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***In vitro* Anti-infective and Antioxidant activity of *Xylopi aethiopia* [Dun.] A. Rich: A comparison of the fruits and leaves extracts**

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Abstract

Xylopi aethiopia (Annonaceae) is a plant widely used in Africa for both culinary and medicinal purposes. This study aims at evaluating by comparison the anti-infective and antioxidant activities of *X. aethiopia* fruit and leaves. Ethanol extracts of the leaves and fruits of *X. aethiopia* were evaluated for antimicrobial, anthelmintic and antioxidant activities. Antimicrobial activity was evaluated by determining the minimum inhibitory concentrations (MIC) against clinical strains of selected microorganisms. Anthelmintic activity was evaluated by determining the effects of the extracts on the paralytic and death times of *Pheretima posthuma*. Antioxidant activity was determined by the DPPH free radical scavenging method. Results revealed that the extracts from the fruits showed better antimicrobial and antioxidant activities than that of the leaves as well as a concentration dependent anthelmintic activity. This could justify the extensive use of the fruits than the leaves in folkloric medicine.

Keywords: *Xylopi aethiopia*; antimicrobial; antioxidant; *Pheretima posthuma*

Introduction

The use of plants and plants extract for medicinal purposes has been ongoing for thousands of years and it has been the source of most useful therapy in both herbalism and folk medicine [1]. According to the World Health Organization [2], traditional medicine, particularly plant medicine, is recognized as an important alternative healthcare delivery system for most of the world's population. It is estimated that local communities have used about ten percent (10%) of all flowering plants on earth to treat various infections, although only one percent (1%) have been identified by modern scientists [3]. In Ghana, traditional medicine, especially plant medicine, provide many citizens with cheap healthcare services [4].

The basic medicinal property of these plants lies in some chemical substances found in them. These chemical substances produce a definite physiological action on the human body and are generally known as phytochemicals. These chemicals are non-nutritive but act as a protective shield against diseases. Some of the important phytochemicals include; alkaloids, flavanoids, tannins and phenolic compounds [5]. The major benefit of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments [6]. Thus the need to discover the medicinal properties of plants. Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [7], hence the need to search for new anti-infective agents.

In continuation of this effort, the present study is to investigate by comparison the antimicrobial, antioxidant and anthelmintic activity of the crude ethanol extract of the fruit and leaves of *Xylopi aethiopia* (Annonaceae).

Xylopi aethiopia is a deciduous tree popularly known as 'African pepper'. It belongs to the family Annonaceae [8]. In Ghana, it is known in Twi and Wasa as "Hwentia", meaning slender nose, referring to the shape of the fruit. *X. aethiopia* is majorly used medicinally in the following countries: In Congo, the infusion of the extract of the bark of the tree is made into palm-wine and used in the treatment of asthmatic attack, stomach aches and rheumatism at dosage rate of one or two glasses per day [9]. Also in Nigeria, the powdered bark of the tree is dusted onto ulcerous wounds and used locally for the treatment of cancer and ulcers [10]. The dried root crushed into powder is used as mouthwash for toothache and pyorrhea in Senegal [11]

the decoction of the leaves and roots is used differently in Nigeria as a general tonic and to treat fever [10]. In Cote D'Ivoire, the fruits are recommended as a source of blood tonic to women after child delivery, for blood replenishment. It is also used as an anthelmintic and an analgesic for chest pain [11]. In Ghana, it is used to treat cough (fruits and roots of the plant), dysentery and biliousness (fruit, stem and bark) [12]. Research conducted on the plant has shown that the phytochemical constituents of the fruits include; cardiac glycoside, flavanoids, phlobatannins, tannins, anthraquinones, saponin and steroids. The methanol essential oil extract of *Xylopiya aethiopic* fruits showed inhibitory effect against two gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), four gram negative bacteria (*Escherichia coli*, *klebsiella pneumoniae*, *proteus vulgaris* and *pseudomonas aeruginosa*) and two fungi (*Aspergillus niger* and *Candida albicans*) based on a study carried out by Hassan *et al.*, (2014) [13] using the cup plate agar method. A similar study on the crude extracts (Aqueous and Petroleum Ether extracts) of the leaves of *Xylopiya aethiopic* (Annonaceae) showed inhibitory effect on *Staphylococcus aureus* and *Escherichia coli* in the disc diffusion and agar well diffusion techniques. Studies conducted by Ekeanyanwu and Etienajirhevwe, (2012) [14] indicated the vermifugal activity of the fruit extracts on earthworms. Odukoya *et al.*, (2008) [15] conducted a study on the aqueous extract on the fresh and dried fruit of *Xylopiya aethiopic* and established that it also possesses antioxidant activity by the ferric thiocyanate method. Another study conducted by N,dri *et al.*, (2009) [16] using the free radical (DPPH) scavenging method on the essential oil extracts from the leaves and fruits of *X. aethiopic* also showed the presence of antioxidant activity. Evidence from the above literature reviewed has established the antimicrobial, antioxidant and anthelmintic properties of the leaves and fruit of *Xylopiya aethiopic* (Annonaceae). However, it has not been established the extent of effectiveness of the individual parts (fruit and leaves) in relation to one another, that is, what part of the plant is more effective? The fruit or the leaves? This is the aim for this current study.

