PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *GONGRONEMALATIFOLIUM* (UTAZI) LEAVES

John Ama IBIAM
Department of Science Laboratory Technology,
Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.
amaibiam2002@gmail.com +2348063803459
Nnam Afamefula OKO
Department of Science Laboratory Technology,
Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.
Chukwuemeka Stanley UGAH
National Institute of Construction Technology, Uromi, Edo State, Nigeria

ABSTRACT

The leaves of Gongronema latifolium (Utazi) plant were examined for their Phytochemical and antimicrobial properties. The Phytochemical Screening was carried out using standard procedures on ethanol extract of the leaves sample. The leaves of the plant sample showed the presence of the following phytochemicals such as Alkaloids (2.55±1.47), Flavonoids (2.63±0.46), Saponins (3.60±0.092), Tannins (2.84±0.032), Phenols (1.49±0.04), Oxalate (0.076 mg/100), Terpenoids (0.28±0.078 mg/l) and Cardiac glycosides (0.02±0.00), Phytate (1.44±0.019). Moreover, the plants extract was screened for their antimicrobial activities against Escherichia coli, Staphylococcus aureus, Candida albican, and Aspergillus niger. The results of the antimicrobial activities were observed as the gradient increased. Escherichia coli (22 mm) and Staphylococcus aureus (25 mm) showed the highest susceptibility to the plants extracts at 250 mg. The plants extract showed an antifungal activity against Candidaalbican and Aspergillus niger had (10 mm) at the 250 mg. The Lowest Minimum Concentration (MIC) was observed in the extract against Staphylococcus aureus at 50 mg/ml while the highest MIC was recorded in the extract against Aspergillus niger at 200 mg/ml. These investigation shows that the Gongronema latifolium leaves extract contain bioactive constituents such as Alkaloids, Flavonoids, Saponins, Terpenoids, Phenol, etc. which may account for the antimicrobial activities recorded and its pharmaceutical constituents.

Introduction

The dependency of man on plants for medicine is as old as the creation of the world. The plant kingdom represents a rich store house of organic compounds, many of which have been used for the development of novel agent having good efficiency in various pathological disorders. Plants are the richest source of drugs for traditional medicine, pharmaceutical intermediates and chemical entities for synthetic drugs. The use of plant products as medicine could be traced as far back as the beginning of human civilization. The earliest mentioned medically used plants in Hindu culture are found in "Rigved" which is said to have been written between 4500-1600 B C (Agwu & Chukwu, 2017).

Phytochemicals such as alkaloids, tannins, steroid, glycosides, saponins, flavonoids, anthraquinones and terpenoids are non-nutritive plant chemicals which occur naturally in plants that have protective or disease preventive properties (Dahiru, Obnubiyi & Umaru, 2006). They are non-

essential nutrients, meaning that they are not required by the plants for sustaining life (DanMalam, Abubakar & Katsayal, 2001). It is well known that plant produces these chemicals to protect them but recent research has shown that they can also protect humans against diseases. Some have shown more promise than others in fighting diseases and illness in humans

Gongronema latifolium commonly called "utazi" in Igbo, South Eastern and "arokeke" in Yoruba South Western part of Nigeria, respectively, is an all-season edible plant with soft and pliable stem, belonging to the family of *Asclepiadaceae* (Ugochukwu & Babady, 2003). It is widely used in the West African sub-region for a number of medicinal and nutritional purposes. It is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Chinedu *et al.,* 2013). A range of pharmacological tests have shown promising hypoglycemic activities and also interesting anti-bacterial, antioxidant, anti-inflammatory, protective, anti-plasmodia, anti-asthmatic, anti-sickling, anti-ulcer, analgesic, and anti-pyretic properties of *Gongronemalatifolium* (Oliver-Bever,1986). The leaves of *Gongronema latifolium* are used as vegetables in preparation of soups to which they add a bitter-sweet flavor (Agbo *et al.,* 2005). Phytochemicals are non- nutritive plant chemicals which occur naturally in plants that have protective or preventive properties. They are non-essential nutrients meaning that they are not required by plants for sustaining life (DanMalam *et al.,* 2001). It is well known fact that plants produces this chemical to protect themselves but recent research has shown that they can also protect humans against diseases. Some have shown more promises than others in fighting diseases and illnesses in humans. Alkaloids, Flavonoid, Tannins, Steroids, Glycosides and Saponins are all examples of Phytochemicals. Most Phytochemicals have antioxidants activities which protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activities includes Alkyl, Sulfides, Carotinoids, Flavonoids etc.

