ANTIMICROBIAL POTENTIAL OF AQUEOUS AND ETHANOL EXTRACT OF TERMITE AND BEE NATURAL PRODUCTS ON *Escherichia Coli* FOR POSSIBLE MEDICINAL PURPOSE

ABSTRACT

Background:arthropodshave been utilized for their socio-economic value as food and medicine for decades in most part of the world. Many traditional healers use insects in their traditional medicine healing system.The idea of utilizing substances collected from insects as medicinal resources, might have originated from the chemical compounds (such as pheromones, venoms, and toxins) sequestered from plants that have shown medicinal value. The study was targeted atat investigating the antimicrobial potential of termite and bee natural products on Escherichia coli for possible medicinal purpose. The insects were collected using different insect traps from farmlands within Agulu and Nanka communities, Anambra State, Nigeria. The sample was identified and authenticated at the Zoology Department. The insect was killed using killing jar technique, air dried, pulverized and macerated for further investigation. The zoochemical properties and antimicrobial activity of the extract was investigated.

Result: It was observed that the constituents, carbohydrate and saponins were present in all the extracts. Tannins, Flavonoids and Terpenoids were present in the ethanol extracts of both insects. The other zoochemical investigated: Anthraquinones. Alkaloids, Cardiac glycoside, and steroids were not observed in all of the extracts. The water extract of both bee and termite exhibits less activity against *E. coli*. The ethanol extract of both insects showed *E. coli* growth inhibition. As distinct zones of inhibition were observed. The ethanol extract of termite showed higher inhibition activity (8.33 ± 0.60 mm zone of inhibition), this was followed by the ethanol extract of bee (7.08 ± 1.18 mm zone of inhibition). The aqueous (water) extract of termite (1.33 ± 0.67 mm zone of inhibition) showed higher activity than that of the bee extract (1.00 ± 0.58 mm zone of inhibition).

Conclusion: The result of the study showed that ethanol extract of termite and bee contain bioactive constituents with antibacterial effect and lends credence to the entomo-ethno medicinal use of insect in the treatment of bacterial infections

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KEYWORDS: Antimicrobial, Zoochemical, Termite, Bee, *E. coli*, Agar well diffusion

INTRODUCTION

For centuries in some parts of the world like Africa East Asia and South America, insects and some arthropods are utilized for their socio-economic value as food and medicine [1]. Due to the long-term traditional use some of these insects have not been experimentally evaluated, while some have been investigated to contain important properties [1]. The idea of utilizing substances collected from insects as medicinal resources, might have originated from the chemical compounds (such as pheromones, venoms, and toxins) sequestered from plants that have shown medicinal value. Besides they are medicinal value some of these insects play mystical and magical rules in some cultures of the world. According to research, between 1961 and 2010, about 939 nature-derived approved drugs were from plants, and few invertebrates like leeches, sponges, and cone snail; none were from insects [2]. This can be attributed to the difficulties and challenges associated with drug development such as species identification, drug toxicity, cost, and large scale production [2]. Following the decline in the rate of novel drug development from modern genomics, in silicon drug design, and high throughput screening, there has been an increasing interest in the traditional screening method of drugs from the large diversity of animals, plants, and microbes available. Notable progress has been recorded from the use of more traditional biochemical screening methods in developing therapeutics from insects and other arthropods. Examples are melittin from bees, alloferon from blowflies, and anticoagulants from tick [3].

Many traditional healers use insects in their traditional medicine healing system. For example, in India, termite has been utilized in the treatment of ulcer by the kurichchan tribe, while the Irular tribe uses it for treating Anemia, body pain, and rheumatism. The black beetle has been associated with the treatment of malaria, mole cricket (Gyrillotalpa) in the treatment of sprain, and the honey bee for treating headache, mouth ulcer, cough, cold, and insect bite [4]. According to Banjo (2003), termite has been reported to exhibit antibacterial activities because of their high cysteine rich antifungal peptide. Esiner et al. (2000), reported contemporary issues in the use of insects in drugs manufacturing and ascertain that insects have very important source of drugs for modern medicine since they possess immunological, analgesic, antibacterial, antidiuretic and antirheumatic properties. The people of Agulu and Nanka community are also not exempted from their use of insects in medicine. This southeastern community in Nigeria has been reported for their large diversity of economic insects which they use as food, in treatment of disease, and for cultural functions [5].

