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THE PHYTOCHEMICAL SCREENING AND TOXICITY OF THE STEM EXTRACTS OF *DRACAENA MANNI* (BAKER)

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Abstract

Dracaena manni Baker (Dracaenaceae) is a plant used in trado – medicine for the treatment of various ailments, however literature is scarce about the efficacy of this medicinal plant. Thus the present study investigates this plant to corroborate the claims in trado – medicine and document our findings.

The phytochemicals were extracted from the stem of *Dracaena manni* with ethyl acetate and then methanol. The screening of the extracts was carried out by brine shrimp lethality test (BST), antimicrobial and antifungal tests using microbes.

The phytochemical screening revealed the presence of tannins, saponins, alkaloids and anthraquinones in the methanolic extract while the ethyl acetate extract contain steroids, anthraquinones and terpenoids. The antimicrobial and antifungal analyses indicate ethyl acetate extract sensitivity towards *Escherichia coli* and *Candida albicans* while methanolic extract was sensitive towards *Staphylococcus aureus* and *Candida albicans*. The extracts were not active against the brine shrimp larvae.

This study indicates that *D. mannii* have potential antimicrobial and antifungal activity but there are slight differences in the activities of the extracts based on the solvents that was used for extraction. The results of our findings have indicated the biological activities of the plant extracts and their usability in the preparation of new drugs. However, further study is required to identify the active constituents responsible for these antibacterial and antifungal activities.

Keywords: *Dracaena Manni*, Alternative Medicine, Antifungal, Antimicrobial

INTRODUCTION

Antimicrobial resistance has become a global problem. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Strategies to improve the current situation include research in finding new and innovative antimicrobials (Freeman, 1997; Panda and Dutta,

2011). This has forced scientists to search for new antimicrobial substances from different sources especially medicinal plants (Thatoi et al., 2008).

Broad use of traditional medicine is often attributable to its accessibility and affordability in developing countries (Bhat et al., 1990; Bodeker, 1994; De Silva, 1994; Mahonge et al., 2006; Sofowora, 2008). As a matter of fact medicinal plants play a key role in the human health care with about 80% of the world population relying on the

use of traditional medicine which is predominantly based on plant materials (WHO, 1993). In 1997, the 30th World Health Assembly adopted a resolution urging interested governments to utilize their traditional systems of medicine with regulations suited to their national health care systems (Panda and Dutta, 2011), because of the fact that traditional medicines are cheaper and safe, with minimum side effects.

The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003), while numerous studies have identified compounds within plants that are effective antibiotics (Basile et al., 2000). Plant based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious disease, simultaneously mitigating many of the side effects that are associated with the synthetic antimicrobials. Medicinal plants represent a rich source of antimicrobial agent (Mahesh and Satish, 2008). The fact that pathogenic microbial infectious agents have developed chemotherapeutic and antibiotic resistance has led to the screening of several medicinal plants for their potential antimicrobial activities (Thatoi et al., 2008). Several studies have been carried out to extract the various natural products for screening antimicrobial activity (Cowan, 1999; Nita et al., 2002; Velickovic et al., 2003; Patil and Gaikwad, 2011).

The family Dracaenaceae (Agavaceae) with more than 480 species has a distribution in the tropic and subtropics dry climate regions throughout the world (Mimaki et al., 1998).

Many species of the genus *Dracaena* are used in African traditional medicine for the treatment of a variety of diseases. *Dracaena mannii* bark's decoction is used by the native people of the western highlands of Cameroon against abdominal pains. Furthermore, the mixture of its roots with "palm wine" is used in Cameroonian ethnomedicine for the treatment of male impotency (Noumi et al., 1998), while its uses in the treatment of sexually transmitted diseases in Owo region of Ondo State, Nigeria has been reported (Kayode et al., 2009). Similarly, Sofowora, and Olaniyi (1975) reported the antibacterial activity of both the light petroleum and ethanol fraction of the stem of the plant.

