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DISCOVERY OF NEW ANTI-FUNGAL PHYTOCHEMICAL PDHC (PROPANE-DIYL-BIS-HEXAHYDRO-ISOCHROMENE) ISOLATED FROM *ALTERNANTHERA SESSILIS* LEAVES

Ranjitha Dhevi V. Sundar [1], Lokesh Ravi [2] and Sathiavelu Mythili [*1]

School of Biosciences and Technology [1], Vellore Institute of Technology, Vellore - 632014, Tamil Nadu, India.
Composite Interceptive Med-Science Laboratories [2], Pvt. Ltd., Bangalore - 560099, Karnataka, India.

Keywords:

Alternanthera sessilis, Antimicrobial activity, *In-vitro* antidiabetic, GC-MS, 3,3'-(propane-2,2-diyl)bis(3,4,5,6,7,8-hexahydro-*1H*-isochromene)

Correspondence to Author:
Dr. S. Mythili

Senior Assistant Professor, Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore - 632014, Tamil Nadu, India.

E-mail: smythili@vit.ac.in

ABSTRACT: This study reports the potential of *Alternanthera sessilis* leaves as antioxidant, antimicrobial, anti-inflammatory and anti-diabetic agent and also the characterization of a novel phytochemical from *A. sessilis* using bio-activity guided extraction. Antimicrobial property was studied against five bacterial pathogens, *Escherichia coli* (MTCC: 1687), *Bacillus cereus* (MTCC: 0430), *Klebsiella pneumonia* (MTCC: 7028), *Staphylococcus aureus* (MTCC: 3160), *Proteus mirabilis* (MTCC: 3310) and against two fungal pathogens, *Aspergillus niger* [MTCC 281] and *Aspergillus flavus* [MTCC 10938]. Petroleum ether extract of *A. sessilis* leaves demonstrated significant antimicrobial property and hence was further purified to identify its active phytochemicals. Silica-gel Column Chromatography followed by antimicrobial screening, lead to the identification of the active fraction, that was characterized by spectroscopic analyses. FT-IR, GC-MS and NMR (C^{13}, and DEPT-45; 90; 135) analysis of the active fraction, characterized the phytochemical to be 3,3'-(propane-2, 2-diyl)-bis-(3,4,5,6,7,8-hexahydro-1*H*-isochromene) abbreviated as PDHC. To the best of knowledge, this is the first report of PDHC due to novelty in its chemical structure. *In-silico* molecular docking was performed for this identified compound to identify the possible mechanism of action. This study opens up opportunities for further research on this valuable anti-fungal phytochemical leading to possible treatment applications.

INTRODUCTION: Medicinal plants are referred to as 'medicinal' or 'officinal' plants as it contains a ridiculous amount of secondary plant products. More than thousands of years, it has been well documented the medicinal uses of plants and different parts of the world still, rely on traditional medicines for treating different maladies [1]. Medicinal plants are considered to be the "spine" of traditional medicine. In less developed nations, more than 3.3 billion individuals use medicinal plants continuously [2].

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Majority of new drugs are mainly acquired from the compounds that are resulting from natural products. Phytochemical screening of medicinal plants plays a vital role in discovering many numbers of new drugs. There are approximately 60% of anti-tumor, and anti-infectious drugs are on the market that is mostly obtained from the natural origin [1].

Medicinal plants studies help in understanding the harmfulness of plant and thereby it protects animals and human from natural poisons [3]. Some chemical compounds produced by plants possess biological functions, and also they offer defense against fungi, insects and anthophagous mammals [4]. It was estimated that about 25-30% of all drugs that are resulting from the natural origin are available as therapeutics [5]. Siddhars had well recognized the medicinal values of several herbs and documented.

Greens posses many numbers of medicinal properties; thereby it is taken by southern part of Indian peoples as their diet [6]. The lowering of blood sugar has established several herb and plants. Thereby there is a need in determining the plant's pharmacology that is used in the system of traditional medicine [7]. Medicines from herbs are widely used because it was believed to be safe as they are derived naturally. And many of these plants have been reported to have medicinal properties interacting anti-coagulant, anti-diabetic, hypotensive effects. Medicines from herbs are mainly consumed as they are affordable, accessible, chronological background [8].

In Ayurvedic medicine, *Alternanthera sessilis* is referred to as Matyakshika. The plant was used for hazy vision, night blindness, and postnatal complaints in some parts of Bihar (India) and their boiled leaves and shoots of this plant were drunk as an antihypertensive remedy [9]. They are also used by people in local areas of Amarkantak region to treat skin diseases, liver diseases, diarrhea, fever and spleen diseases [10]. The plant's aerial parts also have shown a hepatoprotective activity; it also likes the ability to heal the wounds, and their leaves are mainly used for treating the wound, skin, and eye disease [7].

There is a growing need for a non-toxic hepato-protective agent, and most importantly the use of products derived from plants as it was used as the hepatoprotective agent has been documented. Many numbers of plants have been formulated to claim for hepatoprotective action, and approximately 160 phytoconstituents was derived from 101 plants had declared to hold property on protecting the liver [11]. Many antibiotics become susceptible, due to the rise of many pathogens that are resistant to drugs. Thereby it is important to develop new compounds having antimicrobial properties having novel action and structure for new diseases caused by infections. Thus, researchers mainly focus on folk medicines for developing medicines against the infections caused by microbes [12].

Plants produce various chemical constituents that play a vital role in performing the biological function. And also it acts against hunters like insects *etc*. So far, it was approximately estimated that there are 12,000 phytochemical were derived [13]. Based on this literature survey and based on the lack of research articles on the bioactivity of this genus of the plant, this plant was chosen as the plant of interest for this analysis. The present study investigated the antimicrobial, antioxidant and anti-inflammatory activity of leaf extracts from *A. sessilis* and also the phytochemical composition of the extract by the biochemical reaction.

MATERIALS AND METHODS:

Collection of Plant Materials: The fresh leaves of *Alternanthera sessilis* was collected from various locations in Vellore. The plant materials were collected in a sterile bag and transformed into the laboratory. To reduce the chance of contamination, the plant materials were freshly collected and utilized for further studies [14, 15].

Preparation of Plant Extracts: The plant leaves were washed two to three times thoroughly in double distilled water and shade dried for 2-3 weeks. Periodically the moisture level of the leaves was observed. Once it is completely dried, using a motor and pestle, the plant leaves were powdered. The ground samples were stored in a sterile container for further process [14, 15].

Extraction Procedure: The extraction procedure was done by dissolving 15 g of dried powdered leaf in 200 ml of methanol and pet ether solvent respectively. Then the conical flasks were sealed tightly with para-film and kept in a shaker at room temperature for 1-2 days. Using Whatman no.1 filter paper, the content was filtered and evaporated. About 3-5 g of crude extract was obtained in each extract for 15 g of dried powder. The resulting crude extracts were prepared at various concentration and processed further [14, 15].

