Research Article

Comparative phytochemical evaluation, antimicrobial and antioxidant properties of methanolic and ethanolic extracts of *Daedalea elegans*- A Nigerian Mushroom

Aina, D.A[1]* , Olawuyi, O.J[2] , Mensah-Agyei, G.O[1]. , Olaiya, A.R[1]. , Adeoye-Isijola, M.O[1].

[1]*Department of Microbiology, School of Science and Technology, Babcock University, Illisan-Remo, Ogun State P.M.B 21244 Ikeja, Lagos, Nigeria*

[2]*Department of Botany and Microbiology, University of Ibadan, Oyo State, Nigeria*

Received: 2 September 2016 Revised: 10 October 2016 Accepted: 11October 2016

Abstract

Objective: The methanolic and ethanolic extracts of *Daedalea elegans* were evaluated for their phytochemical constituents, antimicrobial and antioxidant capacities. **Materials and methods:** Ethanol and methanol extracts of fruit bodies of *Daedalea elegans* were tested against *Escherichia coli, Salmonella typhi*, *Staphylococcus aureus, Streptococcus pneumoniae, Proteus mirabilis, S. paratyphi, Enterococcus fecalis* and *Candida albicans*. The antibacterial and antifungal activities of the above extracts were determined by well diffusion assay. **Results**: Phytochemical analysis indicated the presence of alkaloids, steroids, flavonoids in both extracts. Both extracts were effective against *E.coli, Streptococcus aureus* and *Candida albican*. The ethanol extract showed higher zone of inhibition than the methanol extract tested. In antioxidant properties both extracts were significantly lower than the ascorbic acid of the DPPH and phenolic content than the total flavonoid content. **Conclusion**: The results indicated that *D. elegan* possess antimicrobial and antioxidant potentials.

***Keywords*:** *Daedalea elegans*, phytochemicals, antioxidant and antimicrobial

Introduction

Mushrooms belong to a special group of macroscopic fungi. Macromycetes arranged in the phylum Basidiomycota and some of them in the Ascomycota are known as the higher fungi (Moradali et al., 2007). Several compounds with important pharmaceutical properties have been isolated from these organisms. Substances that act as anti-aging, in longevity, modulating the immune system, having hypoglycemic activity and to inhibit tumor growth have been isolated from mushrooms, such as polysaccharides. Polysaccharides can interconnect several points forming a wide variety of branched or linear structures, for example, ß glucans (Ooi and Liu, 2000). Furthermore, other bioactive substances such as triterpenes, lipids and phenols have also been identified and characterised in mushrooms with medicinal properties (Maiti et al., 2008). The predominating interest of Nigerians in mushrooms is to use them as food. This is because of their good taste, appetising aroma and nutrient contents (Fasidi and Kadiri, 1993; Jonathan and Fasidi, 2001).

For centuries, mushrooms have been prescribed for treatment of diseases such as gastro–intestinal disorder, bleeding, high blood pressure and various bacterial infections (Stamets, 1993; Brodie, 1998). Inedible mushrooms show bitter and pungent taste, and especially those belonging to Polyporaceae have been used as medicinal drugs (anticancer, etc.) in China from ancient times. The use of natural products isolated from mushrooms against infection and cancer diseases is one of the cornerstones of modern medicine (Mizuno, 1999). Examples of these mushrooms are *Daedalea elegans* ,*Ganoderma applantum* and *Agaricus* spp., which have been claimed to have anticancer properties (Benjamin, 1995). However, little attention has been paid to the chemical constituents of inedible mushrooms.

Daedalea elegans is an inedible mushroom which are highly variable species or cluster of species, perhaps

**Address for Corresponding Author:*
Aina D. A.
Department of Microbiology,
School of Science and Technology, Babcock University, Illisan-Remo, Ogun State P.M.B 21244 Ikeja, Lagos, Nigeria.
Email: ainatayo2002@yahoo.com; tel: +2348033565433

recognized by its elongated, maze-like pores; its thin, tough, whitish to brownish cap with zones of colour; and the tendency of its pore surface to bruise reddish. It is lumpy towards the point of attachment and smoother toward the margin; and its ecological role, serving to decompose the deadwood of hardwoods**.** *D. elegans* has been reported as a source of phytochemicals and antioxidants with medicinal values.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reaction produces free radicals, these radicals can start chain reaction that damage cells. Antioxidants terminate these chains reaction by removing free radicals intermediate and inhibit other oxidation reaction. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiol, ascorbic acid or polyphenols (Sies 1997). This study was set out to examine comparatively the phytochemical constituent, antioxidant and antimicrobial properties of ethanolic and methanolic extracts of *D. elegans*.

