Phytochemical Contents and *In Vitro* Pathogenic Microbial Growth Inhibitory Activities of *Acanthus montanus* Root and Leaf Extracts

G. I. Ndukwe*, O. F. Okoronkwo and I. R. Jack

Department of Chemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria
*Corresponding author. E-mail address: gloria.ndukwe@ust.edu.ng
Mobile phone: +2348033404528

Abstract

The study was directed at extraction, bioactivity analysis and ascertaining phytochemical composition of the leaf and root extracts of Acanthus montanus. Acanthus montanus (Acanthaceae family) is a shrub that is popularly grown in tropical countries of the world. The plant materials were harvested from Choba community in Rivers State, Nigeria. Extracts of Acanthus montanus (28.3, 19.45 and 25.5 g of n-hexane, ethylacetate and ethanol leaf extracts; and 3.45, 11.32 and 12.44 g of n-hexane, ethylacetate and ethanol root extracts respectively) were obtained through sequential maceration and were separately subjected to in vitro bioassay against a wide range of pathogenic microbes using the agar well diffusion method. Fifteen microorganisms were employed in the investigation, eight bacteria which included Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Shigella sonniea, Shigella dysentariae, Escherichia coli, Staphylococcus aureus, Staphylococcus pyogenes and seven fungi which included Candida albicans, Sacharomyces cerevisiae, Rhizopus eligastus, Aspergillus flavus, Aspergillus fumigates, Fusa equisetti and Aspergillus niger. The crude extracts exhibited high microbial growth inhibitory activities with zones of inhibition which ranged between 14 and 34 mm, though there was moderate (19 mm zone of inhibition), or no activity observed for the n-hexane leaf and root extracts. All test organisms were sensitive to the extracts. Standard analytical methods and Gas Chromatography - Flame Ionization Detector (GC-FID) were used for analysis and quantification of the phytochemicals present in the extracts. Major phytochemical contents of the leaf and root extracts included flavonoids (86% - 52.66%), alkaloids (10.66% - 3.25%) and other phenolic compounds (20.52% - 6.38%). In conclusion, the leaf and root extracts of Acanthus montanus are broad-spectrum antimicrobial agents also rich in flavonoids.

Keywords: Antibacterial, antifungal, phytochemical, *Acanthus montanus*, broad-spectrum, antimicrobial

INTRODUCTION

The reliance on plant based medicinal products has become very noticeable, especially in underdeveloped countries of the world. The practice and usage of these plants depend on localities and traditions where some are even used for magical purposes[1]. The high cost of orthodox drugs from

developed countries has provided no choice for the poverty ridden citizens of Africa to rely on traditional medicines for their healthcare needs especially when there are life threatening attendant side effects on these orthodox drugs[2].

It is worth noting that plant toxicity in traditional preparations cannot be overlooked. It is on record that there are some plants that contain phytocompounds that are toxic to human health, hence caution is advised especially when there is no scientific evidence exonerating the said plants from human toxicity. Some plant products have been proven to be poisonous to vital organs of the human body such as the heart, the kidney and the liver[3].

The resistance posed by microorganisms to some of the orthodox drugs has engineered more research in areas of bioactivity and phytochemical analysis of plants that have been overwhelmingly used in traditional medicines, knowing that information evaluating most of these plants are scarce[4].

Acanthus montanus (nees) T. Anderson (Acanthaceae) is commonly called mountain thistle. The uses of *Acanthus montanus* root and leaves in traditional medicine include treatment of skin infections, respiratory tract infections, venereal diseases, arthritis, rheumatism, spontaneous abortion and gastritis[5].

MATERIALS AND METHODS

Extraction

Fresh leaves and roots of *A. Montanus* were collected from Choba, in Obio-Akpor Local Government Area of Rivers State, Nigeria and were identified by Dr Chimezie Ekeke of the herbarium of the University of Port Harcourt, Rivers State, Nigeria.

Some of the harvested leaves and roots were subjected to hydrodistillation for the purpose of extracting essential oil[6-8]. Briefly, fresh leaves of *A. Montanus* were chopped into small pieces and weighed. Chopped leaves (200 g) were loaded into a round bottom flask seated on a heating mantle and distilled water poured into the flask. A Clevenger apparatus was tightly fitted on the flask and the plant material was heated for 2 hours and no oil was collected.