The occurrence of steroidal saponins in several Dracaenaceae plants is well documented (Okunji et al., 1991 and Mimaki et al., 1999). For instance Okunji et al (1991) reported the molluscidal activity of saponins from the fruit pulp of *D. mannii*, while anti-cancer saponin had been isolated from the plant (Yoo et al., 2010). The ethanolic extract of the stem bark of *D. mannii* was also reported to yield anti-inflammatory steroidal saponin (Leon et al., 2008). From the foregoing we decided to investigate the bioactivity of the ethyl acetate and methanolic extract of the stem of *D. mannii*.

Experimental

Plant Materials

The stem of *Dracaena mannii* was obtained from "OjaTuntun", a local market in Ilorin, North Central Nigeria. The plant was identified at the herbarium of the plant Biology Department of the University of Ilorin, Ilorin, Nigeria. The plant was dried at

ambient temperature, powdered using mortar and pestle.

Extraction

A known weight of the powdered stem (106.5 g) was successively extracted with ethyl acetate and methanol, the extract was concentrated and kept in sample bottle for further analysis.

Phytochemical Screening

The crude extracts were subjected to phytochemical, screening, testing for the presence of alkaloids, tannins, anthraquinones, glycosides, carbohydrates, steroids, terpenoids and saponins using standard experimental procedure (Harborne, 1973 and Trease and Evans, 1989).

Brine Shrimp Lethality Test

The modified method of McLaughlin *et al.*, (1998) was employed in this study. Natural sea water from Bar Beach, Lagos was poured into an improvised hatching chamber made of plastic dish, brine shrimp eggs were added at the closed section of the chamber. The open air section of the chamber was then exposed to fluorescent light for 48 hrs. Sample bottles of the same size used were washed and sterilized before use. Different concentrations (10, 100, 1000µg/ml) of each of the extracts were prepared using the extracting solvents. The tests were carried out in triplicates.

After 48 hrs a drop of DMSO and 4 ml of sea water were added to each of the sample bottles containing the extract. Ten brine shrimp larvae were carefully counted into each of the sample bottles with the aid of

dropping pipette and the volume of the sea water was made up to 5 ml.

A control experiment containing 5 ml of sea water, a drop of DMSO and ten brine shrimp larvae was set up. The experiment was maintained at room temperature for 24 hr under light after which number of surviving larvae were counted and recorded; the data obtained were subjected to Finney's Probit analysis to determine the LC₅₀ of each extract. The toxicity is expressed by this LC₅₀ which is defined as concentration of the oil that kills 50% of the larvae within 24 hr. Percentage mortality was also calculated as number of dead larvae divided by initial number of larvae (10) multiply by 100.

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{initial number of live larvae}} \times 100$$

Antimicrobial Analysis

The antimicrobial and antifungal activities of the extracts of *D. mannii* stem were evaluated by pour plate and surface plate (Diffusion methods) at the Pharmaceutical Microbiology Department laboratory, University of Ibadan, Ibadan, Nigeria. The antimicrobial analysis was carried out based on the method reported by Satish *et al* (2008) and National Committee for Clinical Laboratory Standards (NCCLS).

In the method a total of ten organisms comprising three gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis* and *Websillaepneumoniae*; three gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* and four fungi *Candida albican*, *Aspergillusniger*, *Rhizopus stolon* and *Penicillumnitatum* were prepared to test the activities of the extract. Six concentrations (6.25 to 200 mg/ml) of

each of the extract were prepared using the extracting solvent. A positive (10 µg/ml of gentamicin for bacteria and tiocomyola for fungi) and negative (solvents) controls were also prepared.

Pour Plate Method (Bacteria)

An overnight culture of each organism was prepared by taking a spatula full of the organism from the stock and inoculated each into the sterile nutrients and incubated for 24 hours at 37°C. From this culture, 0.1 ml of each organism was measured and poured into 9.9 ml of Sterile Distilled Water (SDW). From the diluted organism 0.2 ml was taken into the prepared sterile nutrient agar at 45°C, then aseptically poured into the sterile Petri dishes and allowed to solidify for 45 minutes.

