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Extraction, Spectral Investigation and Biological Activities of Peltophorum pterocarpum (DC.) K.Heyne flower

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Abstract: Peltophurm petrocarpum possesses amazing pharmacological physiological actions and other uses, according to a comprehensive literature review. Peltophurm petrocarpum also has a variety of physiological benefits. This made us want to extract a novel component from its solvent using the peltophurm petrocarpum flower. An effective technique for isolating chemicals from peltophurm petrocarpum flowers is being developed. Our plan is to use a Soxhlet device at boiling temperature to separate the material by treating it with pure chloroform. Studies using gas chromatography, mass spectrometry and antibiotic activators helped to better identify the chemical. Glass plates coated with silica gel 60 (E.Merck, India Ltd.) were used for Thin Layer Chromatography (TLC). GC-MS (Hewlett-Packerd 6890/5973, equipped with an HP-5, operating at 1000 eV ionization energy) was used to examine the active extract. Standard medications were used to test the isolated compound's antibacterial and antifungal properties against a variety of species.

Keywords: Peltophurm Pterocarpum, Soxhlet Apparatus, Chromatography, GC-MS

1. Introduction

Many of the natural compounds derived from plants served as the foundation for the creation of significant pharmaceuticals. Regretfully, nature frequently produces these chemicals in smaller quantities. When substantial amounts of the physiologically active chemicals are needed, the challenges of isolating them from other less intriguing and co-occurring constituents become problematic. Nonetheless, a helpful technique for structurally characterizing these active ingredients is bioactive guided fractionation [1–10].

Research on the antibacterial activity of *peltophorum petrocarpum* (yellow poinciana) (Figure 1) crude extracts, both organic and aqueous, can be conducted in herbal medicine, and isolated drug discovery must continue in light of newly emerging diseases like SARS and Bird Flu. Herbal medicine and natural products, or phytochemicals, can be found in plants. Guyana is ideally suited for the creation of phytopharm, a farm dedicated exclusively to the cultivation of plants high in phytochemicals and other natural goods. Phytopharm is a botanical enterprise that was founded in the United Kingdom in 1990 [11–20]. The company is creating remedies for appetite suppression and Alzheimer's illness. One benefit of botanicals is that, if there is a history of their use, a business can begin assessing plant extracts for clinical efficacy in treating disease right away. To validate folklore particles, scientific evidence would be needed. An examination of the antibacterial activity of particular plants utilizing the most recent antimicrobial activity of *Peltophorum petrocarpum* is one example of such evidence [21–25].

Figure 1. Peltophurm petrocarpum)

1.1 Description of the species

The description of the species of peltrophurm petrocarpum is given bellow:

KINGDOM	Planate
SUBKINGDOM	Tracheobionta
SUBFAMILY	Caesalpinioideae
DIVISION	Magnoliophya
CLASS	Magnoliopisda
SUBCLASS	Rosidae
ORDER	Euphorbiales
FAMILY	Fabaceac
GENUS	Peltophorum
SPECIES	P. Pterocarpum

1.2 Biological importance of Peltophurm *petrocarpum*

Numerous different researchers have experimentally shown that Peltophurm petrocarpum has a variety of physiological effects. Peltophurm petrocarpum has several advantages. The removal of hemorrhoids is among the potential advantages. Additionally, it relieves constipation and gas. Additionally, it improves the body's ability to absorb nutrients efficiently. Not many people are aware of some of the peltophurm petrocarpum's shown advantages. There is an antibacterial effect of Peltophurm petrocarpum. Additionally, it contains anti-oxidant, anti-microbial, and anti-diabetic properties. Given the trend of newly emerging diseases like SARS and BIRD FLU, research on Peltophorum petrocarpum's antibacterial potential in herbal therapy and drug discovery must continue [26–27].

