MPRI-JMBP | INTERNATIONAL JOURNAL OF MEDICAL, BIOLOGICAL AND PHAR. SCI.
VOL. 11 NO.3 JUN-2020 ISSN: 1999-5650

IN VITRO ASSESSMENT OF ANTIMICROBIAL POTENTIAL OF XYLOPIA AETHIOPICA FRUIT (UDA)

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Abstract

Several methods were employed in this study in extracting and testing the antimicrobial activities of the three extracts of Xylopia aethiopia (Hot water, cold water and ethanolic extracts) against Staphylococcus aureus, Echerichia coli, Pseudomonas aeroginosa, Klebsiella Prieumoniae, and Candida albicans.Staphylococcus aureus showed the greatest susceptibility to the extracts whereas Echerichia coli showed the least susceptibility (MIC-E.coli=50mg/ml, S.aureus=10mg/ml and 20mg/ml). Staphylococcus aureus was also found tobe more sensitive to the hot water extract than the cold water and ethanolic extract (MIC, 10Mg/ml).The results also showed that the ethanolic extract was the most effective of the extracts (MIC: 20, 50, 30, 20, 20). It can also be deduced from the result that Staphylococcus .aureus showed the greatest susceptibility to the extract, while E.coli showed the least susceptibility, followed by Candida. albicans and as such suggest that Xylopia aethiopica will be very effective for the treatment of Staphylococcus.aureus infection. Therefore, there was significant difference in the antimicrobial activities of the three extracts of

KEYWORDS:
Invitro, Antimicrobial and Xylopia aethiopica.

Xylopia aethiopica based on One Way ANOVA analysis carried out. Traditional medicine should be encouraged and modernized so as to increase the utility of new plant based antimicrobials like Xylopia aethiopica.

INTRODUCTION

Xylopia aethiopica is a compression from the Greek words-Xylon pikron meaning bitter wood, aethiopia refers to the origin of the wood(i.e. Ethiopia, though most of it grow in Ghana).So Xylopia aethiopica means " bitter wood from Ethiopia" It belong to the family of plants known as Annonaceae(Josh,2009). Xylopia aethiopica is known by English name as –Negro pepper. It is evergreen, aromatic tree growing up to 20m high with peppery fruit. It is known in igbo language as 'uda" and is native to the low land rainforest and moist fringe in the savannah zones of Africa (Maurice et al., 1999).The fruit look rather like small twisted bean pods. They are dark brown, cylindrical, 2.5 to 5.0cm long and 4 to 6mm thick. The contours of the seeds are visible from outside. Each pod contains 5 to 8 kidney shaped seeds of approximately 5mm. The hull is aromatic and slightly bitter but not the grain (seeds) itself. The fruit are often smoked during the drying process and this resulted to attractive smoky spicy flavor (Josh, 2009).

Xylopia athiopica has been used as a pepper substitute in Europe but with regular imports of black pepper from India starting from 6th century. In later times, Xylopia aethiopica was only traded as a pepper substitute in times of war and short supply (Josh, 2009). It consists of two types of acids namely; diterpenic and xylopic acids. It also contains essential oil. These constituents are present in varied amounts in the leaves,fruit, stem and root bark. For example, the essential oil contents of the fresh fruit is about 3.6%, dried fruit =3.33%, leaf=0.46%, stem bark=0.80% and root bark=0.92% (Fleischer et al., 2008).

In recent review, Xylopia aethiopica was shown to have a wide range of biological activities including insecticidal, anti-tumor, anti-asthmatic, anti-inflammatory hypotensive and coronary vasodilatory effects and these were attributed to the wide variety of secondary metabolites in the plant (Fleischer et al., 2008).They have been shown to have antimicrobial

INTERNATIONAL JOURNAL – MBP
VOL. 9 NO.9 JUN-2019 ISSN: 1999-5650

property against a wide range of gram positive and gram negative bacteria including Bacilus subtilis, Staphylococcus aureus, Pseudomonas aeroginosa. It has also been shown to have some degree of activity against the yeast like fungus known as Candida albicans (Christian, 2007).

This study is aimed at assessing or evaluating the antimicrobial potential of the fruit called Xylopia aethiopica (Uda) using Escherichia coli, Staphylococcus aureus, klebsiella pneumonia, Pseudomonas aeroginosa and Candida albicans as test organisms.

