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Screening of Daniella Oliveri against Three Bacteria and One Fungus

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ABSTRACT

The antimicrobial effects and the phytochemical constituents of Daniella oliveri was investigated against clinical strains of Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and Fusarium oxysporum. The plant was found to be active against the test organisms at concentrations ranging from 50mg/ml to 100mg/ml of the crude extract of the plant. The ethanol extract was more active than the ethyl acetate and the aqueous extracts with Streptococcus pyogenes showing more sensitivity with the highest zone of inhibition of 23± 1.46 at 100mg/ml concentration. While the lowest zone of inhibition recorded was 10 recorded at 80mg/ml against Pseudomonas aeruginosa. The aqueous extract of the plant did not show any activity against Pseudomonas aeruginosa. The phytochemical screening of the plant revealed the plant to contain Saponins, Flavonoids, Tannin, Alkaloids and steroids but Glycosides and Phlobantanin were absent. The antimicrobial screening of antibiotic disc showed the organisms to be sensitive to augmentin, ceftriazone, gentamycin, pefloxacin and ciprofloxacin (Ps. aeruginosa), gentamycin, (Str. Pyogenes) gentamycin, ciprofloxacin (S. aureus.).The thin layer chromatography showed the ethanol extract to contain more constituents than the extracts of the other solvents.

Keywords: Antimicrobial, phytochemical constituents, daniella, staphylococcus species, pseudomonas species, flavonoid, saponin, tannin, alkaloids, steroids, glycosides.

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INTRODUCTION

It is estimated that there are 250,000 – 500,000 species of plants and about 1-10% of these are used for food by both human and animals. It is possible that more are used for medicinal purposes (Moerman, 1996). Mainstream medicine is increasingly receptive to the use of antimicrobials and other drugs derived from plants as traditional antibiotics become ineffective and as new particularly viral diseases remain intractable to these types of drugs (Lewis *et al.,* 1999). Finding healing power in plants is an ancient idea; people on all continents have long applied poultices and imbibed infusions by hundreds if not thousands of indigenous plants dating back to prehistory (Cowan, 1999).

It is estimated that today, plant materials are present in or have provided the models for 50% western drug (Iwu *et al*., 2001).Many commercially proven drugs that are used in modern medicine were initially used in the crude form in traditional or folklore healing practices or for other purposes that suggested potentially useful biological activities (Iwu *et al.,* 2001).There is a need to further investigate the fundamental scientific bases of the use of these medicinal plants by defining and quantifying the percentage crude phytochemical constituents present in these plants. Studies revealed that the leaves and stem of these plants are rich in alkaloids, flavonoids, tannins and saponins (Edeoga, 2006).

It is based on this that investigation into the use of *Daniella oliveri* in the treatment of infections involving *Staphylococcus aureus*, *Streptococcus pyogenes, Pseudomonas aeruginosa* and *Fusarium oxysporum* was carried out and the phytochemical constituents of the plant was elucidated to establish the efficacy of the plant.

Daniella oliveri (Rolfe) Fabiaceae is commonly known as Ilorin balsam (eepo iya) or *copaihu afdricana*. It is an indigenous African tree found extensively in Benin, Cameroon, Gambia and Nigeria. (Onwekaema and Udorh, 1999).The leaves are used traditionally to treat diabetes, yellow fever and as aphrodisiac (Onwekaema and Udorh, 1999).The leaves were found to contain quercitrin, quercameritrin, rutin and the rare flavoured glycoside quercitin-3-methoxy 3-o-rhamnosylpranosyl (I,6)- β- d- Glycopyranoside (Narssine) isolated from n- butanol extract (Onwekaema and Udorh, 1999).

MATERIAL AND METHODS

Collection and preparation of plant sample:

Plant was collected, identified and authenticated at the herbarium of the department of plant science, University of Adoekiti, Ekiti state. The plant was air dried at room temperature, ground into fine powder and stored in air tight container.

Collection of Microorganisms:

Organisms were collected from the University Teaching Hospital Complex, Ibadan and University of Ife Teaching Hospital annex, Ilesa, Osun State.

Extraction procedure:

Twenty (20) gm of plant sample was weighed into three separate conical flasks and 100ml of ethanol, ethyl acetate, and distilled water was added to each conical flask. The flasks were allowed to stand for five (5) days after which each solutions were filtered using No 1 Whatman filter paper. The filtrates were allowed to evaporate to dryness and the crude extracts were reconstituted with 50% dimethyl sulphoxide.

