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COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITIES OF FRUITS OF *Xylopia aethiopica* (Dunal) A. RICH AND BULB OF *Crinum jagus* (Thompson) DANDY

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ABSTRACT

Xylopia aethiopica and Crinum jagus are medicinal plants which had been used traditionally for various ailments such as cough, amenorrhea, stomach ache and urinary ailments. The aim of this study was to compare the antibacterial activities of the ethanolic extracts of fruits of X. aethiopica with ethanolic extracts of bulb of C. jagus. Agar well diffusion method was used against the following organisms: K. pneumonia, E. coli, Ps. aeruginosa, S. aureus, and B. subtilis. Both plant extracts demonstrated concentration dependent activities against all the bacteria tested. Fruits of X. aethiopica was observed to be more active against S. aureus while bulb of C. jagus showed greater activities against E. coli, K. pneumonia and Ps. aeruginosa. The two plant extracts showed almost the same level of activity against B. subtilis. Phytochemical screening revealed the presence of tanins and cardiac glycosides in X. aethiopica which were absent in C. jagus. In conclusion X. aethiopica showed greater activity against Gram-positive bacteria while C. jagus was more active against Gram- negative bacteria.

Keywords: medicinal plants, extracts, Gram-positive, Gram-negative.

INTRODUCTION

Xylopia aethiopica (Dunal) A. Rich (Annonaceae) is a tree of 20 meter high and about 60 - 70 cm in diameter with a clear straight stem. *X. aethiopica* is commonly known in English as Negro pepper, West African pepper tree or Senegal pepper. It is a native to the low land, rain forest and moist fringe forest in the savanna zones of Africa. *X. aethiopica* is largely found in West, Central and Southern Africa (Maurice, 1993; Burkill, 1995, Orwa et al., 2009).The fruits are peppery and often split down one side when ripe. Traditionally the fruits of this plant are commonly used for therapeutic purposes against cough, dizziness, amenorrhea, stomach-aches, lumbago, bronchitis, neuralgia, rheumatism, dysentery, uterine fibroid, and malaria (Burkill, 1995; Soh *et al*., 2013; Woode *et al.,* 2012).

The chemical constituents of *X. aethiopica* include alkaloids, saponins, sterols, glycosides, flavonoids, terpenoids, fixed oils, volatile and aromatic oils (Ezekwesili et al., 2010; John-Dewole *et al*., 2012).

Crinum jagus (Thompson) Dandy (Amaryllidaceae) is a bulbous plant with thin wide spreading, rich green leaves found in tropical and subtropical regions of the world. It is usually cultivated in small patches in the bank of a stream or in a swamp forest. The local names are 'ogbedeodo' (Yoruba), 'alubarhain' (Edo) and 'oyimbakar' (Efik/Ibibio). (Mabbery, 1990; Adesanya *et al,* 1992). Traditionally, the bulb of *C. jagus* is used in urinary ailments, as purgative and remedy for tuberculosis (Akinyemi et al., 1986). The bulb is also effective in curing chronic cough, anti-snake venom activity, antioxidant and antihaemorrhagic activities had also been recorded (Ode and Asuzu, 2006; Ode et al., 2010). Lycorine, an important alkaloid present in *C. jagus* had been found to have a marked antifungal action. Other chemical constituents of *C. jagus* are saponins and alkaloids (Trease and Evans, 1989).

The increased rate of emergence of resistance to antibiotics is alarming despite the continuous increase in discovery of new antibiotics. In fact it has been reported that infectious disease is the major cause of death responsible for approximately one half of all deaths in tropical countries (WHO, 2013). This has given rise to the need for exploring medicinal plant since plant derived medicines are relatively safer than synthetic drugs. They offer profound therapeutic benefits and are more affordable (Anika et al., 2017). Both *X. aethiopica* and *C. jagus* are used traditionally as remedy for cough (Adjanohoum *et al*., 1991).

