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Evaluation of Antibacterial Activities of Fractions from Ethanol-Extracted Residues of *Piper guineense* Leaves on Gram Negative Clinical Isolate

Tharcitus Chilaka Onwudiwe, Prince Chiazor Unekwe, Kingsley Chimsorom Chilaka* and Malachy Ifeanyi Obi

Department of Pharmacology and Therapeutics, Faculty of Medicine, Nnamdi Azikwe University, Nnewi Campus, Nigeria

**Corresponding author*

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A B S T R A C T

The problem of drug resistance to orthodox antimicrobial agents has remained a setback in the treatment of bacterial infections in the modern society. Adverse effects, coupled with scarcity and high cost of orthodox drugs have necessitated interest in the search, development and use of antibacterial agents from plant origin. *Piper guineense* is claimed in traditional medicine as a remedy for gram negative organism-transmitted infections. The leaves of *Piper guineense* plant was collected, washed, dried at room temperature and pulverized. The plant material was extracted with 80% ethanol. The ethanol-extracted residue was subjected to fractionation. Seventeen fractions were obtained, and were pooled together based on their R_f values into five pooled-fractions labeled: PF-1, PF-2, PF-3, PF-4, PF-5. Both the ethanol and fraction extracts were subjected to phytochemical analysis, preliminary antibacterial screening, minimum inhibitory and minimum bactericidal concentrations determination using both clinical isolates and type culture organisms. The yield of ethanol-extracted residue was low (21.08g) when compared to the amount of pulverized plant material (500g). Phytochemical analysis revealed the presence of flavonoids, alkaloids and terpenoids in all the extracts. The extracts produced statistically significant lower zone of inhibition ($p<0.05$) when compared with the standard drug (amoxicillin), it also demonstrated activity against test organisms used in the study. The findings of this study demonstrated that ethanol leaf extract of *Piper guineense* possess antibacterial activities, therefore justifies the traditional claim of the plant.

Introduction

Since their discovery more than 70 years ago, antibacterial drugs have become an essential part of the modern health care landscape, allowing treatment of previously life-threatening bacterial infections[1]. However, the ever-increasing levels of antibacterial resistance threatens the health benefits achieved with antibiotics and this

phenomenon is recognized as a global crisis due to the current and potential impact on global population, costs of health care system and gross domestic product mainly through treatment options [2,3,4]. A recent report suggests that absolute numbers of infections due to resistant microbes are increasing[5].

The problem of drug resistance is partly caused by irrational prescription, indiscriminate use of fake and substandard antibiotics as well as faulty diagnosis which ultimately lead to treatment failure and complication of diseases conditions. In order to overcome this ugly trend, the need to evaluate, develop new and safe antimicrobial agents from plant origin cannot be over emphasized. Contrary to the synthetic drugs, antibiotics of plant origin are not associated with numerous side effects. Besides, plants have been documented to have advantages of toxicity consideration based on their long term use in human[6,7]. For thousands of years, medicinal plants have been used to treat health disorders, and prevent diseases epidemics. Microbial growth in diverse situations has been controlled by plant derived products[8,9].

Piper guineense, commonly referred to as African black pepper or Ashanti pepper belongs to family Piperaceae, is a herbaceous climber plant 4-10m in length, and commonly found in Nigeria and tropical Africa zone. It is locally called "uziza" in Igbo, "iyere" in Yoruba and "monsoro" in Hausa.

The fruits and leaves are commonly sold in Nigeria markets as condiment for food flavouring[10]. Ethnomedicinal uses of *Piper guineense* include: as carminative and eupeptic [11], treatment of respiratory infection and syphilis [12], aphrodisiac [13]. This present work studied the antimicrobial activities of the crude and fraction extracts of the leaves of *Piper guineense*, as part of exploration for new and novel bioactive compounds.