Inoculum preparation:

Barium sulphate ($BaSO_4$) was used as a standard for the preparation of the broth culture of the organisms. One gm of $Bacl_2$ was added to 99mls of distilled water. 1ml of tetra oxosulphate (vi) was added to 99ml of distilled water. 0.5ml of the 1% Sulphuric acid (H_2SO_4) was added to the 99.5ml of $Bacl_2$ solution to form $Baso_4$.

Antibacterial screening:

The sensitivity test was carried out using the agar well diffusion method of Sofowora (1996). Twenty mls of molten agar was poured into each sterile Petri dish and allowed to set. The Petri dishes were inverted and incubated overnight to observe for contamination. Sterile cotton swabs were dipped into the standard inoculum and the excess pressed out on the side of the test tube to avoid over flooding. The swab was used to swab the surface of the set agar plates. Cork borer of 9mm diameter was used to bore holes equidistant from each other on the agar. Extracts of the different solvents were used to fill the bored holes. At the centre of the plate was a hole containing 50% dimethysulphoxide. This serves as the control of the experiment. The plates were thereafter incubated at 37˚C for 24hrs and zones of inhibition were observed and recorded as depicted in table 1.

Antibiotic sensitivity test:

The test was carried out using the standard antibiotics disc agar diffusion method described by Cheesebrough (2000). Sterile cotton swab was used to inoculate the plates as described in 2.5. The antibiotic discs were placed firmly on the inoculated plates using a sterile forceps to ensure firm contact of disc with agar. The plates were incubated as in 2.5 and zones of inhibition were observed and recorded in table 2.

Antifungal screening:

This method was adopted from the methods of Smith (1978). 2ml of plant extract of different concentrations were introduced into sterile Petri dishes. Twenty (20) ml of sterile PDA was added to each plate and the plates were swirled gently to ensure proper mixing and even

distribution of the extract in the plates. The plates were allowed to set and observed for contamination. Mycelial discs (6mm in diameter) were taken from the edge of a 5day old culture of *Fusarium oxysporum* and placed on the already set PDA plates and incubated at 28˚C. The radial mycelial growth was measured every 24hr for 3-5 days and the result is as shown in table 3.

Phytochemical screening:

The plant was screened for phytochemicals using the methods of Odebiyi and Sofowora (2000).

Thin Layer Chromatography:

The plant extract were separated into their constituents using the thin layer chromatography and the constituents were screened for their antimicrobial activities. The Rf values and the antimicrobial activities are depicted in table 4.

RESULTS AND DISCUSSION

Daniella oliveri showed high antimicrobial properties by inhibiting all the test organisms at varied concentrations. The highest zone of inhibition recorded was 23 $\pm$ 1.46 for the ethanol extract at 100mg/ml against *Str. pyogenes*. While the lowest zone of inhibition recorded was 10 $\pm$ 0.84mm for ethanol extract at 80mg/ml against *Ps. aeruginosa*. Although the ethanol extract of plant was able to inhibit the growth *S. aureus* at concentration as low as 50mg/ml. The aqueous extract was not active against *Ps*. *aeruginosa*. The plant however showed antifungal activity against *F. oxysporum* between concentrations of 80mg/ml and 100mg/ml.. This puts the plant in the category of broad spectrum antimicrobial.

The antibiotic screening showed that out of ten antibiotic discs used in the test, *P. aeruginosa* was sensitive to Augmentin (30µg) with zone of inhibition of 10mm, Ceftriazone(30µg) with zone of inhibition of 12mm,Gentamycin (10µg) with a zone of inhibition of 12mm, Pefloxacin (5µg) with a zone of inhibition of 10mm and Ciprofloxacin (10µg) with a zone of inhibition of 8mm.*Str pyogenes* was sensitive to Gentamycin (10µg) with a zone of inhibition of 13mm while *S. aureus* was sensitive to Gentamycin (10µg) with a zone of inhibition of 16mm and ciprofloxacin (10µg) with a zone of inhibition of 14mm. The low activities of the conventional antibiotics against the test organisms could be as a result of resistance of these organisms to the discs, possibly due to previous exposure to the antibiotics since the organisms were clinical strains. This report supported the claims of Smith *et al* (1999) that the emergence of resistant strains of microorganisms threatened to return us to the era before antibiotics. Friedkin *et al* (2002).

