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Full Length Research Paper

Phytochemical and antimicrobial studies on *Detarium microcarpum* Guill and Sperr (*Caesalpinioceae*) seeds coat

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Ninety percent aqueous methanol extract of the seeds coat (32.324%, w/w) of *Detarium microcarpum* was screened against clinical isolates of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomona aeruginosa,* Klebsiella *pneumonia, Salmonella paratyphi* and *Candida albicans*. A broad spectrum antimicrobial activity at the test concentration of 10 mg/ml comparable with the control drugs chloramphenicol and penicillin G for bacteria and nystatin for the mould *C. albicans* was observed. On bio-guided fractionation, the ethyl acetate soluble fraction (ES) retains the observed spectrum of antimicrobial activity. Preparative thin layer chromatographic separation of the active ESF on silica gel 60G using butan-2-one : chloroform : acetic acid : water (40:40:2:1 v/v/v/v) gave eight constituent bands: B1 (R_f = 0.000), B2 (R_f = 0.079), B3 (R_f = 0.250), B4 (R_f = 0.286), B5 (R_f = 0.500), B6 (R_f = 0.686), B7 (R_f = 0.814), and B8 (Rf = 1.000). B1 retained the observed antimicrobial activity spectrum, B2 and B3 showed residual inhibition against *B. subtilis* and B4 - B8 were inactive. Phytochemical screening indicated the presence steroidal saponins and flavonoids in B1, and flavonoids in B2 and B3. The UV-visible spectrum of B2 and B3 revealed the characteristic benzopyrone maxima (236 – 300 nm). Against the selected bacteria, MIC range of B1 of 1.0488 -2.8887 mg/ml was observed comparable to those of standard control (1.2357 - 3.3420 mg/ml) and chloramphenicol (2.0840 -3.9630 mg/ml) which is suggestive of resistant strains. This study revealed the antimicrobial principles in the seed coat of *D. microcarpum* to be steroidal saponins and flavonoids with the possibility of synergistic action.

Key words: *Detarium microcarpum*, seed coat, antimicrobial activity, benzopyrone, flavonoids, steroidal saponins, phytoalexins.

INTRODUCTION

Detarium microcarpum is a member of the *Caesalpinioceae* special family of the larger *Leguminosae*. It is a rain forest and savannah tree of tropical Africa (Keay, et al., 1964; Hopkins and Stanfield, 1966).

Among the Ibos in south eastern Nigeria, it is mythically conceived to be a chip of the primal tree that grows in God's own compound. It symbolizes truth and honesty (Ejizu, 1986). Nutritionally, the seed which is used as a traditional soup thickener contain lipids, carbohydrates, proteins, crude fibre and the essential elements: Na, K, Mg, Ca, S, P and Fe (Abreu and Relva, 2002; Abreu et al., 1998). Saponins, phytates and cyanides are reportedly present as anti-nutrients (Anhwange et al., 2004). In African ethno-medicine, the plant and the closely related species *Detarium senegalense are* used in the treatment of syphilis, dysentery, bronchititis, leprosy, sore throat, pneumonia, diarrhoea, malaria and meningitis (Daziel, 1937; Abreu et al., 1998, 1999; Okwu and Uchegbu,

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Abbreviations: MIC, Minimum inhibitory concentration; **IZD,** inhibition zone diameter; **Rf,** retention factor; **SC,** seed coat of *Detarium microcarpum*; **ES,** ethyl acetate soluble fraction of SC; **EI,** ethyl acetate insoluble fraction of SC; **TLC,** thin layer chromatography; **B1 - B8,** TLC fractions of ES.

2008; Burkil, 1995; Keay et al., 1989). Its antifungal and acetyl cholinesterase inhibitory activities have been reported (Cavin et al., 2006; Adamu et al., 2006). The muco-adhesive properties of the defatted gum extracts have been reported also (Okorie and Chukwu, 2005).

This study is aimed at a scientific investigation into the anti-microbial and phytochemical properties of the seeds coat of *D. microcarpum* with the view of authenticating its ethno medicinal uses

MATERIALS AND METHODS

Materials

The seeds of *D. microcarpum* were purchased from the Nsukka market in south eastern Nigeria and authenticated by Ozioko D of the Botany Department of the University of Nigeria, Nsukka. They were subsequently de-coated to give the seed coat (SC). The reagents, methanol, acetone, chloroform, butan-2one, acetic acid, dimethylsulphoxide and silica gel 60G were of analytical grade products of BDH Chemicals Ltd, England and were obtained commercially. The reference drugs penicillin®, nystatin® and chloramphenicol® are formulated generics of Helm pharmaceuticals, Hamburg, Germany, Pharmamed pharmaceuticals Ltd, India and Yangzhou Pharm, China, respectively. The microorganisms *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella paratyphi* and *Candida albicans* were clinical isolates form the Bishop Shanaham Hospital, Nsukka and were purified by culturing at the Pharmaceutical Microbiology Laboratory of the University of Nigeria, Nsukka, Nigeria.

