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Phytochemical Screening and Antimicrobial Activity of *Xylopia aethiopica* and *Gongronema latifolium* on Common Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RUBE, UOE, UME and EFO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author UOE managed the analyses of the study. Authors EFO, UOE and RUBE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the study was to determine the proximate composition, phytochemical composition and the antimicrobial potentials of the extracts of *Xylopia aethiopica* and *Gongronema latifolium* on some commonly encountered pathogens. The proximate composition results reveal that both plants were very rich in basic nutrients. *X. aethiopica* had 14.5% moisture, 2.41% ash, 1.38% protein, 0.33% fat and 72.1% carbohydrate. *G. latifolium* on the other hand, had 44.1% moisture, 3.43% ash, 9.10% protein, 3.65% fat, 8.60% fiber and 31.3% carbohydrate. Phytochemical screening of both plants showed that they were abundant in phytochemicals such as alkaloids, glycosides, saponins, flavonoids, reducing compounds and polyphenols. However, tannins, phlobatannins, anthraquinones and hydroxymethyl anthraquninones were absent. Crude quantification of the phytochemicals revealed that flavonoids and polyphenols were the most

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abundant of all. Analysis of variance triplicate readings was significant (P < 0.0001). *Esherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed varying zones of sensitivity to the aqueous, ethanol and methanol extracts of both plants. Aqueous extracts of *G. latifolium* gave the highest zone of inhibition of 26 mm with *P. aeruginosa* with while the least inhibition of 7 mm was recorded with methanolic extract against *S. aureus*. The zones of inhibitions for *X. aethiopica* were almost similar for all the test isolates. The result of the study confirms that these plants have tremendous potentials that could further be exploited.

Keywords: Phytochemicals; antimicrobial; proximate analysis; spices.

1. INTRODUCTION

Despite advances in the areas of genomics and drug design only a few antibiotics have been able to make it to the market since the 1970s. Sadly, resistance to available antibiotics is becoming more and more common thus prompting the need for alternative medicine [1]. The World Health Organization report released in April 2014 stated that "*this serious threat is no longer a prediction for the future; it is happening now in every region of the world and has the potential to affect anyone, of any age, in any country*" [2]. As promising alternatives, plants have been exploited for their phytochemicals or bioactive agents to treat infectious diseases. Although 25 to 50% of currently used pharmaceuticals are plant derived, none are used as antimicrobials. Studies have shown that plants are very rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, phenols and flavonoids and these have been found to have *in vitro* antimicrobial properties [3-5].

Gongronema latifolium and *Xylopia aethiopica* are examples of plants that are commonly used in South Eastern part of Nigeria as spices. In addition to their use as spices, they also have medicinal properties, are very rich in nutrients and minerals as well. Also known as *Utazi* by the Igbos, Efiks and Ibibios tribes, *G. latifolium* has been found to contain protein, fibre, lipid, phytochemicals, and minerals including potassium, calcium, phosphorus, iron, zinc, lead, copper, cobalt and magnesium. It is also found to be very rich in valine, aspartic acid, glutamic acid, glycone, essential oils and various fatty acids [6-7]. Aqueous and ethanolic extracts have also been shown to have good antimicrobial activity against *Staphylococcus aureus, Esherichia coli, Salmonella enteritidis, Pseudomonas aeruginosa, Listeria monocytogenes* and *S. typhimurium*. Antifungal, anti-inflammatory, anti-plasmodial, anti-asthmatic, anti-ulcer and gastrointestinal relaxing effect has also been reported [7]. On the other hand, *Xylopia aethiopica* is a plant that is used both as spices and medicine [8]. Studies have shown that it is very potent for curing ailments such as cough, rheumatism, and nerve pains. In addition, it is administered to women who just gave birth to remove blood clots. Furthermore, it has been found to contain phytochemicals such as alkaloids, saponins, flavonoids, tannins, terpenes, steroids and cardiac glycosides [9]. Like *G. latifolium*, it also contains essential oil [10]. *X. aethiopica* have been shown to have good antimicrobial activity against commonly encountered food pathogens such as *Bacillus subtilis, S. aureus, E. coli, Leuconostoc*, *Lactobacillus casei* and *Candida* species [11].

Given the importance of these two important spices and available information on proximate composition, phytochemical components and their antimicrobial activities on common pathogens, we decided to carry out a confirmatory study.

2. MATERIALS AND METHODS

2.1 Source, Collection and Preparation of Medicinal Plant Samples

The freshly harvested plants were obtained locally from a popular market in Uyo, the Akwa State Capital called Urua Akpan Ndem. They were identified at University of Calabar Botanical Garden. The leaves of *G. latifolium* and the fruits of *X. aethiopica* were used in the study.