Phytochemical Analysis: The preliminary quail-tative phytochemical screening was carried out using a standard protocol. Phytochemical analysis was done to determine the presence and absence of phenols, alkaloid, tannins, anthraquinone glycosides, saponins and flavonoids in the five different medicinal plant leaf powder [16, 17].

Acid Extract: To the 1 g different medicinal plant leaf powder, 6 ml of concentrated HCl was added and allowed to stand for 20 min. Then the extracts were filtered using the Whatman paper.

Flavonoids: In two separate test tube, 2 ml of acid extract was taken, 2 ml of NaOH was added to the first tube, and 2 ml of distilled water was added to the second tube. The tube that gives the yellow color indicates positive for flavonoids.

Water Extract: To the 1 g of powdered leaf, 14 ml of distilled water was added and boiled gently using a mantle, then the content was filtered using Whatman filter paper.

Tannin Test: A volume of to the 2 ml of above filtrate few drops of 1M $FeCl_3$ was added. The appearance of green color indicates the presence of condensed tannin, whereas the blue color indicates hydrolyzable tannin.

Saponins: To the test tube about 2 ml of water extract was taken and shaken vigorously. The presence of saponins determines by the froth formation of above 1 cm long.

Phenol Test: In a test tube 1 ml of the water extract was taken and few drops 5% $FeCl_3$ solution was added. The appearance of a dark green color indicates positive for phenol.

Alcohol Extract: About 8 ml of methanol was added to the 1 g of five different plant leaf powder respectively. Then the content was allowed to stand for 30 min. The resultant extracts were filtered and evaporated using mantle. Then it is resuspended in 3 ml of chloroform.

Alkaloid: On a filter paper, few drops of alcohol were placed, and the drangendroff's reagent was sprayed over the filter paper. The presence of alkaloids is determined by the appearance of reddish brown color in the filter paper.

Anthraquinone Glycoside: About 1 ml of ammonia was added to the 2 ml of an alcohol extract and shaken vigorously, the appearance of green color at the bottom and reddish color at the top indicates a positive result.

Terpenoids: To the extract, few drops of Concentrated H_2SO_4, to that acetic anhydride was added to the wall of the test tubes, a reddish brown ring indicates the presence of terpenoids.

Determination of Total Phenolic Content: Folin-ciocalteu method was used to determine the total phenolic content of the plant leaf extract. The methanol and pet ether leaf extracts obtained from different plants were dissolved in methanol (1mg/mL) and mixed with 0.5 ml of reagent. To the content, 1.5 ml of 20% sodium carbonate was added. Under the condition, the mixture was allowed to stand for 60 min. Then the absorbance was read at 650 nm. Gallic acid was used as a standard. From the calibration curve, the total phenolic content of the extract was determined, and the results were expressed as mg of gallic acid equivalent per gram of the extracts. The determination of the total phenolic content was carried out in triplicates [18, 19].

Antioxidant Activity:

DPPH (1- diphenyl 2-picrylhydrazyl) Radical Scavenging Activity: DPPH free radical scavenging a standard method was used to determine the antioxidant activity. The methanol and pet ether leaf extracts of plant leaves were subject to determine their radical scavenging ability. The crude extracts were diluted in methanol to prepare 1 mg /mL concentration. To achieve final concentrations, the dilution was made to 50, 100 and 150 µg/mL. To the different concentrations of the plant extracts were mixed with 2 ml of DPPH solution. Then the reaction mixture is incubated at room temperature for 30 min under dark. The decrease in the absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as a standard. The reaction was done in triplicates, and the free radical scavenging activity was calculated using the following formula [20]

$$\% \text{ DPPH radical scavenging} = Ac - As / Ac \times 100$$

Where Ac is the absorbance of the control, As is the absorbance of the sample.

Reducing Power Assay: The reducing power was performed for each sample extracts (1 mg/mL) at different concentrations (50,100,150 µg/ mL). The extracts were mixed with 2.5 ml of 0.2 M phosphate buffer, pH 6.6 and 2.5 ml 1% potassium ferricyanide. After incubation for 20 min at 50 °C, 2.5 ml 10% trichloroacetic acid was added to the mixture. Then the content was centrifuged at 1000 rpm for 10 min. after centrifugation, the 2.5 ml of the supernatant was added to 2.5 ml distilled water and 0.5 ml 0.1% ferric chloride was added and

vortexed. Then the absorbance of the resulting solution was read at 700 nm against a blank. Ascorbic acid was used as positive control. The reaction was carried out in triplicates [21].

$$\text{Percentage inhibition} = Ac - As / Ac \times 100$$

Where, Ac is the absorbance of the control, As is the absorbance of the sample

***In-vitro* Anti-inflammatory Activity:**

Protein Denaturation Method: Albumin denaturation method was carried out to determine the anti-inflammatory activity of the plant extracts. Different concentration of the methanol and pet ether extracts (100, 200, 300 µg/mL) was prepared and 2 ml of these prepared extracts were added to the reaction mixture containing 0.2 ml of freshly obtained egg albumin from hen's egg followed by 2.8 ml of PBS (phosphate buffered saline) (pH: 6.4) was added. Control used here was double distilled water. At 37 °C, the reaction mixture was incubated for 15 min. After incubation, the content was heated for 5 min at 70 ºC and cooled. The absorbance was measured at 660 nm against blank. Aspirin was used as the reference drug, and the absorbance was taken. The method was carried out in triplicates [13]. The protein denaturation inhibition percentage was determined using the formula,

$$\% \text{ inhibition} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

***In-vitro* Antibacterial Activity:**

Test Microorganisms: Five different bacterial strains such as *Escherichia coli* (MTCC: 1687), *Bacillus cereus* (MTCC: 0430), *Klebsiella pneumonia* (MTCC: 7028), *Staphylococcus aureus* (MTCC: 3160), *Proteus mirabilis* (MTCC: 3310) were used in the present study.

Agar Well Diffusion Method: The antibacterial activity of the leaf extracts was carried out using agar well diffusion method against the selected test organism. To the sterile Petri plates, Muller Hinton agar was prepared and poured, and the plates were allowed to solidify. Using a sterile cotton swab, the bacterial strains were spread uniformly over the medium, about 0.5 cm; wells were made in the medium with the help of cork borer. Three different concentrations (25, 50,100 µg/mL) of the crude extracts. To the wells, 100 µl of different concentration of the extracts were added, and the plates were incubated at 37 ºC for 24 h after the incubation period, the plates were examined, and the zone of inhibition around the wells was measured. Streptomycin was used as positive control [12, 22].