Materials and Methods

Collection and identification of plant material

Fresh leaves of *Piper guineense* (Pipereceae) were collected from a farm land in Naze, Owerri North Local Government Area of Imo State, Nigeria. The plant was identified by Boniface Uwakwem in the Department of Pharmacognosy, Madonna University, Nigeria and was confirmed by Chimezie Ekeke a taxonomist, with a herbarium number UPH/P/251 a voucher specimen was deposited with Herbarium.

Preparation and extraction of plant material

The preparation and extraction of plant material were done according to the procedure specified by [14]. In this procedure, fresh matured leaves of *Piper guineense* were thoroughly washed, air-dried at room temperature for two weeks and coarsely powdered. The powdered material was kept in tightly closed containers until when needed for extraction. About 500g of coarsely powered leaves of *Piper guineense* was macerated in two litres of ethanol (80%) at room temperature with occasional shaking for three days, after which the filtrate was separated from the marc using filter paper (Whatman No. 1). The marc was re-macerated twice. The obtained filtrates were combined and placed in a water bath (40^{o}C) to evaporate the ethanol.

Determination of yield of plant extract of *Piper guineense*

This was carried out according to the method proposed by [15]. The plant extract was evaporated to dryness at temperature of 40^{o}C in a hot air oven using a previously weighed empty beaker. Yield in percent was calculated using the formula:

$$\text{Yield} = \frac{\text{Weight (g) of Residue}}{\text{Weight (g) of marcerated powered material}} \times 100$$

Fractionation of ethanol-extract residue

Two chromatographic techniques (thin-layer and column chromatograph) were employed. The thin-layer chromatography was first used to determine the solvent system that gave best resolution, while column chromatography was used to fractionate the crude extract.

Preliminary TLC was carried out according to procedure proposed by [16] and was conducted on silica gel (F_{254}) plates to determine the solvent system that gave best resolution. The following solvent systems were prepared and in the ratio stated below.

Chloroform/Ethylacetate (9:1)
Chloroform/Ethylacetate (8:2)
Chloroform/Ethylacetate/n-Hexane (7:2:1)
Chloroform/Ethanol (1:1)
Chloroform/Ethylacetate/Ethanol (7:2:1)
Chloroform/n-Hexane (1:1)
Ethylacetate/n-Hexane (1:1)
Ethylacetate/Ethanol (1:1)

The silica gel plates were spotted with ethanol-extract residue about 2cm from the base using capillary tube, and allowed for about 10 minutes to dry. The spotted plate was placed at an angle of 30^{o} in chromatographic tank containing about 20ml of the respective solvent system.

After resolution, the plate from each of the solvent system was air-dried and spots were viewed under UV lamp at 254nm and 365nm wave length for identification of fluorescing spots. The TLC plates were sprayed with Dragendoff's reagent for colour development.

Column chromatography was carried out according to the procedure described by[17], using the solvent system (Chloroform/Ethylacetate/Ethanol (7:2:1) that gave the best resolution in the preliminary thin-layer chromatography. Column chromatography was carried out using a glass column of internal diameter of 20mm and length 19cm (Quick-fit England).

The column was packed with sufficient quantity of wet silica gel (F_{254}) and allowed to stabilize for 24 hours.

Then a 10g amount of crude extract was dissolved in ethanol, placed on the column and then continuously eluted with the solvent system (Chloroform /Ethylacetate Ethanol; 7:2:1) that gave best resolution in the preliminary thin-layer chromatography.

Seventeen-10ml fractions were collected and their TLC mobility (R_f) and colour reaction were determined and compared. TLC mobility (R_f) was calculated using the following formula.

$$R_f = \frac{\text{Distance(cm) travelled by the spot from starting point in TLC}}{\text{Distance(cm) travelled by the solvent front in TLC}}$$

Pooling of fractions

Fractions that showed similar R_f value and colour reaction were pooled together, evaporated to dryness, labeled appropriately and stored in well-sealed containers in the refrigerator until when needed.