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Table 1: The antimicrobial effects of *Daniella oliveri* showing zones of inhibition in mm

	100mg.ml			90mg/ml			80mg/ml			70mg/ml			60mg/ml			50mg/ml			25mg/ml		
	EE	EA	DS	EA	DW	EE	EA	DW	EE	EA	DW	EE	EA	DW	EE	EA	DW	EE	EA	DW	EE
Staphylococcus .aureus	20± 1.19	20± 1.19	17± 1.46	17± 1.46	15± 0.84	18	15± 0.84	12± 1.19	15± 0.54	14± 1.69	11± 0.84	13± 1.46	10± 1.19	10± 1.19	12± 1.19	---	---	---	---	---	---
Pseudomonas. aeruginosa	15± 1.46	13.0	---	10.0	---	10± 0.84	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Streptococcuss. Pyogenes	23± 1.46	21± 1.19	22± 0.84	19± 1.46	18± 0.84	16± 1.19	19.0	17± 0.84	15± 1.6	14± 0.84	15± 1.69	12± 1.19	13± 1.41	10± 1.45	---	----	---	---	---	---	---

Table 2: ANTIMICROBIAL EFFECTS OF ANTIBIOTIC DISCS

Antibiotics	*S. aureus*	*Str. pyogenes*	*Ps. aeruginosa*
Amoxyllin	-ve	-ve	-ve
Ofloxacin	-ve	-ve	-ve
Augmentin	-ve	-ve	10mm
Ceftriazone	-ve	-ve	12mm
Nitrofurantonin	-ve	-ve	-ve
Gentamycin	16mm	13mm	12mm
Pefloxacin	-ve	-ve	10mm
Cotrimozazole	-ve	-ve	-ve
Ciprofloxacin	14mm	-ve	8mm
Tetracycline	-ve	-ve	-ve.

Key: -ve---no activity

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Table 3: Antifungal screening of *Daniella oliveri* in mm

	100mg.ml			90mg/ml			80mg/ml			70mg/ml			60mg/ml			50mg/ml			25mg/ml		
	EE	EA	DS	EA	DW	EE	EA	DW	EE	EA	DW	EE	EA	DW	EE	EA	DW	EE	EA	DW	EE
Fusarium oxysporum	0	0	0		0	0	0	2	2	2	3	3	3	4	5	4	6	5	3	6	7

Table 4: Thin layer chromatography and Rf values of *Daniella oliveri*

Extracts	Extract front	Solvent front	Rf values	Antimicrobial activities			
				S.aureus	*Str. pyogene*	*Ps. aeruginosa*	*F.oxysporum*
D. oliveri a	6,10	17	2.8, 1.7	10	---	---	10
D. oliveri b	3,5,7,10	17	5.7, 3.4, 2.23.	1.7, 5	10,5.	---	7
D. oliveri c	2,5,10	17	8.5,3.4, 2.43.	---	---	5	5

Key; a---Ethanol extract, b ---Ethyl acetate extract, c---Aqueous extract.

Friedkin *et al* (2002) also reported the antimicrobial resistance of all health care associated pathogen. This was also confirmed by the fact that patients prefer to threat infections (particularly skin infections) using herbs rather than conventional methods (Personal communication).

The high antimicrobial properties of the plant extract (Table 1) is not surprising because previous studies have reported ethanol to be a better solvent for extraction (Obi and Onuoha, 2000).The presence of phytochemicals such as Flavonoids, Tannins, Saponins, and steroids are possiblr responsible for the high antimicrobial activity of the plant. These constituents have been known to possess medicinal values as well as exhibiting physiological properties (Oguntokun, 2006).The presence of these bioactive phytochemicals in plants inhibits life processes of microbes (Abiy *et al,* 2005).

The activity and spectrum of the extracts as a result of the nature of solvents lends more weight to the findings of Obi and Onuoha(2000) who reported high recovery of alkaloids and essential iols with ethanol than with any other solvents. Flavonoid is used in medicine as antimicrobial, anti-inflammatory and anti oxidant (Evans, 1996).Phenol have been reported to precipitate protein and render them resistant to proteolytic enzyme attack in the alimentary canal (Nataki, 1994).The presence of steroids and flavonoid in plant have been variously reported by researchers (Okwu, 2001; Matu *et al*., 2003). Steroidal compounds are of utmost importance in medicine. Tannin has been reported to inhibit the growth of HIV and Herpes simplex virus (Okuda *et al.,* 1991).Alkaloids had been found to be useful in the treatment of bruises and superficial wounds. They have been found to interfere with cell division in microorganisms (Noble, 1990). Pmploma Rogers(1999) reported that plants containing these phytochemicals have been useful in treating bacterial and fungal infections

In the chromatographic analysis (Table 5). The higher number of components in the ethanol extract is possibly responsible for the high antimicrobial activity observed for the ethanol extract.

CONCLUSION

The result of these findings confirmed the therapeutic potency of the plat. It can therefore be classified as active in the treatment of infections caused by the above listed microorganisms. This result thus provides the rationale for the continued use of the plant in traditional folkloric medicine. It also forms a good basis for the selection of the plant as candidate specie for further pharmacological studies.

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