Materials and Methods

- **Materials**

Source of Grain Samples: The dried fruit of Xylopia aethiopica was purchased from Ariaria International Market, Aba.

- **Methods**

1. **Preparation of cold water extract:** The dried fruit of Xylopia aethipica were dried in a little further for 10mins in the surgifield hor air oven model SM9023A at 45^{O}C. The aqueous (cold water) extract of the fruit was then prepared using the method described by Christian (2007) and Harborne (1973). The dried fruit were crushed to a powder using Panasonic electric blender model MX-795N. Hundred grams of the crushed fruit was weighed and mixed with four hundred mills of distilled water in a beaker. This was stirred for five minutes, covered and left standing for 24h.Thereafter; it was then shake properly and filtered with Whatman No 1 filter paper in conjunction with a funnel. The residue left after filtration was then dried in the oven and allowed to cool then weighed. The amount of the crushed fruit (in gram) present in the filtrate was determined using the method of Abdulraman et al., (2004).

The mass of the dried residue was subtracted from the mass of the crushed fruit used for the extraction(I.e. 100g).The mass which was in gram was converted to milligram by multiplying by one thousand. The concentration of the crude extract (in milligram per ml) was then determined by dividing

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VOL. 9 NO.9 JUN-2019 ISSN: 1999-5650

the mass calculated above by the volume of the filtrate. The concentration was found to be 55mg/ml.

2. **Dilution of the Extract:** Different concentrations of the extract in (mg/ml) were prepared using the methodology of Eja et al., (2006). From the crude extract, dilutions of 2:20, 2:9, 2:3.5, and 2:0.2 in distilled water, corresponding to 5mg/ml, 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml respectively were prepared. These were calculated based on the ratio. For instance, 2:20 resulted in $\frac{2}{22} \times 55 = 5$mg/ml.

   2:9 resulted in $\frac{2}{11} \times 55 = 10$mg/ml and so on.

At the end of the dilution process, the diluted extracts were stored in different labeled sterile bottles appropriately.

3. **Preparation of the hot water Extract:** The procedures described in (1) and (2) above were repeated for the hot water extract but with hot water (100^{Oc}) as the extracting solvent.
4. **Preparation of the Ethanol Extract**: The procedures described in (1) and (2) above were repeated for the ethanol extract but with 96% ethanol as the extracting solvent.
5. **Preparation of Nutrient broth:** The nutrient broth was prepared from an anhydrous powder according to the manufacturer's specifications and directions. 1.3gram of the anhydrous nutrient broth was weighed and dissolved in 100ml of distilled water in a conical flask. This was covered with aluminum foil and autoclaved for 15mins at 121^{o}C and 15psi. The nutrient broth was brought out of the autoclave, allowed to cool to 45Oc then poured into 5 sterile bottles and corked.
6. **Sub culturing of the test organisms in nutrient broth**: Each of the test organisms were picked from its pure culture in the plate and subculture into nutrient broth in McCartney bottle assigned to each of them using sterile inoculating loop. The bottles were Stoppard and incubated for 24h at 37^{o}C.

7. **Susceptibility test:** The method described by Pelczar and Chan (1977), Chesbrough(1984) was adopted. 45 nutrient agar plates were prepared and allowed to solidify. The broth culture for each of the test organisms was the swabbed into the nutrient agar plates assigned to then using sterile cotton swab.6mm disc were cut from Whatman no 1 filter paper using a paper perforator. They were the poured in a beaker, covered and autoclaved for 15 minutes. They were then transferred to the hot air oven where they underwent drying. 15 of the sterile disc were soaked in each of the diluted cold water extract.

- Five of the swabbed nutrient agar plates (each of which contains one of the test organisms) were selected and unto each of the plates, six extract embedded disc of different cold water extract concentrations were placed using sterile forceps. The disc was then pressed slightly to adhere unto the medium surface with the forceps. This was repeated on another two sets of five swabbed nutrient agar plate to make a three plates of each test.
- The procedure above was repeated for the hot water extract.
- For the ethanolic extract, the Kirby-Bauer method (Katileen, 2008 and Prescott et al., 2005) was applied. The sterile discs were soaked in different ethanol dilutions. The impregnated discs were then arranged on a tile that was disinfected with 96% ethanol. The tile with the disc was then placed in the oven and heated for 5mins at 45Oc to evaporate the ethanol. Thereafter, the dried discs were placed on the swabbed nutrient agar plate just as above stated method. Each of the plates was left to stand for 20mins for proper diffusion of extract to take place. The plates were then incubated at 37Oc for 24h.

