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Analysis of bioactive compounds and antimicrobial screening of *Phyllanthus amarus* Schum. and Thonn.

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ABSTRACT

Phytochemical screening of the extracts in different parts of *Phyllanthus amarus* revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. The presence of various bioactive compounds in the plant parts of *Phyllanthus amarus* helps us to evaluate growth effects of some selected bacteria. The development of resistance against conventional antibiotics by pathogens is of great concern. Leaf, stem and root of *Phyllanthus amarus* were separately extracted with 70 % ethanol and 70 % methanol and the crude extracts were assayed for six pathogenic microorganism's viz. *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Proteus mirabilis* for susceptibility to the various extracts of *P. amarus in vitro.* The zones of inhibition of both plant extracts ranged between 7.5-30.0 mm against the test organisms. Leaf extracts of *Phyllanthus amarus* were most active against the growth of the bacteria. *S. aureus* was the most susceptible organism and the most resistant bacterium was *P. aeruginosa* to the ethanolic extracts basing on MIC values obtained for leaf,stem and root parts of *P.amarus*.. Ciprofloxacin and Chloramphenicol were the standard antibiotics that were used. Antimicrobial study suggests that both methanolic and ethanolic extracts of the plant show approximately the same inhibitory effect on the test pathogenic microorganisms. Hence Ethanolic and methanolic leaf extracts of *P. amarus* were subjected to GC-MS analysis to investigate possible bioactive components in them. Nine compounds were detected in ethanolic leaf extract and seventeen medicinally important bioactive compounds were detected in methanolic leaf extract which confirms the application of *P. amarus* for finding novel drug compound.

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Introduction:

Phyllanthus amarus Schum and Thonn. Fam. *Euphorbiaceae* is a small, erect annual herb that grows 30 - 40 cm high and are highly distributed in most tropical and sub-tropical countries. The extract is a good blood purifier for light malaria fever and anemia, while the root extract is said to have theurapeutic effect on jaundice [1-2]. *P. amarus* primarily contains active constituents including phyllanthin and hypophyllanthin alkaloids and bioflavonoids like quercetin, reported that their functions are diverse which include provision of strength to plants, attraction of insects to pollination and defense against predators, provision of colours while some are simply waste products [3-4]. Plants are important source of potentially useful structures for the

development of new chemotherapeutic agents [5] Phytochemicals such as tannins and phenols from *P. amarus* have been associated with some antimicrobial importance [6]. Past reports on the phytochemicals contents of *P. amarus* have been on the whole plants as it is a small herb and often used in this manner in folk medicine. This study investigated the phytochemical composition of the leaves, stem, seeds and roots and antimicrobial potential of root, stem and leaves of *P. amarus*. From this study, a novel prophylactic or curative ingredients for the control of the test pathogenic microbes can be obtained for the benefit of mankind.

Materials and Methods:

Sample collection and preparation:

Fresh samples of *P. amarus* were collected from various places in Andhra Pradesh, India. The collected materials were air-dried at room temperature .The samples of each of leaves, stem, seeds and roots were crushed using blender. The crushed sample was stored in air-tight containers and analyzed individually in triplicate.

Reagents:

Most of the chemicals used were of the analytical grade. Deionized and distilled water were used throughout the experiment.

Extraction:

Ten grams (10 g) each of the samples were placed in glass bottles and soaked with 100 ml each of methanol, chloroform and ethanol respectively and were allowed to stay for 48h. After this, the mixture was stirred before carefully decanted and filtered to obtain the extract

Phytochemical screening:

The extracts were analyzed for the presence of alkaloid, flavonoids, steroid, terpenes, tannins, saponins, glycosides, anthraquinone in line with the procedures described by Harborne and Sofowora.**[7-9]**

Antimicrobial Screening:

Extraction of plant material:

Adequate quantity of each air-dried sample was mixed separately with 70% ethanol and 70% methanol for 3 days at room temperature with intermittent shaking. The suspensions were then filtered using 40 mesh screens and the filtrate concentrated using rotary evaporator. The concentrated crude extracts were freeze-dried and kept under refrigeration until further use.

Bacterial strain collection:

Standard strains of six pathogenic microorganisms, *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa and Klebsiella pneumonia* were obtained from IMTECH,Chandigarh.

Preparation of test organisms:

Standard strains of *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa and Klebsiella pneumonia* were propagated on nutrient agar (Merck) plates and maintained on the plate at 2-4^{o}C. Isolates were sub-cultured in Mueller-Hinton broth at 37^{o}C for 16 hours prior to antibacterial testing. 20 µl of these cultures were sub-cultured into 3 ml of sterile Mueller-Hinton broth and incubated at 37^{o}C for another 2 hours. The turbidity of the actively growing broth cultures was adjusted with sterile Mueller-Hinton broth to obtain turbidity optically comparable to that of 0.5 McFarland Standard.

