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Investigation of Bulb Extracts of *Crinum jagus* for Antibacterial and Antifungal Activities

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ABSTRACT: This study investigates the bulb extracts of *Crinum jagus* for antimicrobial activities. The bulb samples were dried, ground and subjected to successive extraction using hexane, ethylacetate and methanol. The extracts were screened for activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebisidlae pneumonae*, *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer* at concentrations between 6.25 and 200 mg/ml. Antimicrobial assays werecarried out using agar diffusion method. The Minimum Inhibitory Concentration (MIC) of the extracts was determined. The percentage yields obtained for the hexane, ethylacetate and methanol extracts of the bulbs are 0.28 %, 0.44% and 24.68 % respectively. Results showed that the methanolic extract had better antibacterial and antifungal activities than the other extracts. The methanol extract showed the highest antibacterial activity against *S. aureus* with a zone of inhibition of 28 mm at 200 mg/ml. Also, the methanol extract showed the highest antifungal activities against *C. albicans*, *A. niger* and *P. notatum* with a zone of inhibition of 20 mm at 200 mg/ml. The hexane and ethylacetate extracts had no activity against the microorganisms at 6.25 mg/ml.The methanol extract had the lowest Minimum Inhibitory Concentration (MIC) of 2.50 mg/ml against *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. typhi*. The methanol extract could be a source of potent antimicrobial compounds.

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Microbial infections are known to have profound economic impact on public health in tropical regions especially in immune-deficient or immunosuppressed patients. This challenge has been further compounded by the emergence of multidrug-resistant strains of these organisms. Searching for new antimicrobial agents with mechanisms of action differing from those of the drugs in market is therefore imperative (Alves *et al*., 2000). Plants are used in traditional medicine as decoction, infusion, tincture and crude extracts for the treatment of various diseases. So important are medicinal plants to the world's primary health care that the World Health Organization advocated that countries should interact with traditional medicines with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origin (Kaewseejan *et al*., 2012). Plants are therefore potential sources of potent antimicrobial agents. Many current research efforts have therefore focused on the isolation and characterization of potent antimicrobial compounds from plants. Herbal medicine has witnessed a tremendous surge in acceptance and public interest and is available not only in the drug stores but also in food stores and supermarkets (Zampini *et al*., 2009). Infact, traditional medicine programs are now being incorporated into the primary health care systems in Mexico, the People's Republic of China, Nigeria, and other developing countries (Verpoorte, 2000). However, as the global uses of medicinal products continue to grow, many of them remain untested for efficacy. The current study is a contribution towards bridging this gap. The plant *Crinum jagus*, belongs to the family *Amaryllidaceae*. It is known as '*Ogede-Odo*' in Southwestern Nigeria. It is employed in the treatment of skin wounds and ailments such as rheumatism, tuberculosis, witlow and as an antibacterial agent by herbalists in different parts of Nigeria. Despite the widespread use of the plant by herbalists for the treatment of microbial infections, there are no scientific reports on the antimicrobial activity of the plant. In this study, therefore, extracts of the bulb of *C. jagus* would be screened for antimicrobial activity using suitable assays. The results obtained would confirm if this plant part actually possess antimicrobial properties. If activity is observed, a scientific justification for the use of the

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plant by traditional healers of Southwestern Nigeria would have been provided.

MATERIALS AND METHODS

Sample Collection, Preparation and Extraction: The bulbs of *Crinum jagus* were collected from the Botanical Gardens of the University of Ibadan, Nigeria. The samples were identified by Mr. Owolabi, a taxonomist and the curator of the Botanical Garden. They were then dried under mild sunlight for about 6 weeks and subsequently ground to a particle size of 1000 µm. A 1kg portion of ground sample was extracted sequentially using 2.5 litres each of hexane, ethylacetate and methanol. Each extraction was carried out using the maceration method. The process lasted for three days/ solvent. The extracts were thereafter concentrated to dryness using a rotary evaporator. The percentage yield was calculated using the expression.

$$\%\, Yield = \frac{Weight\ of\ extract}{Weight\ of\ sample}\ x\ 100$$

Preparation of isolates: This was carried out as described by Adeniyi *et al*. (2013). Extracts were screened for antibacterial and antifungal activities against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi* and *Klebisidlae pneumonae*, *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*. All the organisms used are clinical isolates. A loop full of each organism was taken from the stock and inoculated into a sterile nutrient broth of 5 ml. This was followed by incubation for 18-24 hrs at 37ºC. From the overnight culture, 0.1 ml of each organism was taken and put into 9.9 mls of sterile distilled water to get 1:100 (10^{-2}) of the dilution of the organism.

