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Antibacterial Activities of Extracts of Cinnamomum Cassia and Xylopia aethiopica on Clinical Pathogens.

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Abstract
The study was conducted to investigate the antibacterial activities of the mixture of extracts of *Cinnamomum cassia* and *Xylopia aethiopica* against seven clinical bacterial isolates *Bacillus subtilis, Streptococcus faecalis* and *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae* and *Pseudomonas aeruginosa* using the Agar well diffusion method. In addition, the possible synergistic effects when associated with antibiotics were studied. The preliminary phytochemical screening revealed the presence of cardiac glycoside, flavonoids, phlobatannins, tannins, saponin and steroids in varying concentrations. The combined ethanol extracts of S. *cassia* and *X. aethiopica* at all concentrations showed no antibacterial activity against *S. aureus, P. aeruginosa, K. pneumoniae, S. faecalis* and *E. coli.* However, at a concentration of 50mg/ml - 150mg/ml the extracts inhibited the growth of *S. dysenteriae* and *B. subtilis*. The combined ethanol extracts of *S. cassia* and water extract of *X. aethiopica* only had moderate antibacterial activity against *S. dysenteriae* and *B. subtilis* .The antibiotics in this study had low to very high inhibitory effects on the bacteria species tested except Ampicillin that had no effect on *P. aeruginosa.* The combined effect of ethanol extracts of *S. cassia* and *X. aethiopica showed no synergistic effect on all tested organisms;* the combined effect of ethanol extract of *S. cassia* and *X. aethiopica also showed no synergistic effect on all tested organisms*
Keywords: *Cinnamomum cassia, Xylopia aethiopica*, antibiotics, pathogens and Synergism.

1. Introduction

Cinnamon (Cinnamomum cassia) is from the family Lauraceae and is widely grown in tropics. The inner bark is a pungent, sweet, hot herb that stimulates the circulatory system, improves digestion, relieves spasms and vomiting, and controls infections. (Ayfer and Ozlem, 2003). It is also used for colds, influenza, fevers, arthritic and rheumatic complaints. Other medicinal and health benefits of cinnamon include lowering of cholesterol, reduces blood sugar, reduces heart diseases, fighting of cancer among others (Cralg,1999;Vangalapati *et al*.,2012). The essential oil and aqueous preparation of *Cinnamomum cassia* barks have also been reported to possess great antimicrobial properties (Nassia and Perween, 2006*)*.

Xylopia aethiopica (Dunal) belongs to the family Annonaceae, it is a valuable medicinal plant that is widely distributed in the West African. Almost every morphological part of the plant is used in traditional medicine for managing various ailments such as skin infections, candidiasis, dyspepsia, cough and fever (Ghana Herbal Pharmacopoeia, 1992; Mshana *et al.,* 2000). The essential oil as well as the crude extracts (both alcoholic and aqueous) of the plant have been shown to have antimicrobial property against a wide range of Gram positive and Gram negative bacteria, and *Candida albicans* (Thomas, 1989; Tatsadjieu *et al.,* 2003; Asekun and Adeniyi, 2004; Okigbo *et al.,* 2005).

Various parts of the plant have been traditionally employed in different therapeutic preparations. Sometimes, a combination of *X. aethiopica* with other plant types or a combination of different parts of *X. aethiopica* is used to achieve the desired effects (Fall *et al*., 2003; Ogunkunle and Ladejobi, 2006).

This study was carried out to determine the synergistic antibacterial potencies of ethanol and aqueous extracts of both plants, powdered bark of *C. cassia* and *X. aethiopica*., the synergism between the plant extracts and antimicrobial drugs was also investigated.

2. Materials and Methods

2.1 Collection and Identification of Plant Samples

The fruits of *X. aethiopica* and bark of Cinnamon (*Cinnamomum cassia*) were collected from Ago- Iwoye and Sagamu (Ogun State), identified and authenticated in the Elikaf herbarium of the Department of Plant Science in Olabisi Onabanjo University, Ago- Iwoye, Ogun State. The fruits of *X. aethiopica* and bark of Cinnamon (*C. cassia*) were grinded into powdered form and kept in separate airtight containers.

2.2 Extraction method

500g of the powdered bark of cinnamon was macerated in 1litre ethanol; 300g of the powdered fruit of *X. aethiopica* was also macerated in 1litre of a 99.7% ethanol while 200g of *X. aethiopica* was macerated in 1litre of sterile water.

