

**LC-MS CHARACTERIZATION OF ACETONE EXTRACT OF DIPLOCYCLOS
PALMATUS (L. JEFFRY) LEAVES AND ANTI-MICROBIAL S. MARCESCENS
ACTIVITY**

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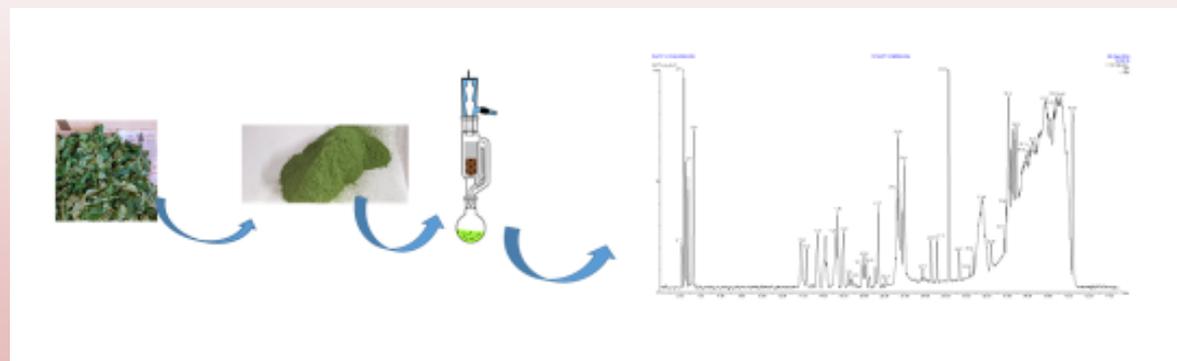
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ABSTRACT

The plant produces secondary metabolism to protect themselves from bacteria these are the phytochemicals stored in the different parts of plants during the growth. We found that the some of secondary metabolites in the D. palmatus leaves (DPL). Acetone soxhlet extract of DPL contains the 2-((3-Cyano-4,6-dimethyl-2-pyridinyl)oxy)acetamide, 4-Methoxytriphenylmethyl cation, D-(+)-Xylose, permethyl- D-(-)-Ribose, permethyl- D-(-)-Lyxose, permethyl- Cyprodinil, Mangiferin, Xipamide, Simvastatin, Catechin, dalbergin, Neochanin glucopyranosiduronic acid, Sativanone, chromanol, Sinapinate, Glucuronide, isoquercetin, Gomisin A, Schizandrin schisanhenol Theaflavin molecules. Acetone extract containing compounds only showing the antibacterial activity against the S. marcescens.



KEYWORDS: LCMS, Soxhlet Extract of Acetone, D. palmatus Leaves, Anti-Microbial-Zone Inhibition Test- S. marcescens.

1. INTRODUCTION

The plants part remedies are widely used by the traditional medicinal practitioners for curing various diseases in their day to day practice. In traditional system of medicine, different parts (Leaves, stem, flower, seeds and even whole plant) of *Diplocyclos palmatus*. *Diplocyclos palmatus* commonly known as Shivalingi is a lesser heard and perennial climber having various biological activity of extract.^[1] The plant *D.palmatus* is found to be useful in several disorders.^[2] Phytochemicals, antioxidant potential and phenolic profiling of leaf and fruit extracts of *Diplocyclos*

palmatus.^[3] Fruit containing bioactive components extracted by continuous shaking extraction (CSE) and showed the highest cucurbitacin content in this plants^[4] it is commonly found in the warmer rainforests of Australia, Malaysia, Africa, India, and South China. The seeds are being used for promoting the conception in women and sperm production in men. Their seeds are utilized in the remedial of cough, asthma, flatulence, skin diseases, and snakes bite. Further, their leaves are also used for inflammation and asthma The data confirm that the component conjugated fatty acid in *D. palmatus* seed oil is octadeca cis-9, trans- I 1, cis-13-trienoic acid

