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Full Length Research Paper

Phytochemical and antimicrobial studies of extract of the fruit of *Xylopia aethiopica* for medicinal importance

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Phytochemical screening of the fruit of *Xylopia aethiopica* confirmed the presence of Saponin, Saponin glycoside, Tannin, Balsam, Cardiac glycoside and Volatile oil. Spectrophotometric analysis for trace metals (such as Mg, Zn, Cu, Ni and Fe), Phosphorus and Sulphur showed that *X. aethiopica* contained Mg(0.370±0.002 mg/100g), Zn (1.020±0.001 mg/100g), Cu (0.274±0.004 mg/100g), Ni (1.099±0.001 mg/100g), Fe (0.690±0.002 mg/100g), P (30.62±0.02 mg/100g) and S (100.50±0.51 mg/100g). The medicinal properties were evaluated *in-vitro* by antimicrobial and antifungal assays. The aqueous and petroleum ether extracts showed growth inhibitory effects on *Staphylococcus aureus* and *Eschericha coli* but *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* were resistant to the fruit extract and the antibiotic controls. The Minimum Inhibitory Concentration (MIC) on *S. aureus* and *E. coli* were 12.50 mg and 6.25 mg respectively. The Minimum Bactericidal Concentration (MBC) of the crude extract against the test organism ranged from 12.50 mg to 25.00mg.

Key words: antimicrobial, herbal, pharmaceutical, phytochemical, *X. aethiopica*.

INTRODUCTION

The use of plants and plant extracts for medicinal purposes has been going on for thousands of years; and it has been the source of much useful therapy in both herbalism and folk medicine (Foye, 1999). The use of medicinal plants in traditional medicine have also generated a lot of interest and concern about their efficacy and safety margin, since 65-70% of the Nigerian population patronize traditional medicine practitioners in their various forms and methods (Bubayero, 1998; Lambo, 1998 and Sofowora, 2001). Plants produce many chemical compounds, which have potential value in the treatment of diseases, but a number of them could also be poisonous. Chemical compounds with beneficial effects have been isolated and biologically assayed to establish their medicinal activity. Modern drugs used in orthodox medicine have also been sourced from plants (Sofowora, 2001). It is therefore not surprising that medicinal plants are vastly employed in the treatment of various ailments which include; snake-bite, eye injuries, conjunctivitis, burns, scalds, abdominal colic, peptic ulcer, diarrhea, dysentery, chronic ulcer, measles, hepatitis, arthritis and rheumatism (Esuoso and Odetokun, 2005). Mere isolation and elucidation of chemical structures of plant extracts may not be too significant, until appropriate bioassays are carried out to establish the biological activity exhibited by the plant extract (Ekong, 2006). *X.aethiopica*, a deciduous tree popularly known as 'African pepper', belongs to the plant family called *Annonaceae* (Keay, 1999). Burkil (1999) and Lajide (1995) reported the medicinal uses of the fruit extract of *X.aethiopica* in the treatment of bronchitis, oedema, dysentery and febrile pains. In Congo, the infusion of the extract of the bark of the tree into palm wine is used in the treatment of asthmatic attack, stomach aches and rheumatism at dosage rate of one or two glasses per day (Tona *et al*., 1999). While in Senegal, the dried root crushed into powder is used as mouthwash for toothache and pyorrhea. In Cote D'ivoire, the fruits are recommended as a source of blood tonic to women, after

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baby delivery, for blood replenishment. It is used as anti-helminthic and also as analgesic for chest pain (Esuoso and Odetokun, 2005). *X.aethiopica* is used locally in Nigeria for the treatment of cancer and ulcers. The powdered bark of the tree is dusted onto ulcerous wounds, while a decoction of the leaves and roots is a general tonic for fever in Nigeria (Burkil, 1999). The crude extract exhibit a strong anti-feedant activity on subterranean termite, *Reticulitermes speratus* (Lajide, 1995). Thus, the aim of this study is to investigate the various phytochemical and anti-microbial properties of the fruit of *X. aethiopica* available for medicinal use.