Fresh leaves and roots were separately washed, air dried and pulverized using an electric blending machine. Non-volatile components of the plant were extracted via

maceration[9]. The pulverized leaves and roots (1.5 kg each) were macerated separately in aspirator bottles with *n*-hexane at room temperature for 72 hours. The extract was filtered into a conical flask using a glass funnel properly fixed with cotton wool and filter paper. The procedure was carried out three more times to obtain the *n*-hexane extract. The extracts were concentrated using a rotary evaporator at 45 ℃. The residue was still subjected to further extraction with ethylacetate and ethanol respectively using the same procedure. The dried extracts were weighed, and their yields recorded.

Biological Assay

The extracts were screened for antimicrobial activity against some disease-causing microbes using standard procedures where agar well diffusion method was employed[10, 11]. Organisms used for this work were previously characterized bacterial and fungal isolates from the Department of Microbiology, Federal Medical Center, Owerri, Imo State. The organisms are eight bacteria which included *Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Shigella sonniea, Shigella dysentariae, Escherichia coli, Staphylococcus aureus, Staphylococcus pyogenes* and seven fungi which are *Candida albicans, Sacharomyces cerevisiae, Rhizopus eligastus, Aspergillus flavus, Aspergillus fumigates, Fusa equisetti and Aspergillus niger*.

The bacterial isolates were reconfirmed using selective media (mannitol salt agar and eosine methylene blue agar) for purity and viability. They were incubated at 37 ℃ for 24 hours. Molten agar was introduced into Petri dishes and allowed to solidify. The isolates were introduced unto the sterile petri dishes containing agar and sterile spreader was used to evenly spread the isolates. A cup borer was used to make 5 mm diameter holes in each dish uniformly and extracts (200 mg/ml) were introduced into the holes aseptically and allowed to stand for one hour for pre-diffusion. Also previously prepared standard antibiotic (cefuroxime, 30 µg/ml) was aseptically introduced into one of the holes in the petri dishes. The petri dishes were labelled and incubated at 37 ℃ for 24 hours. After 24 hours, zones of inhibition were measured using a ruler and recorded in millimeters. Zones of inhibition less than 6 mm were considered insignificant. The agar well diffusion method used here tested the effectiveness of the extracts against specific organisms. Highly effective antibacterial agents will produce a wide range of no

bacterial growth while an ineffective antibacterial agent will show no change in the surrounding bacterial concentration at all. This method is used to determine the best antibacterial agent to use against a new or drug resistant pathogen.

For the antifungal assay, potato dextrose agar medium was employed. The organisms were collected from the cultures previously incubated for 48 hours and were aseptically introduced into sterile petri dishes. Molten agar was also aseptically introduced into the petri dishes and the content allowed to solidify. A cup borer was used to make uniform holes (5 mm) on the petri dishes. The extracts (200 mg/ml) and standard antifungal (30 µg/ml) were aseptically introduced into the holes and allowed to stand for one hour for pre-diffusion and then labeled and incubated at 37 ℃ for 48 hours. After 48 hours, zones of inhibition were measured and recorded in millimeters.

Phytochemical Assay

The analysis of phytochemicals was performed with a BUCK M910 gas chromatography equipped with a flame ionization detector using standard methods[12]. The injector temperature of the GC was 280 ℃ with splitless injection of 2 µl of sample and linear velocity of 30 cm/s. Helium gas was the carrier gas with a flow rate of 40 ml/min. The oven operated initially at 200 ℃, it was heated to 330 ℃ at the rate of 3 ℃ min^{-1} and was kept at this temperature for 5 min. The detector operated at a temperature of 320 ℃. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals; and expressed in µg/g.

RESULTS AND DISCUSSIONS

Leaf and Root Extracts

No essential oil was obtained from the extraction done on the leaves of *Acanthus montanus* through hydrodistillation. It is also evidence that in the traditional use of the plant, there was no mention of its use in oil form in literature.

Sequential extraction carried out on the dried and pulverized leaves and roots of the plant via maceration, resulted in extracts with different yields. The *n*-hexane extract had the highest percentage yield for the leaves with 1.89%, followed by the ethanol extract with 1.70%. The ethylacetate extract had the least yield with 1.30%. However, considering the extracts from the roots, the ethanol extract of the roots had the highest yield of 0.83%,

followed by ethylacetate extract (0.75%). Unlike the leaves, *n*-hexane extract of the roots had the least yield (0.32%), with these results it is clear that more plant materials were needed to obtain reasonable extracts from the roots for experimental and other purposes. The total yield of extracts from the leaves and the roots were calculated to be 4.89% and 1.9% respectively.