Extraction and isolation of plant materials

One hundred and seventy grams (170 g) of the powdered plant materials was macerated in 90% methanol for 24 h and filtered. The residue was repeatedly extracted with more portions of the aqueous methanol until a colourless extract was obtained. The filtrates were combined and concentrated to dryness using the rotary evaporator at 40°C. The colour of the SC extract was reddish-brown. The SC was further fractionated with ethyl acetate to obtain the ethyl acetate soluble (ES) and insoluble portion (EI). The active ESF portion was then separated by thin layer chromatography using 20 x 20 cm glass plate coated to a thickness of 0.5mm with silica gel 60G. Ascending technique was employed with butan-2-one: chloroform: acetic acid: water (40:40:2:1 v/v) after other solvent systems were tried. Detection was by visualization in daylight and UV light. The bands were collected separately and eluted with methanol to give a total of eight constituents B1 – B8.

Phytochemical methods

The presence of alkaloids, terpenoids, steroids, flavonoids, tannins, phenolics, reducing sugars, glycosides and carbohydrates were screened for by using the appropriate standard phytochemical screening reagents (Harbourne, 1998; Trease and Evans, 1985; Sofowora, 1982).

UV spectroscopic methods

The UV spectra of 0.1 mg/ml solution of the constituents B1 - B8 in methanol were determined and their λ-max was recorded. These were compared with literature (Dean, 1963; Harbourne, 1998).

Microbiological methods

The cork boring agar diffusion method was used (Howard et al., 1987; Ebi and Ofoefule, 1997). Mueller Hinton agar was used as growth media for bacterial and Saboraund dextrose agar for the fungi. Penicillin G® and chloramphenicol® were used as standard control drugs for bacterial strains, while nystatin® was used for *C. albicans.* Five fold serial dilutions of the 10 mg/ml test concentration in dimethyl sulfoxide (DMSO) were made and their respective IZD were determined from where the minimum inhibition concentration against the selected pathogenic organisms were determined which is from the intercept of a plot of logarithm of concentration against square of inhibition diameter (Howard et al., 1987; Ebi and Ofoefule, 1997).

RESULTS

The yield of SC was 32.324% (w/w). The result of the antimicrobial screening in Table 2 showed the ES portion of the seeds coat to contain the active principle(s). Thin layer chromatographic separation of the ES portion gave eight constituents: B1 (R_f = 0.000), B2 (R_f = 0.079), B3 (R_f = 0.250), B4 (R_f = 0.286), B5 (R_f = 0.500), B6 (R_f = 0.686), B7 (R_f = 0.814) and B8 (R_f = 1.000) with colour: reddish brown, orange, grey, yellow, yellow, yellowish-green, orange and green, respectively. Fraction B1 showed similar spectrum of activity like the ES, while fractions B2 and B3 inhibited *B. subtilis* only. Fractions B4 - B8 however showed no antimicrobial activity at the test concentration of 10 mg/ml. The result of phytochemical study in Table 1, revealed the presence of steroidal saponins and flavonoids in B1, flavonoids in B2 and B3, while B4 - B8 were insensitive to the used screening reagents. With the exception of B4 and B7 with maximum at 266 and 260nm respectively, the UV absorption spectrum of the other fractions (B2, B3, B5, B6 and B8) showed principal maximum in the region of 236 ± 3 nm in methanol (Table 3). The minimum inhibitory concentrations of B1 in Figures 1 and 2 showed a better inhibition effect against *B. subtilis* and *K. pneumonia* when compared to penicillin G® and also better inhibition of *S. paratyphi* and *S. aureus* when compared to chloramphenicol®.

DISCUSSION

Some flavonoids, saponins and phytosterols are known to be phytoalexin principles in plants (Harbourne, 1998; Dean, 1963; Ishaya et al., 1969; Bailey and Mansfield, 1982; Singh et al., 1982; Arnason et al., 1983; Panda and Khush, 1995; Foley and Moore, 2005). Seed coats are known to accumulate phytoalexins as a defence mechanism against predators and microbial attack on the seed. Their detection in the seed coat of *D. microcarpum* may therefore not be unconnected with this and the

Table 1. Results of phytochemical screening.

