Determination Of Antibacterial Activity And Phytochemistry Of Three Herbal Plants On Clinical Isolates

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Abstract: The antibacterial activity of three plants extract Abrus precatorius, Croton penduliflorus (Seeds) and Nauclea latifolia (Root) were evaluated in an in vitro test on Escherichia coli, Klebsiella pneumonia and Enterococcus faecalis. Twelve extracts consisting of hot ethanol, cold ethanol, hot water and cold water were prepared. They were tested using the agar diffusion method. Efficacy was assessed by measuring the diameter of zone of inhibition around the colonies on Muller Hilton agar medium. E.coli showed the highest susceptibility to all plant extracts this was followed by E. faecalis. K. pneumonia showed the least susceptibility to all the extracts. Across board, the cold ethanol extracts of the plants showed the highest susceptibility while the other extracts exhibited variable antibacterial activities. The minimum inhibitory concentration (MIC) of the eight most active extracts ranged between 3.025mg/ml to 50mg/ml while minimum bactericidal concentration (MBC) was between 6.25mg/ml - 25mg/ml. Commonly used antibiotics such as Augmentin and Amoxillin showed low sensitivity as compared to the extracts with varying inhibition zone of 0-4mm Phytochemical screening of these extracts showed that flavonoid was detected in only A. precatorius, Glycoside was found in all extract except in C. penduliflorus extracts. Alkaloid, saponin and Tannin were found in all the plant extracts. This invitro study demonstrated that folk medicine in addition to being cheaper could be effective as modern medicine in combating pathogenic microorganisms.

Index Terms: Antibacterial, Phytochemical, Antibiotic susceptibility, Plant extracts.

1 INTRODUCTION

Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C and 800 B.C give many references of medicinal plants. One of the remotest works in traditional herbal medicine is “Virikshayurveda”, compiled even before the beginning of Christian era (Himal et al., 2008). Herbal medicine is widely practiced throughout the world today. These medicines are safe and environment friendly. Herbal drugs have become increasingly popular and their use is widespread. Clear-cut proof of their efficacy in microorganisms inducing pathogenesis is yet to be explored (Sankar et al., 2010). Various medicinal plants have been used for years in daily life to treat disease all over the world. The search for new antimicrobial properties of natural products cannot be ignored because they can be found in the most remote part of the world where Doctors are not present. The World Health Organization (WHO) estimates that nearly 80% of the world population depends upon traditional medicine, predominantly originated from plants for their primary Healthcare.

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In Nigeria, there appears to be an overwhelming increase in the public awareness and usage of herbal medical products in the treatments and or prevention of diseases (Oluyege et al., 2010) with this increased usage , the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals. These herbal remedies are often perceived as being natural and therefore safe, but they are not free from adverse effects, which may be due to factors such as adulteration, substitution, contamination, misidentification, lack of standardization, incorrect preparation and dosage, inappropriate labeling and or advertisement (Lau et al., 2003). The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been transmitted orally for centuries is fast disappearing from the face of the earth due to the advent of modern technology and transformation of traditional culture (Ganesan et al., 2004). In these local communities where Medicare is not so easily accessible due to lack of healthcare facilities and the high cost of orthodox treatment, recourse to traditional medicine offers the only hope of staying healthy and alive. In addition, about 200,000 natural compounds are currently known this figure is just a drop of water in the ocean considering the wideness of natural resources. Despite the availability of a vast range of territorial flora, it is estimated that only 5 -15 % of the approximately 250,000 species of higher plants have been investigated chemically and pharmacologically so far. Hence, the potential of large areas of tropical rainforests remains virtually untapped (Maitera et al., 2011). Traditionally, in West and South Africa infusions and decoctions of the stem, bark and leaves of plant are used for the treatment of malaria, stomach ache, fever, diarrhea and nematodes infections in human and animals. The two methods employed by Nigerians to remove debris in the mouth are by tooth brush and paste or by use of parts of various plants native to West Africa, referred to as “African Chewing Sticks” (Bamidele et al., 2013). Abrus precatorius belongs to the Family: Fabaceae, Common Names**:** Crab’s eye, cats eye, Oju ologbo, omisimisi, Indian liquorice, Jequirity and Wild liquorice.A perennial climber, it has pinnately compound leaves and bright red seeds with a black patch on one side of

