

Assessment of *Andrographis paniculata* and *Aframomum melegueta* on Bacteria Isolated from Wounds

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ABSTRACT

Reason: *Andrographis paniculata* and *Aframomum melegueta* are plants commonly used traditionally for the management of skin diseases. The scientific basis for the use of these plants' extracts for wound healing and skin diseases remains poorly understood.

Methods: Powdered dry samples (50 g) of *Andrographis paniculata* leaves and *Aframomum melegueta* seeds were both extracted in ethanol and n-hexane separately. Bacteria isolates were obtained from the Ekiti State University Teaching Hospital, Ado-Ekiti, while organisms were sub-cultured on nutrient agar slants at 37°C for 24 hours before using for this investigation.

Main Findings: The results showed that both ethanol and hexane extracts of *A. melegueta* and *A. paniculata* inhibited the growth of all the bacteria tested. At concentrations of 150 and 200 mg/ml, the *A. paniculata* hexane extract was more potent than the ethanol extract and highly effective against the *Corynebacterium accolens* with zones of inhibition of 8.5 mm, 10 mm and 12 mm while *A. melegueta* ethanol extract was observed to be more potent than the hexane extract at the same concentrations. *Aframomum melegueta* and effective against *Klebsiella pneumoniae* with the highest zones of inhibition of 10.5mm and 12mm.

Conclusion: Both plants can be concluded to have broad spectrum antibacterial activity confirming them as effective antimicrobial plants useful in the management of skin diseases.

KEY WORDS: Antibacterial, *Aframomum melegueta*, *Andrographis paniculata*, Skin diseases.

1. INTRODUCTION

Andrographis paniculata of the Family Acanthaceae, commonly known as king of bitters, is a traditional Chinese, Southeast Asian and Indian herb used for centuries in Ayurvedic medicine. The herb has been revered for treating numerous infectious diseases and also highly regarded as having a preventive effect from many diseases including skin conditions (Hancke, 1995; Melchior, 2004), liver conditions (Churvedi, 1983; Trivedi and Rawal, 2001), infections like leprosy, pneumonia, tuberculosis and HIV/AIDS (Calabrese, 2000) due to its powerful immune strengthening benefits.

Aframomum melegueta belongs to the Family Zingiberaceae of the Angiosperms in the Kingdom Plantae. It is commonly used by many traditional healers in African ethno-medicine as a remedy for variety of ailments especially in the treatment of skin diseases including wounds such as sores, bites, burns and lacerations. In traditional medical practice (TMP), the powdered seed mixed with some other ingredients are mostly applied topically on incisions made by herbal practitioners on their patients. According to Okeke (2006), this form of traditional herbal medicine practice has made a significant contribution to the healthcare provision for rural communities. A high economic value has been placed on both plants due to their high demand in the herbal markets. However, the scientific basis for the use of these plants' extracts for skin related diseases especially wound healing remains poorly understood. The present investigation was carried out to study the antibacterial potential of the extracts of these plants on bacteria isolated from wound infections.

2. MATERIALS AND METHODS

Collection of Bacteria Isolates: Bacteria isolates used were obtained from Ekiti State University Teaching Hospital's Microbiology Laboratory, Ado-Ekiti, Ekiti State, Nigeria from March to April, 2016. The bacteria collected include *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 25923, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Klebsiella pneumoniae* ATTC 43816, *Pseudomonas aeruginosa*, *Corynebacterium accolens* and *Escherichia coli*. The organisms were then sub-cultured on nutrient agar slants, maintained at 37°C for 24 hours and used for investigation in the Microbiology Laboratory of Afe Babalola University, Ado Ekiti.

Collection and Preparation of Plant Materials: *Andrographis paniculata* leaves were sourced from Kogi State, Nigeria. *Aframomum melegueta* seeds were purchased from the herbal market (Oja Oba) in Ado-Ekiti of Ekiti State, Nigeria. Both plant parts were air dried at room temperature and ground to powdered form. The samples were then stored in screw cap containers until use.