Preparation of crude extracts and antibiotics:

Exactly 400 mg of the plant extracts were dissolved in 1 ml 20% (v/v) sterile dimethylsulphoxide (DMSO) in order to obtain a stock concentration of 400 mg ml^{-1}. The stock solution was further diluted using 20 % (v/v) DMSO in two-folds to obtain working concentrations of 200 mg ml^{-1} and 100 mg ml^{-1}. DMSO was used as a negative control. Ciprofloxacin and chloramphenicol were prepared to final concentrations of 0.05 mg ml^{-1} and 0.30 mg ml^{-1} respectively and served as the positive drug controls against the bacteria.

Antimicrobial activity assay:

The agar well diffusion method was used to investigate bacteria growth inhibition potentials of the crude extracts. A sterilized cork borer of an internal diameter of about 6 mm was used to bore holes in the Mueller-Hinton agar plates. The extracts and control antibiotic, 0.05 mg ml^{-1} ciprofloxacin and 0.30 mg ml^{-1} chloramphenicol powder were dispensed into these holes. A triplicate of each plate was made. The plates were kept in a refrigerator for about 4 hours for complete diffusion of the extracts and the control antibiotics. The plates were then incubated at 37^{o}C for 24 hours. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with a sterilized ruler and the mean calculated.

Determination of Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration (MIC) was determined by broth dilution method [10]. Two fold serial dilutions of the crude extracts, with appropriate antibiotic as control were prepared in Mueller–Hinton broth[11]. A direct suspension of microorganisms was prepared in saline water from a 24 h old suspension in Mueller–Hinton broth. The turbidity of the suspension was adjusted to match 0.5 McFarland standard [12] using a spectrophotometer (Shimadzu UV 2450 UV – Vis Spectrophotometer) at 600 nm, which corresponds to 2.4 X 108 cfu/ml. For broth dilution tests, 0.1 ml of standardized suspension of bacteria (10^8 cfu/ml) was

added to each tube at a final concentration of 0.0097 mg/ml – >5.00 mg/ml, and incubated at 37^0C. The lowest concentration of the tube which did not show any visible growth after the macroscopic evaluation was considered as the MIC.

Statistical analysis: Data are expressed as mean ± SD and were analyzed.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis for ethanolic extracts:

GC-MS analysis were carried out on a GC-MS-QP 2010Plus Shimadzu system and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30m x 0.25mm 1D x µl df, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (Split ratio of 10:1) injector temperature-250ºC; ion-source temperature 280ºC. The oven temperature was programmed from 110ºC Isothermal for 2 min.) with an increase of 10ºC / min to 200ºC then 5ºC / min. to 280ºC / min, ending with a 9 min. isothermal at 280ºC. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbo mass. The detection employed the NIST Ver. 2.0 year 2009 library.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis for methanolic extracts:

GC-MS analysis were carried out on a GC-MS - 5975C AGILENT (GC-MS- QP 2010, SHIMADZU)) system and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column DB-5ms Agilent (30m x 0.25mm 1D x µl df, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.9995%) was used as the carrier gas at constant flow rate 1.51 ml/min with a split ratio of 10:1. The oven temperature was operated according to the following oven temperature: 70°C held for 3 min, raising at the rate of 10°C min-1 up to 200°C with no held, raising at the rate of 5°C min-1 up to 300°C with 9 min held, injector temperature and volume 250°C and 1 µl, respectively. The total GC running time was about 36 min. The MS operating conditions were ionization voltage 70 eV, source temperature of 230°C, inlet line temperature of 240°C, mass scan (m/z)-40-700, solvent delay: 5 min, total MS running time-34 min8. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST (2011) library.

Results and Discussion:

Phytochemicals are present in the various parts of the plants. Saponin was present on the methanol, chloroform and ethanol extract in all parts of the plant except in the methanol and ethanol extracts of the seeds (Table 1). The presence of alkaloids in the methanol and ethanol extract of the leaf and in the root extracts of *P. amarus* suggest that it may be used as basic ingredient for antifungal and bactericidal agents. The high content of saponins in the plant may aid in reducing the burden of the liver as regards cholesterol metabolism [13].