Peltophurm petrocarpum is used to treat appetite suppression and Alzheimer's disease. Numerous intestinal and extraintestinal diseases, including gram-negative pneumonia, meningitis, peritonitis, mastitis, urinary tract infections and septicemia, can be brought on by it. Furuncles (boils) and carbuncles (a group of furuncles) are both caused by Staphylococcus aureus. Staphylococcus aureus can cause serious illness in newborns. Skin scalding caused by Staphylococcus aureus. Both pneumonia and Staphylococcal endocarditis (infection of the heart valves) can be lethal. Human opportunistic oral and vaginal infections are caused by the diploid fungus Candida albicans, which is a type of yeast [28–32].

2. Experimental Methods

2.1 Material and Methods

2.1.1 Plant Material

The peltophurm petrocarpum plant is found in Sri Lanka, Tamil Nadu, Karnataka, and Andhra Pradesh. The flowers are gathered on the Coimbatore campus of SRMV College of Arts and Science. After being physically cleaned of stones and other unwanted materials, the flowers used in the study were ground into small particles in an electrically powered grinder.

2.2.2 Chemicals

Petroleum ether was the only chemical that was not of laboratory reagent (LR) quality. All solvents, with the exception of ethanol, were acquired from Merck Chemicals Private Ltd. (India).

2.3.3 Methods of Extraction with pure chloroform using soxhlet apparatus

The powdered *peltophurm petrocarpum* was taken in a soxhlet apparatus. The solution was refluxed for 20 minutes while a water condenser was connected to the flask's top and water was allowed to flow through it to condense the chloroform fumes. After cooling the flask the filtrate was saved. Two drops of this filtrate is taken for TLC analysis.

2.3.4 Thin-Layer Chromatographic Analysis

A small amount of the refined material was put into a tiny vial and dissolved in a few drops of ethyl acetate. To spot the sample, a silica gel thin-layer chromatography plate was made. On the TLC plate, a base line was drawn. A sample of the crude oil was put on the baseline and left to dry with the help of a capillary. The plate was put in a developing jar containing a 3:1 mixture of petroleum ether and ethyl acetate. The solvent front was drawn when the jar was removed. After that, the plates were seen in an iodine chamber. A crude oil sample on several thin-layer plate spots.

3. Result and Discussions

3.1 Spectral analysis

Peltophurm petrocarpum contains several important alkaloids, flavonoids, steroids, etc. These alkaloids and flavonoids are important in medicinal biology. Using a solvent extraction technique, we have attempted to extract significant components from peltophurm petrocarpum in this work. Petroleum ether is a non-polar solvent that we have chosen for our experiment's extraction procedure. In the course of our work, we have embraced the soxhlet apparatus extraction procedure for extraction using pure chloroform. TLC and GC-MS were used to isolate, purify, and identify the extracted product.

3.1.1 Gas Chromatography and Mass Spectrometry (GC-MS)

GC-MS was used to examine the active extract using a capillary column (phenyl methyl siloxane, 25m × 25mm i.d.) with helium as the carrier gas (0.9 ml/min) and a split ratio of 1:1; oven temperatures ranged from

100°C (3 min) to 280°C at 1 to 40°C per minute; and detector temperatures between 250 and 2800°C.

Retention periods of samples injected under identical chromatographic conditions were used to calculate retention indices. By comparing the mass spectra and retention times of the standard and plant samples with those reported in the literature and with the mass spectra of the Wiely library, the chemicals were identified (Table 1-4). Gas chromatography-mass spectrometry with NIST05 library databases was used to identify the chemicals. Over 80,000 electron compact (EI) mass spectra were used in the compilation of these databases. Reverse fit mode matching with a high degree of certainty was the only one that was acceptable.

Peltophurm petrocarpum flower extract contains a number of compounds like alkaloids, terpinoids and flavonoids. Tentatively proposed library compounds match the molecular weight of the sample. From the GC-MS spectrum the molecular weight of library compounds are compared with the mass spectrum of the sample. The molecular weight of 2-methyl-2-[2'-(oxiran-2"-yl)] (M.W=158) more or less matches with the mass spectrum given in the Figure 2 So the compound may be 2-methyl-2-[2'(oxiran-2-yl)]. The molecular weight of flurobda-7-en-17-oic acid (M.W=316) matches with the mass spectrum given in the Figure 3. So the compound may be flurobda-7-en-17-oic acid. The molecular weight 336 in the mass spectrum depicted in Figure 4 matches with cycloshiromodial-8-O-angelate.The molecular weight of nonadecane (M.W=268) is identified in the mass spectrum given Figure.5. The molecular weight of 5-methyl-1-hexene-3, 4-Dione (M.W=126) matches with the mass spectrum given in Figure 6.