In Vitro Pathogenic Microbial Growth Inhibitory Activities of Acanthus montanus Extracts

n-Hexane extracts of both the leaf and root did not inhibit the growth of all the eight pathogenic bacteria assessed (Table 1). Ethylacetate extracts exhibited good activities with zones of inhibition range of 14 – 31 mm and 19 – 33 mm for the leaf and root respectively which were comparable with the standard drug. Though, *E. coli* had the least sensitivity to ethylacetate extracts with zones of inhibition of 14 and 19 mm for leaf and root respectively. None of the tested bacteria was resistant to ethanol extracts, they all showed high sensitivity to the extracts (23 - 29 mm and 19 – 31 mm zones of inhibition for leaf and root respectively.

There were moderate pathogenic fungal growth inhibitory activities observed for the *n*-hexane leaf extracts against *S. cerevisae* (19 mm), *R. oligastus* (19 mm) and *F. equisetti* (9 mm) only; the other fungi were all resistant to *n*-hexane extracts (Table 2). However, ethylacelate and ethanol extracts exhibited outstanding activities against all test fungi with higher zones of inhibition than those of the standard drug. In terms of sensitivity of the test fungi, *F. equisetti* was less sensitive to ethylacetate extracts (14 mm zone of inhibition).

The results of this study are similar to the results of other researchers who reported antibacterial activity of *Acanthus montanus* leaf extract against *staphylococcus species*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*[13, 14]. Consequently, with the pathogenic microbial growth inhibitory activities of *Acanthus montanus* against these test organisms which are the underlining causes of so many diseases, the traditional use of the leaves and roots of *Acanthus montanus* for the treatment of several diseases can be authenticated.

Table 1: Antibacterial Activities of *A. montanus* Leaf and Root Extracts

Organism	Zone of Inhibition (mm)						
	Leaf Extracts			Root Extracts			Standard drug (Cefuroxime)
	EtOH	EtOAc	*n*-Hex	EtOH	EtOAc	*n*-Hex	
S. typhi	24±2	25±0	0	29±2	32±0	0	30±1
S. paratyphi	24±0	25±0	0	31±1	29±2	0	30±0
P. aeroginosa	29±2	25±0	0	19±0	30±0	0	31±0
S. sonniea	29±1	24±1	0	29±1	31±1	0	28±1
S. dysenteriae	23±0	26±0	0	30±0	33±1	0	30±1
E. coli	20±1	14±1	0	29±1	19±0	0	28±1
Staph. aureus	29±1	29±2	0	25±2	33±1	0	32±0
S. pyogenes	28±0	31±1	0	26±1	32±2	0	35±0

Table 2: Antifungal Activities of *A. montanus* Leaf and Root Extracts

Organism	Zone of Inhibition (mm)						
	Leaf Extracts			Root Extracts			Standard drug (Ketoconazole)
	EtOH	EtOAc	*n*-Hex	EtOH	EtOAc	*n*-Hex	
C. albicans	14±0	20±1	0	24±0	16±1	0	30±0
S. cerevisae	33±1	30±1	19±1	32±0	31±1	0	28±0
R. oligastus	34±0	33±0	19±1	29±2	32±0	0	26±0
A. flavus	30±0	32±0	0	24±0	32±0	0	30±1
A. fumigates	31±1	30±0	0	25±0	31±1	0	16±0
F. equisetti	28±0	14±1	9±0	21±1	14±0	0	20±1
A. niger	33±1	31±0	0	29±1	30±2	0	24±1

Phytochemical Contents of Acantus montanus Extracts

The leaf and root extracts contained flavonoids, other phenolic compounds,

alkaloids, and steroids; tannins were absent in the leaf extracts while saponins were absent in the root extracts (Tables 3 and 4). Analysis of the extracts indicated very high percentages of flavonoids (71.25 – 86% and 52.66 – 85.47% for leaf and root extracts respectively). Some of the flavonoids detected were flavon-3-ol, kaempferol, anthocyanins, rutin and proanthocyanidins. The results of this work are corroborated with other reported works which indicated enormous polyphenols and minimal presence of tannins in *Acantus montanus*. Other phytochemicals reported for *Acantus montanus* include steroids, alkaloids, saponins and glycosides[15]. Benefits and activities of these phytochemicals have been widely reported and outlined as follows.