Table 1: Phytochemical screening of the parts of *P. amarus*

S. No.	Phytochemical	Leaves			Stems			Seeds			Roots		
		Met	Chl	Eth	Met	Chl	Eth	Met	Chl	Eth	Met	Chl	Eth
1.	Alkaloids	+	-	+	-	-	-	-	-	-	+	+	+
2.	Anthraquinone	+	-	-	-	-	-	-	-	-	+	+	+
3.	Balsam	+	+	+	+	+	+	+	+	+	-	-	--
4.	Flavonoids	+	+	+	-	-	-	-	+	-	-	-	--
5.	Glycoside	-	-	-	-	-	-	-	-	-	-	-	--
6.	Phlobatanin	-	-	-	-	-	-	-	-	-	_	-	-
7.	Saponins	+	+	+	+	+	+	-	+	-	+	+	+
8.	Saponins glycoside	+	-	-	-	-	-	-	-	-	-	-	--
9.	Steroids	+	+	+	+	+	+	-	+	-	-	--	-
10.	Tannins	+	+	+	+	+	+	-	-	-	+	+	+
11.	Terpenoid	+	+	+	+	+	+	+	+	+	+	+	+

+ = Present; - = Absent

Note: Met. = Methanol; Chl. = Chloroform; Eth. = Ethanol

Flavonoid is completely absent in the methanol, chloroform and ethanol extract of the stem but was present in the methanol, chloroform and ethanol extract of the leaves. Flavonoid was reported to act as antioxidant, inhibitors of cyclooxygenate and 5-lipoxygenase, such that additional anti-inflammatory

and thrombocyte-aggregation inhibitory effect can be expected [14]. Terpenoids was present in the methanol, chloroform and ethanol extract of the stem, root, seed and leaves of the plant. Phlobatanins was completely absent in the leaves,stem ,root and seeds of methanol, chloroform and ethanol extracts. Balsam was present in the stem, seeds and leaves of the methanol, chloroform and ethanol extracts but completely absent in the root methanol, chloroform and ethanol extract. The presence of alkaloids and terpenoids in the *P. amarus* extract indicated it as a natural agent for antibacterial and antifungal botanicals. Further work on the formulation of the extracts for invivo field screening is imperative.

Antimicrobial potential of leaf of *P. amarus* extract:

Results of the present antimicrobial study suggests that both methanolic and ethanolic extracts of various parts (stem, leaf and root) show approximately the same inhibitory effect on the test pathogenic microorganisms. Zones of inhibition of ethanolic and methanolic extracts of the stem, the root and the leaf of *P. amarus* were studied at 100 mg/ ml and 200 mg/ml. When ethanolic extracts were compared at 100 mg/ ml concentration, leaf exhibited highest inhibition zone (19.5±0.5 mm) against *Salmonella typhi,* whereas stem and root extracts exhibited maximum inhibition zones of 13.9±0.0 mm and 14.6±0.6 mm respectively against *Escherichia coli.* There were no inhibition zones for root, stem and leaf ethanolic extracts against *Klebsiella. Pneumonia.* (Table 2, Fig 1). When methanolic extracts were compared at 100 mg/ ml concentration , leaf and stem exhibited highest inhibition zones of 19.0±1.0 mm and 17.5±0.5 mm against *P. aeruginosa, and* root extracts exhibited maximum inhibition zone (15.8±0.0 mm) against *Escherichia coli.* There were no inhibition zones for root, and leaf methanolic extracts against *Klebsiella. Pneumonia.,* but stem extracts exhibited lowest inhibition zone (7.5±0.5 mm) against *S. aureus* (Table 3, Fig 2).On the basis of the MIC values, the leaf extract was most susceptible against *Staphylococcus aureus* with MIC value of 3.125 mg $ml.^{-1}$ Leaf, stem and root extracts of *P. amarus* were however less effective against the growth of *P. aeruginosa* with MIC value of 50 mg/ml (Table 5, Fig 4).Thus *P. aeruginosa* was the most resistant test organism. The variations in the activities (zones of inhibition) of the different extracts on the microorganisms could be due to the extent of distribution of the various phytochemicals present in the plant. Standard antibiotics namely Ciprofloxacin and Chloramphenicol were used as positive control. Ciprofloxacin exhibited excellent activity towards the whole set of pathogenic microorganisms whereas Chloramphenicol did not exhibit activity against *Salmonella typhi* and *Staphylococcus aureus* (Table 4, Fig 3). A problem associated with many *in vitro* antibacterial studies of plant extracts is that, it is often difficult to elucidate the precise mechanism of action of inhibition because such extracts are complex mixtures of many secondary plant products. However, several studies have shown that the most common mechanisms of inhibition include inhibition of cell wall synthesis, inhibition of protein synthesis, and alteration of cell membranes, inhibition of nucleic acid synthesis and anti-metabolite activity. Therefore, *P. amarus* extracts might be acting through one or more of these mechanisms in inhibiting the growth of the susceptible bacteria.