The following compounds present in the flower extract were found to match with the library compounds.

1 2-methyl-2-[2'(oxiran-2-yl)]
2 Flurobda-7-en-17-oic acid
3 Cycloshiromodial-8-O-angelate
4 Nonadecane
5 5-methyl-1-hexene-3, 4-Dione

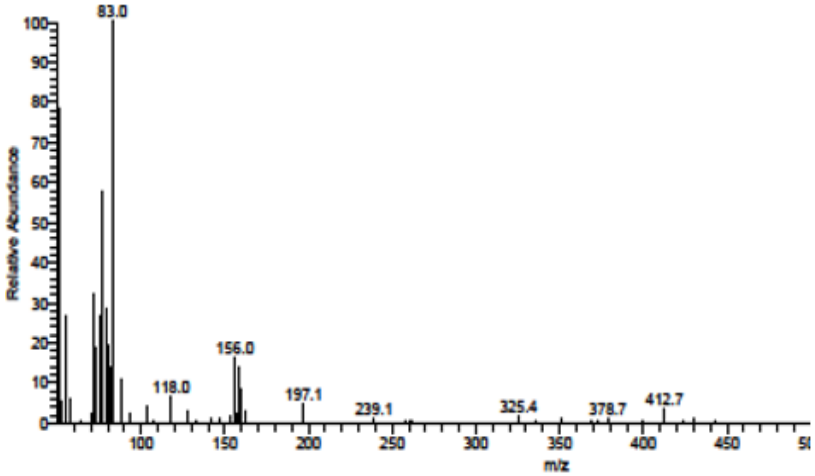

Figure 2. Mass spectrum of 2-methyl-2-[2'-(oxiran-2"-yl)]