Using a sterile cork barrier of 8 mm diameter, eight wells were bored each for the prepared sample concentration and two controls. The plates were left on the bench for two hours to allow the extract to diffuse properly into the nutrient agar and then incubated uprightly for 24 hours at 37°C.

Surface Plate Method (Fungi)

A sterile Sabouraud Dextrose Agar (SDA) was prepared, aseptically poured into sterile plates and allowed to solidify after which 0.2 ml of each organism was spread on the surface of the agar. Eight wells of uniform diameter [8 mm] were made by using a sterile borer and the graded concentrations of the extract were introduced. All the plates were left on the bench for 2 hours to allow the extract to diffuse properly into the agar. The plates were incubated uprightly for 48 hours at 26 – 28°C.

Results and Discussion

Phytochemical Screening

The phytochemical screening of the ethyl acetate extract of the bark of *D. mannii* revealed the presence of steroids, anthraquinones and terpenoids while the methanolic extract was characterised by the presence of tannins, saponins, terpenoids, alkaloids and anthraquinones. Saponins had earlier being reported from the stem bark of the plant (Okunji and Iwu, 1991; Leon et al., 2008; Yoo et al 2010).

Brine Shrimp Lethality Assay

The brine shrimp lethality assay result of the ethyl acetate and methanolic extract of the bark stem of *D. mannii* is presented in Table 1. A general bioassay capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality test (BST). The technique is easily mastered, costs little, and utilizes small amount of test material (Pisutthanant et al., 2004). BST is predictive of cytotoxicity activity and since its introduction this *in vivo* lethality test has been successively employed for bioassay-guide fractionation of active cytotoxic and antitumor agents (Meyer et al., 1982; Zhao et al., 1992). For both extracts the activity increases with increasing concentration. However, the methanolic extract exhibit the higher activity with percentage mortality of 36.70, 23.33 and 16.67 % at 10, 100 and 1000 µg/ml respectively, while for the ethyl acetate at the same concentrations were 10.00, 3.33 and 0.00 %. The LC₅₀ calculated for the two extracts were greater than 10,000 µg/ml indicating that the extracts may not contain cytotoxic compound of significant interest. The brine shrimp assay has been

used successfully for the isolation of bioactive compounds from plant extracts (McLaughlin et al., 1998). The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the microwell scale. A compound is considered to be cytotoxic if it inhibits vital metabolic processes or it causes disorder in living organisms resulting in perversion of behaviour or death (Fatope, 1995). The brine shrimp lethality assay has been a useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities of plant extracts and it has been reported that extracts resulting in LC₅₀ values of less than 250 µg/ml are significantly active (Reiser et al., 1996).

Table 1: Bioactivity of *D. mannii* extracts using brine shrimp (*A. salina*) lethality assay

Extract	Dosage (µg/ml)/Survivals			LC ₅₀ (µg/ml)
	1000	100	10	
EA	10, 10, 7	10, 10, 9	10, 10, 10	> 10,000
MeOH	6, 6, 7	8, 9, 6	9, 9, 7	> 10,000

Key: EA = ethyl acetate extract; MeOH = methanol extract

Antimicrobial Analysis

Antibacterial activity of the extracts of the stem bark of *D. mannii* against different bacterial pathogens is presented in the Table 2. The activity of the methanolic extract is dose dependent, the activity increases with increase in concentration. The most susceptible organism was *S. aureus* being the most inhibited by all the concentration of the methanol extract except at 6.25 mg/ml where none of the organism was affected (Table 2). The least affected organism by the methanolic extract was *B. subtilis* which was resistant to the extract at dose level of 50 mg/ml Table 2. From the Table it is clear that the minimum inhibitory concentration (MIC) for *S. aureus* is 12.5 mg/ml, 25 mg/ml for *E. coli* and *W. Pneumoniae*, 50 mg/ml for *P. aeruginosa* while it was 100 and 200 mg/ml for *B. subtilis* and *S. typhi* respectively, (Table 2).

