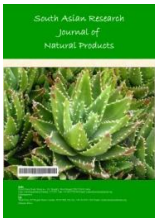

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Comparative Phytochemical and Antimicrobial Analyses of Leaves of *Pterocarpus mildbraedii Harms* and *Xylopia aethiopica* (Dual) A. Rich

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Authors' contributions

This work was carried out in collaboration among all authors. Author ACE designed the study and supervised the work. Authors OEE and KGE carried out the analysis. Authors ACJO and CFM wrote the literature and edited the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To compare the phytochemicals and antimicrobial activities of *Pterocarpus mildbraedii* Harms and *Xylopia aethiopica(Dual) A.Rich*
Methodology: The leaves of *P. mildbraedii* and *X. aethiopica* were collected, washed, air-dried, ground and each extracted with n-hexane, ethyl acetate and methanol. The extracts were analysed for the presence of phytochemicals. Antimicrobial analyses were also carried out on the extracts.
Results: Alkaloids, saponins, flavonoids, phenols and resins were found in all the extracts of both plants. As the polarity of the solvents used increased, the abundance of saponins, tannins and phenols increased in both plants. Also the abundance of steroids decreased as the polarity of solvents used increased in both plants. Saponins, tannins and phenols were found to have high percentage composition in *P. mildbraedii* while alkaloids and flavonoids were very high in *X. aethiopica*. Generally extracts of *X. aethiopica* showed more activity against the bacteria than the *P. mildbraedii. S. aureus* was only susceptible to ethyl acetate leaf extract of *X.aethiopica*. The

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ethyl acetate extract of both plants showed inhibition to the growth of *E. coli.* N-hexane extract of *X. aethiopica* was the only extract which showed against the one of two fungi used.
Conclusion: The two plants contained many metabolites which have been attributed to the antimicrobial activities exhibited by the two plants. These metabolites should be isolated and the subsequent development of the metabolites in formulation of drugs.

Keywords: Pterocarpus mildbraedii; Xylopia aethiopica; phytochemicals; antibacterial; antifungal.

1. INTRODUCTION

Plants serve as an important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional medicine [1]. The traditional medicine system has continued to be practiced in different parts of the world especially in developing countries. However, the prohibitive cost of treatment, side effects of several synthetic drugs and development of resistance to the currently used drugs for infectious diseases have led to an increased emphasis on the use of plant materials in developing countries as source of medicine[2,3,4,5,6,7]. According to WHO, about three-quarter of the world's population currently use medicinal plants as primary health care system[1,3]. They also estimated that about 21,000 plant species have potentials of being used as medicinal plants, whereas only 30% of the entire plant species are already in use [8,9]. The large diversity of medicinal plant species is a huge source of potentially active phytochemicals with novel structures. Approximately 119 pure chemical substances isolated from higher plants are used as medicine throughout the world [1]. These chemical substances can be classified into alkaloids, flavonoids, terpenoids, tannins, steroids, etc which are known as the secondary metabolites. The secondary metabolites act as antibiotics and helps in sustaining the overall health and functional status of the cells within organ systems of the body [10]. In the course of this study, we will be comparing the phytochemical and antimicrobial properties of the leaves of *P. mildbraedii* and *X. aethiopica*.

