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Phytochemical, Antimicrobial and Bio-Active Component Analysis of *Platycerium Superbum (L.)* Methanolic Extract

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Abstract

Plants have been considered for several years to be a valuable source of compounds that can be active against the activity of many pathogenic organisms but lower plants are rarely considered useful in many cases. In this study, the phytochemical analysis, antimicrobial activity and Gas Chromatography-Mass Spectrometer (GC-MS) analysis was carried out on *P. superbum* methanolic extract following standard procedures from earlier reports. The qualitative phytochemical screening result revealed the presence of important phytochemicals like tannin, saponin, alkaloid, flavonoid, protein, terpenoid, oxalate, glycoside, anthraquinone, and phytobiotin in the plant extract. Flavonoid has the highest quantity of 6.69mg/g while terpenoid has the lowest quantity of 0.66mg/g. The result of the antimicrobial activity of *P. superbum* revealed that the plant extract compared with some standard drugs like Ciprofloxacin, Streptomycin, Septrin and Gentamycin had significantly the same level of activity at certain concentrations while the extract showed greater potency against the test microbes at some other concentrations.

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The GC-MS analysis of the extract revealed the presence of essential bioactive compounds like Silanediol, Cyclohexanecarboxylic acid, Methyl ester, Cyclohexacarboxylic acid, Bicyclo [3.1.1] Heptane, Bicyclo [5.2.0] nonane, Methyl palmitate and 7– Octaecenoic which have been reportedly given some therapeutic and industrial credits.

Keywords: *P. superbum*; MRSA (Methicillin-Resistant *Staphylococcus aureus*); Antibiotics; MIC (Minimum Inhibitory Concentration).

1. Introduction

Medicinal plants have been used for centuries as remedies for diseases in humans and so many atimes offer a new source of biologically active chemical compounds as antimicrobial agent [1]. If the toxic effect tested *in-vivo* is low there is a possible chance of introduction of such drug for therapeutic purpose [2]. Plants have abundant ability to produce aromatic secondary metabolite, most of which are phenolic acids, quinones flavones, flavonoids, flavonols, tannins and coumarins. Many reports have it that this group of compound shows antimicrobial effect and serve as plant defence mechanisms against pathogenic microorganisms. According to [3], plants that contain substances which can be used for therapeutic purposes or which can be used as precursors for the synthesis of useful drugs is a medicinal plant. The treatment and control of disease by the use of available medicinal plants among ethnic groups will continue to play significant role in medicinal health care implementation in the developing countries of the world. This is because of their effectiveness, affordability, low toxicity and acceptability. There is also the global threat of resistance by pathogenic organisms to already developed drugs most especially in developing countries, this could be dealt with using the natural and novel antibiotics with diverse mechanisms of action from some new and unique sources from lower plants [4].

P. superbum is a lower plant since it does not have roots and produce spores to reproduce, rather than flowers. It belong to the family *Polypodiaceae* and is propagated from it spores. Apart from it ornamental uses, it has been reported to have wild medicinal uses. In Nigeria, young leaves of *P. superbum* are prescribed as a common antiulcer remedy [5]. The leaf extract of *P. superbum* is reported to have divers uses such as preventing miscarriage in women when taken two months after conception [6] and in the treatment of oedema coughs and hypertension [7]. The isolation and characterization of polysaccharide from *P. superbum* has also been reported by [8]. However, no reports yet on the identification of active principles contained in the extract of *P. suberbum* but [9] reported the identification of bioactive components in the methanolic extract of *Gleichenia pectinata*. This present study however is aimed at evaluating the phytochemical components, antimicrobial activity and the characterization of bioactive components in *P. superbum* methanolic extract.

