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THE PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF OIL FROM THE SEED OF *THEVETIA PERUVIANA* PLANT

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ABSTRACT

The antimicrobial activities of methanol and chloroform extract of oil from the plant *Thevetia peruviana* were investigated using well diffusion method and broth dilution techniques. The oil from the plant was tested against *Staphylococcus aureaus, Streptococcus pyogenes, Corynebacterium ulcerans* and *Bacillus subtilis* Gram positive. The Gram negatives are *Escherichia coli, Neisseria gonorrhoae, Klebsiella pneumonia Salmonella typhi* and *Shigella dysenteriaea.* The only fungus was the yeast *Candida albicans*. The methanol extract exhibited activity against all the organisms tested except *Klebsiella pneumoniae,* with zones of inhibition 10-26mm and 0-10mm respectively. The chloroform extract inhibited only two organism, *Salmonella typhi and Shigella dysenteriae.* The methanol extract show high activity against Bacillus, *Corynebacterium* and *Candida,* zones of inhibition 20-26. The phytochemical screening on the oil revealed the presence of flavonoids, Glycosides, phlobatannins, Saponins, steroids and tannins. This studies shows that the oil from the seed of *Thevetia peruviana* contains compounds that have antimicrobial activity.

Keywords: Phytochemical, antimicrobial, *Thevetia peruviana*.

INTRODUCTION

Resistance to antimicrobial agents is a major global public health problem. Infectious diseases account for approximately one-half of all death in tropics. Despite the progress made in the understanding of microorganisms and their control in industrialized nations , incidents due to drug resistant microorganisms and the emergence of unknown disease causing microbes, posed enormous public health concern [1]. *Streptococcus pyogenes, Stephylococcus aureus* and *Streptococcus pneumoniae,* the organisms that causes respiratory and cutaneous infection, as well as *pseudomonas* and members of *Enterobacteriaceae*, causing diarrhea and urinary tract infections, and sepsis, are now resistant to virtually all of the older antibiotics [2]. This resistance is largely due to indiscriminate use of antimicrobial drugs commonly used on the treatment of these infectious diseases [3]. Furthermore some antibiotics have serious undesirable side effect which limit their application, so there is serious need to develop new antimicrobial agents that are very effective with minimal unwanted side effect and higher plants represent a potential source of novel antibiotic prototypes [4] and [5].

Thevetia peruviana (Yellow oleander, Daffodil tree, Campaninlla, Campanero or Mexico oleander) is a dicot plant from a family of Apocynaeceae. The plant is said to be originated from tropical America, which is now distributed all over the tropical countries. It is propagated by seeds and cuttings. It is a perennial and ever green plant seasonally with size of 6-8 ft; as a tree it can be up to 20 ft tall. It has alternate nearly sessile, linear to linear lanceolate leaves with 6 inches long by ¼ inch wide. The plant has regular, fragrant, yellow or orange flower. The stem can be trained as tree with single trunk. All parts of the plant, particularly the seeds and the milk sap are poisonous owing to the presence of cardiac glycosides or cardiac toxins [6]. *Thevetia peruviana* commonly known as Yellow oleander is an ornamental plant which grows in Nigeria and other parts of the world, such as tropical America, western Asia, Southern Europe, India and tropical African Extracts from the plant contain glycosides whose toxicity against snails, slugs [7], bacteria, insect [8] and human [9] have been documented.

The plant extracts have been reported to have antifungal properties against *Cladosporium cucumerinum* [10]. In Peru the leaves and the flower are used in the treatment of the cases of bones, rheumatism, arthritis, sorcery epilepsy, nerves and heart attacks. The seeds are also used in the case of cancer and menopause. Recent studies have shown that several alcohols extract of various traditional medicinal plants exhibit antimicrobial activities [11] and [6]. The seed oil has also been reported to have antimicrobial, anti-inflammatory properties, anti-parasitic and insect

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anti-feedant or repellant activities [12] and [13]. [14], reported that the plant exhibit great activity against *E. coli*, *Enterobacter aerogens* and *Aligenes faecalis*. Paint made from *T. Peruviana* plant oil extract can protect timber from termites' attack [15].

Therefore with respect to this we report the antimicrobial activities of oil extracted from the seed of *Thevetia peruviana* against some selected pathogenic microorganisms.

