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Differential Antibacterial Activity of the Various Crude Leaf Extract of Eucalyptus Offensiveness Against Selected Pathogenic Bacterial Strains

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Abstract: *The antibacterial activities of Eucalyptus officinalis extracts were studied. The antibacterial efficiency of the above mentioned plant was evaluated according to agar diffusion and broth dilution methods by using Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Proteus mirabilis and Pseuomonas aeruginosa clinical isolates and typed cultures. The most susceptible bacteria were E. coli followed by S. aureus while the most resistant bacteria were Pseud. aeruginosa followed by P. mirabilis and Strept. Pneumoniae. From the screening experiment, the hot water extract produced the highest inhibitory effect 17.22±0.14 and 17.63±0.06 against the clinical isolated and typed culture respectively, hence this plant can be further subjected to isolation of the therapeutic antimicrobials and pharmacological evaluation.*

Keywords*: antibacterial activity, bacterial strains, crude leaf extract, Eucalyptus officinalis, pathogenic,*

I. Introduction

Medicinal plants are a source of great economic value all over the world (Bishmu *et al*, 2009). A large number of diverse types of plants grow in different parts of the country. Nigeria, being rich with all the three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity possess thousands of species with known medicinal value and the use of different parts of these medicinal plants to cure specific ailments has been in vogue since ages.

The increase in antibiotic resistance bacteria is largely due to the widespread use of antibiotics in medicine, in animal care, and in agriculture. The problem is compounded by the lack of new antibiotics to attack bacteria in different ways to circumvent the resistant genes. Decreasing efficiency and resistance of pathogens to antimicrobial drugs made the search of a new antimicrobial agent an important strategy for the establishment of alternative therapies in difficult handling infections.

Plants are potent biochemists and has been components of phyto-medicine, a wondrous assortment of industrial chemicals have been obtained from them (Parekh *et al*, 2006).

However, there has been a rising interest in the research for natural products from plants for the discovery of new antimicrobial and antioxidant agents in recent times (Nascimento *et al*, 2000, Rios and Recio, 2005). Moreso, many of these plants have been known to synthesize active secondary metabolites such as phenolic compounds found in essential oils with established potent insecticidal and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; R'ios and Recio, 2005).

Santo *et al* (1997) remarked that the World Health Organization (WHO) has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs. Some studies have identified and isolated the main active ingredients in the plants responsible for this antimicrobial activity Oloke *et al* 1988; Carson and Riley, 1995). However, the study on medicinal plants will allow for the demonstration of their physiological activity and also catalyze many pharmacological studies that will lead to the development of more potent drugs with no or minimal toxicity and high sensitivity especially towards the emerging microbial agents Fabricant and Fansworth, 2001). The main objective of the research is to screen and evaluate antibacterial activity of crude extracts and to find out minimum bactericidal concentration (MBC) against both gram positive as well as gram negative.

II. Materials and Methods

2.1 Aqueous and ethanolic extraction of the plant parts: Modified Okogun (2000) method of extraction was adopted in the process. Such that the diluents used were 95% ethanol and hot water. 50g of plant material were boiled with 200ml of water and allowed to cool for the aqueous extraction. For ethanol extraction, 15g of powdered plant material was soaked in 150ml of ethanol for 24h. The filtrate was concentrated at 45^{o}C under reduced pressure using a rotary vacuum evaporator. The extracts were kept at refrigeration temperature until required for use.

2.2 Phytochemical screening: Phytochemical screening of the various plant extract was carried out using standard procedure as described by Adegoke and Adebayo (2009a and b) and Trease and Evans (1984) to determine the presence of alkaloids, saponins, tannins, anthraquones, glycoside, flavonoids and reducing sugar.

2.3 Collection of test organism: Test organisms (*Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa*) were obtained from Federal Medical Center Owerri Microbiology Laboratory, while the type cultures were obtained from Federal College of Veterinary and Medical Technology, Vom, Jos, Plateus State. The identity of the test organisms were reconfirmed and stored at 4^oC in nutrient agar slant.

