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Chemomorphological study and antimicrobial activity of *Daedalea quercina*

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ABSTRACT
Chemical and morphological studies were carried out on Daedalea quercina Fr. from Nigeria to determine its characteristics. Fifty specimens of the basidiocarps of Daedalea quercina were observed and collected from the dead log of Mangifera indica in August 2003, Obosi, Nigeria. The specimens were characterized using microscopic and macroscopic parameters and chemical spot test. The antimicrobial activity of the crude n-hexane: diethyl ether, chloroform: acetone and methanol extracts of the polypore were tested using thin layer chromatography agar overlay method. Morphologically, D. quercina was yellowish in colour, broadly sessile, and laterally fused in 2-3 layers. Basidiocarp was leathery with a pleasant smell. The pileus was concentrically zoned, has lamellate pore surface and the hyphal system was trimitic with ellipsoid, hyaline spores. Chemically, the colour of the tissues changed to brown with Ferric chloride, non-reactive with Melzer's reagent, and produced characteristic colours with various reagents used in the test. None of the solvent extracts inhibited the growth of C. herbarium. The three solvent extracts were active against B. subtilis but the growth of P. syringea was inhibited by the hexane: ether and chloroform: acetone extracts of the fungus. Phenolics and terpenes were detected in the three solvent extracts.

Keywords*: Daedalea quercina, Chemical and morphological studies, antimicrobial activity, Phenolics, terpenes, Nigeria*

INTRODUCTION

Daedalea species (elo) are mushrooms in the class basidiomycetes (Kirk *et al*., 2001). Medicinal basidiomycetes represent unlimited source of phytochemicals such as primary and secondary metabolites (Roja and Rao, 1998). According to Lorenzen and Anke (1998) and Mizuno (1999) wide ranges of substances from higher basidiomycetes belonging to different classes of chemical compounds have been described and their biological properties evaluated. Benedict and Brady (19720 reported the presence of polyacetylene in compounds from *Daedalea* species. Five new triterpens 16-o-acetylpolyporenic acid C, 16 alpha- acetoxy-24-methylene-3-oxolanost-8-en-21-ioc acid, (+)-24-methylene-3, 23-dioxolanost-8-en-26-ioc acid, (+)-3beta, 12beta-dihydroxy-24-methyl-23-oxolanost-8-en-26-oic acid, and 12beta, 23-epoxy-3alpha, 23-dihydroxy-24-methyllanost-8-en-26-oic acid and some known fungal constituents have been isolated from *D. quercina* (Rosecke and Konig, 2000). The enzyme laccase purified from the white rot fungus was used in decolorizing synthetic dyes (Baldrian, 2004).Steroids, Lignins, ganomycins, vitamins, nucleosides, alkaloids and amino acids have also been characterized from *Ganoderma* species (Smania *et al*., 1999; Chai *et al*., 1997; Rosecke and Konig, 2000; Mothana *et al*., 2000).

Little has been reported on the taxonomy of polypores from Nigeria (Zoberi, 1972; Ofodile and

Uma, 2001; Ofodile 2006).There is also an increasing problem with pathogens developing resistance to available antibiotics used against them, which has resulted in an increased interest in alternative control strategies. This paper reports the morphological and chemical characteristics of *Daedalea quercina* Fr. and the antimicrobial activity of three solvent extracts of the polypore against *Bacillus subtillis*, *Pseudomonas syringea* and *Cladosporium herbarium*

Material and Methods

Collection and preservation of fungal specimens

Fifty specimens of the basidiocarps of *Daedalea quercina* were observed and collected from the dead log of *Mangifera indica* in August 2003. The length and diameters of the specimens were measured with a ruler and the details of the macroscopic features, colour, smell and texture of the specimens were recorded according to the methods of Ryvarden and Johanson (1980); Laessoe (1998). Dr Peter Roberts, Mycology section, The Royal Botanic Gardens (RBG), Kew, verified the identity of the fungus. Voucher specimens were deposited in the Herbarium of RBG, Kew, UK.

Chemomorphological study of fungal specimens

Morphological and chemical studies of the specimens were carried out according to the methods of Zoberi, (1972); Ryvarden and Johansen, (1980); and De Rosa, (2003).

Morphological examination of fungal specimens Macroscopic examination

The fruiting season, shape, width, length, thickness, texture, and smell of the fruit bodies, colour, texture, shape, surface margin, area of attachment, and behaviour on touch of the pileus were assessed. The texture, colour and diameter of the context, colour changes, diameters of the pore surfaces were determined using the methods of Ryvarden and Johansen, (1980).

Microscopic examination

Tissues and spores were observed under a binocular microscope (Leica DMLB) at ×63 magnification (2mm equivalent to 1µm). Photomicrographs of tissues and spores were taken with a digital camera

(AF micro-Nikon, 60mm f/2.8D), which was connected to a computer (Dell Latitude, Pentium 111).