2. Materials and Methods

2.1 Plant Sample/Microorganism Collection and Processing

P. superbum whole plants were collected from Adeyemi College of Education, Ondo state, Nigeria. It was identified at University of Ilorin Herbarium and voucher number UILH/003/1261 was obtained. The plants were then washed, air dried and crushed. The pure isolates of the bacteria strains were procured from the department

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of microbiology and parasitology, University of Ilorin Teaching Hospital and they were properly identified with the following ID: *Staphylococcus aureus* (Methicillin-Resistant Staphylococcus aureus), *Pseudomonas aeruginosa* (27853) *Bacillus subtilis* (6051), *Klebsiella pnemoniae (13883), Escherichia* coli (25922) and *Proteus mirabilis* (29906). They were further stored in the refrigeration at 4^{o}c before use. However, further sub-culture was done to keep the organism viable. Before antimicrobial sensitivity assay, these isolates were sub-cultured into nutrient broth agar at 37^{0}c overnight. The organisms were made sure to be at their exponential phase of growth before carrying out the sensitivity analysis.

2.2 Extraction

After air drying the plant, the plant extraction was done by maceration at room temperature using methanol as the solvent. The crushed plant sample was placed in a closed vessel and methanol was added at the plant to solvent ratio 1:10 w/v. a suitable solvent (methanol) was added and left for 72 hours with occasional agitation. The liquid was then strained and cleared by filtration; the filtrate was gradually made to undergo rotary evaporation to remove the methanol content.

2.3 Phytochemical Screening

2.3.1 Qualitative Analysis

The *P. superbum* extract of the medicinal plant under study were screened for the presence of tannin, saponin, alkaloid, anthraquinone, flavonoid, protein, terpenoid, oxalate, glycoside, anthraquinone, and phytobiotin using the standard methods as described by [10,11].

2.3.2 Quantitative Analysis

The quantitative phytochemical screening of P. superbum was investigated following the reports of the following researchers; Tannin, [12], Flavonoid [13], Saponin [14], Alkaloid [15], oxalate and glycosides [16], Protein [17] and Terpenoids [18].

2.4 Sterility Test for the Extract

The extract was checked for sterility on Mueiller-Hinton agar by streaking method after sterilization with pasteurization temperature at 70^{0}C for 30 minutes.

2.5 Antimicrobial Sensitivity Assay

The antibacterial activity of the methanolic of *P. superbum* extract was determined using the agar-well diffusion method described by [19]. The bacteria isolated were first grown in nutrient broth for 18 hours before use. Prepared and sterilized Mueller Hinton agar was poured onto petri-dishes which were allowed to solidify. The bacteria cultured in the nutrient broth agar were introduced on the petri-dish containing solidified Mueller Hinton agar with the use of a swab stick. The extract concentrations were prepared as follows: 200mg/ml,

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100mg/ml, 50mg/ml, 25mg/ml and 5mg/ml by serial dilution. Standard broad-spectrum antibiotic drugs which are Gentamicin (100mg/ml), Streptomycin (100mg/ml), Ciprofloxacin (20mg/ml) and Septrin (400mg/ml) were used as positive controls against the test bacteria. Negative controls were also set up in a parallel using the solvent for extraction. Antibiotic discs of 6mm diameter were soaked into the extract at different concentrations and the drugs at the prescribed concentration against the growth and lifecycle of the bacteria for a period of 6 hours. The plates were incubated at 37^0C and observed for zone of inhibition after 24h. A zone of clearance around each well signified inhibition and the diameter of each zone were measured in millimetre (mm) with a transparent ruler. All assays were performed in three independent triplicates. This method followed the report of [20].

2.6 Determination of Minimum Inhibitory Concentration (MIC)

Microorganisms were tested for their ability to produce visible growth on a series of agar plates by agar micro-dilution method containing concentrations; 5-200mg/ml of the plant extract. Each volume of the extract were mixed with already prepared nutrient broth in a test tube and 0.1ml of standard inoculums (1-2 x 10^7 cfu/ml) was added to each tube with tubes without the extract used as control tubes. The MIC endpoint for each strain was taken as the lowest concentration of antibiotic (plant extract) at which there was no visible growth on the agar [21].

