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RESEARCH PAPER

ANTIBACTERIAL ACTIVITY OF EXTRACTS OF MISTLETOE (TAPINANTHUS DODONEIFOLLUS (DC) DANCER) FROM COCOA TREE (THEOBRAMA CACAO)

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ABSTRACT

This study to investigate the antibacterial activities of mistletoe grown on cocoa trees, 4 different extracts (1% HCl, ethanol, acetone and 5% acetic acid) were prepared and tested against some strains of bacterial organisms (*Escherichia coli, Pseudomonas aeruginosa*, *Staphyloccocus aureus* and *Klebsiella aerogenes*). Following standard laboratory procedures, the extracts were prepared and the minimum inhibitory concentration (lowest concentration of extract that did not allowed growth within the incubation period) was determined. The results showed extract of 5% acetic acid to act against the four test organism. Ethanolic extract of mistletoe showed antibacterial potential against *S. aureus, P. aeruginosa* and *K. aerugenes*. Except for *E. coli,* ethanolic extract presented the best antibacterial potential followed by 5% acetic acid, acetone and lastly 1% HCl. The MIC activity ranged from 8.6µg/ml for ethanolic extract on *K. aerogenes* to 150µg/ml for 1% HCl on *S. aureus*. The present study reveals that the antibacterial activities of mistletoe depend on the extraction solution and the bacterial type. This result points to the role mistletoe could play as an antibacterial and as a precursor for the preparation of drugs.

Keywords: Antibacterial, Mistletoe, Microorganisms, Traditional medicine

INTRODUCTION

Bacteria are listed at the first position among the microorganisms causing opportunistic infections (Kone et al., 2004) and this has lead to the use of so many antibacterial agents. According to Berkowitz (1995), the widespread rate of bacteria and indiscriminate use of antibacterial have lead to development of drug resistance among many virulently pathogenic bacteria species. The issue is furthermore problematic considering that many of the currently used antibacterials are associated with adverse effects such as blood cancer, upper gastrointestinal complications, organ damages, toxicity, hypersensitivity, immune-suppression, and tissue residues posing public health hazard (Yusuf et al., 2013). Moreover, the available synthetic broad spectrum antibiotics are costly and in some cases are not within reach. These disadvantages weaken the therapeutic utility of available synthetic broad spectrum antibacterials and hence the need for alternative remedies.

Interestingly, natural plant products have been used for therapeutic purposes since the time immemorial and their use is of a greater demand nowadays (Calixto, 2000). It is widely believed that traditional medicine sometimes called herbalism is the most ancient method of curing diseases (Evans, 2005). They are the first and only true medicines ever used by man. However, in Nigeria, until recently, the practices of the use of herbs was characterised with secrecy and shrouded in dreaded magical incantations, rituals and sacrifices. Considering the fact that therapeutically properties of medicinal plants are very useful in healing various diseases and the advantage of these medicinal plants are 100% natural (Serrentino, 1983), man is now being dedicated to herbal medicine as many academics are now into ethnomedicine.

One plant of interest is the Mistletoe (*Tapinanthus bangwensis*), which belongs to the family *Loranthanceae*. It is a well known evergreen parasitic plant, which grows on deciduous trees in ball-like bush (Kay, 1986; Evans, 2005). The evergreen 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 a semi-parasite that can grow in most parts of the globe; either edible or non-edible trees, with that only those that grow on edible plants are used for medicinal purposes (Evans, 2005).

A wide variety of pharmacological effects have been studied on the varieties of mistletoe and have been reported to be anti-diabetic (Orhan et al., 2005; Shahaboddin et al., 2011), vasodilator (Tenorio-Lopez et al., 2005; 2006), sedative, antiepileptic, antipsychotic (Gupta et al., 2012), antihypertensive (Ofem et al., 2007), anti-inflammatory, immunostimulant (Lavastre et al., 2004), antimutagenic (Hong and Lyu, 2012), anticancer (Sabova et al., 2010; Cetin and Ozcelik, 2007; Burger et al., 2001) as well as antioxidant activities (Orhan et al., 2005; Shahaboddin et al., 2011).