2.4 Sensitivity screening: Agar well diffusion method was used to determine the preliminary sensitivity screening of the crude plant extract. The standardized innoculum of the test organism using Mcfarland standard was seeded on sterile Muller Hinton agar plants. Duplicate wells of 5mm were made using a corck borer. 100ml of 200mg/ml concentration of the various plant extracts was introduced into the wells. The plates were allowed to stand for 1hour for diffusion to take place and then incubated for 24h at 37^oC. Using a ruler the zone of inhibition was measured to the nearest millimeters.

2.5 Determination of the minimum inhibitory concentration (MIC): Using the macro-broth method as described by NCCLS (1998), a two-fold serial dilution of the reconstituted extract was prepared using Mueller Hinton Broth. Each dilution was inoculated with 100µl of the standardized suspension of the test organism, the cultures were incubated at $37^oC$for 24h. The MIC was determined as the highest dilution that showed visible growth.

2.6 Determination of minimum bactericidal concentration (MBC): 0.1ml of all MIC tubes showing no growth was inoculated into sterile Mueller Hinton agar plate incubated at 37^oC for 24h and observed for growth. MBC growth was determined at least concentration showing no growth.

2.7 Statistical analysis: The inhibition zones were statistically analyzed by conducting a paired sample T-test using SPSS (2006) computer application programmes. Means ± standard deviation were considered significant at P = 0.05.

III. Results

Amongst the phytochemicals tested, only anthraquones and glycosides were not detected in both the ethanolic and hot water extract of *E. officinals*. This is shown in Table 1. In Table 2, it was observed that hot water extract of *E. officinalis* had the highest inhibitory effect against both the clinical isolates and typed culture of *Escherichia coli* (17.22±0.14 and 17.63±0.06 respectively). This was followed by the ethanol extract against *Staphylococcus aureus. Proteus mirabilis* was slightly inhibited by both the ethanol and hot water extracts.

From the results of the MIC and MBC of the extracts, it was observed that the ethanol extract exhibited a bactericidal effect against both the clinical isolate and typed culture of *S. aureus* and *Escherichia coli*. The hot water extract also showed a bactericidal effect against *Escherichia coli.* Both extracts, showed a bacteriostatic effect against the clinical isolate and typed culture of *P.mirabilis*. This is as shown in Table 3.

IV. Discussion

Plant extracts are valuable resources for primary health care and complementary health care system. Undoubtedly plant extracts containing substances of medicinal value that are yet to be discovered, though large number of plant extracts are constantly being screened for their antimicrobial effect, these plant extracts may prove to be rich source of compounds with possible antimicrobial activities.

The phytochemical screening of the ethanolic and aqueous extracts of *Eucalyptus officinalis* did show the presence of mainly phenolic compounds (alkaloids, saponins, tannins, flavonoid) which have been found to possess antimicrobial properties (Alma *et al*, 2003). Several studies have described the antioxidant properties of medicinal plant extracts which are rich in phenolic compounds such as flavonoids, Quinones etc. Phenolic compounds have been shown to be toxic to microorganisms. The mechanism thought to be responsible for phenolic toxicity to microorganisms includes enzyme inhibition by the oxidized compound possible through reaction with sulflydral groups or through more nonspecific interactions with the protein (Manson, 1987).

The results of antimicrobial activity and MC arrays showed promising evidence for the antimicrobial activity of *E. officinalis* fresh leaf extracts against some human pathogens. The low zone of inhibition obtained with some extracts particularly the ethanol extract may be due to errors or contamination.

Comparing the sensitivity of the clinical isolates to the typed cultures as shown in Table 2, it can be deduced that the typed cultures exhibited relatively higher degree of sensitivity to the crude extracts. This may be because the clinical isolates may have developed some degree of resistance due to continuous exposure to antibiotic agents. This is in line with the observation of Hugo *et al* (1991), that the continuous presence of antibiotic can cause microorganisms to alter their metabolic pathways or produce an exflux pump system that can pump antibiotic out of the microbial cells.