MATERIALS AND METHOD

Plant Material

The *Thevetia peruviana* kernel seeds were collected from Bansi Village Zing Local Government in Taraba state, Nigeria, in the month of November 2009 and was authenticated at the Herbarium unit of Biological Sciences department, Ahmadu Bello University, Zaria, where a voucher specimen was preserved, the Kernels were removed and the seeds were air dried and made into powder using pestle and mortar. The oil was extracted using methanol and chloroform. Filtered crude oil was stored at 4^0C in a refrigerator

Phytochemical Screening

The methanol and chloroform extracts of the oil were subjected to phytochemical screening for the presence of flavonoids, glycosides, phlobatannins, Saponins, steroids and tannins using standard procedure [16].

The test organisms

The organisms used for this study includes *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium ulcerans,* Gram positive. The Gram negatives are *Escherichia coli, Neisseria gonorrhoae, Salmonella typhi, Klebsiella pneumoniae* and *Shigella dysenteriae.* The yeast *Candida albican*s was also used. All the organisms were obtained from the department of medical microbiology Ahmadu Bello University Teaching Hospital (ABUTH), Zaria Nigeria. All he isolates were checked for purity and were maintained in instants of nutrient agar for the bacteria and slant of sabouraud dextrose agar for the yeast.

Antimicrobial Susceptibility Testing

Preliminary antimicrobial activities of the oil on the two solvents were carried out using well diffusion method as reported by [17]. The oil was tested at an initial concentration of 50mg/ml with Erythromycin a standard antibiotic 50mg/ml as a control drug. The inocula were prepared by inoculating the test microorganism in nutrient broth and incubating at 30^0C for 24hrs for bacteria, while the yeast was incubated at 24^0C for 48hrs. After incubation the broth culture were diluted to 1:1000ml of the diluted culture was inoculated into sterilize Nutrient and sabouraud dextrose agar for the bacteria and the yeast respectively. The media was allowed to dry at 37^0C for 30 minutes, by the used of standard Cork borer of 6mm in diameter a well was made at the centre of each inoculated media and 0.lml of the oil which has a concentration of 50mg/ml was then introduced into each well. The plates were incubated at 37^0C for 24hrs for the bacteria and at 25^0C for 48hrs for the yeast, after which the plates were observed for the zone at inhibition of growth. The zones were measured with a transparent ruler and the result recorded in millimeter. All tests are carried out in duplicate and the antimicrobial activity was expressed as the mean diameter of inhibition zones.

Minimum Inhibitory Concentration (MIC)

MIC was determined on the organisms that exhibited sensitivity against the tested microorganisms using broth dilution techniques of [18], [14] and [19]. 50mg/ml of each of the extracts were reconstituted into nutrient broth in test tubes and the 50mg/ml was taken as the initial concentration. Four more tubes of 5ml nutrient broth were set up and 5ml of 50mg/ml of the extract was taken and used for two-fold dilution of the four tubes of nutrient broth forming concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. The number of each cell at 1.5×10^8cfu/ml using 0.5 Macfarland's standard was prepared in normal saline and inoculated into the broth. The tubes were incubated at 37^oC for 24 hours and 25^oC for 48 hrs for the fungus. The tube with the lowest concentration which has no growth (turbidity) of the microbes was taken to be the minimum inhibitory concentration (MIC).

Minimum Bactericidal/Fungicidal Concentration

Minimum bactericidal/fungicidal concentrations were determined by asserting the test tube content resulting from MIC determination. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient and sabouraud dextrose agar for the bacteria and the fungi respectively, the plates were incubated at 37^0C for 24hrs for the

bacteria and 25^{0}C for 48hrs for the fungi in which the growth of the microorganism was observed. The lowest concentration of the subculture with no growth of the test microorganism was e as minimum t or fungicidal.

RESULTS AND DISCUSSION

The results from table1 shows the phytochemical analysis of the oil, tables 2 & 3 shows antimicrobial and zones of inhibition tables 4 & 5, minimum inhibitory and minimum bactericidal/fungicidal concentrations. The phytochemical analysis of methanol and chloroform extracts from the oil revealed the presence of flavonoids, Glycosides, phlobatannins, steroids, saponins and tannins. Flavonoids and tannins have been reported to possess antimicrobial activity [17] and [13]. The antimicrobial activity of flavonoid is due to their ability to complex with extracellular and soluble protein and to complex with bacteria cell wall, while that of tannins may be related to their ability to inactivate microbial adhesion enzymes and cell envelop proteins [18].