each seed. Common in forest tracts of Nigeria, India and Burma, ascending to the outer Himalayas. Nauclea latifolia Family: Rubiaceae, Common names: English- Pin cushion tree, African peach, Guinea peach, Sierra Leone peach French- Scille maritime, oignon marine, medicinal squill. African vernacular names: Hausa- Tafashiya, tashiyaigia (medicinally useful bark), Igbo- Ubuluinu. Yoruba- Egbesi. Nauclea latifolia is an evergreen multi-stemmed shrub or a tree. It grows up to altitudes of about 200m. Nauclea latifolia has an open canopy and terminal spherical head lined cymes of white flowers. The flowers are joined with their calyces. It is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. Three other related species Nauclea pobeguini, N. diderichii, and N vanderguchtii are forest trees. N. diderichii is planted in Omo forest reserve, Nigeria. (Adjanohoum et al.,1999).

Croton penduliflorus: Family Euphorbiaceae,Common names**:** Aworoso**,** Croton penduliflorus is a tree like shrub. The flowers are unisexual and regular with usually five perianth leaves, stamens are numerous and united. The superior ovary of three carpels forms a triocular capsule. Usually found in tropical forest (Adjanohoum et al.,1999).Experiment evidences shows that the seed extract of Abrus precatorius has protective effect against alcohol induced renal injury and that this effect may be related to a reduction in alcohol- induced lipid peroxidation (Ligah et al., 2009).

2 MATERIALS AND METHOD

Collection and Identification of Plant Samples

Fresh seed samples of Abrus precatorius and Croton penduliflorus plants were purchased from Mushin herbal market in Lagos, also Root sticks of Nauclea latifolia plant were bought from the same place. The plants were authenticated at the Botany department of the University of Lagos, Akoka.

Source and Maintenance of Test Organisms

Pure culture of Enterococcus faecalis, Escherichia coli and Klebsiella pneumonia were obtained from the Medical Laboratory of the Microbiology unit of the Lagos University teaching hospital (LUTH). The organisms were confirmed using biochemical tests.

Antimicrobial testing: Agar well diffusion method

After solidification of the agar, 0.1ml of Escherichia coli, Klebsiella Pneumonia and Enterococcus faecalis of Mc farland standard were introduced unto the surface of the sterile prepared plate and a sterile glass spreader was used for even distribution of the inoculum. Holes were made aseptically with a 7mm sterile cork borer, 0.1ml of the test solution were introduced into the wells. The extract was allowed to dilute into the medium for 1 hour and then incubated aerobically for 24hrs at 37^0C.The plates were examined for zones of inhibition, which indicates the degree of susceptibility of the test organisms. The antimicrobial activity of water and ethanol extract were measured and compared with the control well (well containing only water).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the active extracts was determined by diluting the extracts in nutrient broth to give concentrations of 100, 50, 25, 12.5, 6.25 and 3.125mg/ml. 2ml of sterilize extract was added to the first tube containing 2ml of broth. The tube was shaken and 2ml transferred aseptically to the next tube containing the same quantity of broth. This was done until serial dilution was achieved in the last tube i.e. the sixth tube. The 0.1ml of the bacterial suspension of Mcfarland standard was inoculated into each test tube and they were incubated at 37^0C for 24 hours. The minimum inhibitory concentration was regarded as the lowest concentration of the extract that did not permit any visible growth when compared with the control tube (Alade and Irobi, 1993).

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined by sub culturing test solutions which showed no detectable growth (no turbidity) after 24hrs incubation onto fresh drug free nutrient agar and incubated further for 24hrs to determine the minimum bactericidal concentration of the extracts required to kill the organism. These organisms were indicated by the failure of the test organism to grow on the plate after incubation indicated a bacteriostatic effect, while the plates that do not show growth after incubation indicated a bactericidal effect (Anibijuwon et al., 2010).