Flavonoids are associated with activities such as anti-inflammatory, anti-pyretic, hypoglycemic, antifungal, antibacterial and antitumor. The protective activities of flavonoids are attributed to their ability to transfer electrons to free radicals. Proanthocyanidins and anthocyanidins occur naturally throughout the plant kingdom especially in fruits and vegetables. They are considered condensed tannins[16]. They are known for several activities which endeared proanthocyanidins to enter the natural product market as dietary supplement. Antioxidant property of these components has made them usable in the prevention of many degenerative diseases[17]. Other activities exhibited by these phytochemicals include anticancer, anti-infection, cardio protective, wound healing, antimicrobial, anti-inflammatory, anti-viral, anti-hyperglycemia and anti-allergic[18]. Naringenin, one of the flavonoids, also shows some of the activities which are peculiar to other flavonoids. They are abundantly found in citrus fruits and pigmented vegetables and have been beneficial in the management of cancer, liver injury, cardiovascular diseases, oxidative stress and osteoporosis. It has been recorded to also have anti-diabetic, anti-inflammatory, anti-lipidemia, antioxidant and anti-depressant properties[19]. Flavanones have been proven to possess antioxidant, anti-hyperlipidemia and anti-inflammatory properties while flavones are known to have anti-microbial and anti-fungal properties. They bind to the protein serum albumin which makes its transport in the human plasma easy[20]. Rutin and kaempferol are other flavonoids that are also abundant in the

plant kingdom. Rutin has activities which include antioxidant, cardio protective, anti-cancer, cytoprotective and vasoprotective[21]. Kaempferol possesses anti-diabetic, anti-cancer and anti-inflammatory properties[22]. Catachin and epicatechin are flavonoids which are also available in *Acanthus montanus*. Catechin is known to possess health benefits such as antioxidant, anti-diabetic, cardioprotective, anti-obesity, anti-cancer and vasoprotective while epicatechin possess activities which includes cardioprotective, antidiabetic, antioxidant and anti-carcinogenic. Catechin is abundantly endowed in cocoa while green tea is rich in epicatechin[23].

Tannins are polyphenols which are water soluble and can be found in many plants. They also possess some biological activities which include anti-inflammatory and antioxidant properties. It is also known that this component decreases the value of nutritional intake due to its ability to decrease feed intake and efficiency in experimental animals[24].

Sapogenins are the non-sugar portions of the group of natural products called saponins and they occur naturally in plants. They possess good biological activities as members of the saponins, which may include anti-carcinogenic, anti-hyperlipidemia, anti-viral, anti-bacterial and anti-fungal. However, sapogenins have some harmful properties which include their haemolytic and cytotoxic activities which have been well recorded. They can also induce hypoglycemia by impairing the digestion of proteins, the absorption of vitamins and minerals in the small intestines[25].

Alkaloids are another group of phytochemicals that enormously occur in plants. Their analgesic uses with the discovery of morphine and its derivatives are known. However, the presence of quinine, sparteine and ribalinidine in the leaves and roots of *Acanthus montanus*, must have been responsible for some outstanding activities in their ethnobotanical uses. These alkaloids have been documented to be effective in the management of cancer, malaria, microbes and protozoans. Ribalinidine, which is a strong antioxidant, has been reported for its strong radical scavenging property. Quinine has been used over a long time in the treatment of malaria and as a muscle relaxant.

However, it has a non-narcotic analgesic effect unlike some other alkaloids which are narcotic analgesic such as morphine, codeine, heroine and cocaine[26].

Oxalates which are classified as anti-nutrients were present in the leaves and roots of *Acanthus Montanus* in very small quantities which may be responsible for the unique safety of the plant according to toxicological studies done on the plant[27]. They interfere with the absorption of some nutrients such as calcium by producing calcium oxalate crystals which eventually form kidney stones. This ability of oxalates to bind with essential micronutrients and prevents their proper absorption may cause health defects such as rashes, headache, scurvy and some nutritional deficiencies. The relative safety of *Acanthus montanus* is brought to bear with a very low concentration of oxalate as the only antinutrient discovered in the extracts of the plant[28].