2.7 Characterization of the bioactive components in P. superbum extract using GC-MS

Identification of phytoconstituents in *P. superbum* methanolic extract was done using a GC-MS machine (Model; 7890A GC System, 5675C Inert MSD with triple axis; The column model is Agilent 19091-433HP-5Ms 5% Phenyl methyl silox) equipped with a fused silica capillary column of 30 m length, 0.25 mm diameter, and 250 µm film thickness treated with phenyl methyl silox. The ion source temperature (EI) was 250°C while the interface temperature was 300°C, Pressure 16.2 psia, out time 1.8mm. 1µl injector in split mode with split ratio 1:50 with in jection temperature of 300°C.

The column temperature started at 35°C for 5mins and changed to 150°C at the rate of 4°C/min. The temperature was raised to 250°C at the rate of 20°C/min and held for 5mins. The total elution time was 47.5 mints. The bioactive compounds of *Platycerium superbum* extract were identified by comparing their retention indices and patterns of mass spectra with reference to NIST library.

2.8 Statistical analysis

All samples were performed in three independent experiments and the result of the quantitative phytochemical screening was expressed as mean $\pm$ standard deviation (SD).

The result of the antimicrobial screening of the plant extract in comparison with standard antibiotics were analysed using Analysis of variance (ANOVA) as done by MINITAB statistical software at 95% confidence level to indicate the level of significance between the inhibition zones.

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3. Results

3.1 Qualitative and Quantitative Phytochemical Analysis of P. Superbum Extract

From the result of the qualitative and quantitative phytochemical screening of the extract, it was deduced that the extract is rich in important phytochemical compounds that have reportedly been of vital biochemical values. It was observed that the quantity of flavonoid was the highest while phytobiotin was lowest in quantity. No reducing sugar was found in the extract as at the time of analysis.

Table 1: Qualitative/Quantitative Phytochemical Analysis of *P. Superbum* Extract

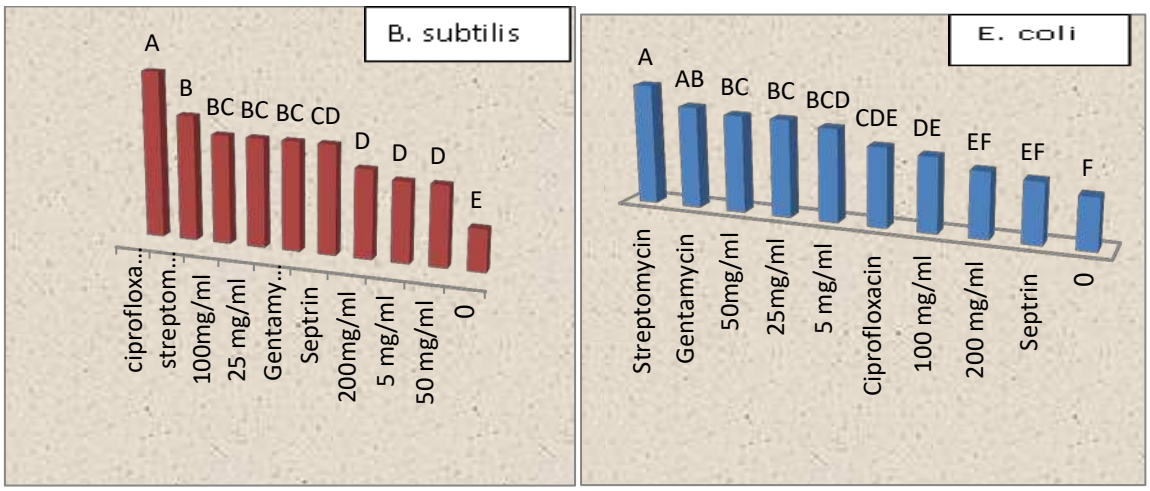
S/N	Phytochemicals	Present/Absent in the extract	Quantity (mg/g)
1	Alkaloids	+	2.50 ±0.014
2	Saponin	++	2.84 ± 0.014
3	Glycoside	+	2.17 ± 0.007
4	Reducing sugar	_	-
5	Tannin	+	1.12 ± 0.014
6	Flavonoid	++	6.69± 0.007
7	Protein	++	2.16 ± 0.057
8	Steroid	+	-
9	Terpenoid	+	0.66 ± 0.014
10	Oxalate	+	1.12 ± 0.034
12	Anthraquinone	+	-
13	Phytobiotin	+	-