Antibacterial assay (Agar - well diffusion method): This was carried out as described by Adeniyi *et al*. (2013). 0.2 ml of the diluted organism (10^{-2}) was taken into the prepared sterile nutrient agar which was at 45^0C. This was thereafter poured aseptically into sterile petri dishes and allowed to solidify for about 40-45 minutes. A standard cork borer, 8 mm in diameter, was used to make wells according to the number of graded concentrations (6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of the extracts. The different concentrations of the extracts were introduced into the wells. The experiments were carried out in triplicates. The plates were allowed to stand on the bench for 2 hrs to allow pre-diffusion. The plates were incubated uprightly in the incubator for 18-24 hrs at 37^0C. The standard drug, Gentamycin (10 mg/ml), was used as the control

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Antifungal assay (Agar diffusion-surface plate method): This was carried out as described by Adeniyi *et al*. (2013). A sterile Sabouraud Dextrose Agar (62 g/l) was prepared, aseptically poured into sterile plates in duplicates and allowed to set properly. The diluted organism (0.2 ml of the 10^{-2}) was used to cover all the surface of the agar using sterile spreader. Wells were made on the plates using a sterile cork borer of the 8mm diameter. In each well, the graded concentrations of the extract and control were introduced. The plates were left on the bench for 20 minutes so as to allow extracts to diffuse properly into the agar. The plates were incubated uprightly in the incubator for 48 hrs at 26-28^0C. The standard drug, Tioconazole (70 %) was used as the control. The experiments were carried out in triplicates.

Determination of Minimum Inhibitory Concentration (MIC): This was carried out as described by Adeniyi *et al*. (2013). Graded concentrations (0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml, 10 mg/ml and 20 mg/ml) of the samples were prepared. Two ml of each concentration was introduced into 18 mls of nutrient agar at 45-50ºC. These were mixed together and poured aseptically into the sterile plates and allowed to set. The organisms were thereafter streaked on the plates at different concentrations in order to determine the minimum concentration that would inhibit/hinder the growth of the organisms. The bacterial plates were incubated at 37^0C for 24 hours while the fungal plates were incubated at 26^0C-28^0C for 48 hours. The plates were observed for the growth of the microorganisms after the incubation period.

RESULTS AND DISCUSSION

The percentage yields of the extracts are shown in Table 1. The solvents used for the extraction of the samples ranged from non-polar to polar (hexane being the least polar and methanol is the most polar). This procedure ensures that compounds in the samples are separated based on differences in their polarities. The highest yields were obtained for the methanol extracts of the bulbimplying that this plant part is very rich in polar compounds.

Table 1: Percentage yield of Extracts

Extract	Yield (%)
CJBHE	0.28
CJBEE	0.44
CJBME	24.68

CJBHE-Hexane Extract of *C. jagus* bulbs; CJBEE-Ethylacetate Extract of *C. jagus* bulbs; CJBME-Methanol Extract *C. jagus* bulbs

Tables 2 and 3 respectively show the zones of inhibition of the bacterial and fungalorganisms the *C. jagus* bulb extracts were screened against. The

methanol extract, CJBME showed a broad spectrum of activity against the test organisms. The zones of inhibition of CJBME ranged between 18 - 28 mm at 200 mg/ml against the bacteria pathogens. Similarly, the extract CJBME had the highest activity against the fungal microorganisms at 200 mg/ml. The hexane and ethylacetate extracts showed very poor or no activity at 6.25 mg/ml against the test organisms. The zone of inhibition is a measure of the degree of susceptibility of the microbes under study to the different extracts. Furthermore, the fact that the effect of an antimicrobial agents vary with target species (Prescott *et al*., 2005) explains the variations in the inhibition zones among organisms tested at the same concentration. The zone of inhibition of an extract against an organism also depends on the initial population density of the organism, their growth rate, their physiological state, nature and the rate of diffusion of the antimicrobial agent (Hugo and Russel, 2007; Pelcza *et al*., 2002). Differences in polarity among various solvents have been reported to account for the differences in solubility of active plant active properties, hence variations in the degree of activity (Ngo *et al*., 2017). This result indicates that most of the phytoconstituents responsible for the observed antimicrobial activities reside in the methanol extracts of the bulb of the plant. The antimicrobial activity of the extracts was concentration-dependent. This is in agreement with the previous reports (Bashir *et al*., 2018). A previous study indicated the phytoconstituents in the methanol extract of the bulb as tannins, resin, sterols, phenols, alkaloids and terpenoids (Alawode *et al*., 2019). Tannins are reported to possess broad antimicrobial properties by means of different mechanisms that include enzyme inhibition, oxidative phosphorylation reduction and iron deprivation, among others (Parekh and Chanda, 2007). The antimicrobial activities of sterols, flavonoids andterpenes have also been reported (Cushnie *et al*., 2014; Kavitaa *et al*., 2014; Cushnie and Lamb, 2005). Another study has reported the presence of several alkaloids in the bulb of *C. jagus* including lycorine, crinamine, pseudolycorine, 6-hydroxycrinamine, hamayne, bowdensine, and demethoxy-bowdensine and morpholine (Nwaehujor *et al*., 2012; Adesanya *et al*., 2012; Edema and Okieimen, 2002; Tram *et al*., 2002). The antibacterial potency of Crinamine against *Bacillus subtilis* and *Staphylococcus aureus* has been reported (Udegbunam *et al*., 2015). The antibacterial and antifungal properties demonstrated by the methanol extracts of the bulb of *C. jagus* could have arisen from the activity of one compound or the synergestic action of many bioactive compounds present in minor proportion in the plant. Udegbunam *et al*. (2015) screened methanol extract of *C. jagus* bulb for antimicrobial activity *in vitro* and *in vivo* in rat wound. In the *in vitro* assay, a concentration-dependent activity against *B. subtilis*, *S. aureus*, and *C. albicans* was obtained. The results are similar to those obtained in the current study.