Table: 2. Antimicrobial activity of ethanolic extracts on selected microorganisms

Test organisms	Concentration (mg/mL)	Mean zones of inhibition		
		Ethanolic extracts		
		Root	Stem	Leaf
S. typhi	100	12.5±0.5	13.5±0.5	19.5±0.5
	200	25.0±0.0	24.3±0.3	23.5±0.5
K. pneumoniae	100	0.0	0.0	0.0
	200	10.5±0.5	10.3±0.3	11.0±0.0
E. coli	100	14.6±0.6	13.9±0.0	17.7±0.0
	200	26.0±0.0	23.5±0.5	25.0±0.0
S. aureus	100	9.0±0.0	8.5±0.5	10.5±0.5
	200	14.5±0.5	10.0±0.0	14.5±0.5
P. mirabilis	100	8.5±0.5	9.0±0.0	9.9±0.9
	200	12.0±0.0	14.0±1.0	13.5±0.5
P. aeruginosa	100	12.0±0.0	13.3±0.3	14.0±1.0
	200	20.0±0.0	20.0±1.0	20.0±0.0

Data expressed as mean ± SD

Table: 3. Antimicrobial activity of methanolic extracts on selected microorganisms

Test organisms	Concentration (mg/mL)	Mean zones of inhibition		
		Ethanolic extracts		
		Root	Stem	Leaf
S. typhi	100	10.0±0.0	15.0±0.0	15.5±0.5
	200	15.0±0.0	25.0±0.0	24.5±0.5
K. pneumoniae	100	0.0	9.0±1.0	0.0
	200	0.0	19.0±1.0	8.0±1.0
E. coli	100	15.8±0.0	14.5±0.5	16.0±1.0
	200	25.5±0.5	26.0±1.0	24.0±0.0
S. aureus	100	8.0±1.0	7.5±0.5	0.0
	200	14.5±0.5	14.0±0.0	9.5±0.5
P. mirabilis	100	8.0±1.0	8.0±0.0	9.0±0.0
	200	12.5±0.5	14.0±1.0	10.5±0.5
P. aeruginosa	100	12.5±0.5	17.5±0.5	19.0±1.0
	200	20.0±0.0	27.0±0.0	24.0±0.0

