic acid, Coproporphyrin-II, Dimethyl sulfone, etc. Our findings provided facts that methanolic extract of these tested plants have medicinally significant bioactive compounds and it justifies their use in the traditional medicines for the dealing of various diseases.***

Keywords: HR-LCMS, *Xanthomonus axonopodis, Pseudomonas syringae* and *Madhuca longifolia*.

1. Introduction

Since prehistoric time plants play an important role in medicine. About 80% of the world's population is completely reliant on plant extract and its derivatives for the healing of a variety of diseases and infections. Bioactive compounds which are isolated from medicinal plants play a very significant role in maintaining people healthy around the world. Traditional healthcare system like Ayurveda, Homeopathy, Siddha and Unani stated about use of plant based products to cure various human ailments [1; 12]. Now days, number of widely used drugs with low or no side effects were isolated from plants. Therefore it is necessary to identify a novel, potent drug molecules from plant products that are safe with low side effects to cure illness [9; 13]. *Madhuca longifolia* is very nutritious plants and it is used as an herbal medication to treat number of diseases such as bronchitis, helminths, acute and chronic tonsillitis [2], pharyngitis and chronic tonsillitis [8], headache, rheumatism, skin diseases, laxative and piles [20]. Sugar is the major constituent of flowers, even though they also contain vitamins, proteins, organic acids, and essential oils [23]. Flowers are used as a remedial agent against numerous diseases such as ulcer, snakebite, scabies, rheumatism, etc. [11]. It is also used in the formulation of pickles and flavoring agent [17]. Alcoholic extract of *Madhuca longifolia* flowers were analyzed against *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus oryzae* and *Aspergillus niger*. It showed considerable antimicrobial activity against tested organisms [7]. Fatma *et al*., [4] studied the Antibacterial Activity of *Madhuca longifolia* stem extract on *Streptococcus mutans*, a bacteria associated to tooth decay. Though the extract showed a high MIC value, it still proved to be effective. This recommends that the plant could be a useful natural medicine for oral problems like dental caries. Flavonoids, riboflavin, triterpene, niacin, carotene, ascorbic acid, biotin, sterol, folic acid, thiamine and inositol these phytochemicals were recorded in this plant [3].

The present investigation was revealed to identify new plant based compounds against *Xanthomonus axonopodis* and *Pseudomonas syringae* using flower extract of *Madhuca longifolia*. In this regard, high resolution liquid chromatography and mass spectrometry (HR-LCMS) was performed to separation and identification of the phytoconstituents based on their retention time and data base difference from the crude extracts showing good antibacterial activity.

2. Materials and Methods

Collection of plant Material:
Fresh and Healthy Flowers of *Madhuca longifolia* were collected from Belora Tal. Mantha Dist. Jalna, during March 2024. The identification is done with the help of standard floras (flora of Marathwada by Naik *et al.,* 1998) [10]. The fresh flowers were washed and shade dried, powdered and stored in airtight container for further study.

Preparation of plant Extract:
Methanolic extract was prepared by using Soxhlet extractor. About 20 gm of flower powder were subjected to soxhlet extraction with 200 ml of methanol at 55-65^0 C up to 72 hours. Solvent was evaporated at 40-50 ^{0}C by using Rotary evaporator. The collected powder was weighted and

dissolved in Dimethyl sulfoxide (DMSO) with 10% concentration. The extracts were preserved in sterile glass bottles at 4^0 C temperatures for further study [5; 22].

Anti Bacterial Activity of Plant Extract:
The assessment of antibacterial activity, agar disc diffusion method was used [24; 6].

