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International Journal of Medicine and Medical Sciences ISSN: 2167-0404 Vol. 3 (5), pp. 009-011, November, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Comparative antibacterial studies of mistletoes growing on two diffrent host plants in Akure North, Nigeria

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Accepted 30 September, 2013

The antibacterial activity of 60 % methanolic leaves extracts of mistletoes (*Viscum album*) growing on cocoa and cola trees were tested on *Bacillus cereus, Pseudomonas aeruginosa,Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi in vitro*. Both the gram-positive and gram-negative organisms showed variable sensitivity to the extracts treatments. The results obtained indicated that extracts from both host plants had some antibacteria activities against the microorganisms when compared with standard antimicrobial agents (ciprofloxacin and sterptomycin) used as positive controls at P < 0.05 significant level. In general,extracts from cocoa (*Theobroma cacao*) plant showed more antimicrobial tendency than those from cola (*Kola nitida*) plant.

Key words: antibacterial activity, methanolic extact, *Viscum album*, *Theobroma cacao*, *Kola nitida*.

INTRODUCTION

The mistletoe plant is an obligate parasite that depends partly on its host to obtain water and minerals but can carry out photosynthesis(Griggs,1991). It is an evergreen semi-parasite that can grow in most parts of the globe. Mistletoe can grow on either edible or non-edible trees, while only those that grow on edible plants are used for medicinal purposes (Evans, 2005). The growth of Mistletoe on different kinds of plants, are of disease curing specificity, for example, mistletoe grown on Guava, Kolanuts and Citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia, while those grown on cocoa is best used for curing diabetes(Ekhaise et al., 2010).

Bacteria are listed at first position among the microorganisms causing opportunistic infections (Kone et al., 2004),so many antibacterial agents are now used in treating bacterial infections.Their widespread and indiscriminate use lead to development of drug resistance among many virulently pathogenic bacterial species (Berkowitz,1995).

Many of the currently used antibacterials are associated with adverse effects such as blood cancer, upper gastrointestinal complications, organ damages, toxicity, hypersensitivity, immunosuppression, and tissue residues posing public health hazard. Also, these synthetic broad spectrum antibiotics are cost prohibitive and are not within the reach of our poor farmers. These disadvantages undermine the therapeutic utility of the currently avilable antibacterials and hence the need for alternative remedies for the treatment of bacterial infections. Natural plant products have been used for therapeutic purposes since the time immemorial and their use is of a greater demand nowadays (Calixto, 2000). So, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases. Mistletoes therefore is one such plant which is reported to possess several medicinal properties.Its use as an anti-cancer, anti-diabetic, anti-hypertensive, and indeed as 'all-purpose herb' has been reported(Kafaru,1993). Mistletoes is used traditionally by the people in Akure North as topical antibiotic in form of pastes for the treatment of wounds and other skin infections. Many of these folkloric uses have already been investigated (Obatomi et al.,1996; Osadebe and Ukwueze, 2004; Ukwueze, 2008). During recent years considerable work has been done to investigate the pharmacological importance of mistletoes on scientific lines but not mush work has been reported so far on comparative pharmacological importance of the plant growing on different host trees.Therefore, it was considered worthy to investigate and compare the antibac-

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terial activity of mistletoes growing on two diffrent host plants in Akure North,Nigeria.

MATERIALS AND METHODS

Plant materials

Fresh leaves of mistletoes (*V.album*) were collected from the parent plant growing on cocoa and cola host trees at Ogbese, Akure North of Nigeria. The leaves were identified and confirmed at the Crop Soil Department of the Federal University of Thechnology Akure

Preparation of plant extract

The leaves were destalked, washed and air dried at a room temperature of $28^0C \pm 2^0C$ for six weeks until a constant weight resulted.The dried leaves were pulverized and kept in a refrigerator in a well labeled air-tight containers for analysis. A 100 g of each sample was weighed (using electric weighing balances) into a beaker containing 1000 ml of 60% methanol.This was homogenized in a warring blender for 72 hrs. until a homogenate was achieved. This was then filtered using Whatman no. 1 filter paper and the filtrate was collected and then concentrated in *vacuo* using a rotary evaporator. It was reconstituted using dimethyl sulphoxide (DMSO) 1:50(w/v) for the analysis.

Test Organisms

Pure cultures of pathogenic strains of *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa,* were obtained from the stock cultures of the University Teaching Hospital, Ibadan, Oyo State, Nigeria. The identities of the organisms were confirmed using standard methods of the morphological and biochemical characteristics of each organism at the Microbiology Department Federal University of Technology, Akure, Nigeria.

Antimicrobial activity assay

The antibacterial activity of the *V.album* leaves methanolic extracts was screened *in vitro* against the four bacteria mentioned above following disc diffusion method (Oluma et al., 2004, Doughari et al., 2007). The concentrated leaf extracts were dissolved in 5% dimethyl sulfoxide (DMSO) and the blank discs of 6 mm diameter were punched from filter paper of uniform thickness and sterilized by heat.The blank discs were separately impregnated with 30 mg/ml of each extract. The discs were carefully and firmly placed on the Agar plates earlier seeded with standardized bacterial suspensions (approximately 1.5×10^6 cfu/ml). Paper discs impregnated with a solution of 30 mg/ml of the standard antibiotics ciprofloxacin and steptomycin were used as controls, for comparison. Filter paper discs dipped into sterile distilled water and allowed to dry were used as control. The plates were then incubated at $37\ ^0C$ for 18 h. Antibacterial activity was determined by measurement of zone of inhibition around each paper disc.

