ANTIBACTERIAL PROPERTIES OF *Boeahaviadiffusa*AND *Huntaria umbellate* ON BACTERIA ISOLATED FROM BLOOD SAMPLES MALARIA PATIENTS.

ABSTRACT

Malaria remains a major infectious disease ravaging the tropical part of the world with more deleterious effects on tropical low- and middle-income nations. Aside fromthe complications, secondary infections by bacteria have become rampant; hence, the need for the antibacterial properties of *Boerhaaviadiffusa* and *Huntaria umbellate* extracts. Bioactive ingredients of the plants were extracted using standard methods and the extracts were subjected to filtration using a membrane-injection filter. Malaria positive blood samples were obtained and the bacteria present in them were isolated and identified before subjecting them to sensitivity test on the extracts. The results showed that steroids and tannin were not detected in both cold and hot water extracts while glycoside was the most abundant in the extracts, followed by terpenoid and phenol. The probable bacteria generally identified are *Acinetobacter, Escherichia, Klebsiella, Staphylococcus, Salmonella, Streptococcus* and *Pseudomonas*. The bacteria with the highest frequency of occurrence were *Salmonella* and *Staphylococcus*. The least occurred bacteria was*Pseudomonas*. Both *Staphylococcus* and *Salmonella* had 28% of occurrence in all the samples. *Salmonella* was most susceptible to ampicillin having a diameter of zone of inhibition of 18.33±0.58 mm, while it was not susceptible at all to nitrofurantoin. *Escherichia* was most susceptible to tetracycline with a zone diameter of 14.33±0.58 mm, while *Pseudomonas* was most susceptible to ampicillin having a zone diameter of 10.00±0.00 mm. At 100mg/mL concentration, the highest diameter of zone of inhibition was 11.33±0.58. The results obtained in this study have shown that both *B. diffusa* and *H. umbellate* extracts have a high antibacterial effect.

Keywords: Antibacterial; Extracts; Malaria; Isolates.

INTRODUCTION

Malaria is a preventable and curable disease, yet it remains a devastating tropical disease, with high infection and mortality statistics. Malaria, a life-threatening disease caused by a parasitic infection of the red blood cells by *Plasmodium* parasites transmitted through a bite of the female *Anopheles* mosquitoes, is undoubtedly the single most destructive and dangerous infectious agent in the developing world, predominantly tropical and subtropical regions, including parts of the Americans, Asian and Africa (Winter *et al*., 2006). Fifth species, *Plasmodium knowlesi*which is a simian parasite has been shown to infect humans but rarely (WHO, 2014).

Boerhaaviadiffusa(Spreading Hogweed in English) named in honour of Hermann Boerhaave, a famous Dutch physician of the 18th century [Chopra, 1969], is mainly a diffused perennial herbaceous creeping weed of India (known also under its traditional name as *B. diffusa*) and of Brazil (known as Erva tostão) and is up to 1 m or more in length, having spreading branches. It belongs to the family *Nyctaginaceae*. The leaves are simple, thick, fleshy, and hairy, arranged in unequal pairs, green and glabrous above and usually white underneath. The shape of the leaves varies considerably ovate - oblong, round, or subcordate at the base and smooth above.

Hunteriaumbellata (K. Schum.) Hallier f. belong to the *Apocynaceae* family, a West African glaborous tree known as Abeere in Yoruba, Southwest Nigeria (Adeneye*et al*., 2009). It has been widely used by different folk, which include the management of infections, diseases, treatment of pain and metabolic disorders such as diabetes mellitus and obesity (Falodun*et al*., 2006). Many genera in the *Apocynaceae* family have been well-studied, especially their chemical composition and economic importance. Studies have revealed that the aqueous seed extract of *H.umbellata*has

been tested on different experimental models of diabetes (Igbe*et al*., 2009) for its anti-hyperglycemic effect coupled with anti-obesity and anti-hyperlipidemic potentials, which are well-documented. Likewise, oral toxicity studies have been conducted that authenticate its safety prior to oral administration in experimental animals (Adeneye*et al.*, 2010).

Collection and authentication of plant materials

Fresh dried seeds of *H.umbellata*and leaves of *B.diffusa* were collected from various locations in Akure and its environs and identified at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The plant samples were authenticated at the Herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

3.2 Preparation of Plant Extracts

The plant samples were air-dried at ambient temperature (25-30°C) for 5-10 days. The dried plant samples were blended and each plant sequentially extracted using cold water, hot water and ethanol (Omoriege*et al*., 2016). The essential oil of the dried *H. umbellata* seed was extracted adopting the method of Rants (2023)

3.3 Phytochemical analysis

The plant extracts were analyzed for the presence and amounts of different classes of secondary metabolites using the technique described by Das *et al*., (2023) as follows:

3.3.1 Phytochemical Analysis of *A. paniculata* leave extract (Qualitative)

The leaves extract of *A. paniculata* was tested for the presence of different phytoconstituents like alkaloids, saponin, tannin, phlobatanin, anthraquinone, flavonoid, steroids, terpenoid and cardiae glycosides using standard methods (AOAC, 2011).