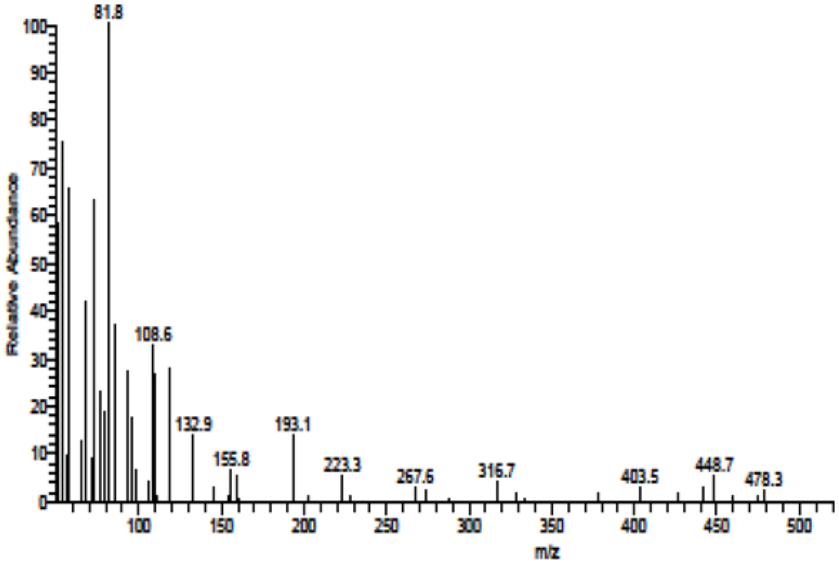

Figure 3. Mass spectrum of flurobda-7-en-17-oic acid

Table 1. Library search compounds

SI	RSI	Compound Name	Molecular Formula	Molecular Weight	Area
393	656	5-methyl-5-(o- iodophenyl)isoxaline	$C_9H_{10}INO$	275	4.80
388	757	5-benzoyl-1,4,5,6- tetrahydropyrimidine	$C_{11}H_{12}N_2O$	188	4.80
354	636	1-cyclohexene-1-methanol	$C_7H_{12}O$	112	4.80
352	639	2-methyl-2-[2`-(oxiran=2``- yl)]-1,3-dioxole	$C_8H_{14}O_3$	158	4.80
317	621	3-methylene-1,4-pentadiene	C_6H_8	80	4.80
190	669	1-cyclopentene-1-aceticacid,3- oxo-2-pentyl-, methyl ester	$C_{13}H_{20}O_3$	224	4.80
279	635	1-amino-1-phenyl-1-ethanol	$C_8H_{11}NO$	137	4.80
186	687	(5Z)-5-ethylidine-4- methylene-N-phenyl-2- oxazolidinone	$C_{12}H_{11}NO_2$	201	4.80

Table 2. Library search results

SI	RSI	Compound Name	Molecular Formula	Molecular Weight	Area
411	776	Methyl-1-methylimmidazole-2- czrboxylate	$C_6H_8N_2O_2$	140	1.08
400	859	Isopropyl-1-methylimmidazole-2- carboxylate	$C_8H_{12}N_2O_2$	168	1.08
318	754	4-cycloprpo-4-pentan-1-ol	$C_8H_{12}O$	124	1.08
314	819	(2R, 3R)-3,7-Dimethyl-2,3-epoxy-6- 0ctane-1-ol	$C_{10}H_{18}O_2$	170	1.08
295	744	(u,1)-1-oxa-4-chlorospirol[2,5]octane	$C_7H_{11}ClO$	146	1.08
285	773	6-hepentonitrile	$C_7H_{11}N$	109	1.08
228	859	4-methyl-1-hexyne-3-one	$C_7H_{10}O$	110	1.08
174	791	2-2(N-phenylaminooxy)pent-4-ene-1-ol	$C_{11}H_{15}NO_2$	193	1.08
133	767	5-(3-carbethoxy-2- butenyl)bicycle[4,3,0]octane	$C_{16}H_{26}O_2$	250	1.08
172	761	Fluroabda-7-en-17-oic acid	$C_{20}H_{28}O_3$	316	1.08