Preparation of Plant Extracts: Powdered samples of *Andrographis paniculata* and *Aframomum melegueta* were extracted using ethanol and hexane. Fifty gram (50 g) of dry powder of *A. paniculata* leaf was soaked in 600 ml of hexane and 600 ml of ethanol respectively. 50 g of powdered *Aframomum melegueta* was also soaked in 600 ml of hexane and ethanol respectively for 72 hours. Both samples were refluxed with 200ml of each solvent for 24 hours,

after which they were filtered properly with muslin cloth and the extracts were collected in separate beakers. The samples were evaporated on water bath at 50°C and kept in the refrigerator until use.

Phytochemical Screening: Qualitative phytochemical screening of the plant extracts was carried out for the presence of secondary metabolites including terpenoids, steroids, tannins, flavonoids, glycosides, saponins, alkaloids, phenols, cardiac glycosides and anthraquinones according to the method of Sofowora, 2008.

Screening for Antibacterial Activity: The agar well diffusion method was employed for the determination of antibacterial activities of the extracts of *A. paniculata* and *A. melegueta* using Mueller-Hinton agar (NCCLS, 1993). The plant extracts were dissolved in Dimethyl sulfoxide (DMSO) at different concentrations of 200, 150 and 100 mg/ml. Wells of 7 mm diameter were bored on agar that was streaked with each bacteria isolate. The different extract concentrations were added into the wells; the plates were left for 15 minutes and incubated at 37°C for 24 hours, after which the zones of inhibition were measured and recorded.

Antibiotic Susceptibility Test: Using the agar disc diffusion method according to Murray (1995), the test organisms were suspended in nutrient broth in comparison to 0.5 McFarland standard and the organisms were incubated for 24 hours. Each of the isolates was inoculated by using a sterile cotton swab stick to streak it evenly on the surface of the sterile Mueller Hinton agar plates. The antibiotic discs were placed aseptically on the surface of the agar plates using sterile forceps. The plates were then inverted and incubated for 24 hours at 37°C. The antimicrobial disc used include the Gram negative disc comprising of Augmentin 30 µg, Amoxicillin 30 µg, Septrin 30 µg, Chloramphenicol 30 µg, Sparfloxacin 10µg, Ciprofloxacin 10 µg, Gentamicin 10 µg, Pefloxacin 30 µg, Tarivid 10 µg and Streptomycin 30 µg and the Gram positive disc comprising of Erythromycin 10 µg, Pefloxacin 10 µg, Gentamicin 10 µg, Ampiclox 30 µg, Streptomycin 30 µg, Ciprofloxacin 5 µg, Zinnacef 20µg, Amoxacillin 30 µg, Rocephin 25 µg and Septrin 30 µg. The diameters of the zones of inhibition of the organisms were measured in duplicates with a ruler and the mean values of each set of readings were obtained.

3. RESULTS AND DISCUSSION

Extracts were gotten from the plant leaf of *Andrographis paniculata* and *Aframomum melegueta* seed using two solvents: Ethanol and Hexane. Their antimicrobial activity was tested against bacterial isolated from wounds obtained from Ekiti State University Teaching Hospital. The antibacterial activity was expressed as the average diameter of the zone of inhibition of bacterial growth around the disc.

A total of eight isolated bacterial species were used, comprising of five gram negatives including *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Klebsiella pneumoniae* ATCC 43816 and three gram positives including *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 25923, and *Corynebacterium accolens*.

The results of this study showed that both ethanol (a polar solvent) and Hexane (a non-polar solvent) extracts of *A. melegueta* and *A. paniculata* respectively inhibited the growth of all the bacteria tested. This suggests that both plants have broad spectrum antibacterial activity.

In the antibacterial study of *A. paniculata* extracts, it was observed that the Hexane extract (Table.1) was more effective than the ethanol extract as it showed a higher zone of inhibition at the concentration of 150 and 200 mg/ml when compared with the ethanol extract (Table.2). This showed that the plant contained active constituents which reside more in the non-polar solvent. The hexane extract was also observed to be more effective against *Corynebacterium accolens*, a gram positive bacteria which was found to be resistant to some of the standard antibiotics unlike other gram positives, having the highest zone of inhibition of 12.0mm, followed by 11.5mm for *Staphylococcus aureus* ATCC 25923. This conformed to the report of Sule (2010) and Kumar (2010), stating that the plant showed significant antibacterial activities against tested Gram-positive bacteria.

The various antibacterial activities of *A. paniculata* in the two different solvents (Ethanol and n- Hexane) as seen in these results confirmed the importance placed on these plants by the local users against variety of infections particularly in the treatment of some skin infections.