(punica).^[5] The results showed that *D. palmatus* methanolic leaf extract effectively inhibited the quorum sensing regulated virulence factor production as well as biofilm formation in *Serratia marcescens* and it also evidenced that that the lifespan extension of photoaged *C. elegans* was accomplished by the actions of antioxidants such as tocopherols and phytol in *D. palmatus* methanolic leaf extract.^[6] The antioxidant activity of the medicinal plants *Diplocyclos palmatus* were estimated by using 2, 2-Diphenyl 1-Picryl Hydrazyl assay. In this study evaluating therapeutic potential of these medicinal plants due to their high free radical scavenging activity^[7] the pharmacognostical characteristics and in vivo anticonvulsant activity of chloroform extracts of *Diplocyclos palmatus* leaves found no significant changes in average body weight of animals, toxicity study and suspension having good stability on storage. The in vivo study reveals the anticonvulsant activity of chloroform extract against MES and PTZ induced convulsions. The chloroform extract is found to be more potent, similar to Phenytoin^[8] The literature found that to determine and compare anthelmintic efficacies of these plants against the commercially available anthelmintic drug, nitazoxamide^[9] the simultaneous HPLC-quantification of phenolic acid(s) in the aerial parts of *Diplocyclos palmatus* and evaluation of their bioactivity potential through in vitro antioxidant assays^[10] The chloroform and methanolic extracts of aerial parts of *Diplocyclos palmatus* contained alkaloids, flavonoids, tannins, saponins, diterpenes, triterpenoids, glycosides and steroids. Phenols were present in methanolic extract only. Petroleum ether extract contained tannins, triterpenoids, glycosides and steroids. Anthraquinones were totally absent in all the three extracts^[11] *Diplocyclos palmatus* Seeds of Aqueous extract contains Glycosides, Tannin, Phenolic compounds.^[12] The seed of *Diplocyclos palmatus* contained 12 % oil and 40 % protein^[13] owing to this literature survey we are mention here to achieve the successive soxhlet extraction of *Diplocyclos palmatus* leaves extraction and carried it anti-microbial activity with the.

2. MATERIAL AND METHODS

The plant *Diplocyclos palmatus* fresh leaf collected from the Bhokardan tahsil near talani village in the month September. The leaf is dried for five to six day under the shadow place without direct contact of sunlight. The dry leaf were crushed and made it into fine powder for extraction. Successive extraction of 10gm dried powder leaves sample where carried out using Soxhlet extractor apparatus at 50°C for eight hours, successively in order to polarity of organic solvent pet ether, ethyl acetate, acetone, ethanol and n-butanol 100 milliliter of solvent used for each time of extraction. Mueller-Hilton Agar (SRL- Chem-Cat no.-24756) (MHA) Plates Bacterial Culture (*S. marcescens*, MTCC 86) Procured from Microbial Type Culture Collection and Gene Bank (MTCC)- Chandigarh Whatman No 1 filter paper discs

(5mm) Solvent (vehicle control)- Acetone Ciprofloxacin (SRL Chem- 78079) Amount Loaded – 10µl.

2.1 Details of technique used

Instrument: Waters, SYNAPT-XS HDMS, UK Model:DBA064 Separation Module: UPLC ACQUITY H CLASS Series System LC Column: C18 Waters, Acquity BEH 2.1*100mm 1.7µm Acquisition: MRM, unit resolution Injection Volume: 5 micro liters Flow rate:0.2 ml/min Run time 45min.

2.2 For Mass spectrometer following parameters were used

Data Acquisition mode: ESI Positive (sensitivity or resolution), Desolvation Gas Flow: 950Lts/Hr Cone Gas Flow: 50 Lts/Hr Desolvation Temperature: 5500C Source Temperature: 1200C Capillary Voltage: 3.22kev Cone Voltage: 50V Collision Energy 4ev Source offset 80 V Mobile Phase used: Solvent A = 0.1% Formic Acid + LC-MS Grade water Solvent B = 0.1% Formic Acid + Acetonitrile.