2.2 Collection, Culture and Identification of Human Pathogens

The test microorganisms were obtained from the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria. The organisms collected were *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* and were identified using standard microbiological techniques [12-13].

2.3 Preparation of Aqueous, Methanolic and Ethanolic Extracts

This was done following the methods of Ebana et al. [4] [11] but with slight modifications. Briefly, both samples were sun dried and then made into a powder. Exactly 200 g of each plant materials were dissolved in 400 ml of sterile distilled water, methanol and ethanol (90%) separately. The aqueous extracts were kept for 72 hours with a foil wrapped around the flask to avoid microbial degradation. After which it was made into a powder and stored at 4℃ until use. The ethanol and methanol extracts were obtained using a Soxhlet extractor and concentrated using a rotary evaporator. The extract was kept at until use at 4℃.

2.4 Phytochemical Screening

The phytochemical screened for were tannins, alkaloids, saponins, glycosides, reducing compounds, polyphenols, anthraquninones, hydroxymethyl anthraquinones and phlobatannins. These were done as previously described [4-5].

2.5 Proximate Analysis Determination of the Diets

The proximate analysis of the plants for the starter and finishers phase were determined using the methods described by AOAC [14].

2.6 Quantification of the Phytochemicals

All the screened phytochemicals that gave positive results were then quantified using methods already described [15].

2.7 Antimicrobial Sensitivity Testing

This was done using methods already described [16]. Briefly, a cork borer was used to cut filter papers into tiny disks. The disks were wrapped with aluminum foil and sterilized in the hot air oven. A colony of each test organisms was sub-cultured on nutrient broth and incubated at 37℃ for 6 hours. They were then inoculated on freshly prepared Mueller-Hinton agar plates. The sterilized filter paper disks were soaked in the respective test extracts and then placed on the plates aseptically. The plates were incubated at 37℃ for 24 hours. After incubation, the zones of inhibition were determined in triplicates for each extracts and isolates.

2.8 Statistical Analysis

Triplicates readings obtained from proximate composition analysis, quantification of phytochemicals and the zones of inhibition for both plants were subjected to one way analysis of variance (ANOVA) and the results expressed as Mean±SD. Both analysis were done using Microsoft Excel 2007 version and at 95% level of significance.

3. RESULTS

Proximate composition of *X. aethiopica* and *G. latifolium* (g/100 g dry matter) are presented in Tables 1 and 2. These reveal the presence of carbohydrate, protein, fat, moisture, fibre and ash. Replicate readings obtained showed significance ($p<0.05$). Table 3 indicates the presence of phytochemicals in both study plant parts with polyphenol being the most abundant phytochemical in all the extracts examined. Tannins, phlobatannins, anthraquninones and hydroxymethyl anthraquinones were absent in both samples. In addition to polyphenol, the extracts of both plants had alkaloids, glycosides, saponins, flavonoids and reducing compounds. The phytochemicals were then quantified and the results presented in the Tables 4 and 5 for *X. aethiopica* and *G. latifolium*, respectively. Both results were significant ($p<0.05$). The result antimicrobial sensitivity of the ethanolic, methanolic and aqueous extracts of both plants are presented in Table 6. The test isolates are *E. coli, P. aeruginosa* and *S. aureus*. The results indicate that the extracts had varying amounts antimicrobial activities. The highest zone of inhibition of 17 mm was shown with *G. latifolium* on *E. coli* using methanolic extract. Ethanolic extracts of both samples did not inhibit *E. coli.* For *P. aeruginosa*, the highest zone of inhibition of 26 mm was seen with aqueous extract of *G. latifolium* and the least inhibition of 11.00 mm was seen in the ethanolic extract *X. aethiopica*. *S. aureus* showed an inhibition of 22.00 mm which was the highest with *G. latifolium*. However, the least inhibition of 7 mm amongst the three isolates was seen in the methanolic extract of *G. latifolium.* On the average, inhibitions recorded for *G. latifolium* were higher than that of *X. aethiopica.*

4. DISCUSSION

X. aethiopica and *G. latifolium* are two of the many spices that are commonly used in Nigeria by many especially pregnant women who just

gave birth in order to help remove blood clots [16-19]. Our study reveals that *X. aethiopica* and *G. latifolium* are rich in protein, carbohydrate, crude fat, crude fiber and ash and this justifies their use as food. *G. latifolium* in our study gave 44% moisture, 3.43% ash, 9.10% protein, 3.65% fat, 8.60% fiber and 31.3% carbohydrate. Offor et al. [16] in their study of phytochemical and proximate composition of *G. latifolium* leaves showed that it had 11.13% moisture, 33.60 protein, 38.55% carbohydrate, crude fat 3.41, 4.20 fiber and 9.11% ash. In another study by Alobi et al. [16] they found that the leaves of *G. latifolium* had the following proximate composition: 43.70% carbohydrate, 15.2% moisture, 6.3% fiber, 33.21% crude protein and 1.6% fat. Compared to both studies, our moisture content was higher but protein content was lower.

