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Phytochemistry and *in-vitro* Antimicrobial Evaluation of *Phragmanthera incana* (Schum.) Balle Extracts on Selected Clinical Microorganisms

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Authors' contributions

This work was carried out in collaboration between all authors. Author OTO designed, carried out the research, wrote and read through the manuscript. Authors ABS and TRF supervised the research. Author FCA carried out the bench work and put the research into writing. Author OAO read through the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Phragmanthera incana*, an African mistletoe hemi-parasitic plant growing on cocoa (*Theobroma cacao*) and kolanut (*Cola nitida*) trees was screened for antimicrobial and phytochemical activities due to their ethno medicinal claims as remedies for gastro intestinal tract infections, wound, diarrhoea, dysentery and skin infections.
Methodology: The antimicrobial activities of hexane, methanol, chloroform, ethyl acetate and aqueous extracts of *P. incana* obtained from cocoa (*Theobroma cacao*) and kolanut (*Cola nitida*) trees were tested *in vitro* against five Gram negative and two Gram positive pathogenic bacteria;

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and three pathogenic fungi species using agar well diffusion technique. Qualitative and quantitative phytochemicals of the plants were determined by chemical methods.
Results: Phytochemical screening of *P. incana* on cocoa and kolanut trees showed the presence of phytochemicals quality and quantity assessments. Varying degrees of inhibitory activities was observed with the solvent extracts of *P. incana* from cocoa and kolanut trees on test bacteria species while the test fungi species were resistant to all the extracts.
Conclusion: This study showed that the methanol and ethyl acetate extracts of *Phragmanthera incana* had more antibacterial activities than other solvents. Antifungal activity was not exhibited by the five extracts of *P. incana* harvested from the two host plants used. *Proteus mirabilis* and *P. aerugiosa* commonly known for their resistant activities to most conventional antibiotics were inhibited by some of the extracts of *P. incana* in this study.

Keywords: Antimicrobial activity; solvent extracts; Phragmanthera incana; phytochemicals.

1. INTRODUCTION

Medicinal plants are frequently used as remedies for many infectious diseases [1]. The treatment and control of diseases by the use of the available medicinal plants in a locality has been helpful and of a priority to majority urban and rural duellers in healing various diseases because of the reliability and stability in plant products for healing [2,3,4]. The use of herbal medicine as alternative therapy has become prevalent throughout the world due to the growing resistance of pathogens to conventional antibiotics [5]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [6,7] to combat the worldwide emergence of multi-drug resistant pathogenic microbes such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and many other ß-lactamase producers; and other intractable diseases of non-microbial infections. Medicinal plants from the tropical and subtropical climates have many medicinal properties against diseases caused by microorganisms.

In plants, the synthesized aromatic substances (metabolites) are used as defensive weapons against predation by microorganisms, insects and herbivores. However, some of these metabolites are involved in pigmentation (tannins and quinines) and formation of plant odour (terpenoids) and flavour (capsaicin). These defensive molecules give plants their medicinal value which is appreciated by human beings because of their importance in health care of individuals and communities [8,9]. Flavonoids, carotenoids, alkaloids, tannins and phenolic compounds widely distributed in plants have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic, etc [10].

The mistletoe plant is an obligate parasite that depends partly on its host to obtain water and minerals but can carry out photosynthesis [11]. It is an evergreen hemi-parasite that can grow in most parts of the globe. It has different families and species that are well known worldwide. They grow primarily on deciduous trees such as oak, elm, fir, pine and apple. They are widely distributed throughout Africa, Europe, North America, and Asia.

In South-Western Nigeria, mistletoe is commonly found growing especially on tree crops like cocoa (*Theobroma cacao* L.), kola [*Cola nitida* (Vent.) Schott & Endl. or *Cola acuminate* (*P. Beauv*.) Schott & Endl.], coffee (*Coffea arabica* L.), bush mango [*Irvingia gabonensis* (Aubrey-Lecomte ex O. Rorke) Baill.] etc. Mistletoe can also be found growing on citrus plants like orange (*Citrus sp*.), guava (*Psidium guajava* L.) etc. [12].

They are highly specialized angiosperms of the Loranthaceae and Viscaceae family. They consist of about 75 genera and 1,000 species of woody plants, many of them are hemi parasites, all of them except three having the mistletoe habit [13,14]. They cause important damages to their hosts with great economic loss [15].