Table 2:*In vitro* antibacterial activity of *Crinum jagus* bulb extracts

EXTRACT	Zones of Inhibition						
	Conc. (mg/ml)	SA	EC	BS	PSA	ST	KP
CJBHE	200	20.3±0.94^{a}	18.3±0.47^{a}	17.3±0.94^{a}	17.7±0.47^{a}	17.7±0.47^{a}	16.3±0.47^{a}
	100	18.3±0.47^{b}	16.0±0.82^{b}	16.7±0.94^{a}	16.0±0.82^{b}	13.6±0.47^{b}	13.7± 0.47^{b}
	50	15.7±0.47^{c}	14.3±1.24^{b}	14.0±0.82^{b}	13.3±0.94^{c}	11.3±0.94^{c}	11.3± 0.94^{c}
	25	14.7±0.94^{c}	11.7±0.94^{c}	10.3±0.47^{c}	9.3±0.94^{d}	10.0±0.82^{c}	9.7±0.47^{c}
	12.5	10.2±0.82^{d}	10.3±0.47^{c}	-	-	-	-
	6.25	-	-	-	-	-	-
CJBEE	200	17.7±0.47^{a}	15.3±0.94^{a}	14.0±0.82^{a}	18.0±0.82^{a}	14.3±1.25^{a}	11.7±0.47^{a}
	100	15.7±0.47^{b}	14.3±0.47^{a}	11.3±0.94^{b}	15.3±0.94^{b}	11.3±0.94^{b}	10.3±1.25^{a}
	50	14.0±0.82^{c}	10.0±0.82^{b}	10.3±0.47^{b}	12.0±0.82^{c}	10.3±1.25^{b}	-
	25	10.0±0.94^{d}	-	-	-	-	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
CJBME	200	28.3±0.47^{a}	24.0±0.82^{a}	19.7±0.47^{a}	28.0±0.82^{a}	25.6±1.25^{a}	18.0±0.82^{a}
	100	24.7±0.94^{b}	20.0±0.82^{b}	18.0±0.82^{b}	23.3±0.94^{b}	20.0±0.82^{b}	16.3±0.47^{b}
	50	19.3±0.94^{c}	17.7±0.47^{c}	15.7±1.25^{b,c}	20.6±0.94^{c}	17.6±0.47^{c}	13.7±1.25^{c}
	25	18.3±0.47^{c}	15.7±0.47^{d}	13.3±0.94^{c,d}	18.0±0.82^{d}	14.3±0.47^{d}	11.3±0.94^{c,d}
	12.5	14.0±0.82^{d}	13.3±0.94^{e}	11.7±0.47^{d}	14.3±0.47^{e}	11.7±1.25^{e}	10.3±1.25^{d}
	6.25	9.7±0.47^{e}	10.0±0.82^{f}	10.0±0.82^{e}	9.3±0.94^{f}	10.0±0.82^{e}	-
CONTROL	-ve	-	-	-	-	-	-
	GENT	38	38	38	40	40	40