Test for Alkaloids

About 0.5g of the extract was stirred with 5mL of 1% aqueous hydrochloric acid (HCl) on a steam water bath for 2mins. One mL of the filterate was treated with a few drops of Dragendorff's reagent. Blue black turbidity was taken as preliminary evidence for the presence of alkaloid.

3.3.3 Test for Saponins

The ability of saponin to produce frothing in aqueous solution was used as a screening test for saponin. About 0.5g of the extract was taken with distilled water in a test tube; frothing which persist on warming was taken as preliminary evidence for the presence of saponin.

3.3.4 Test for Tannins

About 5g of the extract was stirred with 10 mL of distilled water, filteredand ferric chloride reagent was added to the filtrate; a blur black green or blue green precipitate was taken as evidence for the presence of tannin.

3.3.5 Test for Anthraquinone

Borntrager's test was used for the detection of Anthraquinone. 0.5g of the extract was shaken with 10ml of benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of pink red or violet colour in the ammonia layer indicator the presence of free Anthraquinone.

3.3.6 Test for Flavonoid

About 0.5g of the extract was stirred with 20ml of dilute ammonia solution, a yellow colouration was observed, the disappearance of the yellow colour after the addition of 1ml concentrated H_2SO_4indicates the presence of flavonoid.

3.3.7 Test for Steroid

20ml of acetic anhydride was added to 0.5g of the extract and filter, 2ml of concentrated H_2SO_4 was added to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroid.

3.3.8 Test for Terpenoids

About 5g of the extract was mixed with 20ml of chloroform and filtered; 3ml of concentrated H_2SO_4 was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of terpenoid.

3.3.9 Test for Cardiac Glycosides

The following tests were carried out to test for cardiac glycosides.

Legal's Test: The extract was dissolved in pyridine and a few drops of 2% sodium nitroprusside with a few drops of 20% sodium hydroxide (NaOH) were added. A deep red colouration which faded to a brownish yellow indicates the presence of cardenolides (AOAC, 2015).

3.4 Quantitative Phytochemical Analysis

3.4.1 Determination of Tannin

About 2g of finely ground sample was weighed into a 50ml sample bottle. A 10ml of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hours at 30^0C. Each solution was then centrifuge and the supernatant store in ice. Exactly 0.2ml of each solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solutions were prepared from 0.5mg/ml of the stock and the solution made up to 1ml with distilled water. Exactly 0.5ml of folinciocateau reagent was added to both sample and standard followed by 2.5ml of 20% Na_2CO_3; the solution was then vortexed and allowed to incubate for 40 minutes at room temperature, its absorbance was read at 725nm

against a reagent blank concentration of the same solution from a standard tannin acid curve was prepared Makkar and Goodchild (1996).

3.4.2 Determination of Total Flavonoid

The total flavonoid content of the extract was determined using a colourimeter assay developed by Bao (2005). 0.2ml of the extract was added to 0.3ml of 5% $NaNO_3$ at zero time. After 5mins, 0.6ml of 10% $AlCl_3$ was added and after 6min, 2ml of 1m NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rytin equivalent.

3.4.3 Determination of Saponin

The spectrophotometric method of Brunner (1994) will be used for saponin determination. 2g of the finely grinded sample will be weighed into a 250ml beaker and 100ml of Isobutyl alcohol or (But-2-ol) will be added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was filtered using No 1 whatman filter paper into 100ml beaker containing 20ml of 40% saturated solution of ($MgCO_3$). The mixture obtained was then filter through No 1 whatman filter paper; a clean colourless solution was taken into 50ml volumetric flask using pipette, 2ml of 5% iron (iii) chloride ($FeCl_3$) solution was added to make up with mark of the distillated water. Allow to stand for 30mins for the colour to develop. The absorbance is read against the blank at 380nm.

3.4.4 Determination of Phenol

A 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4 mins this was filtered and extract was concentrated on a water bath to one quarter of the original volume.

Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and wash with dilute ammonium hydroxide and then filtered. The residue was then dried and weighed (Harbone, 1973).

3.4.5 Determination of Cardiac Glycosides

The procedure described by Sofowora (1995) was used. 10ml of the extract pipetted into a 250ml conical flask. 50ml chloroform was added and shaken on vortex mixer for 1 hour. The mixture was filtered into 100ml conical flask: 10ml of pyridine and 2ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10mins. 3ml of 20% sodium hydroxide (NaOH) was added to develop a brownish yellow colour. Glycosides standard (Digitoxin). A concentration which range from 0 – 5mg/ml were prepared from stick solution the absorbance was read at 510nm.