The white-berried mistletoe (*Viscum album*) has been documented as a traditional treatment for diabetes and high blood pressure. Currently, it is best used as an adjuvant therapy with cancer chemotherapy or radiotherapy [16,17]. A decoction of the leaves of mistletoe is traditionally used in the treatment of hypertension to alleviate symptoms such as headache, dizziness, palpitation, etc. For centuries, mistletoe also served as a folk medicine for cancer treatment and the plant has sometimes been used in Europe to treat tumours [18]. It is also used as a digestive aid, heart tonic and treatment of arthritis, amenorrhea, wounds,

asthma, bed wetting, infection, hysteria and other mental disturbances [15].

Mistletoe is employed to correct menstrual disorder, excessive menstruation and uterine haemorrhages; it is also used as blood purifier and an excellent and effective remedy for epilepsy. Mistletoe is a diuretic and depurative, therefore, it increases the production of urine and the elimination of metabolic toxic waste such as uric acid [19].

Plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [20].

Phragmanthera incana is a woody parasitic shrub, stems to 2m long; of secondary jungle and bush savanna areas; from Sierra Leone to West Cameroons and Fernando Po Island (in the gulf of Guinea that forms part of Equatorial Guinea) , and extending across the Congo basin to Zaire and Angola [12]. The plant is very variable in form, common and widely distributed [21]. Young parts are more or less densely covered with brown hairs; berries red. The plant is very variable in the shape and size of the flowers and leaves. It's found on *Alchornea castaneifolia*, *Anacardium occidentale, Aleurites molluccana*, *Bauhinia monandra, Bombax sessile*, *Theobroma cacao* and *Cola nitida* (Flora of West Tropical Africa).

Much research has not been done on *Phragmathera incana* therefore making little information available for its use in disease treatment. In this research, we are investigating the antimicrobial activity and phytochemical screening to evaluate its use in folklore medicine.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Phragmanthera incana growing on Cocoa (*Theobroma cacao*) and Kolanut (*Cola nitida*) trees were collected from Alesan Obolode, Owo, Ondo State, Nigeria. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN) herbarium. A voucher specimen of *Phragmanthera incana* with Forestry Herbarium Index (FHI) 108925 was submitted at the Department of Botany herbarium of the University of Ibadan with University of Ibadan Herbarium (UIH) number 22332.

2.2 Collection of Test Organisms

Five Gram negative pathogenic bacteria; *Proteus mirabilis*, *Aeromonas bestiarum*, *Citrobacter youngae*, *Pseudomonas aeruginosa* and *Morganella morgani* and two Gram positive bacteria; *Bacillus cereus* and *Staphylococcus aureus*; three pathogenic fungi; *Aspergillus niger, A. flavus* and *Trichoderma spp*. were obtained from the Department of Microbiology, Afe Babalola University, Ado-Ekiti. Biochemical tests were done for confirmation of microorganisms based on microscopic appearance and characterization was carried out as described by [22]. The isolates were then stored on slants at 4ºC and on plates at 25ºC for bacteria and fungi respectively.

2.3 Extraction of Plant Sample

Leaves of *Phragmanthera incana* harvested from cocoa and kolanut were air-dried and various weights were extracted with hexane, methanol, chloroform, ethyl acetate and water using cold extraction method for 72 hours. The solvents were distilled over using Rotary Evaporator (model number RE300DB) at a low temperature of about 45ºC and further concentrated on water bath at a low temperature of 40ºC [23,24]. The extracts were decanted and concentrated on water bath at a low temperature of 45ºC to give the crude extracts used for the antimicrobial investigation.

2.4 Antibacterial Activities of *P. incana* Extracts

An overnight culture of each bacterial isolate was prepared by taking two wire loops of the organism from the stock and inoculating each into 5 ml sterile nutrient broth and incubated for 24 hours at 37ºC. From overnight culture, 0.1ml of each organism was taken and put into the 9.9 ml of sterile distilled water to get (1:100) of the dilution of the organism. An aliquot (0.1 ml) was taken from the dilution into a sterile Petri dish, prepared Mueller Hinton agar was then poured into the plate, swirled and allowed to solidify. A sterile cork borer (6 mm) was used to create wells inside the sterile agar plates. The crude extracts were reconstituted with olive oil and introduced into the well. The olive oil served as the negative control and was introduced into a separate well while standard antibiotic discs served as positive control. The plates were allowed to stay on the bench for 2 hours before

incubation at 37ºC for 24 hours. The tests were conducted in triplicates. The antimicrobial activity was determined by measurement of zone of inhibition around each well.

