Available online at www.ujpronline.com
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY OF METHANOLIC STEM EXTRACT OF *BOMBAX BUONOPOZENSE* P. BEAUV (SILK COTTON TREE)

Udeh VC[1], Agboeze Emmanuel*[1], Omeje EO[2]
[1]*Department of Industrial Chemistry, Enugu state University of Science and Technology, Nigeria.*
[2]*Department Of Pharmacy, University of Nigeria Nsukka, Nigeria.*

Article Info:

Article History:
Received: 5 August 2023
Reviewed: 11 September 2023
Accepted: 19 October 2023
Published: 15 November 2023

Cite this article:
Udeh VC, Emmanuel A, Omeje EO. Phytochemistry and antimicrobial activity of methanolic stem extract of *Bombax buonopozense* P.beauv (Silk cotton tree). Universal Journal of Pharmaceutical Research 2022; 7(5):57-62.
https://doi.org/10.22270/ujpr.v7i5.840

***Address for Correspondence:**
Agboeze Emmanuel, Department of Industrial Chemistry, Enugu state University of Science and Technology, Nigeria. Tel- +234 903 923 9802; E-mail: *emmanuel.agboeze@gmail.com*

Abstract

Background: The aim and objective of this study was to evaluate methanolic extract of *Bombax buonopozense* (stem) for antimicrobial and phytochemical screening.
Methods: The methanolic stem extract of *B. buonopozense* with documented ethno-medicinal applications were fractionated in different solvents (n-hexane, chloroform, ethyl acetate, n-butanol and water) and subjected to phytochemical screening and antimicrobial activity against some disease-causing microorganisms including gram positive and gram negative (*Staphylococcus aureus*, *Salmonella* spp and *Escherichia coli*). The antimicrobial tests were carried out in triplicates, the data obtained were subjected to one-way ANOVA using statistical package for social science (SPSS).
Results: Phytochemical screening revealed the presence of Alkaloids, saponins, saponin glycosides, Tannins, hydrolysable Tannins, steroids and triterpenoids, flavonoids, phenols and volatile oils. Each fractions of the extract demonstrated antibacterial activity against all the organisms tested. MIC values of each fractions revealed strong inhibition against all the organisms tested. The methanolic stem extract of *B. buonopozense* in chloroform, water, ethyl acetate, n-hexane and n-butanol showed the following MIC values. *S. aureus*. 6.25, 25, 12.5, 6.25, and 12.5. *E. coli* 100, 100, 50, 6.25 and 12.5. *Salmonella* spp. 3.125, 12.5, 25, 3.125 and 12.5 respectively.
Conclusion: This study shows that the extract posses' antimicrobial properties which can be used as alternatives to conventional antibiotics.
Keywords: Antimicrobial activity, *Bombax buonopozense*, Diosquinone, *Escherichia coli*, MIC, phytochemical constituents, *Staphylococcus aureus*.

INTRODUCTION

Natural products, such as plant extracts either as pure compounds or as standardized extracts, provides unlimited opportunities for new drug discoveries because of the unmatched availability of its chemical diversity. The use of plant extracts in the treatment of diseases has become an important interest over the years. This is as a result of the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing effectiveness[1,2]. In view of this, there is an urgent need to find the alternative to chemotherapeutic drugs in disease treatment particularly those of plants origin which are easily available and have considerably less side effects[3,4].

In the past, humans used plant to treat common infectious disease and even long before mankind discovered the existence of microbes, the idea that certain plants had healing potential was well accepted[5]. Researchers are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for their antimicrobial properties[6]. Specifically, the medicinal value of this plant lies in some chemical substances that produce a definite physiological action on the human or animal body[7]. The most important of these bioactive constituents which are mainly: secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds[8,9]. These phytochemicals are toxic to microbial cells. Medicinal plants generally contain a number of compounds which may be potential natural

antibacterial for the treatment of common bacterial infections. Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments[10].