+ =present - =absent

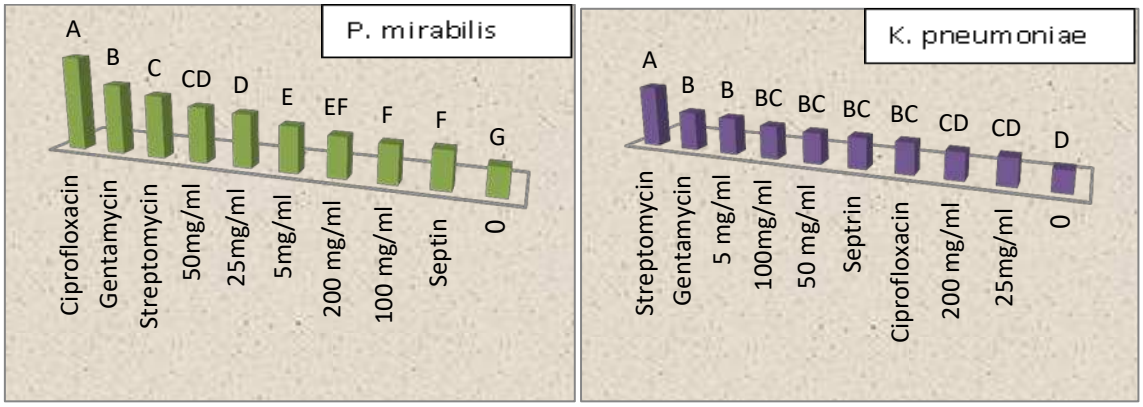
From the figures presented below, the antimicrobial screening investigated at different extract concentrations in comparison with standard antibiotics revealed that *P. Superbum* extract at 25mg/ml and 100mg/ml against *Bacillus subtilis* had the same potency with Septrin and Gentamycin. The extract against E. coli revealed that the extract at 25mg/ml and 50mg/ml were significantly the same in potency with the test drugs. The extract concentrations from 50mg/ml to the least was more active than Septrin against *P. mirabilis* and all the concentrations of the extract had significantly same potency with Ciprofloxacin, Septrin and Gentamycin against *K. pneumonia. P. superbum* extract at 50mg/ml was discovered to show significantly the same activity with Ciproflocacin against *P. aeruginosa* while the extract at 25mg/ml was found to have the higher activity than the test drugs against *S. aureus.*

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(a) **(b)**



(c) **(d)**



(e) **(f)**

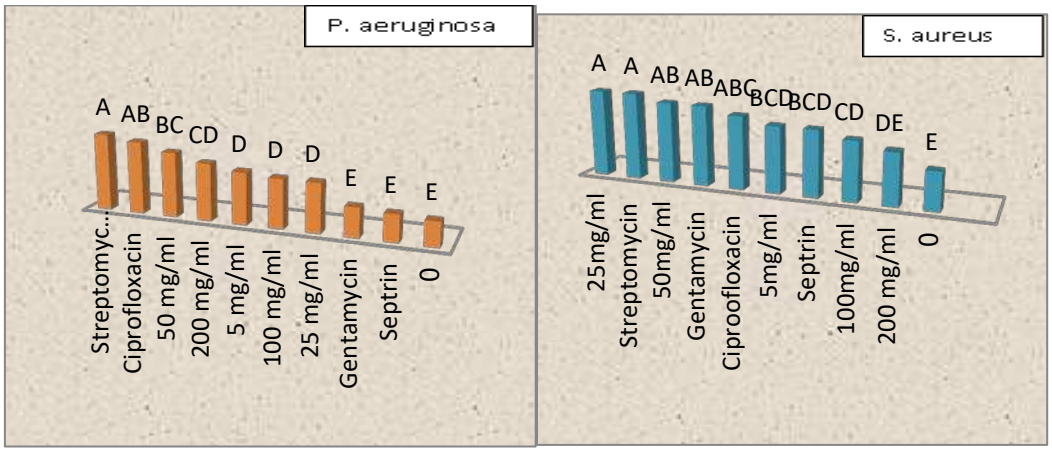


Figure 3.1(a-f): The Antimicrobial Activity of *P. Superbum* methanolic extract against test bacteria strains in comparison with standard drugs: **(a)** *S. subtilis* **(b)** *E. coli* **(c)** *P. mirabilis* **(d)** *K. pneumonia* **(e)** *P. aeruginosa* **(f)** *S. aureus.*