Labeling and storage of the plant extracts

The plant extracts were labeled as follows:
EE = Ethanol Extract
PF-1 = Pooled Fraction-1
PF-2 = Pooled Fraction-2
PF-3 = Pooled Fraction-3
PF-4 = Pooled Fraction-4
PF-5 = Pooled Fraction-5

The labeled containers were stored in the refrigerator until when needed.

Determination of pH of ethanol and fraction extracts

This was carried out using the procedure described by [15] where appropriate amount of the extracted residues (ethanol and fraction extracts) were mixed with demineralized water and pH of the resulting solution determined using pH meter.

Phytochemical analysis of ethanol and fraction extracts.

This was conducted using standard procedures specified by [18]

Sources and purification of test organisms

The organisms used in the antibacterial study were clinical isolates obtained from patient with sexually transmitted disease in Madonna University Teaching Hospital Elele. Standard typed cultures were obtained from Bioresources Development and Conservation Project Center, Nsukka. The test organisms (i.e. the clinical isolates and typed cultures) were repeatedly isolated to purify them according to the procedure described by [19]. The cultures were appropriately stored in agar slant until when needed.

Preparation of culture media

The culture media were prepared according to manufacturers' specification which involves accurate weighing of amounts of pre-formulated product, and dissolved in one litre distilled water. The mixture was distributed in bijou bottle and sterilized in autoclave at 121^{o}C for 15 minutes. The sterilized nutrient agar was maintained in a molten state until when needed, while sterilized nutrient broth was stored in the refrigerator until when needed.

Standardization of bacterial suspension

Bacterial suspension was standardized according to the procedure prescribed by [20] which involves streaking the test organisms on agar plate and incubating for 24 hours at 37^{0}C, the emergent discrete colonies were incubated in agar broth until turbidity of 0.5 Mcfarland was achieved, which can be determined using Mcfarland standard ($5x10^5$ cfu/ml for gm-ve, and $1x10^5$ cfu/ml for gm +ve organisms).

Preliminary antibacterial screening of plant extracts and standard antibiotic (amoxicillin)

This test was done to determine the doses of the extracts (ethanol and fractions) and standard drug (amoxicillin) that may have antibacterial activity. The preliminary sensitivity screening was conducted according to procedure described by [20].

In this test, each of the test organisms were seeded into agar plates and subsequently, different concentration of the extracts (ethanol and fractions) and standard drug were introduced into duplicate well of agar culture. After incubation for 24 hours at 37°C, zones of inhibition (mm) were measured and mean zone inhibition (MZI) determined. Only the samples that showed significant zone of inhibition were selected for further antimicrobial studies.

Determination of minimum inhibitory concentration of the plant extracts and standard antibiotic (amoxicillin)

In a method described by[20], two fold serial dilutions of plant extracts (ethanol and fractions) and Amoxicillin were prepared, each in ten test tubes containing nutrient broth. Each dilution was seeded with 100 microlitres (0.1ml), (representing $5x10^5$ for gm-ve) of the standardized suspension of test organisms previously incubated for 24 hours at 37°C (i.e. 24 hour broth culture).

Minimum inhibitory concentration (MIC) was determined as the highest dilution or the least concentration that produced no visible growth.

Determination of minimum bactericidal concentration of the plant extracts and standard antibiotic (amoxicillin).

This was also done using a procedure described by [20]. In this method, 0.1ml volume of dilution showing no visible growth were inoculated on sterile agar and incubated for 24 hours at 37°C. The Minimum bactericidal concentration (MBC) was determined as the highest dilution showing no visible growth.

Statistical Analysis

The figures obtained from the preliminary sensitivity test were expressed as ± standard error of mean (SEM). The mean MICs for the extracts and standard drug (amoxicillin) were tested for statistical significance using one-way analysis of variance (ANOVA), Chi-Square (X^2), followed by Turkey's multiple comparison tests using SPSS software version 24. $P<0.05$ was considered as significant.