Inspire the numerous researches on insects' natural products and their potential medicinal properties in the treatment of human diseases, there have been additional advances in this field. This present research therefore is aimed at investigating the antimicrobial potential of termite and bee natural products on *Escherichia coli*for]] possible medicinal purpose.

MATERIAL AND METHOD

Sample Collection and Identification

The insects were collected using different insect traps from farmlands within Agulu and Nanka communities, Anambra State, Nigeria. The sample was identified and authenticated at the Zoology Unit, Department of Biological sciences, Ahmadu Bello University, Zaria. A voucher specimen number was deposited.

Preparation of Extract

The insects (Termite and Bee) were killed using a killing jar, air dried, pulverized and stored at room temperature. The powdered material (20 g) was macerated using water and ethanol for 3 days with occasional agitation, the extract was concentrated using water-bath at 40^{o}C.

Stock concentration of 10 mg/mL was prepared by dissolving 80mg each of water and ethanol extracts in 8 mL of dimethyl sulfoxide (DMSO) and two-fold serial dilution was performed to obtain three more concentrations of 5, 2.5 and 1.25 mg/mL.

Test organism

Clinical isolates of Escherichia coli were obtained from the Department of Clinical Microbiology, National Institute of Pharmaceutical Research and Development. All isolates were checked for purity and maintained in a Nutrient agar.

Zoochemical screening

Biochemical screening was carried out according to the procedure as described by Trease and Evans [6].

Antimicrobial Activity

The antibacterial assay for the ethanol extracts was carried out using the agar well diffusion assay as described by [7] with slight modifications. The bacterial suspensions were adjusted to 0.5 McFarland turbidity standards and inoculated onto previously sterilized Mueller-Hinton Agar plates (diameter: 90 mm). A sterile cork-borer was used to make five wells (8 mm in diameter) on each of the MHA plates. Aliquots of 80 µl of each extract dilutions were applied in each of the wells in the culture plates previously seeded with the test organisms. Streptomycin (S), Ampicillin (PN), Ceporex (CEP), Tarivid (OFX), Nalidixic Acid (NA), Peflaxin (PEF), Gentamycin (CN), Augmentin (AU), Ciprofloxacin (CPX), and Streptomycin (SXT) served as the positive controls. The cultures were incubated at 37 ^{o}C for 24 h. The antimicrobial potential for each extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each of the crude extract, three replicates were conducted against each organism.

Statistical Analysis

The results obtained were reported as mean ± standard error of the mean.

RESULTS AND DISCUSSION

Zoochemical Analysis: the zoochemical screening of the water and ethanol extract of Termite and Bee is summarized in Table 1. It was observed that the constituents, carbohydrate and saponins were present in all the extracts. Tannins, Flavonoids and Terpenoids were present in the ethanol extracts of both insects. The other zoochemicals investigated: Anthraquinones. Alkaloids, Cardiac glycoside, and steroids were not observed in all of the extracts (Table 1). Similarly, Yahaya et al. [1] reported the presence of carbohydrate, steroids, saponins, tannins, flavonoids, and alkaloids in crude extract of ground beetle. The use of some insects and arthropods as medicine can be attributed to the zoochemical properties identified in these arthropods [8].

