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Anti-microbial activities of Raw and Boiled *Thevetia peruviana* Seed on some selected microorganisms

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Abstract: *A research work entitled: Anti-microbial activities of raw and boiled Thevetia peruviana seed on some selected microorganisms was carried out in the chemistry laboratory of Federal University of Technology Akure (FUTA), Ondo State, Nigeria. The seed was obtained from Lafe Area of Akure in Ondo State. The study investigated the powdered sample of the raw and boiled seeds of Thevetia peruviana; and the seed extracts were analysed for anti-microbial activities in the treatment of some bacteria diseases. The study also examined the effect of boiling on the sample against the microorganism. Adia et al. (2001) method was adopted for the study. The oil from the samples were cultured over each nutrient agar (ASA) plates at 37 0C for 24 hours and were tested against some organisms such as: Escherichia coli, Psuedomonas syringine, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus, Xanthomonas oximopoides and Candida albican. The study revealed that the n-hexane extract exhibited activity against all the organisms tested with zones of inhibition 9-14mm and 0.2-3mm respectively except Escherichia coli, and Pseudomonas syringine that showed no zone of inhibition. The n-hexane extract showed a significantly high activity against Bacillus subtilis and Pseudomonas aeruginosa. Thus, out of the seven selected microorganisms, the raw and boiled seed extract was able to control four of the microbes at 0.5ml concentration used. These microbes are Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, and Xanthomonas. oximopoides. While boiling does not have any effect on the sample against the microorganism at the same concentration used. The study concluded that the presence of anti-microbial activities in the seed revealed high medicinal properties which can be used to treat the infectious diseases created by the tested microorganisms. The study therefore recommends that further studies should be carried out on Thevetia peruviana seed against other microorganism that are not used in this study.*

Keywords: *Thevetia peruviana, anti-microbial, microorganisms, agar disc diffusion, raw boiled, milk bush, ornamental tree.*

INTRODUCTION

Thevetia peruviana also called Olomiojo in Yoruba is a small ornamental tree which widely grows throughout the tropical and sub-tropical regions. (Samal *et al.*, 1992) The entire parts of the plant with the fruit and seed are used medicinally for treatment of diverse of diseases and sicknesses as it contained both micro and macro mineral elements which are essential for normal functioning of the body. (Akintelu and Amoo, 2016) The seeds, leaves, fruits and roots of the plants are regarded as potential sources of biologically active compounds (Antimicrobial) such as insecticides, fungicides and bactericides. (Ambang *et al.*, 2010; Oji and Okafor, 2000; Tewtrakul *et al.*, 2002)

Thevetia peruviana is planted as large flowering shrub in gardens and parks in temperate climates. Generally, the plants have served as models in drug development. (Priya and Deepika, 2015) The entire plant contains milky juice which is poisonous. The root paste is used against tumours. The plant has Cardiac glycosides and is considered to be a promising drug for congestive heart failure. (Sandhya and Biradar, 2014) Antimicrobials (commonly known as antibiotics) are agents that inhibit the growth of microorganisms. Smith and Fyte (Smith-Palmer *et al.*, 1998) reported that the agents are grouped according to the` microorganisms they act primarily against and can also be classified according to their function. Antimicrobials that kill microbes are called microbicidal; those that merely inhibit their growth are called microbiostatic. (Smith-Palmer *et al.*, 1998)

This research work aimed to investigate the anti-microbial activities of *Thevetia peruviana*, raw and boiled seed extracts in the treatment of some bacteria diseases if affected by plants and mammals.

Antimicrobial Pesticides

According to the Environmental Protection Agency and the Federal Insecticide, Fungicide, and Rodenticide Act, antimicrobial pesticides are used in order to:

1. Control growth of microbes through disinfection, sanitation, or reduction of development.
2. Protect inanimate objects (for example floors and walls), industrial processes or systems, surfaces, water, or other chemical substances from contamination, fouling, or deterioration caused by bacteria, viruses, fungi, protozoa, algae, or slime.

Materials and Method

Collection of plant material

The milk bush seed (*Thevetia peruviana*) used in this study was obtained from Lafe Area in Akure Ondo State. 400g of the seed gotten from the fruits were air dried, cracked and the seed were removed from the fruit; 200g of the seeds were boiled while the remaining was left unboiled (raw). Both raw and boiled seeds were sundried at room temp and ground to powder, packaged in airtight sample plastics bags and stored in the refrigerator at 4ºC prior to laboratory analysis.

Preparation of extracts

30grams powder of the seed was extracted with n-hexane as a solvent by using Soxhlet apparatus until the extractive was cleared. The extracts were separated from the solvent through distillation process and were stored in a bottle for further use.

Chemical Reagents

All reagents were of analytical grades and they were obtained from the Department of Chemistry, Federal University of Technology Akure (FUTA).