2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) was determined for each of the plant extract showing antimicrobial activity against the test isolates using broth micro dilution method [25]. The MIC values were taken as the lowest concentration of the extracts in the well of the test tube that showed no turbidity after incubation. The turbidity of the wells in the test tube was interpreted as visible growth of microorganisms. The minimum bactericidal concentration (MBC) was determined by sub culturing from each well showing no apparent growth. Least concentration of the extract showing no visible growth on sub culturing was taken as MBC [26,27].

2.6 Agar Well Diffusion Method

Aliquots of spore were prepared by mixing loopful of fungal spores in sterile distilled water. Spore suspension (0.1 ml) was put into a sterile Petri dish, prepared Potato Dextrose agar was then poured into the plate, swirled and allowed to solidify. A sterile cork borer (6 mm) was used to create wells inside the sterile agar plates. The crude extracts were reconstituted with olive oil and introduced into the well. The olive oil served as the negative control and was introduced into a separate well as appropriate while nystatin, a standard antifungal drug served as positive control. The plates were allowed to stay on the bench for 2 hours before incubation at 27ºC for 48 hours. The tests were conducted in triplicates. The antifungal activity was determined by measurement of zone of inhibition around each well.

2.7 Qualitative Phytochemical Determination

2.7.1 Tannins test

Five grams each of plant extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. One millilitre of the filtrate was treated with few drops of Draggendoff's reagent. Blue-black turbidity serves as preliminary evidence of alkaloids [28].

2.7.2 Flavonoids test

Five grams of each extract was shaken with distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence of the presence of saponins [28].

2.7.3 Anthraquinones test

Five grams of each extract was stirred with 100 ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins [28].

2.7.4 Cardiac glycosides (keller-killiani test)

Disposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid determines the presence of phlobatannins [28].

2.7.5 Saponin test

Five millilitres of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of concentrated H_2SO_4. A yellow coloration observation determines the presence of flavonoids [28].

2.7.6 Alkaloids test

A total of 0.5 g of the extract was shaken with 100 ml of benzene and filtered. Five millilitres of 10% ammonia solution was added to the filtrate. The mixtures were shaken and the presence of pink, red or violet colour in the lower phase of the ammonia indicates the presence of free anthraquinones [28].

2.8 Quantitative Phytochemical Determination

2.8.1 Determination of total phenol contents

The total phenolic content of the extracts was determined using a modified Folin-Ciocalteu method [29,30]. 200 µl of sample was mixed with 2.6 ml of distilled water, 200 µl of Folin-Ciocalteu's phenol reagent was added to each tube. The content was vortexed and incubated for 5 min. Then 2 ml of 7% Na_2CO_3 was added to each tube. The content in the tube was vortexed and incubated for 2 hours with intermediate shaker.

The absorbance of samples was in spectrophotometer at 752 nm. Total phenol

contents were expressed as milligrams of Gallic acid per gram of dry extract.

2.8.2 Determination of total flavonoids content

The content of flavonoids was determined using quercetin as a reference compound. Stock solution (0.50 µl) of each extract was mixed with 50 µl of aluminium trichloride and potassium acetate. The absorption at 415 nm was read after 30 minutes at room temperature. Standard quercetin solution was prepared from 0.01 g quercetin dissolved in 20 ml of ethanol. All determinations were carried out in duplicate. The amount of flavonoids in extracts was expressed as quercetin equivalent (QE) /gram dry weight [31,32].

2.8.3 Saponin determination

Spectrophotometric method of [33] was used. Two grammes of finely ground sample was weight into a 250 ml beaker and 100 ml of Isobutyl alcohol was added. The mixture was shaken in a shaker water bath for 5 hours to ensure uniformity in the mixture. The mixture was filtered with No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was again filtered with filter paper to obtain a clean colourless solution. 1 ml of the colourless solution was pipetted into 50 ml volumetric flask, 2 ml of 5% ferric chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 minutes for colour development. The absorbance was read against blank at 380 nm.

