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PHYTOCHEMICAL SCREENING AND BIOACTIVITY OF CHLOROFORM, ACETONE AND ETHYL ACETATE EXTRACTS OF *HAEMATOSTAPHIS BARTERI*

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ABSTRACT
The plant *Haematostaphis barteri* is commonly used traditionally for the treatment of diarrhoea, wound, headache, malaria, dysentery and fevers. The aim of this work was to carry out the phytochemical screening and antimicrobial activities chloroform, acetone and ethyl acetate extracts of *H. barteri*. The results showed that flavonoids, terpenes, terpenes, tannins, and saponins are detected in all the leaf extracts. The result of the antimicrobial activity obtained from the chloroform, acetone and ethyl acetate extracts of the leaf of *H. barteri* revealed that all the crude extracts chloroform, acetone and ethyl acetate leaf extracts exhibited antibacterial activity against *Salmonella paratyphi, Staphylococcus aureus, Escherichia coli,* and *Penicillium spp.* All the three extracts did not inhibit *Aspergillus niger*.

Keywords: Bioactivity, *Haematostaphis barteri,* inhibition, Organism

INTRODUCTION

Medicinal plants play a significant role in the health of humanity (Sofowora., 1993). Most conventional medicines, food supplements, folk medicines and pharmaceutical intermediate are derived from medicinal plants (Ogbonnia *et al.,* 2013). The utilization of the medicinal plants is often based on ancestral experience, limited scientific evidence regarding safety and efficacy to support the continued therapeutic application of some of these herbal remedies exists compared to such evidence for synthetically formulated drugs (Ojelere *et al*., 2014). Several studies have reported elemental contents in plant extracts which are consumed as herbal health drink or in orthodox medicine (Tadzabia *et al.,* 2013). Plant-derived substances have recently become of great interest owing to their versatile applications (Tiwari *et al.,* 2011). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non-nutritive chemicals that have protective or disease preventive property (Subhashini *et al.,* 2010). Phytochemicals give plants natural defense against diseases and they perform similar function for humans (Tadzabia *et al.,* 2013). Correlation between the phytoconstituents and the bioactivity of plants is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well (Pandey *et al.,* 2013). *H. barteri* popularly known as blood plum is a member of anacardiaceae family. The Hausa name is Jinin kafiri. It is found wild in Taraba, Adamawa and Borno states of Nigeria. The fresh tender leaves are edible. The fruit has oily seed which is edible (Bokhari and Ahmed 1979). The phytochemical screening of the crude extracts obtained by sequential solvent extraction of dried stem bark of *H. barteri* showed the presence of flavonoids, cardiac glycosides and tannins as the major secondary metabolites in the extracts (Ezekiel *et al*., 2016).

Stem bark of *H. barteri* have been used by traditional healers in northern Nigeria for the management of ailments such as, cancer (Kubmarawa *et al.,* 2007), stomach ache, and vomiting (Rabo and Sanusi, 2001), anemia and hemorrhoid (Ezekiel *et al*., 2016). The free radical scavenging properties of the extracts were determined quantitatively by the use of 2, 2- diphenyl- 1-picrylhydazyl (DPPH) and result revealed that only acetone, ethanol and aqueous methanol extracts were weakly active with percentage inhibition of DPPH activities of 49.9%, 51.3% and 48.2% respectively (Ezekiel *et al*., 2016).

H. barteri is one of the plants which have been used in traditional medicine for many years. The bark is astringent, bitter and febrifuge. An infusion of the plants leaves is used to treat diarrhea and fevers. To the best of our knowledge little or no work has been done on the plant *H barteri* in Taraba, Nigeria. This work is designed to enrich the available scientific data on the phytochemistry and antimicrobial activities of *H barteri* leaves. This paper reports the phytochemistry and antimicrobial activities of *H barteri* leaves on some bacterial and fungal isolates.

MATERIALS AND METHODS

Sample Collection and Preparation

H. barteri leaves were collected from their natural habitat of Zing Local Government Areas of Taraba state, Nigeria. The samples were air-dried for two weeks and then milled into fine powder using a milling machine. The extracts of the leaves were prepared by soaking 100 g of the sample in 250 ml chloroform for four days with frequent agitation. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation using rotatory evaporator, kept in a vacuum oven over night at room temperature to remove all the solvent and weighed. The procedure was repeated on the residue using ethyl acetate and acetone sequentially in order of polarity. The extracts were stored in a desiccator until required for testing.