B. buonopozense (Bambacaceae) is commonly known with local names (Hausa: gurjiya, Igbo: Akpu, Nupe: Kutukpachi; Yoruba: Ogbolo). It is a large tropical tree that grows up to 40 meters in height with large buttress roots that can spread 6 meters down[11]. The individual leaf has entire margin and quite large, measuring from 8 to 23 cm in length by 3 to 7.5 cm in width with the under sides of the leaf being conical buds which contain many seeds that are 5 to 6 mm in length, all of which have a cotton-like fiber covering[12]. It is a wild plant whose edible floral parts are used as vegetable by the inhabitants of North Central, Nigeria and as medicine due to its nutritive and therapeutic properties[11]. The plant is widely distributed in West African countries such as Ghana, Gambia, Cote D'ivoire, Nigeria and others. Hot decoction of the dried stem bark of the plant is taken orally for the treatment of malaria in Ghana. The bark is used to treat chest pain in Gambia. Decoction of the leaves is used to manage stomach ulcers and burns in Ghana. Aqueous extract of the leaves is claimed to be effective in the treatment of diarrhea and dysentery. The immature fruits are prepared as an emollient for skin, decoction of the young leaves is used as a warm bath for febrile children (Irvine F. R, 1961). The grounded bark is taken by pregnant women to increase lactation; the extract from the bark is drunk on the head for dizziness. The gum resin from the bark is pulverized, mixed with oil and used to manage skin disease[7].

This study was designed to evaluate fractionated methanolic extract of *B. buonopozense* (stem) for antimicrobial and phytochemical screening against multi-drug pathogenic organisms to ascertain its potentiality in treating infections caused by micro-organisms.

MATERIALS AND METHODS

Collection of samples

The fresh stem of *B. buonopozense* were collected and identified by a taxonomist Mr. Ozioko of INTERCEED Nsukka. The stem of *B. buonopozense*was washed and air dried for two weeks.The dried stem was ground to powder and stored in an air tight polyethylene bag ready for extraction.

Preparation of plant extracts/extraction

The method in our previous work was used with little modification [3]. Extraction was done by cold maceration with 95% methanol with continuous stirring and agitation, extracts were removed intermittently and fresh solvent added to ensure for neat extraction, this was done for 72 hrs. After 72 hrs the extract was pooled together and filtered using Whatman 2 filter paper. The extract was evaporated to dryness in vacuum using rotary evaporator at 40±0.1°C. The extract was subsequently weighed and stored in glass sample bottles at 4°C.

Test organisms for microbial analysis

Clinical isolates of *Staphylococcus aureus* (Gram positive), *Salmonella* spp (Gram positive), and *Escherichia coli* (Gram negative) obtained from the microbiology department, Enugu State University Teaching Hospital (ESUT) Enugu were used for this study. The organisms were sub cultured in nutrient broth at 37ºC for 6h prior to antimicrobial testing.

Solvent partitioning/fractionation

The methanolic stem extract of *B. buonopozense* were partitioned using solvent extraction method.Five solvents were used in solvent partitioning (water, n-hexane, chloroform, ethyl acetate and n-butanol).The extract was uniformly dispersed in 100 ml of water and stirred with a magnetic stirrer to achieve uniform mixture or homogeneity, it was then poured into a separating funnel, then aliquots of n-hexane was added, the separating funnel was agitated before being allowed to settle. Heavier lower layer (aqueous) was carefully tapped off and then the upper layer (n-hexane) was tapped off subsequently, this was done continuously until the n-hexane aliquots were reasonably clear, it was tapped off and stored. The same procedure was repeated for other immiscible solvents (chloroform, ethyl acetate and n-butanol), leaving behind in the separating funnel, the water portion of the extract, which was also collected and stored in reagent bottle at 4ºC[10].

Phytochemical screening

The extract was subjected to qualitative phytochemical screening according to standard methods using the n-hexane, chloroform, ethyl acetate, n-butanol and water fractions of the extract. This was done to determine the presence of saponins, Alkaloids, Saponin Glycosides, Tannins, Hydrolysable tannin, steroids and Triterpenoids, flavonoids, Anthraquinone, Phenols, glycosides and volatile oils[11].

The tests are as follows:

Test for saponins

i. 2.5 cm^3 of each fraction of extracts was vigorously shaken with 10 cm^3 of water for 2 minutes in a test tube. Total 2 cm^3 of olive oil was then added. It was observed for persistent frothing and emulsion formation and result recorded.
ii. 1 ml of each fraction of extracts was treated with 1% lead acetate solution. Formation of white precipitate indicates the presence of saponins[12].

Test for saponin glycosides

2.5 cm^3 of mixture of Fehling's solution A and B was added to 2.5 cm^3 of each fraction of extracts in a test tube and observed for bluish green precipitate and observation recorded.

Test for steroids and triterpenoids (Libermann Burchard test)

Total 2 cm^3 of acetic anhydride was added to 2 cm^3 of each fraction of extract in a test tube and cooled well in ice. Total 3 cm^3 concentrated sulphuric acid was carefully added and a change from violet to blue to green colour was observed and recorded[13].

