Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Antimicrobial Activity, Bioactive Constituents, and Functional Groups in Aqueous Methanol Extract of *Polyalthia longifolia* (Sonn.) Thwaites Leaves

Feyisara Banji Adaramola[1], Roger Murugas Cooposamy[2], Olufunmiso Olusola Olajuyigbe[2,3]

[1]Department of Basic Sciences, Chemistry Unit, School of Science and Technology, Babcock University, Ilisan Remo, Ogun State, Nigeria, [2]Department of Nature Conservation, Faculty of Natural Sciences, Mangosuthu University of Technology, Durban, South Africa, [3]Department of Microbiology, School of Science and Technology, Babcock University, Ilisan Remo, Ogun State, Nigeria

Submitted: 03-Mar-2020 Revised: 15-Apr-2020 Accepted: 15-Jul-2020 Published: 11-Nov-2021

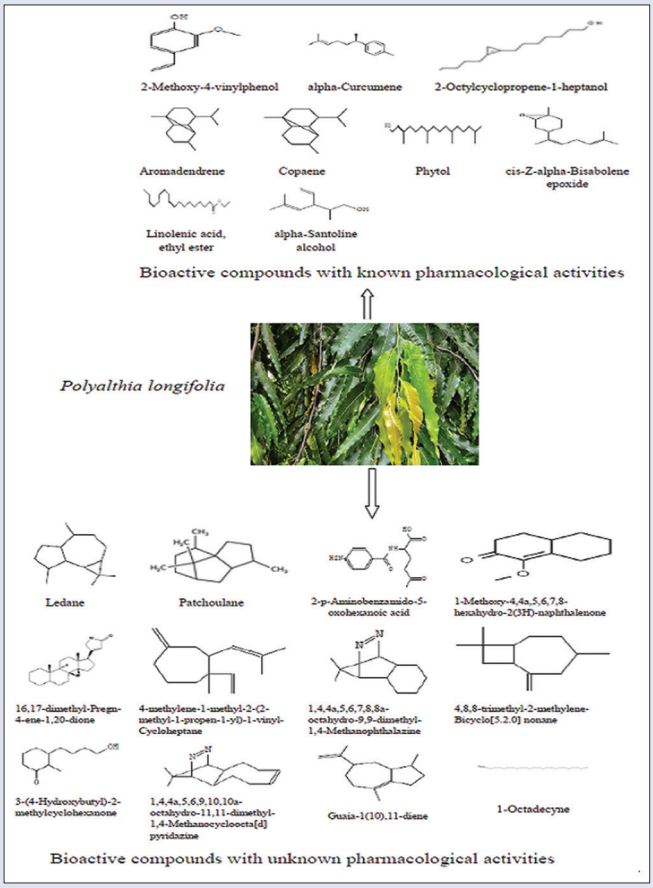
ABSTRACT

Background: *Polyalthia longifolia* is an ornamental plant with various applications in traditional medicine. There is a need to identify its bioactive compounds to justify its medicinal use. **Objectives:** In this study, the functional groups and bioactive compounds in aqueous methanol extract of *P. longifolia* leaves and its antimicrobial potential were investigated. **Materials and Methods:** Bioactive constituents of the extract were assayed with gas chromatography-mass spectrometry (GC-MS) and functional groups by Fourier transform infra-red (FT-IR), while its phytochemical screening and antimicrobial activity were investigated *in vitro* using standard protocols. **Results:** Qualitative phytochemical screening showed the presence of therapeutically important phytochemicals, namely, alkaloids, steroidal and cardiac glycosides, saponins, triterpenes, steroids, phytosterols, resins, phenols, flavonoids, tannins, and diterpenes. The extract showed promising bactericidal and fungicidal properties against the test organisms. *Staphylococcus aureus* was the most resistant of all the test organisms, while other test organisms were significantly inhibited by the extract in a concentration-dependent fashion. FT-IR analysis showed different characteristic peak values confirming the presence of important functional groups, namely, alcohol, alkane, ester, alkene, nitro compounds, amine, carboxylic acid, aromatics, and alkyl halide in the extract. GC-MS analysis identified 25 bioactive compounds in the leave extract. Some of these compounds include 2-methoxy-4-vinylphenol, copaene, aromadendrene, alpha-curcumene, caryophyllene oxide, palmitic acid methyl ester, cis-Z-alpha-Bisabolene epoxide, phytol, alpha-Santoline alcohol, and linolenic acid ethyl ester reportedly possessing antimicrobial, antioxidant, antigenotoxic, antiproliferative, anti-inflammatory, anticarcinogenic, cardio-protective, insecticidal, pesticidal, nematicidal, antiandrogenic, antiviral, and analgesic activities. **Conclusion:** This study underscores the vast therapeutic importance of *P. longifolia* leaves as an important source of potentially useful bioactive principles.

Key words: Aqueous methanol extract, antimicrobial, bioactive compounds, functional group, *Polyalthia longifolia*

SUMMARY

- The leaves of *Polyalthia longifolia* could be used in the treatment of infections in ethnomedicine
- There are bioactive compounds of therapeutic values in its extract
- The potential effectiveness of this plant in the treatment of microbial infections could be related to the pharmacological activities of the identified bioactive compounds.
- Investigating the pharmacological activities of unknown bioactive compounds such as 4,8,8-trimethyl-2-methylene-Bicyclo[5.2.0] nonane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-Cycloheptane, 3-(4-Hydroxybutyl)-2-methylcyclohexanone, 1,4,4a, 5,6,9,10,10a-octahydro-11,11-dimethyl-1,4-Methanocycloocta[d] pyridazine, and Guaia-1 (10),11-diene could be a baseline for further study in the discovery of novel drug compounds.
- The study could form the basis for selecting this plant species for further investigation in the discovery of novel drug compounds to be used in the treatment of infections.



Abbreviations used: FT-IR: Fourier transform-infrared; KBr: Potassium bromide; GC-MS: Gas chromatography-mass spectrophotometry; NIST: National Institute of Standards and Technology; DMSO: Dimethyl sulfoxide.

Correspondence:

Dr. Olufunmiso Olusola Olajuyigbe,
Department of Microbiology, School of Science and Technology, PMB 4005, Babcock University, Ilisan-Remo, Ogun State, Nigeria.
E-mail: funmijuyigbe12@yahoo.com

DOI: 10.4103/pm.pm_66_20

Access this article online
Website: www.phcog.com
Quick Response Code:

Cite this article as: Adaramola FB, Coopoosamy RM, Olajuyigbe OO. Antimicrobial activity, bioactive constituents, and functional groups in aqueous methanol extract of *Polyalthia longifolia* (Sonn.) Thwaites leaves. Phcog Mag 2021;17:594-604.