Phytochemical Screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Ushie *et al*., 2013 and Ushie *et al*., 2016.

Bioassay

This is the study of antimicrobial activity of the crude or purified extracts against micro-organism. It was used as a guide to determine the active components of the leaves of *H barteri.* The crude extracts were tested for antibacterial and antifungal activities. The test organisms were collected from Bauchi Specialist Hospital, Bauchi State, Nigeria. The antibacterial assay was carried out using methods described by Ushie *et al*., (2013) with modifications.

Preparation of varying concentrations of the extracts.

Various concentrations of the extracts were prepared ranging from 50 to 400 mg/mL; this was obtained by measuring 1 mg of the extract and dissolved in 10 mL dimethyl sulphur oxide (DMSO), a solvent that dissolved the extract (100 mg/mL). A serial dilution of the dissolved extract (100 mg/mL) was carried out into three different bottles containing DMSO to obtain concentrations of 400, 200 100 and 50 mg/mL respectively.

Sensitivity test of the crude extract using Agar Well Diffusion Method

The organisms used were standardized using McFarland turbidity standard scale l, to obtain a bacterial cell density of 10^6 colony forming unit per millilitre (cfu/mL). The standardized inoculate were uniformly streaked (swabbed) into freshly prepared Mueller Hinton agar and potato dextrose agar plates respectively for the bacterial and fungal growth. Five wells were made on the inoculated plates with a cork borer (8 mm in diameter). The wells were properly labeled according to different number of the concentrations prepared. The wells were then filled up with the extracts about 0.2 mL per well. The plates were allowed to stay on the bench for 1 hour for the extract to diffuse on the agar. The Mueller Hinton agar plates for bacterial were incubated at 37^{o}C for three days while the potato dextrose agar plates for fungi were incubated at room temperature (drawer) for three days. At the end of incubation period, all plates were observed for any evidence of inhibition, which will appear as clear zones that were completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured with a transparent ruler calibrated in millimeter (mm).

RESULTS AND DISCUSSION

RESULTS

Table 1 presents the results of phytochemical screening of leaf solvent extracts of *H. barteri*. The phytochemical screening of crude extracts of chloroform, ethyl acetate, acetone extracts of *H. barteri* which revealed the presence of flavonoids, terpenoids and saponins in the leaf extracts.

Table 1: Phytochemical Screening Chloroform, ethyl acetate, acetone extracts of *H. barteri*

S/N	Phytochemicals	CE	EAE	AE
1	Alkaloids	-	-	-
2	Phlobatanins	-	-	-
3	Flavonoids	+	+	+
4	Saponins	+	+	+
5	Cardiac glycosides	-	-	-
6	Terpenoids	+	+	+
7	Steroids	-	-	-
8	Tannins	++	++	++

CE = Chloroform, AE = Acetone extract, EAE = Ethyl acetate extract,
+: Present, - : Not present

Table 2: The Mean Zone of Inhibition of Chloroform, ethyl acetate, acetone extracts of *H. barteri*

Organisms	Conc. (Mg/ml)	CE	EAE	AE	C (+)	DMSO (-ve)
Pseudomonas aeruginosa	400	08	08	09	25	00
	200	05	06	05	19	00
	100	00	00	00	14	00
	50	00	00	00	10	00
Staphylococcus aureus	400	10	21	18	29	00
	200	09	10	11	23	00
	100	05	07	9	19	00
	50	02	04	04	12	00
Escherichia coli	400	12	21	22	32	00
	200	09	13	18	25	00
	100	06	10	11	18	00
	50	00	6	08	13	00
Aspergellius Niger	400	06	02	04	15	00
	200	03	00	03	10	00
	100	00	00	00	00	00
	50	00	00	00	00	00
Penicillium Spp	400	16	06	15	28	00
	200	12	03	12	20	00
	100	09	00	09	13	00
	50	04	00	04	09	00