Screened phytochemical	Extracts/fractions/TLC Fractions										
	MESC	EI	ES	B1	B2	B3	B4	B5	B6	B7	B8
Alkaloids	-	-	-	-	-	-	-	-	--	--	-
Phenolic	++	+++	+++	++	++	++	tr	-	-	-	-
Flavonoids	++	++	+++	++	++	++	tr	-	-	-	-
Tannins	-	-	--	-	-	-	-	-	-	-	-
Saponins	++	+	+	++							
Triterpene/ steroids	++	+	+	++	-	-	-	-	-	-	-
Reducing sugars	+	+	+	+	+	-	-	-	-	-	-
Glycosides	+++	+++	+++	+++	+	-	-	-	-	-	-
Carbohydrates	++	++	++	+	+	-	-	-	-	-	-

+ = Moderately present; ++ = present; +++ = highly present; tr = trace amount and - = not present.

Table 2. Antimicrobial sensitivity of extracts and fractions of *D. microcarpum* seeds coat.

Samples at 10 mg/ml	Zone of inhibition (mm) against micro-organisms						
	S. aureus	*B. subtilis*	*K.p*	*E. coli*	*S.p*	*P.a*	*C.a*
SC	20	15	18	18	15	18	18
ES	20	16	23	14	15	16	18
EI	-	-	--	-	-	-	-
B1	23	16	26	15	15	20	17
B2	-	12	-	-	-	-	-
B3	-	10	--	-	-	-	-
B4	-	-	--	-	-	-	-
B5	-	-	--	-	-	-	-
B6	-	-	--	-	-	-	-
B7	-	-	--	-	-	-	-
B8	-	-	--	-	-	-	-
Penicillin G	47	10	16	-	14	-	-
Chloramphenicol					18		_
Nystatin	-	-	--	-	-	-	30

- Sign means no zone of inhibition, *P.a*, *K.p*, *C.a* and *S.p* stand for *P. aeruginosa, K. pneumonia, C. albicans* and *S. paratyphi*, respectively; SC = seeds coat of *D. microcarpum,* while ES and EI are the ethyl acetate soluble and insoluble fractions of SC, respectively; B1 - B8 are the TLC fractions of ES.

observed antimicrobial activities. The high MIC values of the penicillin G® (1.2357 – 3.3420 mg/ml) and chloramphenicol® (2.0840 – 3.9630 mg/ml) could be ascribed to resistant strains and/or content of active non-uniformity. Generally, MICs greater than the breaking point values of 16 µg/ml for chloramphenicol and 16 ug/ml for penicillin-G against strains of microorganism means that the microorganism is resistant to the drug (Howard et al., 1987). The breaking point of an antimicrobial agent is the concentration that can achieve optimal therapy in the serum of patients (Howard et al., 1987). MIC values ≥0.25 µg/ml for pure penicillin G against staphylococci bacteria has been associated with resistant strains (Baxter, 1987). An MIC range of 0.2 – 1.0 µg/ml has been reported for pure penicillin-G, against *S. aureus* (Baxter, 1987). MIC values ≥2 mg/l for penicillin-G against pneumococcal bacteria have been associated with resistant strains (McGowan and Metchock, 1985). An MIC range of 0.1 – 5 mg/ml for chloramphenicol against resistant strains of *Salmonella enterica serovar typhi* have been reported (Mandal et al., 2004). An MIC value as high as 10.8 mg/ml, have been reported for chloramphenicol against *Salmonella* spp. (Ajali and Chukwurah, 2004).

Conclusion

This study reveals the potentials of phytoalexins in the

Table 3. The ultra-violet (UV) absorption maxima of the TLC fractions of ES.

TLC fraction	λmax (nm)	Absorbance A	Relative intensity
B1	206	0.595	21.948
	260	2.711	100
	302	2.69	99.521
B2	206	0.426	21.735
	236	1.941	99.031
	278	1.96	100
	358	0.442	22.551
	371	0.418	21.327
B3	206	0.237	17.087
	236	1.387	100
	278	0.933	67.268
	368	0.467	33.67
	386	0.445	32.084
B4	266	2.252	100
	392	0.591	26.243
B5	236	1.605	91.872
	266	1.747	100
	398	0.306	17.516
B6	212	0.74	26.561
	239	2.786	100
	278	2.254	80.905
B7	260	2.583	100
	302	2.536	98.1894
B8	233	2.829	100
	275	1.593	56.31

λmax (nm) = Maximum wavelength in nanometer; TLC = thin layer chromatography.