Table 2: The antimicrobial activity of the extracts of *D. mannii* on some Gram – positive and Gram – negative bacteria

Concentration (mg/ml)	Zone of Inhibition (mm)											
	Ethyl Acetate extract						Methanol extract					
	SA	EC	BS	PA	WP	ST	SA	EC	BS	PA	WP	ST
200	16	24	18	16	14	12	20	16	14	18	16	10
100	12	20	14	14	10	10	18	14	10	14	14	--
50	10	10	12	12	--	--	14	12	--	10	12	--
25	--	12	10	10	--	--	12	10	--	--	10	--
12.5	--	10	--	--	--	--	10	--	--	--	--	--
6.25	--	--	--	--	--	--	--	--	--	--	--	--

KEY: SA = *S. aureus*; EC = *E. coli*; BS = *B. subtilis*; PA = *P. aeruginosa*; WP = *W. Pneumoniae*; ST = *S. typhi*

The activity of the ethyl acetate extracts of the stem bark of *D. mannii* against different bacterial pathogens followed the same trend as reported for the methanolic extract. However, the most susceptible organism to the extract was *E. coli* while *W. Pneumoniae* and *S. typhi* were the most resistant organisms, Table 2. The minimum inhibitory concentration recorded for the organism were 12.5 for *E. coli*, 25 mg/ml for *B. subtilis* and *P. aeruginosa* 50 mg/ml for *S. aureus* and 100 mg/ml for *W. Pneumoniae* and *S. typhi*, Table 2.

Antifungal activity of the extracts of the stem bark of *D. mannii* against different fungi pathogens is presented in the Table 3. From Table 3 it is clear that the extracts were active against all the fungi pathogens at 200 mg/ml, however, the activity decreases with decrease in concentration of the extracts. The most inhibited fungus was *C. albicans* being very sensitive to both the ethyl acetate and the methanolic extracts and with MIC of 12.5 mg/ml each. The other fungi *A. niger*, *R. stolon* and *P. nitatum* have MIC of 50 mg/ml each with the ethyl acetate extract

while the MIC for *A. niger*, and *R. stolon* was 100 mg/ml and 200 mg/ml for *P. nitatum* with the methanolic extract, Table 3.

Table 3: The antifungal activity of the methanolic and ethyl acetate extract of *Dracaena mannii*

Concentration (mg/ml)	Zone of Inhibition (mm)							
	MeOH				EA			
	C A	A N	R S	P N	C A	A N	R S	P N
200	20	12	12	10	20	14	14	16
100	18	10	10	--	16	12	12	12
50	16	--	--	--	14	10	10	10
25	14	--	--	--	12	--	--	--
12.5	10	--	--	--	10	--	--	--
6.25	--	--	--	--	--	--	--	--

KEY: CA = *C. albicans*; AN = *A. niger*; RS = *R. stolon*; PN = *P. nitatum*; MeOH = methanolic extract; EA = ethyl acetate extract

The knowledge of extent and mode of inhibition of specific compounds which are present in plant extracts, may contribute to the successful application of such natural compounds for treatment of infection disorder like fungal and bacterial diseases. The present status of medicinal plants and their products provide opportunity for the developing countries to benefit from the emerging marks as the developing countries possess most biodiversity of medicinal plants.

Conclusions

The presence of saponins and alkaloids has been reported to be responsible for various pharmacological properties. This study indicates that *D. mannii* have potential antimicrobial and antifungal activity but there are slight differences in the activities of the extracts based on the solvents that are used for extraction, it is natural that there are differences in their biological activities since the solvents was of different polarity and different compounds are extracted. The important thing is the determination of the biological activities that plants have and their usability in the preparation of new drugs. Further study is required to identify the active constituents responsible for these antibacterial and antifungal activities.

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