Inoculums preparation:
Bacterial inoculums were prepared by inoculating a loopful of target bacterial colony (24 h old culture) in 5 ml nutrient broth and incubated at 27 ± 1^0C for 10 hours till a moderate turbidity was developed. The turbidity was adjusted to 0.5 McFarland standard (WHO Drug Information, 1993) and the cultures were diluted with sterile distilled water if necessary which corresponds to the cell density of 1.5 X 10^8 (CFU/mL) [25]. Testing of antibacterial activity was done *in vitro* in 9 cm Petri plates with 20 ml nutrient agar medium. Surface of the nutrient agar was inoculated with bacterial culture using lawn culture method. The surface was allowed to dry for 10 minutes sterilized 5 mm paper disk were soaked in extracts which were dried and placed on the surface of nutrient agar using sterile forceps. For comparison the antibiotic Streptomycin (1000 ppm) was used. All the plates were incubated at 37 ± 1^0 C for 48 hours test was carried out in triplicate and results were recorded in terms of diameter of the zone of inhibition in mili-meters.

High Resolution Liquid Chromatography and Mass Spectrometry (HR-LCMS) profiling:
The extract was prepared in methanol and then subjected to HR-LCMS profiling. The HR-LCMS of plant extract was carried out in Sophisticated Analytical Instrument Facility (SAIF), IIT Powai, Mumbai (MS). Chemical finger prints of *Madhuca longifolia* plant extracts were prepared by Agilent High resolution liquid chromatography and mass spectrometry model-G6550A with 0.010% mass resolution. The acquisition method was set to be MS- minimum range 50 *(M/Z)* and maximum range 1100 *(M/Z)* with scanning rate each spectrum per second. Gas chromatography had maintained at 250^{0}C with gas flow 13 psi/minute. HiP sampler with model- G4226A was used with auxiliary speed 100.0 µL / minute, ejection speed 100.0 µL / minute, flush out factor 5µl and 8µl injection volume used for HR-LCMS. Binary pump, model-G44220B was used with high pressure limit 1200 bar with 30 minute of acquisition method [16].

a) A-100% Water
b) B-5% Acetonitrile, used as a solvent.

Identification of components:
Interpretation on mass spectrum HR-LCMS were conducted using the database of Sophisticated Analytical Instrument Facility (IIT, Bombay) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the SAIF library. The compound name, molecular weight and structure of the components of the test materials were ascertained on the basis of retention time.

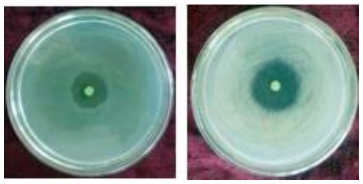
3. Result and Discussion

The present work was carried out for HR-LCMS analysis and antibacterial activity of methanolic extract of flower of *Madhuca longifolia.* The study reveals that the presence of number of secondary metabolites such as Dihydrodeoxystreptomycin, Protorifamycin-I, Coproporphyrin-II, Quercitrin, Dihydromyricetin, Betaxolol etc. (Table-2). These metabolites were very important to control bacterial diseases [16]. According the recorded antibacterial activity (Table-1), methanolic extract of flower showed very effective against both the tested bacteria as compared with standard antibiotics Streptomycin (1000 ppm). Maximum percent inhibition over control was recorded against *X. auxonopodis* (14 ± 0.1) even very good result showed against *P. Syringae* (15 ± 0.2). Through the study the results clearly reveals that the plant extract play the important role in controlling the plant diseases. Kalaivani and Jagsdeesan (2013) screened antimicrobial activity of leaf and flower extract of *M. longifolia* against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, they showed that the flower extract were very potent against all tested bacteria [7]. Rohit kumar Bargah (2015) revealed that the bioactive compound plays a significant role in the cure of diseases without any side effects; there is a need to explore new drugs from natural sources [18]. India is a rich source of variety of traditional medicine that relay to a very large area on native plant species for new drug materials. Therefore, now there is a need to look back towards traditional medicine which can serve a novel therapeutic agent [19]. The pharmacognostical evaluations also give valuable information which is necessary to standardize the drug.