P. mildbraedii Harms belonging to the family of *Papilionaceae* is majorly found in African countries like Nigeria, Ghana, Liberia, Cameroon, Sierra Leone, Equatorial Guinea and Tanzania. The *Pterocarpus* genus includes some species like *P. tinctorius Welw., P. osun Craib, P. mildbraedii Harms., P. santalinus L. F.* and *P. erinaceus* Poir distributed in Africa. In ethnomedicine reports, showed that *Pterocarpus* genus is used to treat inflammation, pain, infectious, cardiovascular, gastrointestinal and skin diseases in Africa, Asia and Latin America. It commonly known in Nigeria with different local names such as "Oha" in Igbo, "Madobiyar rafi" in Hausa, "Urube" in Edo, "Geneghar" in Ijaw and "Kakupupu" in Urhobo[11,12]. In Eastern Nigeria, *Pterocarpus* has two more different species: *Soyauxii Taub* (Oha) and *Santalinoides L`Herit* (Uturukpa)[13]. *P. mildbraedii* leaves are nutritional vegetables often harvested from the wild and consumed in Southern Nigeria. According to [14], the level of sodium, hydrogen cyanide and oxalate in the vegetables are low while the amino acid profile indicates that the leaves is rich in essential amino acids. The tree is also local source of wood, they are rarely exploited for their timbers in Tanzania and used in making mortars. In Ghana, the trees are planted in cocoa plantation to provide shades [15]. The leaves extracts are used in the treatment of headache, pains, fever, convulsion, respiratory disorders and as antimicrobial agents [11]. The leaves of *P. mildbraedii* have been recommended for consistent use to prevent diabetics [16,17]. The result of analysis carried by Ezekwesili et al, 2016 [17], showed that *P. mildbraedii* is also good source of beneficial chemicals having antioxidant, hypocholesterolemic, chemoprotective and antibacterial properties. The leaf extract of *P. mildbraedii* was reported to have no apparent *in vivo* toxicity on the kidney and heart. Hamzah et al, 2018)'s [11] work also showed that the hepatoprotective compounds present in the plant can be exploited for the prevention and treatment of liver damage. Proximate, mineral, anti-nutritive and phytochemical screening of leaf of *P. mildbraedii* revealed that the leaf contained 20.63 ± 0.03% ash, 13.33 ± 0.01% moisture, 26.45 ± 0.03% crude protein, 8.66 ± 0.01% fat, 12.33 ± 0.02% crude fibre, 18.61 ± 0.44% Carbohydrate, Ca, Na, Mg, Zn, K, P, Fe, Mn, 0.47 ± 0.47% tannic acid, 0.23 ± 0.00% polyphenol, 5.49 ± 0.02% saponin, 4.65 ± 0.02% alkaloids, 3.66 ± 0.01% flavonoids, 3.33 ± 0.09mg/g oxalate, 6.38 ± 0.58mg/g phytin phosphorus and 22.65 ± 2.06 mg/g phytic acid[18]. Some compounds like *fagasterol or lupeol, oleic acid, palmitic acid, 1,2,3,4 Butanetetrol or Erythritol, N,N Dimethyl-*

2-propyn-1- amine, 1,2-Benzenediol, 4-Hydroxypiperidine, and n-Hexadecanoic acid have been isolated from ethanol leaf of *P. mildbraedii* [19].

X. aethiopica (Dunal) A.Rich belonging to the family of *Annonaceae*, is commonly known as 'Negro pepper', 'grains of Selim', 'Ethiopian pepper', 'Guinea pepper' or 'Negro pepper'. In Nigeria, it is known as 'Uda' in Igbo, 'Eeru' in Yoruba, and 'Chimba' in Hausa. It grows naturally in the savanna region of Africa, particularly in Ghana, Nigeria, Cameroon, Ethiopia, Sudan, Angola and Senegal [20,21]. *X. aethiopica* has played a key role in African traditional medicine for several countries owing to its wide array of therapeutic indications. Almost every morphological part of the plant is used as medicine especially the fruits. They are used in the treatment of cough, stomachache, dizziness, amenorrhea, bronchitis, dysentery, headache, neuralgia, female sterility, purgative, rheumatism, biliousness, malaria, hemorrhoids, uterine fibroid, diabetes, boils, diarrhea, stomach disorder, menstrual disorder, naso-pharyngeal infections, arthritis, sores, wounds and cuts among others [20,21,22,23,24]. They are also used as medicine for managing various ailments including skin infections, candidiasis, syphilis, dyspepsia etc [25].

According to [20], the decoction of the seeds is used by traditional birth attendants to induce placental discharge postpartum due to its abortificient effects. The powdered roots are used in local treatment of cancer. The decoction of the leaves is used as anti-emetics [26]**.** The stem bark is used in combination with other medicinal plants for treatment of postpartum breast infections. In Ivory Coast, *X. aethiopica* is taken to promote fertility and ease of childbirth [27]. Studies have shown that the plant possess antibacterial, antifungal, antiplasmodial, analgesics, anti-inflammatory, antidiabetic and antimicrobial properties, anti-hypertensive and diuretic effects [28]. [25,29,23] reported that the essential oil of the plant consist of mainly monoterpenoids and sesquiterpenoids like β-pinene, 1,8-cineol, α-terpineol, terpinene-4-ol, bisabolene. [20] also isolated oxoaporphine alkaloids and lysicamine from the methanol and ethyl acetate extracts of the plant respectively.

The two plants are used in South Eastern part of Nigeria in many soups and decoctions used in various traditional medicines. The work aims to compare the antimicrobial activities of the plants and also compare the metabolites in some extracts of the plants which could be responsible for the antimicrobial activities.

2. METHODOGY

The Leaves of *Pterocarpus mildbraedii* and *Xylopia aethiopica* were collected from Awka, Anambra State and were identified by taxonomists in the Department of Botany, Nnamdi Azikiwe University, Awka. Both were air-dried for 3 weeks and pulverized to powder. The powdered samples were stored to be used for analysis.