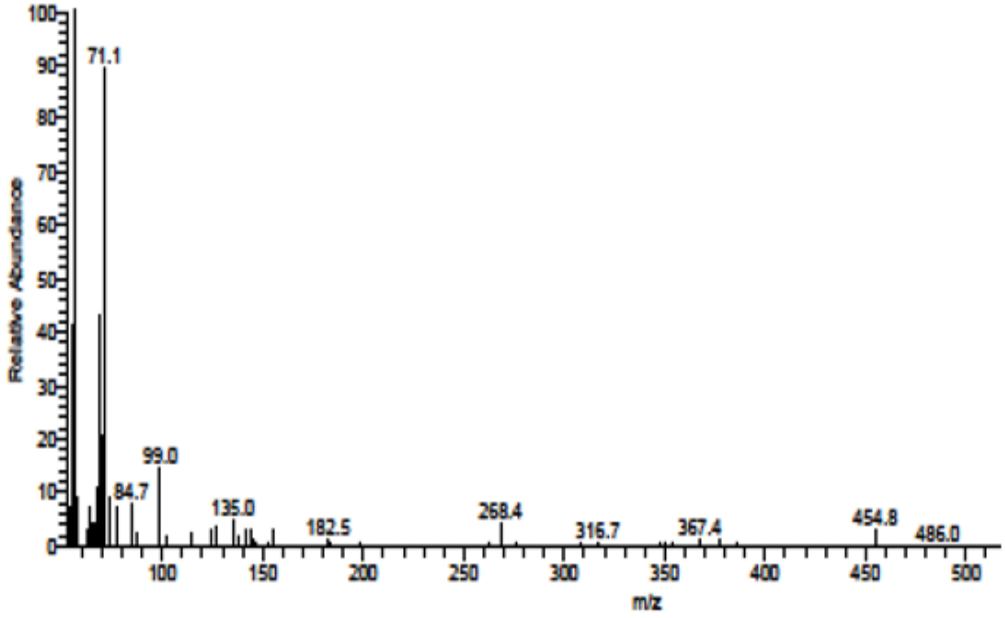

Figure 4. Mass spectrum of cycloshiromodial-8-O-angelate

Table 3. Library Search Compounds

SI	RSI	Compound Name	Molecular Formula	Molecular Weight	Area
372	783	1,1-dipropoxy-3-(2`- hydroxyethoxy) propane	$C_{11}H_{24}O_4$	220	1.97
312	979	Shiromodiol-8-O-angelate	$C_{20}H_{32}O_4$	336	1.97
312	979	Cycloshiromodiol-8-O-angelate	$C_{20}H_{32}O_4$	336	1.97
133	971	Methyl-4-nitrohex-4-enoate	$C_7H_{11}NO_4$	173	1.97
117	860	2-methyl-3-pentene-2-ol	$C_6H_{12}O$	100	1.97
115	846	(E)-2-methyl-3-pentene-2-ol	$C_6H_{12}O$	100	1.97
111	803	2,3-dimethylbut-3-en-2-ol	$C_6H_{12}O$	100	1.97

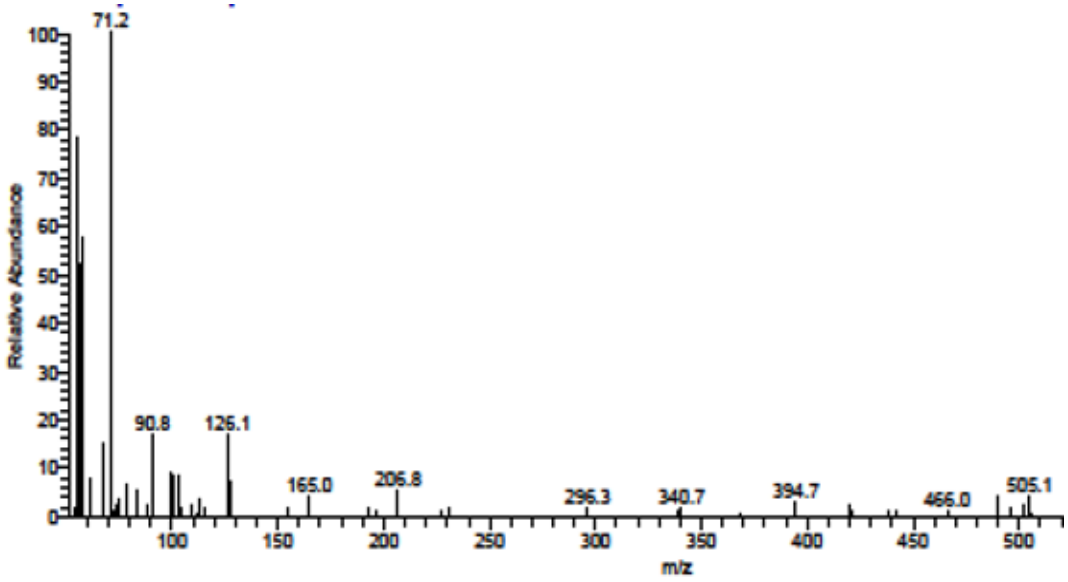

Figure 5. Mass spectrum of nonadecane

Table 4. Library Search Compounds

SI	RSI	Compound Name	Molecular Formula	Molecular Weight	Area
575	912	Octacosane	$C_{28}H_{58}$	394	0.69
546	890	2-Propyldecan-1-ol	$C_{13}H_{28}O$	200	0.69
541	910	Hexacosane	$C_{26}H_{54}$	366	0.69
518	951	Hexadecane,2-methyl-(CAS)	$C_{17}H_{36}$	240	0.69
515	957	Tricosane	$C_{23}H_{48}$	324	0.69
511	950	Nonadecane	$C_{19}H_{40}$	268	0.69
478	957	1-(2-hydroxyethoxy) tridecane	$C_{15}H_{32}O_2$	244	0.69