Antifungal Activity:

Agar Well Diffusion Method: The antifungal activity of the leaf extracts was carried out using agar well diffusion method against the selected fungal pathogens *Aspergillus niger* [MTCC 281] and *Aspergillus flavus* [MTCC 10938]. The fungal pathogens were maintained using sabouraud dextrose agar (SDA) [Hi-media]. SDA was prepared and poured into the Petri plates. After solidification, the fungal spores were spread on to the media plate using a sterile cotton swab. Using cork borer the wells were punctured. For antifungal activity, 100 mg/mL concentration of the crude extract was used. To this well, 100 µl of the extracts were added, and the plates were incubated at 37 ºC for 48 h. After the incubation period, the zone of inhibition was measured. Fluconazole was used as positive control [23].

Pure Compound Identification and Extraction:

Thin Layer Chromatography (TLC): TLC was done to analyze the variation in bioactive chemical constituents. The pet ether extract obtained from the leaf of *A. sessilis* was subjected to Thin Layer Chromatography. The optimal solvent system for TLC was confirmed by testing with different solvents at various proportions. The extract was spotted on the TLC sheet, and the ratio of (8:2) petroleum ether: acetone solvent system was used. Using visible light and Deep Vision-TLC Chamber at a UV wavelength of 254 and 365 nm, the band that is separated can be viewed. For isolation of pure compound silica gel chromatography (Silica 60-120, Hi-Media, India) was done [24].

Silica Gel Column Chromatography: Silica gel Column Chromatography (60-120 Hi-Media) was used for purification. The petroleum ether crude extract obtained from *A. sessilis* was subjected to silica gel column chromatography. About 15 g of silica gel was heated in a hot air oven at 100 ºC for 20-30 min. To the silica gel, about 75 ml of petroleum ether was added and mixed. By wet packing method, it was packed into the column

without forming any air bubbles. Then weigh 2 g of crude extract *i.e*, petroleum ether crude extract of *A. sessilis* and mixed with silica gel and pack the mixture into the column tightly. The solvent system used was petroleum: acetone (8:2). Then the column was washed with 250 ml of the solvent system, and the resulting fractions were collected in test tubes at a 1 ml min^{-1} flow rate. And the contents were dried and used for further studies [25].

GC-MS Analysis: The petroleum ether crude extract and the compound isolated from the petroleum ether extract of *A. sessilis* was subjected to (GC-MS) analysis. The extracts were analyzed in a Perkin Elmer Clarus 680 equipped with Mass Spectrometer Clarus 600 (electron ionization) fitted with elite - 5 MS capillary column (30 m, 0.25 mmID, 250 μmdf). The GC oven was maintained with the initial temperature of 60 °C for 2 min, ramp 10 °C/min – 300 °C, the temperature was maintained at 300 °C for 6 min. Helium was used as a carrier gas with a constant flow rate of 1 mL/min, mass transfer line and source temperature were set at 240 °C. The spectral analysis was analyzed using Turbo mass version 5.4.2 software. The identification of components was accomplished using computer searches in NIST. By comparing the mass spectral patterns to the NIST-2008 library, the structures were determined [24].

FT-IR and NMR Analysis: The compound that has been isolated from the *A. sessilis* (petroleum ether leaf extract) was subjected to FT-IR (Fourier Transform Infrared spectrophotometry) and NMR (Nuclear Magnetic Resonance). FT-IR analysis was done to determine the different functional groups present in the sample. Perkin Elmer Spectro-photometer system was used for FT-IR analysis at a resolution of 1 cm^{-1} with a scan range of 450-4000 cm^{-1}. Using AV500 FT-NMR Spectrometer $1H^1$, C^{13} NMR were analyzed to determine the positions of hydrogen and carbon in the pure compound [24].

***In-silico* Docking Analysis:** The 3D structure of 3 common antifungal targets proteins (14 α-sterol demethylases, mevalonate 5-diphosphate decarboxylase, and N-myristoyltransferase) was downloaded from the Protein data bank website (www.rcsb.org). The protein structures that are downloaded were analyzed in PyMOL tool, and non-amino acid residues were removed from the structure using PyMOL. The PDB-ID of the downloaded structures is; 1E9X, 1FI4, 1NMT. The 3D structure of the ligand was drawn using ChemSketch-11. Using AutoDock-4.2 (MGL Tools), the ligand and protein interaction was studied. PyMOL is used for viewing the results obtained from AutoDock [24].

RESULTS:

Phytochemical Analysis: The powdered leaf of *A. sessilis* was subjected to the qualitative phytochemical analysis to identify the presences of phytochemical constituent's phenols, alkaloid, tannins, anthraquinone glycosides, saponins, and flavonoids. Results were positive for the presence of phenols, alkaloids, tannins, and flavonoids whereas saponinswasabsent in *A. sessilis*. The results of the phytochemical study were summarized in **Table 1**.

TABLE 1: QUALITATIVE PHYTOCHEMICAL COMPOSITION

Phytochemicals	*A. sessilis*
Phenol	+
Flavonoids	+
Tannins	+
Terpenoids	+
Saponins	-
Anthraquinone glycosides	+
Alkaloids	+

'+' – Positive; '-' – Negative

Determination of Total Phenolic Content: The methanolic extract of *A. sessilis* contained 10.79 ± 0.001 mg of GAE/g of extract, and petroleum ether extract consisted of 10.62 ± 0.002 mg of GAE/g of extract. The results were presented in **Table 2**.

TABLE 2: TOTAL PHENOLIC CONTENT OF THE LEAF EXTRACTS

Name of the plants	Total phenolic content (mg of GAE/g of extract)	
	Methanol	Petroleum ether
A. sessilis	10.79 ± 0.001	10.62 ± 0.002

Values are expressed as the mean ± standard deviation (n = 3)

Antioxidant Activity:

Diphenyl-Picrylhydrazyl (DPPH) Assay: Both the extracts demonstrated significant antioxidant activity. The methanol leaf extract showed the strongest free radical scavenging activity 86.03 ± 0.97% than that of pet ether extract 69.58 ± 1.23% at a concentration of 150 μg/mL. Percentage of DPPH radical scavenging activity of plant leaf extracts and ascorbic acid is shown in **Fig. 1**.

Reducing Power Assay: Petroleum ether extract of *A. sessilis* demonstrated higher reducing power of 86.09 ± 1.14% than the methanol extract 84.31 ± 1.14% at a concentration of 100 µg/mL. ethanol extract demonstrated Results of reducing power assay are represented in **Fig. 2**.