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From the result of the Minimum Inhibitory Concentration of the extract against the test bacteria, it was observed that the extract had the lowest MIC against the activity of *K. pnuemoniae* and *P. mirabilis* while it had the highest MIC against the activity of *P. aeruginosa.*

Table 2: The Minimum Inhibitory Concentration of *P. superbum* extract against the test bacteria strains

Microrganism	Organism ID	MIC (mg/ml)
B. subtilis	ATCC 6051	35
E. coli	ATCC 25922	25
K. pneumonia	ATCC 13883	5
P. aeruginosa	ATCC 27853	50
P. mirabilis	ATCC 29906	5
S. aureus	MRSA	25

The table below shows the obtained GC-MS result for the plant extract and this reveals some essential chemical compounds like Silanediol, Cyclohexanecarboxylic acid, Methyl ester, Cyclohexacarboxylic acid, Bicyclo [3.1.1] Heptane, Bicyclo [5.2.0] nonane, Methyl palmitate and 7 – Octadecenoic acid. The compound with the highest percentage area was Cyclo-hexane carboxylic acid with 61.2% while Silanediol, dimethyl has the lowest percentage area of 2.24.

Table 3: GC-MS Analysis of *P. Superbum* extract revealing the bioactive components

S/N	RETENTION TIME	AREA %	NAME OF COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT
1.	5.22	2.24	Silanediol, dimethy-tert-butyldimethylsiyl	H_40_2Si	64.1
2	15.79	6.12	Cyclohexane carboxylic acid,methyl ester	$C_{10}H_{18}O_2$	172.22
3	19.48	61.21	Cyclohexanecarboxylic acid	$C_7H_{12}O$	128.1
4	38.66	3.42	Bicyclo(3.1.1)heptanes	C_7H_{12}	96.1
5	39.05	4.73	Bicyclo(5.2.0) Nonane, Ethanol	C_9H_{16}	124.2
6	39.42	14.59	Methyl palmitate	$C_{17}H_{34}O_2$	270.5
7	40.56	7.69	7-Octadecenoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5

4.1 Discussion and Conclusion

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The Qualitative phytochemical analysis of *P.superbum* methanolic extract as shown in the table above revealed the presence of phytocompounds like Saponin, Tannins, Alkaloids, Flavonoid, Protein, Terpenoid, Oxalate, Glycoside, Anthroquinine, and Phytobiotin which are known to be biologically active. These secondary metabolites exert antimicrobial activity through different mechanisms. For instance, alkaloids have toxicity against cells of foreign organisms and this aids the activity of the compound being potent against human cancer cell lines [22]. It is also used widely in the production of powerful pain killer medications [23]. Reference [24] reviewed the activity of saponin in managing inflammation. Flavonoid has been reported to exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, anti- allergic cytostatic and anti-oxidant [25]. Reference [26] reported that tannins are important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery [27]. The presence of these important phytochemicals and others probably is responsible for the activity of the plant extract against a wide range of test pathogenic bacteria strains. Ethnomedicinally, young leaves of *P. superbum* have been prescribed as a common antiulcer remedy [5], miscarriage prevention [6] and treatment of oedema coughs and hypertension [7]. The antibacterial potency of methanol extract of *P.superbum* using agar dilution method has been reported by [28]. According to the obtained result of the comparative study of the antimicrobial activity of P. superbum extract and the standard drugs, it could be deduced that the extract showed remarkable activity against the test microbes even competing well and better in some cases than certain test drugs. This is an evidence that the plant extract as a source of vital phytochemical compounds have therapeutic potentials against some pathogenic conditions.