3.4.6 Determination of Terpenoid

The procedure described by Sofowora (1995) was used. Five gram of finely grounded sample was weighed into a 50ml conical flask. 20ml of chloroform: methanol 2:1 was added the mixture was shaken thoroughly and allowed to stand for 15mins at room temperature. The suspension was centrifuged at 3000rpm; the supernatant was discarded and the precipitate was re-washed with 20ml chloroform: methonal 2:1 and then re-centrifuge again the precipitate was dissolved in 40ml of 10% SDS solution. 1ml of 0.01m ferric chloride was added and allowed to stand for 30mins before taking the absorbance at 510nm. The STD Terpenoid (alpha terpineol). Concentration ranging from 0.5mg/ml from the stock solution.

3.4.7 Determination of Steroid

A quantitative determination of steroid was determined by weighing a 5gm of the finely powdered sample into 100ml conical flask and 50ml of pyridine was added to it, and shake for 30mins at room temperature; 3ml of 250mg/ml metallic copper powder or copper(i)oxide and allow to incubate for 1hr in the dark and the absorbance was measure at 350nm against reagent blank. (Sofowora, 1995).

Collection of Blood Samples

The blood was collected using Anaesthetization methods as described by Bada *et al*., (2023).The blood was carefully transferred into EDTA bottles for haematological assays and lithium heparin bottles for determination of liver functioning and biochemical parameters respectively (Baker *et al*., 2020).

Collection of human malaria positive blood samples

The collection of human malaria positive blood samples was done using the method of Kayiba *et al*. (2021) after obtaining ethical clearance from the Ondo State Hospital Management Board, Akure. The blood samples were collected from the Federal University of Technology Medical centre and the University of Medical Science Teaching Hospital laboratories with the scientist on duty filling a simple questionnaire (Sample at the Appendix I) by asking the patient few questions.

3.5 Bacteria isolation from blood samples

The bacteria isolation was done using standard microbiological methods outlined by Prescott *et al*. (2022). Purification and identification of the isolates was done using colonial morphology and biochemical tests (Jawetz*et al*., 2021).

3.15 Standardization of test bacteria

A loopful of the bacterial culture was aseptically inoculated into freshly prepared sterile nutrient broth and incubated for 24 hours. Zero-point-two millimetre was pipette from the 24 hours broth culture of the test organism and was dispensed into 20 ml sterile nutrient broth and incubated for another 4 hours to standardise the culture to 0.5 McFarland's standard (10^6cfu/ml) before use as described by Prescott *et al*. (2021).

3.6Antibiotic sensitivity profile

The antibiotic sensitivity profile was investigated in order to compare the sensitivity of the microorganisms to the different conventional antibiotics as described by Baker *et al*. (2021). The disc diffusion method was used to determine the susceptibility and resistance of the organisms to the antimicrobial drugs. Twenty millilitres of sterile Mueller-Hilton agar was aseptically poured into sterile Petri dishes and allowed to gel. Each plate was seeded with the test organism before aseptically introducing the antibiotic disc with sterile forceps onto the surface of the solidified Mueller Hilton agar plate and incubated at 37 ^{o}C for 24 hours. After incubation, clear zones around the disk were measured in millimetres and recorded as the zones of inhibition. Diameters of zone of inhibition was measured with a calibrated ruler and then compared with Clinical and Laboratory Standards Institute (CLSI) standard for their sensitivity or resistance. Seeded plates without antibiotic disks served as the control. The antibiotic sensitivity profile was carried out in triplicates.

3.7 Preparation of Plant Extracts

The air-dried sample of *H, umbellata*and *B. diffusa* were powdered in a table model grinder for extraction. The powdered materials were extracted using methanol. Three hundred gram (300 g) of the grinded samples was weighed into three different containers, and was labeled. Then 2000ml of the solvents was added, covered, shaken and mixtures were left for about 72 hours,

after which the solvents along with the extracts were drained out with muslin cloth, filtered with no 1 Whatman filter paper and semi-solid extracts were obtained in vacuo using rotary evaporator.

