ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL COMPOSITION OF CRUDE ETHYL ACETATE EXTRACTS OF *ANISOPUS MANNII*ON SOME SELECTED CLINICALLY IMPORTANT MICROORGANISMS.

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ABSTRACT - *Anisopusmannii*(Asclepiadaceae) –is a glabrous twining shrub, strong climber with greenish flower in globose, lateral umbelliform cymes it is widely used as an anti-diabetic agent in Northern and southern Nigerian traditional medicine, Studies were conducted to determine the phytochemical constituents of leaf and bark ethyl acetate extracts of *Anisopusmannii* and to evaluate its antimicrobial activity, using the agar well diffusion technique using standard phytochemical analysis procedures, results revealed the presence of saponins, alkaloids, flavonoids, glycosides, and tannins. steroids and anthraquinones were not detected in the extracts. Antimicrobial activities were assayed using the concentrations of 60mg/ml, 30mg/ml, 15mg/ml and 7.5mg/ml. the Extracts dose dependently inhibited the growth of *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiellapneumoniae, and pseudomonas aeruginosa.* The minimal inhibitory concentration (M.I.C) values were 15 mg/ml for *Escherichia coli,* 30mg/ml for *Staphylococcus aureus, Salmonella typhi,* and 60mg/ml for *Klebsiellapneumoniae* and *Pseudomonas aeruginosa*. The proximate analysis showed that the plant contained low fat (6.50%, 6.18%), high carbohydrate content (42.00%, 40.23%) in leaves and bark extracts respectively.These results suggest thatthe leaf and bark extracts of *Anisopusmannii* could be promising in its potential usefulness for treatment ofbacterial induced ailments and a good dietary source of nutrients.The purpose of this research work is to determine the antimicrobial activities on selected clinical organisms and element composition, Anti-nutrients, Proximate contents of *Anisopusmannii*

KEYWORD - Antimicrobial activity, phytochemical activity, proximate composition, elemental constituent.

INTRODUCTION

Nature has been a source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world's developing countries. (Zolfaghari*et al.,* 2000)Plants are the basis of traditional medicine in Africa and have been used for thousands of years.These plants that possess therapeutic properties or exert beneficial pharmacological effects on animal body are generally designated as "Medicinal Plants".Zolfaghari*et al.,* 2000 . According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals ('phyto: from Greek – *phyto*meaning 'plant') or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests (Abo *et al*.,1991, Liu 2004; Nwezee*t al.,* 2004; Dougharie*t al*., 2009).

Anisopusmannii is a species of flowering plant in the family Asclepiadaceae. It is native to the tropical Americas, including the west indies. It is rarely cultivated, this plant is readily common around us in north part of Nigeria (Hausa) and are commonly used in folk medicine. The plant *Anisopusmannii*(Family Asclepiadaceae) is a perennial herb with leaves spread and petiole 1.3 - 2 cm long, glabrous twining shrub, strong climber with greenish flower in globose, lateral umbelliform cymes and horizontally opposite follicle 6-8inch long and about half inch thick, tapering to a slightly hooked point at the apex (Hutchinson and Dalziel 1963). It is a glabrous twining shrub with leaves petiolate, elliptic, ovate and shortly cuspidate at apex up to 15 cm or more long and

12 cm broad, and the stem twining to a height of 3.7 - 4.6 cm It is known as 'Sakayau' or 'Kashezaki' (meaning sweet killer) among the Hausas of the northern Nigeria, where a cold decoction of the stem is traditionally used as remedy for hyperglycemia (Sani*et al.*, 2009). It is a familiar herb in the traditional medicinal preparations in northern Nigeria, where a decoction of the whole plant is used as a remedy for *diabetes*, diarrhea and pile.Previously, the proximate composition, mineral elements and anti-nutritional factors of *Anisopusmannii* was reported (Aliyu*et al*., 2009).