Pseudomonas aeroginosa resistance to antimicrobials is through alteration of cell membrane permeability possibly by modification of protein the outer membrane, so that it is difficult for an antimicrobial to enter the bacterial cell and also its tendency to colonize surfaces in a biofilm form thereby making the cell impervious to therapeutic concentration of antibiotics. The observed insensitivity of *Pseudomonas aaeruginosa* to most of the plant extracts agreed with similar work done by Bishu *et al* (2009) and Shittu *et al* (2006) using alcoholic and aqueous plant extracts.

Considerable antibacterial activities of the plant sample were noted in the various extracts as compared to the standard antibiotic penicillin. The results revealed the presence of medicinally important constitutes in the *E. officinales* leaf extracts.

V. Conclusion

Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore these *E. officinales* leaf extracts could be seen as a good source for useful drugs and have great potentials as antimicrobial agents against selected pathogens and they can be used as alternative medicine in the treatment of infections caused by these strains of bacteria.

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TABLE 1: Phytochemical screening result of *Eucalyptus offinalis* extracts

Phyto constituents	Ethanol extract	Hot water extract
Alkaloid	+	+
Saponins	+	+
Flavonoids	-	+
Tannins	+	-
Anthraquone	-	-
Glycosides	-	-
Reducing sugar	+	+

Key: - absence, + = present

TABLE 2: Mean zone of inhibition of *Eucalyptus officinalis* extracts

Organisms	**Ethanol extract**	**Hot water extract**	**Pecicillin**
Escherichia coli			
Clinical isolate	7.05 ± 0.01	17.22 ± 0.14	24.78 ± 0.08
Typed culture (ATCC 1775)	9.06 ±0.01	17.63 ±0.06	
Staphylococcus aureus			
Clinical isolate	8.81 ±0.08	0.00±0.00	13.17±0.09
Typed culture (ATCC 12600)	11.09±0.01	1.48±0.74	
Streptococcus pneumoniae			
Clinical isolate	0.00 ±0.00	0.00 ±0.00	13.73±0.06
Typed culture (ATCC 6674)	2.15±0.21	0.70±0.07	
Proteus mirabilis			
Chemical isolate	1.12 ±0.06	1.46±0.07	9.07±0.02
Typed culture (NCTB 67)	1.65±0.04	2.17±0.03	
Pseudomonas aeruginosa			
Clinical isolate	0.00 ±0.00	0.00 ±0.00	4.49±0.01
Typed culture (ATCC 10145)	0.19±0.01	0.00 ±0.00	

TABLE 3: Minimum inhibitory concentration (MI) and minimum bactericidal concentration (MB) of the crude extracts of *Eucalyptus officinals* (mg/ml)

Organisms	**Extracts**					
	Ethanol extract			**Hot water extract**		
	MIC	MBC	MIC/MBC	MIC	MBC	MIC/MBC
Escherichia coli						
Clinical isolates	25	50	0.50	100	100	1.00
Typed culture (ATCC 1775)	25	50	0.50	50	50	1.00
Staphylococcus aureus						
Clinical isolates	100	100	1.00	> 200	> 200	< 0.50
Typed culture (ATCC 12600)	100	100	1.00	> 200	> 200	< 0.50
Streptococcus pneumoniae						
Clinical isolates	> 200	> 200	< 0.50	> 200	> 200	< 0.50
Typed culture (ATCC 6674)	100	> 200	< 0.50	200	> 200	< 0.50
Proteus mirabilis						
Clinical isolates	100	> 200	<0.05	100	> 200	<0.50
Typed culture (NCTB 67)	100	> 200	<0.05	100	> 200	<0.50
Psedomonas aeruginosa				> 200	> 200	< 0.50
Clinical isolates	> 200	> 200	< 0.50	> 200	> 200	< 0.50
Typed culture (ATCC 10145)	> 200	> 200	< 0.50			