3.8 Reconstitution of the *H. umbellata and B. diffusa*extract

One gram of the extracts was dissolved in 10 ml of 30% Dimethyl sulfoxide DMSO

3.9Antibacterial assay of *H. umbellata and B. diffusa*extract

The potential antibacterial activity of *H. umbellata and B. diffusa*extracts were carried out has described by Geetha *et al*. (2017) with slight modification. The sterile petri dishes were filled with 25ml of Muller Hinton agar and allowed the agar to get solidified. Prior to streaking the plates with bacterial culture, 5mm diameter wells were punched in the medium using a sterile borer. After the agar gets solidified the bacterial cultures were inoculated by spreading in the petri plates using sterile cotton swabs. Then 0.1ml of plant extract in peptone water was directly applied to the well made on the surface of Muller Hinton agar containing bacterial lawn. Positive control was maintained with antibiotic ciprofloxacin and wells containing solvent alone was maintained as negative control. The inoculated plates were incubated overnight at 37oC for allowing the bacterial growth and the diameter of zone of inhibition was measured in mm.

3.10 Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) on window 10 and means were separated using Duncan's New Multiple Range Test (DNMRT). Statistical software Package for Social Science (SPSS) version 26 was applied in analysing the data.

RESULTS

Qualitative and Quantitative Phytochemical properties of selected medicinal plants

The result of the qualitative phytochemical analysis showed that different constituents were present in the different extract of the plants. In the *B.diffusa*extracts, only phenol and saponin were detected in the cold-water extract while the hot water extract showed the terpenoid, flavonoid and glycosides in addition to the two detected in cold extract. The result is shown in Table 1. Table 2 shows the result of the quantitative phytochemical analysis of the same extract. Steroids and tannin in were not detected in both cold and hot water extracts while glycoside was the most abundant in the extracts followed by terpenoid and phenol.

Table 1: Qualitative phytochemical result of *B. diffusa* extract

Compounds	Hot water	Cold water	Ethanol
Saponins	+	++	+
Terpenoids	+	-	+
Steroids	-	-	+
Tannins	-	-	+
Flavonoids	+	-	+
Glycosides	+	-	+
Phenol	+	+	+
Anthraquinones	-	-	-

Key: += Present; - = Absent

Table 2: Phytochemical composition of the *B. diffusa* extracts

Compounds	Hot water	Cold water	Ethanol
Saponins	3.18	4.209	5.63
Terpenoids	4.689	-	25.95
Steroids	-	-	7.04
Tannins	-	-	4.29
Flavonoids	2.821	-	0.68
Glycosides	1.376	-	26.36
Phenol	2.907	4.301	24.54

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Figure 1 shows the quantitative phytochemical compounds of *H. umbellate* (Abeere and represented as ABH). Also, glycoside was only present in the hot water extract and was the most abundant phytochemical in the extract. Only flavonoid, phenol and saponin was present in the

cold-water extract. However, the hot water extract had in addition to the three four more phytochemicals in abundant and they are glycosides, steroid, tannin and terpenin.