2.1. MATERIALS AND METHODS

2.1.2. PLANT COLLECTION/SOURCE OF *ANISOPUS MANNI*

The leaf and bark of the selected plants were obtained from a location in the southwestern part of Nigeria, in the tropical rainforest of Ibadan, Oyo State Nigeria at 6.20an on the 12th March, 2016. The plant was authenticated by a certified botanist at the herbarium unit of Department of Plant science and Biotechnology, AdekunleAjasin University, AkungbaAkoko, Ondo State, Nigeria and ObafemiAwolowo University, Ile-Ife. Osun state, Nigeria. The leaves, bark and stem were washed thoroughly with distilled water, stored in air tight containers and kept at room temperature prior to use (Vats and Sardana 2011).and a voucher number AAUA 2132 was assigned to the plant for future reference.

2.1.3. TEST ORGANISMS

The test bacteria used in this study were *Salmonella typhi, Klebsiellapneumoniae, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. They were obtained from the AdekunleAjasin University, AkungbaAkoko, Ondo State, Nigeria and ObafemiAwolowo University, Ile Ife, Osun state, Nigeria. They were isolated on sterile nutrient agar slants and taken to the microbiology laboratory of the AdekunleAjasin University, AkungbaAkoko, Nigeria. All slants of test organisms were kept at -4^{o}C prior to bioassay of the extracts. Extensive biochemical tests were carried out to further confirm all the test bacterial strains (Osuntokun &Olajubu 2014), (Barrow 1995).

2.1.4. PREPARATION OF *ANISOPUS MANNII* EXTRACTS FOR EXTRACTION

All the plant materials obtained were first washed thoroughly with sterile distilled water and air dried at room temperature for about two weeks to ensure that the samples lose most of their moisture content. The following extractions were carried out: aqueous and Ethanol. For each extraction, 250g of each dried plant material was weighed separately into conical flasks containing 750ml each of distilled water and ethanol, the mixtures were initially shaken rigorously and left for 9 days. All mixtures were filtered using sterile Whatman filter papers and the filtrates were collected directly into sterile crucibles. All filtrates obtained were introduced into sterile reaction tubes and heated continuously in water bath at the following temperatures: 78^{O}Cfor ethanol extraction, and 105^{O}C for distilled water. The residues obtained were kept at room temperature (Osuntokun 2015).

2.1.5. STANDARDIZATION OF *ANISOPUS MANNII* EXTRACTS

Using aseptic condition, the extracts is reconstituted by adding 1.2g of each extracts with 5ml of dimethylsulphoxide (DMSO) and 15ml of sterile distilled water making it 60mg/ml. For each extracts,7.5ml of distilled water is measured into three sterile bijou bottle. In bijou bottle A 7.5ml from 60mg/ml extracts was added and in bijou bottle B 2.5ml from 60mg/ml extracts was added and bijou bottle C 2.5ml from bijou bottle A was added. A is 30mg/ml, B is 15mg/ml, C is 7.5mg/ml respectively. (Osuntokun 2014)

2.1.6. STANDARDIZATION OF INOCULUM

Slants of the various organisms were reconstituted using an aseptic condition. Using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, transfer 0.1ml of the isolated colony using a sterile needle and syringe into 9.9ml of sterile distilled water contained in each test tube and then mixed properly. The liquid now serves as a source of inoculum containing approximately 10^{6}cfu/ml of bacterial suspension. (El Astal, 2005).