Steroids are found in animals and plants. However, plant steroids which are secondary metabolites have been documented to possess several biological activities. These activities include anti-inflammatory, anti-bacterial, antitumor, anti-fungal, antitussive, hepatoprotective, cardiotonic, antipyretic, anti-allergic, analgesic and anti-arthritis[29].

Table 2: Phytochemical Contents of *Acanthus montanus* Leaf Extracts

Group	**Phytochemical**	**EtOH extract (µg/g)**	**EtOAc extract (µg/g)**	***n*-hex extract (µg/g)**
Flavonoids	Proanthocyanidins	14.8753	-	0.1822
	Anthocyanin	20.1829	10.5186	9.9326
	Naringenin	9.2892	7.9053	4.9840
	Flavonones	7.6818	6.5762	8.2694
	Epicatechin	-	10.7381	-
	Flavone	5.4690	5.4788	-
	Catechin	6.1248	6.2928	-
	Kaempferol	33.3588	20.7971	42.5210
	Naringin	10.4207	7.5953	-
	Flavon-3-0l	18.9348	16.3985	-
	Rutin	-	-	0.1863
Total flavonoids (µg/g)		**126.3373**	**84.7054**	**66.0755**

Percentage composition of flavonoids (%)		**86**	**80.24**	**71.25**
Alkaloids	Ribalinidine	3.9333	3.9338	4.8874
	Quinine	-	-	5.0014
	Sparteine	0.8342	0.7203	-
Total Alkaloids (µg/g)		**4.7675**	**4.6541**	**9.8888**
Percentage compositions of Alkaloids (%)		**3.25**	**4.41**	**10.66**
Saponins	Sapogenin	-	-	2.0257
Total Saponins (µg/g)		**0**	**0**	**2.0257**
Percentage compositions of Saponins (%)		**0**	**0**	**2.18**
Other Phenolics	Phenol	6.1863	6.1876	4.3028
	Resveratrol	3.1798	4.0840	6.4668
Total Other Phenolics (µg/g)		**9.3661**	**10.2716**	**10.7696**
Percentage compositions of Other Phenolics (%)		**6.38**	**9.73**	**11.61**
Anti-nutrients	Phytate	-	-	-
	Oxalate	1.2970	0.8008	2.5635
Total Anti-nutrients (µg/g)		**1.2970**	**0.8008**	**2.5635**
Percentage compositions of Anti-nutrients (%)		**0.88**	**0.76**	**2.76**
Tannins	Tannins	-	-	-
Steroids	Steroids	5.1225	5.1282	1.4205
Total Steroids (µg/g)		**5.1225**	**5.1282**	**1.4205**
Percentage compositions of Steroids (%)		**3.49**	**4.86**	**1.53**

Table 3: Phytochemical Contents of *Acanthus montanus* Root Extracts

Group	Phytochemical	EtOH extract (µg/g)	EtOAc extract (µg/g)	*n*-hex extract (µg/g)
Flavonoids	Proanthocyamidins	15.8176	8.9404	-
	Anthocyanins	11.4905	7.6228	8.0681
	Naringenin	8.1907	8.1795	7.6226
	Flavonones	11.3191	11.3092	-
	Epicatecinhin	12.9953	12.5281	11.7306
	Flavones	5.4690	5.4788	11.2245
	Catechin	5.5023	5.4915	4.9036
	Kaempferol	17.0354	13.2651	4.9418
	Naringin	8.3065	8.2995	-
	Flavon-3-ol	17.6544	17.6809	-
	Rutin	-	-	7.5327
Total Flavonoids (µg/g)		**113.7808**	**98.7958**	**56.0239**
Percentage compositions of Flavonoids (%)		**85.47**	**83.16**	**52.66**
Alkaloids	Ribalinidine	3.9333	3.9338	-
	Quinine	-	-	5.9213
	Sparteine	0.8342	0.7203	-
Total alkaloids (µg/g)		**4.7675**	**4.6541**	**5.9213**
Percentage composition of Alkaloids (%)		**3.58**	**3.92**	**5.57**
Saponins	Sapogenin	-	-	-
Other phenolics	Phenol	6.1863	6.1876	21.8288
	Resveratrol	4.0939	4.0840	-
Total Other Phenolics (µg/g)		**10.2802**	**10.2716**	**21.8288**
Percentage composition of Other Phenolics (%)		**7.72**	**8.65**	**20.52**
Anti-nutrients	Phytate	-	-	-
	Oxalate	0.8005	0.8006	-
Total Anti-nutrients (µg/g)		**0.8005**	**0.8006**	**0**