The increase in diseases that affect humans and other living organisms and the needs to seek remedies to this disease conditions and improve the overall health conditions of man necessitated the study. This study aims at carrying out the Phytochemical Screening and Antimicrobial activities of ethanol extracts of *Gongronema latifolium* leaves.

Materials and Methods

Collection, Identification and Preparation of Sample

The Fresh and healthy leaves of the plant species, *Gongronema latifolium* were collected randomly after maturation from the agricultural garden, department of agricultural technology, Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State. They were identified by Mr Obinna Chinwike of the Botanic unit of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State. The plant sample were separated from their stalk, washed and air dried in an for five hours. The dried sample were grounded into fine powder and weighed. It was then stored in an air-tight container for further use.

Preparation of Nutrient Agar

Mueller Hinton AGAR: Exactly 3.04 g of powdered Mueller Hinton Agar was weighed and dissolved in 80 ml of distilled water.

POTATO DEXTROSE AGAR (PDA): Exactly 3.04 g of powdered phosphate dextrose Agar was weighed and dissolved in 80 ml of distilled water.

Extraction of *Gongronema latifolium* (Utazi) Leaves Using Ethanol

Exactly 50 g of the dried ground *Gongronemalatifolium* leaves were soaked with 250 ml of ethanol in a round bottomed flask for 48 hours at room temperature (for thorough extraction). The extracts were filtered using Whatman filter paper (No. 42) and the ethanol was evaporated to dryness using a hot water bath. The ethanol extracts of the leaves sample yielded a dark-greenish residue. The extracts were preserved in the refrigerator for further investigation for Potential Phytochemical Screening and Antimicrobial properties.

Methods for Phytochemical Screening.

The Phytochemical Screening was performed using a standard procedure (Evans, 2002; Ayoola *et al*., 2008)

Preparation of the Plant Extracts

The ethanol extracts of the leaves of *Gongronema latifolium* were prepared by soaking 20 g of powered leaves sample in 200 ml of ethanol and were allowed to stand for 72 hours. The residues were dissolved to obtain the desired plant extract for the antimicrobial test.

Sources of Pathogen

The pathogen used in this study were *Candidaalbican, Escherichia coli., Staphyloccus aureus, and Aspergillus niger.* They were obtained from the stock culture of the microbiology laboratory, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State. Viability test of each isolate were carried out by resuscitating the organism cultured in the nutrient agar medium and incubated at 37^0 C for 24 hours.

Statistical Analysis

The data obtained were expressed as mean ± standard error for the diameter of inhibition in each group. The data obtained were analyzed by using the one-way analysis of variance (ANOVA) in all cases, statistical significance was established at values ($P<0.05$).

Results and Discussion

The results of the Phytochemical Screening and Antimicrobial Activities of *Gongronemalatifolium* leaves extract are presented in the Tables below:

Table 1: The Results of the Qualitative Phytochemical Screening of *Gongronema latifolium* Ethanol leaves extract.

TEST	*Gongronema latifolium* leaves extract.
Alkaloids	+ve
Flavonoids	+ve
Saponins	+ve
Tannins	-ve
Terpenoids	+ve
Phenol	+ve
Cardiac Glycosides	+ve

Key: +ve = Present, -ve = Absent.