Test for glycosides (general)

i. Dilute sulphuric acid (2.5 cm^3) was added to 5 cm^3 of fractions of extract in a test tube and

boiled for 15 minutes. Then 2 cm^3 of 10% sodium hydroxide and 5 cm^3 of mixed Fehling's solution A and B were added. The formation of brick red precipitate is a positive test.

ii. The fractions of extracts were hydrolyzed with HCL for few hours on a water bath and the hydrolysate was subjected to Bontrager's test to detect the presence of glycosides.

(a) Bontrager's Test: Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink colour, shows the presence of glycosides[5].

Test for Tannins

i. Ferric Chloride Test: To 1-2 ml of each fraction of extracts a few drops of 5% aqueous $FeCl_3$ solution was added. A violet colour formation indicates the presence of tannins.

ii. Lead acetate test: In a test tube containing about 5.0 ml of each fraction of extracts a few drops of 1% lead acetate was added. A yellow precipitate indicates the presence of tannins[6].

Test for hydrolysable tannins

Total 4 cm^3 of 10% ammonia solution was added to 4 cm^3 of each fraction of extracts in a test tube and shaken very well and observed for the formation of an emulsion and the result recorded.

Test for flavonoids

i. Shinoda's Test: In a test tube containing 0.5 ml of each fraction of extracts, 5-10 drops of diluted HCL and small piece of magnesium (magnesium ribbon) were added and the solution was boiled for a few minutes. A reddish pink colour indicates the presence of flavonoids

ii. Alkaline Reagent Test: To 1.0 ml of each fraction of extracts, few drops of dilute sodium hydroxide were added. An intense yellow colour produced in the extracts which becomes colourless on addition of a few drops of dilute acid indicates the presence of flavonoids[7].

Test for alkaloids

i. **Dragendroff's Test:** To 1 ml of each fraction of extracts 4 ml of Hcl was added. To the acidic medium 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

ii. **Wagner's Test:** Two drops of Wagner's reagent was added to 2 cm^3 of each fraction of extracts in a test tube and observed for a deep brown precipitate and observation recorded.

iii. **Mayer's Test:** Three drops of Mayer's reagent was added to 2 cm^3 of each fraction of extracts in a test tube and observed for a reddish precipitation or colouration.

iv. **Tannic Acid Test:** Two drops of 10% (W/v) tannic acid was added to 2 cm^3 of each fraction of extracts in a test tube and observed for a cream colouration and observation recorded[8].

Volatile oil test

Six drops of ferric chloride solution were added to a mixture of 2 cm^3 of each fraction of extracts in a test tube and 2 cm^3 of 90% (v/v) ethanol. The resulting mixture was observed for green coloration and the result recorded[9].

Test for phenols

To 3 ml of each fraction of extracts, 3 ml of 5% w/v ferric chloride solution was added. The blue-black colour indicates the presence of phenol

Test for anthraquinones

Total 5 ml of each faction of extracts was hydrolyzed with dil. Sulphuric acid (H_2So_4) and extracted with benzene. Total 1 ml of dilute ammonia was added to it. Rose pink colouration indicated the positive response for anthraquinones[10].

Antimicrobial screening

The agar diffusion technique as described in a previous study was used to determine the antimicrobial activity of the plant extract[13]. To test antibacterial activity of the stem extract of *B. buonopozense* 0.8 g of extract was dissolved in DMSO and then varying concentration of the extract (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.562 mg/ml) were obtained. Standard inoculums of $1.5x10^8$ cells which matched, 0.5 McFarland standard was spread on the surface of a sterile Muller Hinton agar plates in duplicates. A sterile6mm cork borer was used to make a hole on the Muller Hinton agar plates in which 0.1 ml each of the plant extracts were added. The plates were incubated at 37°C for 24 hrs. The antimicrobial activity was detected by measuring zones of inhibition in millimeters[9].

Determination of Minimum Inhibitory Concentration (MIC)

This was determined using broth dilution method as described in a previous study[13]. The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganism. Varying concentrations of the extracts (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.562 mg/ml) were prepared. 0.1 ml of standar-dized test organisms was inoculated into the tubes containing the different concentrations of the extract and controls were equally set up by using solvents and test organisms without extract. These were incubated for 24 hrs at 37°C. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration[9].