INTRODUCTION

For several years, plants and plant products have played significantly positive roles in the health of humans and their environment. They have continually provided human with oxygen for breathing and provided nutrients through edible plants parts and bioactive ingredients as medicine for human health care. Human's continuous search for safer, cheaper, and a more readily available medicine with little or no side effects has made medicinal plants of very great importance. This is because medicinal plants possess several phytochemicals, which have shown laudable efficacy in combating both infectious and lifestyle diseases. It is, therefore, not surprising that in spite of the current huge scientific development, the role of medicinal plants remains significant in providing health care for about 80% of human population, especially in the developing nations.[1] In fact, medicinal plants are a source of inspiration for many modern-day drug formulations. In the pharmaceutical industry, medicinal plants form an essential part of research and developments focusing on isolation or direct use of bioactive principles, the development of semi-synthetic drugs, or active screening of natural products to obtain synthetic pharmacologically potent compounds.[2] While their medicinal properties have been attributed to the presence of phytochemicals exerting definite physiological influence on human body,[3] various pharmacological studies have recognized the relevance and importance of medicinal plants as potential sources of bioactive compounds.[4,5] Consequently, Mukherjee *et al*.[6] and Sheeja and Kuttan[7] reported that plant-sourced bioactive compounds have resulted in the discovery of new drugs effective for protection against and treatment of various diseases including cancer and Alzheimer's disease although beneficial pharmacological effects of plants and plant materials typically result from the combined effects of bioactive compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids, which are present in different plants' tissues including leaves, flowers, bark, seeds, fruits, and roots.[8]

Polyalthia longifolia belonging to the family *Annonaceae* is one of such plants with both ornamental and numerous medicinal importance. The genus *Polyalthia* has about 120 species widely distributed in Africa, South-Eastern Asia, Australia, and New Zealand.[1] Although this plant is ornamental, *P. longifolia* finds its reference in Indian medicinal literature owing to its popular Hindi name Ashoka[1] and a number of biologically active principles that have been identified in this plant.[1,9] In traditional medicine, different parts of the plant have been exploited for the treatment of many ailments such as septic infection, diarrhea, cancer, and hepatosplenomegaly.[10] In India, fever, mouth ulcer, uterus-related ailments, gonorrhea, and heart diseases are being treated with the leaves, while the stem bark are used to treat hypertension and diabetes.[11] As an antipyretic agent in traditional medicine,[12] it is pharmacologically active as antimicrobial[13-15] and cytotoxic[16,17] with hypotensive effect.[18] Furthermore, Osuntokun *et al*.[19] reported that its fruits, stem bark, roots, and leaves have been used in ethnomedicine for their potent antibacterial, antifungal, antidiabetic, anti-inflammatory, and antioxidant activities in Nigeria. Considering ethnotherapeutic relevance of this plant, identifying potentially bioactive compounds of therapeutic importance in its parts becomes essential. This study, therefore, investigated the bioactive principles and antimicrobial potentials of aqueous methanol extract of *P. longifolia* leaves *in vitro*.

MATERIALS AND METHODS

Collection of plant sample

Fresh and mature leaves of *P. longifolia* were obtained from its tree within Babcock University campus where it was growing in its natural habitat. The leaves were thoroughly washed with copious amount of water and dried at room temperature for 2 weeks. They were subsequently pulverized with a LEXUS MG-2053 OPTIMA laboratory grinder and kept in polyethylene bags for further use.

Extraction of leaf sample

Fifty grams of the pulverized leaves was macerated in 400 mL of methanol (80%) for 72 h with intermittent shaking. The mixture was filtered at three consecutive times with Whatman No 1 filter paper plucked with cotton wool and the filtrates were combined. The combined filtrate was evaporated to dryness under reduced pressure with rotary evaporator EYELA N-1001 at 40°C. The dried extract was kept in an airtight glass tube and stored in the refrigerator at 4°C for further use.

Functional group analysis by Fourier transform infrared

In order to determine the chemical bonds/functional groups present in the methanol extract of *P. longifolia* leaves, the extract was subjected to Fourier transform infrared (FT-IR) spectroscopic analysis. Ten mg of the powdered extract was then encapsulated in 100 mg of potassium bromide (KBr) pellet to obtain translucent sample discs. The encapsulated powdered sample was loaded in a Bruker FT-IR spectrophotometer (PerkinElmer, City, Country Waltham, MA, USA) for analysis by scanning at wavelengths ranging from 300 to 3500 cm^{-1} at a resolution of 4 cm^{-1}. The percentage of transmission was recorded against the wave numbers. Peak values were recorded, and the functional groups were predicted.

Gas chromatography mass spectrometry analysis of the extract

Bioactive compounds in methanol extract of *P. longifolia* leaves were identified by gas chromatography mass spectrometry (GC-MS) equipped with GCMS-QP2010 : SHIMADZU (Shimadzu Corporation). The GC-MS was equipped with a split injector and an ion-trap mass spectrometer detector with a fused-silica capillary column of 1.00 µm thickness, 30 mm × 0.25 mm dimension, and a programmed temperature ranging from 60°C to 250°C at 3.0°C/min. The injector temperature was 250°C, while the detector temperature was 200°C. Helium served as the carrier gas at 46.3 cm/s flow rate. Comparing average peak area of individual compound with the total area, the relative percentage of each identified compound was determined. Identification of components was done through computerized matching of their spectra with the spectra of known compounds from the spectra Library of National Institute of Standards and Technology (NIST, 2009). The fragmentation patterns of the compounds eluted were identified by comparison with known data from the database.