2.2. ANTIMICROBIAL ASSAY OF *ANISOPUS MANNII* USING AGAR WELL DIFFUSION METHOD.

All antibacterial assays for the plant extracts were carried out by well diffusion technique. All the test organisms were sub-cultured onto sterile Mueller Hinton Agar plates and incubated at 37^{O}C for 18-24h. Five distinct colonies for each organism were inoculated onto sterile Mueller Hinton broth and incubated for 3-4h. All inocula were standardized accordingly to match the 0.5 McFarland standard and this standard was used for all susceptibility tests. All the extracts were reconstituted accordingly into the following concentrations: 60, 30, 15 and 7.5mg/ml; using the Dimethyl Sulphoxide (DMSO). The susceptibility testing was investigated by the Agar well diffusion method. A 0.1 ml of 1: 10,000 dilutions (equivalent to 10^{6}cfu/mL) of fresh overnight culture of the clinical isolates grown in Muller Hinton agar and potato dextrose agar was seeded into 40 mL of Muller Hinton agar, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile cork-borer of 4 mm diameter, equidistant wells were made in the agar. Drops of the re-suspended, (2mL per well) extracts with concentrations between 60 to 7.5 mg/mL were introduced into

the wells till it was filled. Ciprofloxacin 2mg/mL were used as the control experiment. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation at 37^{o}C for 24 hours. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. All experiments were performed in duplicates (Osuntokun 2015). & (Osuntokun 2014). (Osuntokun &Ayodele 2014)

2.3. DETERMINATION OF QUALITATIVEBIOACTIVE COMPONENT (PHYTOCHEMICAL SCREENING), OF*ANISOPUS MANNI*

(i) **Test for Alkaloids**

About 0.2gram was warmed with 2% of H_2SO_4 for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the present of Alkaloids. (Ahmedulla, 1999).

(ii) **Test for Tannins**

One milliliter of the filtrate was mixed with 2mi1 of Fec1$_{3,}$A dark green colour indicated a positive test for the tannins. (Edeoga, 2005)

(iii) **Test for Saponin**

One milliliter of the plant filtrate was diluted with 2 mil of distilled water; the mixture were vigorously shaken and left to stand for 10minutes, during which time, the development of foam on the surface of the mixture lasting for more than 10minutes, indicates the presence of Saponins. (Edeoga, 2005)

(iv) **Test for Anthraquinones**

One milliliter of the plant filtrate was shaken with 10mil of benzene; the mixture was filtered and 5 mil of 10 % (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test (Edeoga, 2005)

(v) **Test for Flavonoid**

About 5 mL of each aqueous extracts was added with 1% NH3 solution. A positive test result was confirmed by the formation of a yellow coloration or turbidity (Ekpo, 2009).

(vi) **Test for Cardiac Glycoside**

About 5 mil of the extract was mixed with 2 mil of glacial acetic acid containing one drop ferric chloride solution. To this, 1 mil of concentrated sulphuric acid was slowly underplayed to the sample mixture. A positive test result was confirmed by the presence of a brown ring at the Interface (Ekpo, 2009).

(vii) Test for steroids

10 mil of each ethanol extract are evaporated to insipient dryness over a steam bath and cooled to room temperature. It was then defatted repeatedly with hexane. The defatted aqueous layer was then warmed over a steam bath to remove the residual hexane. To this, 3 mil of $Fecl_3$ reagent was added and 1 mil of concentrated sulfuric acid was then slowly added. A positive test was evident when a reddish brown coloration occurred. (Guevarra et al 2005).

(viii) Test for Phenols- Total Phenol (Spectrophotometric Methods)

2 g of each sample, 1 ml of diethyl ether was added for defatting. The fat free samples were boiled with 50 ml of ether for 15 min to obtain the phenolic components which were measured at 505 nm following the standard method (Osuntokun 2016)

2.4. QUANTITATIVE METHOD OF ANALYSES OF *ANISOPUS MANNI*

(i) **Saponins**

The grinded plant samples (20 g) were extracted with 20% aqueous ethanol by using a water bath maintained at 55°C, for 4 hour with stirring. After filtration the residue was re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml volume separately (water bath temperature was 90°C). Diethyl ether (20 ml) was used for extraction. The process was repeated three times. The ether layer was removed and 60 ml of n-butanol was added to the water layer. Butanol extract was washed with 5% NaCl aqueous solution. After evaporation, the samples were dried in oven to a constant weight; the saponin content was calculated as percentage of the starting material Guevarra, 2005), (Elias 2007).