Percentage compositions of Anti-nutrients (%)		**0.60**	**0.67**	**0**
Tannins	Tannins	-	-	12.1806
Total Tannins (µg/g)		**0**	**0**	**12.1806**
Percentage compositions of Tannins (%)		**0**	**0**	**11.45**
Steroids	Steroids	4.2729	4.2776	10.4423
Total Steroids (µg/g)		**4.2729**	**4.2776**	**10.4423**
Percentage compositions of Steroids (%)		**3.20**	**3.60**	**9.81**

CONCLUSION

The phytochemical contents of the leaf and root extracts of *Acanthus montanus* are clear indications of its numerous uses in traditional medicine. The extracts of a plant so loaded with many flavonoids, other phenolic compounds, steroids and alkaloids, must be a plant of interest for many other research. Even though its use for the treatment of malaria has not been popularly reported in literature, however the presence of quinine in the *n*-hexane root extract of *Acanthus montanus* is an indication that it may have anti-malarial activity and a non-narcotic muscle relaxant activity which are associated with quinine. The extracts exhibited high broad-spectrum *in vitro* inhibitory activities against the growth of tested pathogenic microbes.

REFERENCES

1. Akerele, O., Medicinal plants and primary healthcare: An agenda for action, *Fitoterapia*, **1988**, 59, 355-363.

2. Okenwa, U. I., Donatus, E. O., Chemical composition and antimicrobial activities of adventitious root sap of Musanga cecropioides, *Der Pharma Chemica*, **2013**, 5*(1)*, 224-228.

3. Melchart, D., Hagar, S. M., Albrecht, S., Teschkes, R., Jingzhang, D., Weidenhammer, W., Herbal

traditional Chinese medicine and suspended liver injury: A prospective study, *World Journal of Hepatology*, **2017**, 9*(29)*, 1141-1157.

4. Akpanabiatu, M. I., Umoh, I. B., Eyong, E. U., Edet, E. E., Uboh, F. E., Influence of Rauwolfia vomitoria root bark extract on cardiac enzymes of normal wistar albino rats: Recent progress in medicinal plants, *Biopharmaceuticals*, **2006**, 14*(13)*, 273-278.

5. Jiofack, I. C., Fokunang, N., Gueje, V., Kemeuze, E., Ethnobotany and phytomedicine of the upper Nyong Valleyforest in Cameroon, *African Journal of pharmacy and pharmacology*, **2009**, 3*(4)* 144-150.

6. Ndukwe, G. I., Ekong, R. E., Odazie, P. G., Baridam, S. S., Chemical profiling and antimicrobial potential of volatile leaf oils isolated from three mangroves growing in Rivers State, Nigeria, *Journal of Chemical Society of Nigeria*, **2022**, 47*(1)*, 094-100.

7. Jack, I. R., Ekong, R. E., Ndukwe, G. I., Phytochemical constituents of dichloromethane fraction and essential oil of *Napoleonaea imperialis* rind, *Journal of Pharmacognosy and Phytochemistry*, **2020**, 9*(5)*, 59-66.

8. Ndukwe, G. I., Okhiku, J. O., Chemical composition and anti-microbial activity of the volatile oils of *Averrhoa carambola* L. (star fruit) grown in Nigeria, *Journal of Chemical Society of Nigeria*, **2018**, 43*(2)*, 141-150.

9. Ndukwe, G. I., Clark, P. D., Jack, I. R., *In vitro* antioxidant and antimicrobial potentials of three extracts of *Amaranthus hybridus* L. leaf and their phytochemicals, *European Chemical Bulletin*, **2020**, 9*(7)*, 164-173.

10. Ndukwe, G. I., Garba, S. Y., Adelakun, E. A., Activity-guided isolation and antimicrobial assay of a flavonol from *Mitracarpus verticillatus* (Schumach. & Thonn.) Vatke, *IOSR Journal of Applied Chemistry*, **2016**, 9*(9)*, 118-131.

11. Ndukwe, G. I., Ojinnaka, C. M., Oyedeji, A. O., Nxasana, N., Apalata, T., Antibacterial activity of the fruit of *Napoleonaea imperialis* P. Beauv, *Journal of Innovation and Research in Health Sciences & Biotechnology*, **2015**, 1*(1)*, 1-11.