Anti-microbial Analysis: The anti-microbial analyses were carried out using standard methods as indicated below:

Antibacterial screening test using the Agar Diffusion method.

The micro-organisms of choice used for the investigation are *Echericha coli, Pseudomonas syringine, Xanthomonas oximopoides, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus,* and *Candida albican.* The isolates were separately cultured over each nutrient agar (ASA) plates. Sterile cup borer of 8 mm diameter was used to make wells on the solidified agar into which about 0.5 ml of each of the oil were aseptically introduced. The plates were incubated at 37 ^{0}C for 24 hrs. Zones of inhibitory around the wells were measured by the use of vernier callipers. Results were quoted as the radii (mn) of the zones of inhibition around the wall. Control plates were also set-up using standard antibiotics (Streptomycin sulphate). (Aida *et al.*, 2001)

Results and Discussion

Table 1: Inhibitory effect of extract on some selected micro-organism.

Zone of Inhibition (Growth) in mm after 24hrs incubation			
Micro-organisms	**Raw** (0.5 ml/well)	**Boiled** (0.5 ml/well)	**Streptomycin Sulphate (as standard)** 0.5ml/well
B. subtilis	3.00	3.00	9
E. coli	0.00	0.00	12
C. albican	1.50	0.00	7
B. cereus	2.00	1.20	11
P. aeruginosa	3.00	3.00	14
P. syringine	0.00	0.00	9
X. oximopoides	0.50	0.20	14

Figure 1: Nutrient agar (ASA) plates showing zone of inhibition on selected microorganisms.

The result in the above table showed the anti-microbial activity of *Thevetia peruviana* seed oil on some organisms with streptomycin sulphate as standard. Out of the seven (7) selected pathogens that were used for the analysis, the result revealed that n-hexane extract of the seed had inhibitory activity against four (4) micro-organisms in both raw and boiled *Thevetia peruviana.* The microbes as shown in figure 1 includes: *Bacilus subtilis*, *Bacilus cereus*, *Pseudomonas aeruginosa* and *Xanthomonas oximopoides* at the concentration used, indicating that the oil of *Thevetia peruviana* have anti-microbial activity and can be used to control the microbes. However, figure 2 showed that *B.subtilis* and *P. aureginosa* had maximum inhibitory activity while *X. oximopoides* had minimum inhibitory activity.

The result also revealed that the seed oil for both raw and boiled did not inhibit the growth of *Echericha coli* and *Pseudomonas syringine*. By implication, the oil cannot be used to control the microbes. The result finally revealed that the raw seed oil inhibit *Candida albican* while the boiled seed oil had no effect on the microbe. This means that the raw oil can be used to control *Candida albican* infection. Earlier, studies showed evidence of similar observation from root and bark extract of *Nerium oleander*. (Hussain and Gorsi, 2004) Therefore the results established that *Thevetia peruviana* seed is medicinal and the oil will be good as antimicrobial in the treatment of microbial infections.

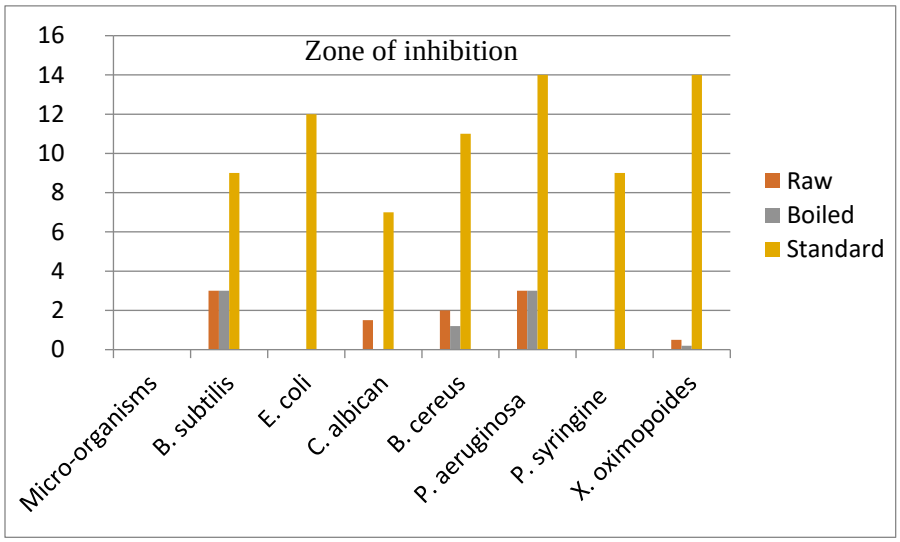

Figure 2: Inhibition zone Diameter (mm) of the microorganisms

Conclusion

This study has given insight into the anti-microbial analysis of the seed of *Thevetia peruviana*. The presence of anti-microbial activities in the seed is evidence that it has high medicinal properties. Therefore, these results are useful to treat the infectious diseases created by tested microorganisms.

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