However, the growth of Mistletoe on different kinds of plants exhibit disease curing specificity. For example, Ekhaise et al. (2010) observed that 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. Also, the phytochemical profile and bioactivity of mistletoe have been reported to depend on the host trees (Luczkiewicz et al., 2001; Vicas et al., 2011). Considering the assertions by Ekhaise et al. (2010), Luczkiewicz et al. (2001) and Vicas et al. (2011), this study is aimed at investigating the antibacterial activity of different extracts of mistletoe grown on cocoa tree.

MATERIALS AND METHODS

Processing of plant samples: Plant materials were collected from in and around Ekpoma, Edo State, Nigeria. The mistletoe leaves were washed in tap water, rinsed in sterile distilled water and dried for 5 days at 60^0 C in Lab 1 of the Department of Microbiology, Ambrose Alli University, Ekpoma.

Preparation of extracts: Two hundred and fifty grams (250g) of the dried mistletoe leaves were weighed and blended to powder with a clean kitchen blender. It was then stored in airtight glass containers and kept in the laboratory cupboard, until required for preparation.

5 grams of the grounded mistletoe leaves was weighed into 100ml reagent bottle and 95ml of extraction solvent (1% HCl, 5% acetic acid, acetone and ethanol) was added and left to extract on a mechanical shaker overnight at room temperature. This was done using all the four extraction solvents (1% HCl, 5% acetic acid, acetone and ethanol).

The extract solution was filtered aseptically into another 100ml reagent bottle using a watt-man No 1 filter paper. All the filtrate were screened for purity by inoculation unto MacConkey agar and nutrient agar plates and incubated at 37^0C for 48 hours. Filtrates yielding growth of any organism was re-filtered and rescreened for purity until a sterile extract solution was obtained, following the methods outlined by Orhue (2004).

Micro organism preparation/growth: The test organisms used are all human pathogenic organisms of clinical origin. They include *Pseudomonas aeruginosa, Eschenchai coli*, *klebsiella aerogenes* and *Staphylococcus aureus*. They were obtained from the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma-Nigeria, where they were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmation.

Determination of Minimum Inhibitory Concentration (MIC): Using a 50ml specific gravity bottle, the density of the extract solution was determined. In a similar manner, the density of the plain solvent was also determined.

To determine the concentration of the extract, the density of the plain solvent was subtracted from that of the extract solution. This was done for all four extraction solvents. With the known extract concentrations and the four clinical isolates of *Pseudomonas aeruginosa, Eschenchai coli*, *klebsiella aerogenes* and *Staphylococcus aureus*, the MIC of the extract solutions were determined. The lowest concentration of extract that did not allow growth within the incubation period was taken to be the minimum inhibitory concentration. The experiments were performed in 3 repetitions for each of the extraction solvents and the average was calculated.

Data analysis: Data were keyed into SPSS (version 16) and the mean of each determined MIC was then presented in suitable table for simple descriptive comparison. The MICs of the mistletoe extract in the different extraction solutions were compared using ANOVA (LSD) at 95% level of confidence.

RESULTS

The four different extracts of mistletoe prepared with four kinds of solvent (1% HCl, 5% acetic acid, acetone and ethanol) were tested for antibacterial activity (Table 1). Antibacterial activity against the four test organism (*S. aureus, E. coli, P. Aeruginosa and Klebsiella aerogenes*) was observed with extract of 5% acetic acid. Ethanolic extract of mistletoe was observed to show antibacterial potential against *S. aureus, P. aeruginosa* and *K. aerugenes* and except for *E. coli,* it presented the best antibacterial potential followed by 5% acetic acid, acetone and lastly 1% HCl. On the other hand, 1% HCl and acetone extracts of mistletoe only showed antibacterial activity to *S. aureus* and *P. aeruginosa* only.

