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Evaluation of Extracts of Leaves of *Crinum jagus* for Antimicrobial Properties

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ABSTRACT: Plants have been used in ethno-medicine for ages in the treatment of various diseases. In the current study, the leaves of *C. jagus* are investigated for antimicrobial activities. The leaves were dried and extracted successively with hexane, ethylacetate and methanol. The concentrated 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 using the agar diffusion method. The Minimum Inhibitory Concentration (MIC) was also determined. The percentage yields obtained were 0.92 %, 1.20 % and 25.2 % for the hexane, ethylacetate and methanol extracts respectively. The zones of inhibition of the organisms by the extracts generally increased with the concentrations. The methanol extract showed the best activity of the three extracts tested. The methanol extract had values ranging between 20 mm and 26 mm against the bacteria at 200 mg/ml. This extract also showed values between 18 mm and 20 mm against the fungi at 200 mg/ml. The lowest MICs values (of 2.5 mg/ml) were obtained against *S. aureus*, *E. coli*, *B. subtilis* and *S. typhi*. The polar constituents in the leaves of the plant are likely responsible for the antimicrobial properties observed. None of the extracts showed activity comparable to those of the standard drugs, however the methanol extract showed some potential as a source of antimicrobial compounds.

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The emergence and spread of multidrug resistant strains of microorganisms arising from misuse of antibiotics are a threat to the current antimicrobial therapy. This has necessitated a continuous search for cheap and more effective antimicrobial drugs. Medicines derived from plants have made significant contributions to human health as about 80% of the world's population rely on traditional medicine which is predominantly plant-based (Bourhia *et al*., 2019). Plants are therefore potential sources of new active ingredients which may be used as template for the synthesis of new antimicrobial drugs (Agarwal *et al*., 2016; Cheesman *et al*., 2017). In recent times, a resurgence of research aimed at isolating the bioactive compounds from plants has been observed. This is because antimicrobials derived from plants are safer than their synthetic counterparts (Cheesman *et al*., 2017). A huge number of novel drug candidates have been isolated from plant sources. Phytochemicals are known to be responsible for the medicinal properties displayed by plants. For example, flavonoids, quinones, tannins, coumarins, terpenoids, alkaloids, lectins and polypeptides (Othman *et al*., 2019) are all known to possess antimicrobial activities.

Plant extracts have been reported severally to demonstrate antimicrobial activities against different strains of microorganisms. Extracts of the plants, *Oxalis corniculata*, *Artemisia vulgaris*, *Cinnamomum tamala*, and *Ageratina adenophora* showed activity against *Rhizopus* spp., *Escherichia coli* and *Stapylococcus aureus* by standard methods (Manandhar *et al*., 2019).

The ethanolic leaf extracts of *Baeckea frutescens* showed antibacterial activity against Methicillin-Resistant *Staphylococcus aureus*. The observed activity was attributed to the presence of alkaloids, flavonoids, steroids, terpenoids, phenols, and carbohydrates in the extracts (Othman*et al*., 2019).*Crinum jagus*, is used by traditional medical practitioners in the treatment of chronic cough, rheumatism, tuberculosis and witlow In addition, it is employed as an anthelminthic, purgative, rubefacient and an emetic. Despite the widespread use of the plant by herbalists, no information exists in scientific literature on the antimicrobial properties of the leaves of the plant, hence this study.

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MATERIALS AND METHODS

Sample Preparation and Extraction: Leaf samples were collected from the Botanical Gardens of the University of Ibadan, Ibadan, Nigeria. The plants were identified by Mr. Owolabi, a taxonomist and the curator of the Botanical Gardens. The samples were dried under mild sunlight for 6 weeks and ground to powder. Thereafter, 1000 g of sample was subjected to successive extraction using hexane, ethylacetate and methanol. Extractions were carried out using the maceration method. The extracts were concentrated to dryness using a rotary evaporator. The percentage yield was calculated using the expression.

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

Microorganisms used for antimicrobial assay: Clinical strains of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi* and *Klebisidlae pneumonae*, *Candida albicans*, *Aspergillus niger*,*Penicillium notatum* and *Rhizopus stolonifer* were used in the current study.