Qualitative and Quantitative tests were carried out on the samples to determine the presence of the phytochemicals in the powdered samples. Antimicrobial analysis was also carried out on the sample.

2.1 Extraction of the Phytochemicals

10 g of the powdered samples were soaked separately in 100 ml of each of the three solvents: methanol, ethyl acetate and n-hexane. Each of the three solutions was shaken and the mixtures were left to stand at room temperature for 48 hours after which they were filtered with Whatman No. 1 filter paper. The filtrates were collected and concentrated by heating on a rotary evaporator. The concentrated extracts were then used for the analysis.

Qualitative and quantitative analyses were carried out using the standard methods described by [30,31,32], to ascertain the presence and quantity of metabolites such as tannins, alkaloids, flavonoids, steroids, terpenoids, saponins, cardiac glycosides, proteins, phenols and resins.

2.2 Determination of Antimicrobial Activity

2.2.1 Antimicrobial screening tests

The crude extracts and fractions of the leaves of the two plants were tested against 24 hours broth cultures of *Escherichia coli, Staphylococcus aureus, Streptococcus faecalis, Candida albican, Aspergillus niger* and *Salmonella app* by following the procedure of [33] Nester et al., 2002.

2.3 Bacterial Susceptibility Test

Susceptibility test were performed on the crude extracts to ascertain their activity or not against *Escherichia coli, Staphylococcus aureus, Streptococcus faecalis, Candida albican,*

Aspergillus niger and *Salmonella typhi*. Higher concentrations of extracts were used (50 mg/ml, using methanol as solvent). In the test tube, 20 ml nutrient agar (in a test tube) was melted at 100°C and stabilized at 45°C for about 15 minutes. About 0.1 ml inoculums were added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the agar. The loop was flamed before it was used each time.

The content of the test tube was poured into a Petri dish and allowed to set. The Petri dishes were then labelled with the respective organisms (inoculums) and dates. By means of a 10 mm cork borer, three cups were bored, well separated and equidistant from each other in the agar. The cups were labelled with three crude extracts. Each cup was filled with its corresponding extract to about three-quarters full. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The plates were then incubated aerobically at 37°C and examined for any zone of inhibition after 24 hours.

2.4 Determination of Minimum Inhibitory Concentration

Four different concentrations of the antimicrobial agents were prepared (40, 20, 10 and 5 mg/ml) from the crude extracts, aqueous and also from the chloroform fractions of the various extracts. The working area was disinfected using phenol before the start of the work.

20 ml nutrient agar was melted at 100°C and stabilized at 45°C for about 15 minutes in a test tube. About 0.1 ml *staphylococcus aureus* was added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculum thoroughly with the agar. The loop was flamed before it was used each time.

The content of the test tube was poured into a Petri dish (which was previous autoclaved at a pressure of 15 lb/in2 for 20 minutes) to set. The Petri dish was then labelled with the name of the inoculum and date. By means of a 10 mm sterile cork borer, four cups were bored well separated and equidistant from each other in the agar. The cups were labelled with the four concentrations of the crude aqueous extract. Each cup was filled with its corresponding extract to about three-quarters full.

The Petri dish was quickly covered and then kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into agar).

The same procedure was followed for the different extracts and fractions, with the same microorganism and the other organisms. Thus, each extract was tested against each of the test organism, using chloramphenicol as the control for each organism.

The plates were incubated aerobically at 37°C for 24 hours and examined for any zone of inhibition. The reading was done against a dark background under reflected light. The diameters of the zones of inhibition of growth were measured with the help of a pair of dividers and rule from the underside of the covered plates for spots with inhibitions. The average of the diameters was taken. The actual zones were calculated by subtracting the diameter of the cups (10 mm) from the total zone of growth.

The zones of inhibition obtained were plotted against the log of concentrations to determine the minimum concentrations at which these extracts can inhibit the growth of the test organisms. The minimum inhibitory concentrations were obtained by determining the concentration at which the zone of inhibition was zero.

3. RESULTS AND DISCUSSIONS

The results of the phytochemical screening of the leaves of *P. mildbraedii* and *X. aethiopica* are summarized in the tables.