Key: CE= Chloroform, EAE= Ethyl acetate extract, AE = Acetone extract, Values greater than 7 mm indicate activity and 00 means no activity

DISCUSSION

Phytochemicals

The phytochemical screening of crude yields of the chemical constituents of *H. barteri* showed the presence of flavonoids, tannins, terpenoids and saponins in all the extracts are present in all the leaf extracts. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen 1997). Okoli and Okere (2010) pointed out that flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and inhibit tumor growth. Saponins causes complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis on intravenous injection (Francis *et al.*, 2002). Tannins can be used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods, and diarrhea, and as antidote in heavy metal poisoning (Ogunleye and Ibitoye.,2003). Liu, *et al.* (2003) reviewed the biological activities of tannins and observed that tannins have remarkable activity in cancer prevention and anticancer.

The plants extracts of *H. Barteri* was investigated to evaluate the antimicrobial activity. The evaluation of this antimicrobial activity of these plant extract was reported using the Table 2. which revealed that all the crude extracts of the leaf inhibited antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli.* and also illustrated in Figure 1 to Figure 5.

Pseudomonas aeruginosa

The result of *Pseudomonas aeruginosa* presented Figure 1. Shows that the extract inhibited *(*08 - 09 mm zone diameter of inhibition) by the concentration of 400 mg/100ml while at the concentration of 50, 100 and 200 was below the 07mm zone diameter which is an indication that at this level of concentration of the extract *Ps. Aeruginosa* cannot be used to control the organism.

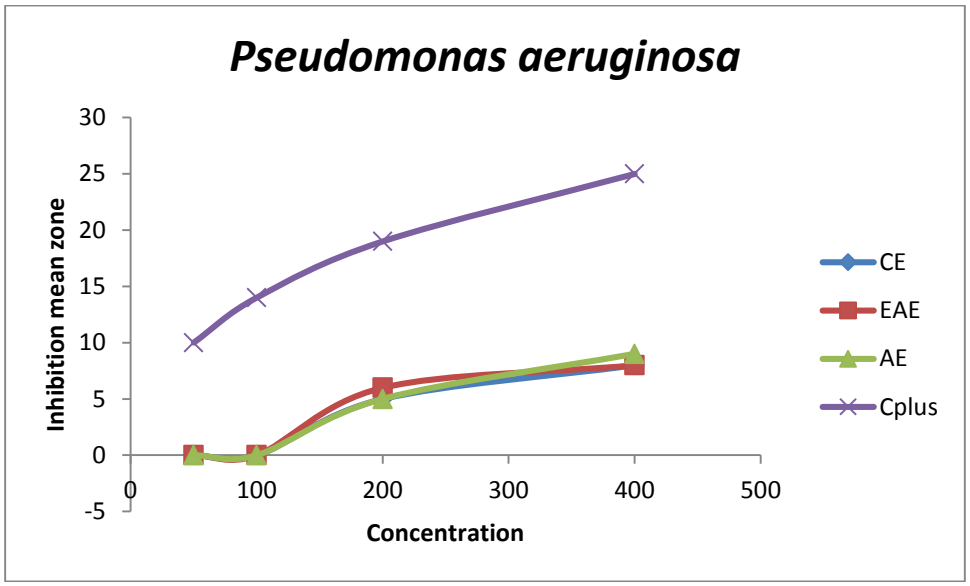

Fig. 1: Graph of extracts of *H. barteri* on *Pseudomonas aeruginosa*

Staphylococcus aureus

Figure 2 is the results of *Staphylococcus aureus* which revealed that plant extracts potentially suppressed the microbial growth at the concentration of 200 and 400 mg/100ml at the range of (09 - 21 mm zone diameter) while at the concentration of 50 and 100 the extract was unable to retarded *St. aureus* with the ethyl acetate extract demonstrating the highest activity of 21 mm zone diameter of inhibition.