Aframomum. melegueta extracts unlike that of *A. paniculata* revealed that the ethanol extract was more potent than the hexane extract especially at concentrations of 150mg/ml and 200 mg/ml (Table.4). This suggests that the antibacterial constituents of the plant is likely present in the polar solvent than in the non-polar solvent. It has been reported that Gram-positive bacteria are more susceptible to the action of some drugs due to the simple structure of their cell wall compared to that of Gram-negative bacteria which have greater complexity of the dual cell wall (Rios & Recio, 2005; Cos, 2006). In this study, the Gram-negative bacteria exhibited different characteristics. They were susceptible to the ethanol extract of *A. melegueta* in the same manner they exhibited to the standard drugs (Table.6). This result implied that *A. melegueta* ethanol extract was more effective than the Hexane extract where the zones of inhibition include 11.5 mm for *E. coli*, 14.0mm for *Pseudomonas aeruginosa* and 11.5 mm for *Klebsiella pneumoniae*, which are all pathogenic organisms (Table.4). Adetutu (2011) has reported the importance of *A. melegueta* in the treatment of measles and leprosy, disinfections and other phytomedicinal uses such as purgative, galactagogue, antiparasitic and also for incisions made on the skin. The significant results obtained in this study

justified the antibacterial potential of the two plants investigated, and the value bestowed on their popular usefulness in the treatment of skin infections.

The phytochemical screening of both plant extracts showed the presence of phyto-constituents including phenols, tannins, saponins, flavonoids, glycosides and alkaloids (Table.5). Although, the phytochemical result of *Andrographis paniculata* revealed the presence of glycosides, alkaloids and cardiac glycosides only with both solvent extracts which might be attributed to the antibacterial activity of plants in this study. The antimicrobial effect of *A. paniculata* extracts may be attributed to the presence of these phyto-constituents as shown in Table.5. The biological functions of glycosides include their use in storing up proteins which might be very important in the recovery process of wounds. Cardiac glycosides are useful in the treatment of congestive heart failure and cardiac arrhythmia (Ambrosy, 2014), whereas, alkaloids may act as reservoir for protein syntheses. They may act as protective substances against the animal or insect attacks. They may also function as plant stimulants or regulators in activities such as cell reproduction, growth and metabolism which may aid wound recovery (Devika and Justin, 2014). Saponins have hemolytic, expectorant, anti-inflammatory and immune-stimulating activity and beyond that, saponins demonstrate antimicrobial properties particularly against fungi and additionally against bacteria and protozoa.

Aframomum melegueta ethanol extract composed of phenols, tannins and saponins while the Hexane extract composed of terpenoids, flavonoids and cardiac glycosides. The presence of terpenoids corroborates the report of (Owokotomo, 2014; Ajaiyeoba and Ekundayo, 1999) who reported that *Aframomum melegueta* possesses essential oils in all its parts of plants including stem, root, leaf and seed, in different quality and quantity. A sesquiterpene hydrocarbons known as α -caryophyllene was identified to be the main components followed by β -caryophyllene which may support its usage for wounds, cuts or incisions in order to accelerate their healing. Other secondary metabolites present may be responsible for the usage of the plant in the management of other forms of diseases.

Moreover, previous study has attributed activity of *Aframomum melegueta* to its phenolic contents stating that its phenols and phenolic compounds have been extensively used in disinfection, remained the standard with which other bactericides were compared (Doherty, 2011). Phenolic compounds are believed to be chemo-preventive compounds that may decrease the risk of developing cancer. They also have anti-oxidant effects, they react with and capture dangerously reactive free radicals before the free radicals can react with other biomolecules and cause serious damage. These results can be complemented with the observations of previous work that plants are made up of substances that have antimicrobial properties (Olukoya, 1993; Oladunmoye and Dada, 2007; Sule, 2010; Kumar, 2010; Hannah, 2014).