(ii) Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath. The dried extracts were weighed andthe test procedure defined by Mahato and Sen 1997 was followed

(iii)**Tannins**

About 500 mg of the plant sample was weighed into a 50 ml plastic bottle. 50 mil of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 mil volumetric flask and made up to the marked level. Then, 5 mil of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M Hcl and 0.008 M potassium ferrocyanide. The absorbance was measured at 550 nm within 10 minutes. The tannins content was calculated using a standard curve of extract. (Mahato, 1997).

(v) **Alkaloids**

Five grams of the plant sample was weighed into a 250 mil beaker and 200mil of 10% acetic acid in ethanol was then be added, the reaction mixture was covered and allowed to stand for 4 hour. These were filtered and the extract will be 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 is complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass. (Mary, 2008)

2.5. DETERMINATION OF PROXIMATE ANALYSIS OF *ANISOPUS MANNI*

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists Methods.

1. Determination of moisture content was done by drying samples in oven (WiseVen, WON-50, Korea) at 110 °C until constant weight was attained (Horwitz, 2003).

2. Nitrogen estimation was carried out by the micro-Kjeldahl (BUCHI, KjelFlex K-360, and Switzerland) method with some modification. (Hussain*et al*., 2011).

3. The crude proteins were subsequently calculated by multiplying the nitrogen content by a factor of 6.25.(Hussain*et al*., 2011) The energy value estimation was done by summing the multiplied values for crude protein,

4 Crude fat and carbohydrate respectively at Water Factors (4, 9 and 4). Crude fats were determined by Soxhlet apparatus using *n*-hexane as a solvent.

5. The ash values were obtained by heating samples at 550 °C in a muffle furnace (Wise Them, FHP-03, Korea) for 3 h.(Hussain*et al*,.2011).

6. The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash content and crude fat from the total dry matter (Horwitz ,2003)..

7. Crude fiber was estimated by acid-base digestion with 1.25% H_2SO_4 and 1.25% NAOH solutions Al-(Harrasi*et al*.,2012).

3.0. RESULTS

In table 3, the yield result of extraction showed that leaf was extracted than bark with values of 9.2g and 8.4g for leaf and bark respectively.

In table 4, *Staphylococcus aureus*had zones of inhibition varied from 17mm (60mg/ml) to 4mm at concentration 7.5mg/ml, *Escherichia coli* showed a zone of inhibition from 15mm to 5mm at 60mg/ml and 7.5mg/ml respectively, *Salmonella typhi*which showed highest zone of inhibition at 60mg/ml and 2mm at concentration 7.5mg/ml, *Pseudomonas aeruginosa*also showed varied zones of inhibition from16mm to 3.0mm at concentration 60mg/ml and 7.5mg/ml respectively, similarly *Klebsiellapneumoniae*showed varying lowest zone of inhibition at varying concentration 12mm to 0mm at 60mg/ml and 7.5mg/ml respectively.

In table 5, *Staphylococcus aureus*had zone of inhibition that varied from 16mm (60mg/ml) to 2mm (7.5mg/ml), *Escherichia coli* 12mm (60mg/ml) to 1mm (7.5mg/ml), *Salmonella typhi*16mm (60mg/ml) to 2mm (7.5mg/ml) *Pseudomonas aeruginosa*14mm at 60mg/ml to 7.5mg/ml, Similarly, *Klebsiellapneumoniae*showed a varied zone of inhibition 14mm (60mg/ml) and 2mm at 7.5mg/ml.

In table 6, The minimal inhibitory concentration (M.I.C) values were 15 mg/ml for *Escherichia coli*, 30mg/ml for *Staphylococcus aureus*, *Salmonella typhi*, and 60mg/ml for *Klebsiellapneumoniae*and *Pseudomonas aeruginosa*.

In table 7, Alkaloid, cardiac Glycoside, Tannins and Flavonoids were confirmed positive in both *Anisopusmannii*leaves and bark extracts while, Phenol and Steroid was confirmed negative in both extracts. Also, Anthraquinones and saponins was positive in *Anisopusmannii*leaf, but were not detected in bark extracts.