Phytochemical screening

The presence of secondary metabolites including alkaloids, tannins, flavonoids, phenolics, steroids, glycosides, saponins, terpenoids, reducing sugar, fixed oil, resin, anthraquinone, phlobatannins, and phytosterols in the aqueous methanol extract of *P. longifolia* leaves was determined by preliminary screening of its phytochemical according to the methods of Trease and Evans[20] and Rajeshwari *et al*.[21]

Antimicrobial activity assay

The antimicrobial activity of *P. longifolia* leave aqueous methanol extract against clinical isolates of Gram-negative and Gram-positive bacteria and a fungus was carried out using agar diffusion method. These organisms including *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATTC 10702R, *Klebsiella pneumoniae*

ATCC 10031, *Proteus vulgaris* ATCC 6830R, *Pseudomonas aeruginosa* ATCC 19582, and *Candida albicans* obtained from Babcock University Teaching Hospital Laboratory, Ilisan-Remo, Ogun State, Nigeria. The test bacteria were grown in nutrient broth for 24 h, while the fungus was grown in potato dextrose broth for 5 days. The inocula of the test organisms were prepared using the colony suspension method.[22] Colonies picked from 24-h-old cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by transferring 0.1 ml of the bacterial suspension to 9.9 ml of sterile nutrient broth before being used. This assay was carried out according to the method described by Srinivasulu *et al*.,[23] Olajuyigbe and Afolayan,[24] and Adaramola *et al*.[25] Briefly, sterile Mueller–Hinton agar (Oxoids Ltd., Basingstoke, Hampshire, UK) plates were swabbed with the resultant saline suspension of each adjusted bacterial strain, while sterile potato dextrose agar plates were swabbed with the adjusted *C. albicans*. The wells were then bored into the agar medium using a heat-sterilized 6-mm cork borer. The wells were filled with 100 µl of each extract concentration along with 100 µL of ciprofloxacin (2.5 µg/ml) and fluconazole taking care not to allow spillage of the solutions onto the surface of the agar. The culture plates were allowed to stand on the laboratory bench for 1 h to allow proper diffusion of these solutions before being incubated at 37°C for 24 h for the bacterial isolates and 3–5 days for the fungus. Dimethyl sulfoxide served as the blank, while ciprofloxacin (for bacteria) and fluconazole (for fungi) were used as standard drugs. After the incubation period, the diameter of each zone of inhibition was recorded and expressed in millimeter. Minimum inhibitory concentration of the extract on the organisms was carried out at lower concentrations as described above. The entire microbial assay was conducted under strict aseptic conditions, and all analyses were carried out in triplicates.

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the leave extract as presented in Table 1 showed the presence of pharmacologically important phytochemicals such as alkaloids, steroidal and cardiac glycosides, saponins, triterpenes, steroids, phytosterols, resins, phenols, flavonoids, tannins, diterpenes, and carbohydrates, which could account for the antimicrobial activites of the plant. However, anthranol glycosides, fixed oil, anthraquinone, and phlobatannins were not detected in the leaf extract.

Table 1: Phytochemicals in aqueous methanol extract of *Polyalthia longifolia* leaves

Phytochemical	Test	Results
Alkaloids	Mayer's test	+
	Wagner's test	+
Glycosides		
Anthranol glycoside	Modified Bontrager's test	–
Steroidal glycoside	Lieberman Buchard test	+
Cardiac glycoside	Legal's test	+
Saponins	Foam's test	+
Triterpenes/STEROIDS	Salkowski's test	+
Phytosterols	Lieberman Buchard test	+
Fixed oil	Stain test	–
Resins	Acetone-water test	+
Phenols	Ferric chloride test	+
Flavonoids	Alkaline test	+
	Lead acetate test	+
	Shinoda test	+
Anthraquinone	Anthraquinone test	–
Phlobatanins	Phlobatanin test	–
Tannins	Tannin test	+
Diterpenes	Copper acetate test	+
Carbohydrates	Fehling's test	+
	Molish's test	+

+: Present; –: Absent

Fourier transform infra-red analysis

The FT-IR spectrum of aqueous methanol extract of *P. longifolia* leave is presented in Figure 1, while the peak value data and the probable functional groups present in the extract are presented in Table 2. The FT-IR analysis indicated the presence of O–H (alcohol), –C–H (alkane), C=O (ester), C=C (alkene), N–O (nitro compound), C–N (amine), O–H bending (carboxylic acid), C–H bending (aromatics), and C–Cl (alkyl halide) in methanol extract. The presence of these functional groups could be responsible for the various pharmacological properties of *P. longifolia* leave.

Antimicrobial analysis

In the present study, the growth inhibitory ability of *P. longifolia* leaf methanol extract against test organisms is shown in Table 3. The results showed that *S. aureus* was the most resistant of all the test organisms because it showed no inhibition zones from 10 to 40 mg/mL concentrations but produced 15 ± 1.00 mm inhibition zone at 50 mg/mL extract's concentration. However, other test organisms were significantly inhibited by the extract at the concentrations ranging between 10 and 50 mg/L and mostly in a concentration-dependent trend. Meanwhile, inhibition zones of the test organisms ranged from 22 to 25 ± 1.00, 20–25 ± 1.00, 15–21 ± 1.00, 18–26 ± 1.00, 21–25 ± 1.00, and 18–26 ± 1.00 mm for *E. faecalis*, *B. cereus*, *P. aeruginosa, K. pneumoniae*, *P. vulgaris,* and *C. albicans,* respectively. The MIC values were obtained as 5 mg/mL for *S. aureus* and *P. vulgaris;* 3 mg/ml for *E. faecalis*; 1 mg/mL for *B. cereus*, *P. aeruginosa,* and *K. pneumoniae;* and 0.5 mg/mL for *C. albicans.*

Gas chromatography-mass spectrometric analysis

Figure 2 shows the GC-MS chromatogram of the extract, with peaks indicating the presence of 25 different compounds. The individual compounds with their respective properties are presented in Table 4, while their biological activities are presented in Table 5. The most predominant compound identified in the leaf extract was 1,4-methanocycloocta[d] pyridazine, 1,4,4a, 5,6,9,10,10a-octahydro-

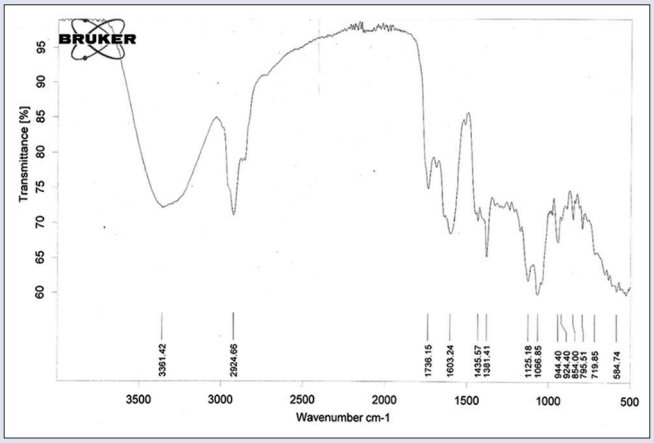


Figure 1: Fourier transform infrared spectrum of aqueous methanol extract of *Polyalthia longifolia* leaves