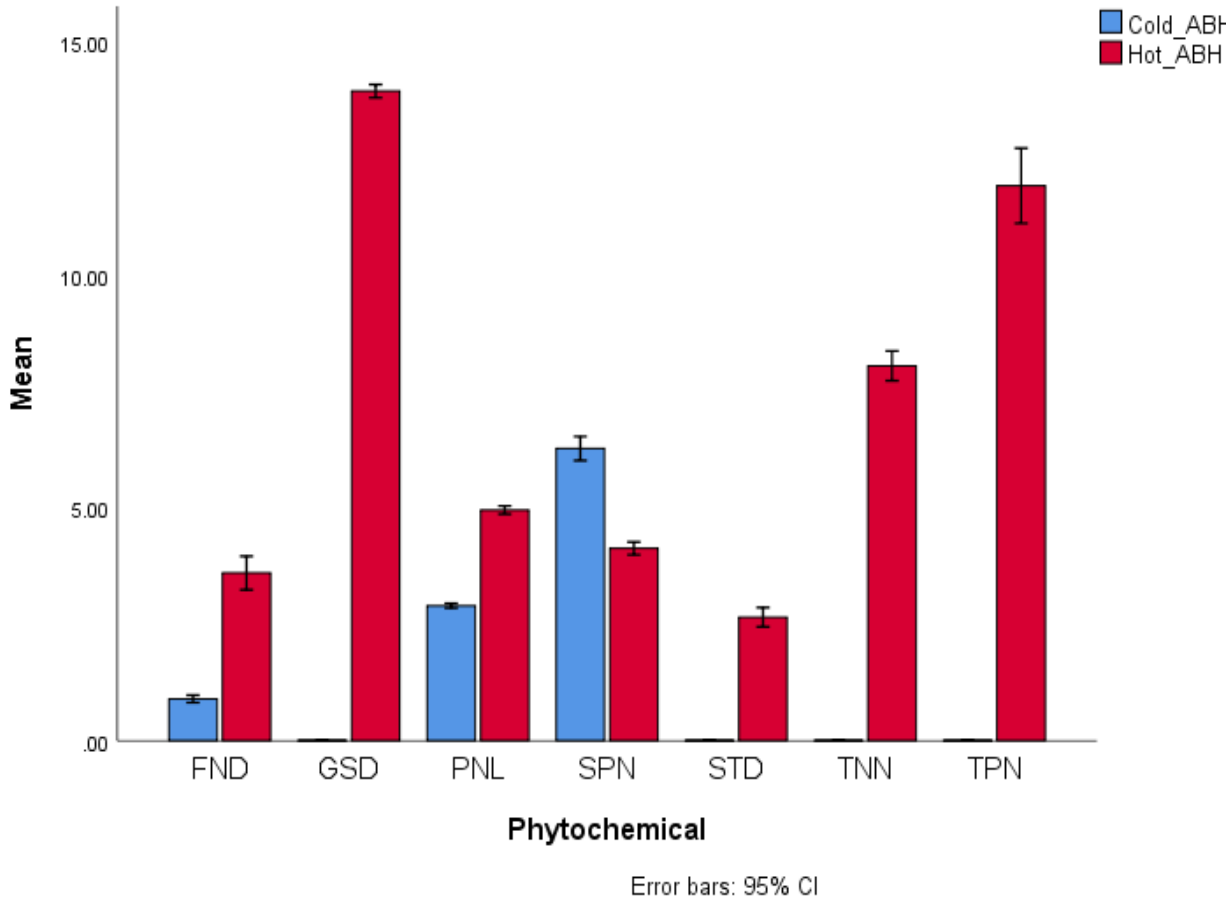

Figure 1: Quantitative Phytochemicals in Extracts of *H.umbellata*

Keys: PNL= Phenol; GSD= Glycoside; STD=Steroid; TNN=Tannin; TPN=Terpinene; SPN=Saponin; FND= Flavonoid

A total of fifty malaria positive blood samples were obtained with basic information obtained from anonymous donors. Table 11 shows the age and gender distribution of the samples collected. The highest number of samples were obtained from age 0-10 years, while the least

number of samples were obtained from age 51 and above. On the basis of gender distribution, more samples were obtained from females than males with each of them amounting to ratio 8:17 respectively. Also, the number of males decreased with increase in age. Table 12 shows the educational distribution of the samples collected with the highest number of the samples obtained from children in primary schools and the least from those without any form of education background respectively.

Table 3 shows the result of the isolation of bacteria from blood samples of malaria infected patients. Out of the fifty blood samples, forty-two had bacteria while eight were sterile. Table 13 shows the probable identity of the bacteria after subjecting them to various biochemical tests. A total of seven bacteria were identified from the thirty-two blood samples that have bacteria using different media, biochemical tests and sugar fermentation tests. The probable bacteria general identified are *Acinetobacter, Escherichia, Klebsiella, Staphylococcus, Salmonella Streptococcus* and *Pseudomonas*. The bacteria with the highest frequency of occurrence were *Salmonella* and *Staphylococcus*. The least occurred bacteria was*Pseudomonas*.

Table 4 shows the percentage occurrence of each bacterial isolate from the blood samples. Both *Staphylococcus* and *Salmonella* had the highest percentage of occurrence in the samples with 28% of occurrence in all the samples. The bacteria with the least percentage of occurrence were *Pseudomonas* with a percentage of 4% occurring in two samples only. *Escherichia coli* also recorded 24% along with *Streptococcus, Klebsiella* only had 22% percentage occurrence.

Table 3: Age and gender distribution of samples collected

S/N	Age	Male	Female
1	0-10	7	13

2	11-20	5	5
3	21-30	2	4
4	31-40	2	7
5	41-50	0	3
6	51 and above	0	2
7	Total	16	34

Table 4: Educational distribution of samples

S/N	Educational qualification	Number
1	Primary	18
2	Secondary	12
3	Tertiary	17
4	None	3
5	Total	50

Table 5: Morphological characteristics of bacterial isolates

Isolate no	Pigmentation/color	Shape	Edge	Optical characterized	Consistency	Colony surface	Spore formation	Gram's reaction	Motility
1	White	Circular	Entire	Translucent	Butyrous	Smooth	Negative	-ve rod	+
2	White	Irregular	Lobate	Translucent	Viscid	Smooth	Negative	-ve rod	+

3	Milky white	Circular	Entire	Opaque	Butyrous	Smooth	Negative	-ve rod	+
4	Milky white	Circular	Entire	Translucent	Swarmy	Smooth	Negative	+ve cocci	+
5	Black	Circular	Entire	Translucent	Butyrous	Smooth	Negative	-ve rod	+
6	Milky white	Circular	Entire	Opaque	Butyrous	Smooth	Negative	+ve cocci	+
7	Green	Circular	Entire	Translucent	Butyrous	Smooth	Negative	-ve rod	+