Thereafter, the zones of inhibition produced by the extracts were measured with a transparent meter rule and the MIC (minimum inhibitory concentration) of each of the extracts against each of the test organisms determined and the results tabulated.

Result

The following results were obtained at the end of the research work. Table 1 shows the results of the 15 biochemical tests used to identify the organisms. Table 2, 3 and 4 contain detailed results of the susceptibility test carried out for different extracts of Xylopia aethiopica.

Table 1- Biochemical test results for the test organisms (Bacteria)

Parameter	Results			
	Escherichia coli	Klebsiella pneumonia	Staphylococcus aureus	Pseudomonas aeroginesa
Gram reaction	-ve	-ve	+ve	-ve
Motility	+ve	-ve	-ve	+ve
Catalase	+ve	+ve	+ve	+ve
Coagulase	-ve	-ve	+ve	-ve
Indole	+ve	-ve	+ve	+ve
Urease	-ve	+ve	+ve	+ve
Nitrate	+ve	+ve	+ve	-ve
Citrate	-ve	+ve	+ve	-ve
Methyle red	+ve	-ve	-ve	-ve
Voges-proskauer	-ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	-ve
Lactose	+ve	+ve	+ve	-ve
Maltose	+ve	+ve	+ve	-ve
Mannitol	+ve	+ve	+ve	+ve

Table 2- Susceptibility test Result for Ethanol Extract of Xylopia aethiopica

Test organisms	Conc.(mg/ml)	Zones of inhibition(mm)				MIC(mg/ml)
		A	B	C	MEAN	
Staphylococcus aureus	5	-	-	-	-	-
	10	-	-	-	-	-
	20	11.0	10.5	11.5	11.0	20

	30	11.0	13.0	12.0	12.0	-
	40	12.5	12.0	12.0	12.5	50
	50	13.5	14.50	15.0	15.0	-
Escherichia coli	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	-	-	-	-	-
	40	-	-	-	-	-
	50	9.0	8.0	8.5	8.5	50
Candida albicans	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	8.0	8.0	8.0	8.0	30
	40	10.5	10.0	11.0	10.5	-
	50	12.0	11.0	13.0	12.0	-
Klebsiella Pneumonia	5	-	-	-	-	-
	10	-	-	-	-	-
	20	8.5	9.5	9.0	9.0	20
	30	10.0	10.3	9.5	10.0	-
	40	10.3	12.3	11.5	11.5	-
	50	12.3	13.0	14.0	13.0	-
Pseudomonas aeroginesa	5	-	-	-	-	-
	10	-	-	-	-	-
	20	9.0	9.0	10.5	9.5	20
	30	11.0	12.0	10.0	11.0	-
	40	13.0	12.5	10.5	12.0	-
	50	14.5	14.0	15.0	14.5	-

Table 3- Susceptibility test Result for cold Extract of Xylopia aethiopica

Test organisms	Conc.(mg/ml)	Zones of inhibition(mm)				MIC(mg/ml)
		A	B	C	MEAN	
Staphylococcus aureus	5	-	-	-	-	-
	10	-	-	-	-	-

	20	9.0	10.0	8.0	9.0	20
	30	11.5	10.0	11.5	11.0	-
	40	12.0	11.5	12.5	12.0	-
	50	13.5	11.5	12.5	12.5	-
Escherichia coli	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	-	-	-	-	-
	40	-	-	-	-	-
	50	-	-	-	-	-
Candida albicans	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	-	-	-	-	-
	40	8.0	9.5	9.5	9.0	40
	50	10.5	9.5	11.50	10.5	-
Klebsiella Pneumonia	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	10.5	10.5	9.0	10.0	30
	40	11.0	10.5	10.0	10.5	-
	50	13.0	12.0	11.0	12.0	-
Pseudomonas aeroginesa	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	10.0	9.0	11.0	10.0	30
	40	10.5	11.0	11.5	11.0	-
	50	13.5	12.5	13.0	13.0	-

Table 4- Susceptibility test Result for Hot water Extract of Xylopia aethiopica

Test organisms	Conc.(mg/ml)	Zones of inhibition(mm)				MIC(mg/ml)
		A	B	C	MEAN	
Staphylococcus aureus	5	-	-	-	-	-
	10	8.5	8.0	9.0	8.5	10

