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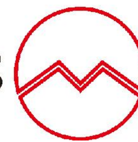

The Efficacy Of Methanol, Dichloromethane And N-Butanol Extracts Of *Anonna Muricata* Leaves On Selected Bacteria And Fungi

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Abstract: In Nigeria, *Annona muricata* is locally being use as source of medicine for curing various infections. This research was aimed at determination of the potency of the leaves against selected bacterial and fungal strains. The antibacterial and antifungal activities of methanol, dichloromethane and n-butanol extracts of Annona muricata leaves against selected bacterial and fungi strains were examined. Methanol, dichloromethane and n-butanol extracts of Annona muricata leaves showed high inhibitory activities between (24-29 mm) against Bacillus subtilis, (25-29mm) against Clostridium sporogenes, (25-29) against Entrococcus faecalis (30-31) against Klebsiella pneumonia, (26-31) against and (26-30) against Staphyloccus aureus. The antifungal activities of methanol, dichloromethane and n-butanol extracts of Annona muricata leaves showed broad inhibition zones against the growth of Aspergillus flavus, Candida albican, Fusarium oxysperium and Penicillium camemeri. These study provide scientific information and justification that support the local use *Annona muricata* leaves as medicinal therapy and equally revealed *Annona muricata* leaves as a major ingredient to bank on for the design of novel antibiotics.
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Introduction

The search for novel antibiotics with efficiency to terminate the growth of microbial infections across the world has led to the increase in the use of plants with good medicinal properties as important treatment alternatives (Abba, et al 2009). The examination of the antimicrobial efficiency of many plant extract has been embarked on recently to discern new antimicrobial agents with less side effect on human and better efficiency against microbes (Sarita, et al 2019). *Annona muricata* is a plant from Annonaceae family whose medicinal value is commended in Nigeria and many Africa countries. The pharmacological investigations on the extracts of *Annon amuricata* plant shows its potency and efficiency to cure ulcer, inflammatory and bacterial (Tai et al 2014). Information from researchers also stated that *Annona muricata* fruit has been used for the treatment of cough, skin rashes and parasites infections (Haidan et al 2016).

Traditional healers claim that herbs made from medicinal plants are more potent and possess less side harm when compared with conventional medicines. In undeveloped nations, people with low revenue from the remote societies use herbs for the cure of disease causes by microbial infections (Haidan et al 2016). Therefore, the use of herbal drugs could serve as an alternate in treatment of sicknesses instigated by multidrug resistant bacteria antifungal (Ramesh et al 2017).

Many antibiotics have lost their usage in combating microbes due to multidrug-resistant strains and other harms they produce when used (Peter et al 2018). Literatures have shown the microbial resistance to antibiotics such as tetracycline, erythromycin and penicillin (Timothy 2017). Also the resistance developed by some human pathogens against conventional antibiotics that are readily available in the market and pharmaceutical stores calls for adoption of other alternatives from other sources including medicinal plants. This fact then trigger us to embark on the study of the efficacy of Annona muricata leaves extract on some microorganism.

Materials and methods

Analytical grade reagents are used and they

include n-hexane, methanol, dichloromethane, n-butanol, aluminum oxide, silica gel, diethyl ether and methanol and apparatus use are autoclave, incubators, pipette, petri-dish canister, inoculating loop, spatula, drying oven and weighing balance.

Sample collection and Extraction process

Annona muricata leaves were obtained from Okitipupa town in Ondo state. The leaves were authenticated in the botanist from Obafemi Awolowo University, Ife, Nigeria. They were properly rinsed to remove dust and other impurities and air dried. The dried leaves were then pulverized. About 2 kg of powdered *Annona muricata* leave was extracted with 10 L of methanol for 48 hours with adequate agitation at the intervals of 3 hours. The extracting solvent was decanted and filtered after 48 hours of extraction, the extract was partitioned with methanol, dichloromethane, and n- butanol. Each of the fractions was concentrated on a digital rotary evaporator (Heidolphlaborata 4010) to obtain various extracts of the *Annona muricata* used.

Table 1.0 Microorganism used

Bacteria	Fungi
Bacillus subtilis	*Aspergillus flaws*
Clostridium sporogenes	*Candida albican*
Entrococcusfaecalis	*Fusariumoxysperium*
Klebsiella pneumonia	*Penicilliumcamemeri*
Pseudomonas aeruginosa	
Staphyloccusaureus	

Determination of the antifungal activities of the extracts of the *annonamuricata* leaves.