3. RESULTS AND DISCUSSION

Sample	Ec MIC	Ec MBC	Kp MIC	Kp MBC	Ef MIC	Ef MBC
G	25	50	*		*	
C	*		*		12.5	-
K	*		50	-	*	
L	*		12.5	25	*	
H	*		3.025	6.25	*	
I	6.25	12.5	*		*	
E	12.5	25	*		*	
F	12.5	25	*		*	

Table1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) as expressed in mg/ml

Sample	Ec		Kp		Ef	
	MIC	MBC	MIC	MBC	MIC	MBC
G	25	50	*		*	
C	*		*		12.5	-
K	*		50	-	*	
L	*		12.5	25	*	
H	*		3.025	6.25	*	
I	6.25	12.5	*		*	
E	12.5	25	*		*	
F	12.5	25	*		*	

LEGEND

Ec = Escherichia coli Kp = Klebsiella pneumonia

Ef = Enterococcus faecalis - = No MIC/ MBC * = Not tested.

Table2: Result of phytochemical properties of the aqueous and ethanol extract of the plant extracts.

Extracts	Alkaloids	Glycosides	Saponins	Tannins	Flavonoids
A	+	++	+	+	++
B	+	++	+	+	++
C	+	++	+	+	++
D	+	++	+	+	++
E	++	-	+	+	-
F	+	-	+	+	-
G	++	-	+	+	-
H	+	-	+	+	-
I	++	+	+	+	-
J	++	+	+	+	-
K	++	+	+	+	-
L	+	-	+	+	-