MATERIALS AND METHODS

The fruits of *X.aethiopica* were bought as dried fruits from traditional herb sellers at *Oje* Market in Ibadan, Nigeria. The plant species was later identified and authenticated by the department of Botany, University of Ilorin, Kwara State. The fruits were further dried at room temperature for two weeks.

Sampling

The dried bulk samples of the fruits were pulverized using pestle and mortar, and sieved through a $2mm^2$ wire mesh to obtain a fine powder. The powdered samples were mixed together and quartered to obtain a representative sample of mass of 150g.

Aqueous Extracts

20g of the powdered fruits of *X.aethiopica* was weighed into 250ml conical flask. 150ml of distilled water was added to the sample in the flask. The solution was then stirred with a glass rod and allowed to soak for 24hrs. The aqueous extract was filtered thrice through a plug of adsorbent cotton wool embedded in a glass funnel. The filtrate was then filtered through 11cm Rund-filter paper MN713. The solution was concentrated by gentle evaporation on a heating mantle and poured into 100ml beaker.

Methanolic Extracts

200ml of methanol was measured into the round bottom flask of the soxhlet. 20g of the powdered fruit was placed in the sample container (i.e. thimble) of the soxhlet. The apparatus was coupled and the system was switched on at thermostat temperature of 65^{o}C. The sample was continuously extracted under reflux for three hours, and the extract was poured into 100ml flask. Methanolic extract of the sample was concentrated by gentle evaporation on a heating mantle.

Petroleum Ether Extracts

200ml of petroleum ether was measured into the round bottom flask of the soxhlet. 20g of the powdered fruit of *X.aethiopica* was placed in the thimble of the soxhlet. The apparatus was coupled and the system was switched on at thermostat temperature of 60^{o}C. The sample was continuously extracted under reflux for three hours and the extract was poured into 100ml beaker after some of the petroleum ether had been recovered. The 100ml extract of the sample was concentrated by gentle evaporation on a heating mantle.

Table 1. Phytochemical Compounds in Fruit Extract of *X. aethiopica*

Phytochemical Compounds	Remarks
Alkaloids	-ve
Anthracene	-ve
Balsam	+ve
Cardiac glycoside	+ve
Saponin	+ve
Saponin glycoside	+ve
Tannin	+ve
Volatile oil	+ve

Key: +ve = positive
-ve = negative

Phytochemical Screening of Crude Extracts

Phytochemical screening of the crude extract for saponin, saponin glycoside, tannin, phenolic acid, anthracene, alkaloid, volatile oil, balsam and cardiac glycoside were carried out by the methods described by Evans (1989), Harbone (1993) and Sofowora (1999).

Spectroscopic analysis of crude extracts

Methods of Howtz (1990), Skoog et al. (2006) and Pavial et al. (1992) were used for spectroscopic analysis of the samples, using Atomic Absorption Spectrophotometer (A200).
Colorimetric determination of Phosphorus was done using Vanadomolybdate (Yellow) method (AOAC, 2000). Spectrophotometric determination of Sulphate was done using Turbidometric method (AOAC, 2000). Antimicrobial assay of crude extracts of *X. aethiopica* was done using the methods described by Egwari (1999), Ntiejumokwu and Kolawole (1990), and WHO (1991) to test the effects of crude extracts on the following pathogenic microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Saccharomyces cerevisiae*. Determination of antibiotic activity and antibiotic control was done by using the Disc Diffusion and Agar Diffusion techniques as described by WHO (1991). Determination of Minimum Inhibitory Concentration (MIC) of the crude extracts was done by using Tube Dilution method as described by Rotimi *et al*. (1999).