Statistical analysis

The tests were carried out in triplicates, the data obtained were subjected to one-way ANOVA using statistical package for social science (SPSS), version 23. Means were separated using Least Significant Difference (L.S.D), considered statistically significant at $p<0.05$.

RESULTS

Phytochemical screening result

Phytochemical analysis of the fractionated methanolic stem extract of *B. buonopozense* were conducted using the fractions of each of the solvent. The table below shows the positive and negative reactions of the extract fraction to the following secondary metabolites:

Alkaloids, Flavonoids, Tannins, Saponins, Saponin glycosides, hydrolysable tannins, phenols, anthrax-quinones, steroids/triterpenoids, volatile oils and glycosides. The result of phytochemical analysis of the stem extract is shown in Table 1.

Determination of antimicrobial activities of the extract

The result of the antimicrobial activity of the fractions of methanolic extract of *B. buonopozense* against the test organisms, namely, *S. aureus*, *E. coli* and *Salmonella spp* are showed in tables 2 to 6 while table 7 shows the result of minimum inhibitory concentration of the extract on the test organisms. The extract showed varying degrees of growth inhibition against the isolates.

Table 1: Phytochemical profile of the fractions of *B. buonopozense* stem extract.

Phytoconstituents	Solvents				
	n-hexane	Chloroform	Ethyl acetate	n-butanol	Water
Alkaloids	+++	+++	++	-	-
Saponins	-	-	+++	+	+++
Saponin glycosides	-	-	+	+++	+++
Tannins	+	-	-	-	+
Hydrolysable tannins	+++	++	+	-	+
Steroids & triterpenoids	+	+	-	-	-
Flavonoids	-	-	+	-	-
Anthraquinone	-	-	-	-	-
Phenols	-	+	+++	++	-
Glycosides	-	-	-	-	-
Volatile oils	-	++	-	+	-

(+)=suspected/slightly present, (++)= present, (+++)= very present, (-)= absent

Table 2: Antimicrobial activity of aqueous (water) fraction of *B. buonopozense* stem extract.

Test Organisms	Different concentration (mg/ml)/zones of inhibition (mm)						
	100	50	25	12.5	6.25	3.125	1.56
S. aureus	12±1.73	12±0.57	6±1.73	4±1.15	2±0.57	0.0±0.0	0.0±0.0
E. coli	5±0.57	3±0.57	0.0±0.0	0±0.00	0.0±0.0	0.0±0.0	0.0±0.0
Salmonella SPP	14±0.57	12±1.73	10±1.15	5±1.73	2±0.57	0.0±0.0	0.0±0.0

Table 3: Antimicrobial activity of the chloroform fraction of *B. buonopozense* stem extract.

Test Organisms	Different concentration (mg/ml)/zones of inhibition (mm)						
	100	50	25	12.5	6.25	3.125	1.56
S. aureus	17±1.15	13±0.57	11±2.88	6±1.15	6±1.73	2±0.57	0.0±0.0
E. coli	6±0.57	5±1.15	2±0.57	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Salmonella SPP	30±2.88	26±2.30	18±1.73	13±1.15	11±2.30	6±1.73	4±0.57

DISCUSSION

The results of this study have x-rayed valuable evidence in support of *B. buonopozense* as potent antibacterial agent. The data presented in this study narrowed down the phytochemicals into specific fractions of the extract. Result of the phytochemical screening of *B. buonopozense* stem extract showed the presence of these phytochemicals in each of the fractions of the extract.

- n-hexane fraction contains (Alkaloids, tannins, hydrolysable tannins and steroids/triterpenoids)
- chloroform fraction contains (Alkaloids, Hydrolysable tannins, steroids/triterpenoids, phenols and volatile oils)
- Ethyl acetate fraction contain (Alkaloids, saponins, hydrolysable tannins, flavonoids and phenols).
- n-butanol fraction contains (saponin glycosides, saponins, phenols and volatile oils).
- Water fraction contain (saponins, saponino-glycosides, tannins and hydrolysable tannins).