2.3 Test Microorganisms

The test organisms used in this study: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Shigella dysenteriae, and Streptococcus faecalis* obtained from Nigeria Institute of Medical Research Centre (NIMR), Lagos, Nigeria. The bacterial isolates were first sub cultured on a nutrient agar and incubated at 37°C for 24hrs.

2.4 Preliminary Phytochemical Screening

Phytochemical screening for major constituents was undertaken using qualitative methods in accordance with Trease and Evans (1989) and Sofowora (1993). The plant materials were screened for the presence of flavonoids, phenol, terpenoids, phlobatannins, anthraquinones, cardiac glycoside, tannins, saponin, steroids and alkaloids.

2.5 Combined Effects of the Plant Extracts on the Test Organisms:

Antibacterial effect of the combined plant extracts was determined by the agar well diffusion technique. An overnight bacterial culture was obtained by sub culturing from the steady agar slope and inoculating into 5ml of sterile nutrient broth. The inoculated broth culture was incubated into sterile nutrient agar in petri-dishes using flood method. A sterile cork borer of 6mm diameter was used to bore wells into the seeded solidified agar and 2drops each of 25mg/ml, 50mg/ml, 100mg/ml and 150mg/ml (in which half of both *C. cassia* and *X. aethiopica* extracts were measured to give each concentration) of plants extracts were pipetted into the wells including the controls i.e. 70% of ethanol and water in separate wells.

The plates were left in order to allow proper pre-diffusion of the plant extracts and controls into the medium after which the plate were incubated at 37^{o}C for 24hrs. The zones of inhibition were then measured and recorded.

2.6 Effect of Combination of Plant Extracts and Antibiotics on Test Organisms

The cultured bacteria was poured and spread on the nutrient agar using flood method. A sterile cock borer of 6mm diameter was used to bore wells into each media. The mixture of different antibiotic drugs (Erythromycin, Ampicillin, Ciprofloxacin and Gentamicin) at the same concentration each (6.25mg/ml) and plant extracts were introduced into the wells. Plates were incubated at 37^{o}C for 24hrs and the zone of inhibition were measured and recorded.

3.0 Results

The phytochemical constituents of cinnamon bark powder revealed the presence of cardiac glycoside, flavonoids, phenol, phlobatannins, anthraquinones, tannins, saponin, steroids and alkaloids and absence of terpenoids while the fruit of X. showed the presence of cardiac glycoside, flavonoids, phlobatannins, tannins and steroids but absence of phenol, terpenoids, anthraquinones and alkaloids (Table 1).

Table 1: Phytochemical constituents of cinnamon bark powder and fruits of *X aethiopica*

Phytochemical Tests	Cinnamom spices	Xylopia aethiopica
Cardiac glycoside	++	+++
Flavonoids	+++	++++
Phenol	++++	-
Terpenoids	-	-
Phlobatannins	+++	+++
Anthraquinones	++	-
Tannins	++++	++++
Saponin	++	++
Steroids	++++	+++
Alkaloids	++	-

- = absent **+++ = present**

++ = slightly present **++++ = strongly present**

Table 2 shows the combined effect of extracts of *C. senna* and *X. aethiopica* on tested organisms, the ethanol extract of plants had no inhibitory effect on *P. aeruginosa, K. pneumoniae, S. faecalis* and *E. coli.* However, at 50mg/ml and above; the growth of *S. dysenteriae* and *B. subtilis* were inhibited. The combined effect of ethanol extracts of cinnamon and aqueous extract of X. showed antibacterial activity at 100mg/ml and 150mg/ml against *B. subtilis* and *E. coli* while no zone of inhibition was recorded for other tested organisms.

Table 2: Combined effect of extracts of *C. cassia* and *X. aethiopica* on tested organisms

Extracts	Conc. mg/ml	*S. aureus*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. dysenteriae*	*S. faecalis*	*E. coli*
Combination of ethanol extract of cinnamon and ethanol extract of *X. aethiopica*	25	6.00	6.00	6.00	6.00	6.00	6.00	6.00
	50	6.00	6.00	6.00	20	17	6.00	6.00
	100	6.00	6.00	6.00	20	17	6.00	6.00
	150	6.00	6.00	6.00	20	17	6.00	6.00
	Ethanol	6.00	6.00	6.00	6.00	6.00	10	15
Combination of ethanol extract of cinnamon and water extract of *X. aethiopica*	25	6.00	6.00	6.00	6.00	6.00	6.00	6.00
	50	6.00	6.00	6.00	6.00	6.00	6.00	6.00
	100	6.00	6.00	6.00	14	6.00	6.00	10
	150	6.00	6.00	6.00	15	6.00	6.00	10
	water	6.00	6.00	6.00	6.00	6.00	6.00	6.00