Gradient used

Time (min)	Flow(µL/min)	%A	%B	Curve
0.00	0.200	95.0	5.	6
5.00	0.200	95.0	5.	6
30.	0.2	10.0	90.0	6
35	0.2	10.0	90.0	6
36.0	0.200	95.0	5.0	6
45	0.200	95.0	5.0	6

3. RESULTS AND DISCUSSIONS

3.1 Anti-microbial activity assay

The fruits of *D. palmatus* were revealed as a potent antioxidant, anti-diabetic and anticancer bio-resource.^[4] The ethanolic extract of *D. palmatus* fruits was shown analgesic activity and it was found to be more remarkable.^[14] The presence of organic constituents like alkaloids, triterpenoids, flavonoids, saponins, steroids and proteins in the dried fruit. This provides impetus to conduct advanced research on this fruit to uncover its vast medicinal potential.^[15] The ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds possess analgesic, anti-inflammatory and antiarthritic activities in animal model^[16] The Antibacterial activity was checked by following Zone Inhibition Method (Kirby-Bauer method).

3.1.1 Anti-Microbial-Zone Inhibition Test- *S. marcescens*

The MHA plates were inoculated by spreading with 100 µl of Bacterial culture, *S. marcescens* (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx cell density (1.5 X 10⁸ CFU/mL from Mueller- Hilton Broth) and followed by placing the discs containing 10 µl of different concentration (0 to 100 mg/ml). One disc in each plate was loaded with solvent alone which served as vehicle control and Ciprofloxacin disc (10µg) were taken as positive control. The plates of *S. aureus*, *E. faecalis* *P. aeruginosa* *S. marcescens* were incubated (Basil

Scientific Corp. India) at 37 °C for 24 hrs. The clear zones created around the disc were measured and recorded.^{[17],[18]} Based on the results obtained from this study, acetone extract was found effective to Anti-Microbial-Zone Inhibition Test- *S. marcescens*.

Ciprofloxacin (PC) Effective Amount 10µg shows Zone at Effective Concentration up to 32 mm as a standard. Acetone extract amount 50µg loaded to agar plate showing Zone at Effective Concentration 6.33mm fig.1 & 2.

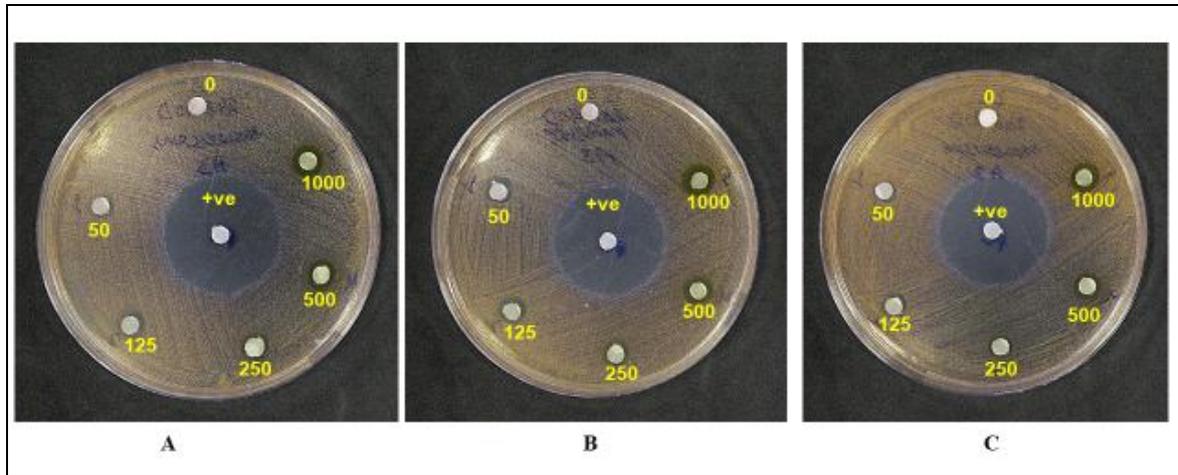


Fig.1. *S. marcescens*.