4. DISCUSSION

The results of the qualitative phytochemical analysis showed that alkaloids, flavonoids, phenols, resins and saponins were present in all the extracts of both *Pterocarpus mildbraedii* and *Xylopia aethiopica* plants. The presence of alkaloids in *X. aethiopica* could be the basis of

Table 1. Results of qualitative phytochemical Analysis of leaf of *Pterocarpus mildbraedii*

Phytochemical constituents	Hexane	Ethylacetate	Methanol
Alkaloids	+	+	+++
Saponins	+	++	++
Tannins	-	-	++
Flavonoids	+++	++	++
Steroids	+	+	-
Terpenoids	-	-	-
Cardiac glycosides	++	-	+
Proteins	-	-	-
Phenols	+	+	+++
Resins	+	+	+

Table 2. Results of qualitative phytochemical analysis of leaf of *Xylopia aethiopica*

Phytochemical Constituents	Hexane	Ethylacetate	Methanol
Alkaloids	+	+	++
Flavonoids	+++	+++	+
Saponins	+	++	++
Cardiac glycosides	+	+	++
phenols	++	++	+++
steroids	++	+	-
Terpenoids	+	-	+
Tannins	_	+	++
Resins	+	+	++
Proteins	+	+	-

Key: + = low abundance, ++ = moderate abundance, +++ = high abundance and – = absent.

Table 3. Result of quantitative determination of phytochemcial constituents of Leaf of *P. mildbraedii*

Phytochemical constituent	Quantity (%)
Flavonoids	2.52
Phytate	0.580
Alkaloids	2.76
Saponins	14.44
Tannins	9.1
Phenolics	9.25
Oxalate	1.20

Table 4. Results of quantitative determination of phytochemical constituents of leaf of *X. aethiopica*

Phytochemical constituents	Quantity (%)
Alkaloids	6.38
Flavonoids	9.27
Saponins	4.12
Tannins	4.96
Phenolics	0.61
Phytate	0.35
Cardiac glycosides	2.84

Table 5. Results of antimicrobial analysis of leaf extracts of *P. mildbraedii*

Organism	n-hexane extract	Ethyl acetate	Methanol extract	control
Staphylococcus aureus	-	-	-	++ (10mm)
S. faecalis	-	++ (11mm)	+ (9mm)	++ (11mm)
E. coli	-	++ (10mm)	-	+ (8mm)
Salmonella typhi	-	-	+ (8mm)	++ (10mm)
Candida albicans	-	-	-	++ (10mm)
Aspergillus niger	-	-	-	++ (10mm)

Table 6. Results of antimicrobial analysis of leaf extracts of *X. aethiopica*

Organism	n-hexane extract	Ethyl acetate	Methanol extract	control
Staphylococcus aureus	-	++(12mm)	-	++ (10mm)
S. faecalis	-	+(8mm)	-	++ (11mm)
E. coli	++ (11mm)	++ (13mm)	-	+ (8mm)
S. typhi	-	-	++ (10mm)	++ (10mm)
Candida albican	++ (15mm)	-	-	++ (10mm)
Aspergillus niger	-	-	-	++ (10mm)

Values are DZI = Diameter of inhibition zone; ++ = high activity (highly Sensitive); += low activity (slightly Sensitive); - =No activity or growth (Resistant)

Table 7. Result of MIC analysis of leaf extracts of *P. mildbraedii*

Extract	Organism	MIC VALUE(µg/ml)
Methanol	*S. faecalis*	1
Ethylacetate	*E.coli*	1
Ethylacetate	*S. faecalis*	2

Table 8. Result of MIC analysis of leaf extracts of *X. aethiopica*

Extract	Organism	MIC value (µG/ML)
Ethyl-acetate	*Staphylococcus aureus*	2
Ethyl acetate	*E. coli*	1
N-hexane	*E. coli*	1
Methanol	*S. typhi*	2

the therapeutic use of the plant as an antimalaria drug [34]. Flavonoids were more abundant in the n-hexane which is against the report of Chanda et al, [35] that flavonoids were found more in polar solvent, and phenols were more in polar solvent which is in line with Ghasemzadeh et al, [36] and Widyawati et al, [37] reports. The presence of saponins which increased with increase in the polarity of the solvent used is in line with the report of Üstündağ and Mazza, [38], that polar solvents are the common extraction solvents for saponins. Tannins were found present only in the methanol extract of *P. mildbraedii* and methanol and ethyl acetate extracts of *Xylopia aethiopica,* which is not surprising since it has been reported that solubility of phenolic compounds increased with increase in solvent polarity [39]. The anti diarrhea, anti diabetic and wound healing properties shown by *X. aethiopica* could be attributed to the presence of tannins found in ethyl acetate and methanol extracts of the plant [40,41,42]. Steroids were present in the n-hexane and ethyl acetate extracts of both plants and absent in the methanol extracts, which is not surprising because steroids are essentially hydrophobic [43]. Terpenoids and Proteins were absent in all the extracts of *P. mildbraedii* but present in the n-hexane and ethyl acetate extracts of *X. aethiopica*. The presence of terpenoids in the extracts of *X. aethiopica* is in line with findings of [20,23,44] that many monoterpenoids and sesquiterpenoids were found in the plant. Cardiac glycosides were present in all the extracts of both plants except the ethyl acetate extract of *P. mildbraedii*. Cardiac glycosides have been reported to have antibacterial, antifungal, analgesic, anti inflammation, antihypertensive, muscle relaxation and anticancer activity, as heart tonic, diuretics

and emetics, and many of the ethno medicinal uses of both plants could be as a result of the presence of the cardiac glycosides [45,46].