Size of cork borer: 6.00mm

Table 3 is the combined effect of extracts of Cinnamon, X. *aethiopica* and antibiotics on tested organisms. The antibacterial effect of antibiotics was positive against all test organisms except Ampicillin that had no activity against *P. aeruginosa*. The combined effect of ethanol extracts of *C. senna*, X. *aethiopica* and antibiotics showed greater zone of inhibition against all test organisms except, for Ampicillin that recorded lower zones of inhibition against *B. subtilis* and *S. dysenteriae*. The table also showed the combined effect of ethanol extract of cinnamon, aqueous extract of X. *aethiopica* and antibiotics; Erythromycin and the extracts did not have effect on *S. faecalis, B. subtilis,* and *K. pneumoniae*. Ampicillin and the extracts did not have effect on *P. aeruginosa*.

Table 3: Combined effect of extracts of *C. cassia*, *X aethiopica* and antibiotics on tested organisms

Plant extracts	Antibiotics	*S. aureus*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. dysenteriae*	*S. faecalis*	*E. coli*
	Erythromycin	20	15	18	43	16	32	42
	Ciprofloxacin	40	40	36	43	18	40	49
	Ampicillin	21	6.00	22	18	12	20	20
	Gentamicin	29	29	21	30	28	23	29
Ethanol extract of Cinnamon+ ethanol extract of *X. aethiopica* + antibiotics	Erythromycin	27	21	16	18	14	16	21
	Ciprofloxacin	30	36	21	32	32	33	39
	Ampicillin	15	16	29	20	16	15	38
	Gentamicin	18	30	22	21	28	18	31
Ethanol extract of Cinnamon+ aqueous extract of *X. aethiopica* + antibiotics	Erythromycin	11	14	6.00	6.00	14	6.00	15
	Ciprofloxacin	35	35	30	35	12	30	35
	Ampicillin	15	6.00	10	12	11	19	11
	Gentamicin	25	33	20	20	15	25	22

Size of cork borer: 6.00mm

4.0 Discussion

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the

combinations of secondary products present in the plant.

In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids which are capable of producing definite physiological action on body. (Joshi *et al.,*2009). Plants with anti-bacterial effect are rich in polyphenolic substances such as tannins, alkaloids, steroids and polyphenolic acids. These phenolic substances as well as the alkaloids in plants have been listed as the most important bioactive constituents of natural products (Edeoga *et al.,* 2005) which are useful supplements used for the maintenance of human health (Kumar *et al.,* 2005) and sometimes with remarkable therapeutic potentials.

The presence of these metabolites suggests great potential for *C. cassia* and *X. aethiopica* as a source of useful phytomedicine. The presence of Tannins shows it is an astringent, which help in wound healing and anti-parasitic. Alkaloids are known to possess anti-malaria property (Ronan *et al*., 2009). Plant is a good source of anti-malaria for which it is already traditionally being used for. The presence of Phenols contributes to the development of color, taste and palatability, as well as the defense system of plants (Tarnai *et al*., 1994), including their antimicrobial activity (Ejechi *et al*., 1998). Literature search reveals that spices are able to prevent or retard the growth of microorganisms (Nychas, 1995). The mechanisms responsible for the antimicrobial activity of phenol include adsorption and disruption of microbial membranes, interaction with enzymes and substrate coupled with metal ion deprivation (Fattouch *et al*., 2007). The presence of Cardiac glycosides shows their use in the treatment of congestive heart failure and cardiac arrhythmia. The presence of Flavonoid compounds exhibit inhibitory effects against multiple viruses and other microorganisms. The presence of tannins, saponins, anthraquinones, alkaloids and phenols reported by Okogun (1986) implicated these and other chemicals in the curative values of many Nigerian medicinal plants in use. Thus, from the overall results obtained, it is evident that the two plants screened possess anti-microbial agents active against some pathogenic organisms associated with skin infections. They therefore justify their popular use by local herbalist in the treatment of skin diseases.