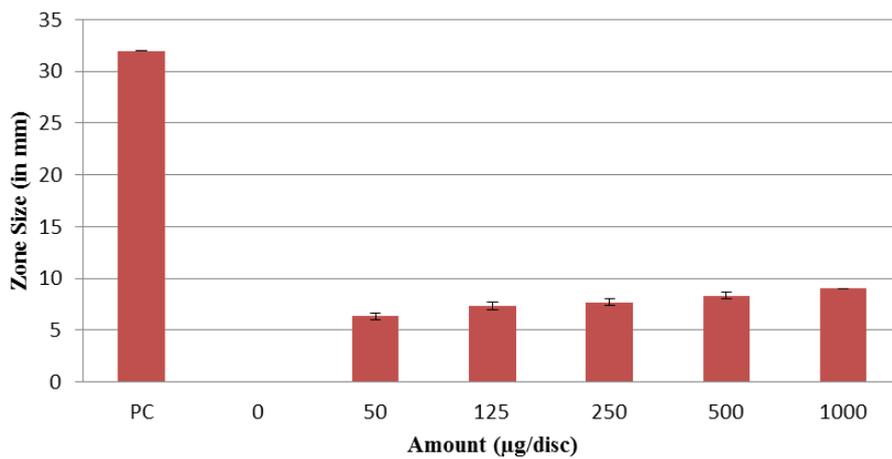


fig.2. Antibacterial Activity- *S. marcescens* at different concentration of acetone extract DPL

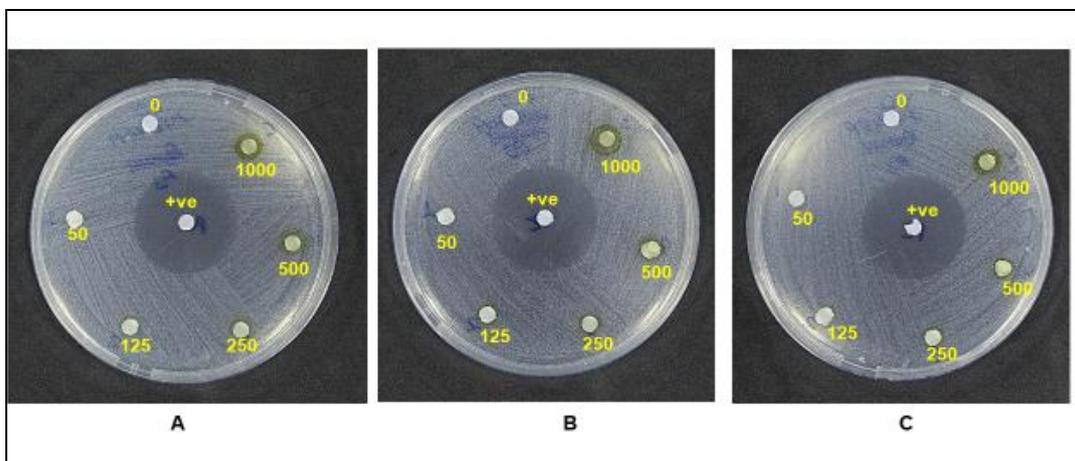
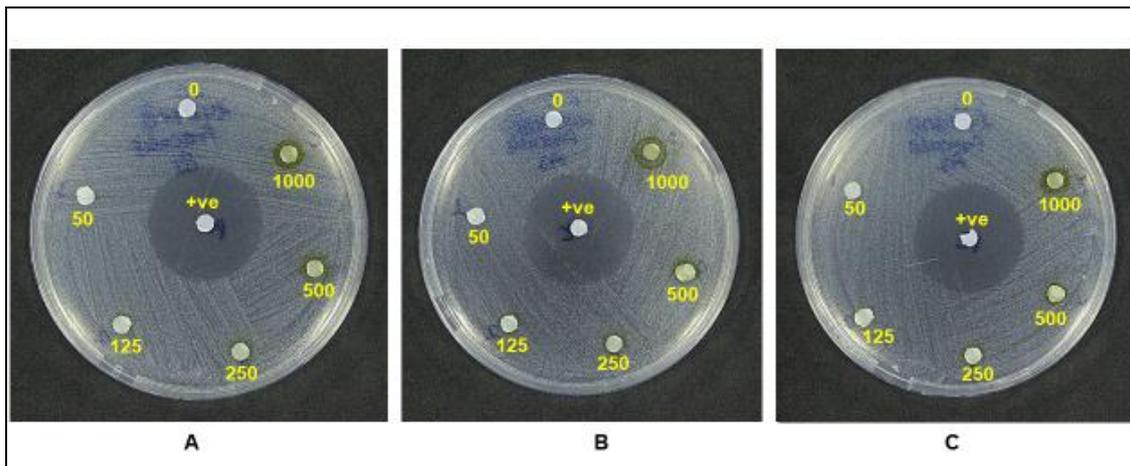
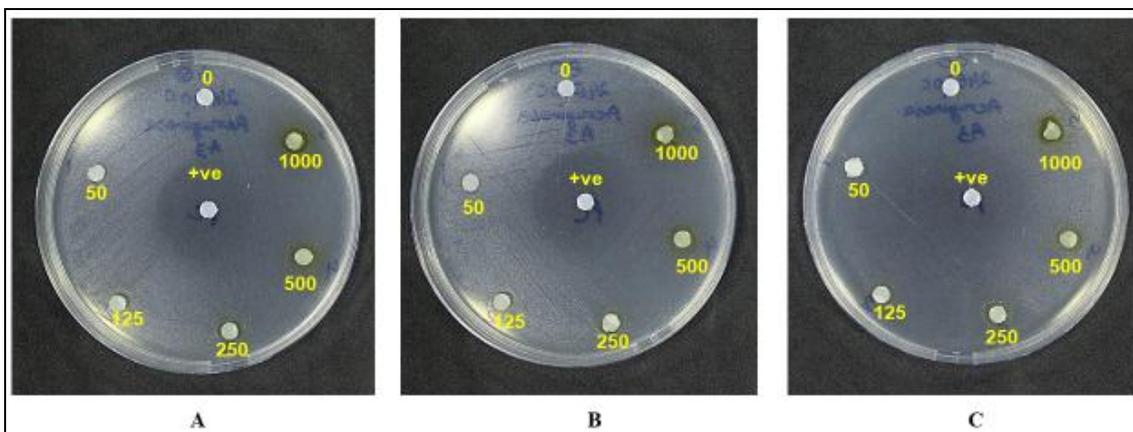