***In-vitro* Anti-inflammatory Activity:**

Protein Denaturation Method: Inhibition of protein denaturation was performed to evaluate the anti-inflammatory activity. Methanolic leaf extract demonstrated a maximum inhibition of 47.66 ± 1.52% at a concentration of 100 µg/mL whereas, petroleum ether extracts demonstrated a higher activity of 62.33 ± 1.52% which is similar to that of the standard. The result of anti-inflammatory activity is shown in **Fig. 3**.

***In-vitro* Antidiabetic Activity:**

Inhibition Assay for *α*-amylase Activity: Petroleum ether extract of *A. sessilis* demonstrated significant α-amylase inhibition of 67.79 ± 1.80% at the lowest concentration of 25 µg/mL which is similar to that of the standard acarbose showing 69.39 ± 1.50% inhibition. Inhibition activity was shown in **Fig. 4**.

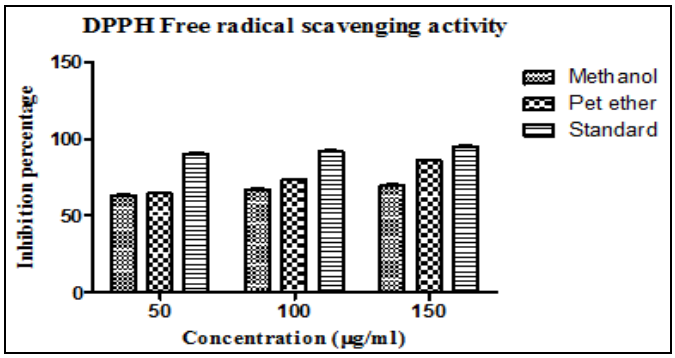


FIG. 1: DPPH FREE RADICAL SCAVENGING OF METHANOL AND PETROLEUM ETHER LEAF EXTRACTS

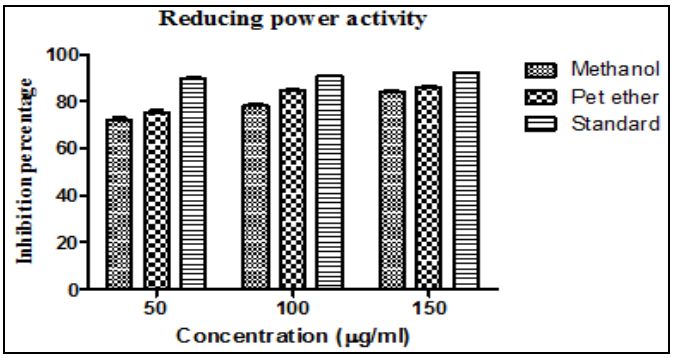


FIG. 2: REDUCING POWER ASSAY OF METHANOL AND PETROLEUM ETHER LEAF EXTRACTS

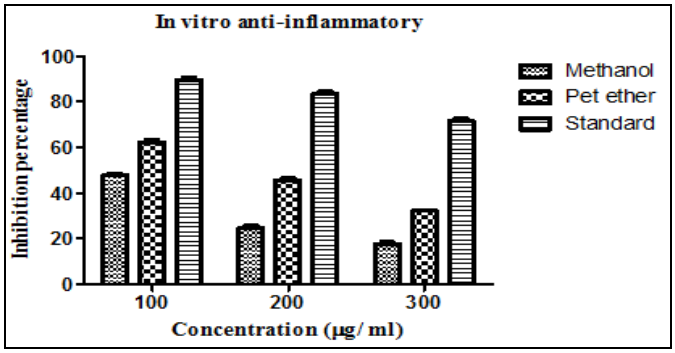


FIG. 3: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF METHANOL AND PETROLEUM ETHER LEAF EXTRACTS. Values are expressed as the mean ± standard deviation (n = 3).

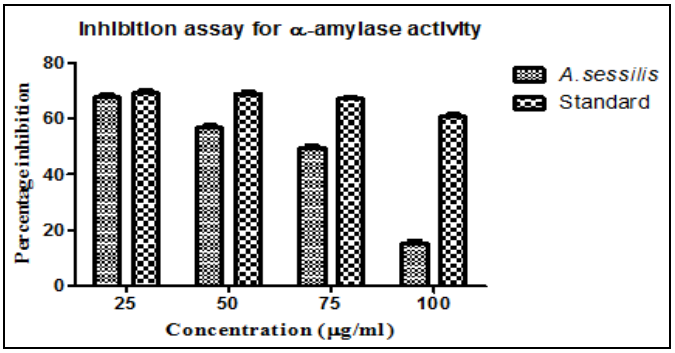


FIG. 4: *α*-AMYLASE INHIBITION ACTIVITY OF PETROLEUM ETHER LEAF EXTRACT OF *A. SESSILIS*

Antibacterial Activity: Both the extract showed activity against the test pathogens *E. coli*, *B. cereus*, *Klebsiella pneumonia*, *S. aureus*, and *P. mirabilis*. As the concentration of the extract increases, the antibacterial activity of the extract also increased. Observed zone-of-inhibition for the studied extracts are represented in **Table 3**.

TABLE 3: ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS AGAINST TEST ORGANISMS

Extracts	Concentration (µg)	Inhibition zone diameter (mm)				
		K. pneumoniae (MTCC: 7028)	*E. coli* (MTCC: 1687)	*B. cereus* (MTCC: 0430)	*P. mirabilis* (MTCC: 3310)	*S. aureus* (MTCC: 3160)
Methanol	25	-	-	-	-	-
	50	-	-	-	-	-
	100	-	17	18	13	12
Petroleum ether	25	-	-	11	11	12
	50	12	12	14	12	15
	100	18	13	16	14	17
Streptomycin	10 mg	16	22	20	25	17

The petroleum ether extract *A. sessilis* showed the highest activity against *K. pneumoniae* with 18mm zone of inhibition at its 100 µg/mL concentration. The methanol extract showed activity against the test organism at 100 µg/mL concentration.

Antifungal Activity: The petroleum ether of *A. sessilis* showed the significant activity of about 10 mm and 22 mm of the zone of inhibition against *A. niger and A. flavus* respectively, whereas the methanol extract showed activity only against *A. niger*.

Antifungal activity of *A. sessilis* leaf extracts are tabulated in **Table 4** and images of agar well plate method is shown in **Fig. 5**.