In table 8, quantitative analyses of minerals present in plant extracts showed that calcium had the highest value at 30.45mg/100g Lead was not detected in leaf extracts and copper showing the lest value at 0.03mg/100g, potassium showed the highest 29.11mg/100g and copper showed the lowest value at 0.02mg/100g in bark extracts of *Anisopusmannii*.

In table 9, Quantitative Analyses of anti-nutrients present in the plant extracts showed that phylate had the highest value at 15% and tannin showed lowest value of 1.32% for leaf extracts. Alkaloids showed the highest value at 5.20% and Oxalate showed the lowest value at 1.50%

In table 10, quantitative analyses of proximate nutrient composition of the plant extracts was determined, carbohydrates had the highest value in both extracts (42%, 49.23%) and fats showed the lowest value (6.50%, 6.18%)

Table 3: Yield result of *Anisopusmannii* leaves and bark extraction.

Plant part used	**Initial weight**	**Volume of solvent**	**Ethyl acetate**
Leaf	400g	1200ml	9.2g
Bark	400g	1200ml	8.4g

Table 4: Antimicrobial activity of Ethyl Acetate Crude Leaf Extracts

Test Microorganism	Zones of Inhibition (mm)				
	60mg/ml	30mg/ml	15mg/ml	7.5mg/ml	Control
Staphylococcus aureus	17.0	11.0	7.0	4.0	25.0
Escherichia coli	15.0	10.0	6.0	5.0	24.0
Salmonella typhi	18.0	10.0	6.0	2.0	24.0
P. aeruginosa	16.0	11.0	6.0	3.0	22.0
K. pneumonia	12.0	8.0	2.0	0.0	26.0

Zones of inhibition measured in mm

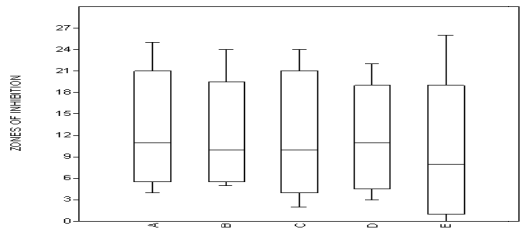

Fig.1 Antimicrobial activities of the *Anisopusmannii* leaf extracts on selected organisms.

Key A: *Staphylococcus aureus*, B: *Escherichia coli* C: *Salmonella typhi*D: *Pseudomonas aeruginosa* E: *Klebsiella*

Table 5: Antimicrobial activity of Ethyl Acetate Crude Bark Extracts

Test organisms	Zones of inhibition (mm)				
	60mg/ml	30mg/ml	15mg/ml	7.5mg/ml	Control
Staphylococcus aureus	16.0	8.0	4.0	2.0	27.0
Escherichia coli	12.0	6.0	3.0	1.0	26.0
Salmonella typhi	16.0	8.0	4.0	2.0	23.0
Pseudomonas aeruginosa	14.0	10.0	4.0	1.0	22.0
Klebsiellapneumoniae	14.0	7.0	4.0	2.0	23.0

Zones of inhibition measured in mm.

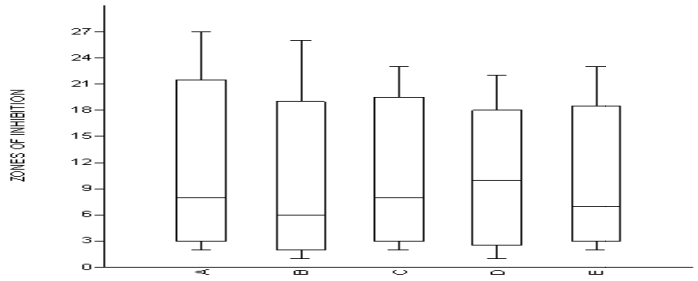

Fig. 2.0 Antimicrobial activities of bark ethyl acetate extracts of Anisopusmannii on selected microorganism.