Data expressed as mean ± SD

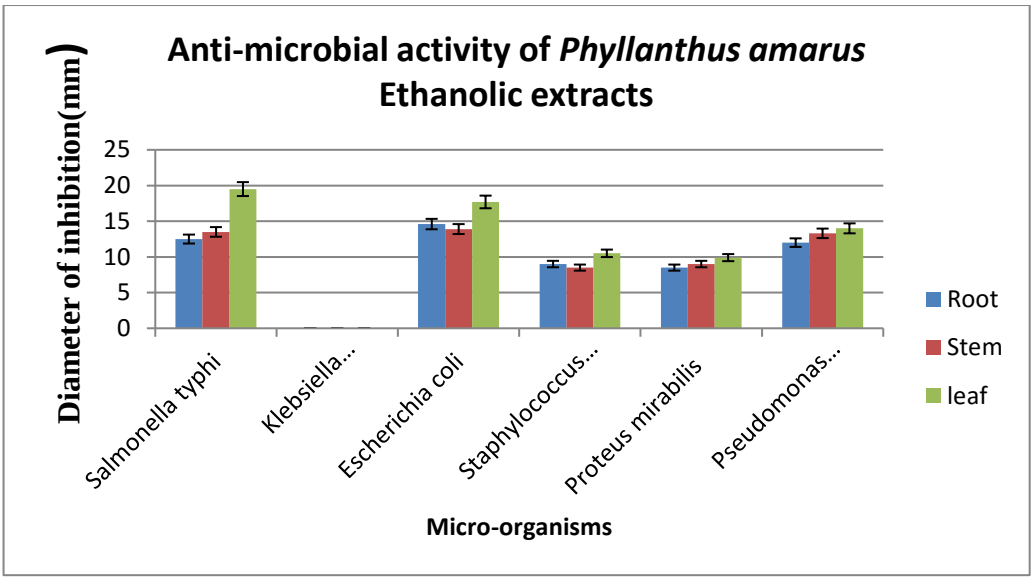

Fig. 1: Anti-microbial activity of *Phyllanthus amarus* ethanolic extracts

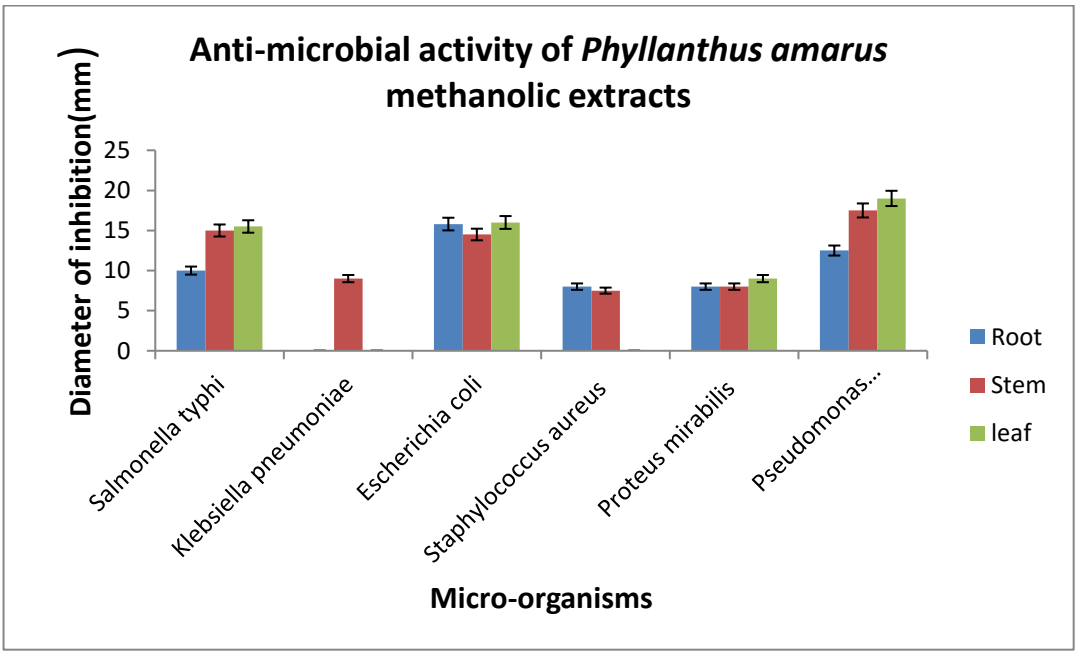

Fig. 2: Anti-microbial activity of *Phyllanthus amarus* Methanolic extracts

Table: 4. Zones of inhibition of commercially available antibiotics on selected microorganisms

Test organisms	Mean zones of inhibition(mm)		
	DMSO 20 % V/V	Ciprofloxacin 0.05 mg/ mL	Chloramphenicol 0.30 mg/mL
S. typhi	0.0	44.3±0.30	0.0
K. pneumoniae	0.0	30.0±0.00	21.0±0.00
E. coli	0.0	34.5±0.50	15.3±0.30
S. aureus	0.0	40.0±0.00	0.0
P. mirabilis	0.0	35.3±0.30	11.0±0.00
P. aeruginosa	0.0	33.5±0.50	19.0±0.00