Slides of the specimen were prepared using drops of KOH solution on a clean, grease- free slide. Minute pieces of tissue were removed from the pileus surface, the cortex, and tubes of the basidiocarp with the aid of a forceps, placed in the mounting solution and covered with a clean slip. The cover slip was gently tapped to spread out the tissue, so that the structures could be easily observed. Spores were scraped from the pore surface into the mounting solution for observation. Types of hyphal system, number, colour, diameter, presence or absence of cystidia were assessed. Hyphal diameters (20 each) were measured with caution avoiding collapsed hyphae. The diameters, shape of spores were also measured as above. Caution was taken to avoid very young and immature spores. The averages of the sizes obtained were regarded as the diameter of the hyphae and spores.

Chemical spot test on fungal specimens

The chemical tests employed were methods of De Rosa (2003); and Zoberi (1972)

Ferric chloride test:

Five drops of 5% solution of ferric chloride in water were applied to a mass of tissues (20mg) of the sample and allowed to stand under observation and the reaction was recorded.

Melzer's reagent test:

Chloral hydrate (20 grams) was mixed with iodine (0.5 gram), potassium iodide (1.5 gram) and water (20 millilitres). The mixture was warmed with stirring to dissolve. Five drops were mixed with a mass of tissues (20mg) of the sample and was allowed to stand for 10-20 minutes and reactions were recorded.

10%Ammonium Hydroxide (NH_4OH) test:

Ammonium hydroxide (10%) was mixed in enough water to make 100 milliliters of solution. Five drops were then applied to a mass of tissues (10mg) of the sample and were allowed to stand for 10-20 minutes and reaction was recorded.

Sulphuric acid test:

Two drops of concentrated sulphuric acid (18 molar) were applied to a mass of tissues (20mg) of the sample and was allowed to stand for 10-20 minutes and reaction was recorded.

Ferrous sulphate test:

Five drops of 5% ferrous sulphate solution was applied to a mass of tissues (20mg) of the sample and

was allowed to stand for 10-20 minutes and reaction was recorded.

Antimicrobial activity

Assays were performed against two bacterial species *Bacillus subtilis* IMI347329 and *Pseudomonas syringae* IMI34748, which were grown on the nutrient agar (Oxoid CM3) at 37°C for 24h and stored at 4°C. The fungus used was *Cladosporium herbarum* IMI 300461 and was grown on Malt extract (Oxiod L39, 2%) agar (1.5%) medium at 25°C. The sporulating cultures were kept at 4°C.

Preparation of fungal specimen

Ground fungal sample was sequentially extracted with three combinations of solvents to ensure selective extraction of compounds based on their polarity. Sample (1.5g) of the polypore was first extracted with n-hexane: diethyl ether (1:1) overnight at room temperature. The n-hexane: diethyl ether extract was filtered using Whatman No. 1 filter paper and air-dried. Chloroform; acetone and 80% methanol extracts were further obtained from the residue. They were filtered into weighed vials using Whatman No 1 filter paper. The weight of each extract was determined and the final residue discarded.

Thin layer chromatography (TLC) bioassay

The TLC agar overlay method (Rahalison *et al*., 1991) was used to test the activity of the fungal extracts. The hexane: diethyl ether extracts were reconstituted in chloroform, while the chloroform: acetone and methanol extracts were reconstituted in acetone and methanol respectively.

Antibacterial assay

Aliquots (100µg) of each extract were spotted on the precoated TLC plates (1.05554 aluminum sheets, 20 by 20cm, silica gel 60 F_{254}, Merck). The chromatograms were obtained using the following mobile phases (i) the n-hexane/diethyl ether extract with 100% chloroform (ii) the chloroform: acetone extract with chloroform: acetone (9:1) and (iii) the 80% methanol extract with chloroform: acetone: water (75:25:2).

An aliquot (1µl) of a chloramphenicol solution (1mg ml^{-1}) was used as a positive control. Nutrient agar (2.8g ml^{-1}) seeded with test organism adjusted to × 10^7 was evenly spread on the air dried TLC chromatograms fixed in assay trays (22×22cm) and excess medium quickly poured out of the trays. TLC plates without control or antimicrobial agent were used as negative controls. Plates were incubated overnight at 37°C after which a p-iodonitrotetrazolium violet solution (0.05mg ml^{-1} in 5% aqueous EtOH) was spread evenly on the plates and incubated further for 1hour at 37°C. Growth inhibition zones were observed as clear spots against pink background.

Antifungal assay

The antifungal activity (Homans and Fuchs, 1970) was carried out on the species of polypore by spraying the conidia of *Cladosporium herbarium* suspension in a malt extract (2%) solution. Aliquots (100µg) of extracts were spotted on the precoated TLC plates (1.05554 aluminum sheets, 20 by 20cm, silica gel 60 F_{254}, Merck) and eluted as above. Nystatin (1µg) was used as a positive control while the TLC plates without extract or antifungal agent were regarded as the negative control. The seeded plates were incubated in moist, sealed container for 72hours at 25°C. Growth inhibition zones were observed as above.