Results and Discussion

Yield and pH of Plant Extracts (Ethanol and Fraction)

The quantitative yield of the ethanol extract and fraction extracts were relatively low when compared to respective amount (500g) of pulverized plant material macerated and the amount (10g) of ethanol extract (EE) fractionated. Column chromatographic separation yielded a total of seventeen (17) fractions. Both the ethanol and fractions extracts possessed low acidity as shown in table 1.

Phytochemical Analysis of Plant Extracts (Ethanol and Fractions)

The ethanol extract (EE) and fraction extracts (PF-1, PF-2, PF-3, PF-4 and PF-5) contained flavonoids, while glycosides were absent in pooled fraction-2 and pooled fraction-4. There were absence saponins in pooled fraction-1 (PF-1), pooled fraction-2 (PF-2) and pooled fractions-5 (PF-5), while tannins and phenols were absent in pooled fraction – 3 (PF-3) as shown in Table 2 below.

Preliminary Antibacterial Screening of Plant Extracts (Ethanol and Fractions) at 50mg/ml and Standard Drug (Amoxicillin) at 50mcg/ml.

The result of preliminary antibacterial screening revealed that the plant extracts (ethanol and fractions) and standard drug produced significant mean zone of inhibition diameter (mm) at concentration of 50mg/ml and 50mcg/ml respectively on test organisms with exception of *Pseudomonas aeruginosa* as shown in Table 3 below. The zone inhibition produced by PF-1 was small.

Minimum Inhibitory Concentration of the Plant Extracts (Ethanol and Fractions) and Standard Drug (Amoxicillin)

From the result in Table 4, it is shown that with exception of pooled fraction-1 (PF-1) and pooled fraction-2 (PF-2), the rest of the extracts produced inhibition on the clinical isolate and type cultures used in the study. They did not produce inhibition on *P. aeruginosa* but pooled fraction-4 (PF-4) at 100mg/ml produced inhibition of *P. aeruginosa*

Minimum Bacterial Concentration (MBC) of Plant Extracts (Ethanol and Fractions) and Standard Drug (Amoxicillin)

The result of minimum bacterial concentration revealed that pooled fraction-4 (PF-4) did not produce bactericidal activity on *E. coli* clinical Isolate. The pooled fraction-1 (PF-1), pooled

fraction-2 (PF-2) ethanol extract did not show bactericidal activity on different organism as shown in Table 5.

In this study, ethanol was used in the extraction of plant material because it gave the highest yield when compared to other solvents in the preliminary extraction. Moreso, it was reported[21]that ethanol maceration is a more applicable method of extraction for research because of its effectiveness and low cost when compared to other extraction methods. In addition, most traditional herb preparations were done with ethanol as solvent. The ethanol preparations were used traditionally on account of their efficacy [22]. Judging by the amount of pulverized plant material (500g) in this study, crude extract yield obtained was low (21.08g). This correlated with the finding by [23], that biologically active compounds usually occur in low concentration in plants. Both ethanol and fraction extracts possess low acidity as shown in Table 1. Medicinal plants are safe, cheap, effective and available source of biologically active molecules[24,25]. Phytochemical screening of crude and fraction extracts of *Piper guineense*s in this study showed the presence of Saponins, Flavonoids, Tannins, Glycosides, Alkaloids, Phenolic acid and Terpenoids. This observation is supported with those report from other studies which indicated the presence of these phytochemicals in plant extracts [26]. One or more of these plant secondary metabolites may be responsible for antiulcer activities of the plant.

Flavonids are natural metabolites present in different fruits and vegetables have been reported to possess antimicrobial activities[27].