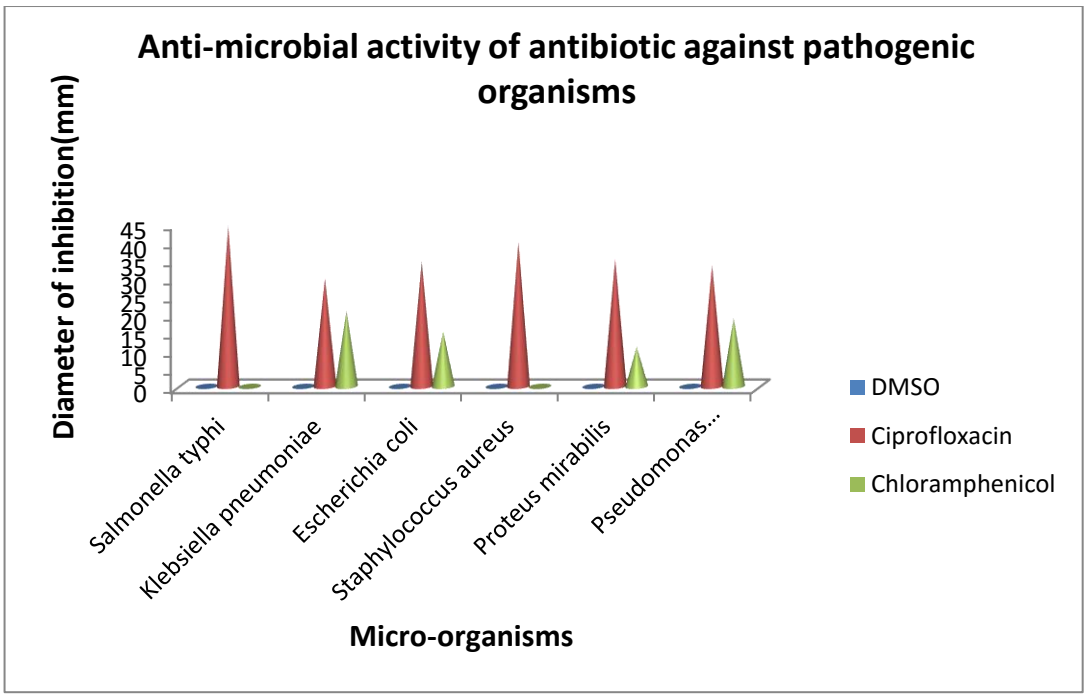

Fig. 3: Anti-microbial activity of antibiotics on microorganisms

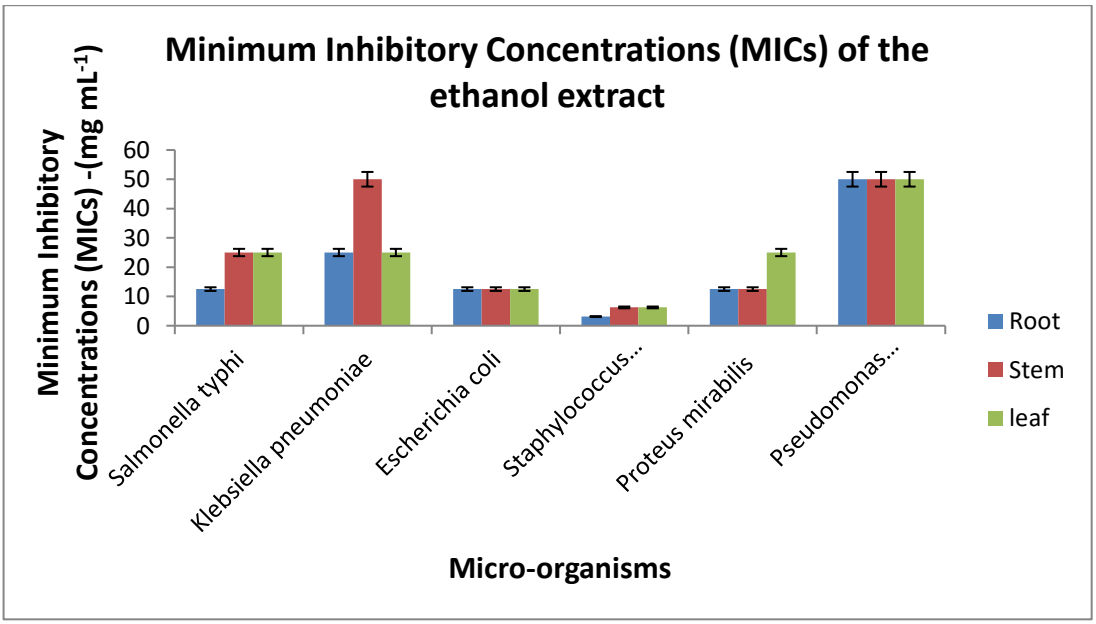

Fig. 4: Comparative study of Minimum Inhibitory Concentrations (MICs) of the ethanol extract of *Phyllanthus amarus*

Table: 5. Minimum Inhibitory Concentrations (MICs) of the ethanol extract of *Phyll anthus amarus:*

Test organisms	MIC (mg mL^{-1})		
	Leaf	**Stem**	**Root**
S. typhi	12.5	25.0	25.0
K. pneumoniae	25	50	25
E. coli	12.5	12.5	12.5
S. aureus	3.125	6.25	6.25
P. mirabilis	12.5	12.5	25
P. aeruginosa	50	50	50

GC-MS analysis for ethanolic leaf extracts:

The compounds present in the ethanolic extract of leaves of *P. amarus* were identified by GC-MS (Fig 5).The active principles with their retention time (RT), molecular formula and percentage composition in the ethanol extract of leaves of *P. amarus* is presented in Table 6. Phytocompounds and its biological activities obtained through the GC-MS study of leaves of *P. amarus* have been tabulated (Table 7). The activity of phytocomponents identified in *P.amarus* by GC-MS is based on Dr. Duke's phytochemical and ethnobotanical databases.