It has been reported by Ezeifeka *et al*., (2004) that the ethanol extract of *X aethiopica* leaves had activity against *S. aureus, E. coli* and *P. aeruginosa* but the aqueous extract of the same had no effect on all the bacteria except *S. aureus* while in this study the synergistic antibacterial effect of the mixture of ethanol extract of cinnamon and ethanol extract of X. *aethiopica* also showed activity against *S. aureus* but had no effect against *E. coli* and *P. aeruginosa* but the synergistic antibacterial effect of the mixture of ethanol extract of cinnamon and aqueous extract of xylopia showed activity against *E. coli* and had no effect against *S. aureus* and *P. aeruginosa*.

This work also agrees with the work of Ezeifeka *et al.,* (2004) who concluded that the ethanol extracts of the *X. aethiopica* plant parts showed more inhibitory effects than the aqueous extracts which indicated that the active ingredients of the plants were better extracted with ethanol than water. This observation was also seen in the extracts of *C. cassia*, in that the synergistic antibacterial effect of the mixture of ethanol extract of cinnamon and ethanol extract of X. *aethiopica* showed more inhibitory effects than the synergistic antibacterial effect of the mixture of ethanol extract of cinnamon and aqueous extract of *X. aethiopica*

The ability of plants extracts of *C cassia* and *X. aethiopica* to inhibit microorganisms explains why they are used in folk medicines in Nigeria, Ghana and other tropical countries (G.H.P., 2007).

This study agrees with Nweze and Onyishi (2009) who reported that *P. aeruginosa* showed resistance to Ampicillin while the other conventional antibiotics Gentamicin and Ciprofloxacin had inhibitory effects on the same organism.

The synergistic antibacterial effect of the mixture of ethanol extract of cinnamon and ethanol extract of *X. aethiopica* with antibiotics showed more antibacterial activity than the synergistic antibacterial effect of the mixture of ethanol extract of cinnamon and ethanol extract of *X. aethiopica* without antibiotics. Also the synergistic antibacterial effect of the mixture of the ethanol extract of cinnamon and aqueous extract of *X. aethiopica* with antibiotics showed more antibacterial activity than the ethanol extract of cinnamon and aqueous extract of *X. aethiopica* without antibiotics.

5.0 Conclusion

This work has justified the ethno -botanical uses of both plant extracts due to their chemical constituents and also encourages the combination of drugs with the plant extracts since synergism in this study produced higher inhibitory effect on the organisms tested.

REFERENCES

Asekun, O. T.and Adeniyi, B. A. (2004). Antimicrobial and cytotoxic activities of the fruit essential oil of Xylopia aethiopica from Nigeria , *Fitoterapia,* 75: 368-370

Ashish S, Mohit S.M andSharma, K. (2011) Antibacterial activity of commercial and wild cinnamon species. *Journal of phytology* 2011, 3(2): 102-106.

Ayfer,A. D and Ozlem, T.E.(2003). Antimicrobial Activities of Various Mediciolinal and Commercial Plant

Extracts. *Turk J Biol*. 27 (2003) 157-162.

Azumi, S., Tanimura, A., and Tanamoto, K. (1997). A novel inhibitor of bacterial endotoxin derived from cinnamon bark. *Biochem. Biophys. Res. Commun*. 234:506-510.

Cralg, W.J. (1999). Health- promoting properties of common herbs. *Amer. J. Clin. Nutr*., 70(3): 4915-4995.

Edeoga,H.O, Okwu, D.E and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants, *Afr. J. Biotechnol.* 4(7): 685-688

Ejechi, B. O., Souzey, J. A and Akpomedaye, D. E. (1998). Microbial stability of mango (*Mangiferaindica* L.) juice preserved by combined application of mild heat and extracts of two tropical spices. *J. Food Prot.*, 61, 725–727 (1998).

Ezeifeka, G. O., Orji, M. U., Mbata, T. I. & Patrick, A. O. (2004). Antimicrobial Ativities

of Cajanus cajan, Garcinia kola and Xylopia aethiopica on Pathogenic Microorganisms, *Biotechnology*, 3(1): 41 - 43

Fall, D., Badiane, M., Ba, D., Loiseau, P., Bories,C., Gleye, C., Laurens,A and Hocquemiller, R .(2003). Antiparasitic activities of Senegalese Annonacae used in traditional medicine. *Dakar Med*. 48(2): 112-116.

Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C. I. G., Angioni, A., Dessi, S.Marzouki, N. and Cabras, P. (2007). Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *J. Agric. Food Chem.*, 55; 963–969

Ghana Herbal Pharmacopoeia (1992). Policy Research and Strategic Planning Institute (PORSPI) The Advent Press, Accra, 150-152

Ghana Herbal Pharmacopeia (2007). Science and Technology Policy Research Institute and Council for Scientific and Industrial Research. Qualitype Ltd, Accra, pp. 15-17, 30-34, 83-86, 108-111, 118-121, 226-229

Joshi, B., Lekhak, S. and Sharma A. (2009). Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu University Journal of Science, Engineering and Technology*, 5(1): 143 – 150.

Kumar, R.S., Sivakuma, T, Sunderem, R.S, Gupta, M., Murugesh, K., Rajeshwa, Y.,

Kumar, M.S and Kumar, K.A. (2005). Antioxidant and antimicrobial activities of Bauhinia recemosa L. stem bark. Braz. *J. Med. Biol. Res.* 38: 1015-1024.

Mshana, N. R., Abbiw, D. K., Addae-Mensah, I., Adjanouhoun, E., Ahyi, M. R. A., Ekpere, J. A., Enow-Orock, E. G., Gbile, Z. O., Noamesi, G. K., Odei, M. A., Odunlami, H., Oteng-Yeboah, A. A., Sarpong, K., Sofowora, A. and Tackie, A. N. (2000). Traditional Medicine and Pharmacopoeia, Contribution to the revision of ethnobotanical and Floristic Studies in Ghana, OAU/STRC Technical Report, 67.

Nassia,M.A.C and Perween,T.(2006)Antimicrobial activity of Cinnamomum cassia against diverse microflorawith its nutritional and medicinal imparts. *Pak. J.Bot.*, 38(1):169-174

Nweze, E.I. and Onyishi, M.C. (2009). In vitro antimicrobial activity of ethanolic and methanolic fruit extracts of *Xylopia aethiopica* and its combination with disc antibiotics against clinical isolates of bacteria and fungi.. *J Rural Trop Public Health* 9:1–6

Nychas, G. J. E. (1995). *Natural antimicrobials from plants.* In: *New Methods of Food Preservation*;Gould, G. W., Ed.; Blackie Academic & Professional, London, U. K., 1995; pp. 58–89.

Ogunkunle, A.T.S and Ladejobi, T.A (2006). Ethnobotanical and phytochemical studies on some species of Senna in Nigeria. *Afr. J. Biotechnol.* 5(21): 2020-2023

Okigbo, R.N., C.S. Mbajiuka and C.O. Njoku (2005). Antimicrobial Potentials of (UDA) *Xylopia aethopica* and *Ocimum gratissimum* L. on Some Pathogens of Man, Intern. *J. Mol. Med. Advance Sci.* 1 (4): 392-397

Okogun, A. (1986). The Chemistry of Nigeria Medicinal Plants. In: The state of medicinal plants in Nigeria (Ed.) Abayomi Sofowora, Ibadan University press, Nigeria, p. 31 – 52.

Sofowora A (1993).Medicinal plants and Traditional Medicine in Africa; Spectrum Books Limited, Ibadan Nigeria. pp:289.

Tarnai, E. A., Pagliuca, G., Piretti, M. V., Cipollone, M. (1994). Systematic investigation ofpolyphenol compounds from different parts of cherry tree (*Prunus avium*), *Fitoterapia*, 65:541–548.

Tatsadjieu, L.N., Essia Ngang, J.J., Ngassoum, M.B and Etoa ,F.X (2003). Antibacterial and antifungal activity of *Xylopia aethiopica, Monodora myristica, Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* from Cameroon, *Fitoterapia*.74: 469-72

Thomas, O. O. (1989). Re-examination of the antimicrobial activities of *Xylopia aethiopica, Carica papaya, Ocimum gratissimum* and *Jatropha curcas*. *Fitoterapia,* 60: 147-156

Trease, G.E and Evans,W.C. (1989) Textbook of Pharmacognosy. 12th Edn. Balliere, Tinadl London.

Vangalapati, M.Satyan,S., SuryaPrakash,D.V and Avanigadda,S.(2012).A review of

pharmacological activities and clinical effectd of Cinnamon species. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.*3(1):653-663