11,11-dimethyl-(17.87%), while the least predominant compound was copaene (0.38%). Other compounds identified with relatively high concentration include aromadendrene (3.68%), caryophyllene oxide (3.40%), patchoulane (4.47%), 1-Octadecyne (3.12%), 16, 17-dimethyl-Pregn-4-ene-1, 20-Dione (2.74%), 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-Cycloheptane (4.05%), phytol (5.68%), 1,4,4a, 5,6,7,8,8a-octahydro-9,9-dimethyl-1,4-Methanophthalazine, (3.60%), 4,8,8-trimethyl-2-methylene-Bicyclo [5.2.0] nonane (14.72%), 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-Cycloheptane (6.90%), and 3-(4-Hydroxybutyl)-2-methylcyclohexanone (11.68%). The antimicrobial activity shown by the extract was further confirmed by the presence of therapeutically important compounds such as 2-Methoxy-4-vinylphenol, phytol, palmitic acid methyl ester, caryophyllene oxide, alpha-Curcumene, alpha-Santoline alcohol, aromadendrene, and copaene with reported antimicrobial properties, as shown in Table 5.

DISCUSSION

Phytotherapy, the use of plants and plant products in the management of disease, has gained significant attention in recent times. This could be attributed to their effectiveness as therapeutic tools in combating myriads of diseases.[41] Medicinal plants are considered a repository of a vast array of bioactive principles such as ascorbic acid, carotenoids, alkaloids, tannins, flavonoids, phenolics, sterols, steroids, glycosides, saponins, terpenoids, and antocyanins, among others. The medicinal properties of plants are justifiably attributed, among other things, to these bioactive constituents, which have been shown to possess biological and therapeutic activities such as antimalaria, anti-inflammatory, anticancer, antibacterial, antifungal, antiviral, insecticidal, antioxidant, immune system stimulation, platelet aggregation reduction, and hormone metabolism modulation properties.[42,43] In the present study, hydro-methanol extract of *P. longifolia* leaf assayed for the presence of various phytochemicals indicated the presence of specific bioactive compounds, functional groups, as well as its antimicrobial potential against clinical isolates.

Although phytochemicals are essential and required for sustenance of human life, they also possess important properties to prevent and protect human from all kinds of diseases.[44] Alkaloids are a crucial drug source and reportedly possess antimicrobial, antioxidant, cytotoxic, anti-arrythmic, antihypertensive, anticancer, hypoglycemic, and antimalaria activities.[45,46] While glycosides are employed in the treatment of congestive heart failure and cardiac arrhythmia,[19] saponins possess hypocholesterolemic, antioxidant, antidiabetic, anti-inflammatory, immunostimulant, hypoglycemic, anticarcinogenic, and antifungal properties.[47,48] Terpenes have excellent antioxidant activity.[49] Steroids possess analgesic properties,[50] and phytosterols have been reported to possess anti-inflammatory, anti-neoplastic, anti-pyretic, and immuno-modulating activities.[49] Resins are known to possess antiseptic and antibacterial activities.[51] Phenolic compounds possess several biological functions such as antioxidant, anticarcinogenic, and anti-inflammatory properties, which are attributed to their fundamental reducing abilities.[52] Flavonoids have been studied extensively as antioxidant agents playing crucial functions in decreasing the risk of coronary heart disease, diabetes, hypertension, and certain forms of cancer in human beings.[53,54] Tannins are used in antidiarrheal, hemostatic, and anti-hemorrhoidal formulations. The presence of these biologically important phytochemicals in *P. longifolia* leaf extract, therefore, underscores its medicinal values. However, the FT-IR spectroscopy which has proven to be a sensitive and valuable tool for identifying

Table 2: Functional groups in aqueous methanol extract of *Polyalthia longifolia* leaves

Peak values	Functional group
3361	0-H stretching (alcohol)
2924	-C-H stretching (alkane)
1736	C=O stretching (ester)
1603	C=C stretching (alkene)
1435	-C-H bending (alkane)
1381	N-O asymmetric stretching (nitro compound)
1125	C-O stretching (alcohol)
1066	C-N stretching (aliphatic amine)
944	=C-H bending (alkene)
924	O-H bending (carboxylic acid)
854	C-H bending (aromatics)
795	C-Cl stretching (alkyl halide)
719	C-Cl stretching (alkyl halide)
584	Alkyl halide

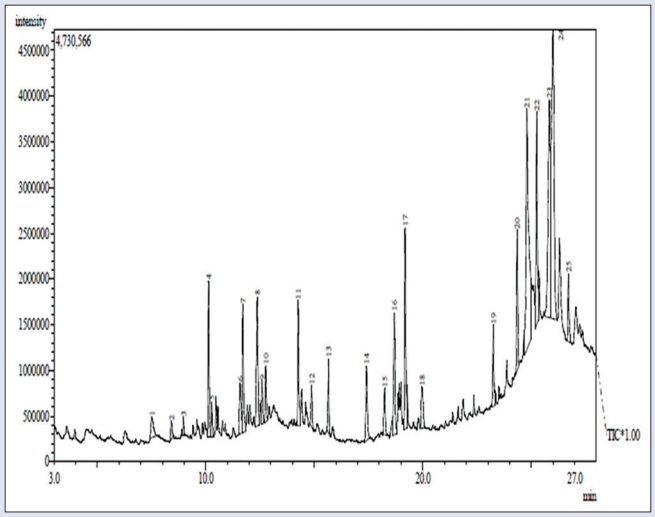


Figure 2: Gas chromatography-mass spectrometry chromatogram of aqueous methanol extract of *Polyalthia longifolia* leaves

Table 3: Susceptibility of test microbial isolates to aqueous methanol extract of *Polyalthia longifolia* leaves

Organism	10 mg/mL	20 mg/mL	30 mg/mL	40 mg/ml	50 mg/mL	Ciprofloxacin/ fluconazole (0.5 mg/mL)	MIC (mg/mL)
Staphylococcus aureus ATCC 6538	NI	NI	NI	NI	15±1.11	28±0.00	5.00
Enterococcus faecalis ATCC 29212	22±0.51	22±0.22	23±0.43	24±1.00	25±1.00	26±1.13	3.00
Bacillus cereus ATTC 10702 R	20±1.10	21±0.99	22±1.21	23±0.46	25±0.23	29±1.10	1.00
Pseudomonas aeruginosa ATCC 19582	15±0.32	17±0.22	18±0.31	18±1.24	21±1.22	25±0.55	1.00
Klebsiella pneumoniae ATCC 10031	18±1.14	22±1.00	23±1.10	23±0.08	26±0.05	26±1.23	1.00
Proteus vulgaris ATCC 6830 R	21±0.25	21±0.55	21±0.54	25±0.35	25±1.33	29±1.28	5.00
Candida albicans	18±0.54	20±0.34	20±0.55	20±1.33	26±1.04	27±0.98	0.50