Cough is an upper respiratory tract infection, the respiratory tract can be infected by a variety of bacteria both Gram positive and Gram negative with the disease ranging from mild to severe (Sorde et al., 2011).

The aim of this study was to compare the antibacterial activities of ethanol extracts of fruits of *X. aethiopica* and bulb of *C. jagus* against some Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Collection, authentication and preparation of plant samples

Fruits of *Xylopia aethiopica* and the bulb of *Crinum jagus* were obtained from Oje market in Ibadan and were authenticated at the Forestry Research Institute of Nigeria (FRIN), Idi-Ishin, Ibadan, Oyo State, Nigeria with Forest Herbarium Institute No 12458 and 60898 respectively. The fruits and the bulbs were carefully dried in the oven at 44.5^{o}C for 4 hours. This method was used because it is one of the easiest and rapid thermal processing that can preserve phytochemicals (Azwanida, 2015). The dried samples were then grounded into fine powder using an electric blender. The powdered samples were stored in different containers and labelled appropriately.

Extraction of powdered sample

Fifty grams each of powdered fruits of *X. aethiopica* and bulb of *C. jagus* were extracted separately in soxhlet apparatus with ethanol for five hours. The extracts were concentrated to dryness and were kept at 4^{0} C for further use.

Phytochemical Screening

Phytochemical screening was carried out according to standard procedure as described by Trease and Evans, (1989) and Sofowora, (1993). The samples were screened for anthraquinone glycosides, cardiac glycosides, saponins, tanins, flavonoids and alkaloids.

Test Organisms

The test organisms: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* were obtained from Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Ibadan while *Klebsiella pneumonia* was obtained from Medical Microbiology Laboratory, University College Hospital, Ibadan. The cultures of these test organisms were purified and confirmed by microscopy and repeated sub-culturing on selective media.

Preparation of culture media

The culture media were aseptically prepared according to the manufacturer's instructions and autoclaved at 121 ^{0}C for 15 min.

Antibacterial activity

Agar well diffusion method was used. Discreet colonies of the test organisms on nutrient agar plates were emulsified in 3ml of sterile physiological saline and the turbidity adjusted to 0.5 McFarland standard (this is the barium sulphate standard against which test organisms turbidity are compared. (Cheesbrough, 2004). Using sterile swab sticks, the surface of Mueller Hinton agar (MHA) in a 90mm diameter plate was inoculated with the prepared inoculum by streaking the surface of agar in three directions, rotating the plate approximately 60 $^{\circ}$ to ensure even distribution. At equidistant positions, using a cork borer of 6mm diameter, six wells were made in the seeded Mueller Hinton agar plates. Different concentrations of plant extracts used was 80mg/ml, 40mg/ml, 20mg/ml and 10mg/ml; 0.2ml of the different concentrations were introduced into the wells and the plates were kept at room temperature for 1 hour to allow diffusion of the extracts into the agar surrounding the wells. The plates were incubated at 37 ^{o}C for 18 hours. Gentamicin 20µg/ml (broad spectrum antibiotic) and ampicillin 40µg/ml (narrow spectrum antibiotic) were used as reference while ethanol was used as control. All the tests were performed in duplicate and the antimicrobial activity was expressed as the mean diameter (mm) of clear zone produced by the plant extracts.

Statistical analysis

One-way ANOVA was used for the statistical analysis, the significant value was measured at P value > 0.05.

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening showed that the fruits of *X. Aethiopia* and bulb of *C. jagus* both had saponin, alkaloids and flavonoids as their chemical constituents. Cardiac glycosides and tanins were absent in *C. jagus* (Table 1). Previous reports on the chemical constituents of *X. aethiopica* which corresponds with the findings of this study include Harigan et al. (1994), Esekhiagbe et al. (2009), Ezekwesili et al. (2010), John Dewole et al. (2012), Nwaichi and Igbinobaro (2012). These chemical components may be responsible for the various pharmacological effects of these plants. Research has shown that saponin reduces blood cholesterol, boost oxidant activity and reduces risk of cancer (Hoost and Williams, 2008). Cardiac glycosides are used for management of heart problems (Prassas and Diamondis, 2008, Manunta and Ferrand, 2008) while flavonoids had been reported to have anti allergic, anti-cancer, anti-inflammatory and anti-viral activities (Morris and Zhang, 2006, Lee et al, 2005).