In vitro Antimicrobial Assay on Extracts: This was carried out as described by Adeniyi *et al.* (2013). A 0.2 ml of 1:100 dilution of the overnight culture of each bacterial and fungi isolates were used to seed sterile nutrient and Sabouraud Dextrose Agar which were at 45ºC respectively. One (1) gram of each extract was reconstituted with 50% methanol and diluted serially to give the different 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 used in the current study.

A standard cork borer of 8 mm diameter was used to make wells according to the number of graded concentrations Gentamycin (10 mg/ml) and Tioconazole (70 %) were used as the positive control for the bacterial and fungi isolates respectively while methanol (50%) was used as the negative control. The plates were incubated at 37ºC for 24 hours after which the diameters of the zones of inhibition were measured. 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 added to 18 mls of nutrient agar at 45-50ºC. These were mixed together and poured asceptically into the sterile plates. The plates were allowed to set. After this, the organisms were streaked on the plates at different concentrations in order to determine the minimum concentration that would inhibit/hinder the growth of the organisms. All the plates were incubated appropriately (bacterial plates at 37^0C for 24 hours and fungal plates 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

Table 1 shows the percentage yields of the extracts. The polarity of the solvents used range from non-polar (hexane) to slightly polar (ethylactetate) to polar (methanol). Since solvents dissolve compounds with like polar characteristics, the extraction method employed ensured the separation of phytoconstituents in the sample into non-polar, medium polar and polar compounds for hexane, ethylacetate and methanol extracts respectively. The methanol extract (CJLME) had the highest yield (25.20 %) while very low yields were obtained for hexane (0.92 %) and ethylacetate (1.20 %) respectively. This result implies that the leaves of the plant are very rich in polar constituents.

Table 1: Percentage yield of Extracts

EXTRACT	Yield (%)
CJLHE	0.92
CJLEE	1.20
CJLME	25.20

CJLHE-Hexane Extract of C. jagus leaves; CJLEE-Ethylacetate Extract of C. jagus leaves; CJLME-Methanol Extract C .jagus leaves

Tables 2 and 3 show the zones of inhibition obtained when the extracts were screened for antibacterial and antifungal activities against the test organisms. Generally, it can be observed that the zones of inhibition increased with increasing concentrations of the extracts. This is in agreement with previous reports (Homaida *et al*., 2019).

The extracts showed differing antimicrobial activities against the test organisms. For example, at 200 mg/ml, the zones of inhibition of the bacteria under investigation ranged between 16 and 18 mm for hexane extract whereas the values ranging between 20 and 26 mm were obtained for the methanol extract. These must be due to the differences in the nature of the phytoconstitutents present in each extract.

Differences in polarity among various solvents have been reported to account for the differences in solubility of active plant active principles, hence variations in the degree of activity (Ngo *et al*., 2017).