Data are expressed as mean±SD of three replicates. NI: No inhibition; SD: Standard deviation; MIC: Minimum inhibitory concentration

Table 4: Bioactive compounds identified in aqueous methanol extract of *Polyalthia longifolia* leaves

Peak number	Retention time	Compound name	Molecular formula	Molecular weight	Area (%)
1	7.524	2-p-Aminobenzamido-5-oxohexanoic acid	$C_{13}H_{16}N_2O_4$	264	1.24
2	8.412	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.60
3	8.967	Copaene	$C_{15}H_{24}$	204	0.38
4	10.129	Aromadendrene	$C_{15}H_{24}$	204	3.68
5	10.273	alpha-Curcumene	$C_{15}H_{22}$	202	0.86
6	11.581	1-Methoxy-4,4a, 5,6,7,8-hexahydro-2(3H) naphthalenone	$C_{11}H_{16}O_2$	180	2.06
7	11.71	Caryophyllene oxide	$C_{15}H_{24}O$	262	3.40
8	12.374	Patchoulane	$C_{15}H_{26}$	206	4.47
9	12.592	Ledane	$C_{15}H_{26}$	206	1.31
10	12.765	Z, Z, Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	206	1.45
11	14.259	1-Octadecyne	$C_{18}H_{34}$	250	3.12
12	14.877	2-Octylcyclopropene-1-heptanol	$C_{18}H_{34}O$	296	1.14
13	15.651	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	270	1.75
14	17.408	16,17-dimethyl-Pregn-4-ene-1,20-dione	$C_{23}H_{34}O_2$	342	2.74
15	18.239	cis-Z-alpha-Bisabolene epoxide	$C_{15}H_{24}O$	220	1.48
16	18.686	4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-Cycloheptane	$C_{15}H_{24}$	204	4.05
17	19.188	Phytol	$C_{20}H_{40}O$	296	5.68
18	19.958	alpha-Santoline alcohol	$C_{10}H_{18}O$	154	1.80
19	23.253	Linolenic acid, ethyl ester	$C_{20}H_{34}O_2$	206	2.18
20	24.35	1,4,4a, 5,6,7,8,8a-octahydro-9,9-dimethyl-1,4-Methanophthalazine	$C_{11}H_{18}N_2$	178	3.60
21	24.799	4,8,8-trimethyl-2-methylene-Bicyclo[5.2.0] nonane	$C_{12}H_{22}$	178	14.72
22	25.256	4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-Cycloheptane	$C_{15}H_{24}$	204	6.90
23	25.834	3-(4-Hydroxybutyl)-2-methylcyclohexanone	$C_{11}H_{20}O_2$	184	11.68
24	26.002	1,4,4a, 5,6,9,10,10a-octahydro-11,11-dimethyl-,1,4-Methanocycloocta[d] pyridazine	$C_{13}H_{20}N_2$	204	17.87
25	26.716	Guaia-1 (10),11-diene	$C_{15}H_{24}$	204	1.94

and characterizing functional groups or chemical bonds present in an unknown mixture of natural products[30] and GC-MS able to identify various bioactive compounds in plant materials have helped in identifying the chemical makeups of the methanolic extract of *P. longifolia*. The therapeutic properties of these important bioactive constituents including antioxidant, antigenotoxic, antiproliferative, anti-inflammatory, anticarcinogenic, cardioprotective, insecticidal, pesticidal, nematicidal, antiandrogenic, antiviral, and analgesic based on reports from other studies underscore the veracity of this plant's usefulness in traditional medicine, while other compounds identified in the extract without biological activities found in literature could also contribute individually or synergistically to the pharmacological activities of the extract.

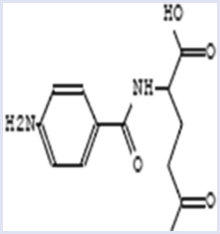
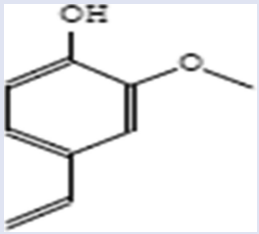
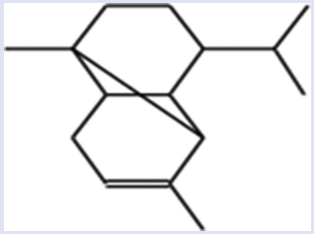
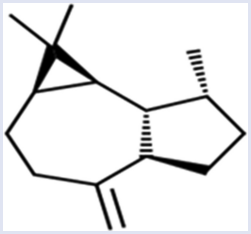
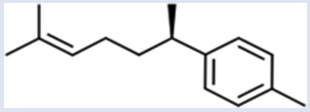
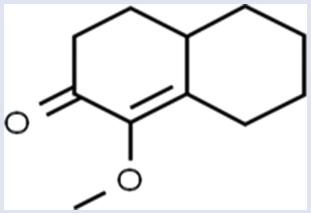
As it is crucial to investigate the potential pharmacological importance of these compounds to further identify more potential therapeutic activities of the extract, the ability of the extract to show antibacterial activity against multidrug-resistant clinical bacterial isolate is significant as the emergence and high prevalence of multidrug resistance among various strains of micro-organisms have become a serious public health concern. This is due to the inappropriate and continuous use of commercially available synthetic antimicrobials without prescriptions in treating infectious diseases. In addition to causing multidrug resistance necessitating the search for better alternative medicines from plants, these synthetic drugs are not without other adverse effects including addiction and high toxicity. However, plant-based remedies have provided enormous relief as their bioactive constituents have shown significant therapeutic potentials with minimal or lesser side effects as compared to synthesized drugs. Consequently, plant-based therapy has become a crucial component of traditional medical practices while serving as a major source of bioactive compounds for several major pharmaceutical drugs used in treating numerous diseases. Thus, plants are considered important sources of potentially useful bioactive principles for the development of new therapeutic agents.[55]

Considering the level of poverty and knowledge about the relevance of medicinal plants in ethnomedicine especially in the developing nations, the use of medicinal plants as an alternative approach to therapy is complementary in achieving primary health-care delivery. While several biological activities including antimicrobial potential of plant extracts have been attributed to the presence of secondary metabolites which are known for their various pharmacological efficacies, their useful medicinal properties have been ascribed to the combined effects of these secondary metabolites.[8] Antimicrobial activity of various extracts of *P. longifolia* leave have been reported by several authors.[56,57] From this study, the bactericidal and fungicidal activities indicated by the extract could be associated with the presence of bioactive principles such as alkaloids, phenols, flavonoids, saponins, and tannins in the extract.