	20	9.0	10.5	9.9	9.5	-
	30	11.0	11.0	11.0	11.0	-
	40	12.5	10.5	11.5	11.5	
	50	13.0	12.0	14.0	13.0	-
Escherichia coli	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	-	-	-	-	-
	40	-	-	-	-	-
	50	-	-	-	-	-
Candida albicans	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30					
	40	9.0	11.5	9.5	10.0	40
	50	12.0	11.5	11.0	11.5	-
Klebsiella Pneumonia	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	9.5	11.0	9.5	10.0	30
	40	11.5	10.5	11.0	11.0	-
	50	12.0	12.0	12.0	12.0	-
Pseudomonas aeroginesa	5	-	-	-	-	-
	10	-	-	-	-	-
	20	-	-	-	-	-
	30	9.0	10.0	11.0	10.0	30
	40	11.5	12.0	10.5	12.0	-
	50	12.5	13.5	13.0	13.0	-

From the MIC values obtained for tables 2, 3 and 4, it is evident that the ethanolic extract is the most effective of the extracts.

Table 5- Comparison of the minimum inhibitory concentration (MIC) of the three extracts against the test organisms.

Test Organisms	Minimum Inhibitory Concentration(mg/ml)		
	Ethanol extract	Hot water extract	Cold water extract
S.aureus	20	10	20
Ecoli	50	-	-

C.albicans	30	40	40
K. pneumoniae	20	30	30
P.aeroginesa	20	30	30

The table 5 above shows the comparison of the antimicrobial effectiveness of the three extracts of Xylopia aethiopica in terms of its minimum inhibitory concentration (MIC). It is also an evident that the test organisms were most susceptible to the ethanolic extract. They exhibited the same level of sensitivity to the two aqueous extracts (cold water and hot) except Staphylococcus aureus which showed a very high sensitivity to the hot water extract.

Table 6- Comparison of the minimum inhibitory concentration (MIC) of the three extracts against the test organisms using one way ANOVA.

Source of variation	Sum of squares	Degree of Freedom	Mean of squares	Fcal	Fcrit
Between samples	10.5	4	2.625	64.7	
Within samples(Enor)	1358.7	8	169.8		
Total	1369.2	12			

Discussion

The antimicrobial activities of the cold water, hot water and ethanolic extracts of Xylopia aethiopica was tested against a species of Gram positive bacteria (Staphylococcus aureus) and species of Gram negative bacteria Klebsiella Pneumoniae, Pseudomonas aeroginesa, Esherichia coli) and yeast –like fungus (Candida albicans).It can be seen from the result in table 5 that ethanol extract exhibited lower MICs against the test organisms than their corresponding cold water and hot water extracts. The exception being S.aureus which showed greater susceptibility to hot water extract. The findings suggest that the antimicrobial agent in Xylopia aethiopica that affects S.aureus is more soluble in hot water than in cold water and ethanol.

The result also showed that E.coli was resistance to the extracts and all the dilutions except the ethanol extract of concentration 50mg/ml (see table 5). This poor sensitive of E.coli to the extracts confirms previous reports (Maurice et al., 1999, Ijeh et al., 2005 and Fleisher et al., 2008) and further suggest that the ethanol extract could be used only at very high concentration in the treatment of infection caused by E.coli. Also, the cold water extract and hot water hot extract exhibited similar MIC against the test organisms except on S. aureus where the hot water extract exhibited lower MIC. But from the data in tables 3 and 4, some of the hot water dilutions produced a slightly larger zone of inhibition than their corresponding cold water dilutions, implying that the hot water extract has a slight higher antimicrobial activity than the cold water extract.

It can also be deduced from the result that S.aureus showed the greatest susceptibility to the extract, while E.coli showed the least susceptibility, followed by C. albicans. These agrees with the findings of (Fleisher et al., 2008) and the report of Maurice et al., 1999) and suggest that Xylopia aethiopica will be very effective for the treatment of S.aureus infection. Therefore, there is significant difference in the antimicrobial activities of the three extracts of Xylopia aethiopica (see table 6).

Conclusion

In conclusion, Xylopia aethiopica extract are very effective antimicrobial agent against a wide range of microorganisms (both bacteria and fungi).Therefore if the active ingredients would be extracted it could serve as a broad spectrum antimicrobial drug that would be used for treating many kinds of disease like candidiasis etc.

Acknowledgement

The authors acknowledged the Almighty God for wisdom and knowledge.

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