Table 2: *In vitro* antibacterial activity of the *Crinum jagus* Leaves Extracts

Extracts	Conc. (mg/ml)	SA	EC	BS	PSA	ST	KP
CJLHE	200	17.7 ± 0.47^{a}	18.0 ± 0.82^{a}	16.0 ± 0.82^{a}	17.6 ± 0.47^{a}	15.3 ±0.94^{a}	16.3 ± 1.24^{a}
	100	15.7 ± 0.47^{b}	16.3 ± 0.47^{b}	13.7 ± 0.47^{b}	16.0 ± 0.82^{b}	14.0± 0.82^{a}	14.0 ±0.82^{a}
	50	13.3 ± 0.94^{c}	14.7± 0.94^{b}	11.7 ± 0.94^{c}	14.0 ± 0.82^{c}	11.3±0.94^{b}	11.3 ± 0.94^{b}
	25	11.7±0.47^{c}	12.6 ± 0.47^{c}	10.6 ± 0.94^{c}	11.3 ± 0.94^{d}	10.0±0.82^{b}	10.3 ±0.47^{b}
	12.5	9.3 ± 0.94^{d}	9.7 ± 0.47^{d}	-	9.3 ± 0.94^{d}	-	-
	6.25	-	-	-	-	-	-
CJLEE	200	16.0 ± 0.82^{a}	17.3 ± 0.94^{a}	14.7 ± 0.94^{a}	16.0 ± 0.82^{a}	13.3±0.94^{a}	14.0 ± 0.82^{a}
	100	13.7 ±0.47^{b}	15.7 ± 1.24^{a}	11.7 ±0.47^{b}	13.7 ±0.47^{b}	12.0±0.82^{a}	11.3 ± 0.94^{b}
	50	12.3 ± 0.47^{c}	12.0 ±0.82^{b}	10.0 ± 0.82^{c}	11.3 ± 0.94^{c}	9.7 ± 0.47^{b}	9.3 ±0.94^{b}
	25	10.0 ±0.82^{d}	9.3 ± 0.47^{c}	-	10.3 ±0.47^{c}	-	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
CJLME	200	25.7 ±0.47^{a}	24.0 ± 0.82^{a}	25.3 ±0.94^{a}	20.3 ± 1.24^{a}	20.0 ±0.82^{a}	19.3±0.94^{a}
	100	20.0 ± 0.82^{b}	19.0 ± 1.41^{b}	22.0 ± 0.82^{b}	18.0 ±0.82^{a}	17.3 ± 0.94^{b}	16.0 ±0.82^{b}
	50	18.7±0.94^{b}	17.3± 0.94^{b,c}	18.3 ± 1.24^{c}	13.7 ± 0.47^{b}	16.3 ±0.94^{b,c}	14.3± 0.94^{b,c}
	25	14.0 ±0.82^{c}	16.0 ± 0.82^{c}	13.6 ±0.47^{d}	11.3 ± 0.94^{c}	14.3 ±1.24^{c}	12.0± 1.41^{c,d}
	12.5	11.3 ± 0.94^{d}	13.3 ± 0.94^{d}	12.0 ± 0.82^{e}	10.0 ± 0.82^{c}	10.0 ± 0.82^{d}	10.0 ±0.82^{d}
	6.25	10.0 ± 0.82^{d}	9.7 ±0.47^{e}	10.3 ± 1.24^{e}	-	-	-
Control	-ve	-	-	-	-	-	-
	GENT	40	38	38	38	38	40

CJLHE-Hexane Extract of C. jagus leaves; CJLEE-Ethylacetate Extract of C. jagus leaves; CJLME- Methanol Extract C. jagus leaves; 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 pneumoniae; CA-Candida albicans; AS-Aspergillus niger; PN-Penicillium notatum and RH- Rhizopus stolonifer; -=No zone of inhibition; NT= Not tested; values with different superscripts are significantly different (p < 0.05)

Table 3:*In vitro* antifungal activity of the *Crinum jagus* Leaves Extracts

Extracts	Conc. (mg/ml)	CA	AN	PN	RS
CJLHE	200	17.3 ± 0.94^{a}	16.0 ±0.82^{a}	15.7±0.47^{a}	15.7 ±1.24^{a}
	100	14.0 ±0.82^{b}	14.3± 1.24^{a,b}	14.3±0.47^{b}	13.3 ±0.94^{a,b}
	50	11.3 ± 1.24^{c}	12.0± 0.82^{b}	12.3±1.24^{b}	11.7 ± 0.47^{b}
	25	9.7 ± 0.47^{c}	9.3 ±0.94^{c}	9.3 ±0.94^{c}	10.0 ± 0.82^{c}
	12.5	-	-	-	-
	6.25	-	-	-	-
CJLEE	200	16.3±1.24^{a}	14.3 ±1.24^{a}	13.7 ±1.24^{a}	13.7±1.24^{a}
	100	14.0 ±0.82^{a}	12.3 ± 0.47^{a}	12.7 ± 0.94^{a}	12.0 ±0.82^{a}
	50	11.6 ±0.47^{b}	10.0 ± 0.82^{b}	9.6 ±0.47^{c}	9.3 ±0.47^{b}
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
CJLME	200	19.7 ± 1.24^{a}	20.0 ±0.82^{a}	17.6 ± 1.24^{a}	18.3 ± 0.47^{a}
	100	17.7 ±0.47^{a}	18.3 ± 0.47^{b}	14.3 ±0.47^{b}	14.0 ± 0.82^{b}
	50	14.0 ± 0.82^{b}	15.3 ±0.94^{c}	12.0 ±0.82^{c}	11.3 ±0.94^{c}
	25	11.3 ± 0.94^{c}	14.0 ± 0.82^{c}	10.3 ±0.47^{d}	10.7 ± 0.94^{c}
	12.5	9.7 ±0.47^{c}	11.7 ± 0.47^{d}	-	-
	6.25	-	10.3± 0.47^{e}	-	-
Control	-ve	-	-	-	-
	TIOC	28	28	28	28