CONCLUSION

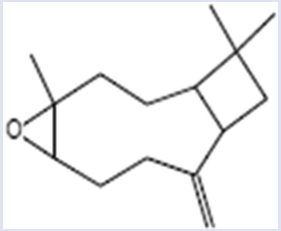
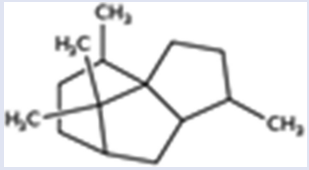
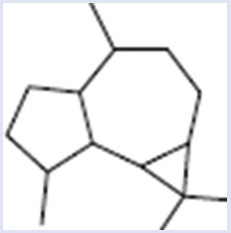
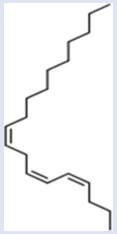
This study confirmed the presence of various pharmacologically important principles with a wide range of bioactivities including antimicrobial, antioxidant, antigenotoxic, antiproliferative, anti-inflammatory, anticarcinogenic, cardio-protective, insecticidal, pesticidal, nematicidal, antiandrogenic, antiviral, and analgesic activities in the aqueous methanol extract of *P. longifolia* leaves. Some of these compounds include 2-methoxy-4-vinylphenol, copaene, aromadendrene, alpha-curcumene, caryophyllene oxide, palmitic acid methyl ester, cis-Z-alpha-Bisabolene epoxide, phytol, alpha-Santoline alcohol, and linolenic acid ethyl ester. In addition, the antibacterial and antifungal activities of the extract against the test clinical isolates may be attributed to the presence of the aforementioned compound identified in the extract. This study underscores the medicinal veracity of *P. longifolia* leaf and thus suggests that the plant would make a good prospect for the isolation of bioactive compounds essential in drug formulation and its development.

Table 5: Bioactivity of compounds identified in aqueous methanol extract of *Polyalthia longifolia* leaves

Compound name	Structure	Nature	Bioactivity	Reference
2-p-Aminobenzamido-5-oxohexanoic acid		Unknown	Unknown	
2-Methoxy-4-vinylphenol		Phenolic (Guaiacol)	Antioxidant, antimicrobial and anti-inflammatory	Jeong *et al.*[26]
Copaene		Sesquiterpenoid	Antioxidant, antigenotoxic, anti-proliferative activity	Türkez *et al.*[27]
Aromadendrene		Sesquiterpene	Anti-tumor, analgesic antibacterial, anti-inflammatory, sedative, fungicide	Mulyaningsih *et al.*[28] Pakkirisamy *et al.*[29]
alpha-Curcumene		Sesquiterpene	Antimicrobial	Santos da Silva *et al.*[30]
1-Methoxy-4,4a, 5,6,7,8-hexahydro-2 (3H)-naphthalenone		Unknown	Unknown	

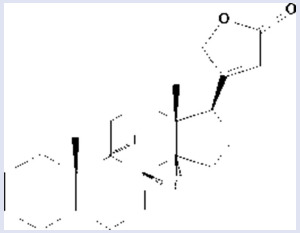
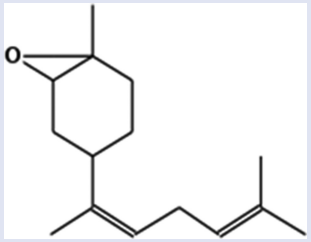
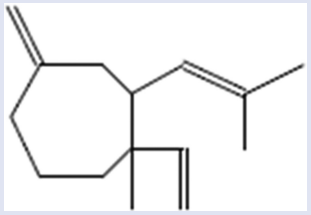
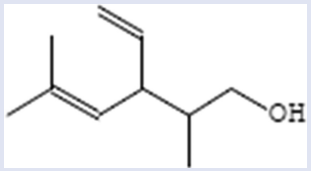
Contd...

Table 5: Contd...

Compound name	Structure	Nature	Bioactivity	Reference
Caryophyllene oxide		Epoxide	Anti-inflammatory antioxidant, antiviral, anticarcinogenic, and analgesic properties	Tung *et al.*[31] Hammami *et al.*[32] Zheng *et al.*[33]
Patchoulane			Uknown	-
Ledane		Unknown	Unknown	
4,6,9-Nonadecatriene		Triene	No activity reported	
1-Octadecyne		Alkyne	No activity reported	-
2-Octylcyclopropene-1-heptanol		Alcohol	Antibacterial activity	Omnia Gamal *et al.*[34]
Palmitic acid methyl ester		Fatty acid ester	Antimicrobial, pesticidal, nematicidal, antioxidant, antiandrogenic	Duke *et al.*[35] Chandrasekaran *et al.*[36]

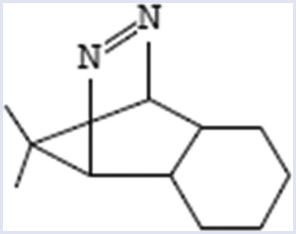
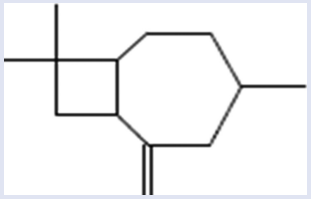
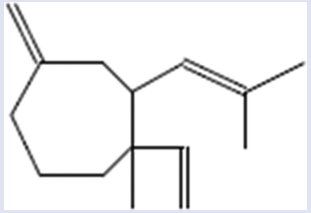
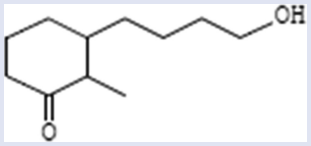
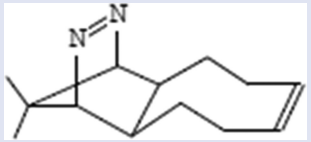
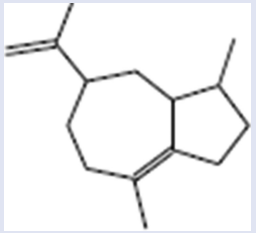
Contd...