Most of the bioactive compounds identified in P. superbum extract by GC-MS have reportedly been known to be of vital biological usefulness. For example, Cyclo-hexane carboxylic acid having the highest percentage is used for drug, industrial production of Adipic acid and caprolactam as well as precursor to nylon [29]. This suggests the possible usefulness of the plant for the industrial production of nylon. Methyl-palmitate has been reported to have anti inflammatory and anti fibrotic effect. 7-Octadecenoic acid is another compound which is used commercially in the preparation of oleates and lotion and as a pharmaceutical solvent [30]. Cyclo-hexane carboxylic acid methyl ester is said to be used in making drugs to treat throat disorder anti asthmatic agent, bronchiodialator, expectorant and also used in making drugs for genital disorder urinary tract bladder and kidney disorder [30]. Bicyclo (5.2.0) nonane used in making drugs for genital or sexual disorder, urinary tract, prostate, bladder and kidney [31] .Silanediol, dimethyl is reportedly successful in the treatment of dermatological disorder, acne immunological disorder and viral infection [32]. With this extract being source to these important therapeutic and industrial compounds, it explains the potency of the extract against the activity of pathogenic microbes like *E. Coli*, *K. Pneumonia* and *P aeruginosa* which are responsible for disease conditions that the compounds have been known to show activity against. It is however concluded that further isolation and purification of these bioactive compounds will enhance their antimicrobial properties and create an avenue for the detection of a possible novel type of any of the identified bioactive compounds. Although, this study is limited in that the particular active principle was not isolated and identified.

5. Recommendation

It is therefore recommended that further research and identification on the particular isolated active ingredient

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that is responsible for the antimicrobial activity of P. superbum extract against the test microorganisms should be conducted.

References

[1]. K. A. Hammer, C.F. Carson and T.V. Riley. "Antimicrobial activity of essential oils and other plant extracts'. Journal of applied microbiology. Vol. 86 (6), pp.985-994, 1995.

[2]. I.N. Ibeh. General and Reproduction Toxicology: united press, Nigeria, 1998, pp. 68.

[3]. W. H. O. Resolution-promotion and Development of training and Research in Traditional Medicine: WHO document, 1997, no.30-49.

[4]. A.G. Femi-Adepoju, A.O. Adepoju and A.T. Ogunkunle. "Assessment of fungicidal and fungistatic properties of some Nigerian moss plants". Global J. Biol.Agric.Health.Sci., Vol.3(1), pp.126-131, 2014

[5]. R. W. Pemberton. "The common staghorn fern, Platycerium bifurcatum, naturalises in southern Florida". American Fern Journal, 93(4), pp. 203-206, 2003.

[6]. I. N. Flora and C.O. Ubah. In Hand book on Herbs and your Health, 2nd ed., SNAP Publishers, Nigeria, 2006, pp.10.

[7]. J. K. Mensah, C. Odunbaku, and O. Sonibare. "Medicinal Herbs used for managing some ailments among Esan people of Edo State, Nigeria". Pakistan Journal of Nutrition, Vol. 6, pp. 490-497, 2006.

[8]. E. O. Omeje, M. U. Adikwu, C. O. Esimone, W. O. Obonga, G. B. Okide and O.C. Eberendu. " Characterisation of Polysaccharides from the fern Platycerium bifurcatum with expected biological activity". Journal of Phytomedicine and Therapeutics, Vol. 12, pp. 66-75, 2007.

[9]. A. G. Femi-Adepoju. "Ethnobotanical Studies of Some Selected Ferns" Ph.D, University of Ilorin, Ilorin, Nigeria, 2017.

[10]. E. A. Sofowora. Medicinal plants and traditional medicine in West African.Second Edition, John Willey and son Ltd. New York, 1993, pp. 289.

[11]. D. E. Okwu and O. Okeke. "Phytochemical screening and mineral composition of chewing sticks in south Eastern Nigeria". Global Journal of pure and Applied science, Vol. 9(2), pp. 235 -238, 2003.

[12]. G. Padmaja. "Evaluation of techniques to reduce assayable tannin and cyanide in cassava leaves". J Agric. Food Chem., Vol. 37, pp 712-716, 1989.