TABLE 4: ANTIFUNGAL ACTIVITY OF THE *A. SESSILIS* LEAF EXTRACTS

Name of the plants	Extracts	Concentrations (100 mg)	
		Zone of inhibition (mm)	
		***A. niger* [MTCC 281]**	***A. flavus* [MTCC 10938]**
A. sessilis	Methanol	20	-
	Pet. ether	10	22
Positive control	Fluconazole	-	-

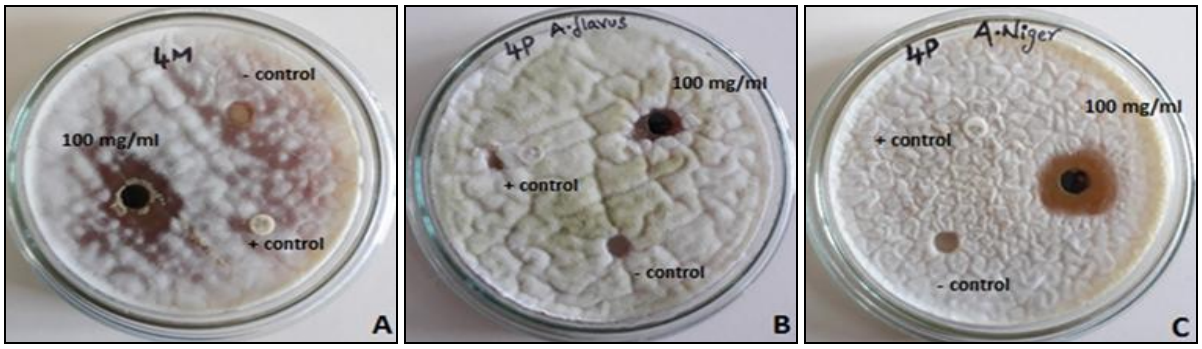
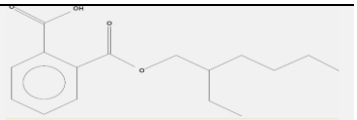
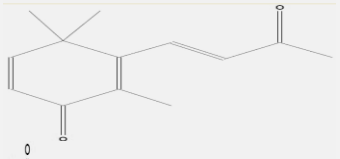
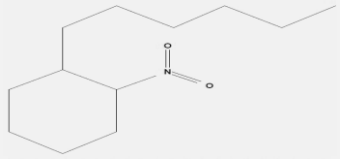
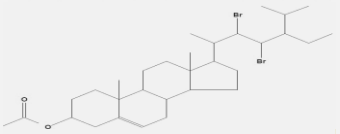


FIG. 5: ANTIFUNGAL ACTIVITY OF *A. SESSILIS*; A) METHANOL EXTRACT (*A. NIGER*); B) PETROLEUM ETHER EXTRACT (*A. FLAVUS*); C) PET. ETHER EXTRACT *(A. NIGER*)

GC-MS Analysis of the Crude Extract: The petroleum ether crude extract obtained from the *A. sessilis* was subjected to GC-MS analysis (Gas Chromatography-Mass Spectrometry). The compounds found in Gas Chromatogram were subjected to Mass Spectrometry, and the results are matched with known compounds in the NIST library. Gas Chromatogram revealed the presence of 19 known compounds. The identified compounds with reference retention time, chemical structure, molecular weight, and physiological activities were given detail in **Table 5.** Chromatogram of crude extract was shown in **Fig. 6.**

TABLE 5: LIST OF NIST LIBRARY MATCHES FOR COMPOUNDS PRESENT IN *A. SESSILIS* PETROLEUM ETHER CRUDE EXTRACTS

Retention time	Forward	Reverse	Mol. Wt	Compound Name	Structure
2.84	589	789	158	1-propanamine, 2-methyl-N-(2-methylpropyl)-N-nitroso-	
15.57	701	896	322	11-Tricosene	
16.56	949	964	296	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	
17.04	955	974	296	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	
17.33	790	874	296	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	
19.80	637	934	322	11-Tricosene	
21.39	819	903	396	1-Heptacosanol	
21.48	656	917	322	11-Tricosene	

22.68	925	964	278	1,2-Benzenedicarboxylic acid, Mono(2-Ethylhexyl) Ester	
22.97	850	930	490	17-Pentatriacontene	
23.63	488	829	204	2,3-Dehydro-4-Oxo-.Beta.-Ionone	
24.28	928	964	506	Octacosyl Trifluoroacetate	
24.49	787	907	396	1-Heptacosanol	
25.82	522	926	213	1-Hexyl-2-Nitrocyclohexane	
26.28	719	866	618	Tetratetracontane	
26.62	676	829	612	22,23-Dibromostigmasterol acetate	
27.88	947	973	380	Heptacosane	
28.97	723	890	690	Octatriacontane, 1,38-Dibromo-	
30.19	970	985	604	Tritetracontane	

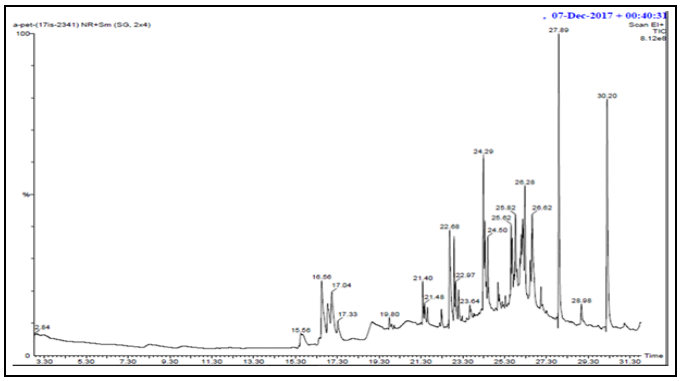


FIG. 6: GAS CHROMATOGRAPHY-MASS SPECTRO-METRY CHROMATOGRAM OF PETROLEUM ETHER CRUDE EXTRACT OF *A. SESSILIS*

Purification of Phytochemical by Column Chromatography: The petroleum ether crude extract of *A. sessilis* produced distinctive bands in the silica gel TLC sheet in a solvent system of Petroleum Ether: Acetone (8:2 v/v) (Shown in **Fig. 7B**. The crude extract was subjected to Silica gel column chromatography in the same solvent system (Shown in **Fig. 7A**. Total of 46 different fractions was obtained, from that, the fractions which have similar TLC signature were pooled together (35-46) and concentrated. The fraction was again tested for purity in TLC (as shown in **Fig. 7C**. The bioactive fraction was subjected to spectral studies to identify/characterize the phytochemical present. Total of 18 mg of the pure compound was obtained.