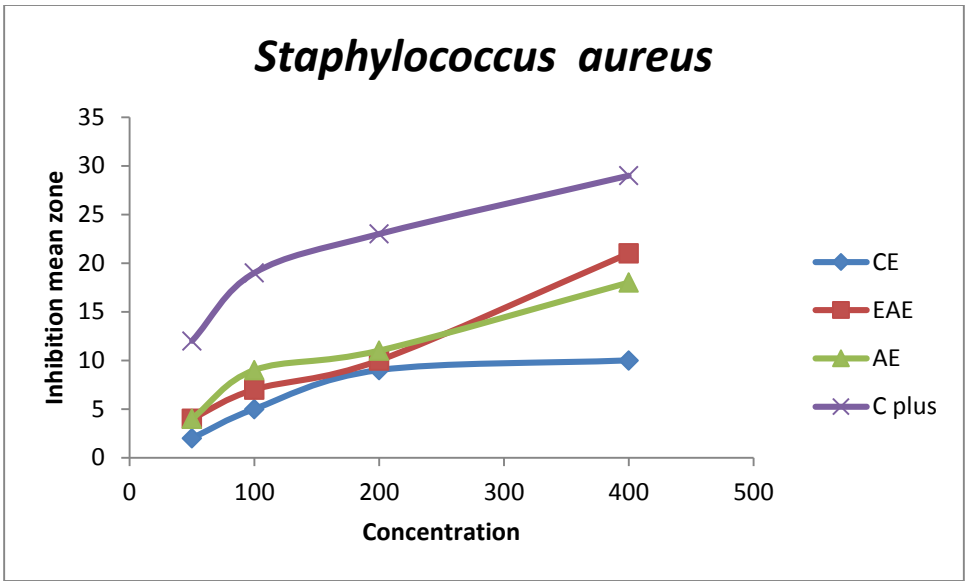

Fig. 2: Graph of extracts of *H. barteri* on *Staphylococcus aureus*

Escherichia coli

Escherichia coli is inhibited by all the extracts (09 - 21 mm zone diameter of inhibition) at the concentration of 200 and 400 mg/100ml while the concentration of 50 and 100 did not inhibit *E. coli* with the ethyl acetate extract demonstrating the highest activity of 22 mm zone diameter of inhibition illustrated in Figure 3.

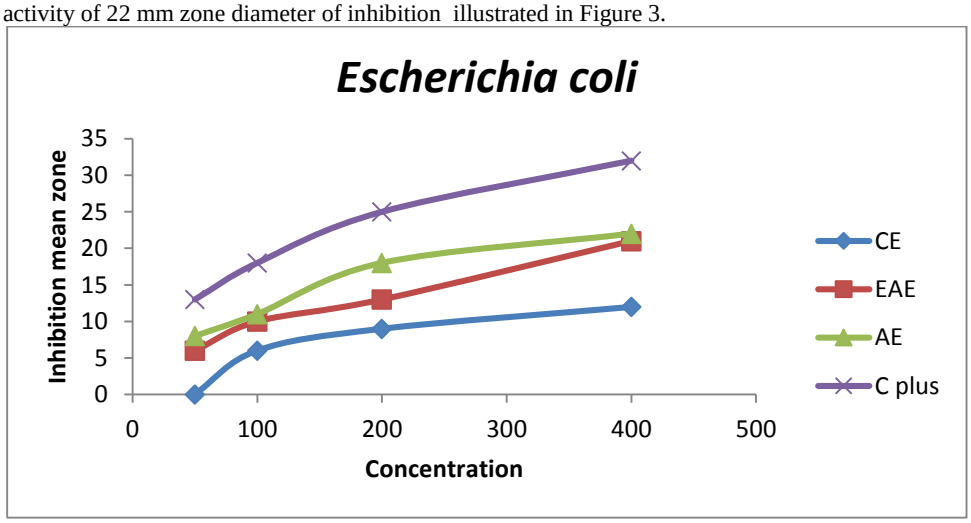

Fig. 3: Graph of extracts of *H. barteri* on *Escherica coli*

Aspergellius niger and Penicillium spp

The leaf extracts of *H. barteri* did not suppressed antifungal activity on *Aspergellius niger* and *Penicillium spp* except for the chloroform and acetone extracts that inhibited (12 - 16 mm zone diameter of inhibition) shown in Figure 4 and 5.