Method

Collection of plant material

The leaves of *Xylopiya aethiopic* (XAL) was collected in the month of September 2015 from the Physique Garden of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi and then dried. The fruits of *X. aethiopic* (XAF) was bought from the Kumasi central market, Ghana in September 2015. The plant samples were authenticated in the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana.

Collection of worms

Adult Indian earthworms (*Pheretima posthuma*; class Annelida; subclass Megascolecidae) which have anatomical and physiological resemblance to human intestinal roundworms [17, 18] were used to study. The earthworms were obtained from nearby swamps in and around Central University campus, Accra, Ghana in the month of February, 2016. The worms were washed with normal saline to remove all traces of faecal matter, dirt and waste surrounding their body.

Preparation and extraction of plant samples

The leaves and fruits of *X. aethiopic* were air dried at room temperature (25 to 28 °C). The dried plant sample were ground into powder using a laboratory mill machine (Christy and

Noris, UK). Extraction of the plant samples was done using the cold maceration method. With the aid of an analytical balance, 300g and 200g of the dried and grounded plant powders of the fruits and leaves of *X. aethiopic* (Annonaceae) was weighed respectively and separately extracted in 1000 mL of 70% v/v ethanol in a stoppered container with occasional shaking at 120 rpm (revolutions per minute) for 15 min using a Stuart Mini-orbital Shaker (SSMI) at room temperature for 72 h. The mixture was filtered using a what-man filter paper with the aid of a suction pump in order to obtain a clear filtrate. The filtrate was further concentrated using a rotary evaporator (Buchi, Germany) at 40 °C. The crude extract was then dried in an oven at 40 °C. It was then stored in a desiccator.

Phytochemical analysis

The crude extracts were screened for the present some secondary plant metabolites such as; tannins, glycosides, alkaloids and flavonoids according to the standard procedure [19]

Evaluation of Antimicrobial activity

Test organisms

Clinical strains of two gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), three gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and one fungus (*Candida albicans*) were used for the studies.

Preparation of test organisms

The organisms were cultured in nutrient broth at 37 °C for 24 h prior to the experiment. The turbidity of the actively growing broth cultures was adjusted with sterile water to obtain turbidity optically comparable to that of 0.5 McFarland Standard

MIC determination by micro-titre broth dilution method

Using a 96 well micro-titre plate [20], the plates were initially filled with 100 µL double strength nutrient broth (Oxford, UK), calculated volumes of the plant extracts (XAF and XAL) with sample concentrations of 2, 4, 8, 16 and 32 mg/mL, and that of ciprofloxacin and ketoconazole with concentrations of 0.0125, 0.025, 0.05, 0.1 and 0.2 mg/mL, and 1, 2, 4, 8 and 16 mg/mL respectively. A volume of 20 µL of the 24 h organism suspension (0.5 McFarland standard) was also incorporated into the wells. Calculated volumes of sterile water was finally added to obtain a final volume of 200 µL. The plate was then incubated at 37 °C for 24 h. The MIC was determined as the lowest concentration of the plant extract and standards that inhibited the growth of the organisms which was indicated by the absence of purple coloration upon addition of some volumes of tetrazolium dye MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide).