Table.1 Yield and pH of Plant Extracts (Ethanol and Fractions)

Extracts	Yield(g)	Percent Yield	pH
EE	21.08	-	6.2
FE-1	0.41	4.1	6.4
FE-2	0.47	4.7	6.3
FE-3	0.35	3.5	6.0
FE-4	0.54	5.4	6.3
FE-5	0.38	3.8	6.1
FE-6	0.47	4.7	5.8
FE-7	0.62	6.2	6.0
FE-8	0.75	7.5	6.1
FE-9	0.55	5.5	5.9
FE-10	0.74	7.4	5.8
FE-11	1.22	12.2	6.2
FE-12	0.67	6.7	6.4
FE-13	0.63	6.3	5.9
FE-14	0.40	4.0	6.0
FE-15	0.56	5.6	6.2
FE-16	0.73	7.3	5.9
FE-17	0.42	4.2	6.3

EE = Ethanol Extract
FE = Fraction Extract

Table.2 Phytochemical Analysis of Plant Extracts (Ethanol and Fraction)

Test	CEE	PF-1	PF-2	PF-3	PF-4	PF-5
Carotenoids	+	-	-	+	-	-
Phenols	+	+	+	-	+	+
Saponins	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	-	+	+
Glycosides	+	+	-	+	-	+
Alkaloids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+

+ = Present; - = Absent

Table.3 Preliminary Antibacterial Screening of Plant Extracts (Ethanol and Fractions) at 50mg/ml and Standard Drug (Amoxicillin) at 50mcg/ml

Test organism	EE (MZI)	PF-1 (MZI)	PF-2 (MZI)	PF-3 (MZI)	PF-4 (MZI)	PF-5 (MZI)	Amoxicillin (MZI)
E .coli	2.14±0.81	0.26± 0.32	1.41 ± 1.0	14.6± 0.56	8.5 ±1.1	8.2 ±0.14	28.4 ±1.01
Salmonella spp	2.12±0.66	0.28±0.11	1.6 ±0.04	16.2 ±0.30	5.7 ±0.61	6.8 ±0.17	21.85 6
P. aeruginosa	0.00±0.0	0.00±0.00	0.00	0.00	0.00	0.00	0.00
E. coli (ATCC 11775)	5.175±1.07	0.29±0.89	11.9 ±1.2	14.1 ±0.11	9.06 ±1.4	7.0 ±1.6	30.05 ±1.6
S. Kintambo (SSRL 115)	3.52±0.46	0.24±0.81	11.8±0.71	12.3 ±0.79	6.1 ±0.82	9.4 ±0.12	22.2 ±0.81
P.aeruginosa (ATCC10145)	2.24±0.0	0.00±0.0	0.00	0.00	0.00	0.00	0.00

Table.4 Minimum Inhibitory Concentration (MIC) of Plant Extracts (Ethanol and Fractions) and Standard Drug (Amoxicillin)

Test organism	EE (mg/ml)	PF-1 (mg/ml)	PF-2 (mg/ml)	PF-3 (mg/ml)	PF-4 (mg/ml)	PF-5 (mg/ml)	Amoxicillin (mcg/ml)
E. coli	100	-	-	12.5	25.0	25.0	100
***Salmonella* spp**	100	-	-	50.0	25	12.5	100
P. aeruginosa	-	-	-	-	-	-	-
***E. coli* (ATCC 11775)**	50	-	-	6.25	25	25	50
***S. kintambo* (SSRL 115)**	100	-	-	6.25	12.5	50.0	50
***P. aeruginosa* (ATCC10145)**	-	-	-	-	100	-	100

Table.5 Minimum Bactericidal Concentration (MBC) of Plant Extracts (Ethanol and Fractions) and Standard Drug (Amoxicillin)

Test organisms	EE (mg/ml)	PF-1 (mg/ml)	PF-2 (mg/ml)	PF-3 (mg/ml)	PF-4 (mg/ml)	PF-5 (mg/ml)	Amoxicillin (mcg/ml)
E. coli	-	-	-	100	-	100	100
***Salmonella* spp**	-	-	-	100	100	50	100
P. aeruginosa	-	-	-	-	-	-	-
***E. coli* (ATCC 11775)**	-	-	-	200	50	100	50
***S. kintambo* (SSRL 115)**	-	-	-	100	100	50	50
***P. aeruginosa* (ATCC10145)**	-	-	-	-	-	-	-