RESULTS

Table 1 gives the phytochemical compounds present in crude extract of the fruit of *X. aethiopica*. The extracts were positive for some of the following compounds;

Table 2. Trace Metal content in mg/100g

Elements	Conc. (mg/100g)
Manganese	0.370 ± 0.002
Zinc	1.020 ± 0.001
Copper	0.274 ± 0.004
Cobalt	ND
Cadmium	ND
Nickel	1.099 ± 0.001
Iron	0.650 ± 0.001
Lead	0.065 ± 0.001

Table 3. Phosphorus and Sulpur concentration of the extract

Elements	Conc. (mg/100g)
Phosphorus	30.62 ± 0.02
Sulphur	100.50 ± 0.51

The value represents mean ± SD

Table 4. Inhibitory Effects of Fruit Extract of *X.aethiopica* (15mg)

	Zone diameter (mm) of growth inhibition				
Pathogens	**Aqueous**	**Methanol**	**Pet. Ether**	**Ampicillin Control**	**Tetracycline Control**
Staphylococcus aureus	10	0	12	22	26
Pseudomonas aeruginosa	0	0	0	0	0
Escherichia coli	0	0	20	26	27
Saccharomyces cerevisiae	0	0	0	0	0

alkaloids, balsam, cardiac glycoside, saponin, saponin glycoside, tannin and volatile oil indicating their presence in the extract.

Table 2 shows the trace metal contents of the plant extract in mg/100g. The plant samples contained Manganese, Zinc, Copper, Nickel and Iron.

The value represents mean ± SD
ND = Not Detectable

Table 3 shows the concentration of Phosphorus and Sulphur content of the extract in mg/100g. Table 4 gives the inhibitory effects of extract of *X. aethiopica* fruit at 15mg. *Staphylococcus aureus* and *Escherichia coli* were sensitive to petroleum ether extract of *X. aethiopica* with zone diameter of 12mm and 20mm respectively. However, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* (a fungus) were resistant (i.e. shows no growth inhibition) to extract of *X. aethiopica* and the antibiotic controls. *Staphylococcus aureus* and *Escherichia coli* were sensitive to the antibacterial effects of Tetracycline hydrochloride (15mg) and Ampicillin trihydrate (15mg) which were used as positive controls, with zone diameter of inhibition of 22mm and 26mm (for ampicillin) and 26mm and 27mm (for tetracycline) respectively. However, the active compounds in the plant extract seem far less soluble in methanol.

Table 5 and 6 show the Minimum Inhibitory Concentrations (MIC) of the extract on pathogens. The MIC of extract of *X. aethiopica* on both *Staphylococcus aureus* and *Escherichia coli* was 6.25mg.

DISCUSSION

The crude extracts of the fruit of *X.aethiopica* was chemically and microbiologically assayed for the presence of phytochemical compounds which could be responsible for their medicinal use in traditional medicine, as anti-amoebic, anti-diarrhea, anti-helminthic and treatment of Broncho-pneumonia (Tona 1999; Sofowora, 1999 and Owonubi, 1996). The study showed the aqueous extract of *X. aethiopica* tested positive for the presence of cardiac glycoside, saponin and saponin

Table 5. The MIC of *X.aethiopic*a on *Staphylococcus aureus*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g)
A1	25.00	Nil	
A2	12.50	Nil	
A3	6.25	+	6.25
A4	3.13	+	
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

Table 6. The MIC of *X. aethiopica* on *Esherichia coli*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g)
A1	25.00	Nil	
A2	12.50	Nil	
A3	6.25	+	6.25
A4	3.13	+	
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

glycoside, tannin, volatile oil and balsam. This result agrees with similar research done by Kela *et al*. (1999), Menut *et al*. (2002) and Okogun (1996). Most saponins are nitrogen-free glycosides, each containing a sapogenin and sugar molecule. Schuster *et al*. (1999) and Egwari (1999) have isolated steroidal glycosides such as Hecogenin, Progesterone, Testerone and Diosgenin from plants. These steroidal glycosides are used therapeutically as hormones and contraceptives in medicine. Cardiac glycosides, digitoxin and digoxigenin have varying effects in the cardiovascular systems of human. They are used in the treatment of heart disorders and high blood pressure (Groth, 1994 and Stenlake, 1997). Tannins are polyphenolic compounds also used for medicinal purposes e.g. catechol, hydroquinine and resorcinol are phenolic salicylates used as analgesics, antipyretics and as internal antiseptics in medicine and surgery (Bello, 1999 and Stenlake, 1997). Lajide (1995) also isolated diterpens, phenolic amides and lignamides from the seed of *X.aethiopica*, with anti-feedant activity on subterranean termites, *Reticulitermes speratus*. The petroleum ether extract of *X.aethiopica* was found in this research to be most active against the growth of *Staphylococcus aureus* and *Escherichia coli*. It is therefore important to infer that the fat-soluble diterpenes, phenolic amides and lignamides such as ent-kurane diterpenes E-3-(4-dihydroxyphenyl)-N-(4-hydroxyphenylethy)-2-propenamide may be responsible for the antimicrobial properties of *X.aethiopica*. Thus, the presence of these phytochemicals could be responsible for the observed pharmacological effects of the plant extract.