Table 1. Phytochemical constituents of fruits of *X. aethiopica* and bulbs of *C. jagus*

Phytochemical Tests	***X. aethiopica***	***C. jagus***
Anthraquinone Glycoside	-	-
Cardiac glycoside		
i. Kedde test	+	-
ii. Keller -killiani test	+	-
Saponin		
i. Frothing	+	++
ii. Emulsification	+	++
iii. Hydrolysis	+	++
Tanins	+	-
Alkaloids	++	++
Flavonoids	++	++

Key: (+) = fairly present (++) = present in abundance (-) = absent

Antibacterial activity

In this study the ethanolic extracts of the fruits of *X. aethiopica* and bulb of *C. jagus* showed significant activities against all the bacteria tested. As shown in Table 2, the activities of the plant extracts were concentration dependent that is the antibacterial activity increased with increase in concentration. Fruits of *X. aethiopica* was observed to be more active against *S. aureus* while bulb of *C. jagus* showed greater activities against *E. coli, K. pneumonia* and *Ps. aeruginosa*. Figure 1 shows the detailed presentation of the antibacterial activities of the two plant extracts. The two plant extracts showed almost the same level of activity against *B. subtilis*. The statistical analysis showed that there was no significant difference in the activities of the plant extracts at the concentrations tested (P value > 0.05).

Table 2: Antibacterial activities of ethanol extracts of fruits of *X. aethiopica, C. jagus* and the standards showing diameter zones of inhibition (mm)

Extract	**Concentration (mg/ml)**	**Kp**	**Ec**	**Pa**	**Sa**	**Bs**
X. aethiopica	80	14	15	15	37	17
	40	14	14	14	30	17
	20	13	13	12	28	15
	10	12	12	12	25	12
C.jagus	80	20	20	18	30	16
	40	16	18	16	28	15
	20	15	17	15	26	13
	10	14	15	14	25	12
Ampicillin	40µg/ml	-	-	-	24	-
Gentamicin	20µg/ml	22	20	15	27	19

Key (-) = No zone of inhibition

Kp = *Klebsiella pneumonia,* Ec = *Escherichia coli,* Pa = *Pseudomonas aeruginosa,* Sa = *Staphylococcus aureus,* Bs = *Bacillus subtilis*

