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Synergism between ethanol leaf extract of Canarium schwenfurthii and antimicrobial drugs on some pathogenic microbes

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The antimicrobial activity of ethanol leaf extract of *Canarium schwenfurthii* was tested against five bacteria (*Staphyloccocus aureus, Staphylococcus albus, Klebseilla pneumonia, Pseudomonas aeruginosa and Proteus mirabilis*) and six fungi (*Rhizopus sp, Penicillin oxalicum, Aspergillus tamari, Aspergillus niger*, *Fusarium oxysporum* and Fusarum *vacilitus*). The result revealed that the plant had inhibitory activity against all the tested microbes. The protein synthesis inhibitors were more active against the organisms than the cell wall and nucleic acid inhibitors. The antifungal drugs produced varied inhibitory reactions against the fungi tested. The synergetic effect of plant extract and antimicrobial drugs produced greater inhibitory effect than the plant extract or drugs alone. The phytochemical screening of the plant showed the presence of Alkaloid, Cardenolide, Saponin, and Tannin while Anthraquinone was absent.

Keywords: C*anarium schwenfurthii, synergism antimicrobial, pathogenic microbes.*

INTRODUCTION

Infection disease still represent an important cause of morbidity and mortality among human especially in developing countries, even though pharmaceutical industries have produced a number of antimicrobial drugs in the last years, resistance to these drugs by microorganisms have increased. In general, some microorganisms especially bacteria have the genetic ability to transmit and acquire resistant to drug used as therapeutic agents. As a result of an increased demand for alternative medicine, renewed interest in drugs of plant origin has been growing steadily (Obisesan and Adeyemo, 1998) and there is no gain saying in the fact that Nigeria possesses very rich and diverse genetic resource medicinal plants which have both immediate and long term potentials of economic and social attraction (Gbile, 1988).

Canarium schewenfurthii belongs to the family Burseraceae. It is a large forest tree with its crown reaching the upper canopy of the forest. The fruit palp contains 30 to 50% of oil used for manufacturing of shampooing and biofuel (Techiegang *et al.,* 2001; Ajiwe *et al*. 2000). The rhizomes and leaves are as stimulant and against fever, constipation, malaria, diarrhea, sexual infections, post-partum pain and rheumatism.

MATERIALS AND METHODS

Plant Samples: *C. schwenfurthii* were collected from Ago-Iwoye and voucher specimens were deposited at the Herbarium Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria. The leaves of the plant were dried and triturated in a mechanical mill.

Preparation of Plant Extracts

Triturated plant material was extracted using Soxhlet extraction apparatus. 70% of ethanol was used for the extraction. The filtrate was concentrated on a rotary evaporator at 45^0c and the extract was then kept in sterile bottle under refrigerator conditions until use.

Organism Used

Pure culture of *Staphylococcus aureus, Staphyloccocus albus, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Rhizopus spp. Penicillin oxalicum, Aspergillus tamari, Aspergillus niger, Fusarium oxysporum and Fusarum vacilitus* were obtained from the Department of Medical Microbiology, Ogun State University Teaching Hospital, Sagamu and kept in McCartney bottles.

Phytochemical Screening

Phytochemical screening for major constituents was undertaken using quantitative as described by Odebiyi and Sofowora (1990) .The plant extract was screened for the presence of Alkaloid, Tannin, Cardenolide anthraquinone and saponin.

Bacterial Inoculation and Incubation with Extracts

Nutrients agar and nutrient broth (oxoid) were prepared according to the manufactures' recommendation. The agar-well diffusion method was used for the inoculation of the bacteria. Plates containing 30ml of sterile nutrient agar each were inoculated with standardized innocula (1.5 x 108 cells/ml) (Olafimihan and Fawole, 2003) using sterile pasteurized pipette. Wells of 5mm diameter were made at the centre of each plate and 0.15ml of the various concentration of the plant extract was dispensed into each well. The extract was allowed to diffuse into the medium for 1 hour at room temperature. This was then incubated at for 24 hours at 37^0c after which the zones of growth inhibition was measured and recorded in millimeter. The control was set up in a similar manner except that the extract was replaced with sterile distilled water.

ANTIBIOTIC ASSAY

The selected antimicrobial drugs were obtained from a chemist. These drugs, in their high concentration were diluted with sterile water reducing them into a lower concentration (0.4mg/ml). Wells were bored on the prepared agar with a cork borer and with the use of sterile needle and syringe; the antibiotics were poured into the wells. The zone of inhibition was observed after 24hours and recorded.

ANTIFUNGAL ASSAY

Antifungal effects of the extracts were tested using the agar dilution method described by Collins and Lyne (1970) 500 mg/ml of the extract was prepared and incorporated into potato dextrose agar. The plates were incubated at 25^0c for 48 hours and inhibition of growth was noted.

SYNAGISTIC ASSAY

0.4mg/ml of antimicrobial drugs selected were mixed together with the ethanol extract. Wells (6mm) were bored on the agar seeded plates; using cork borer, the mixture of drugs and plant extract were poured into the wells and the zones of inhibition were observed and recorded.