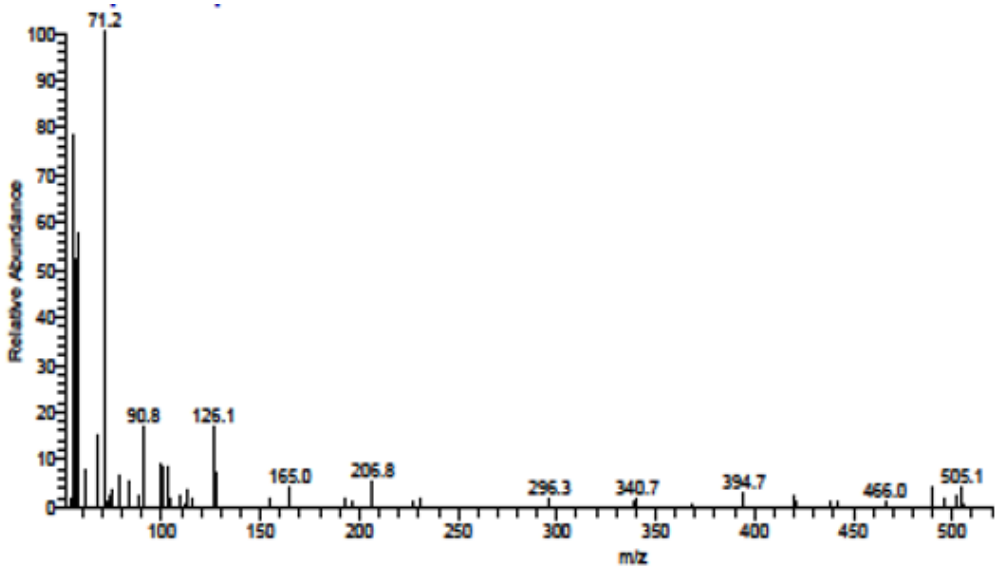

Figure 6. Mass spectrum of 5-methyl-1-hexene-3, 4-Dione

Table 5. Library Search Compounds

SI	RSI	Compound Name	Molecular Formula	Molecular Weight	Area
487	900	2,2-dimethyl-propyl 2,2 propanesulfinal sulfane	$C_{10}H_{22}O_3S_2$	254	2.08
487	875	3-hydroxy-4,4-dimethyldihydro(2- 13C) furan-2-one	$C_6H_{10}O_3$	130	2.08
458	793	2-methyldodecan-1-ol	$C_{13}H_{28}O$	200	2.08
456	803	1-butanol, 4-butaxy-(CAS)	$C_8H_{18}O_2$	146	2.08
445	802	1-N-isobutyl-N-octadecyl- aminosuccinic anhydride	$C_{26}H_{47}NO_4$	437	2.08
246	802	(E)-(S)-(+)-5-hydroxyoct-6-enyl pivalate	$C_{13}H_{24}O_3$	228	2.08
285	784	5-methyl-1-hexene-3,4-dione	$C_7H_{10}O_2$	126	2.08

3.2 Antimicrobial Activities

3.2.1 Materials and methods

3.2.1.1 Collection of plant materials

The Botanical Survey of India (BSI) identified and verified the plant materials. After being properly cleaned with running tap water, the plant bloom was rinsed with distilled water and allowed to dry in the shade. They were kept at room temperature after being pounded into a powder.

3.2.1.2 Preparation of extracts

In a Soxhlet apparatus, 50g of dried leaf powder was extracted in stages using 250mL of methanol, ethyl acetate, and hexane. To obtain the crude, the extracts were dried using a rotating vacuum evaporator at decreased pressure. They were then kept below 4°C until they were needed again.

3.2.2 Biological activity

3.2.2.1 Anti-microbial activity

Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa were among the four bacterial species and four fungi against which the in vitro antimicrobial activity of different alcoholic extracts of the study species' flowers was tested. These microbial strains were all acquired from Kongunadu Arts and Science College's Department of Biotechnology. At 4°C, the fungal and bacterial stock cultures were kept on potato dextrose agar slants and nutritional agar slants, respectively.