The antibacterial activity range from 8.6 µg/ml weight of ethanol extract on *K. aerugenes* to 150ug/ml weight of HCl extract on *Staphylococcus aureus. E.coli* was only sensitive to acetic acid extract (22.5µg/ml). *Staphylococcus aureus* and *P.aeruginosa* were sensitive to all the extracts, with MIC ranging from 70µg/ml to 150µg.ml and 17.5µg/ml to 75µg/ml respectively.

Table I: Minimum Inhibitory Concentration of Mistletoe extract in different extraction Solvent (µg/ml)

Test Organism	1% HCl	5% Acetic Acid	Acetone	Ethanol
Staphylococcus aureus	150	90	90	70
Escherichea coli	0	22.5	0	0
Pseudomonas aeruginosa	75	22.5	22.5	17.5
Klebsiella aerogenes	0	45	0	8.6

DISCUSSION

The results of this study showed that different extract of mistletoe grown from cocoa tree have antibacterial potentials at varying degrees and to different bacterial organisms (table 1). In line with the present findings, some studies have reported the antibacterial activities of several extracts of mistletoes such as n-hexane extract (Erturk et al., 2003) aqueous and methanolic extracts (Sengul et al., 2009), ethanolic and aqueous crude extracts (Oguntoye et al., 2008) aqueous, ethanol, methanol, acetone, ethylacetate, chloroform and petroleum ether crude extracts (Hussain et al., 2011).

Specifically, Huaasin et al (2011) showed that ethylacetate and methanol extracts exhibited prominent activities against both Gram positive and Gram negative bacteria. Interestingly, an antibacterial activity of mistletoe has been reported to compare well with standard antibacterial drugs such as Gentamycin and Cloxacillin (Oguntoye et al., 2008). In this regards, it was reported that the antibacterial activity of mistletoe extracts correlated well with its traditional uses (Pamplona-Roger, 1999).

Also, the antibacterial potentials of mistletoe may be due to its constituents, as according to Hajto et al. (1989) and Schaller et al. (1996), it contains thermo-labile compounds such as lectins and viscotoxins. Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponin, steroid and flavonoid (Ekhaise et al., 2010). These are believed to be responsible for the observed antibacterial effects of plant extracts (Nwze et al., 2004), for the treatment of several infections in Africa, indigenous medicinal plants are often the only means (Fennell et al., 2004).

Thus mistletoe may possess some active ingredients that can inhibit the growth of microorganisms. Also, Cowan (1999) reported that water-soluble compounds, such as polysaccharides and polypeptides, including fabatin and various lectins, are commonly more effective as inhibitors of pathogen adsorption but have no real impact as antimicrobial agents.

The presence of antibacterial properties in mistletoe is of great importance in healthcare delivery system, since it could be used as an alternative to orthodox antibiotics, in the treatment of infections due to the microorganisms,

especially as orthodox antibiotics frequently develop resistance to known biotics (Singleton, 1990) and will reduce the cost of obtaining health care.

The present study reveals that the antibacterial activities of mistletoe depend on the extraction solution and the bacterial type. This result points to the role of mistletoe grown on cocoa tree could play as an antibacterial and as a precursor for preparation of drugs. Conclusively, the findings of this study suggests that extracts of mistletoe grown on cocoa tree may do well as an antibacterial for patients prone to bacterial infections. However, the need for further studies in this direction, for a better development of host antibacterial drugs cannot be over emphasized.

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AUTHORS' CONTRIBUTIONS

The experiment was conceived and designed: Jonathan E Emordi, Steve O Ogbonnia , Sunday O Olayemi. Performed the experiments: Jonathan E Emordi, Steve O Ogbonnia , Sunday O Olayemi , Emmanuel N Anyika, Chioma Dozie-Nwanna. Wrote the paper: Jonathan E Emordi, Steve O Ogbonnia