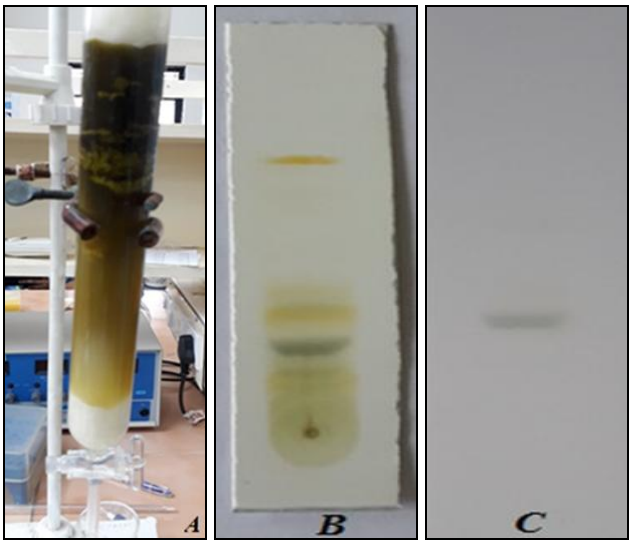


FIG. 7: A: PURIFICATION OF THE CRUDE EXTRACT BY SILICA GEL COLUMN CHROMATOGRAPHY, B: TLC OF THE CRUDE EXTRACT, C: TLC OF PURE COMPOUND

Structure Elucidation of Pure Compound: The compound that was eluted using silica gel chromatography was subjected to spectral analysis. To identify the structure of the compound, the purified compound was subjected to GC-MS, FT-IR, ^{1}H NMR, and ^{13}C NMR, DEPT-45, DEPT-135, DEPT-90 analysis.

GC-MS analysis revealed a single peak which indicates the compound was pure. Gas chromatography of the pure compound was shown in **Fig. 8**. Mass Spectrum analysis of pure compound shown in **Fig. 9**. The mass spectrum showed that the pure compound was of 316.42 g/mol molecular weight.

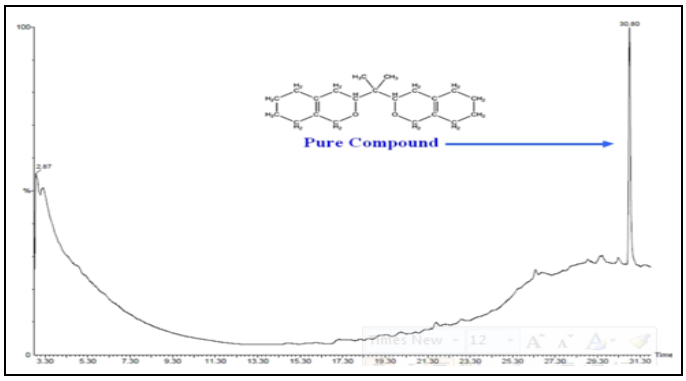


FIG. 8: GAS CHROMATOGRAPHY OF PURE COMPOUND

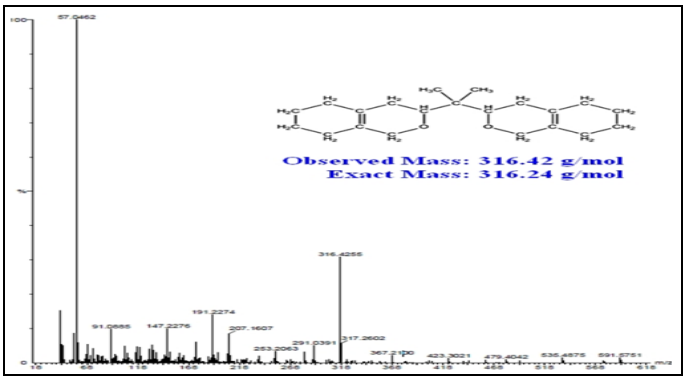


FIG. 9: MASS SPECTRUM ANALYSIS OF PURE COMPOUND

FT-IR and NMR Analysis: The pure compound was subjected to Fourier-Transform Infrared spectroscopy analysis and the functional group of the components were separated based on its peak ratio. FT-IR analysis of the pure compound was shown in **Fig. 10**. The FT-IR analysis of the pure compound demonstrates that in its structure, the compound contains only C-H (Alkanes) group and C=O (Carbonyl) group. Mass spectrum analysis reveals the molecular weight of the compound. The molecular weight of the molecule was detected to be 316.24 g/mol. C^{13}-NMR shifts are shown in **Fig. 11**.