RESULTS

Table 1: Result of Phytochemical Screening of *Canarium Schwenfurthii* leaves

	TEST	OBSERVATION	INFERENCE
A	**ALKALOIDS**		
I	Drangeduff's reagent	**Redish brown ppt**	**+**
Ii	Mayer's reagent	**Pale cream ppt**	**+**
Iii	Wagner's reagent	**Brownish red ppt**	**+**
B	**CARDENOLIDES**		
i.	Keller-killani test	**Appearance of resist brown ring at interface and blue to green colour in acetic ring**	**+**
Ii	Kedde's test	**Violet colour**	**+**
C.	**ANTHRAQUINONES**		
i.	**Chloroform (Ammonia test)**	**Absence of rose pink cherry red colour**	**-**
D	**SAPONINS**		
	Frothing	**Persistent frothing**	**+**
E	**TANNINS**	**Blue-black ppt**	**+**

+Present

- Absent

Table 2: Antibacterial activity of Ethanol Leaf Extract of Canarium Schwenfurthii on some human pathogenic organisms

	S. aureus	S.albus	K.pneumonia	P.aeruginosa	Proteus mirabilis
Canarium schwenfurthii	++	++	+	++	++
Gentamycin	+	++	+	++	+
Erythsomycin	+	++	+	++	+
Samtrin	+	+	-	+	+
Chloramphenicol	++	+	+	+	+
Tetracycline	+	++	+	+	+
Ampicillin	+	-	-	-	-
Penicillin	+	++	-	++	+
Streptomycin	++	++=	++	++++	+++
Plant + Gentamycin	++	++	++	++	++
Plant + Erythsomycin	++	++	++	++	++
Plant + Samtrin	++	++	++	++	++
Plant + Chloramphenicol	++	++	++	++	++
Plant + Tetracycline	++	++	++	++	++
Plant + Ampicillin	++	++	++	++	++
Plant + Streptomycin	++	+++	+++	+++	+++
Plant + Penicillin	++	++	++	++	++

Table 3: Antifungus activity of *Canarium scheweinfurthii* on some fungi

	Rhizopus spp.	P. oxalicum	A. tarnarii	A. niger	F. oxysporm	E. vacitilus
Canarium schweinfurthi	+	++	+	-	+	
Samtrin	+	++	+	-	-	
Fulain	+	-	-	-	-	
Ciposil	+	-	-	+	+	
Flagyl	+	+	+	-	-	-
Plant + Samtrin	++	++	++	+	+	+
Plant + Fulain	+	+	+	+	+	+
Plant + Ciposil	+	++	++	++	++	++
Plant + Flagyl	++	++	++	++	+	++

1 - 10 low inhibition (+)

11 - 20 moderate inhibition (++)

21 - 30 high inhibitor (+++)

31 - 40 very high inhibition (++++)

Table 1 is the phytochemical screening of C. *schwenfurthii*. The plant tested positive to alkaloid, cardenolide, saponin, and tannin tests and negative to anthraquinone test

Table 2 is the antibacterial effect of C. *schw*enfurthii on five human pathogenic microbes. The ethanol plant extract had inhibitory effect on all microbes tested and the least activity was against *K. pneumonia*. The

antibiotic drugs showed variable reactions. Ampicillin only had inhibitory action against *S. aureus* while Streptomycin had inhibitory against all the microbes tested and its effect on P. *aeruginosa* was higher than that of the plant extract.

Synergism between the plant extract and the drugs produced higher inhibitory effect on all the organisms tested.

Table 3 is the antifungal of C. *Schwenfurthii* against 6 fungi. The plant had inhibitory effect on all the fungi and the least effect was against *A. tamari* and *F. vacitilus*. The antifungal drugs also showed varied activities against the fungi. However the synergism reaction between the plant extract and the drugs produced higher inhibitory activity against the fungi growth.

Discussion

The antibacterial activity of *C. shwenfurthii* on the five bacteria tested revealed that the extract inhibitory effect on the gram positive (*S. aureus and S. albus*) and gram negative (*K. pneumonia, P. aeruginosa, P. mirabilis*) bacteria. This shows that the plant has compounds(s) that was able to breakthrough the outer membrane surrounding the cell wall of gram negative bacterial through its lipopoly saccharide covering (Vaaraa, 1992, Ratledge and Wilkinson, 1988).

The phytochemical screening of the plant revealed the presence of tannin, cardenolide, among others and these two classes of compounds are known to be shown curative activity against several pathogens and therefore could explain its use traditionally for treatment of wide array illnesses (Hassan *et al.,* 2004, Usman and Osuji 2007).

The extract exhibited considerable activity against *S. aureus*, a pyrogenic gram positive bacterium known to play a significant role in invasive skin diseases including superficial and deep follicular lesion (Usman and Osuji, 2007). *P. aeruginosa* is known to cause pulmonary, urinary tract infections, burns, wound and also causes other blood infections. Hence, this study has shown that *C. shwenfurthii* can be used to treat these diseases.

The plant also justifies its use in treating gonorrhea and other sexually transmitted diseases caused by *S. aureus*.

The antifungal activity of the plant revealed that the plant could be used to treat aspergillosis; a disease of ear which can cause pain, temporary hearing loss and in severe cases, damage to the ear canal and tympanic membrane.

Streptomycin amongst the antibiotic showed the highest inhibitory activity against the bacteria tested. The inhibitory activity against the gram negative were higher than the plant activity. Ampicillin only had inhibitory against *S. aureus* and this could be that other organisms have become resistant to it. (Mukherjee *et al.,* 2002, Ajaiyeoba, 2005).

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