12. Ugoeze, K. C., Oluigbo, K. E., Chinko, B. C., Phytochemical and nutraceutical benefits of the GC-FID quantified phyto-components of the aqucous extract of *Azadirachta Indica* leaves, *Journal of Pharm Pharmcol Research*, **2020**, 4*(4)*, 149-163.

13. Ebana, R. U. B., Edit, U. O., Ekanemesang, U. M, Noble, M. K., Phytochemical screening and antimicrobial activity of three medicinal plants against urinary tract infection pathogens, *Asian Journal of Medicine and Health*, **2016**, 1, 1-7.

14. Igwe, O. U., Nnaji, J. C., Chemical characterization and investigation of the Bio-effects of the leaves of *Acanthus montanus* (Acanthaceae) on some selected micro-organisms, *International Journal of Chemtech Research*, **2014**, 6*(14)*, 5554-5561.

15. Joshua, E. P., Ukegbu, Y. C., Nwike, P. C., Ikpe, U., Ezeani, E., Orhonigbe, O. I., Nweje-Anyalowu, C. P., Phytochemical and toxicological studies of methanol and chloroform fractions of *Acanthus Montanus* leaves, *Journal of Biological Sciences*, **2021**, 21*(2)*, 52-58.

16. Wang, Y., Chung S. J., Song, W. O., Chun, O. K., Estimation of daily proanthocyanidin intake and major food sources in the U.S diet, *Journal of Nutrition,* **2011**, 141, 447-454.

17. Han, X., Shen, T., Iou, H., Dietary polyphenols and their biological significance, *International Journal of Molecular Science*, **2007**, 8, 950-988.

18. Rauf, A., Imran, M., Abu-Izneid, T., Ul-Haq, F., Patel, S., Pan, X., Naz, S., Silva, A.S., Saeed, F., Suleria, H. A. R., Proanthocyanidins: A Comprehensive review, *Journal of Biomedicine and Pharmacotherapy*, **2019**, 116*(10)*, 89-99.

19. Patel, K., Singh, G. K., Patel, D. K., A review of pharmacological and analytical aspects of naringenin, *Chinese Journal of Intergrative Medicine*, **2018**, 24, 551-560.

20. Jiang, N., Doseff, A. I., Grotewold, E., Flavones: From biosynthesis to health benefits, *Plants,* **2016**, 5, 27-35.

21. Ganeshpurkar, A., Saluja, A. K., The Pharmacological Potential of runtin, *Saudi Pharmacological Journal*, **2017**, 25, 149-164.

22. Chen, A. Y., Chen, Y. C. A., Review of the dietary flavonoid Kaempterol on human health and cancer chemoprevention, *Food Chemistry*, **2013**, 138, 2099-2107.

23. Praskash, M., Basavaraj, B. V., Murthy, K. C., Biological functions of epicatechin: plant cell to human cell health, *Journal of Functional Foods*, **2019**, 52, 14-24.

24. Ghosh, D., Tannins from foods to combat diseases, *International Journal of Pharmaceutical Research and Review*, **2015**, 4*(5)*, 40-44.

25. Chen, Z., Duan, H., Tong, X., Cytotoxicity, hemolytic toxicity and mechanism of action of pulsatilla saponin D and its Synthetic derivatives, *Journal of Natural Products*, **2017**, 81, 465-474.

26. Alvesde-Almeida, A. C., de-Faria, F. M., Dunder, R. J., Recent trends in Pharmacological activity of alkaloids in animal colitis: potential use for inflammatory bowel disease, *Journal of Evidence-Based Complementary and Alternative Medicine*, **2017**, 6*(3)*, 301-305.

27. Iwueke, A. V., chukwu, E. C., Osuocha, K. U., Onupha, O. U., Toxicological studies on *Acanthus montanus* leaf extracts in male and female albino rats, *African Journal of Biological Sciences*, **2021**, 3*(1)*, 34-51.

28. Popova, A., Mihaylova, D., Antinutrients in plant-based foods: A review, *The Open Biotechnology Journal*, **2019**, 13, 68-76.

29. Govindana, S., Viswanathan, S. B., Vijayasekaran, V. B., Alagappan, A. R., Further studies on the clinical efficacy of solanum xanthocarpum and Solanum trilobatum in bronchial asthma, *Phytotherapy Research*, **2004**, 18, 805-809.