The methanol extract showed zone of inhibition of growth ranging from 10 – 26 mm against the test microorganisms with exception of *Klebsiella pneumoniae* which was resistant to the oil, while the chloroform extract had no effect on almost 80% of the test microorganisms. The methanol extract showed strong activity against *Candida albicans*, this organism is known to play role in the mucus membrane infection of the mouth and vagina, the result has indicate that the plant can be a source of compound that can be effective against infection caused by his microbe.

The methanol extract was found to have MIC values ranging from 12.5mg/ml and MBC of 50mg/ml, while the chloroform have the MIC of 6.25mg/ml and the MBC of 25mg/mc on the microorganisms they inhibited. The antimicrobial activities observed with the methanol extract suggest the presence of bioactive compound(s) which can serve as antimicrobial agent or lead compound for the synthesis of an effective and less toxic antimicrobial agent.

Table 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF THEVETIA PERUVANA OIL

Secondary Metabolites	Methanol	Chloroform
Flovonoids	+	+
Ghylosides	-	-
Phlobatanins	+	-
Steroids	+	+
Saponins	+	+
Tannins	+	+

Key: + ⟶ present, - ⟶ absent

Table 2: ANTIMICROBIAL ACTIVITIES OF THEVETIA PLANT EXTRACTS AGAINST

Test Organism	Methanol	Chloroform
Bacillus subtilis	S	R
Corynebacterium ulcerans	S	R
Stapylococus aureus	S	R
Streptococcus pyogenes	S	R
Escherichia coli	S	R
Klebsiella pneumoniae	R	R
Neisseria gonorrhoeae	S	R
Salmonella typhi	S	S
Shigella dysenterae	S	S
Candida albicans	S	R

Key: S ⟶ Sensitive, R ⟶ Resistance

Table 3: ZONE OF INHIBITION OF THE EXTRCTS AGAINST THE TEST MICROORGANISM

Test Organism	Methanol	Chloroform
Bacillus subtilis	20	0
Corynebacterium ulcerans	20	0
Staphylococcus aureus	17	0
Streptococcus pyogenes	10	0
Escherichia coli	11	0
Klebsiella pneumonia	0	0
Neisseria gonorrhoeae	14	0
Salmonella typhi	16	20
Shigella dysenteriae	15	23
Candida albicans	26	0

Table 4: MINIMUM EXHIBITION CONCENTRATION OF THE EXTRACTS AGAINST THE TEST MICROBES

Test organism	Methanol					Chloroform				
	50mg/ml	25mg/ml	12.5mh/ml	6.25mh/ml	3.125mg/ml	50mg/ml	20mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
Bacillus subtilis	-	-	-	O*	+					
Conynebacterium ulcerans	-	-	-	O*	+					
Staphylococcus aureus	-	-	O*	+	++					
Streptococus pyogenes	-	-	O*	+	++					
Klebsiella pneumoniae										
Neisseria gonorrhoeae	-	-	O*	+	++					
Salmonella typhi	-	-	O*	+	++	-	-	-	O*	+
Shigella dysenteriae	-	-	O*	+	++	-	-	-	O*	+
Candida albicans	-	-	-	O*	+					

KEY - ⟶ No growth, O* ⟶ MIC + ⟶ Turbid (Light growth) ++ ⟶ Moderate turbidity (No turbidity)

Table 5: MINIMUM BACTERICIDAL/FUNGICIDAL CONCENTRATION OF THE EXTRACT AGAINST THE MICROBES

Test Organism	Methanol					Chloroform				
	50mg/ml	25mg/ml	12.5mh/ml	6.25mh/ml	3.125mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
Bacillus subtilis	-	O*	+	++	+++					
Conynebacterium ulcerans	-	O*	+	++	+++					
Staphylococcus aureus	-	O*	+	++	+++					
Streptococus pyogenes	O*	+	++	+++	++++					
Escherichia coli	O*	+	++	+++	++++					
Klebsiella pneumoniae										
Neisseria gonorrhoeae	O*	+	++	+++	++++					
Salmonella typhi	O*	+	++	+++	++++	-	O*	+	++	+++
Shigella dysenteriae	O*	+	++	+++	++++	-	O*	+	++	+++
Candida albicans	-	O*	+	++	+++					

KEY: - ⟶ No colony growth, O* ⟶ MBC/MFC + ⟶ Scanty colonies growth
++ ⟶ Moderate colonies growth +++ ⟶ Heavy colonies growth

CONCLUSION

The study showed that the methanol extract of the oil extracted from the seed of *Thevetia peruviana* had more antimicrobial properties than the chloroform extract. We therefore suggest more work should however be required to isolate and characterized the active ingredients and possibly their mechanism of biological action.

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