Table 2: Results of the Quantitative Analysis of the Phytochemicals

Parameters	1	2	3	Mean	STDEV	SEM	COVAR
Flavonoid	2.7	2.5	2.7	2.63	0.094	0.54	0.036
Alkaloid	2.6	2.5	2.55	2.55	0.041	1.47	0.016
Tannin	2.92	2.8	2.8	2.84	0.057	0.032	0.02
Saponin	3.8	3.4	3.6	3.6	0.163	0.092	0.045
Cardiac glycoside	0.02	0.02	0.02	0.02	0.00	0.00	0.00
Phenol	1.57	1.4	1.5	1.49	0.070	0.04	0.047
Phytate	1.46	1.4	1.45	1.44	0.027	0.016	0.019
Terpenoids	0.3	0.29	0.25	0.28	0.022	0.012	0.078

Table 4: The Mean of Diameter of Zone of Inhibition of Ethanol Leaves extracts of *Gongronema latifolium* against Bacterial Isolates.

Organisms	Diameter of zone of inhibition (mm)					
	Crude Extract	**200 mg**	**100 mg**	**50 mg**	**25 mg**	**Control (Ciprofloxacin)**
Escherichia Coli	**22**	**15**	**10**	**-**	**-**	**25**
Staphylococcus aureus	**25**	**18**	**11**	**5**	**-**	**30**

Key: - = Not detected

Table 5: The Mean of the Diameter of Zone of Inhibition of Ethanol extracts of *Gongronemalatifolium* against Fungi isolate.

Organism	Diameter of Zone of inhibition (mm)				
	Crude Extract (mg)	**200 mg**	**100 mg**	**50 mg**	**25 mg**
Candidaalbican	**10 mm**	**10 mm**	**7 mm**	**-**	**-**
Aspergillus niger	**10 mm**	**10 mm**	**-**	**-**	**-**

Key: - = Not detected

Table 6.: The Minimum Inhibitory Concentration (MIC) of the ethanol extracts of *Gongronemalatifolium* leaves against Bacteria and Fungi isolates.

Organism	MIC (mg/ml)
Escherichia Coli	**100 mg/ml**
Staphylococcus aureus	**50 mg/ml**
Candidaalbican	**100 mg/ml**
Aspergillus niger	**200 mg/ml**

Discussion

The results of the phytochemical screening of *Gongronema latifolium* ethanol leaves extracts showed the absence of tannins while Alkaloids, Flavonoids, Saponins, Terpenoids, Phenols and Cardiac glycosides were present. The Phytochemical screening was in agreement with the works of Nduche *et al*. (2018), who carried out the phytochemical screening and antimicrobial activities of four Nigerian medicinal plants and obtained a similar result.

Results in Table 2. Shows that the mean concentration of flavonoids and phenolics was 2.63± 0.094 and 1.49±0.070 respectively. Flavonoids and phenolics which have been reported as free radical scavengers that prevent oxidative cell damage and have strong activities (Pourmorad *et al*. 2006). This is in line with the works of Pourmorad *et al*. 2006, which carried out the antioxidant activities of phenol and flavonoid content of some selected Iranian medicinal plants and obtained a similar result.

The mean concentration of alkaloids was 2.55±0.041. Alkaloids which are beneficial chemicals to plants serving as repellent to predators and parasites. According to Pourmorad *et al* (2006) several alkaloids containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Gurib-Fakin, 2005).

The mean concentration of saponins was 3.6±0.163. According to Roa *et al*. (1995), Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and inhibitory effect on inflammation (Okwu and Emineke, 2006). This is in agreement with the works of Okwu and Emeneke, (2006), on the evaluation of the phytonutrients and vitamin contents of citrus fruits.

The presence and mean concentration of cardiac glycosides was 0.02±0.00. Cardiac glycosides are important naturally occurring drugs whose action helps in the treatment of congestive heart failure and in the treatment of cardiac infections along with other ailments such as cough and chest pain (Usunobun *et al*. 2014). The presence of these cardiac glycosides is in line with the works of Usunobun *et al*. (2014), on the Phytochemical screening and proximate composition of *Annona muricata* leaves.