3.2.2.2 Media used

The bacterial and fungal cultures were conducted using freshly made nutrient agar medium (Muller-Hinton) and potato dextrose agar medium, respectively.

3.2.2.3 Composition of Nutrient agar medium

Peptone – 10g

Leaf extract –10g

Sodium chloride –3g

Agar –15g

Distilled water – 500mL

3.2.2.4 Composition of PDA medium

Potato – 200.0 g

Dextrose- 20.0g

Agar- 15.0g

Distilled water- 100 mL

pH- 5.6±0.2

3.2.2.5 Agar disc diffusion assay

The microbial activity was tested by disc diffusion method. Each bacterial strain and fungal strains were suspended in broth and incubated for 24 hrs at 37°C. The culture media were prepared and autoclaved at 121°c at 15 p.s.i. for 20 min and stored in refrigerator. The media were melted before the process of inoculation. The clean dry sterile Petri-dishes were poured with nutrient agar medium (for bacteria) and potato dextrose agar medium (for fungi).

Then the inoculums were spread over respective agar medium with sterile glass spreader. Small circular paper discs (5mm diameter) impregnated in each extract was placed up on the surface of the inoculated plated separately. Then plates were kept at room temperature for absorption of extract in the medium and then incubated at 37°C in the incubator for 24 – 48 hrs. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone (mm). Tetracycline is used as control and triplicate were maintained for all experiments.

4. Statistical Analysis

One-way analysis of variance (ANOVA) and post hoc Duncan's multiple range test were performed using SPSS (version 9, SPSS Inc., Chicago, USA) after all results were summarized as mean ± standard deviation (SD) of three determinations. The threshold for statistical significance was set at $p<0.05$.

4.1 Anti-bacterial activity

Table 5 and Figure 7 show the results of screening the isolated chemical for its in-vitro growth inhibitory activity against various bacterial strains, including *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and Staphylococcus aureus.* The substance exhibits strong antibacterial action against *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and Staphylococcus aureus*. When compared to Tetracycline standard medications, all extracts showed moderate activity.

Table 6 shows the compound's and tetracycles' in vitro antifungal efficacy as determined by diameter of inhibition on a panel of fungus, including *Aspergillus niger*, A. flavus, *A. baumanii*, and *Fusarium oxysporum*. The identified chemical demonstrated antifungal efficacy against all fungal strains, with a diameter of inhibition of 9.18± 0.68 in petroleum ether extract and 11.75± 0.28 in methonal extract. In the isolated compound extract, 9.23± 0.49 and 15.79± 0.66 µg/ml were shown to be effective against *Fusarium oxysporum and Aspergillus niger*, respectively. The remaining isolated compound extract showed no impact against any of the tested strains.

Table 6. Antibacterial activity of certain alcoholic flower extracts of the species, peltocarphum petrocarpum

Bacteria	Diameter of inhibition zone (mm)			
	Standard (Tetracycline)	Solvent extracts		
		Petroleum ether	Methanol	Water
Staphylococcus aureus	8.13±0.57	15.19±0.38	14.16± 0.56	12.83±0.45
Bacillus subtilis	9.03 ± 0.45	14.17±0.33	15.23± 0.71	11.34±0.23
Escherichia coli	22.43±0.38	10.71 ±0.55	9.05±0.33	10.56±0.33
Pseudomonas aeruginosa	23.03 ± 0.54	9.07 ±0.65	10.11±0.45	9.41 ±0.66

Experiments were perfor med in triplicates and represented as mean±standard deviation (SD).