2.8.4 Tannin determination

A weight of 0.2 g of finely ground sample was placed into a 500 ml sample bottle. 100 ml of 70% aqueous acetone was added and properly covered. The bottles were kept in shaker water bath for 2 h at 30℃. Each solution was then centrifuged and the sediment was stored in ice. 0.2 ml of each solution was pipitted into test tubes and 0.8 ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folin-ciocateau reagent was added to both sample and standard followed by 2.5 ml of 20% Na_2CO_3 the solutions were then shaken vigorously and allowed to incubate for 40 minutes at room temperature, its absorbance was then read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared [34].

2.8.5 Alkaloid determination

Five grammes sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4minutes. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is then considered as alkaloid which was dried and weighed [35].

$$\text{Alkaloids (\%)} = \frac{W3 - W2}{W1} \times 100$$

3. RESULTS

The antimicrobial activities of hexane, methanol, chloroform, ethyl acetate and aqueous extracts of *P. incana* obtained from cocoa (*Theobroma cacao*) and kolanut (*Cola nitida*) were tested *in vitro* against five Gram negative pathogenic bacteria viz*; Proteus mirabilis, Aeromonas bestiarum*, *Citrobacter youngae*, *Pseudomonas aeruginosa* and *Morganella morgani*; two Gram positive bacteria viz *Bacillus cereus* and *Staphylococcus aureus*; three pathogenic fungi *Aspergillus niger, Aspergillus flavus, Trichoderma* species using agar well diffusion technique. In the qualitative phytochemical screening, mistletoe from Theobroma cacao possessed more of the phytochemical compounds than C*ola nitida*. However, alkaloids content was abundantly more in all plant samples, followed by tannins and cardiac glycoside has the least quality in phytochemical constituents screened from the samples (Table 1).

Table 2 illustrates the quantity of screened phytochemicals from the plant samples. Highest constituents was recorded in alkaloids (2940 mg/100 g), followed by flavonoids (2553 mg/100 g) and least in cardiac glycoside (114 mg/100 g) among the phytochemicals screened. However, in the plant samples, highest phytochemical quantity was recorded in mistletoe from *Theobroma cacao* leaf extract (TCL) and was

least in *Theobroma cacao* stem bark extract (TCS) among other extract samples.

The antibacterial potency of the plant samples is presented in Table 3. Highest inhibition of 21.5 mm was observed on *B. cereus* with methanol extract of *P*. *incana* from kolanut tree, followed by *P*. *mirabilis* with zone of 17.5 mm from ethyl acetate extract. Inhibition of 15.5 mm each was created on *P*. *mirabilis* and *A*. *bestiarum* with ethyl acetate extracts of *P*. *incana* from cocoa tree (CEA) and kolanut tree (KEA) respectively. Least inhibition of 3.5 mm was observed on *P*. *aeruginosa* with *P*. *incana* from kolanut tree (KEA). *S*. *aureus* was resistant to all the plants' sample extracts. However, all test bacterial species were resistant to Cocoa mistletoe hexane extract (CHE), Cocoa mistletoe chloroform extract (CCF), Kolanut mistletoe hexane and chloroform extracts (KHE & KCF), Cocoa mistletoe aqueous extract (CAQ) and Kolanut mistletoe aqueous extract (KAQ).

Inhibition created by conventional antibiotics used as positive control is interpreted in Table 4. Highest inhibition of 34.5 mm each was exhibited on *A*. *bestiarum* and *C*. *youngae* by ofloxacin, followed by 20.5 mm each exhibited on *M*. *morgani* and *S*. *aureus* by gentamycin and streptomycin respectively. However, least inhibition of 7.5 mm each was exhibited on *A*. *bestiarum* and *C*. *youngae* by nitrofuratoin among other drugs. While *P*. *mirabilis* was resistant to all conventional drugs, all test bacterial species were resistant to augumentin, tetracycline, contrimozazole, cloxacilin and erythromycin (Table 4).

Nystatin an antifungal used as positive control drug most inhibited *A*. *flavus* with zone of 31.5 mm, followed by *A*. *niger* with 28.00 mm and Trichoderma sp. with 27.00 mm. However, all employed solvent extracts of *P*. *incana* was not effective on any of the test fungal species in this study (Table 5).