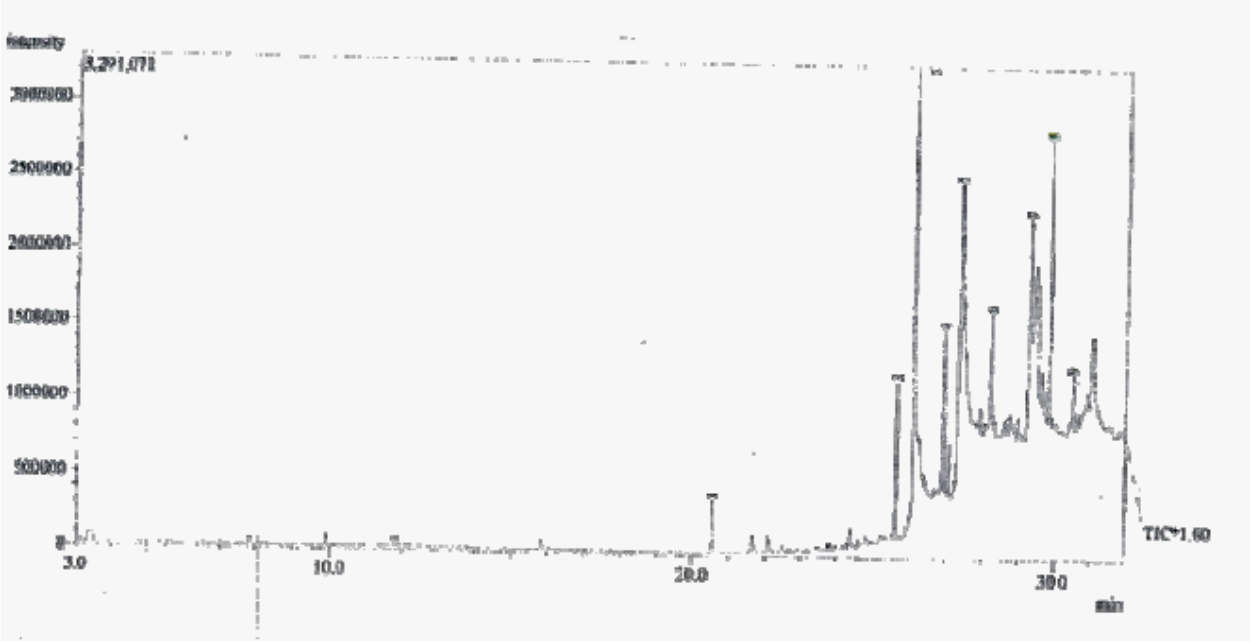

Fig. 5: GC-MS Chromatogram of ethanol Extract of leaves of *P. amarus*

Table: 6. Components detected in *P. amarus* ethanolic leaf extract

S. No.	RT (min)	Name of compound	Molecular formula	Percentage composition
1.	20.5	3,5-di-t-butyl phenol	$C_{14}H_{22}O$	1.2
2.	25.6	Methyl 14-methyl pentadecanoate	$C_{17}H_{34}O_2$	4.1
3.	26.1	Palmtic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	11.8
4.	26.9	10-octadecanoate	$C_{19}H_{36}O_2$	5.5
5.	27.3	9-hexadecenol	$C_{16}H_{32}O$	9.0
6.	28.2	Glycerol 1,3-dipalmitate	$C_{35}H_{68}O_5$	5.7
7.	29.3	2, 13-Octadecadiene-1-ol	$C_{18}H_{34}O$	8.2
8.	29.8	Dioctyl ester	$C_{24}H_{38}O_4$	10.1
9.	30.0	Heptanoic acid (9-dece-1-yl ester)	$C_{17}H_{32}O_2$	4.6

Table: 7. Activity of phytocomponents identified in the ethanolic leaf extra of *P. amarus*

S. No.	Name of compound	Nature of compound	Activity [15]
1.	3,5-di-t-butyl phenol	Phenolic compound	Antimicrobial Antioxidant Anti-inflammatory
2.	Methyl 14-methyl pentadecanoate	Ester	Antifungal
3.	Palmtic acid (Hexadecanoic	Fatty acid	Antimicrobial

	acid)		Antidiarrhoeal
4.	10-octadecanoate	Ester	Antimicrobia
5.	9-hexadecenol	Aldehyde	Antimicrobia
6.	Glycerol 1,3-dipalmitate	Ester	Antimicrobia
7.	2, 13-Octadecadiene-1-ol	Alcoholic compound	Antimicrobia
8.	Dioctyl ester	Ester	Antimicrobia
9.	Heptanoic acid (9-dece-1-yl ester)	Ester	Antimicrobia

GC-MS analysis for methanolic leaf extracts:

The compounds present in the methanolic extract of leaves of *P. amarus* were identified by GC-MS (Fig 6).The active principles with their retention time (RT), molecular formula and percentage composition in the ethanol extract of leaves of *P. amarus* is presented in Table 8. Phytocompounds and its biological activities obtained through the GC-MS study of leaves of *P. amarus* have been tabulated (Table 9).