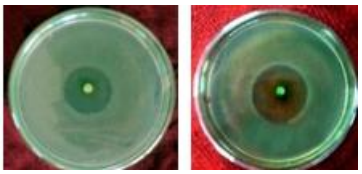
Table 1: Antifungal activity of methanolic flower extract of *Madhuca longifolia.*

Plant	Zone of inhibition (mm) *X. auxonopodis*	Zone of inhibition (mm) *P. Syringae*
Madhuca longifolia	14 ± 0.1	15 ± 0.2
Streptomycin	15 ± 0.1	16 ± 0.2

Photo plate: 1 Antibacterial activity of flower extract of *M. longifolia* compared with Streptomycin (1000ppm) over control



X. axonopodis flower extract Streptomycin



P. syringae flower extract Streptomycin

Table 2: Common Bioactive compounds of Methanol extract of flower of *M. longifolia*

Sr. No	Name of Compounds	Formula	Retention Time	Mol. Weight g/mol	DB Diff, ppm
1	6-hydroxy-2-hexynoicacid	$C_6H_8O_3$	0.564	128.1	5.64
2	2-acetyl-1-pyrolline	C_6H_9NO	0.903	111.1	4.22
3	4-Trimethyl-ammoniobutanal	$C_7H_{16}NO$	1.746	146.2	2.26
4	Mebeverine metabolite (Veratricacidglucuronide)	$C_{15}H_{18}O_{10}$	1.789	358.3	5.7
5	Bis (2-hydroxypropyl) amine	$C_6H_{15}NO_2$	1.886	133.1	6.72
6	Thr-Ala-His	$C_{13}H_{21}N_5O_5$	2.424	327.3	1.39
7	Ethosuximide	$C_7H_{11}NO_2$	2.464	141.1	8.73
8	4-Hydroxy-L-threonine	$C_4H_9NO_4$	2.593	135.1	0.63
9	Neuraminic acid	$C_9H_{17}NO_8$	2.593	267.2	1.73
10	2S-Aminoheptanoic acid	$C_7H_{15}NO_2$	4.148	145.2	5.07
11	N-Methyl-2-pyridone-5-carboxamide (Nudifloramide)	$C_7H_8N_2O_2$	6.471	152.1	8.63
12	Leucine	$C_6H_{13}NO_2$	6.929	131.1	1.6
13	Glu-Asp-Arg	$C_{15}H_{26}N_6O_8$	7.197	418.4	1.95
14	Salicylacyl glucuronide	$C_{13}H_{14}O_9$	8.352	314.2	1.69
15	2-Amino-3-methyl-1-butanol	$C_5H_{13}NO$	8.523	103.1	9.42
16	(S)-Reticuline	$C_{19}H_{23}NO_4$	9.374	329.4	5.02
17	Bergenin	$C_{14}H_{16}O_9$	9.415	328.2	0.98
18	Chlortetracycline	$C_{22}H_{23}C_lN_2O_8$	9.416	478.9	10.24
19	Quercitrin	$C_{21}H_{20}O_{11}$	9.486	448.4	4.17
20	Dihydromyricetin	$C_{15}H_{12}O_8$	9.486	320.2	6.16
21	4-hydroxy pelargonic acid	$C_9H_{18}O_3$	10.067	174.2	3.04