Determination of antioxidant activity

Using the method described by Agyare *et al.* (2015) [21], the free radical scavenging activity of the plant extracts was determined using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). Solutions of XAF and XAL of concentrations of 1000, 300, 100, 30 and 3 µg/mL and reference antioxidant (Ascorbic acid) solution concentrations of 4.74, 1.184 and 0.592 µg/mL were prepared in methanol. DPPH solution of concentration 0.0002 %w/v (5.0 M) was prepared in methanol in a dark room from a stock DPPH solution of 0.02 %w/v. Three milliliters of the DPPH solution was added to 1.0 mL of the methanol extract and reference antioxidant solutions. The tubes were kept in the dark for 30 min after which absorbance (A₁) of excess DPPH

in the extracts and standard solutions were measured at a wavelength of 517 nm using a UV spectrophotometer (Jenway, USA). The absorbance (A_0) reading for a blank solution containing equal volume of methanol and DPPH served as the control [22]. The percentage of free radical scavenged by the test solutions was calculated using the equation [% inhibition = $(A_0 - A_1)/A_0 \times 100$]. Inhibition concentration IC_{50} was determined as the concentration of samples which scavenged 50 % of free DPPH radical.

Evaluation of anthelmintic activity

The *in-vitro* anthelmintic activity was determined by the method described by Bhawar *et al.* (2009) [23]. Earthworms of 4.0 to 22.0 cm in length and 0.2 to 0.6 cm in width were used. Solutions of the extracts of concentrations of 300, 100 and 30 mg/mL were prepared using sterile distilled water. Reference standards used were albendazole (ABZ) at a concentration of 20 mg/mL and piperazine citrate (PZN) at a concentration of 150 mg/mL. A solution of normal saline (0.9%) was used as the negative control. The earthworms were placed in petri dishes (3 worms per petri dish) and treated with one of the following: PZN (150 mg/mL), ABZ (20 mg/mL), XAF and XAL extracts (300, 100 and 30 mg/mL). Observations were made for the times taken for the various extracts to cause paralysis and death of the individual worms. Paralysis was noted when no movement of any sort could be observed except

when the worms were shaken vigorously or dipped into about 50 °C of water. Death was noted when the worms lost motility followed by a fading away of their body colour.

Statistical analysis

All results and graphs were plotted and analysed using the Graph Pad Prism 5.0 for windows (Graph Pad software, San Diego, CA, USA) and analysed by two-way ANOVA followed by Bonferroni post-test analysis which recognizes $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ as statistically significant.

Results

Phytochemical screening

Phytochemical screening of the plant extracts revealed the presence of tannins, saponins, glycosides, flavonoids and alkaloids.

Antimicrobial activity

XAL and XAF showed antibacterial and antifungal activity against all the test organisms with MIC ranging from 2 to 8 mg/mL for the XAF and 8 to 32 mg/mL for XAL with activities of various extracts comparable to those of standard antibacterial (Ciprofloxacin with MIC of 0.0125 mg/mL) and antifungal (Ketoconazole with MIC of 16 mg/mL) agent (Table 1).

Table 1: MIC of the ethanol extract of the leaf and fruit of *X. aethiopica*

Organisms	Minimum Inhibitory Concentration (mg/mL)			
	Extracts		Ciprofloxacin	Ketoconazole
	XAL	XAF		
Staphylococcus aureus	8.0	2.0	0.0125	Nd
Salmonella typhi	8.0	2.0	0.0125	Nd
Escherichia coli	32.0	2.0	0.0125	Nd
Pseudomonas aeruginosa	16.0	2.0	0.0125	Nd
Streptococcus pyogenes	16.0	4.0	0.0125	Nd
Candida albicans	16.0	8.0	Nd	16.0

Nd: not determined

Antioxidant activity (free radical scavenging activity)

Both XAF and XAL exhibited free radical scavenging activity. XAF demonstrated relatively high antioxidant activity than

XAL as shown in the IC_{50} values in table 2. The lower the IC_{50} , the more potent the antioxidant activity.

Table 2: IC_{50} of the leaf and fruit extract of *Xylopiya aethiopica* and Ascorbic acid