On the basis of spectrum of activity, ethanol and fraction (PF-3, PF-4 and PF-5) extracts of *Piper guineense* when compared with controlled drug (amoxicillin), showed antibacterial activity against wide range and Gram-ve organisms used in this study. This observation may explain why the extracts of the plant is widely applied in traditional medicine, particularly in the treatment of stomach disorders and sexually transmitted diseases. However while amoxicillin was effective in microgram amounts, the extracts were effective in milligram amounts. The ethanol and fraction (PF-3, PF-4 and PF.5) extracts at different concentrations showed bacteriostatic and bactericidal activities against different organisms in the study. *Pseudomonas aeruginosa* is generally known to be resistant to most antibiotics, the fraction (PF-4) extract at 100mg/ml exhibited bacteriostatic activity against the organism (ATCC 10145) and hence making it a good candidate for further investigation.

Although the fraction (PF-3, PF-4 and PF-5) extracts showed wide range of antibacterial activity, but comparing their mean zone of inhibition with those produced by ethanol extract, this study observed increase in antibacterial activity. This may suggest that fractionation of the extracts might have led to increased antibacterial activity which correlates with the finding of [28] that purification increases the efficacy of secondary metabolites

References

1. Mark, L., Ali, S. and Khondaker, M. R. (2019). Antibiotic resistance breaker: current approaches and future directions, *FEMS Microbiology Reviews*; 43(5), 490-576.
2. Ventola, C. L. (2015) The antibiotic resistance crisis: part 1: causes and threats; *PT*, 277-283
3. Tadesse, B. T., Ashley, E. A., Ongarello, S., Havummaki, J., Wijegoonewardena, M., Gonzalez, I. J. and Dittrich, S. (2017). Antimicrobial resistance in Africa: a systematic review.*BMC infectious Disease*; 17(616), 416-421.
4. Naylor, N. R, Atun, R., Zhu, N., Kulasabanatha, K., Silva, S., Chetterjee, A,. Knight G. M. Robotham, J. V (2018). Estimating the burden of antimicrobial resistance ; a systematic literature review. *Antimicrobial Resistance Infection Control*; 7(58), 20.
5. European Food Safety Authority (2016). European union summary report on antimicrobial resistance in antimicrobial

resistance in zoonotic and indicator bacteria from humans, animals and food in European union in 2014. *European Food Safety Authority Journal; 14, 1-207*.