22	7-Epiloganin tetraacetate	$C_{25}H_{34}O_{14}$	10.164	558.5	1.73
23	Tyr-Cys-Asn	$C_{16}H_{22}N_4O_6S$	10.488	398.4	3.58
24	Dimethylsulfone	$C_2H_6O_2S$	10.574	94.1	1
25	Barbituric acid, 5-ethyl-5-(2-hydroxyethyl)-	$C_8H_{12}N_2O_4$	10.576	200.1	0.86
26	16-hydroxy-5-hexadecenoicacid	$C_{16}H_{30}O_3$	11.803	270.4	0.28
27	Racepinephrine	$C_9H_{13}NO_3$	12.295	183.2	1.95
28	C16 Sphinganine	$C_{16}H_{35}NO_2$	12.453	273.4	8.33
29	Dihydrodeoxystreptomycin	$C_{21}H_{41}N_7O_{11}$	12.877	567.6	1.13
30	Swietenine	$C_{32}H_{40}O_9$	12.88	568.7	6.6
31	Deoxysappanone B 7,3'-dimethyl ether	$C_{18}H_{18}O_5$	12.974	314.3	0.16
32	Protorifamycin-I	$C_{35}H_{45}NO_{10}$	13.388	639.7	5.66
33	Dihydrosphingosine	$C_{18}H_{39}NO_2$	13.786	301.5	6.64
34	Arg- Ala-Thr	$C_{13}H_{26}N_6O_5$	13.863	346.3	5.62
35	Hexadecanedioic acid	$C_{16}H_{30}O_4$	14.003	286.4	0.85
36	2-isopropyl-hexanoicacid	$C_9H_{18}O_2$	14.39	158.2	2.73
37	Cetylpyridinium	$C_{21}H_{38}N$	14.903	304.5	7.07
38	(9R,13R)-1a,1b-dinor-10,11-dihydro-12-oxo-15-phytoenoica cid	$C_{16}H_{26}O_3$	15.111	266.3	1.32
39	13-methyl-pentadecanoic acid	$C_{16}H_{32}O_2$	18.773	256.4	0.33
40	2- docosanamido ethanesulfonic	$C_{24}H_{49}NO_4S$	19.289	447.7	6.65
41	1alpha,25-dihydroxy-22-thia-20-epivitaminD3/1alpha,25-dihydroxy-22-thia-20-epicholecalciferol	$C_{26}H_{42}O_3S$	19.292	434.7	1.41
42	9-hydroxy-12-oxo-10-octadecenoic acid	$C_{18}H_{32}O_4$	19.637	312.4	7.06
43	4Z-Decenedioic acid	$C_{10}H_{16}O_4$	20.414	200.2	0.57
44	Avocadene	$C_{17}H_{34}O_3$	20.803	286.4	0.47
45	3-hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	20.811	272.4	0.16
46	Khivorin	$C_{32}H_{42}O_{10}$	23.75	586.7	1.73
47	Harderoporphyrin	$C_{35}H_{36}N_4O_6$	24.418	608.7	4.24
48	Coproporphyrin-II	$C_{36}H_{38}N_4O_8$	24.956	654.7	3.3
49	Ramiprilglucuronide	$C_{29}H_{40}N_2O_{11}$	25.009	592.6	4.85
50	Deoxykhivorin	$C_{32}H_{42}O_9$	26.207	570.7	1.57