[13]. A. Kale, S. Gaikwad and K. Mundhe. "Quantification of phenolics and flavonoids by spectrophotometer from Juglans regia". Int J Pharm Biol Sci., Vol. 1, pp 1-4, 2010.

[14]. H. Makkar, P. Siddhuraju and K. Becker. Plant secondary metabolites. Humana Press Incorporation, Totowa, NJ, USA. 2007, pp 129.

[15]. D. Singh, B. Srivastva and A. Sahu. (2004). "Spectrophotometric determination of Rauwolfia alkaloids, estimation of reserpine in pharmaceuticals". Anal Sci., Vol 20, pp 571-573, 2004.

[16]. C.S. Ezeonu and C.M. Ejikeme. "Qualitatative and Quantitative Determination of Phytochemical contents of Indigenous Nigerian Softwoods". Hindawi, Vol. 2016, 9 pages. 2016.

[17]. M. Strickland, M.E. Johnson and J.R Broadbent. "Qualitative and quantitative analysis of proteins and peptides in milk products by capillary electrophoresis". Electrophoresis, Vol. 22(8), pp1510-1517, 2001

[18]. Z. Ladan, J.O. Amupitan, O.A. Oyewale, R.G. Ayo, E. Temple and E.O. Ladan. "Phytochemical

Electronic copy available at: https://ssrn.com/abstract=4465009

screening of the leaf extract of Hyptis spicigera plant". Afr. J. Pure Appl. Chem., Vol. 8(5), pp 83-88, 2014.

[19]. O.N. Irobi, M. Moon-Yong, W.A. Anderson and S.A. Daramola. "Antimicrobial activity of bark extracts of Bridelia ferruginea (Euphorbiaceae)". J. Ethnopharmacol., Vol. 43, pp185-190, 1994.

[20]. S.E. Abah and L.O. Egwari. "Method of antimicrobial susceptibility testing of plant extract". African Journal of Basic & Applied Science, Vol. 3(5), pp 205-220, 2010.

[21]. NCCLS (National Committee for Clinical Laboratory Standards), Approved Standard. NCCLS M38-A. Villanova, PA, USA, 2002.

[22]. T. Nobori, K. Miurak, D. Wu, L. Takabayashik and D. Carson. "Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers". Nature, Vol. 368 (6473), pp 753-756, 1994.

[23]. P.C. Kam and S. Liew. "Traditional Chinese herbal medical and anaesthesia". Anaesthesia, Vol. 57(11), pp1083-1089, 2002.

[24]. M.J. Just, M.C. Recio, R.M. Giner, M.J. Cueller, S. Manez, A.R. Bilia, and J.L. Rios. "Anti-inflammatory activity of unusual lupine saponins from Bupleurum fruticescens". Planta Medica, Vol. 64, pp 404-407, 1998.

[25]. P. Hodek, P. Trefil and M. Stiborova. "Flavonoid-potent and versatile Biologygically active compounds interacting with cytochrome p450". Chemico-Biol. Interm., Vol. 139(1), 1-21, 2000.

[26]. J. Parekh and S. Chanda. "In vitro antibacterial activity of crude methanol extract of Woodfordia fruticosa Kurz flower (Lythacease)". Braz. J. Microbiol., Vol. 38, pp 2, 2007.

[27]. S. Dharmananda. Gallnuts and the user of tannins in Chinese medicine, In: Proceeding of institution for traditional medicine, Portland, Oregon, 2003

[28]. O.O. Ojo, A.O. Ajayi, and I.I. Anibijuwon. " Antibacterial potency of methanol extracts of lower plants". Journal of Zhejiang University of Science B, Vol. 8(3): 189–191, 2007

[29]. B.S. Michael. Organic Chemistry: An- Acid-Base Approach, Taylor and Francis, 2011, pp 302.

[30]. N.S. Thomas. Organic Chemistry, University Science Books, 2006, pp 99.

[31]. R.C. Emmaunelle, J.M. Martin and C. Florine. Chemical Review 2016, Vol. 116(19), 11654-11684, 2016

Electronic copy available at: https://ssrn.com/abstract=4465009