Materials and methods

Fruiting bodies of *Daedalea elegans* was bought from Ibode market in Ibadan and was identified by Prof. Gbolagade Jonathan of the department of Botany, University of Ibadan, Nigeria. The fruit bodies of the test mushrooms was cut into bits and dried. These dried carpophores were pulverized in a moulinex blender. 30.0g of the powdered samples was weighted into conical flasks. Methanol and ethanol were added separately to make it up to 300ml (10g/100ml). The flasks were covered with aluminium foil and allowed to stand for a day before extraction. The solution was filtered through Whatman filter paper no 1 and the filtrate obtained was concentrated in a rotary evaporator. The methanol and ethanol was recovered and the extract was collected and dried using the methodology of Jonathan and Fasidi (2003). The methanolic and ethanolic extracts were reconstituted in methanol and ethanol for antioxidant activity assays and in DMSO for antimicrobial activity assays.

Chemical tests were carried out on the extracts for qualitative determination of the phytochemical constituent using the standard procedures as described by Harbone (1998) and Sofowora (1991).

Test organisms (*Enterococcus fecalis, Salmonella typhi, S. paratyphi, Escherichia coli, Proteus mirabilis, Staphlococcus aureus, Streptococcus pneumonia* and *Candida albicans*) were obtained from the Department of Biotechnology and Molecular Biology of the Nigerian Institute of Medical Research (NIMR), Yaba-Lagos. Stored isolate of *C. albicans* was recovered by subculturing on Sabraud Dextrose Agar and incubated at 30ºC for 24hours. While stored bacterial cultures were recovered by subculturing the isolates on Mueller Hinton Agar and incubated at 37ºC for 24hours, except in the case of *Streptococcus pneumoniae* which was cultured on Chocolate Agar.

Antimicrobial Assay

The antimicrobial activity of the extracts were tested using well diffusion method (Bauer et al., 1996). Colonies of each microbial isolates except *S. pneumoniae* were emulsified in 10ml of sterile normal saline to obtain a turbidity equivalent to 0.5 McFarland. Colonies of *S. pneumoniae* were emulsified in 10ml of sterile normal saline to obtain turbidity equivalent of 1.0 McFarland. 0.1ml of the microbial suspension was pipette on solidified media in Petri dishes containing Chocolate Agar for *Streptococcus pneumoniae*, Mueller Hinton Agar for other bacteria isolates and PDA for *Candida albican.* Four wells were made on each agar plate using a sterile 8mm cork borer and filled with 0.2ml 10mg/ml, 20mg/ml and 30mg/ml of *D. elegans* in such a way that both extracts were tested against all the organisms at different concentrations. A well containing 0.2ml of Dimethyl – sulfoxide (DMSO) was used as a positive control well in every assay. Bacteria plates were incubated at 37˚C for 24 hours and 30˚C for fungi. Growth inhibition was observed and measured in zones of inhibition to the nearest 0.1mm.

Antioxidant Assay

Determination of total phenolic content (TPC): The TPC was estimated by Folin-ciocalteu calorimeter method based on the procedure of Singleton and Rossi (1965). The extract (50mg) was mixed with folin-Ciocalteu reagent (0.5ml) and deionized water (7.5ml). The mixture was kept at room temperature for 5 minutes and then 10ml of 7% sodium carbonate was added to the mixture and then incubated for 90 minutes at room temperature. After incubation, the absorbance against the reagent blank was determined at 760nm. The TPC of the plant was expressed as Gallic acid equivalent (g/100g dry weight). All samples were analysed in triplicates.

Determination of total flavonoid content (TFC): The TFC was measured following a spectrophotometer method (Dewanto et al., 2002).

1ml (100ug/ml) of the extract was diluted with water (4ml) in a 10ml volumetric flask. Initially, 5% $NaNO_2$ solution (0.3ml) was added to each volumetric flask. After 5minutes, 10% $AlCl_3$ (0.3ml) was added and 2.4ml water was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510nm. TFC were determined as quetrcetin equivalents (g/100g of dry weight). Three readings were taken for each sample and the

result averaged.