Chemical analysis

The reconstituted n-hexane:diethyl ether, chloroform:acetone and methanol extracts of the fungal sample were spotted on silica gel TLC plate (20×20cm) and developed with solvents as above. To screen the extracts for terpenoids and phenolics the chromatograms were sprayed with anilsaldehyde-sulphuric acid solution as used by Ofodile *et al* (2005)

Results and Discussions

The results of chemical spot test and antimicrobial activity of *D. quercine* are presented in Tables 1 and 2 respectively. Some of the morphological features of the polypore are also presented in Plates 1 and 2.

The fruiting body of *Deadalea quercina* was perennial, single and broadly sessile; yellowish in colour and laterally fused in 2-3 layers, semi circular, 8-40cm wide and 3-11cm thick, very leathery and soft toughens with dryness with pleasant smell and strongly attached to the substrate. The pileus is flat to slightly concave with a raised base, smooth and concentrically zoned (Plate 1A and B). The margin was wavy and ochraceous, with deeper brown to greyish inner parts. Pore surface was flat and lamellate (gilled) with irregular hymenophore (Plate1 A_1 and B_1)**.** Context was 0.5-1cm thick, ochraceous. The hyphal system was trimitic, skeletal, thick walled, light brown and 2.5-6.0 µm in diameter (Plate 2a). Generative hyphae were thin walled, hyaline

with clamps at the septa, 1.5-4.0cm in diameter (Plate 2b). The binding hyphae was with short branches having solid thick yellowish brown walls, light golden in colour with the diameter of 3.5-6.0 cm. Spores were ellipsoid, hyaline, thick walled, smooth and 5.5-7 x 2.5-3.5µm in diameter with grey spore print.

An earlier description by Ryvarden and Johanson, (1980) and Pamasto, (2001) is in conformity with the present observation of the species except in the possession of smaller spore size.

Table 1: Reaction of tissues of *Deadalea quercina* with various reagents(how was each added and in what concentration?

Reagent	Reaction
$FeCl_3$	Colour changed to brown immediately
Melzer's reagent	Retained colour of reagent
KOH	Colour changed to light brown
NH_4OH	Colour changed to light brown
$FeSO_4$	Colour changed to brown
H_2SO4	Colour changed to blood red

The colour change of the tissues of *D. quercina* in to brown with ferric chloride is an indication that it could contain phenolic compounds (De Rosa, 2003). The polypore procuced specific colours with reagents (Table 1). According to Zoberi (1973); De Rosa (2003) response of tissues to chemicals can serve as means of identification among macrofungi

Table 2:Antibacterial activity of extracts of *Deadalea quercina* (100µg) on *Bacillus subtilis* and *Pseudomonas syringae* sprayed on TLC plates

Extraction solvent	**Hex:Eth**		**Chl:Ace**		**Meth**	
Bacterial species						
	Rf	Activity	Rf	Activity	Rf	Activity
Bacillus subtilis	0.00	+	1.00	+	0.95	+
	0.75	++				
Pseudomonas syringae	0.00	+	ni	ni	0.65	+

Hex:Eth; hexane:diethylether, Chl:Ace; chloroform: acetone, Meth; methanol, +; activity, ++; strong activity; ni; no activity, Rf value is the movement of the compound relative to the solvent front

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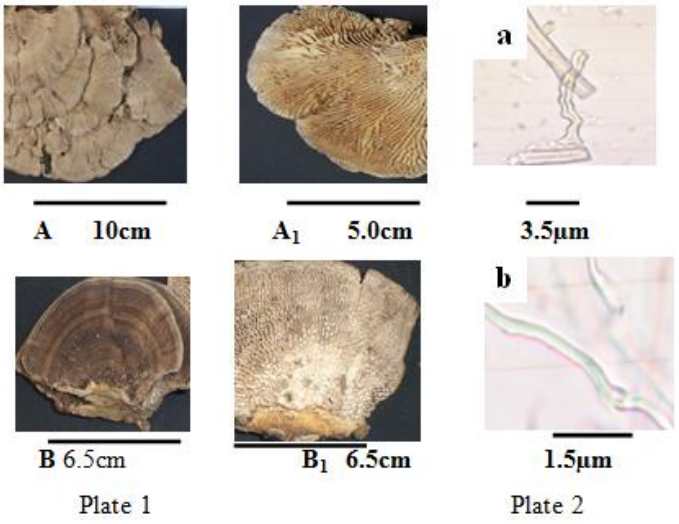

Plate 1 right: A and B; pileus surface and A_1 and B_1; pore surface of *Deadalea quercina*.

Plate 2 left: Photomicrographs of the hyphal system of *Deadalea quercina* (a) skeletal hyphae; (b) generative hyphae.

Table 2 shows that the three solvent extracts of *D. quercina* inhibited the growth of *B. subtilis*. The TLC and preliminary chemical analysis showed that the polypores produced violet, blue to purple colours on the TLC plates when stained with anisaldehyde in sulphuric acid and could contain terpenes (Stahl and Kaltenbach 1961; Lisba, 1964). Moreover, triterpens have been reported as the major constituents of *D. quercina* (Rosecke and Konig, 2000). Therefore, the antifungal activity of the polypore could be attributed to some metabolites namely terpenes and phenolics detected in the different solvent extracts. The chemomorphological features of the polypore will provide information for its identification, which could be useful in therapeutic applications.

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