The antibiotic susceptibility test of the organisms to the conventional antibiotics was presented in Table.6 and Table.7. In the antibiotic susceptibility test, significant resistance was shown by *Corynebacterium accolens* against the antibiotics, erythromycin, pefloxacin, zinacef, ampiclox and gentamycin. Also, *Klebsiella pneumoniae* was highly resistant to the antibiotics, tarivid, pefloxacin, gentamicin, augumentin and amoxicillin, whereas, the *Andrographis paniculata* extracts were highly effective against the *Corynebacterium accolens* with zones of inhibition, 8.5 mm, 10.0mm and 12.0mm (n-hexane extract) and 8.5 mm, 9.0mm and 9.5 mm (ethanol extract). *A. melegueta* extract happens to be the most effective for *Klebsiella pneumoniae* with the highest zones of inhibition of 10.5mm and 12.0mm (for ethanol extract), 7.0mm and 7.5mm (for n-Hexane extract). Although the antibiotics are also highly effective against bacteria isolated from wound infections, these extracts could be considered more effective against *Klebsiella pneumoniae* and *Corynebacteria accolens* making them promising antimicrobial agents for these organisms rather than the less potent antibiotics used.

Table.1. Antimicrobial Activity of n- Hexane Extract of *Andrographis paniculata*

Micro- Organisms	Concentrations			Control
	200 mg/ml	150 mg/ml	100 mg/ml	
<i>Escherichia coli</i>	8.5	8	7	-
<i>Proteus mirabilis</i>	9	7.5	7	-
<i>Pseudomonas aeruginosa</i>	6.5	6	6.	-
<i>Klebsiella pneumonia</i>	8	6	2	-
<i>Klebsiella pneumonia ATCC 43816</i>	9	6.5	6	-
<i>Staphylococcus aureus</i>	10	9.5	8.5	-
<i>Staphylococcus aureus ATCC 25923</i>	11.5	10.5	19	-
<i>Corynebacterium accolens</i>	12	10	8.5	-

Key~ Values represent Mean of three replicates

- : No inhibition

Table.2. Antimicrobial Activity of Ethanol Extract of *Andrographis paniculata*

Micro- Organisms	Concentrations			Control
	200 mg/ml	150 mg/ml	100 mg/ml	
<i>Escherichia coli</i>	2	1.5	0.5	-
<i>Proteus mirabilis</i>	8.5	7.5	7	-
<i>Pseudomonas aeruginosa</i>	7.5	7	5	-
<i>Klebsiella pneumoniae</i>	4	2.5	2	-
<i>Klebsiella pneumonia ATCC 43816</i>	6.5	6	5.5	-
<i>Staphylococcus aureus</i>	7	4	3	-
<i>Staphylococcus aureus ATCC 25923</i>	6	4.5	2.5	-
<i>Corynebacterium accolens</i>	9.5	9	8.5	-

Key~ Values represent Mean of three replicates

- : No inhibition

Table.3. Antimicrobial Activity of Ethanol Extract of *Aframomum melegueta*

Micro-organism	Concentrations			Control
	200 mg/ml	150 mg/ml	100 mg/ml	
<i>Escherichia coli</i>	11.5	11	7	-
<i>Proteus mirabilis</i>	7	6	5.5	-
<i>Pseudomonas aeruginosa</i>	14	9	7	-
<i>Klebsiella pneumoniae</i>	12	10.5	6	-
<i>Klebsiella pneumoniae ATCC 43816</i>	11.5	8.5	4.5	-
<i>Staphylococcus aureus</i>	5.5	5	4.5	-
<i>Staphylococcus aureus ATCC25923</i>	6.5	5	4	-
<i>Corynebacterium accolens</i>	7.5	7	5.5	-

Key ~ Values represent Mean of three replicates

- : No inhibition

Table.4. Antimicrobial Activity of n- Hexane Extract of *Aframomum melegueta*.

Micro- organism	Concentrations			Control
	200 mg/ml	150 mg/ml	100 mg/ml	
<i>Escherichia coli</i>	6	5.5	5.5	-
<i>Proteus mirabilis</i>	4	3.5	3	-
<i>Pseudomonas aeruginosa</i>	7	6	5.5	-
<i>Klebsiella pneumoniae</i>	7.5	7	6	-
<i>Klebsiella pneumonia ATCC 43816</i>	7.5	6.5	5	-
<i>Staphylococcus aureus</i>	4	4	4	-
<i>Staphylococcus aureus ATCC 25923</i>	4.5	4	3	-
<i>Corynebacterium accolens</i>	7	6	5.5	-

Key ~ Values represent Mean of three replicates

- : No inhibition

Table 5. Phytochemical Properties of *Andrographis paniculata* and *Aframomum melegueta*