The result of this study showed that the fractionated methanolic stem extracts of *B. buonopozense* demonstrated reasonable inhibition on the gram positive bacteria (*S. aureus* and *Salmonella spp*) and the n-hexane, ethyl acetate and n-butanol fractions exhibited reasonable sensitivity on the gram negative bacteria (*E. coli*) while the chloroform and n-butanol fractions has only little sensitivity. It is believed that the antibacterial activity of this plant is due to the presence of the phytochemicals. Phytochemicals such as alkaloids, tannins, essential oils, saponins steroids, which are actually the defensive mechanism of the plants against pathogens. The MIC values of the extract was found to have various range, thus indicating that evaluation of MIC is sufficient for measuring bacterial activity[12]. The test organisms used in this study are associated with various forms of human infections. *E. coli* are the most numerous aerobic commensal inhabitants of the large intestine. Certain strains cause diarrhea and all can cause infection when they invade sterile sites[13], like the urinary tract and wound infections in the elderly and young male often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores[14]. *Salmonella* spp typically cause diarrhea and sometimes cause a more serious infection, typhoid fever.

Table 4: Antimicrobial activities of ethyl acetate fraction of *B. buonopozense* stem extract.

Test Organisms	Different concentration (mg/ml)/zones of inhibition (mm)						
	100	50	25	12.5	6.25	3.125	1.56
S. aureus	21±4.61	14±0.57	12±1.15	11±2.30	5±1.15	0.0±0.0	0.0±0.0
E. coli	16±1.15	15±2.30	13±0.57	9±0.57	8±1.73	3±0.57	0.0±0.0
Salmonella SPP	26±2.30	19±0.57	15±1.73	12±3.46	7±1.15	3±1.15	2±0.57

Table 5: Antimicrobial activity of n-hexane fraction of *B. buonopozense* stem extract.

Test Organisms	Different concentration (mg/ml)/zones of inhibition (mm)						
	100	50	25	12.5	6.25	3.125	1.56
S. aureus	22±4.04	18±2.88	17±1.15	10±2.88	6±1.15	2±0.57	0.0±0.0
E. coli	18±2.30	13±3.46	13±1.15	7±0.57	5±1.15	3±0.57	0.0±0.0
Salmonella SPP	25±0.57	21±3.46	17±2.88	11±1.73	10±2.30	4±1.15	2±0.57

S. aureus constitute a major public health threat, being one of the common causes of hospital and community acquired infections[15-17]. The demonstration of activity against both gram positive and gram-negative bacteria is an indication that the plants can be a source of bioactive substances that could of broad spectrum of activities. Some synthetic drugs cause varying degrees of side effects, hence the need for the development of plant-based compounds which could be useful in meeting the demand for newer drugs with minimal side effects[13]. Apart from anti-microbial activities, *B. buonopozense* extract are also exploited for therapeutic purpose to cure several disorders.

Limitations of the study

This study is limited to Phytochemistry and antimicrobial activity of methanolic stem extract of *B. buonopozense* (Silk Cotton Tree).

Table 6: Antimicrobial activities of n-butanol fraction of *B. buonopozense* stem extract.

Test Organisms	Different concentration (mg/ml)/zones of inhibition (mm)						
	100	50	25	12.5	6.25	3.125	1.56
S. aureus	13±1.15	11±2.88	6±0.57	5±1.73	5±1.15	0.0±0.0	0.0±0.0
E. coli	10±1.73	7±0.57	7±1.15	5±1.73	2±0.57	0.0±0.0	0.0±0.0
Salmonella SPP	18±2.30	12±3.46	12±0.57	9±2.30	3±0.57	0.0±0.0	0.0±0.0

Table 7: Minimum inhibitory concentration of chloroform, Aqueous (water), ethyl-accetate, n-hexane and n-butanol fraction of *B. buonopozense* extracts.

Test Organisms	Minimum inhibitory concentration (mg/ml)				
	Chloroform	Water aqueous	Ethyl acetate	n-hexane	n-butanol
S. aureus	6.25	25	12.5	6.25	12.5
E. coli	100	100	50	6.25	12.5
Salmonella SPP	3.125	12.5	25	3.125	12.5

CONCLUSION

The methanolic leaf extract of *B. buonopozense* was found to possess antidiarrheal, antinoceptic, anti-inflammatory, antipyretic anti malaria activities which however, justifies the scientific use of these plants in traditional medicine in the treatment of infections caused by the test organisms.

ACKNOWLEDGEMENTS

The authors would like to appreciate staff members of Pure and Applied Chemistry, University of Nigeria Nsukka and Enugu State University of Science and Technology teaching hospital, Enugu State, Nigeria, for their help during the study, interpretations andsample collection.

AUTHOR'S CONTRIBUTION

Udeh VC: performed analysis, manuscript drafting. **Emmanuel A:** performed analysis and prepared the manuscript. **Omeje EO:** supervision. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest.

