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Phytochemical screening and antimicrobial activity of *Haematostaphis barteri*

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Abstract

The aim of this work was to carry out the phytochemical screening and antimicrobial activities of *Haematostaphis barteri*. The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. The results showed that flavonoids, terpenes, terpenes, tannins, and saponins are present in all the leaf extracts. The result of the antimicrobial activity obtained from the extracts of the leaf of *Haematostaphis barteri* revealed that all the crude extracts of the leaf inhibited or exhibited antibacterial activity against *Salmonella paratyphi, Staphylococcus aureus, Escherichia coli,* and *Penicillium spp.* All the extracts did demonstrate antimicrobial activity against *Aspergillus niger*. The plant *H. barteri* is commonly used traditionally for the treatment of diarrhoea, wound, headache, malaria, dysentery and fevers. The overall results confirm the significance of the use of the plant in traditional medicinal treatment of diarrhea, wound, headache, malaria, dysentery and fevers, in line with reported claims.

Keywords: Antimicrobial; *Haematostaphis barteri:* Phytochemistry; Zing**;** Sensitivity and Inhibition

1. Introduction

Medicinal plants play a significant role in the health of humanity [1]. Most conventional medicines, food supplements, folk medicines and pharmaceutical intermediate are derived from medicinal plants [2]. 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 [3]. Several studies have reported elemental contents in plant extracts which are consumed as herbal health drink or in orthodox medicine [4]. Plant-derived substances have recently become of great interest owing to their versatile applications [5]. 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 [6]. Phytochemicals give plants natural defense against diseases and they perform similar function for humans [4]. Correlation between the phyto-constituents 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 [7]. *Haematostaphis barteri* popularly known as blood plum is a member of anacardiaceae family. The Hausa name is Jininkafiri. 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 [8]. The phytochemical screening of the crude extracts obtained by sequential solvent extraction of dried stem bark of *Haematostaphis barteri* showed the presence of flavonoids, cardiac glycosides and tannins as the major secondary metabolites in the extracts [9].

Stem bark of *H. Barteri* have been used by traditional healers in northern Nigeria for the management of ailments such as, cancer [10], stomach ache, and vomiting (Rabo and Sanusi, 2001), anemia and hemorrhoid [9]. The free radical

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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 DPP activities of 49.9%, 51.3% and 48.2% respectively [9].

*Haematostaphis barteri*i 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* leaf extracts on some bacterial and fungal isolates.

2. Material and methods

2.1. Sample Collection and Preparation

Haematostaphis 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.

2.2. Method of Extraction

The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. The extracts of the leaves was prepared by soaking 100 g of each in 250 ml hexane for four days with frequent agitation until soluble matter is dissolved. 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 methanol. The extracts were stored in a desiccator until required for testing.

2.2.1. Phytochemical Screening Assay

Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by [1], [10], [11] and [12].

2.3. Test for Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue solution indicated the absence of tannins in distilled water and dark green colour indicating presence in methanol.

2.4. Test for Saponins

About 0.2g of plant extract was mixed with distilled water and heated to boil. Frothing (appearance of creamy mix of small bubbles) showed the presence of saponins in methanol while red in distilled water.

2.5. Test for Terpenoids

The extract (0.2g) was mixed with 2ml of chloroform, and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids on both extract.

2.6. Test for Steroids

Acetic anhydride (2 ml) was added to 0.5g of the extract in a test tube. It was then followed by the addition of 2 ml of sulfuric acid. A colour change from violet to blue or green indicated the presence of steroids on both extract.

2.7. Test for Flavonoids

About 0.2g of the extract was dissolved in dilute sodium hydroxide solution, and equal amount of hydrochloric acid was added. A yellow solution that turned colourless indicated the presence of flavonoids on both extract.

2.8. Test for Alkaloids

The aqueous (3ml) was stirred with (3ml) of 1% HCl on a steam bath. Meyer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as positive evidence of alkaloids

2.9. Test for phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Disposition of red precipitate determines the presence of phlobatannins.

2.10. Test for Anthraquinones

About 0.5g of the extract was boiled with 2ml of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3 solution was added to the mixture and heated. Formation of rose pink colour indicated the presence of anthraquinones on both extract.

2.11. Test for Cardiac glycosides

10 cm_3 of 50% H_2S0_4 was heated in boiling water for 5 min. 10 cm_3 of Fehlings solution (5 cm_3 of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycoside was observed.

2.12. 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 [13] Ochi *et al*., (2015) with modifications.

2.13. 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.

2.14. 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).

2.15. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB). Each tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism was used as a control. All tubes were then incubated at 37^{o}C for 24 hours. After the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion.The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

2.16. Determination of Minimum Bactericidal Concentration (MBC)

The results from the Minimum Inhibitory Concentration (MIC) were used to determine the Minimum Bactericidal Concentration (MBC).A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test, it was then streaked unto a freshly prepared sterile nutrient agar plates. The plates were incubated at 37^{o}C for 24 hours. After the incubation period the plates were then examined for the presence or absence of growth. This was done to determine if the antimicrobial effect of the extract was bactericidal or bacteriostatic.