Table 1. Zoochemical screening of the water and ethanol extract of Termite and Bee

Constituents	**Test**	**Termites**		**Bee**	
		Water	Ethanol	Water	Ethanol
Carbohydrate	Molisch	+	+	+	+
Anthraquinones	Bontragar	-	-	-	-
Tannins	Geletin test	-	+	-	++
	Ferric Chloride	-	++	-	++
Flavonoids	Shinoda	-	++	-	+
Alkaloids	Wagner	-	-	-	-
	Dragendoff	-	-	-	-
Saponins	Frothing	+	+	+	+

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Cardiac glycoside	Keller-Killiani	-	-	-	-
	Liebermann's test	-	-	-	-
Steroids	Liebermann-Burchard's test	-	-	-	-
Terpenoids	Salkowski's test	-	+	-	+

Key: - absent; + present in trace quantity; ++ present in moderate quantity

Antimicrobial Activities

The results of the antimicrobial activities of the insect extracts are summarized in Figure 1. The water extract of both bee and termite exhibit less activity against *E. coli*. The ethanol extract of both insects showed *E. coli* growth inhibition. As distinct zones of inhibition was observed Appendix). The ethanol extract of termite showed higher inhibition activity (8.33 ± 0.60 mm zone of inhibition), this was followed by the ethanol extract of bee (7.08 ± 1.18 mm zone of inhibition). The aqueous (water) extract of termite (1.33 ± 0.67 mm zone of inhibition) showed higher activity than that of the bee extract (1.00 ± 0.58 mm zone of inhibition) (Figure 1). The result of the antimicrobial activity of termite and bee extract against *E. coli* indicated that the extract possess medicinal potential. The higher antimicrobial effect of ethanol extract over the other solvent can be attributed to its higher solubility. The higher antimicrobial effect of ethanol extract over the other solvent can be attributed to its higher solubility. Yahaya et al. [1] also reported methanol extract to be effective against *E. coli*. This can be because these solvents are organic solvent and can dissolve organic compounds adequately [9]. Also, most of the compounds identified from plants and animals active against microorganisms are aromatic or saturated compounds that are often obtained through methanol or ethanol extraction [9]. The zones of inhibition observed with the insect extract were lesser than the zones observed for standard antibiotic drugs (Appendix).

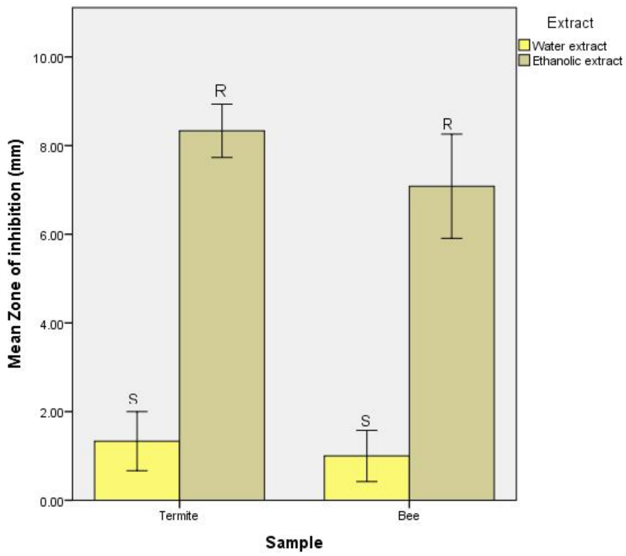

Figure 1: Antimicrobial activity of insect extract on *E. coli* showing the average zone of inhibition

CONCLUSION

The result of the study showed that ethanol extract of termite and bee contain bioactive constituents with antibacterial effect and lends credence to the entomo-ethno medicinal use of insect in the treatment of bacterial infections such as dysentery, typhoid fever, and enuresis. Isolation and characterisation of the bioactive compounds is being investigated.

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APPENDIX

Plate 1.

Control plate: zone of inhibition for standard multiple antimicrobial disc

Plate 2.

Sample plate: Zone of inhibition for aqueous extract of both insect samples

Plate 3.

Sample plate: Zone of inhibition for ethanol extract of both insect samples