Trace Elements are essential components of the body enzymes, haemoglobon, vitamin B_{12} and thyroxin which are important for life processes and metabolism; and are sourced mainly from plants. Analysis of trace metals, sulphur and phosphorus content revealed Mg(0.370±0.002 mg/100g), Zn (1.020±0.001 mg/100g), Cu (0.274±0.004 mg/100g), Ni (1.099±0.001 mg/100g), Fe (0.690±0.002 mg/100g), P (30.62±0.002 mg/100g) and S (100.50±0.51 mg/100g) (See Table 2 and 3). The concentration (mean±SD) of the elements analyzed showed Sulphur > Phosphorus > Zinc > Nickel > Iron > Manganese > Copper. The high concentration of sulphur could be responsible for the plant's antimicrobial properties. The clinical effectiveness of sulphanilamides in the control of bacterial infection has led to their effective use against pneumonia and *streptococci* infection (Groth, 1994 and Stenlake, 1997). Sulphanilamides interfere with the synthesis of folic acid, and important bacterial growth factor, by utilization of *para aminobenzoic acid* (PABA) necessary for the synthesis of trihydrofolic acid. The study showed that aqueous and petroleum ether extracts of the fruit of *X.aethiopica* possess antimicrobial effect against the growth of pure isolates of *Staphylococcus aureus* and *Escherichia coli*. This is similar to what is reported by Egwari (1999). The result of zone diameters of inhibition

of the plant extract on the growth of *Staphylococcus aureus* and *Escherichia coli* (Table 4) compared favourably with that of standard antibiotic controls consisting of *Tetracycline hydrochloride* (15mg) and *Ampicillin trihydrate* (15mg) (WHO, 1991 and Cheesebrough, 2000). *Pseudomonas aeruginosa* was resistant to all the plant extract and antibiotic controls. This observation agrees with that of Timothy and Nelson (1992) and Cheesebrough (2000). The plant extract had no antifungal activity against *Saccharomyces cerevisiae*.

Conclusion

The phytochemical screening of the fruit extract of *X.aethiopica* tested positive for the presence of saponin, saponin glycoside, tannin, balsam, volatile oil and cardiac glycoside. The concentration (mean±SD) of elements analysed in mg/100g showed S > P > Zn > Ni > Fe > Mn > Cu. The high concentration of sulphur and phosphorus is an index for the plant's medicinal properties. The medicinal properties of the plant as evaluated *in-vitro* by antimicrobial assay revealed that aqueous and petroleum ether extract showed growth inhibitory effects on *Staphylococcus aureus* and *Escherichia coli*. However, *Pseudomonas aeruginosa* was resistant to the plant extract and antibiotic controls. The plant extracts have no antifungal effects on *Saccharomyces cerevisiae.*

RECOMMENDATIONS

Further work is recommended on isolation and characterization of active chemical compounds responsible for the antimicrobial/antibacterial properties of the plant. The antibacterial effect of the methanol extract and the antifungal effects of the plant extract should be re-evaluated. Medicinal plants are also known to exhibit seasonal variation in chemical properties and bioactivity, which could also affect their medicinal properties at any given period of time. Therefore, there should be an investigation to mitigate the seasonal chemical properties variation of this plant.

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