Fig. 3: *S. aureus*.

Fig. 4: *E. faecalis*.Fig. 5. *P. aeruginosa*.

Based on the results obtained from this study, fig.3, fig.4, and fig.5 does not seem to impose an antimicrobial effect till 1000 µg/well.

3.2 Characterization of extract by Mass Spectra with Liquid Chromatography

Phytochemical analyses revealed that the percentage yield of petroleum ether extract was found to be more than when compared to other extracts^[15] Tannins, diterpenes, glycosides, Alkaloids, triterpenoids, flavonoids, saponins, steroids and proteins are phytochemical constituents of leaves and seeds of *Diplocyclos palmatus*.^[3] Leaves and seeds extract of *Diplocyclos palmatus* were subjected to evaluate their biological activities.^[16] These phytochemicals of *Diplocyclos palmatus* leaves and seeds are extracted using different solvents which are mentioned above, in future the crude extract sample is to be subjected for different solvent fractions, and these could be effective for the biological activities apart from single extract fraction. Its facts due to the polarity and interactions of solvents has ability to extract different components from the crude sample.^[19] The conflict of this review is that the *Diplocyclos palmatus* extract contains compounds also select for molecular docking study^[20] LCMS chromatogram run for the time 45 minutes the acetone

extracts of *Diplocyclos palmatus* showing different metabolites present in the acetone extracts are as follows with their fragmented mass spectra graph at respective retention time noted.