LEGEND

++ = strongly positive + = positive - = Not detected

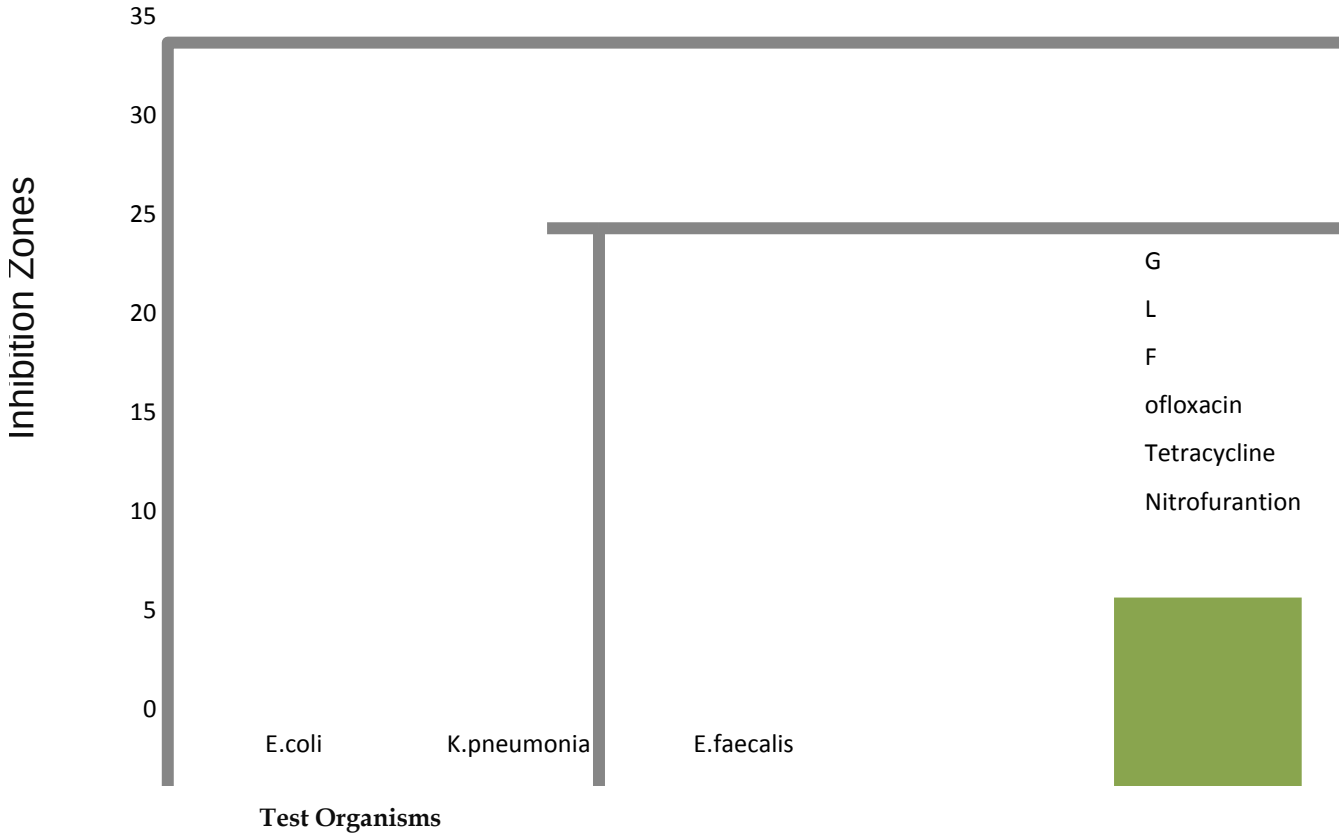

Fig 1: *Comparism between active extracts and commonly used convectional antibiotics on test organisms.*

Legend
G = Cold ethanolic extracts of Croton penduliflorus.
L = Hot ethanolic extract of Nauclea latifolia.
F = Hot water extract of Croton penduliflorus.
= Ofloxacin
= Nitrofurantion
= Tetracycline

4 DISCUSSION

It has been found that for Enterobacteriacea to be regarded as sensitive to any antimicrobial agent, it must produce a zone of inhibition of greater or equal to 14mm. Samples G, C, K, L, H, E and F are therefore effective antimicrobial agent based on the above theory. The plant extracts showed low activity against Klebsiella spp this can be due to the possession of outer membrane by gram negative bacteria that serves as an effective barrier (Girish et al., 2008) and also Klebsiella pneumonia is characterized with the possession of capsule (Podschun et al., 1998) all these can increase the resistance of the bacteria. Although, E.coli is gram negative bacteria, it still showed a high susceptibility to all the extracts, this justifies the work of Maitera et al. 2011. Enterococcus faecalis was generally susceptible to the ethanolic extracts of the plants as seen in the general susceptibility of gram positive bacteria Girish et al. 2008, probably due to their naked cell wall. Croton penduliflorus has showed an active sustainable effect against E.coli, which is a common cause of diarrhea, this buttress the facts of Joy et al., 2009 that C.penduliflorus as a constituent of the tri-herbal pill called Jedi-Jedi pill acclaimed by traditional practitioners and hawkers in Lagos Nigeria as an anti-diarrheal capabilities is partially true.The presence of Tannins in the plants extracts indicates their antimicrobial activity as tannins have been associated with antimicrobial abilities (Rotimi et al., 1987 and Maitera et al., 2011) The value of the minimum inhibitory concentration (MIC) is usually an adequate guide for the treatment of most infections (Olorundare et al., 1992). From the result in table 1, the minimum inhibitory concentration value of the eight extracts that showed the most sensitivity where lower than the minimum bactericidal concentration suggesting that the plant extract where bacteriostatic at lower concentration but bactericidal at higher concentration. The large differences between the minimum inhibitory concentration and the minimum bactericidal concentration indicate that the bioactive components may be present in much lower concentration (Ejeichi, 1996). Commonly used antibiotics such as Tetracycline, Nitrofurantoin and Ofloxacin showed a varying inhibition zone of 14-30mm. This indicates that the antibiotics Tetracycline, Nitrofurantion and Ofloxacin are more effective on a broad spectrum. However, other antibiotics such as Augmentin and amoxillin showed low antibacterial activity as compared to the extracts with varying inhibition zone of 0-4mm.Abrus precatorius has been shown to possess a high presence of

glycosides (Table 2) but its glycoside constituent is been related to antifertility effects on sperm production and the DNA integrity in adult male mice by Sarwat et al., 2009. Rajadurai et al, 2009 as also reported A. precatorius flavonoid constituents has been used in the treatment of scorpion and snake bites by traditional healers of Pachamalai Hills, Tamilnadu, in India. Supportive evidences by Ligha et al. 2009 also shows that the seed extract of Abrus precatorius has protective effect against alcohol induced renal injury.The organisms tested are known to cause, diarrhea, tuberculosis, skin infection, and other health associated infections. The results are a good indication that these plants could be utilized for possible antimicrobial agents. Little wonders that N. latifolia, C. penduliflorus and A. precatorius attend to many diseases. Thus this result justifies the use of the plant parts in ethno medicine. The obtained results may provide a support to use of the plant in traditional medicine. Based on this, further toxicological, Stability test and purification of N. latifolia, C. penduliflorus and A. precatorius should be done.

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