Table 1. Proximate composition of Atta (*Xylopia aethiopica*) in g/100 g dry matter

Proximate composition	Mean±SD (%)	Probability value
Moisture	16.91±0.01 (14.5)	<0.0001 [a]
Ash	2.81±0.02 (2.41)	
Protein	1.61±0.02 (1.38)	
Fat	0.39±0.01 (0.33)	
Fiber	10.91±0.01 (9.35)	
Carbohydrate	84.20±0.00 (72.1)	

[a] *Represents significant Mean±Standard deviation at 95% significance level.*

X. aethiopica on other hand had 14.5% moisture, 2.41% ash, 1.38% protein, 0.33% fat, 9.35% fiber and 72.1% carbohydrate. In their study of 20 wild edible plants used as spices in Cameroon, Bouba et al. [18] found that the plant fruit (g/100 g) had 9.6 moisture, 9.5 ash, 7.9 protein, 0.29 non-protein nitrogen, 33.7 fat and 0.4 carbohydrate. Abolaji et al. [19] reported 16.0% moisture, 4.37% total ash, 12.14% crude fiber, 9.55% total fat, 2.10% crude protein and 55.80% carbohydrate. In these studies, our carbohydrate was much higher but our protein less. In addition to these basic nutrients, both plants have also been found to contain vitamins A, C, E, niacin and thiamin [16] and Ca, Mg, Na, K, Fe, Se, Mn, Zn, Cu [16-19].

Table 2. Proximate composition of Utazi *(Gongronema latifolium)* in g/100 g dry matter

Proximate composition	Mean±SD (%)	Probability value
Moisture	78.50±0.10 [b] (44.1)	<0.0001 [b]
Ash	6.10±0.10 (3.43)	
Protein	16.20±0.10 (9.10)	
Fat	6.20±0.10 (3.65)	
Fiber	15.40±0.10 (8.60)	
Carbohydrate	55.68±0.00 (31.3)	

[b] *Represents significant Mean±Standard deviation at 95% significance level.*

The screening of both plants parts for the presence of phytochemical bases indicates that both were rich in important phytochemical bases. Both ethanolic and aqueous extracts were found to contain alkaloids, glycosides, saponins, flavonoids and polyphenol but not tannins, phlobatannins, anthraquinones, hydroxymethyl anthraquninones. In a study by John-Dewole et al. [19] they found that they *X. aethiopica* was rich in phytochemical such as tannins, alkaloids, anthracene, balsam, cardiac glycosides, saponins and volatile oil. Crude quantification revealed that polyphenol and flavonoids were the most abundant phytochemicals in both plants with values of 4.57%, 8.70%, and 8.40%, 6.94%, for *G. latifolium* and *X. aethiopica*, respectively. Offor et al. [16] reported a crude quantification of

Table 3. Phytochemical screening of atta and utazi using ethanol and aqueous extracts

Phytochemicals	Atta ethanol	Aqueous	Utazi ethanol	Aqueous
Alkaloids	++	+*	+	+
Glycosides	++	+	+	+
Saponins	+	++	+	+
Tannins	-	-	-	-
Flavonoids	++	+	++	+
Reducing compounds	++	+	+	+
Polyphenols	++	+++	++	+
Phlobatannins	-	-	-	-
Anthraquinones	-	-	-	-
Hydroxymethyl Anthraquinones	-	-	-	-

* *+ = present, ++ = present in excess, +++ = present in much excess and - = absent*

18.11% saponins, 16.23% tannins, 11.11% tannins, 11.11% phenols, 11.13% flavonoids and 0.12% alkaloids for *G. latifolium.* Alobi et al. [17] did not detect alkaloids and cardiac glycosides but 23.30 anthraquninones, 18.20 saponins, 1.61 tannins and 11.00 flavonoids (mg/100 g) in *G. latifolium*. Ekpo et al. [9] found alkaloids, saponins, flavonoids, tannins, steroids and cardiac glycosides either in moderate (++) or high concentration (+++). These differences could be due to the difference locations they were obtained from.