CJLHE-Hexane Extract of C. jagus leaves; CJLEE-Ethylacetate Extract of C. jagus leaves; CJLME-Methanol Extract C. jagus leaves; 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 pneumoniae; CA-Candida albicans; AS-Aspergillus niger; PN-Penicillium notatum and RH-Rhizopusstolonifer; - = No zone of inhibition; NT= Not tested; values with different superscripts are significantly different (p < 0.05)

Furthermore, Tables 2 and 3 shows that the bacteria and fungi tested were most susceptible to the methanol extract (CJLME). This is further supported by the result of the Minimum Inhibitory Concentration (Table 4) which shows the lowest concentration of the antimicrobial agent that inhibits microbial growth after 24 hours of incubation. The methanol extract, CJLME showed the best activity against *S.aureus*, *E. coli*, *B. subtilis*, and *S. typhi* with MIC as low as 2.50 mg/ml. The extract also showed moderate activity against *P. aeruginosa*, *K. pnuemoniae* and *C. albicans* with MIC value of 5.00 mg/ml. The hexane extract also shows moderately high activity against *S.aureus*, *E. coli* and *B. subtilis* (MIC = 5.00 mg/ml). The results indicated that methanol extract (CJLME) contains most of the active principles responsible for the

antimicrobial activities displayed by this plant part. In a previous study, we have reported the classes of phytoconstituents present in the extract to include tannins, glycosides, saponins, flavonoids, carbohydrates, alkaloids and terpenoids (Alawode*et al*., 2019). Several compounds from these phytochemical groups have been reported to possess antibacterial and antifungal properties (Othman*et al*., 2019). Further studies however need to be carried out to isolate and characterize the specific compounds responsible for the observed activities.

Drug-resistant strains of some of the organisms under investigation have been reported.For example, *Escherichia coli* has been reported to show resistance against the cephalosporin and fluoroquinolones; *Klebsiellapneumoniae* against cephalosporin and carbapenems; and *Staphylococcus aureus* against methicillin (Bidell*et al*., 2016; Ding *et al.*, 2019). These organisms are gram-negative bacteria which are known for their ability to restrict the diffusion of hydrophobic compounds through their membranes. The activity shown by the methanol extract against these organisms could indicate that they likely contain compounds which could assist in handling the problem of drug resistance. Antifungal resistance by *C. albicans* has also been reported to occur with long-term antifungal use and with recurrent infections, such as those with chronic mucocutaneous candidiasis or recurrent oropharyngeal candidiasis with uncontrolled human immunodeficiency virus infection (Arendrup andPatterson, 2017). The antimicrobial activity observed against the test organisms could be due to the interactions of the plant active metabolites with the organism's cytoplasmic membrane leading to the leakage of intracellular components and precipitation of cytoplasmic contents. Furthermore antibacterial activity could have arisen from inhibition of macromolecular synthesis, especially DNA at higher concentrations (Thangamani*et al*., 2016).