Table 5: Contd...

Compound name	Structure	Nature	Bioactivity	Reference
16,17-dimethyl-Pregn-4-ene-1,20-dione		Unknown	Unknown	
cis-Z-alpha-Bisabolene epoxide		Epoxide	Anti-inflammatory	Hameed *et al.*[37]
4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-Cycloheptane		Unknown	Unknown	
Phytol		Diterpene alcohol	Antinociceptive, antioxidant and anitibacterial	Santos *et al.*[38] Rajab *et al.*[39]
alpha-Santoline alcohol		Alcohol	Antimicrobial, insecticidal	Dr. Duke's Phytochemical and Ethnobotanical Databases[35]
Linolenic acid, ethyl ester		Fatty acid ethyl ester	Cardio-protective	Balamurugan *et al.*[40]

Contd...

Table 5: Contd...

Compound name	Structure	Nature	Bioactivity	Reference
1,4,4a, 5,6,7,8,8a-octahydro-9,9-dimethyl-1,4-Methanophthalazine		Unknown	Unknown	
4,8,8-trimethyl-2-methylene-Bicyclo[5.2.0] nonane		Unknown	Unknown	
4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-Cycloheptane		Unknown	Unknown	
3-(4-Hydroxybutyl)-2-methylcyclohexanone		Unknown	Unknown	
1,4,4a, 5,6,9,10,10a-octahydro-11,11-dimethyl-1,4-Methanocycloocta[d] pyridazine		Unknown	Unknown	
Guaia-1 (10),11-diene		Sesquiterpene	Unknown	

Acknowledgements

The authors hereby acknowledge each contribution made toward the successful completion of this study

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Katkar KV, Suthar AC, Chauhan VS. The chemistry, pharmacologic, and therapeutic applications of *Polyalthia longifolia*. Pharmacogn Rev 2010;4:62-8.
2. Singh R. Medicinal plants: A review. J Plant Sci Special Issue Med Plants 2015;3:50-5.
3. Sammbamurty AV, Subrahmanyam NS. A Text Book of Modern Economic Botany. Vol. 8. CBS Publishers and Distributors; Pvt. Ltd., India; 1998. p. 239.
4. Prusti A, Mishra SR, Sahoo S, Mishra SK. Antibacterial activity of some Indian medicinal plants. Ethnobot Leaflets 2008;12:227-30.
5. Olajuyigbe OO, Onibudo TE, Coopoosamy RM, Ashafa AO, Afolayan AJ. Bioactive compounds and *in vitro* pharmacological effects of ethanol stem bark extracts of *Trilepisium madagascriense* DC. Int J Pharmacol 2018;14:901-12.
6. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phytother Res 2007;21:1142-5.
7. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide. Immunopharmacol Immunotoxicol 2007;29:81-93.
8. Tonthubthimthong P, Chuaprasert S, Douglas P, Luewisuttichat W. Supercritical CO2 extraction of nimbin from neem seeds an experimental study. J Food Eng 2001;47:289-93.
9. Anupam G, Das BK, Chatterjee SK, Chandra G. Antibacterial potentiality and phytochemical analysis of mature leaves of *Polyalthia longifolia* (Magnoliales: Annonaceae). South Pac J Natural Sci 2009;26:68-72.
10. Kuo RY, Chang FR, Wu YC. Chemical constituents and their pharmacological activities from Formosan Annonaceous plants. Chin Pharma J 2002;54:155-73.
11. Sundaresan S, Senthilkumar B. A survey of traditional medicinal plants from the Vellore District Tamil Nadu, India. Int J Ayurveda and Herbal Med 2013;3:1347-55.
12. Raghunathan K, Mitra R, editors. Pharmacognosy of Indigenous Drugs. New Delhi: Central Council for Research in Ayurveda and Siddha; 1982. p. 127-36.
13. Faizi S, Khan RA, Azher S, Khan SA, Tauseef S, Ahmad A. New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. pendula. Planta Med 2003;69:350-5.
14. Faizi S, Mughal NR, Khan RA, Khan SA, Ahmad A, Bibi N, *et al.* Evaluation of the antimicrobial property of *Polyalthia longifolia* var. pendula: Isolation of a lactone as the active antibacterial agent from the ethanol extract of the stem. Phytother Res 2003;17:1177-81.
15. Faizi S, Khan RA, Mughal NR, Malik MS, Sajjadi KE, Ahmad A. Antimicrobial activity of various parts of *Polyalthia longifolia* var. pendula: Isolation of active principles from the leaves and the berries. Phytother Res 2008;22:907-12.
16. Chen CY, Chang FR, Shih YC, Hsieh TJ, Chia YC, Tseng HY, *et al.* Cytotoxic constituents of *Polyalthia longifolia* var. pendula. J Nat Prod 2000;63:1475-8.
17. Chang FR, Hwang TL, Yang YL, Li CE, Wu CC, Issa HH, *et al.* Anti-inflammatory and cytotoxic diterpenes from formosan *Polyalthia longifolia* var. pendula. Planta Med 2006;72:1344-7.
18. Saleem R, Ahmed M, Ahmed SI, Azeem M, Khan RA, Rasool N, *et al.* Hypotensive activity and toxicology of constituents from root bark of *Polyalthia longifolia* var. pendula. Phytother Res 2005;19:881-4.
19. Osuntokun OT, Olanbiwonnu AA, Orimolade GF. Assessment of antibacterial, phytochemical properties and GCMS profiling of crude *Polyalthia longifolia* extract. Int J Med Pharm Drug Res 2017;1:12-27.
20. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002.
21. Rajeshwari S, Balakrishnan A, Thenmozhi M, Venckatesh R. Preliminary phytochemical analysis of *Aegel marmelos*, *Ruta graveolens*, *Opuntia dellini*, Euphorbia royleana and Euphorbia antiquorum. International J Pharmaceut Sci Res 2011;2:146-50.
22. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Dieases (ESCMID). EUCAST Definitive Document E.DEF 3.1, June 2000: Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clin Microbiol Infect 2000;6:509-15.
23. Srinivasulu B, Prakasham RS, Jetty A, Srinivas S, Ellaiah P, Ramakrishna SV. Neomycin production with free and immobilized cells of Streptomyces marinensis in an airlift reactor. Process Biochem 2002;38:593-8.
24. Olajuyigbe OO, Afolayan AJ. *In vitro* antibacterial and time-kill evaluation of the *Erythrina caffra* Thunb. extract against bacteria associated with diarrhoea. ScientificWorldJournal 2012;2012:738314.
25. Adaramola B, Benjamin G, Otuneme O, Fapohunda S. Antimicrobial and antioxidant activities of crude methanol extract and fractions of *Andrographis paniculata* leaf (Family: Acanthaceae) (Burm. f.) Wall. Ex Nees. Jordan J Biol Sci 2018;11:23-30.
26. Jeong JB, Hong SC, Jeong HJ, Koo JS. Anti-inflammatory effect of 2- methoxy-4-vinylphenol via the suppression of NF-κB and MAPK activation and acetylation of histone H3. Arch Pharmacol Res 2011;34:2109-16.
27. Türkez H, Celik K, Toğar B. Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells *in vitro*. Cytotechnology 2014;66:597-603.
28. Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. Phytomedicine 2010;17:1061-6.
29. Pakkirisamy M, Kalakandan SK, Ravichandran K. Phytochemical screening, GC-MS, FT-IR Analysis of methanolic extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacog J 2017;9:952-6.
30. Santos da Silva GN, Pozzatti P, Rigatti F, Hörner R, Alves SH, *et al.* Antimicrobial evaluation of sesquiterpene α-curcumene and its synergism with imipenem. J Microbiol Biotechnol Food Sci 2015;4:434-6.
31. Tung YT, Chua MT, Wang SY, Chang ST. Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. Bioresour Technol 2008;99:3908-13.
32. Hammami S, Jmii H, El Mokni R, Khmiri A, Faidi K, Dhaouadi H, *et al.* Essential Oil composition, antioxidant, cytotoxic and antiviral activities of *Teucrium pseudochamaepitys* growing spontaneously in Tunisia. Molecules 2015;20:20426-33.
33. Zheng GQ, Kenney PM, Lam LK. Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. J Nat Prod 1992;55:999-1003.
34. Omnia Gamal ED, Eman S, Amal H, Rokia A, Abdallah M, Nabil A, *et al.* Phytochemical and biological investigation of *Spergularia marina* (L.) Griseb. growing in Egypt. Nat Prod Sci 2014;20:152-9.
35. Duke JA, Bogenschutz-Godwin MJ, DuCellier J, Duke PK. Phytochemical and ethnobotanical databases. Dr. Duke's phytochemical and ethnobotanical databases. In: Handbook of Medicinal Herbs. 2nd ed. Boca Raton; 2002. Available from: http://www.ars-grin.gov/cgi-bin/duke. [Last accessed on 2019 Mar 14].
36. Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. Eur Rev Med Pharmacol Sci 2011;15:775-80.
37. Hameed IH, Altameme HJ, Idan SA. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. Res J Pharmaceut Biol Chem Sci 2016;7:1843-68.
38. Santos CC, Salvadori MS, Mota VG, Costa LM, Cardoso de Almeida AA, Lopes de Oliveira GA, *et al.* Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci J 2013;2013:949452.
39. Rajab MS, Cantrell CL, Franzblau SG, Fischer NH. Antimycobacterial activity of (E)-phytol and derivatives: A preliminary structure-activity study. Planta Med 1998;64:2-4.
40. Balamurugan A, Michael ER, Parthipan B, Mohan VR. GC-MS analysis of bioactive compounds from the ethanol extract of leaves of *Neibyhria apetala* dunn. Int R J Pharm 2017;8:72-8.
41. Balick JM, Cox PA. Plants, People and Culture: The Science of Ethnobotany. New York, NY: Scientific American Library; 1996.
42. Andre CM, Larondelle Y, Evers D. Dietary antioxidants and oxidative stress from a human and plant perspective: A review. Curr Nutr Food Sci 2010;6:2-12.
43. Kose EO, Akta, Deniz IG, Sarik C. Chemical composition, antimicrobial and antioxidant activity of essential oil of endemic Ferula lycia Boiss. J Med Plant Res 2010;4:1698-703.
44. Holst B, Williamson G. Nutrients and phytochemicals: From bioavailability to bio-efficacy beyond antioxidants. Curr Opin Biotechnol 2008;19:73-82.
45. Rahman S, Akbor MM, Howlader A, Jabbar A. Antimicrobial and cytotoxic activity of the