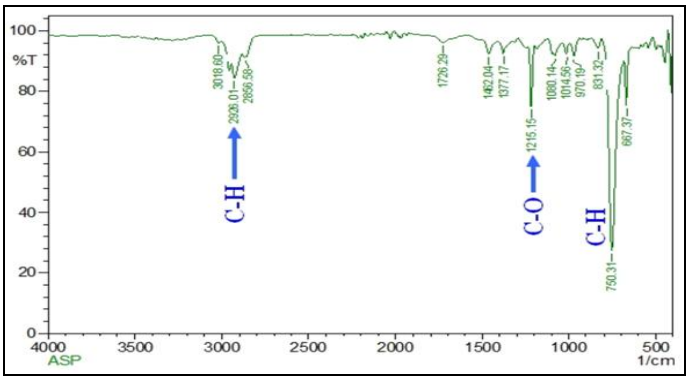


FIG. 10: FT-IR SPECTRUM ANALYSIS OF PURE COMPOUND

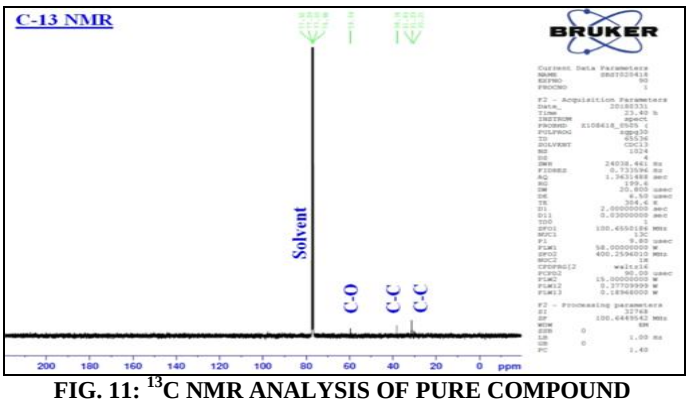


FIG. 11: ^{13}C NMR ANALYSIS OF PURE COMPOUND

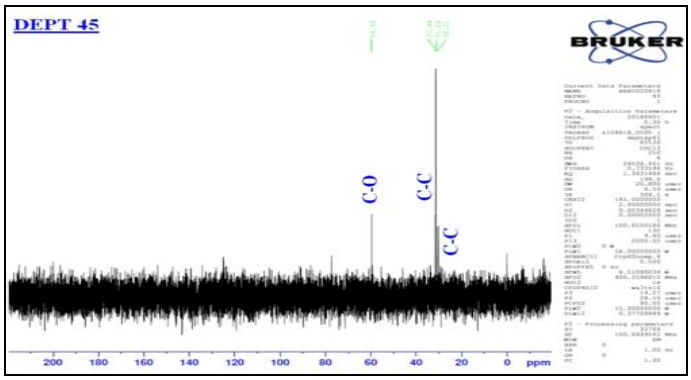


FIG. 12: DEPT-45 OF PURE COMPOUND

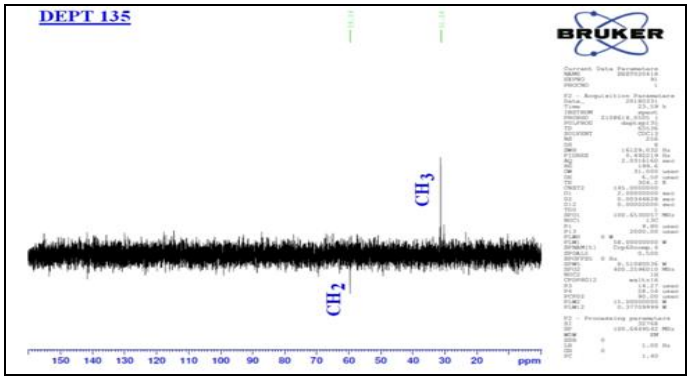


FIG. 13: DEPT-135 OF PURE COMPOUND

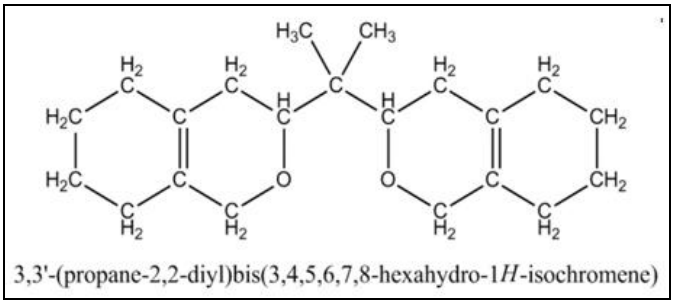


FIG. 14: IDENTIFIED STRUCTURE OF THE PURE COMPOUND PDHC 3, 3'-(propane-2, 2 -diyl) bis (3, 4, 5, 6, 7, 8-hexahydro-1*H*-isochromene

And the DEPT-45 and DEPT-135 were represented in **Fig. 12** and **13**. The pure compound has been identified by combining the different data that are obtained from FT-IR, GC-MS, 1H NMR, 13C NMR, Dept-45, Dept-90 and Dept-135. Identified structure of the pure compound was 3,3'-(propane-2, 2 -diyl) bis (3, 4, 5, 6, 7, 8-hexahydro-1*H*-isochromene and it has the molecular weight of 316.24 g/mol. The identified chemical structure is shown in **Fig. 14**.

***In-silico* Antifungal Activity of PHDC:** Anti-fungal activity of PHDC was predicted by molecular docking studies. The interactions of PHDC with 3 common antifungal targets proteins (14 α-sterol demethylases, mevalonate 5-diphosphate decarboxylase, and N-myristoyltransferase) were analyzed. Among the 3 targets, PHDC exhibited the highest affinity towards 14-Alpha-Sterol Demethylase with free binding energy of -9.5 Kcal/mol with the formation of 7 hydrogen bonds. Results of the protein-ligand docking studies of PHDC with 3 fungal drug target proteins are given in **Table 6**. Graphical representation of the interaction of PHDC with 14 alpha-sterol demethylase protein is shown in **Fig. 15** and **16.**

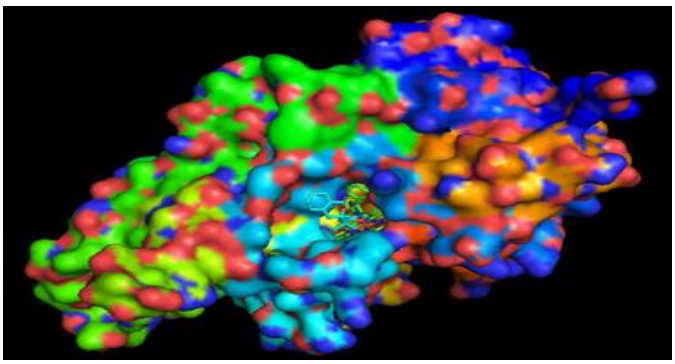


FIG. 15: INTERACTIONS OF PHDC WITH 14 ALPHA-STEROL DEMETHYLASE PROTEIN

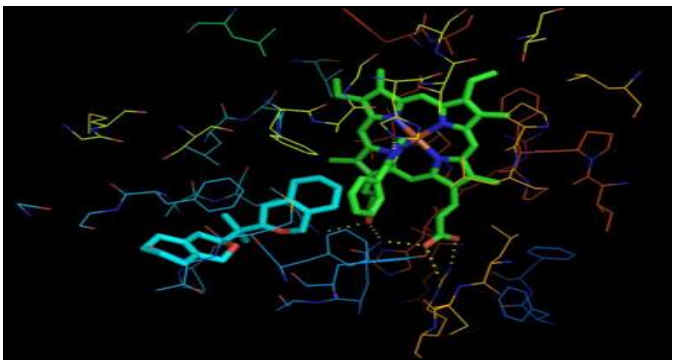


FIG. 16: INTERACTIONS OF PHDC WITH 14 ALPHA-STEROL DEMETHYLASE PROTEIN (PyMOL)

TABLE 6: AUTODOCK RESULTS OFPHDC WITH FUNGAL DRUG TARGET PROTEINS

Target proteins	PDB ID	Binding affinity (Kcal/mol)	No. of H-Bonds
14-alpha-sterol demethylase	1E9X	-9.5	7
N-myristoyltransferase	1NMT	-8.9	38
Mevalonate 5-diphosphate decarboxylase	1FI4	-8.1	1

DISCUSSION: The present studies show the bioactivity and the antimicrobial potential of leaf extracts obtained from *A. sessilis*. Thomas M. Walter *et al* reported the antibacterial activity of the ethanol extract obtained from the different parts of *A. sessilis* (leaves, internodal segments, calli derived from the intermodal segments). Test organism used were *B. substilis, S. typhii, S. pyrogens* and *P. vulgaris*. Significant activity was seen concerning that of Ampicillin and Gentamycin [6]. R. Sivakumar *et al.*, reported the antifungal activity of ethanol leaf extract obtained from *Alternanthera sessilis* was more sensitive to *C. albicans* [26].

The emphasis of our work was focussed on obtaining two different extracts from five different plant leaves. The solvents chosen were Methanol and petroleum ether. Each of the extracts was examined for the presence of various phytochemicals, total phenolic content, antioxidant, *in-vitro* anti-inflammatory, antibacterial and anti-fungal activity. The petroleum ether extract was found to have effective antimicrobial activity, and so it was chosen for further study. Using chromatography technique a pure compound was isolated, and from GC-MS, NMR, and FT-IR data it was found to be PHDC (3,3'-(propane-2,2-diyl)bis (3,4,5,6,7,8-hexahydro-1H-isochromene).