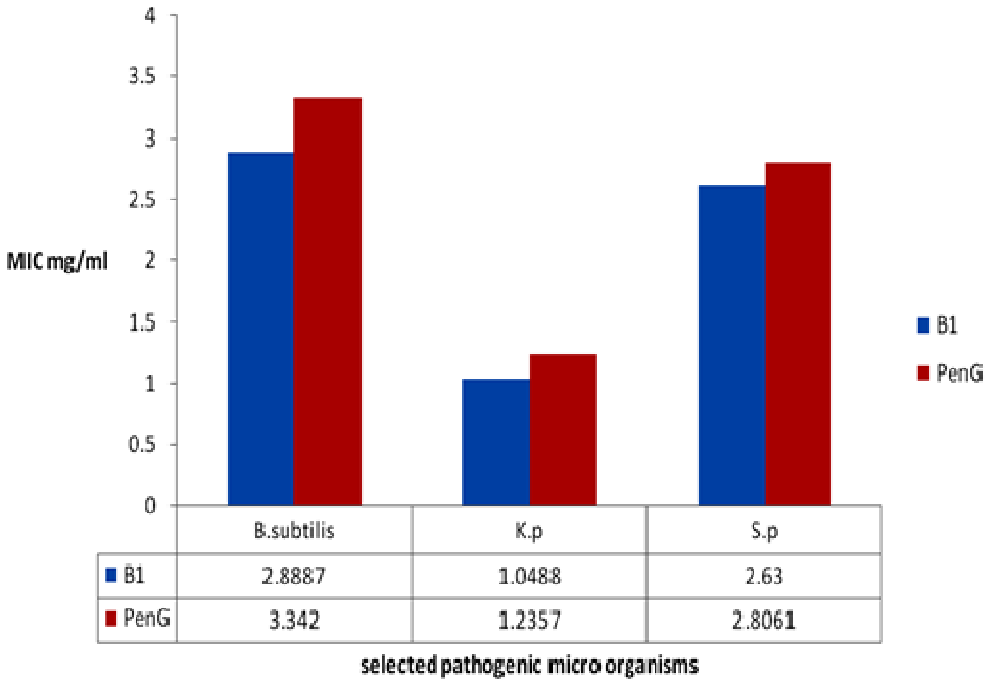

Figure 1. Minimum inhibitory concentration (MIC) values of B1 and penicillin G.

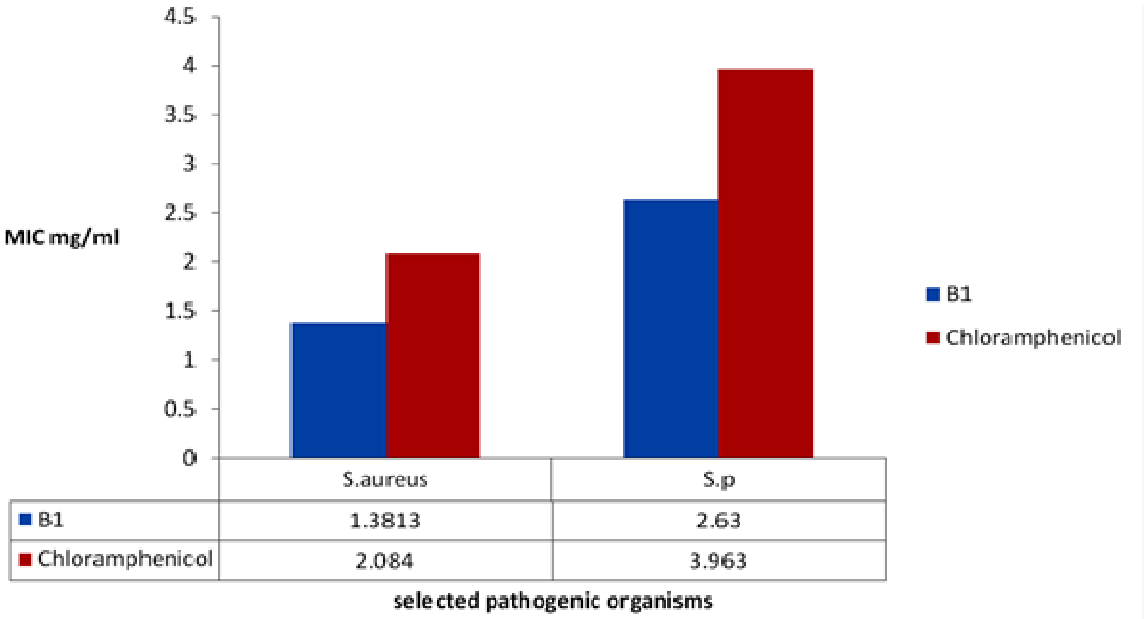

Figure 2. Minimum inhibitory concentration (MIC) values of B1 and chloramphenicol.

seeds coat of *D. microcarpum* and by extension, other seeds in the management of infectious diseases. It also confirms the reported ethno medicinal uses (Daziel, 1937; Abreu et al., 1998). Investigative work is on-going to purify and characterise the active principles.

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