Results in Table 3 shows the mean diameters of zone of inhibition of the plant extract. The inhibition diameter for the ethanol extracts of the plant sample against *Escherichia coli* ranged from 22.0 mm to 10.0 mm among the zones of crude extract, 200 mg and 100 mg while that of 50 mg

and 25 mg showed none. The inhibition diameter for the ethanol extracts of the plant sample against *Staphylococcus aureus* ranged from 25.0 mm to 5.0 mm among the zones of crude extract, 200 mg, 100 mg, and 50 mg. These show that ethanol leaves extract of *Gongronema latifolium* is more potent for microbial isolates. The findings of this research work is in consonance with the findings of Morebise and Fafunso (1998), who reported the antimicrobial activity of *Gongronema latifolium*(Utazi) on *Escherichia coli* and *Staphylococcus aureus*.

Results in Table 4 shows that the fungi isolate (i.e. *candida albican* and *Aspergillus niger*) which shows inhibitions at the crude extract and 200 mg and no inhibitions at 50 mg and 25 mg. These collaborate with the observations of Adaramola-Ajibola *et al*. (2017), on the antimicrobial activities of polar and non-polar solvents leaf extracts of *Gongronema latifolium*.

The minimum inhibitory concentration (MIC) is the least concentration of the extract that inhibits growth of organism. It is an important diagnostic tool since it helps in confirming resistance of microorganisms to antimicrobial agents. The lowest MIC was observed in ethanol extract against *staphylococcus aureus* (50 mg/ml), followed by *Escherichia coli* and *candida albican* at 100 mg/ml and the least MIC was *Aspergillus niger* (200 mg/ml). The zone of inhibition was recorded as *staphylococcus aureus* indicating the inhibitory effect in gram-positive bacteria. This result is in agreement with the observation of Omogbai, *etal*. (2019), who reported the inhibitory effect of ethanol extract on some microorganisms were *Staphylococcus auerus* exhibited high minimum concentration. However, the level of inhibition of the pathogen by the test extracts shows the extract to more appreciable potent. Generally, the activity of plant extracts against diseases causing microorganisms and use in traditional remedies is considered to be a function of the Phytochemicals in the plants (Sofowora, 1993).

Recommendations

Due to the prevalence of many tropical diseases occurring in many developing countries of Africa and Asia and the need to develop new set of drugs that will alleviate these diseases burdens, it is recommended that: Further studies should be carried out on the possible isolation and purifications of the Phytochemicals present in *Gongronema latifolium* leaves.

Further studies should be carried out on the nutritional potentials of *Gongronema litifolium* leaves with the aim of incorporating the leaves into the daily diet of people especially those living in developing countries.

Increase in cultivation or planting of *Gongronema latifolium* in homes because of the enormous potential benefits that could be possible derived from the plants if further research is successfully carried out on these plants.

Conclusion

From the results obtained in this study, Ethanol extracts of *Gongronema latifolium* leaves contain bioactive Phytochemicals like alkaloids, Flavonoids, Saponins, Terpenoids, Phenol, Cardaic glycosides. Moreover, the extracts possess antimicrobial activity at higher concentrations against the bacterial and fungal species tested. The presence of these bioactive constituents in the leaves extracts of this plant is an indication that the plant, if properly screened and painstakingly purified could yield drugs of pharmaceutical significance.

References

Adaramola-, K.M.; Oyetayo, A.M.; Bada, S.O. and Ibitoye, F.O. (2017). Phytochemical Screening and Antimicrobial Activities of Polar and non-polar Solvent Leaf Extract of *Gongronema latifolium(Asclepiadaceae). Asian Journal of Research in medicaland pharmaceutical sciences.* 1(3):1-9.

Agbo, C.U.; Baiyeri, K.P. and Obi, I.U. (2005). Indigious knowledge and utilization of *Gongronema latifolium* benth. A case of women in university of Nigeria, Nsukka. *Biological Reversed Journal*. 3: 66-69.

Agwu, I.G. and Chukwu, P.A. (2017). Phytochemical Screening of *moringa Olerifera* (Moringa) Seeds. National Diploma Project. Akanu Ibiam Federal Polytechnic, Unwana, Afikpo. Pp. 1-3.