Table 6: Biochemical characteristics of bacterial isolates

Isolate no	Cat	Oxi	Ind	H_2S	Nit red	Ure	Lact	Fruc	Malt	Gala	Glu	Arab	Raf	Man	MR	VP	Identified organism
1	+	-	+	+	-	+	-	+	-	-	+	-	-	-	+	-	*Acinetobacter* spp
2	+	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	*Escherichia coli*
3	-	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+	*Klebsiella pneumonia*
4	+	+	-	+	+	+	-	+	+	+	-	+	+	-	+	+	*Staphylococcus aureus*
5	+	+	-	+	-	-	-	+	+	-	+	-	-	+	+	+	*Salmonella typhi*
6	-	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+	*Streptococcus pneumonia*
7	+	+	-	+	-	-	-	+	+	-	+	-	-	+	+	+	*Pseudomonas aeruginosa*

Table 7: Percentage occurrence of isolates

S/N	Name of bacteria	No of samples positive	No of samples negative	Percentage of occurrence
1	*Enterobacter* spp	8	42	16%
2	*Escherichia coli*	12	38	24%
3	*Klebsiella pneumonia*	11	39	22%
4	*Staphylococcus aureus*	14	36	28%
5	*Salmonella typhi*	14	36	28%
6	*Streptococcus pneumonia*	12	38	24%
7	*Pseudomonas aeruginosa*	2	48	4%

Table 8 is the result of the antibiotic sensitivity test of Gram-negative bacteria on commercial antibiotics they were subjected to. *Salmonella* was most susceptible to ampicillin having a diameter of zone of inhibition of 18.33±0.58 mm, while it was not susceptible at all to nitrofurantoin. Escherichia was most susceptible to tetracycline with a zone diameter of 14.33±0.58 mm, while *Pseudomonas* was most susceptible to ampicillin having a zone diameter of 10.00±0.00 mm. Klebsiella on the other hand was most susceptible to chloramphenicol, with a zone diameter of 15.18±0.58.

The Gram-positive bacteria were also most susceptible to ampicillin with the two bacteria (*Staphylococcus* and *Streptococcus*) having zones of inhibition of 16.33±0.58 and 10.67±0.58 mm diameter respectively. *Staphylococcus* was completely unsusceptible to colistin sulphate and

nitrofurantoin antibiotics while *Streptococcus* was not susceptible to kanamycin. Generally, the Gram-positive bacteria were not susceptible to streptomycin. The result for the Gram-positive bacteria is presented in Table8.

Table 8: Antibiotic sensitivity test of Gram-negative isolates on commercial antibiotics

Antibiotics	*Salmonella typhi*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Ampicillin (25µg)	18.33±0.58^{e}	12.67±0.58^{e}	10.00±0.00^{e}	12.33±0.58^{b}
Chloramphenicol (30µg)	13.33±0.58^{c}	9.67±0.58^{d}	9.33±0.58^{b}	15.18±0.58^{d}
Colistin sulphate (30µg)	10.00±0.00^{a}	6.33±0.58^{c}	8.33±0.58^{c}	8.33±0.58^{b}
Kanamycin (30µg)	10.33±0.58^{c}	12.00±0.00^{a}	0.00±0.00^{d}	11.33±0.58^{c}
Nalidixic acid (30µg)	12.33±0.58^{c}	10.00±0.00^{e}	8.67±0.58^{c}	4.33±0.58^{a}
Nitrofurantoin (30µg)	0.00±0.00^{a}	6.67±0.58^{c}	4.33±0.58^{a}	8.33±0.58^{b}
Streptomycin (30µg)	6.67±0.58^{b}	14.00±0.00^{b}	6.33±0.58^{c}	12.33±0.58^{b}
Tetracycline (30µg)	10.33±0.58^{d}	14.33±0.58^{c}	6.00±0.00^{c}	12.00±0.00^{b}

Table 9: Antibiotic sensitivity test of Gram-positive isolates on commercial antibiotics

Antibiotics	*Enterobacter* spp	*Staphylococcus aureus*	*Streptococcus Pneumoniae*

Ampicillin (25µg)	11.67±0.58^{e}	16.33±0.58^{e}	10.67±0.58^{e}
Chloramphenicol (30µg)	4.67±0.58^{b}	12.33±0.58^{c}	8.67±0.58^{d}
Colistin sulphate (30µg)	5.33±0.58^{c}	0.00±0.00^{a}	6.33±0.58^{c}
Kanamycin (30µg)	0.00±0.00^{a}	12.33±0.58^{c}	0.00±0.00^{a}
Nalidixic acid (30µg)	0.00±0.00^{a}	12.33±0.58^{c}	10.00±0.00^{e}
Nitrofurantoin (30µg)	4.67±0.58^{b}	0.00±0.00^{a}	6.67±0.58^{c}
Streptomycin (30µg)	0.00±0.00^{a}	6.67±0.58^{b}	4.00±0.00^{b}
Tetracycline (30µg)	7.33±0.58^{d}	14.33±0.58^{d}	6.33±0.58^{c}