3. Results

Table 1 presents the results of phytochemical screening of leaf solvent extracts of *H. barteri*. The phytochemical screening of crude extracts of *H. barteri* revealed the presence of flavonoids, terpenoids and saponins in all the extracts are present in all the leaf extracts.

Table 1 Phytochemical Screening of *Haematostaphis barteri*

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

HE = Hexane extract,
ME = Methanol extract, L: Leaves, **+**: Present, **-** : Not present

Table 2 Mean Zone of Inhibition of *Haematostaphis barteri*

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

	100	00	00	00	00
	50	00	00	00	00
Penicillium spp	400	13	16	28	00
	200	13	14	20	00
	100	13	10	13	00
	50	06	05	09	00

Key: HE = Hexane extract, ME = Methanol extract, Values greater than 7 mm indicate activity and 00 means no activity,

Table 3 Showing Minimum Inhibitory concentration (MIC) and Minimum Bacteriacidal Concentration in milligram per mililitre (mg/ml) of *Haematostaphis barteri*

MIC AND MBC (mg/ml)						
Organism	**ME**		**HE**		**Msc**	**OVC**
Pseudomonas aeruginosa	MIC	MBC	MIC	MBC	+	+
	200	400	200	400		
Staphylococcus aureus	100	200	200	400	+	+
Escherichia coli	200	400	200	400	+	+
Aspergellius Niger	200	400	200	400	+	+
Penicillium spp	100	200	200	400	+	+

Key :-Msc = Media Sterility Control, OVC = Organism Viability Control

4. Discussion

The phytochemical screening of crude yields of the chemical constituents of *Haematostaphis 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 [14]. Flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and inhibit tumor growth [15] 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 [16]. Tannins can be used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods,anddiarrhea,andas antidote in heavy metal poisoning [17]. Biological activities of tannins were reviewed and observed that tannins have remarkable activity in cancer prevention and anticancer [18].

The result of the antimicrobial activity as shown in Tables 2 and table 3 obtained from the extract of the leaves of *Haematostaphis barteri* revealed the following; all the crude extracts of the leaf inhibited or exhibited antibacterial activity against *Pseudomonas aeruginosa, Salmonella paratyphi, Staphylococcus aureus,* and *Escherichia coli.* All the extracts demonstrated antimicrobial activity against both the test bacteria and fungi with the methanol extracts demonstrating the highest activity for *Staphylococcus aureus* test (29 mm zone diameter of inhibition), followed by the methanol extracts for *Staphylococcus aureus* and *Escherichia coli* (25 and 22 mm zone diameter of inhibition) while the antimicrobial test for fungi did not demonstrate any reasonable activity against *Aspergillus niger* but demonstrated little in *Penicillium spp.* for the extracts of hexane and methanol respectively. The methanol extracts were active against *E. coli* (17 mm zone diameter of inhibition) *S. typhi* (18 mm zone diameter of inhibition), *S. aureus* (20 mm zone diameter of inhibition). The hexane extracts were active against *E. coli* (09 mm zone diameter of inhibition) *S. typhi* (16 mm zone diameter of inhibition), *S. aureus* (17 mm zone diameter of inhibition) and *Penicillium chrysogenum* were active at (13mm and 16mm zone diameter of inhibition) for hexane and methanol extracts while *A. niger*did not inhibit reasonably at 200 mg/ml. Augmentin and Mycotin demonstrated the highest activities against both bacteria and fungi respectively.

The MIC and MBC of the extracts ranged from 50-200 mg/mL, with the acetone extracts demonstrating the lowest values (MIC 50 mg/mL: MBC 50 mg/mL each) against *E. coli, S. aureus* and *S. typhi* followed by the chloroform extracts against

E. coli (MIC 50 mg/mL, MBC 50 mg/mL), ethyl acetate demonstrating the lowest values (MIC 100 and 50 mg/mL: MBC 100 and 50 mg/mL each) against *E. coli.* Hexane demonstrated the lowest values (MIC100 and 50 mg/mL: MBC 50 mg/mL each) against *E. coli,* with almost all demonstrating the lowest values (MIC 200, 100 and 50 mg/mL: MBC 200, 100 and 50 mg/mL each) against *A. niger* and *Penicillium* spp. Most of the MIC values were lower than the MBC values indicating that the extracts could be bactericidal in action. Low MIC and MBC values are also an indication of high efficacy. 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.

5. Conclusion

The bioactive components extracted from the leaves of *Haematostaphis barteri* include; flavonoid, steroids, terpenes, glycosides, saponins and tannin were detected in both hexane and methanol extracts, these component are naturally occurring in most plant materials, and are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property to plants. All the extracts demonstrated antimicrobial activity against both the test bacteria and fungi.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome

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