Fig. 1: Chromatogram of Methanolic extract of flower of *Madhuca longifolia*

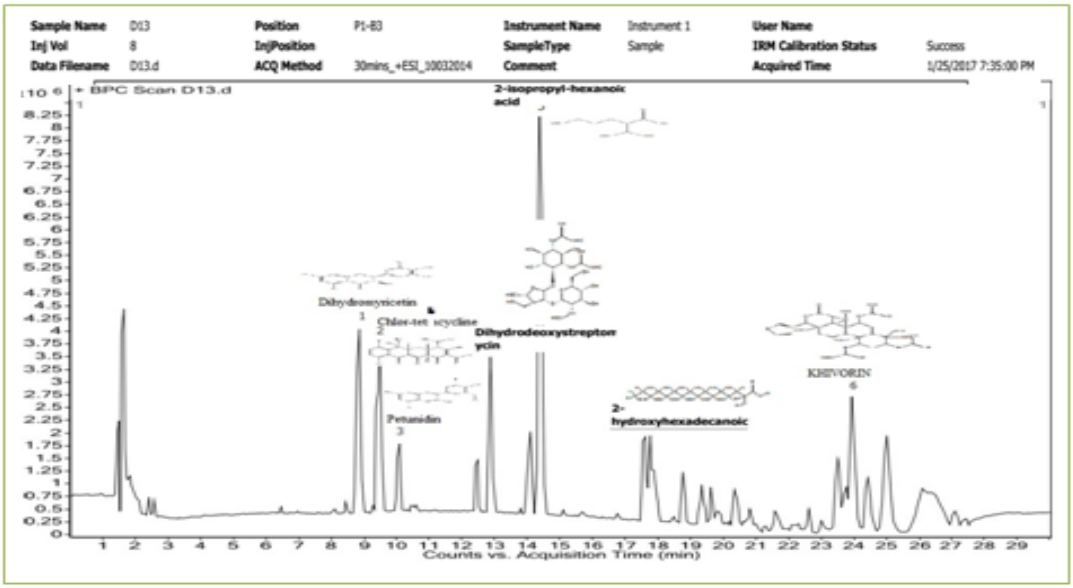
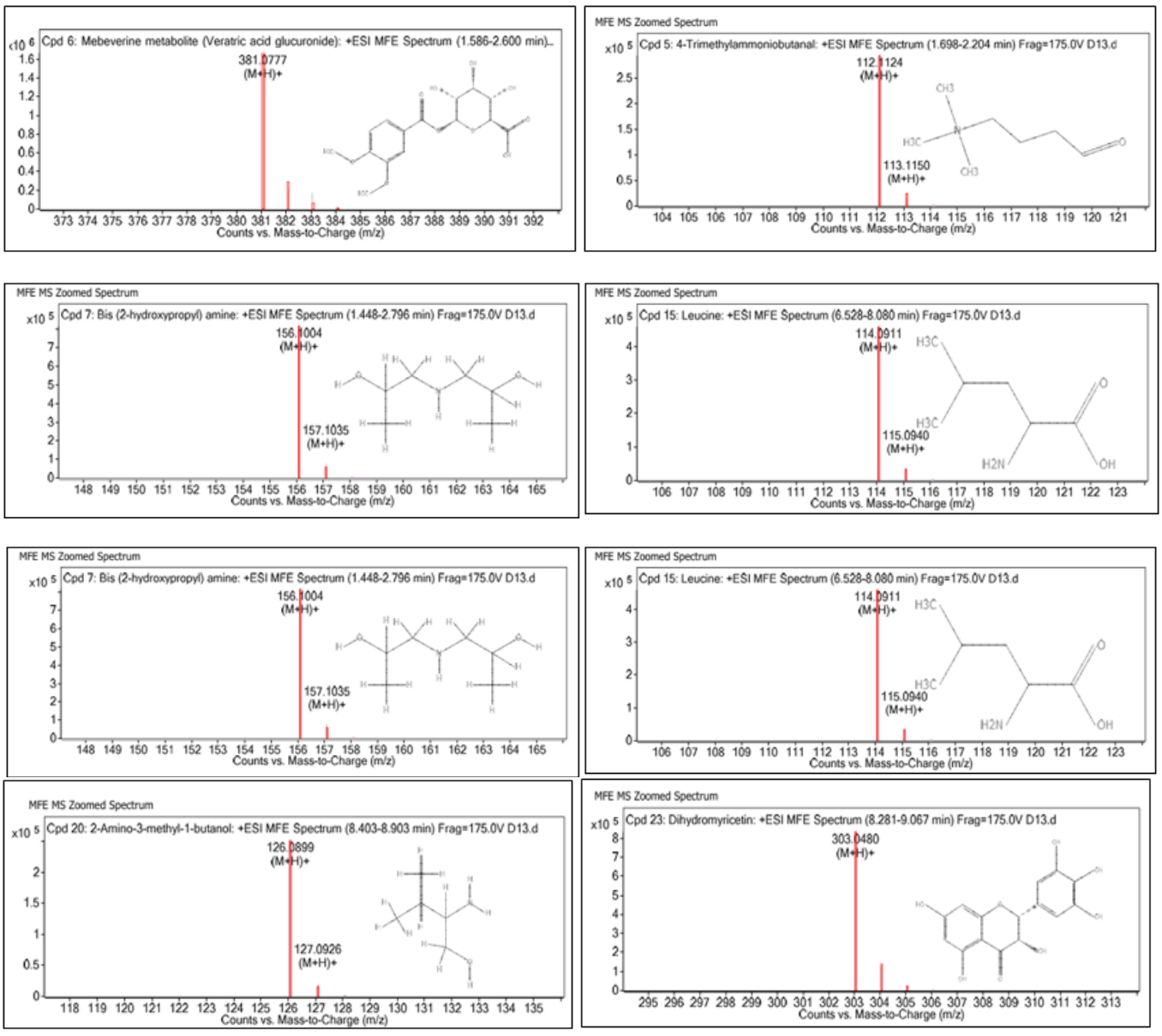
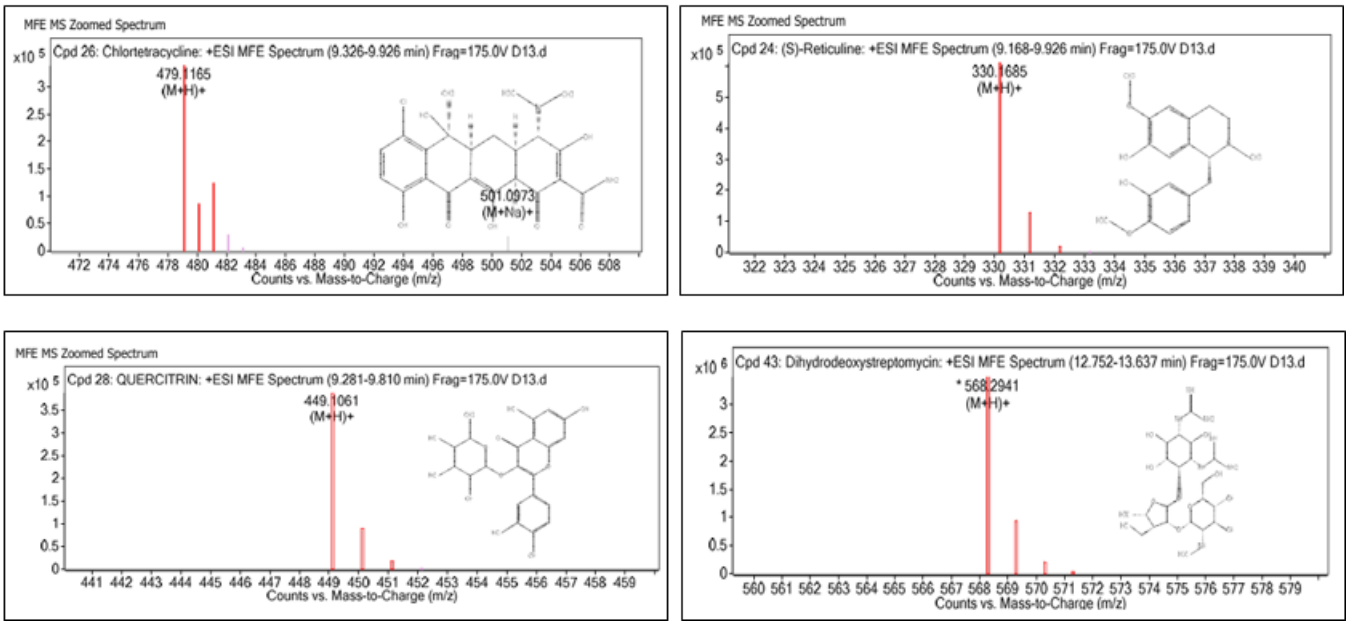


Figure 2: HR-LCMS zoomed spectrum of purified compound isolated from Methanolic flower extract of *M. longifolia*





4. Conclusion

The result of HR-LCMS analysis revealed that the methanolic extract of flower of *Madhuca longifolia* includes precious bioactive compounds which have different medicinal properties that can be helpful for the healing of various diseases [15]. The studies expose the crucial roles of phytochemicals, which are released in the form of secondary metabolites, in controlling the bacterial plant diseases without affecting the environment. Therefore, medicinal plant extracts were very safer and eco-friendly alternative to control plant diseases. Thus, it is concluded that the given plant extracts were very potent against various bacterial diseases.

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