Key A: *Staphylococcus aureus*, B: *Escherichia coli* C: *Salmonella typhi*D: *Pseudomonas aeruginosa* E: *Klebsiella*

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for Bacteria MIC =7.5mg/ml MBC=3.75 mg/ml Minimum inhibitory concentration (MIC Key: + = Growth - = No Growth

Concentration(mg/ml)	**Test organisms**				
	S typhi	*S aureus*	*E coli*	*K pneumoniae*	*P aeruginosa*
60	-	-	-	-	-
30	-	-	-	+	+
15	+	+	-	+	+
7.5	+	+	+	+	+
3.75	+	+	+	+	+
1.87	+	+	+	+	+
0.94	+	+	+	+	+

TABLE 7: Qualitative Analysis of the Phytochemical Screening of *Anisopusmannii*

SAMPLE	**Alkaloid**	**Cardiac Glycoside**	**Steroid**	**Anthraquinone**	**Phenol**	**Tannins**	**Saponin**	**Flavonoids**
Anisopusmannii leaf	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
Anisopusmannii bark	+ve	+ve	-ve	-ve	-ve	+ve	ND	+ve

TABLE 8- Quantitative Analyses of Minerals Present in *Anisopusmannii* Extracts (mg/100g)

Plant sample used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn
Anisopusmannii leaf	22.73	29.11	30.45	26.05	28.04	7.88	ND	0.03	6.00
Anisopusmannii bark	20.56	22,34	21.89	22.10	20.89	5.92	ND	0.02	6.34

TABLE 9: Quantitative Analyses of Anti-Nutrients Present in *Anisopusmannii* Extracts Result in Percentage (%)

Parameters	Anisopus*mannii* bark	Anisopus*mannii* leaf
Tannin	ND	1.32
Phenol	ND	2.50
Phylate	2.25	15.
Oxalate	1.50	6.59
Saponin	1.67	9.67
Flavonoid	ND	6.41
Alkaloids	5.20	N.D

Key

ND:- Not detected.

TABLE 10: Quantitative Analyses of Proximate Nutrient Composition of *Anisopusmannii*Extracts

S/N	% Ash	% MC	% CP	% Fat	% Fibre	%CHO
Anisopusmanni bark	10.53	9.00	14.45	6.50	10.37	42.00
Anisopusmannii leaf	13.27	13.78	10.45	6.18	23.21	49.23

Key:

MC: Moisture Content , CP: Crude Protein , CHO: Crude Protein

4.0. DISCUSSION

Medicinal plants are showing tremendous promise for preventive intervention in the pathogenesis of many diseases, as well as in their treatment (Atawodi, 2005). This study had used phytochemical, and microbial indices to evaluate the biochemical potentials of *Anisopusmannii*, a local botanical plant used in herbal medicinal practice in some states in Northern Nigeria. The results of the phytochemical analysis of *Anisopusmannii* revealed that the leaves and bark contain saponins, flavonoids, alkaloids, glycosides, terpenes and tannins, while Steroid and anthraquinones were not detected.The above constituents are bioactive compounds with different effects. (Egwaikhide*et al.*, 2008; Sofowora, 1993) This is similar to the findings of Okoli*et al*.,2000) who detected the bioactive compounds such tannins, saponin, flavonoids, cardiac glycoside, alkaloids in *Euphorbia hirta*. Alkaloids present have been reported as one of the largest group of phytochemicals in plant with amazing effects on humans, (Akinpelu*et al.,* 2006) and have been used for treatment of intestinal infections associated with AIDS and hypertension (Mcdevitt*et al., 1996;* Parekh *et al.,* 2007).