Table 4. Quantitative estimation of crude phytochemical components in utazi *(Gongronema latifolium*) (%)

Phytochemical components	Mean±SD	Probability value
Alkaloids	2.13±0.06	<0.0001 [c]
Glycosides	1.61±0.00	
Saponins	1.16±0.02	
Tannins	-	
Flavonoids	8.70±0.10	
Polyphenol	4.57±0.01	
Reducing compound	1.74±0.02	

[b]Represents significant Mean±Standard deviation at 95% significance level

Table 5. Quantitative estimation of crude phytochemical components in atta (*Xylopia aethiopica*)

Phytochemical components	Mean±SD	Probability value
Alkaloids	2.40±0.01	<0.0001 [d]
Glycosides	1.51±0.01	
Saponins	1.82±0.01	
Tannins	-	
Flavonoids	6.94±0.01	
Polyphenol	8.40±0.10	
Reducing compound	4.57±0.01	

[d b]Represents significant Mean±Standard deviation at 95% significance level

G. latifolium and *X. aethiopica* are important medicinal plants that are either as vegetables or as spices or both. Studies have linked a number of hypoglycaemic activities, and interesting antibacterial, antioxidant, anti-inflammatory, hepato-protective, anti-plamsmodial, anti-sickling, anti-ulcer, analgesic, and ant-pyrectic activity to these plants [6,8,10,20,21,22,23]. Our study indicates that all the three extracts aqueous, methanolic and ethanolic had varying degree of antimicrobial inhibitory activity. Aqueous extract of *X. aethiopica* showed consistent activity against *E. coli, P. aeruginosa, and S. aureus*. The highest inhibition was seen on *P. aeruginosa* with an inhibitory zone of 26.00 mm was observed with aqueous extract of *G. latifolium* and the least was seen by methanolic extract of *G. latifolium on S aureus* with methanolic extract*.* In a study by John-Dewole et al. [19] using aqueous, petroleum ether, methanol extracts of *X. aethiopica* to examine the antimicrobial action on *E. coli, P. aeruginosa, S. aureus* and *Saccharomyces cerevisiae* found that the later and *P. aeruginosa* were completely resistant all the extracts used. The ampicillin and tetracycline antibiotics used as control in this study were far better than the extracts except for petroleum extract on *E. coli.* In another study by Ilusanya et al. [11] the antimicrobial effect of the ethanolic and aqueous extracts of *X. aethiopica* and those of the antibiotics gentamicin, ampicillin, erythromycin and ciprofloxacin against *P. aeruginosa, E. coli, Bacillus subtilis, S. aureus, Klebsiella Pneumonia,* and *S. faecalis* were examined. They found that ethanolic extract was active against *P. aeruginosa, B. subtilis* and *S. aureus* but not against *E. coli* and *K. pneumonia*. Greater synergism was seen with gentamicin, aqueous and ethanolic extracts. However, the study recommended that the current practice of using *X. aethiopica* and conventional antibiotics should be discouraged.

Eleyinmi [6] using *G. latifolium* showed that all their extracts had no activity against *E. faecalis, Y. enterolytica, E. aerogenes, B. cereus* and *E. agglomerans*. Methanolic extracts were active against *S. entertidis, S. cholerasius ser typhimurium* and *P. aeruginosa* with the highest inhibition being 7mm and was the least inhibition obtained in our study. In the same study, aqueous extract showed against *E. coli* while methanolic extracts were active against *P. aeruginosa* and *L. monocytogenes*. Fleischer [10] showed that the fresh and dried fruits, leaf, stem bark and root bark essential oil of *X. aethiopica* showed various degrees of activity against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *C. albicans* but not *E. coli.* Furthermore, the zones of inhibition shown in our study are comparable to that obtained in a recent study by Ebana [24] using leaves of *Lasianthera africana* and *Dennettia tripetala.* The highest zone of inhibition shown was 25 mm with the ethanolic extract of the leaves of *L. africana* on *E. coli* and was lower than our highest of 26.00 mm on *P. aeruginosa.*

Table 6. Antimicrobial sensitivity (Mean±SD) of ethanolic, methanolic and aqueous extracts of atta (*Xylopia aethiopica)* and utazi (*Gongronema latifolium*) (mm)

Microorganisms	Et	*X. aethiopica* Me	Aq	Et	*G. latifolium* Me	Aq
E. coli	-	13.00±0.71	12.00±0.41	-	17.00±1.41	-
P. aeruginosa	11.00±0.71	13.50±2.12	12.00±2.83	-	19.00±1.41	26.00 ±0.71
S. aureus	12.00±1.41	-	12.00±2.83	22.00±1.41	7.00±1.41	14.00±1.41

Et = ethanolic, Mt = methanolic and Aq = aqueous respectively.

5. CONCLUSION

The findings in this study reveal that both plants are very rich in basic food nutrients, phytochemicals and with excellent antimicrobial activity against commonly encountered microorganisms. There is therefore a need for both plants to be exploited further for their antibacterial potentials.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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