Table 9 shows the result of the susceptibility test of *B. diffusa* hot water and ethanol extracts at 50mg/ml and 100mg/ml respectively. The hot water extract at 50mg/ml was not that effective against the bacteria isolates. For example, *Pseudomonas* was not susceptible at all to the extract at this concentration, while at 10mg/ml concentration the susceptibility value was only 4.33±0.58 mm diameter zone of inhibition. At 100mg/ml concentration, the highest diameter of zone s of inhibition was 11.33±0.58; which was obtained for *Klebsiella* while the lowest level of diameter of zones of inhibition was 0.00 obtained for the hot water extract. The ethanol extract was however, more effective invitro

Table 10: Susceptibility test of *B. diffusa* extracts isolates

*B. diffusa*extracts	*S pneumoniae*	*S aureus*	*K. pneumoniae*	*S typhi*	*E. coli*	*P. aeruginosa*	*E. aeroginosa*
50mg/ml (H)	6.33±0.58[a]	4.33±0.58[a]	5.00±0.00[a]	3.33±0.58[a]	3.33±0.58[a]	0.00±0.00[a]	7.33±0.58[a]
100mg/ml (H)	10.33±0.58[b]	9.67±0.58[b]	11.33±0.58[b]	5.33±0.58[b]	8.67±0.58[b]	4.33±0.58[b]	15.67±0.58[b]
50mg/ml (E)	8.00±0.00[c]	6.67±0.58[c]	8.33±0.58[b]	8.00±0.00[c]	6.33±0.58[c]	6.00±0.00[c]	8.33±0.58[c]
100mg/ml (E)	14.33±0.58[d]	12.33±0.58[d]	14.67±0.58[b]	12.33±0.58[d]	12.00±0.00[d]	9.33±0.58[d]	16.00±0.00[d]

Keys: H= Hot; E= Ethanol

Table 11: Susceptibility test of *H. umbellata* extracts isolates

B. diffusa extracts	*S pneumoniae*	*S aureus*	*K. pneumoniae*	*S typhi*	*E. coli*	*P. aeruginosa*	*E. aeroginosa*
50mg/ml (H)	4.33±0.58[a]	5.33±0.58[a]	4.00±0.00[a]	5.33±0.58[a]	5.33±0.58[a]	2.33±0.58[a]	5.13±0.58[a]
100mg/ml (H)	9.3±0.58[b]	10.67±0.58[b]	9.33±0.58[b]	9.33±0.58[b]	9.67±0.58[b]	6.33±0.58[b]	13.37±0.58[b]
50mg/ml (E)	6.00±0.00[c]	6.67±0.58[c]	5.33±0.58[b]	6.00±0.00[c]	10.33±0.58[c]	4.00±0.00[c]	6.33±0.58[c]

100mg/ml (E)	11.33±0.58 d	13.33±0.58[d]	11.67±0.58 b	10.33±0.58 d	15.00±0.00[d]	8.33±0.58[d]	14.00±0.00[d]

Keys: H= Hot; E= Ethanol

DISCUSSION

A total of fifty malaria positive blood samples were obtained with basic information obtained from anonymous donors. Table 11 shows the age and gender distribution of the samples collected. The highest number of samples were obtained from age 0-10 years, while the least number of samples were obtained from age 51 and above. On the basis of gender distribution, more samples were obtained from females than males with each of them amounting to ratio 8:17 respectively. Also, the number of males decreased with increase in age. Table 12 show the educational distribution of the samples collected with the highest number of the samples obtained from children in primary schools and the least from those without any form of education background respectively.

Table 3 shows the result of the isolation of bacteria from blood samples of malaria infected patients. Out of the fifty blood samples, forty-two had bacteria while eight were sterile. Table 13 shows the probable identity of the bacteria after subjecting them to various biochemical tests. A total of seven bacteria were identified from the thirty-two blood samples that have bacteria using different media, biochemical tests and sugar fermentation tests. The probable bacteria general identified are *Acinetobacter, Escherichia, Klebsiella, Staphylococcus, Salmonella Streptococcus* and *Pseudomonas*. The bacteria with the highest frequency of occurrence were *Salmonella* and *Staphylococcus*. The least occurred bacteria was*Pseudomonas*.