Another constituent of leaves *Anisopusmannii*was tannin. (Parekh *et al.*, 2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment ailment of inflamed or ulcerated tissues hence, confirming the studies of Musa, *et al*., 2009 and also it's usage in folkloric medicine as Anti-inflammatory agent Also, the tannin content (1.32%) may be partly responsibility for the bitter principle associated with the leaves. Dharmananda (2003) reported that herbs that have tannin as their main components are astringent in nature and are used for treating disorder such as diarrhea and dysentery, Presence of Tannin in plant has been reported by Igboko, (1983) to have astringent properties, hastens the healing of wounds and inflamed mucous membrane, haemorrhoid, frost-bite and burn in herbal medicine (Igboko, 1983; Maduyi, 1983), thereby giving credence to its use as treatment for haemorrhoids. Studies have shown that saponin which was also detected have been used for treatment of hyperglycaemia and that dietary source of saponins offer preferential chemopreventive strategy in lowering the risk of human cancer (Olaleye*et al., 2007;* Hodek *et al.,* 2002*).*

Also,Saponin has relationship with sex hormones thereby giving credence to its use as local Aphrodisiac agent. Flavonoids, another constituent of *Anisopusmannii*extracts have been reported of wide range of biological activities like antimicrobial, anti-inflammatory, analgesic, anti-allergic, cytostatic and antioxidant properties. (Das *et al., 1989*).The result of minerals composition, clearly shows that the plant is rich in useful mineral elements such as K, Na, Mg, Ca, in this study lead was not detected.

The microbial inhibitory effects of the extracts produced a dose dependent zone of inhibition in all the organisms tested. However, the effects observed were less than those produced by the standard agent (Ciprofloxacin).The antimicrobial activity exhibited by this leaf and bark was due to the presence of certain phytochemicals reported in this phytochemical screening such as alkaloids, saponin, tannin, flavonoids and cardiac glycoside. This is similar to the findings of okoli*et al*.,2000, who reported that methanolicleaf extracts of *Synclisiascabrida*exhibited significant activity against the pathogens tested due to the presence of high amount of flavonoids and alkaloids that are also known to possess antimicrobial activity

The minimal inhibitory concentration (M.I.C) values were 15 mg/ml for *Escherichia coli*, 30mg/ml for *Staphylococcus aureus*, *Salmonella typhi*, and 60mg/ml for *Klebsiellapneumoniae* and *Pseudomonas aeruginosa*. The organic constituents present in the extracts have been reported to possess some medicinal uses (Blumgerter, 1989; Ogundipe*et al.,* 1998; Abdulrahaman and Onyeyili, 2001). Flavonoids were reported to possess biological activity against microbes (Kinsella, 1993). Extracts of the seeds of *Tetracarpidiumconophorum*was reported to possess antimicrobial activity which was associated with its alkaloids, saponins, tannins, flavonoids, and glycosides contents (Isaac and Chinwe, 2001). (Sanni*et al.,* 2007) reported that the antibacterial activity of the aqueous leaf extracts of *Ocimumbasilicum*was due to the presence of flavonoids in the plant. The antibacterial activity of *Anisopusmannii*as recorded in this study may therefore be due mainly to the presence of flavonoids, saponins, glycosides and alkaloids in the extracts.

5.0. CONCLUSION

This present study has shown the antimicrobial activity *and* phytochemicals, and minerals composition of *Anisopusmannii* leaves and bark extracts. This partly shows the use of this plant in herbal medicine. As a rich source of phytochemicals, coupled with the presence of essential minerals.It has been concluded that the bark and leaves ethyl acetate extracts of *Anisopusmannii*possess antibacterial activity for treating diseases and infection. Its potential application in the treatment of bacterial infection would therefore be promising.Further studies have to be carried out to isolate, characterize and elucidate the structure of the bioactive compounds from the plant for industrial drug composition formulation.

RECOMMENDATION

It is thereby recommended that the medicinal plant such as *Anisopusmannii* and other types of medicinal plants should be studied and exploited for future use.

CONSENT - It is not applicable.

ETHICAL APPROVAL -It is not applicable.

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COMPETING INTERESTS - Authors have declared that no competing interests exist.

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