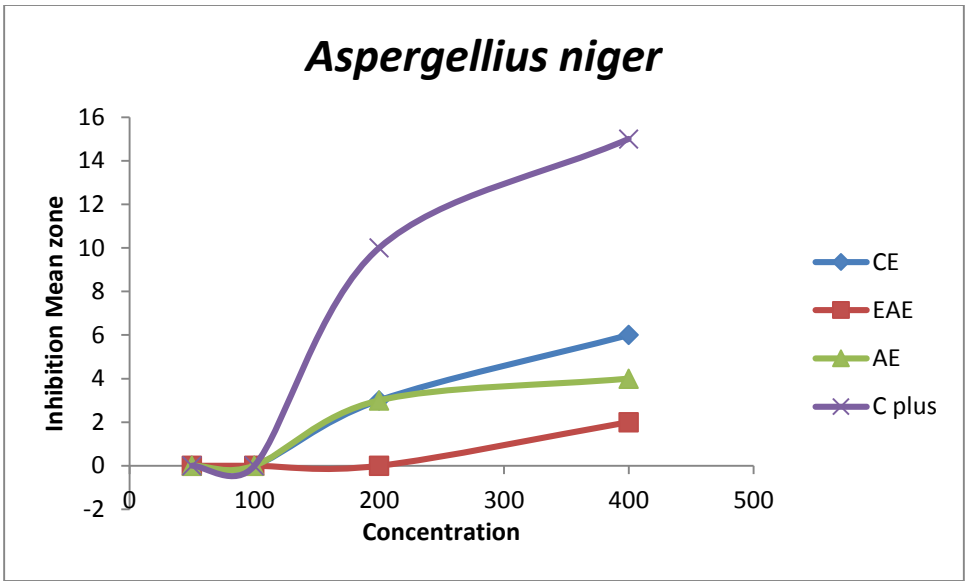

Figure 4: Graph of extracts of *H. barteri* on *Aspergillus niger*

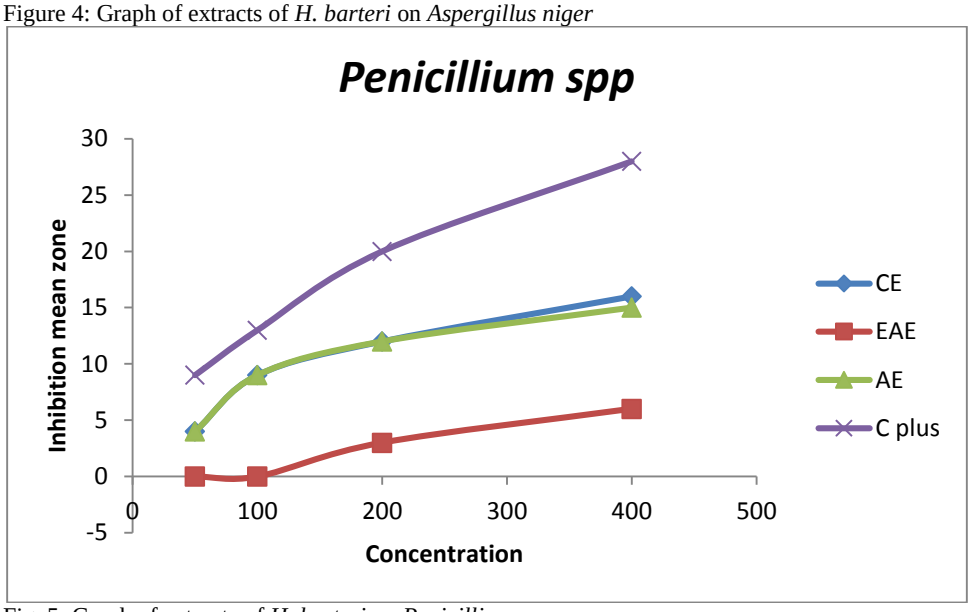

Fig. 5: Graph of extracts of *H. barteri* on *Penicillium spp*

CONCLUSION

The demonstration of activity against both gram-negative and gram-positive bacteria and fungi is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against laboratory isolates is also an indication that it can be a source of very potent antimicrobial substances that can be used against drug resistant microorganisms prevalent in hospital environments.

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