Samples	IC_{50} (μ g/mL)
XAF	0.3612
XAL	138.1
ASA	6.298×10^{-5}

IC_{50} concentration of agent that scavenged 50% of DPPH

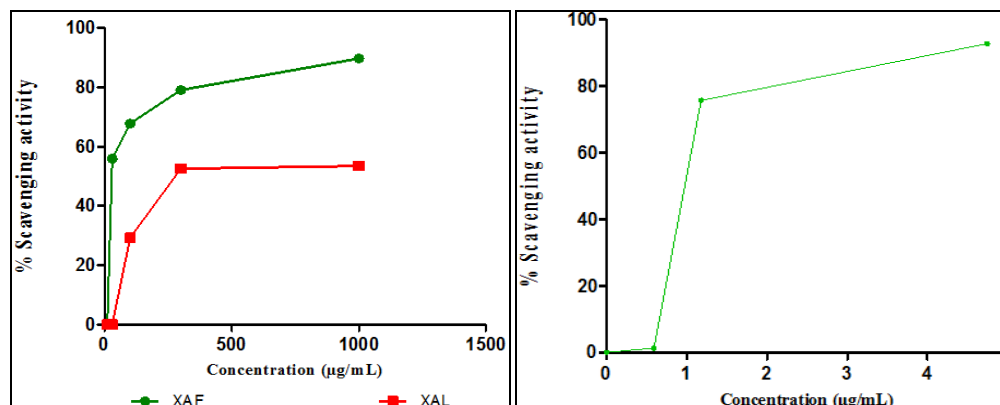


Fig 1: Antioxidant activity of XAF, XAL and ASA at concentrations tested

Anthelmintic activity

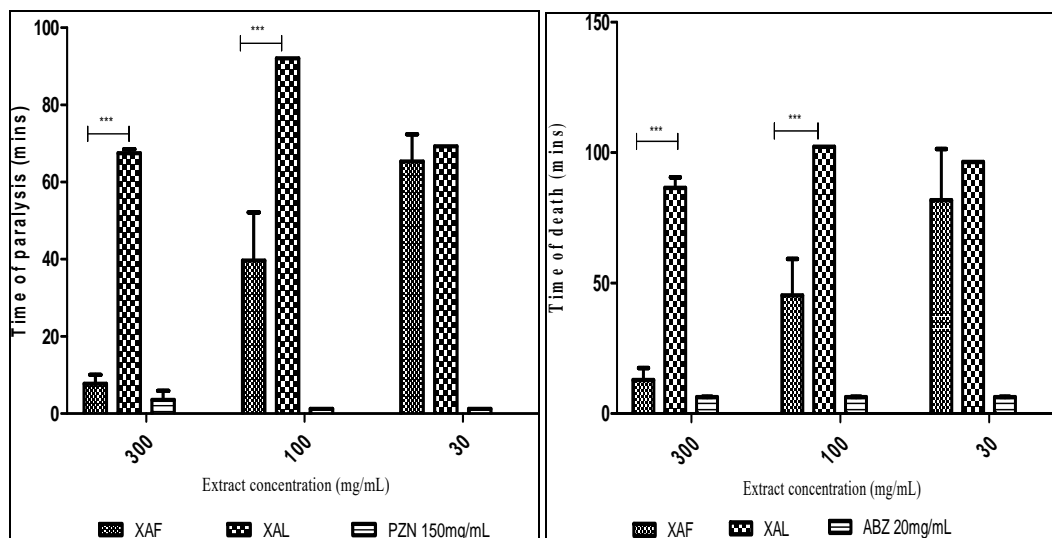
The anthelmintic activity of XAF was more potent than XAL. Both extracts demonstrated a concentration dependent activity

with XAF demonstrating significant paralytic and death times ($p < 0.001$) at concentrations of 100 and 300 mg/mL as compared to XAL at similar concentrations (Table 3).

Table 3: Anthelmintic activities of the leaf and fruit extract of *X. aethiopica*

Treatment	Concentration (mg/mL)	Groups	Time of paralysis (min) (mean±SEM)	Time of death (min) (mean±SEM)
Normal Saline		1	>120.000±0.000	>120.000±0.000
ABZ	20	2	Nd	6.300±0.100
PZN	150	3	3.570±2.330	Nd
XAF	300	4	7.760±2.290***	12.895±4.575
	100	5	39.650±12.500***	45.270±13.990
	30	6	65.340±7.050	81.715±19.625
XAL	300	7	67.490±1.040	86.510±3.990
	100	9	92.080±0.000	102.280±0.000
	30	10	69.270±0.000	96.390±0.000

SEM: Standard error mean, *** $p < 0.0001$

**Fig 2:** Anthelmintic Influence of XAF and XAL on *Pheretima posthuma*.**Discussion**

Phytochemicals produce a definite physiological action on the human body and they are non-nutritive but act as a protective shield against diseases, some of the important phytochemicals identified were; alkaloids, flavonoids, tannins and phenolic compounds [5]