Parameters	<i>Andrographis paniculata</i>		<i>Aframomum melegueta</i>	
	Ethanol extract	n-Hexane extract	Ethanol Extract	n-Hexane extract
Phenols	-	-	++	--
Tannin	-	-	++	--
Saponin	-	+	+	-
Glycosides	+	++	-	-
Flavonoids	-	+	-	++
Steroids	-	-	-	-
Anthraquinones	-	-	-	-
Terpenoids	-	+	-	++
Cardiac Glycosides	-	+	-	+
Alkaloids	++	-	-	-

Key ~ +: present; ++: highly present; -: absent

Table.6. Antibiotic Susceptibility Test (Gram Positive Isolates)

ORGANISMS	Amoxacillin (30µg)	Rocephin (25µg)	(Ciprofloxacin (10µg)	Streptomycin (30µg)	Septin (30µg)
<i>Staphylococcus aureus</i>	24 (S)	28 (S)	30 (S)	32 (S)	30 (S)
<i>Corynebacterium accolens</i>	16 (S)	34 (S)	40 (S)	32 (S)	12 (S)
<i>Staphylococcus aureus</i> ATCC 25923	22 (S)	27 (S)	28 (S)	30 (S)	32 (S)

ORGANISMS	Erythromycin (10µg)	Pefloxacin (10µg)	Zinnacef (20µg)	Ampiclox (30µg)	Gentamycin (10µg)
<i>Staphylococcus aureus</i>	26 (S)	26 (S)	22 (S)	22 (S)	24 (S)
<i>Corynebacterium accolens</i>	R(0)	R(0)	R(0)	R(0)	R(0)
<i>Staphylococcus aureus</i> ATCC 25923	27 (S)	24 (S)	23 (S)	26 (S)	28 (S)

Key: S: Susceptible R: Resistant

Values represent Mean of three replicates

Table.7. Antibiotic Susceptibility Test (Gram-Negative Isolates)

ORGANISM	Steptomycin(30µg)	Tarivid (10µg)	Pefloxacin (30µg)	Gentamycin(10µg)
<i>Escherichia coli</i>	21 (S)	22 (S)	24 (S)	21(S)
<i>Klebsiella pneumoniae</i>	34 (S)	R	R	R
<i>Proteus mirabilis</i>	24 (S)	32 (S)	34 (S)	30 (S)
<i>Pseudomonas aeruginosa</i>	26 (S)	28 (S)	28 (S)	18 (S)
<i>Klebsiella pneumoniae</i> ATCC 43816	32 (S)	34 (S)	36 (S)	30 (S)

ORGANISM	Amoxacillin (30µg)	Ciprofloxacin (10µg)	Sparfloxacin (10µg)	Chloramphenicol (30µg)	Septin (30µg)
<i>Escherichia coli</i>	11 (S)	31 (S)	21 (S)	20 (S)	22 (S)
<i>Klebsiella pneumoniae</i>	R	28 (S)	26 (S)	28 (S)	32 (S)
<i>Proteus mirabilis</i>	26 (S)	28 (S)	24 (S)	22 (S)	28 (S)
<i>Pseudomonas aeruginosa</i>	26 (S)	34 (S)	32 (S)	30 (S)	24 (S)
<i>Klebsiella pneumoniae</i> ATCC 43816	22 (S)	26 (S)	24 (S)	26(S)	30 (S)

Key: S: Susceptible R: Resistant

Values represent Mean of three replicates

4. CONCLUSION

This research was able to establish the antibacterial activity of *Andrographis paniculata* and *Aframomum melegueta* against organisms that affect the skin. It can be concluded that the *Andrographis paniculata* and *Aframomum melegueta* both have broad spectrum antimicrobial activities making them effective antimicrobial plants useful in the management of skin diseases or wound infections. Apart from the use of antibiotics, *Andrographis paniculata* and *Aframomum melegueta* can be considered promising plants with antimicrobial properties especially in the management of wound infections. They can possibly be a substitute for antibiotics that are not very highly effective against some bacteria that cause wound infections. However, further investigation is needed to be carried out on isolation and characterization of the active principle(s) that may be specifically responsible for the antibacterial activity.

Conflicts of interest: The authors report no conflicts of interest existed in this study.

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