CJBHE-Hexane Extract of C. jagus bulbs; CJBEE-Ethylacetate Extract of C. jagus bulbs; CJBME-Methanol Extract C .jagus bulbs; GENT-Gentamycin (10mg/ml); TIOC- Tioconazole (70%); Microorganisms: SA- Staphylococcus aureus; EC- Escherichia coli; BS-Bacillus subtilis; PA- Pseudomonas aeruginosa; ST- Salmonella typhi; KP-Klebsiella pneumonia; -=No Activity; NT= Not tested; values with different superscripts are significantly different ($p < 0.05$)

Table 3: *In vitro* antifungal activity of *Crinum jagus* bulb extracts

EXTRACTS	Conc. (mg/ml)	Zone of Inhibition CA	AN	PN	RS
CJBHE	200	16.3±0.47[a]	16.3±1.25[a]	18.7±0.94[a]	15.3±0.94[a]
	100	14.0±0.82[b]	13.7±0.47[b]	15.7±0.47[b]	14.3±0.47[a]
	50	11.3±0.94[c]	11.3±0.94[c]	14.0±0.82[c]	11.7±1.25[b]
	25	9.7±0.47[c]	10.0±0.82[c]	11.7±0.47[d]	10.3±0.47[b]
	12.5	-	-	9.7±0.47[e]	-
	6.25	-	-	-	-
CJBEE	200	13.7±0.47[a]	12.7±0.94[a]	14.0±0.82[a]	12.0±0.82[a]
	100	12.0±0.82[b]	9.7±0.47[b]	12.0±0.82[b]	10.0±0.47[b]
	50	9.7±0.47[c]	-	9.6±1.25[b]	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
CJBME	200	19.7±0.47[a]	20.0±0.82[a]	20.3±0.47[a]	17.7±0.47[a]
	100	18.0±0.82[b]	17.7±0.47[b]	18.3±0.47[b]	15.3±0.94[b]
	50	15.7±0.47[c]	13.3±0.94[c]	16.0±0.82[c]	14.0±0.82[b]
	25	14.3±0.47[d]	12.0±0.82[c]	14.6±0.94[c]	12.0±0.82[c]
	12.5	11.7±1.25[e]	-	11.7±0.47[d]	9.3±0.94[d]
	6.25	10.0±0.82[e]	-	9.7±1.25[d]	-
CONTROL	-ve	-	-	-	-
	TIOC	28	28	28	28

CA-Candida albicans; AN-Aspergillusniger; PN- Penicilliumnotatum and RS-Rhizopusstolonifer; -=No Activity; values with different superscripts are significantly different ($p < 0.05$)

Table 4: Minimum Inhibitory Concentration of *C. jagus* Bulb extracts (mg/ml)

Organisms	CJBHE	CJBEE	CJBME
S. aureus	10.00	20.00	2.50
E. coli	10.00	20.00	2.50
B. subtilis	5.00	20.00	2.50
P.aeruginosa	10.00	10.00	2.50
S. typhi	10.00	10.00	2.50
K. pneumonia	10.00	10.00	5.00
C. albicans	10.00	10.00	5.00
A. niger	10.00	10.00	10.00
P. notatum	10.00	10.00	10.00
R. stolonifer	10.00	10.00	10.00

The MIC values for the organisms are shown on Table 4. The lowest MICs were obtained for the methanol extract CJBME with MIC values of 2.50 mg/ml against *S. aureus*, *E. coli*, *B. subtilis, P. aeruginosa, C. albicans* and *S. typhi*). Higher MIC values (between 10.00 mg/ml and 20 mg/ml) were obtained when the ethyl acetate and hexane extracts of the bulb of *C. jagus* were screened against the test organisms. The results show that the methanol extracts of the bulb extracts of *C. jagus* extracts (CJBME) compared to other *C. jagus* extracts, showed the highest antimicrobial activities. The extracts showed significant activities against *S. typhi*, *P. aeruginosa* and *K. pneumoniae* which are gram negative bacteria. Gram negative bacteria are typically less susceptible to antibacterials due to the presence of an outer membrane surrounding their cell walls which restricts diffusion of hydrophobic compounds through their lipopolysaccharide covering (Ullah *et al*., 2013). Furthermore, the drug resistant strains of *E. coli*, *K. pneumonia* and *S. aureus* have been reported (Djuric *et al*., 2016). The significant activity shown by the methanol extract against these microorganisms implies that they could possibly assist in combating these challenges.

*Conclusion:*The results of the current study shows that the methanol extract demonstrated better antimicrobial activity against the test organisms than the hexane and ethylacetate extracts. Most of the antimicrobial agents likely reside in the methanol extract of the leaves of the plant. The results have also supported the use of the leaves of the plant in ethnomedicine as antimicrobial agents.

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