6. Ibrahim, M. B, Sowemimo A. A., Sofidiya., M. O., Kunle, O. F., Badmos, K. B., Fageyinbo, M. S., Abdulkareem. F. B., Odukoya. A. O.,(2016). Sub-acute and chronic toxicity profile of *Markhamiatomentosa* ethanolic leaf extract in rats, *Journal of Ethnopharmacology, 193, 65-75.*
7. Augustine, N. R., Madhavan, G. and Nass, S. J. (2017). Committee on Ensuring Patient Access to Affordable Drug Therapies; Board on Health Care Services, Health and Medicine Division; A National Imperative. Washington (DC) United States: National Academic Press, PP.167-173.
8. Refaz, A. D., Molid, S. and Parvaiz, H. Q. (2017). General overview of medicinal plants: a review. *The Journal of Phytophamacology*; 6(6), 349-35.
9. Surette, M. D. and Wright, G. D. (2017). Lessons from the environmental antibiotic resistance. *Annual Review in Microbiology*. 71, 309-329
10. Chibuzor O. and Assumpta O. (2014). Nutritional evaluation of some selected spices commonly used in South-Easthern part of Nigeria. *Journal of Biology and Agriculture*; 4(5), 56-60.
11. Echo, I. A., Osuagwu, A. N., Agbo, R. B., Okpako, E. C. and Ekanem, B. E. (2012). Phytochemical composition of *Aframomummelegueta* and *Piper guineense* seeds.*World Journal of Applied Environmental Chemistry*; 2(1), 17-21.
12. Okigbo, K. N. and Igwe, D. I. (2007). Antimicrobial effects of *Piper guineense* and *Phylantrusamanus* on *Candida albican, Streptococcus feacalis*. *Acta Microbiological Hiugarican*; 54(4) 353-366.
13. Noumi, E., Amvan, Z. P. H. and lontis, D. (1998). Aphrodisiac plant used in Cameroon. *Fitoter*; 69: 5-34.
14. Girma, S., Gidan, M., Erko, B. and Mamoh, H. (2015). Effect of crude leaf extract of *Osyris, Quadripartite* on *Plasmodium berghei* in Swiss albino mice, *British Medical Journal of Complementary and Alternative Medicine*, 15, 184.
15. Okoli, A. S., Okeke, M. I., Iroegbu, C. U. and Ebo, P. U.(2010). Extraction and Evaluation of antibacterial principles of *Harunganamadagascariensis* leaf, *Phytotherapy Research*, 16, 183-186.
16. Stahl, E. (1969). *Thin-layer chromatography. a laboratory handbook*. 1st edn. Berlin: Springer, PP. 52-55.
17. Harbone, J. B. (1991). *Phytochemical methods: a guide to modern techniques of plant analysis*. 4th edn, London: Chapman and Hall Ltd, PP. 212-232.
18. Harbone, J. B. (1998). *Phytochemical methods: a guide to modern techniques of plant analysis*. 5th edn, London, UK: Chapman & Hall, PP..146.
19. Cheesbrough, M. (2000). District laboratory practice in tropical countries (part 2) Cambridge University Press New York, pp. 134-135.
20. National Committee for Clinical Laboratory Standard (2015). *Methods of dilution in antimicrobial susceptibility test. National Committee for Clinical Laboratory Standard.* Villanova, PP. 22-29.
21. Azwanida, N. N. (2015). A review of extraction methods used in medicinal plants; principles, strength and limitation. *Medicinal and Aromatic plant*, 4 196

22. Ibrahim, M. B., Owonubi, M. O., Onaolapu, J. A. (1998). Antimicrobial effects of extracts of leaf, stem and root bark of *Anogiessusleicarpuson Staphylococcus aureus (NCTC 8198). E.coli (NCTC 1041), Proteus vulgaris (NCTC 4636) Nigerian Journal of*

Pharmaceutical Research and Drug Development.2, 20-26.
23. Tushar, F. G.(2017) Effect of extraction method on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*, *Arabian Journal of Chemistry*. 10(51) 51200-51203.
24. Sharif-Rad, M, Varoni, E. M., Salehi, B, Matthew, K. R., Ayatollahi, S. A., Zakaria, Z. A. (2017). Plant of the genus *Zingibe*r as a source of bioactive phytochemicals from tradition to Pharmacy.*Molecules*. 22, 2145
25. Sharifi-Rad, M., Nazaruk, J., Polito, L., Rocha, J. E., Coutinho, H. D. M., Tabanelli, G. and Montanarri, C. (2018*). Matricaria* genus as a source of antimicrobial agents: from farm to pharmacy and food applications, *Microbiology Research*, 215, 76-78.
26. Tyagi, T. and Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistiastratiotes* and *Eichhomiacrassipes*, *Journal of Pharmacognosy and Phytochemistry*, 6, 195-206.
27. Rancy, A. T. and Krishnakumari, S. (2015). Phytochemical profiling of *Myristica fragans* seed extract with different organic solvents, *Asian Journal of Pharmacy and Clincal Research*, 8, 303-302.
28. Wagner, H., Bladt, S., and Zgainski, E. M. (1984). Plant drug analysis.*a thin layer chromatography atlas*. Translated by T. A. Sedt, Berlin: Springer-Verlag, PP. 51-90, 125-178.

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