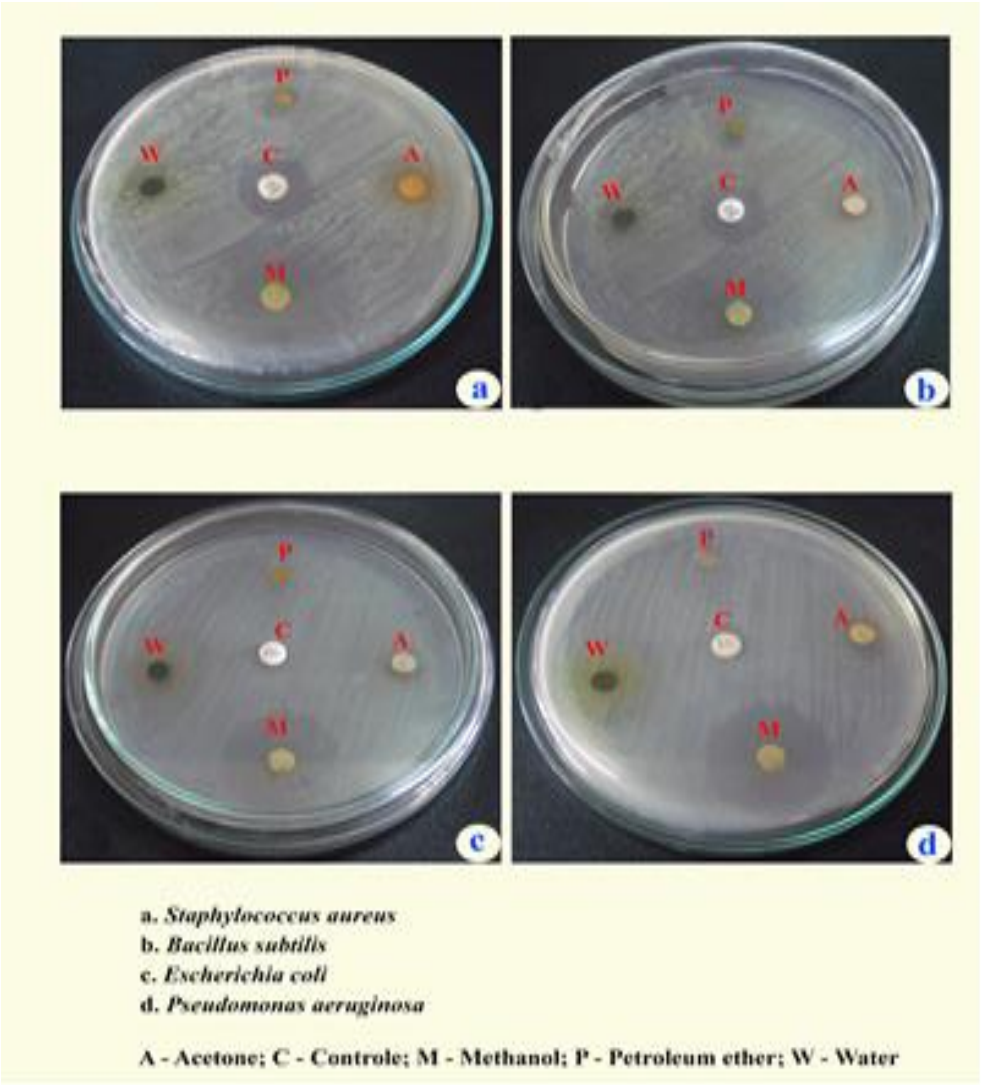

Figure 7. Anti bacterial activity of isolated compound extract Anti-fungal activity

5. Conclusion

In conclusion, this study is a complementary survey to phytochemical and spectral studies carried out on extract oil. This project work involves flower extraction of the plant, peltophurm petrocarpum with chloroform. TLC report also gives an indication that the extract contains a number of compounds. Gas chromatography and Mass Spectrometry spectral data

Indicate that the compounds are tentatively 2-methyl-2-[2'-(oxiran-2"-yl)], flurobda-7-en-17-oic acid, cycloshiromodial-8-O-angelate, nonadecane and 5-methyl-1-hexene-3, 4-Dione. Further pharmacological studies like anti-microbial, and anti-fungal activities studies are required to find out the complete physiological and pharmacological action of the compounds. The identified compounds may be alkaloids, terpinoids and flavonoids. This extraction method is a quick, straight forward and new method of extraction of the alkaloids, terpinoids and flavonoids from the flower of peltophurm petrocarpum.

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Conflict of interest

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