Ayoola, G. A.; Coker, H. A. B.; Adesegun, S. A.; Adepoju-Bello, A. A.; Obaweya, K.; Ezennia, E. C. and Atangbayila, T. O. (2008). Phytochemical Screening and Antioxidant activities of some selected medicinal plants used for material therapy in South-western Nigeria. *Tropical jounal of phamatics. Resverol.* 7:1019-1024.

Cespedes, C.L.; Salazar, J. R.; Martinez, M. and Aranda, E. (2005). Insects growth and regulatory effects of some extracts and sterol from myrtillocactus geometrizes (*Cactacaceae*) against spodoptera frugiperda and tenebio molitor. *Phytochemistry*. 66:2481-2493.

Chinedu, I.; Uhegbu, F.O.; Imo, C.K. and Ifeancho, N.G. (2013). Ameliorating effect and haematological activities of methanol leaf extract of *Gongronema latifolium* in acetaminophen-induced hepatic toxicity in waster albino rats. *International Journal of Biological science*. 3: 183-188.

Dahiru, D.; Obnubiyi, A. and Umaru, H. A. (2006). Phytochemical Screening and Anti-ulcerogenic Effect of *moringa oleifera*. *African Journal of Traditional Complimentary and Alternatives Medicines.* 3: 70-75.

DanMalam, H.u.; Abubakar, Z. and Katsayal, U.A. (2001). Pharmacognostic studies on the leaves of *Moringa oleifera*. *NigeriaJournal of National Products and Medicine.* 5:45-49, 105.

Erickson-Otto, P. (1998). Phytonutrients: Putting a dent in disease. *Food Product Design.* 8:16,

Evans,W.C.(2002). Trease and Evans Pharmacognosy.15th Edition, Elsevier, India.

Gurib-Fakim, A. (2006). *Medicinal plants:* Traditions of yesterday and drugs tomorrow. Module Asphalt Medicine. 27:1-93.

Morebise, O. and Fafunso, M.A. (1998). Antimicrobial and Phytotoxic Activities of Saponin Extracts from two Nigerian Edible Medicinal Plants. Biokemistri. 8(2):69-77.

Nduche, M.U.; Egbucha, K.C. and Amakwe, O..C. (2018). Phytochemical Screening and Antimicrobial Activities of Four Nigerian Medicinal Plants. *International Journal ofResearch in Pharmacy and Bioscience*. 5(2):15-27.

Okwu, D.E. and Emenike, I.N. (2006). Evaluation of the Phytonutrients and Vitamin Contents of Citrus Fruits. *International Journal of Molecular Medicine*. Advanced Science. 2:1-6.

Oliver-Bever, B. (1986). Medical Plants in Tropical West African Combined University press, London. Pp. 89-90.

Omogbai, B. A.; Eigheenrio, O.S.; Obazenu, E.I.; Iduoriyekemwen, A.S. and Ubaka, L.N. (2019)

Poulose, S.M.; Miller, M.G. and Shukitt-Hale, B. (2014). Role of Walnuts in Maintaining Brain Health with age. *Journal of Nutritional.* 144: 5615-5665.

Pourmorad, F.; Hosseinielir, S. J. and Shahabimajd, N. (2006). Antioxidant activity, Phenol and Flavonoid Content of some selected Iranian Medicinal Plants. *African Journal ofbiotechnology*. 5: 1142-1145.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in African. Chischester John Willey & Sons New York.

Sofowora, A. (1993). Screening Plants for Bioactive Agents in Medicinal Plants and Traditional Medicine in Africa. Spectrum books limited, Ibadan. Pp. 128-161.

Ugochukwu, N.H. and Babady, N.E. (2003). Anti-hyperglycemic effects of Aqueous and Ethanol extracts of *Gongronemalatifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin induced diabetic rats. *Life science.* 73:1925-1938.

Usunobun, U.; Okolie, N.P.; Anyanwu, O.G. and Adegbegi, A,J. (2014). Phytochemical Screening and Proximate Composition of *Annonamuricata* leaves. *European Journal of Botany plant and Pathology*. 2(1). 18-28.