Table 1. Qualitative analysis of the phytochemicals in *P. incana* (Schum.) Balle mistletoe and host plants

Parameter	TCL	TCM	TCS	KNL	KNM	KNS
Saponins	++	++	++	++	+++	+
Alkaloids	+++	++++	+++	++	++	++++
Tannins	++++	+++	+++	+	+++	+++
Flavonoids	+++	-	+	++	++	-
Anthraquinones	++	+++	+++	++	+++	++
Cardiac glycosides	+	++	+	++	+	+
Carotenoids	++++	++	+	+	+++	-

Legend: TCL = Theobroma cacao leaf extract, TCM = Mistletoe from Theobroma cacao tree extract, TCS = Theobroma cacao stem bark extract, KNL = Cola nitida leaf extract, KNM = Mistletoe from Cola nitida tree extract, KNS = Cola nitida stem bark extract

+ = Trace, ++ = Present, +++ = Abundant, ++++ = Much Abundant, - = Absent

Table 2. Mean values of quantitative phytochemicals analysis of *P. incana* (Schum.) Balle from cocoa and kolanut trees

Parameters	TCL	TCM	TCS	KNL	KNM	KNS
Saponins (mg/100 g)	612	520	250	493	1642	80
Alkaloids (mg/100 g)	2547	3647	1420	1650	1523	2940
Tannins (mg/100 g)	169	143	522	66	134	124
Flavonoids (mg/100 g)	2553	20	107	1733	1817	12
Anthraquinones (mg/100 g)	98	158	293	113	174	90
Cardiac glycosides (mg/100 g)	14	85	32	114	14	26
Carotenoids (µg/100 g)	1957	947	253	337	1723	53

Values are expressed in dry weight of plant samples

Legend: TCL = Theobroma cacao leaf extract, TCM = Mistletoe from Theobroma cacao tree extract, TCS = Theobroma cacao stem bark extract, KNL = Cola nitida leaf extract, KNM = Mistletoe from Cola nitida tree extract, KNS = Cola nitida stem bark extract

Table 3. Zones of inhibition (mm) of extracts of *P. incana* from Cocoa and Kolanut trees on test bacteria species

	CHE	CME	CCF	CEA	KHE	KME	KCF	KEA	CAQ	KAQ
Proteus mirabilis	-	10.5	-	15.5	-	-	-	17.5	-	-
Aeromonas bestiarum	-	-	-	15.0	-	-	-	15.5	-	-
Citrobacter youngae	-	-	-	6.5	-	-	-	10.5	-	-
Pseudomonas aeruginosa	-	12.0	-	13.0	-	-	-	3.5	-	-
Morganella morgani	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-
Bacillus cereus	-	14.5	-	-	-	21.5	-	-	-	-

Legend: CHE = Cocoa mistletoe hexane extract, CME = Cocoa mistletoe methanol extract, CCF = Cocoa mistletoe chloroform extract, CEA = Cocoa mistletoe ethyl acetate extract, KHE = Kolanut mistletoe hexane extract, KME = Kolanut mistletoe methanol extract, KCF = Kolanut mistletoe chloroform extract, KEA = Kolanut mistletoe ethyl acetate extract, CAQ = Cocoa mistletoe aqueous extract, KAQ = Kolanut mistletoe aqueous extract

Table 4. Zones of Inhibition (mm) of conventional drugs (control) on test bacteria species

Bacteria species	GAM	CHM	OFL	NAL	NIT	AUG	TET	COT	CXC	ERY	STREP
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-
Aeromonas bestiarum	14.5	-	34.5	19.5	7.5	-	-	-	-	-	-
Citrobacter youngae	10.5	-	34.5	20.5	7.5	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	19.5	-	-	-	-	-	-	-	-
Morganella morgani	20.5	-	21.5	-	-	-	-	-	-	-	-
Staphylococcus aureus	15.5	12.5	-	-	-	-	-	-	-	-	20.5
Bacillus cereus	14.5	10.5	-	-	-	-	-	-	-	-	12.5

Legend: GAM = Gentamycin (10 µg), CHM = Chloramphenicol (10 µg), OFL = Ofloxacin (5 µg), NAL = Nalidixic acid (5 µg), NIT = Nitrofuratoin (300 µg), AUG = Augmentin (30 µg), TET = Tetracyclin (10 µg), COT = Cotrimozazole (25 µg), CXC = Cloxacillin (30 µg), ERY = Erythromycin (10 µg), STREP = Streptomycin (10 µg)