REFERENCES

1. Montefiore D, Rotimi VOAdeyemi-Doro FAB. The problem of bacterial resistance to antibiotics among strains isolated from hospital patients in Lagos and Ibadan, Nigeria. J Antimicrob Chemother 1989; 23(4):641-651. *https://doi.org/10.1093/jac/23.4.641*
2. Isibor C, Ahonkahi I. Comparative *in-vitro* activity of fluoroquinolones against clinical bacterial pathogens isolated from central hospital Warri. Nigerian J Sci Res 2022; 21(1): 1-10.
3. Udeh VC, Agboeze E, Omeje EO, Chime CC, Ike OC. Evaluation of the phytochemical and antimicrobial activity of the fractionated methanolic leaf extract of *Diospyros pruessii* Gurke. J Chem Soc Nigeria 2022; 47(3):6-23.

https://doi.org/10.46602/jcsn.v67i3.754
4. Pratap R, Ram VJ. Natural and synthetic chromenes, fused chromenes, and versatility of dihydrobenzo [h] chromenes in organic synthesis. Chem Rev 2014; 114(20):10476-10526. *https://doi.org/10.1021/cr500075s*
5. Ma RH, Ni ZJ, Zhu YY, *et al*. A recent update on the multifaceted health benefits associated with ginger and its bioactive components. Food Funct 2021; 12(2):519-542. *https://doi.org/10.1039/d0fo02834g*
6. Christian EO, Obumneme OC, Okey EM, *et al*. Effect of *Citrus aurantifolia* juice on bodyweight and haematological indices of wistar rats. African J Food Sci 2022; 151-159. *https://doi.org/10.5897/AJFS2022.2175*
7. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African J 2005;685-688. *https://doi.org/10.5897/AJB2005.000-3127*
8. Anyanwu CU, Nwosu GC. Assessment of the antimicrobial activity of aqueous and ethanolic extracts of *Piper guineense* leaves. J Med Plants Res 2014; 8(10):436-440. *https://doi.org/10.5897/JMPR12.976*
9. El-Hack A, Mohamed E, El-Shall NA, *et al*. The use of black pepper (*Piper guineense*) as an ecofriendly antimicrobial agent to fight foodborne microorganisms. Environment Sci Pollution Res 2022; 1-4, 2022. *https://doi.org/10.1007/s11356-021-17806-7*
10. Kareem KT, Kareem SO, Adeyemo OJ, Egberongbe RK. *In vitro* antimicrobial properties of *Bridelia ferniginia* on some clinical isolates. Agric Biol J North America 2010; 1(3):416-420.
11. Mann A, Gbate M, Umar AN. Medicinal and economic plants of Nupeland. Jube Evans books and publications, 2003.
12. Marshall AR, Platts PJ, Gereau RE, *et al*. The genus Acacia (Fabaceae) in East Africa: Distribution, diversity and the protected area network. Plant Ecol Evol 2012; 145(3):289-301. *https://doi.org/10.5091/plecevo.2012.597*
13. Cushnie TT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicro Agents 2014; 44(5):377-386. *https://doi.org/10.1016/j.ijantimicag.2014.06.001*
14. Ema FA, Shanta RN, Rahman MZ, Islam MA, Khatun MM. Isolation, identification, and antibiogram studies of *Escherichia coli* from ready-to-eat foods in Mymensingh, Bangladesh. Vet World 2022; 15(6): 1497. *https://doi.org/10.14202/vetworld.2022.1497-1505*
15. Raweh SM, Alhaj HA, Al-KafAG. *In vitro* assessment of antimicrobial and antioxidant activities of extracts of Yemeni *Fagonia schweinfurthii* Hadidi. Universal J Pharm Res 2022; 7(4):26-31. *https://doi.org/10.22270/ujpr.v7i4.806*
16. Al-Kaf AG, Taj Al-Deen AM, Ali ALhaidari SA, Al-Hadi FA. Phytochemical analysis and antimicrobial activity ofcolocasia esculenta(taro) medicinal plant leaves used in folk medicine for treatment of wounds and burns in Hufash district al Mahweet Governorate–Yemen. Universal J Pharm Res 2019;4(2):29-33. *https://doi.org/10.22270/ujpr.v4i2.254*
17. Al-Mahbashi H, Moharram BA, Al-Maqtari T. Phytochemical, anti-inflammatory, analgesic, antipyretic and acute toxicity of Psiadiapunctulata growing in yemen. Universal J Pharm Res 2020; 5(5):61-66. *https://doi.org/10.22270/ujpr.v5i5.489*