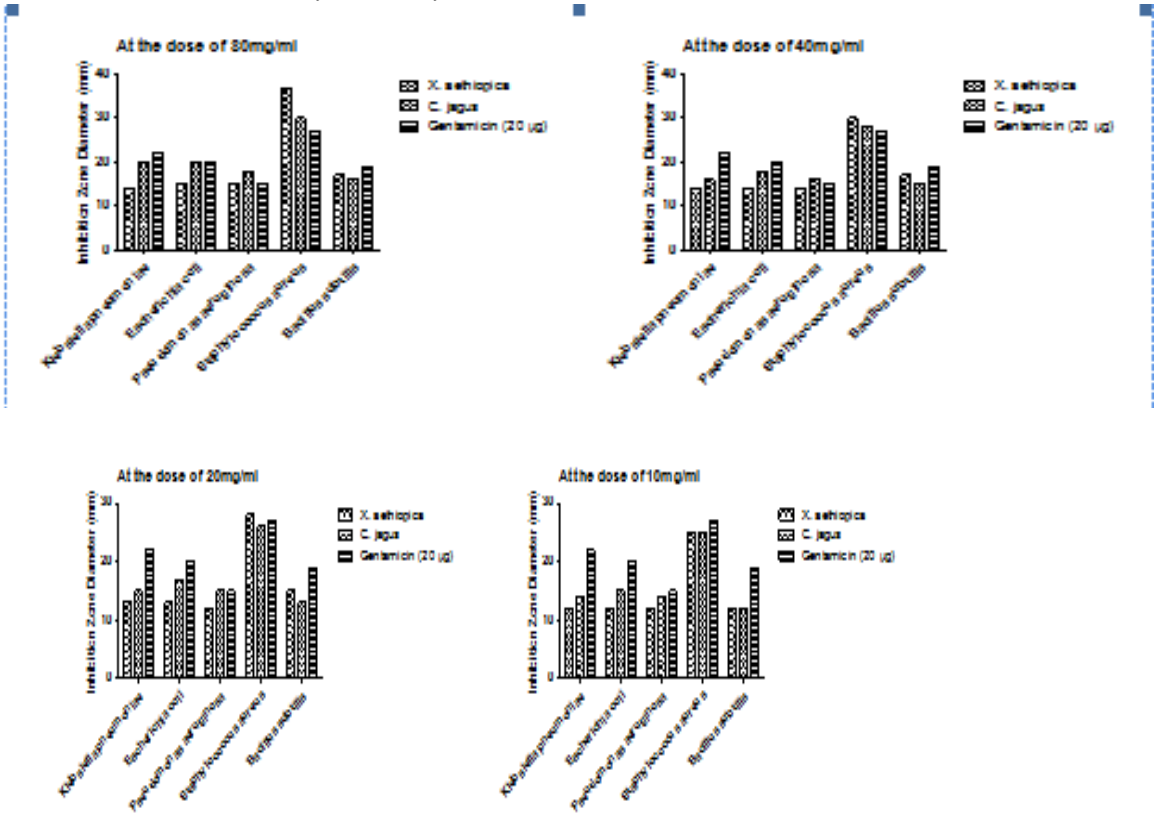

Figure 1: Antibacterial activities of ethanol extracts of fruits of *X. aethiopica* and bulbs of *C. jagus*

The activities of the ethanolic extracts of *X. aethiopica* against *S. aureus* is consistent with previous studies by Anika et al. (2017), Hassan et al. (2014), Ezeifeka et al. (2004), Asekun and Adeniyi(2004). However in a study by Ilusanya et al. (2012)on fruits of *X. aethiopica*, no activity was observed against *E. coli* and *K. pneumonia* while activity against *S. aureus* was only at a higher concentration of 150 mg/ml. Also the activities of the ethanolic extracts of *C. jagus* observed in this study was similar to that reported by Akintola et al. (2016) but no activity was reported against *E.coli.*

Ethanolic extracts of bulb of *C. jagus* showed more activity against Gram negative bacteria (*K. pneumonia, E. coli* and *Ps. aeruginosa*) compared with the fruits of *X. aethiopica* while greater activity was observed with *X. aethiopica* against Gram positive bacteria (*S. aureus* and *B. subtilis*). Comparing the standards used, Gentamicin showed broad spectrum activity against both the Gram positive and Gram negative bacteria. The difference between Gram positive and Gram negative bacteria is in their cell wall structure. Gram positive bacteria do not have an outer cell membrane of lipopolysaccharide and protein found in Gram negative bacteria also the cell wall of Gram positive bacteria is high in peptidoglycan (David and Peter, 2009). This difference in cell wall structure is also responsible for the difference in their susceptibility to the plant extracts and antibiotics.

The difference observed in the antibacterial activities of these two plant ethanolic extracts can be attributed to the difference in their chemical constituents. Both plants contained saponins, flavonoids and alkaloids, while tanins and cardiac glycosides were present in *X. aethiopica* but were absent in *C. jagus*.

CONCLUSION:

Ethanolic extract of the fruits of *X. aethiopica* and bulb of *C. jagus* showed concentration dependent activities against all the bacteria tested. However, bulb of *X. aethiopica* was more active against Gram positive bacteria while *C. jagus* showed greater activity against Gram negative bacteria.

Conflict of interest: There is no conflict of interest between the authors.

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