Phytochemical Screening

The methods of Harbone (1973) and Trease and Evans (1983) were adopted for the determination of the phytochemicals in the extracts. The total phenol content of the extract was determined using the method of Singleton et al.,(1999).

RESULTS AND DISCUSSION

Table 1 shows the qualitative phytochemicals in both dry and wet samples of *V.album.* Phenol, Saponins, flavonoids, Terpenoid, Phytate and Cardiac glycosides were present in both samples but Alkaloid was not detected in that from cola tree. However a positive result was obtained for Keller Killiani test, Salkwoski test, and Kedde's test.Table 2 shows the quantitative phytochemical screening of *V. album* samples. The dry samples show higher levels than the wet samples in all the phytochemicals detected.Table 3 compares the antibacterial properties of the extract with the standard antibiotics.

This work revealed on the general look that the antibiotics were more effective in inhibiting the test organisms than the extracts (Table 3).The bioactive components and concentration of the antibiotics may be a contributing factor as antibiotics are products of large scale industrial fermentation,the product of such process are usually pure due to good manufacturing practices (GMP) and quality control which guarantee standard.The molecular size of the antibiotics aids their solubility in dilluents.This could also enhance their penetration through the cell wall into the cytoplasm of the test organisms (Mailard, 2002).The presence of bioactive molecules (Table 1 and Table 2) seem to be responsible for the antimicrobial activities of the extracts.Though, these compounds are known to be present in most angiospermic plants,but their approximate composition in plants determine to a large extent,the relative biological efficacy of such plants (Ogundare and Onifade, 2009). The hydroxyl group in phenols are thought to be responsible for the toxicity of the compounds to microbes.It inhibits enzymes through reaction with sulphydryl group or through non-spesific interaction with proteins (IPAN News,2003). The observed variation in the

Table 1. Result of the qualitative phytochemicals of dry and wet samples of *V. album.*

Phytochemicals	Sample A	Sample B
Phenol	+	+
Alkaloid	+	-
Saponins	+	+
Tannins	-	-
Phlobatannins	-	-
Flavonoids	+	+
Steroid	-	-
Terpenoid	+	+
Phytate	+	+
Cardiac glycosides	+	+
Keller Killiani test	+	+
Legal test	+	+
Salkwoski test	+	+
Lieberman test	-	-
Kedde's test	+	+

sample A: *V. album* extract from cocoa. sample B: *V. album* extract from cola
+ =present, - =absent.

Table 2. Quantitative phytochemicals data of dry and wet samples of *V.album.*

PCh	Sample A dry	Sample A wet	Sample B dry	Sample B wet
Terpinoid	0.636 ± 0.106	0.225 ± 0.014	0.119 ± 0.028	0.100 ± 0.001
Saponins	22.742 ± 0.383	17.398 ± 0.806	20.478 ± 0.095	15.662 ± 0.553
Phytate	49.06 ± 0.071	42.485 ± 0.346	46.015 ± 0.983	40.377 ± 0.051
Oxalate	5.785 ± 0.056	3.700 ± 0.017	3.892 ± 0.030	2.896 ± 0.032
Flavonoids	4.554 ± 0.332	3.097 ± 0.023	2.534 ± 0.049	2.058 ± 0.905
Alkaloids	3.541 ± 0.059	2.417 ± 0.134	0.00	0.00
Cardiac glycosides	0.609 ± o.017	0.339 ± 0.028	0.146 ± 0.006	0.110 ± 0.015
Phenol	3.487 ± 0.105	2.517 ± 0.133	2.678 ± 0.003	2.002 ± 0.013

PCh =Phytochemicals; SampleA: *V.album* from cocoa tree. SampleB: *V.album* from cola tree. Result is mean of replicates ± SEM (n=3).

Table 3. Result comparing the antibacterial properties of the extract with two broad spectrum antibiotics.

Organisms	sample A	sample B	streptomycin	Ciproflox
E. coli	12.17 ± 0.17	11.33 ± 0.33	18.67 ± 0.17	13.00 ± 0.05
P. aeruginosa	10.33 ± 0.33	9.33 ± 0.33	12.67 ± 0.07	11.00 ± 0.05
Salmonella typhi	12.17 ± 0.75	9.00 ± 0.58	15.00 ± 0.07	12.15 ± 0.05
Bacillus cereus	13.33 ± 0.88	11.00 ± 0.58	17.67 ± 0.58	15.67 ± 0.09
E.faecalis	9.67 ± 0.33	7.33 ± 0.67	18.33 ± 0.58	10.67 ± 0.25
Staph. aureus	11.17 ± 0.44	10.50 ± 1.04	13.33 ± 0.18	11.67 ± 0.09

Sample A = *V. album* extract growing on coca tree Sample B = *V. album* extract growing on cola tree. Result is mean of replicates ± SEM (n=3).

activities of these extracts may be due to the host plant from which the samples were collected.It was observed that *V.album* from cocoa was more antimicrobial than that from cola.The detection of Alkaloids in *V.album* from cocoa and its absence from that from cola might be a contributing factor. Ademiluyi and Oboh, (2008) had reported higher total phenol content in *V.album* from cocoa than that from cashew trees in Nigeria.

CONCLUSION

The results of the verification of the folkloric utilisation of the leaves of the *V.album* as an all-purpose antimicrobial agent is further coroborated. The presence of the identified phytochemicals makes the leaves pharmacologically active and responsible for their usefulness in the management and treatment of various diseases.

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