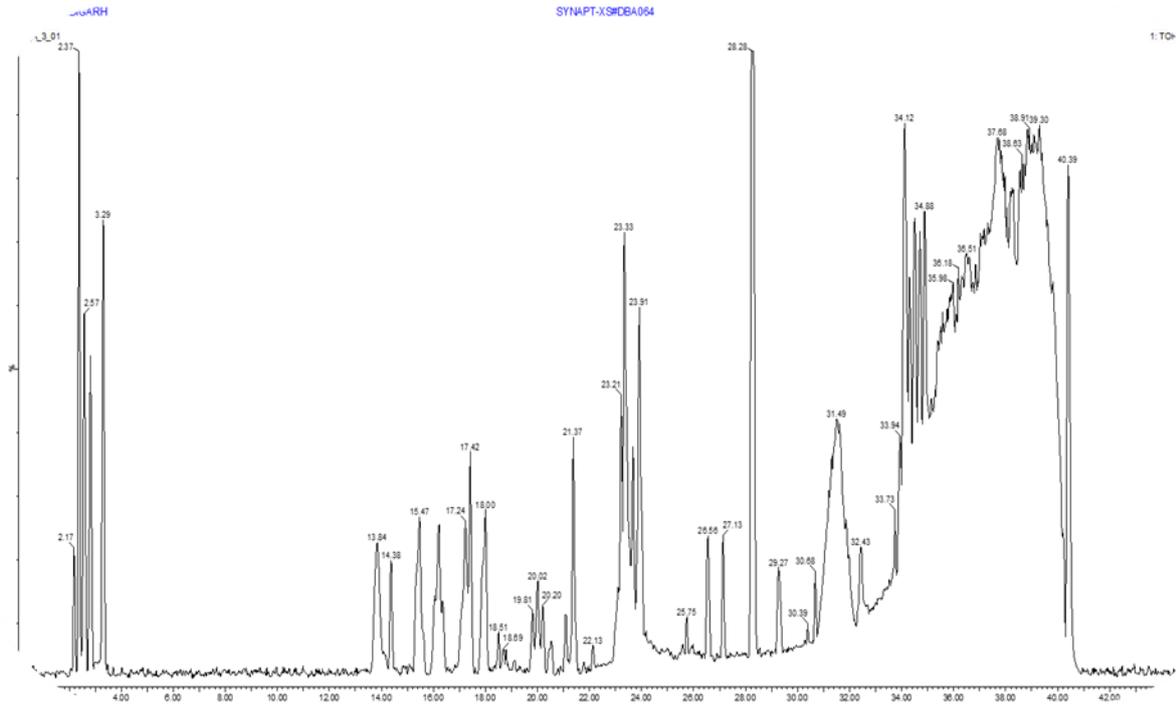


Fig.6. LCMS Chromatogram of positive ion species in Acetone extract

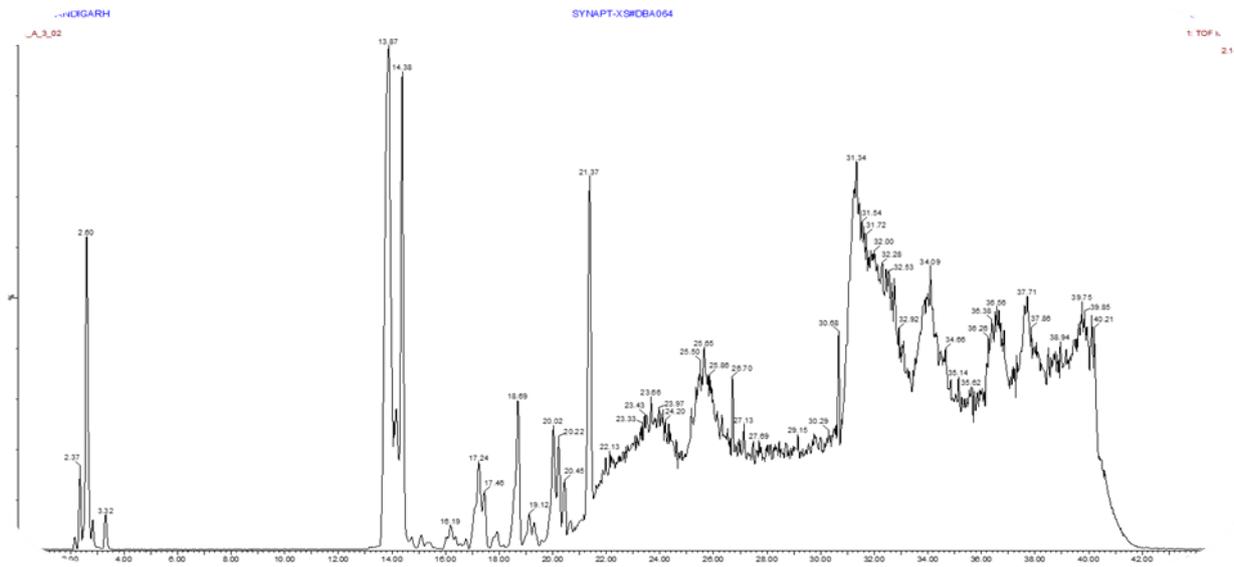


Fig.7. LCMS Chromatogram of Negative ion species in Acetone extract.