So far, there have been no reports on this compound. To identify a possible mode of action, *in-silico* protein-ligand docking study was performed using Autodock-4.2. *In-silico* molecular docking studies revealed the antifungal potential of PHDC by inhibiting the 14 α-sterol demethylase protein. It showed the free binding energy of -9.5 Kcal/mol with the formation of 7 hydrogen bonds.

CONCLUSION: Evaluating the chemical constituent, ability, effectiveness, side effect, and their therapeutical spectrum are important to ensure the safety and exact use of herbal medicines. The presence of phyto-compound in the extracts including, (steroid, phenol terpenoids, flavonoids, glycosides, saponins) may be responsible for these activities. Besides their strong antimicrobial activities, wide distributions and medicinal functions make them promising sources of natural bioactive compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

Our results support the use of the studied plant as traditional medicine, and thereby it suggests that some of these plant extracts possess compounds with good antibacterial and antifungal properties thus it can be used as antimicrobial agents in search of new drugs. Thereby our findings can support for developing fungicidal agent from the *A. sessilis* plant. This study opens up opportunities for further research on this valuable anti-fungal phytochemical leading to possible treatment applications

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REFERENCES:

1. Kumar S, Paul S, Walia YK, Kumar A, Singhal P and Chowk N: Therapeutic potential of medicinal plants : A Review. Journal of Biological and Chemical Chronicles 2015; 1: 46-54.
2. Ajlan A: Medicinal plants: A review. Natural Products: An Indian Journal 2016; 12: 1-6.
3. Singh R: Medicinal plants : A review. Journal of Plant Sciences 2015; 3: 50-55.
4. Thalluri J: A review of medicinal plants. Research and Reviews: Journal of Medicinal and Organic Chemistry 2016; 3: 157-165.
5. Petrus AJ: *Mukiamader aspatana* (L.) M. Roemer-A review of its global distribution, phytochemical profile and antioxidant capacity. Asian Journal of Chemistry 2012; 24: 2361-2368.
6. Thomas W, Merish S and Tamizhamuthu: Review of *Alternanthera sessilis* concerning traditional Siddha medicine. International Journal of Pharmacognosy and Phytochemical Research 2014; 6: 249-254.
7. Mrinmay D, Ashok K, Mastanaiah K and Arup D: Evaluation of Anti-diabetic activity of ethanolic extract of *Alternanthera sessilis* Linn. in streptozotocin-induced diabetic rats. International Journal of Pharma Sciences and Research 2015; 6: 1027-1032.
8. Aziz MM, Raza MA, Saleem H, Wajid M, Bashir K and Ikram M: Medicinal values of herbs and plants, the importance of phytochemical evaluation and ethnopharmacological screening: An Illustrated review essay. Journal of Pharmaceutical and Cosmetic Sciences 2014; 2: 6-10.
9. Anitha R and Kanimozhi S: Pharmacognostic Evaluation of *Alternanthera sessilis* (L.) R.Br.ex.DC. Pharmacognosy Journal 2012; 4: 31-34.
10. Hossain AI, Faisal M, Rahman S, Jahan R and Rahmatullah M: A preliminary evaluation of anti-hyperglycemic and analgesic activity of *Alternanthera sessilis* aerial parts. BMC Complementary and Alternative Medicine 2014; 14: 1-5.
11. Saleem MTS, Chetty MS, Ramkanth S, Rajan VST, Kumar MK and Gauthaman: Hepatoprotective herbs - A review. International Journal of Research in Pharmaceutical Sciences 2010; 1: 1-5.
12. Mahalingam R, Bharathidasan R, Ambikapathy V and Panneerselvam A: Studies on antibacterial activity of some medicinal plant against Human pathogenic Micro Organism. Pelagia Research Library 2011; 1: 86-90.
13. Sangeetha G and Vidhya R: *In-vitro* anti-inflammatory activity of different parts of *Pedalium murex* (L.). International Journal of Herbal Medicine IJHM 2016; 4: 31-36.
14. Ghosh A, Das BK, Roy A, Mandal B and Chandra G: Antibacterial activity of some medicinal plant extracts. Journal of Natural Medicines 2008; 62: 259-262.
15. Ali Jamali NM: Study the effect of medical plant extracts in comparison with antibiotic against bacteria. Journal of Scientific and Innovative Research 2013; 2: 843-845.
16. Narendra K, Sowjanya KM, Swathi J and AKS: Phytochemical evaluation and antimicrobial efficiency of threatened medicinal plant of Andhra Pradesh *Pterospermum xylocarpum* (Thada tree). International Research Journal of Pharmacy 2013; 4: 155-160.
17. Sundar RDV, Segaran G, Shankar S, Settu S and Ravi L: Bioactivity of *Phoenix dactylifera* seed and its phytochemical analysis. International Journal of Green Pharmacy 2017; 11: 1-11.
18. Baba SA and Malik SA: Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaemajacquemontii Blume*. Journal of Taibah University for Science 2015; 9: 449-454.
19. John B, Sulaiman CT, George S and Reddy VRK: Total phenolics and flavonoids in selected medicinal plants from Kerala. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6: 406-408.
20. Sahu RK, Kar M and Routray R: DPPH free radical scavenging activity of some leafy vegetables used by Tribals of Odisha, India. Journal of Medicinal Plant Studies 2013; 1: 21-27.

21. Loganayaki N, Siddhuraju P and Manian S: Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helictere sisora* L. and *Ceiba pentandra* L. Journal of Food Science and Technology 2013; 50: 687-695.
22. Ravi L, Manasvi V and Lakshmi PB: Antibacterial and antioxidant activity of saponin from *Abutilon indicum* leaves. Asian Journal of Pharmaceutical and Clinical Research 2016; 9: 344-347.
23. Prince L and Prabakaran P: Antifungal activity of medicinal plants against plant pathogenic fungus *Colletotrichum falcatum.* Asian Journal of Plant Science and Research 2011; 1: 84-87.
24. Ravi L and Krishnan K: Cytotoxic potential of n-hexadecanoic acid extracted from *Kigelia pinnata* leaves. Asian Journal of Cell Biology 2017; 12: 20-27.
25. Tang Z, Qin J, Xu X, Shi G,Yang H and Liang Y: Applying silica gel column chromatography purify resveratrol from extracts of *Morus alba* L. Leaf. Journal of Medicinal Plants Research 2011; 5: 3020–3027.
26. Sivakumar R and Sumathi D: Phytochemical screening and antimicrobial activity of ethanolic leaf extract of *Alternanthera sessilis* (L.) R. Br. Ex Dc and *Alternanthera Philoxeroides* (Mart.) Griseb. European Journal of Pharmaceutical and Medical Research 2016; 3: 409-412.

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