In this study on the leaves and fruit of *X. aethiopica*, the phytochemical screening of the powdered plant samples revealed the presence of tannins, saponins, glycosides, flavonoids and alkaloids. The various phytochemicals that have previously demonstrated potent bioactivities and have been identified as important were present in the leaf and fruit extract. The presence of these metabolites therefore suggests the great potential of the leaves and fruit of *X. aethiopica* as a source of useful phyto-medicine. Both the ethanol leaf and fruit extract of *X. aethiopica* showed broad spectrum antibacterial and antifungal activity against the test organisms (Table.1). XAF extract exhibited the highest activity against the test organisms as compared to XAL. The results obtained corroborates with the work done by Ilusanya *et al*, (2012) [24] on the fruits of *X. aethiopica* [25]. The activity of the ethanol fruit and leaf extract of *X. aethiopica* against *Escherichia coli* contravenes the studies done by Iwu, (1993); Konning *et al*, (2004); Nweze and Onyishi, (2009) [26-28] with all the authors reporting lack of activity of the plant extracts against *E. coli* and attributed it to the fact that *E. coli* being a Gram negative bacterium, has an extra outer membrane that may be impermeable to the plant extract. Extracts with MIC values

below 8 mg/mL are classified as possessing potent antimicrobial activity [29, 21]. With reference to this study, the antimicrobial activity of XAF extract may be classified as potent whilst the antimicrobial activity of XAL extract may be classified as low. Tannins and flavonoids are bioactive components which are known to be bactericidal, pesticidal or fungicidal in nature [30] and their presence in the plant may have attributed to the antibacterial and antifungal activity possessed by the plant.

The ethanol fruit and leaf extract of *X. aethiopica* demonstrated anthelmintic activity. However, the fruit extract (XAF) demonstrated better anthelmintic activity than the leaf extract (XAL). For a drug to be considered a good anthelmintic drug, it must be able to penetrate the cuticle of the worm or gain access to the alimentary tract [14]. Phytochemicals such as tannins, alkaloids, flavonoids and saponins have been demonstrated to possess anthelmintic activities. Chemically, tannins are polyphenolic compounds [31]. Some synthetic phenolic anthelmintics include; niclosamide, oxiclozanide and bithionol. Tannins have the ability to bind to free proteins in the gastrointestinal tract of the host animal glycoprotein on the cuticle of the parasite and cause death to it [32, 33]. Alkaloids may also have acted on the central nervous system of the earth worms causing paralysis [34]. Saponins are also known to cause changes in membrane permeability and pore formation of the earthworm, similar with two conventional anthelmintic drugs such as praziquantel and toltrazuril [35]. Therefore, the presence of these

phytochemicals suggests the ethanol fruit and leaf extract of *X. aethiopica* as a good source of anthelmintic drug. However, the study revealed that the anthelmintic activity of XAL extract is weaker (giving long paralysis and death times) than XAF extract which showed highly significant anthelmintic activity at concentrations of 100 and 300 mg/mL ($p < 0.001$). The main characteristic of an antioxidant is its ability to trap free radicals thus protecting cells against damages caused by these free radicals. According to Esimone *et al.*, (2009) [36], the antioxidant activity of a plant is principally due to the presence of tannins, flavonoids and polyphenols, therefore the presence of these phytochemicals (tannins and flavanoids) in the leaf and fruit extract of *X. aethiopica* may have accounted for the antioxidant activity.

The IC₅₀ values clearly depict the extent of antioxidant activity of the various extracts (Table 2). The lower the IC₅₀ value, the better the free radical scavenging activity and vice versa, therefore it is evident that ascorbic acid (reference sample) demonstrated the highest free radical scavenging activity (IC₅₀ 6.298x 10⁻⁵ µg/mL) followed by the fruit extract (IC₅₀ 0.3612µg/mL) which demonstrated more free radical scavenging activity than the leaf extract (IC₅₀ 138.1µg/mL). Demonstration of the antioxidant activity of the plant parts, suggests *X. aethiopica* as a potential source of an antioxidant compound.

In general, the high biological activity of ethanol extracts of *X. aethiopica* fruits (XAF) as seen in the study validates the extensive use of the dried fruits of *X. aethiopica* readily obtainable from local markets in various disease conditions by traditional health practitioners.

Conclusion

Ethanol extracts of leaves and fruits of *X. aethiopica* exhibit anti-infective and antioxidant activity. The fruits exhibit profound biological activity than the leaves

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Conflict of interest

Authors declare they have no competing interests

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