Determination of DPPH radical scavenging activity: This was carried out according to the 2,2.diphenyl-2-picrylhydrazyl (DPPH) assay system (Mensor et al.,2001). 1ml of 0.3mm DPPH methanol solution was added to a 2.5ml solution of the extract and allowed to react at room temperature for 30minutes. The absorbance of the resulting mixture was measured at 518nm and converted to percentage antioxidant activity (AA%) using the formula

AA% = [(Abs blank – Abs sample) x 100]

Abs = Absorbance.

Statistical Analysis

Data obtained were analysed by one way analysis of variance, and means were compared by LSD's tests (SPSS 15.0 version)

Results

Phytochemical Screening

The phytochemical analysis revealed the presence of alkanoid, flavonoids and steroids in both methanolic and ethanolic extracts of *Daedalea elegans,* Terpenoids was present in the methanolic extract, Saponine was present in ethanolic extract while Phlobatannis, antraquinones, tannins were absent in both extracts.

Table 1. Phytochemical analysis of methanolic and ethanolic extracts of *D. elegans*

Tests	Methanolic extract	Ethanolic extract
Tannins	_	_
Alkaloids	+	+
Phlobatannis	_	_
Steroids	+	+
Flavonoids	+	+
Anthraquinones	_	_
Terpenoids	+	_
Saponine	_	+

+ = present **- = absent**

Antimicrobial Assay

The extract did not inhibit the growth of *Proteus mirabilis, Enterococcus fecalis, Samonella typhi, Salmonella paratyphi, Streptococcus pneumonae* at any of the concentration used. There were zone of inhibition on *E. coli* plate for both extracts while *S. aureus* showed zones of inhibition for methanolic extract only. The highest zone of inhibition was exhibited on *S. aureus* with a zone diameter of 13mm at 0.3ml of the methanolic extract while the lowest zone of inhibition was exhibited on *E.coli* with a zone diameter of 2mm at 0.1ml. The lowest zone of inhibition of the ethanolic extract was 5mm at 0.1ml on *E. coli* plate. Zones of inhibition were exhibited for *Candida albican.* This study shows the antibacterial and antifungal activity of the extracts against multidrug resistant bacterial pathogens.

The sensitivity test shows that there was inhibition in *S. typhi, S. paratyphi, E. coli, P. mirabilis, E. fecalis* and *S. aureus* but there was no zone of inhibition in *S. pneumoniae* and *Candida albican.*

Antioxidant Assay

All samples were analysed in triplicates. Ascorbic acid was used as the control and the result was averaged for each sample. The mean and standard deviation was calculated using the SPSS evaluation

Discussion

Daedalea elegans used in this study was found to exhibit various degrees of antimicrobial effects against the tested microorganisms. This was evidenced by the clear zone of inhibition exhibited around the tested organisms.

Phytochemical screenings of *Daedalea elegans* extract indicate the presence of alkaloid, steroids and flavonoids in both methanolic and ethanolic extracts. Terpenoids was present in the methanolic extract and saponin was present in the ethanolic extract but phlobatannins, tannin and anthraquinones were absent in both extracts (table 1). The presence of saponin suggests that *D. elegans* may possess

Table 2. The antimicrobial activity of of methanolic and ethanolic extract of *D. elegans* against bacterial and fungal pathogen

Names of pathogens	Zones of inhibition (mm)					
	Ethanolic			Methanolic		
	0.1ml	0.2ml	0.3ml	0.1ml	0.2ml	0.3ml
S. pratyphi	NI	NI	NI	NI	NI	NI
E. coli	5mm	9mm	10mm	2mm	3mm	8mm
E. fecalis	NI	NI	NI	NI	NI	NI
S. aureus	NI	NI	NI	12mm	12mm	13mm
S. pneumonae	NI	NI	NI	NI	NI	NI
S. typhi	NI	NI	NI	NI	NI	NI
P. mirabilis	NI	NI	NI	NI	NI	NI
C. albican	7mm	9mm	10mm	2mm	3mm	7mm

NI: No inhibition, mm: millimetre

Table 3. The mean, standard deviation and p value of DPPH for methanolic and ethanolic extract of *Daedalea elegans*

Extracts	Mean ± standard deviation	*P* value
Methanol extract	17.17±11.25	0.04
Ascorbic acid	36.40±0.52	0.10
Ethanol extract	45.43±8.11	0.13
Ascorbic acid	36.40±0.52	0.19

Table 4. Mean, standard deviation and p value of the total phenolic content

Extracts	Mean± Std. Deviation	P value
Methanol	0.68±0.01	0.00
Ascorbic acid	2.61±0.25	0.01
Ethanol	0.69±0.02	0.00
Ascorbic acid	2.61±0.25	0.01