The results of the quantitative analysis showed that Alkaloids were more present in the *X.aethiopica* sample (6.38%) than the *P. mildbraedii* sample (2.76%). Flavonoids are also more present in *X. aethiopica* (9.27%) than *P. mildbraedii* (2.52%). Saponins however, are more present in the *P. mildbraedii* (14.44%) than *X. aethiopica* (4.12%). The very high quantity of saponins in *P. mildbraedii* is in line with high quantity reported by Akinyeye et al [18] and could be the basis of the pharmacological use of the plant as hepatoprotective, anti diabetic agent [11,16,17,38,47]. Tannins are also more present in *P. mildbraedii* sample (9.1%) than *X. aethiopica* (4.96%). *P. mildbraedii* sample has more percentage of phytate (0.58%) than *X. aethiopica* (0.35%). Phenols are also more present in *P. mildbraedii* (5.987%) than *X. aethiopica* (0.61%). Oxalate are only present in *P. mildbraedii* (0.243mg/kg) while cardiac glycoside in *X. aethiopica* (2.84%). This shows that *X. aethiopica* has greater amount of alkaloids and flavonoids while *P. mildbraedii* has greater amount of saponins and tannins.

The results of the Antimicrobial activity showed that *S. aureus* was resistant to all extracts of *P. mildbraedii*. However, it was highly sensitive to the ethyl acetate extract of *X. aethiopica,* which is in line with the report of Padalia et al, [48] that *S. aureus* was susceptible to semi polar extracts. Streptococcus was resistant to the n-hexane extract of *P. mildbraedii* but was highly sensitive and slightly sensitive to the ethyl acetate and methanol extracts respectively. For *X. aethiopica* sample, streptococcus was slightly sensitive to the ethyl acetate extract and resistant to the rest. *E. coli* was highly sensitive only to the ethyl acetate extract of *P. mildbraedii* whereas it was highly sensitive to the n-hexane and ethyl acetate extract of *X. aethiopica*. The susceptibility of the gram-negative *E. coli* to three extracts was in line with findings of Tamfu et al, [49] and against the report of Singariya et al, [50] who reported that extra lipo-polysaccharide and protein cell wall of gram negative bacteria provides a permeability barrier to the antibacterial agent, and thus makes them less sensitivity to plant extracts. *S. typhi* was only sensitive to the methanol extracts of both plant samples (high activity in *X. aethiopica* and low activity in *P. mildbraedii*) which supported the findings of Ibrahim and Kebede, [51], that methanol extracts showed high inhibition to growths of many bacteria including *S. typhi*. *Candida albican* was resistant to all the extracts of *P. mildbraedii*. However, it was highly sensitive to the n-hexane extract of *X. aethiopica* and resistant to the other extracts. *Aspergillus niger* was resistant to all the extracts of the two plant samples. The inhibition against some of the bacteria and could be attributed to the presence of metabolites like alkaloids, tannins and flavonoids [50]. The sensitivity of *S. aureus* to extract of *X. aethiopica* can be said to be as a result of greater percentage of alkaloids and flavonoids in the plant sample as identified in the quantitative analysis. According to Compean and Ynalvez [52], alkaloids and flavonoids have antibacterial activities against *Staphylococcus aureus*. The high activity of *P. mildbraedii* on *Streptococcus* may be attributed to greater percentage of tannins in *P. mildbraedii*. Its activity in *X. aethiopica* can be attributed to the presence of terpenoids. Studies have shown that tannins and terpenoids have antibacterial activities against streptococcus. *X.aethiopica* showed greater antimicrobial activities than *P. mildbraedii* and this can be attributed to greater phytochemicals present as identified in the qualitative analysis.

5. CONCLUSION

It is evident that plant cells produce variety of phytocompounds for defense mechanism against bacteria. The two plants were shown to have contained many metabolites which have been attributed to the antimicrobial activities exhibited by the two plants. These metabolites should be isolated and the subsequent development of the metabolites in formulation of drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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