Table 1: The Main peak appeared in LCMS Chromatogram.

Sr.no	Probable Compounds	Retention time (in minutes)	m/z ratio
1.	Tetrahydro-5-(4-Hydroxy-3-Methoxybenzyl)-2-Furanone	2.503217	203.0707
2.	5-(Dihydroxyphenyl)- Valerolactone 3-O-Glucuronide	2.628233	383.1002
3.	-Epicatechin	2.655383	289.0704
4.	D-(+)-Catechin	2.655383	289.0704
5.	Dalbergin	2.7261	249.057

6.	Neochanin	2.7261	249.057
7.	1-O-[(2E)-3-(4-Hydroxy-3-Methoxyphenyl)-2-Propenoyl]- β -D-Glucopyranuronic Acid	2.834683	351.0729
8.	5-[(E)-2-Carboxyvinyl]-2-Methoxyphenyl β -D-Glucopyranosiduronic Acid	2.834683	351.0729
9.	1-O-[(2E)-3-(3-Hydroxy-4-Methoxyphenyl)-2-Propenoyl]- β -D-Glucopyranuronic Acid	2.834683	351.0729
10.	4-[(E)-2-Carboxyvinyl]-2-Methoxyphenyl β -D-Glucopyranosiduronic Acid	2.834683	351.0729
11.	(R)-Sativanone	2.834683	321.073
12.	3-(4-Hydroxyphenyl)-6-Methoxy-7-Chromanol	3.3183	253.086
13.	1-(2,4-Dihydroxyphenyl)-2-(4-Methoxyphenyl)Propan-1-One	3.3183	253.086
14.	(3S)-3-(4-Hydroxy-3-Methoxyphenyl)-7-Chromanol	3.3183	253.086
15.	(3S)-3-(4-Hydroxyphenyl)-6-Methoxy-7-Chromanol	3.3183	253.086
16.	Sinapinate	13.53953	223.0604
17.	5-(3,4,5-Trihydroxybenzyl)Dihydrofuran-2(3H)-One	13.53953	223.0604
18.	(2Z)-2-({[(6S,7S)-7-Hydroxy-2,3,10,11,12-Pentamethoxy-6,7-Dimethyl-5,6,7,8-Tetrahydrodibenzo[8]Annulen-1-Yl]Oxy}Methyl)-2-Butenal	14.76752	537.191
19.	Epigallocatechin-Glucuronide	15.07183	463.0901
20.	Isoquercetin	15.07183	463.0901
21.	Epicatechin-7-Glucuronide	15.5826	465.1052
22.	3-(4-Hydroxyphenyl)-4-Oxo-3,4-Dihydro-2H-Chromen-7-Yl -D-Glucopyranosiduronic Acid	19.78307	431.0999
23.	Trimethylsilyl N-(Trimethylsilyl)-N-{2-[(Trimethylsilyl)Oxy]Benzoyl}Glycinate	23.277	392.1528
24.	Gomisin A	24.37997	397.1668
25.	Schizandrin	25.80368	469.1629
26.	Trimethylsilyl N-(Trimethylsilyl)-N-{2-[(Trimethylsilyl)Oxy]Benzoyl}Glycinate	26.36875	410.164
27.	Gomisin A	29.602	397.164
28.	Epicatechin-7-Glucuronide	32.4873	465.1048
29.	Schisanhenol	34.26962	423.1807
30.	Schisandrin A	39.08942	437.1946
31.	Theaflavin	40.38812	585.0996

Conflict of this work is to carry the *D. palmatus* acetone extract of leaf is utilized for column chromatography to purify the desired compounds for achieving the pharmaceutical formulation.

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