Table 5. Zones of inhibition (mm) created by *Phragmanthera incana* extracts harvested from cocoa and kolanut trees on different fungi species

Fungi species	NST	CHE	CME	CCF	CEA	KHE	KME	KCF	KEA	CAQ	KAQ
Aspergillus niger	28.0	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	31.5	-	-	-	-	-	-	-	-	-	-
Trichorderma spp.	27.0	-	-	-	-	-	-	-	-	-	-

Legend: NST = Nystatin (control), CHE = Cocoa mistletoe hexane extract, CME = Cocoa mistletoe methanol extract, CCF = Cocoa mistletoe chloroform extract, CEA = Cocoa mistletoe ethyl acetate extract, KHE = Kolanut mistletoe hexane extract, KME = Kolanut mistletoe methanol extract, KCF = Kolanut mistletoe chloroform extract, KEA = Kolanut mistletoe ethyl acetate extract, CAQ = Cocoa mistletoe aqueous extract, KAQ = Kolanut mistletoe aqueous extract

4. DISCUSSION

The study of plant extracts have helped in many ways to identifying the active principle for physiological and pharmacological responses for the development of therapeutic drugs for the use in humans and animals. Valuable antibacterial was observed with extracts of *P. incana* on some of the test bacterial species while the fungal species employed in this study were all resistant. The observed antibacterial activities of the various crude extracts against the test bacteria may be as a result of the presence of secondary metabolites which are known to possess antimicrobial activities [36]. It may also have involved complex mechanisms like the inhibition of the synthesis of cell wall, cell membrane, nucleic acid and proteins as well as the inhibition of the metabolism of nucleic acids [37]. Outside the presence of secondary metabolites and mechanisms of action of antimicrobial agents on test bacteria, the antimicrobial action of the extracts could also be ascribed to the anionic components such as thiocyanate, nitrate,

chlorides and sulphates besides other water soluble components which are naturally occurring in the plant material [38].

However, the antibacterial and phytochemical indices obtained from the extracts further justified plants as important source of potentially useful structures for the control of bacteria from disease manifestation. With its antibacterial activity and phytochemical screening, it suggests that development of new chemotherapeutic agents may be possible. The first step towards this goal is the *in vitro* antibacterial activity assay where inhibition of test crude plant extracts were comparable with some of purified commercial antibiotics used as positive control. Many reports are available on the ethnomedicinal uses of *P*. *incana* for antiviral, antibacterial, antifungal, anthelmintic, anti-Mollusca and anti-inflammatory [39,40,41,42]. Secondly, the phytochemicals such as flavonoids, phenols, tannins and saponins which have been reported of their innumerable values in health care system and harmless to human system. Some few examples are the tannins which helps in blocking the activity of cancer causing agent and helps to inhibit hormone related cancer such as the ovarian cancer, phenol helps to protect the human system against cancer in the stomach, while flavonoid helps in the adsorption of vitamin C and its prevention from oxidation [43]. Alkaloids are the most efficient therapeutic plant substance. Both natural and synthetic alkaloids are used as basic medicinal agent because of their analgesic, antispasmodic and antibacterial properties [44,45].

The phytochemical analysis carried out in the course of this study showed that *P. incana* contained alkaloids, tannins, flavonoids, phenols and cardiac glycosides which are similar to the findings of [46].

The methanol and ethyl acetate extracts of the mistletoe leaves were better than the hexane (non polar), chloroform (moderately polar) and aqueous solvents because they dissolve more organic compounds which are polar and moderately polar in nature, leading to the liberation of greater amounts of active antimicrobial components [47].

5. CONCLUSION

This study on *Phragmanthera incana* extracts from two economic plants showed antimicrobial potency on test microorganisms proving its utilization as antimicrobial agent in ethno medicine. The Ethyl acetate and methanol extracts of *P. incana* harvested from kolanut showed more inhibitory potentials than the cocoa counterpart; though, the antimicrobial potencies from both plants demonstrated competitive inhibitory activities on most of the tested microbes. Further research is required for the isolation and characterization of the bioactive compound(s) present in these extracts. The test plants extracts fared better in their antibacterial activity than antifungal activities as emphasized with the results of study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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