Table 4 shows the percentage occurrence of each bacterial isolate from the blood samples. Both *Staphylococcus* and *Salmonella* had the highest percentage of occurrence in the samples with 28% of occurrence in all the samples. The bacteria with the least percentage of occurrence were *Pseudomonas* with a percentage of 4% occurring in two samples only. *Escherichia coli* also recorded 24% along with *Streptococcus, Klebsiella* only had 22% percentage occurrence.

Table 5 is the result of the antibiotic sensitivity test of Gram-negative bacteria on commercial antibiotics they were subjected to. *Salmonella* was most susceptible to ampicillin having a diameter of zone of inhibition of 18.33±0.58 mm, while it was not susceptible at all to nitrofurantoin. Escherichia was most susceptible to tetracycline with a zone diameter of 14.33±0.58 mm, while the *Pseudomonas* was most susceptible to ampicillin having a zone diameter of 10.00±0.00 mm. Klebsiella on the other hand was most susceptible to chloramphenicol, with a zone diameter of 15.18±0.58.

The Gram-positive bacteria were also most susceptible to ampicillin with the two bacteria (*Staphylococcus* and *Streptococcus*) having zones of inhibition of 16.33±0.58 and 10.67±0.58 mm diameter respectively. *Staphylococcus* was completely unsusceptible to colistin sulphate and nitrofurantoin antibiotics while *Streptococcus* was not susceptible to kanamycin. Generally, the Gram-positive bacteria were not susceptible to streptomycin. The result for the Gram-positive bacteria is presented in table 6.

Table 7 shows the result of the susceptibility test of *B. diffusa* hot water and ethanol extracts at 50mg/ml and 100mg/ml respectively. The hot water extract at 50mg/ml was not that effective against the bacteria isolates. For example, *Pseudomonas* was not susceptible at all to the extract at this concentration, while at 10mg/ml concentration the susceptibility value was only 4.33±0.58 mm diameter zone of inhibition. At 100mg/ml concentration, the highest diameter of zone s of

inhibition was 11.33±0.58; which was obtained for *Klebsiella* while the lowest level of diameter of zones of inhibition was 0.00 obtained for the hot water extract. The ethanol extract was however, more effective invitro

Worthy of note is the fact that the number of males decreased with increase in age of the blood donors and this trend is similar to the trend observed by Hassannia*et al*., (2020) in which the male sex folks were seen as refusing to go for medical care, except in severe cases where they hve to be rushed to the hospital. Similarly, Chisang*et al*., 2023 observed a similar result in Zambia where the males refused to go to the hospital for treatment even when they are down with malaria. Bofu*et al*., 2023 who also observed a similar result in Tanzania where males believe that herbs are more appropriate for malaria and refuses to go to hospitals. In both references above, the educational qualifications were said to be minimal.

Out of the fifty blood samples, forty-two had bacteria while eight were sterile, which means no form of septicaemia. The probable identity of the bacteria after subjecting them to various biochemical tests showed that all the bacteria isolated were common cause of blood bacteremia (Prescott *et al*., 2022). The total of seven bacteria identified from the forty-two blood samples that have bacteria using different media, biochemical tests and sugar fermentation tests were all common cause of bacteremia, and most importantly co-infecting bacteria with *Plasmodium* (Baker *et al*., 2021). The probable bacteria general identified are *Acinetobacter, Escherichia, Klebsiella, Staphylococcus, Salmonella Streptococcus* and *Pseudomonas*. The bacteria with the highest frequency of occurrence were *Salmonella* and *Staphylococcus*. The least occurred bacteria were *Pseudomonas*.

The bacteria with the least percentage of occurrence were *Pseudomonas* with a percentage of 4% occurring in two samples only, while *Escherichia coli* also recorded 24% along with

Streptococcus, Klebsiella only had 22% percentage occurrence. This result is similar to the results obtained by Adejo*et al*., (2023). In which a similar study was conducted on malaria infection of neonates with emphasis on four days recall feeding habits.

The susceptibility test of *B. diffusa* hot water and ethanol extracts at 50mg/ml and 100mg/ml respectively which showed that the hot water was not that effective against the bacteria isolates. According to Ma *et al*., (2020), the susceptibility of a bacteria to a substance or an extract should be determined by using a standard such as the clinical laboratory standard. For example, *Pseudomonas* was not susceptible at all to the extract at this concentration, while at 10mg/ml concentration the susceptibility value was only 4.33±0.58 mm diameter zone of inhibition and this is still not susceptible to the extract by certain standards. However, *Klebsiella* was most susceptible to most of the extract.

Conclusion

The results obtained in this research have shown that both *B. diffusa* and *H. umbellate* extracts have high antibacterial effect against the bacteria isolates coinfecting patients that are down with malaria.The plants could also be a good sources of novel medicine that could help curb antibiotic resistance of the bacteria that were susceptible to the extracts.

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