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

Organisms	CJLHE	CJLEE	CJLME
S. aureus	5.00	10.00	2.50
E. coli	5.00	10.00	2.50
B. subtilis	5.00	20.00	2.50
P.aeruginosa	10.00	20.00	5.00
S. typhi	10.00	20.00	2.50
K. pneumoniae	10.00	20.00	5.00
C. albicans	10.00	20.00	5.00
A. niger	10.00	20.00	10.00
P. notatum	10.00	20.00	10.00
R. stolonifer	10.00	20.00	10.00

CJLHE-Hexane Extract of C. jagusleaves; CJLEE-Ethylacetate Extract of C. jagus leaves; CJLME-Methanol Extract C. jagus leaves

*Conclusion:*The extracts of the plant under investigation demonstrated antimicrobial activities against the test organisms. The methanol extract proved to be the most effective among the three extracts studied. None of the extracts demonstrated activities comparable to those of the standard drugs, however, the methanol extract showed some potential as a source of antimicrobial compounds.

REFERENCES

Adeniyi, BA; Izuka, KC; Odumosu, B; Aiyelaagbe, OO (2013). Antibacterial and antifungal activities of methanol extracts of *Desmodium adscendens* root and *Bombax buonopozense* leaves. *Int. J. Biol. Chem. Sci.* 7(1): 185-194

Agarwal, P; Agarwal, N; Gupta, R; Gupta, M; Sharma, B (2016). Antibacterial Activity of Plants Extracts against Methicillin-Resistant Staphylococcus aureus and Vancomycin-Resistant *Enterococcus faecalis*. *J Microb Biochem Technol.* 8(5): 404-407.

Alawode, TT; Lajide, L; Owolabi, BJ; Olaleye, MT (2019). Studies on *In vitro* Antioxidant and Anti-Inflammatory Activities of *Crinum jagus* Leaves and Bulb Extracts. *Int J Biochem Res Rev. 28(1): 1-9.*

Arendrup, MC; Patterson, TF (2017). Multidrug-Resistant *Candida*: Epidemiology, Molecular Mechanisms, and Treatment. *Int J Infect Dis.* 216: 3(15): S445–S451.

Bidell, MR;Palchak, M; Mohr, J;Lodise, TP (2016). Fluoroquinolone and Third-Generation-Cephalosporin Resistance among Hospitalized Patients with Urinary Tract Infections Due to *Escherichia coli*: Do Rates Vary by Hospital Characteristics and Geographic Region? *Antimicrob Agents Chemother*. 60(5): 3170–3173.

Bourhia, M; Shahat, AA; Almarfadi, OM; Naser, FA; Abdelmageed, WM; Said, AAH; El Gueddari, F; Naamane, A; Benbacer, L ; Khlil, N (2019). Ethnopharmacological Survey of Herbal Remedies Used for the Treatment of Cancer in the Greater Casablanca-Morocco. *Evid. Based Complementary Alter. Med*. 1- 9.

Cheesman, MJ; Ilanko, A; Blonk, B; Cock, IE (2017). Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution? *Pharmacogn Rev.* 11(22): 57-72.

Ding, Y; Wang, Y; Hsia, Y; Sharland, M; Heath, PT (2019). Systematic review of carbapenem-resistant Enterobacteriaceae causing neonatal sepsis in China. *Ann Clin Microbiol Antimicrob.* 18:36

Homaida, MA; Haran, Y; Omer, EOM, Hassan, ME; Goja, AM (2019). Antimicrobial Activity of the Seed Extracts of *Carthamus tinctorius*, *Moringa oleifera* and *Jatropha curcas* on Pathogenic Bacteria. *Pak. J. Nutr*., 18 (1): 20-24.

Manandhar, S; Luitel, S; Dahal, RK (2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *J Trop Med.* 1895340, 5

Ngo, TV; Scarlett, CJ; Bowyer, M.; Ngo, PD; Vuong, QV (2017). Impact of Different Extraction Solvents on Bioactive Compounds and Antioxidant Capacity from the Root of *Salacia chinensis* L. *J. Food Qual.* 1: 1-8.

Othman, L; Sleiman, A; Abdel-Massih, RM (2019). Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Front Microbiol*. 10: 911.

Thangamani,S; Mohammad, H;Abushahba, MFN; Sobreira,TJP; Hedrick,VE; Paul, LN; Seleem, MN. (2016). Antibacterial activity and mechanism of action of auranofin against multi-drug resistant bacterial pathogens. *Sci Rep* 6, 22571