Clinical isolated fungi obtained were grown on Sabouraud dextrose agar at 25^{0}C. They were monitored untill they sporulated. The fungi spores were gathered into the broth and properly standardized. 20 mL of Sabouraud dextrose agar was seeded with 200 µL of the standardized broth into Maccartney bottles and was transferred into a sterile petri dish after swirling. Several wells of 6 mm diameter were made on the agar with sterilized cork borer. 1 mg/L of the each extract was transferred into a well and another well was filled with 1 mg/L of amphotericin B as a positive control. Proper diffusion of compound into the agar was ensured. The incubation of the plates at 25 ^{0}C for 96 hours was done. The zones of inhibition around the wells were determined. This experiment was repeated twice.

Determination of the Antibacterial activities of the extracts of the *Annona muricata* leaves.

The test bacterial were inoculated into tubes containing peptone water and incubated for 18 hours at a temperature of 37 ^{0}C before usage. Each of the cultures was adjusted to standard of 0.5 Mcfarland turbidity. 20 mL of sterile molten Mueller Hinton agar was seeded with 200 µL of standardized cell suspensions into the Maccartney bottles and was gently transferred into the sterile plates. Wells with 6 mm diameter were made on the plates via cork borer. 0.2 mL of 2mg/L of each extract were transferred. About 1 mg/L of the positive control (Streptomycin) were transferred into the remaining wells. Proper diffusion of the cultured plates was monitored and the plates they were incubated at 35 ^{0}C for 24 hours. The zones of inhibition were recorded. This procedure was repeated for two more times.

Results and Discussion

The result obtained from the antifungal activities of the methanol, dichloromethane and n-butanol extract of *annona muricata* leaves on the selected fungi shows that the methanol extract has highest Inhibition zone on *Aspergillus flaws* (27mm) and lowest Inhibition zone on *Fusarium oxysperium* (25mm), The dichloromethane only has an inhibition zone on *Aspergillus flaws* (27mm) while the n-butanol extract has highest inhibition zone against *Penicillium camemeri* (31mm) and lowest inhibition zone against *Candida albican* (25mm). The extracts has no inhibition against the growth of *Candida albican*, *Fusarium oxysperium, Penicillium camemeri* in dichloro methane extract and *Aspergillus flaws* in the n-butanol fraction as shown in table 2.

The methanol, dichloromethane and n-butanol extract possess higher activity against the standard antifungal amphotericin B used according to their zones of inhibitions supporting the usefulness of *Annona muricata* leaves in folklore remedies for the treatment of diseases caused by these pathogen.

Table 2.0 Result of the antifungal activities of the methanol, dichloromethane and n-butanol extract of *annona muricata* leave

Tested Fungi	Inhibition zone (mm)			
	methanol 2 mg/L	dichloromethane 2 mg/L	n-butanol 2 mg/L	Amphotercin B 1 mg/L
Aspergillus flaws	27	26	0.0	20
Candida albican	26	0.0	25	22
Fusariumoxysperium	25	0.0	26	21
Penicilliumcamemeri	26	0.0	31	23

Table 3.0 Result of the antibacterial activities of the methanol, dichloromethane and n-butanol extract of *annonamuricata* leave

	Inhibition zone (mm)			
Bacteria isolates	methanol 2 mg/L	dichloromethane 2 mg/L	n-butanol 2 mg/L	Steptomycin 1 mg/L
Bacillus subtilis	27	26	25	27
Clostridium sporogenes	29	26	25	24
Entrococcusfaecalis	27	28	25	26
Klebsiella pneumonia	31	0.0	30	27
Pseudomonas aeruginosa	26	28	31	25
Staphyloccusaureus	26	29	30	24

Antimicrobial analysis (Table 3.0) show that the methanol, dichloromethane and n-butanol extract of *Annona muricata* leave were active against all the clinical isolated bacterial strains except in dichloromethane extract against *Klebsiella pneumonia* where no inhibition zone was observed. The extracts showed inhibition zones comparable to streptomycin used as control in this study. The antibacterial activity of this study was found higher than that obtained from the study of (Yohannes et al 2019). The bioactive compounds present in this plant may be responsible for the antibacterial activity of these extracts (Avato et al 2006; Mwitari et al 2013; Alfredo et al 2016; Manikkuwadura et al 2019) also reported that the antibacterial properties of plants are due to the presence of phytochemicals such as carotenoids, flavonoids, lycopene, phenolics and ß-carotene present in them.

Conclusion

The extract of *Annona muricata* shows a broad spectrum against the selected fungi and bacterial which shows the potency of *Annona muricata* as a remedy for treating diseases cause by pathogens. The antimicrobial property displayed by these extracts validate the need for further study on isolation and characterization of active phytochemicals that can fight microbial resistance exhibited by majority of existing antibiotics from the leaves of *Annona muricata*.

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