alkaloids of amalaki (*Emblica officinalis*). Pak J Biol Sci 2009;12:1152-5.

46. Dholi SK, Raparla R, Mankala SK, Nagappan K. *In vivo* antidiabetic evaluation of Neem leaf extract in alloxan induced rats. J Appl Pharmaceut Sci 2011;1:100-5.
47. Ros E. Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis 2000;151:357-79.
48. Nyamai DW, Arika W, Ogola PE, Njagi EN, Ngugi MP. Medicinally important phytochemicals: An untapped research avenue. Res Rev J Pharmacog Phytochem 2016;4:35-49.
49. Prakash D, Gupta C, Sharma G. Importance of phytochemicals in nutraceuticals. J Chin Med Res Dev 2012;1:70-8.
50. Selvan RT, Mohideen AK, Sheriff MA, Azmathullah NM. Phytochemical screening of *Acalypha indica* L. leaf extracts, Int J Appl Technol 2012;3:158-61.
51. Shuaib M, Ali A, Ali M, Panda BP, Ahmad MI. Antibacterial activity of resin rich plant extracts. J Pharm Bioallied Sci 2013;5:265-9.
52. Stan SD, Kar S, Stoner GD, Singh SV. Bioactive food components and cancer risk 648 reduction. J Cell Biochem 2008;104:339-56.
53. Cieślik E, Greda A, Adamus W. Contents of polyphenols in fruits and vegetables. Food Chem 2006;94:135-42.
54. Prakash D, Kumar N. Cost effective natural antioxidants. In: Watson RR, Gerald JK, Preedy VR, editors. Nutrients, Dietary Supplements and Nutriceuticals. USA: Humana Press, Springer; 2011. p. 163-88.
55. Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Anti-amoebic and phytochemical screening of some Congolese medicinal plants. J Ethnopharmacol 1998;61:57-65.
56. Jain T, Sharma K. Assay of antibacterial activity of *Polyalthia longifolia* Benth. and Hook. leaf extracts. J Cell Tissue Res 2009;9:1817-20.
57. Sharker SM, Shahid IJ. Assessment of antibacterial and cytotoxic activity of some locally used medicinal plants in Sundarban mangrove forest region. Afr J Pharm Pharmacol 2010;4:66-9.