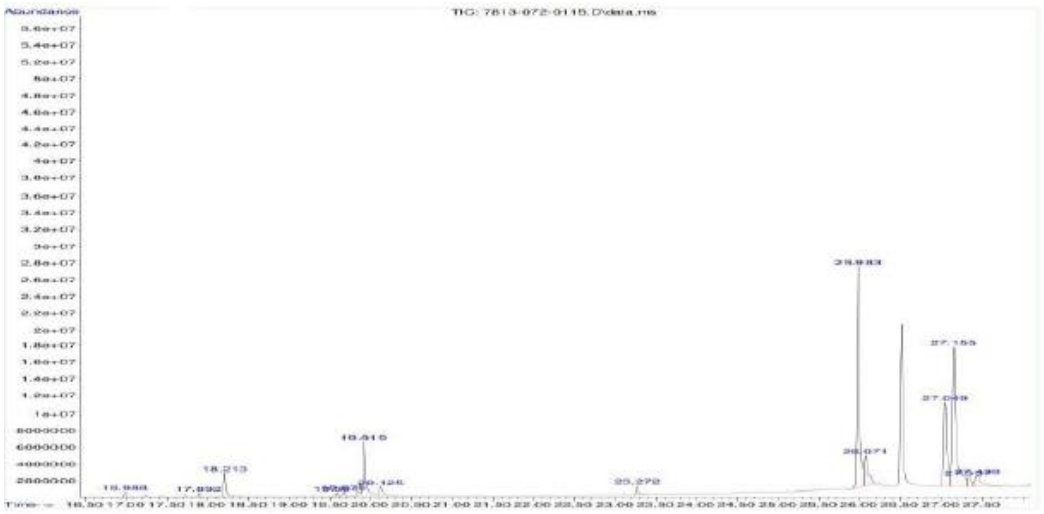

Fig. 6: GC-MS Chromatogram of methanolic Extract of leaves of *P. amarus*

Table: 8. Components detected in *P. amarus* methanolic *leaf* extract

S. No.	RT (min)	**Name of compound**	**Molecular formula**	**Percentage composition**
1.	4.78	Glycerin	$C_3H_8O_3$	4.41
2.	11.29	1, 2, 3 Bezenetriol	$C_6H_6O_3$	2.17
3.	14.22	Diethyl phthalate	$C_{12}H_{14}O_4$	0.68
4.	16.98	Z-12- Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	0.63
5.	17.88	Hexadecanoic acid	$C_{17}H_{34}O_2$	0.39
6.	18.22	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.59
7.	19.58	9, 17- Octadecadienal, (Z)	$C_{18}H_{32}O$	0.43
8.	19.58	Phytol	$C_{20}H_{40}O$	0.61
9.	19.58	9, 12, 15- Octadecatrienoic acid (Z,Z,Z) (linolenic acid)	$C_{18}H_{30}O_2$	9.10
10.	20.12	Octadecanoic acid(stearic acid)	$C_{18}H_{36}O_2$	1.92
11.	23.27	Phthalic acid	$C_{24}H_{38}O_4$.	1.25
12.	19.58	Benzene, 4-butyl-1, 2-dimethoxy benzenamine	$C_{12}H_{18}O_2$	26.21
13.	26.07	Gamma Tocopherol	$C_{28}O_2$	6.04
14.	27.05	Manganese-(II), bis (N,N,N'-trimethyl-ophenylenediamine)-	C_{18} $H_{26}MnN_4$.	12.64
15.	27.15	Ethanone(thioisomaltol)	$C_6H_6O_2$	25.61
16.	27.34	9, 10- Methanoanthracen-11-ol	$C_{18}H_{18}O$.	1.74
17.	27.34	Oxazolone	$C_3H_3NO_2$	3.59
		Total % composition	100	

Table: 9. Activity of phytocomponents identified in the methanolic leaf extra of *P. amarus*

S. No.	Name of compound	Nature of compound	Activity
1.	n-Hexadecanoic acid	unsaturated fatty acid	Anti-bacterial [16] anti-fungal
2.	9, 17- Octadecadienal, (Z)	unsaturated olefin aldehyde	Anti-bacterial[17]
3.	9, 12, 15- Octadecatrienoic acid (Z,Z,Z) (linolenic acid)	fatty acid	Anti-bacterial and anti-fungal activities.[16]
4.	Octadecanoic acid(stearic acid)	fatty acid	anti-microbial[18]
5.	Gamma Tocopherol	phenolic compound	anti-microbial[19]
6.	Oxazolone	hetero cyclic compounds	anti-microbial[20]

Conclusion:

Extracts of various parts of *Phyllanthus amarus* have shown significant broad-spectrum antimicrobial properties against some selected bacteria. Leaf extract of *P. amarus* demonstrated the greatest antibacterial activity with the root extract being the least active. The antibacterial activities shown by the various extracts may be attributed to the some of the phytochemicals present. In conclusion, results from the present study supports folkloric use of *P. amarus* in the treatment of bacterial related infections and also presents the plant as a credible candidate for subsequent isolation of novel antibacterial compounds. We intend to further evaluate the antibacterial activity of the plant against a wider range of microbes. Investigation leading to subsequent isolation and characterization of the bioactive compounds in the plant is desired for the effective management of drug resistant pathogens.

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