Table 5. Mean standard deviation and p value of total flavonoid content

Extracts	Mean± Std. Deviation	P value
Methanol	0.20±0.01	4.00
Ascorbic acid	0.29±0.02	3.84
Ethanol	0.16±0.01	4.00
Ascorbic acid	0.29±0.02	2.58

haemolytic, anti-inflammatory, antioxidative, immunostimulant activities. (Lovkova, 1999). The presence of alkaloids suggest potentials of the extract to help in the white blood cells to dispose harmful microorganisms and cell debris and also improve cardiac condition by reducing blood pressure, increasing circulation and inhibiting the accumulation of arteriosclerosis plaque and blood clots (Jeffery and Harborne, 2000). Terpenoids have been implicated as the phytoconstituent responsible for the antibacterial activity of mushrooms including *Cuminum cymium* and *Carum carvi* (Lacobellis et al., 2005).

In this study, the antibacterial and antifungal activities of the ethanolic and methanolic extracts were determined by well diffusion assay (Bauer et al., 1996). Table 2 indicates that ethanolic extract of *D. elegans* has a higher antibacterial activity. The extracts (methanolic and ethanolic) did not have any activity against *Salmonella typhi, Proteus mirabilis, Salmonella typhi, Streptococcus pneumonae* and *Enterococcus fecalis*. The methanolic extract of *D. elegans* showed its ability to inhibit both Gram negative and Gram positive organisms as well as fungi (in vitro) which shows its broad spectrum activity while the ethanolic extract showed ability to inhibit Gram negatives and fungi. This is in constract with the work of Aina et al., (2012) which showed that ethanolic extract of *Cantharellus cibarius* has a broad spectrum, and Barros et al., 2008 reported the methanolic extract to be narrow spectrum. The reason for this discrepancy may be that the activity of these mushrooms differs. The solvent used had no inhibitory effect on the test isolates indicating the effectiveness of the extracts. This may be due to the presence of phytochemicals such as saponin, phlobatannis, flavonoids and alkaloids in the mushroom extracts (Suksamran et al., 2004).

Result from antioxidant assays (in vitro) in this investigation (Tables 2-5) showed that the plant extracts possess antioxidant activity. The ethanolic extract of *Daedalea elegans* showed higher phenolic content than the methanolic extract. Although the scavenging power of both extracts were significantly low compared to standard ascorbic acid. Ethanolic extract showed higher radical scavenging activity than the methanolic extract. The total flavonoid content of both extracts is low compared to the ascorbic acid.

References

Bauer HW, Kirby WMM, Slerris JC and Truck M. 1996. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 493-496.

Benjamin DR. 1995. Mushrooms: Poisons and Panaceas; W. H. Freeman and Company: New York.

Brodie HJ. 1978. Fungi – Delight of Curiosity. University of Toronto Press, Toronto.

Dewanto VX, Wu KK. Adom, Liu RH. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry, 50: 3010-3014.

Fasidi IO, Kadiri, M. 1993. Effect of sporophore maturity on chemical composition of *volvariella essculenta* (mass) singer, a Nigerian mushroom. Die Nahrung, 37 (3) 269- 273.

Harborne JB. 1998. A Guide to Modern Technique of Plant Analysis, 3rd ed. Chapman and Hall, London. 285

Jeffery B, Harborne D. 2000. Arsenal for survival: Secondary plant products. Taxonomy, 49:435-449.

Jonathan SG, Fasidi IO. 2001. Effect of Carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom. Food Chemistry, 72: 479

Jonathan SG. IO Fasidi. 2003. Antimicrobial activities of two Nigerian edible macrofungi *Lycoperdon pusilum* (Bat. Ex) and *Lycoperdon gigantum* (Pers). African Journal of Biomedical Research, 6: 85-90

Lacobellis NS, Lo Cantore P, Capasso F, Senatore F. 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. Journal of Agricultural and Food Chemistry, 53, 57-61.

Lovkova MI, Sokolova SM, Buzuk GN, Bykhovskii VI, Ponomareva SM. 1999. Prikl. Biokhim Mikrobiol, 1999, 35(5), 578-589.

Maiti S, Bhutia SK, Mallick SK, Niyati Khadgi AK, Matti TK. 2008. Antiproliferative and immunostimulatory protein fraction from edible mushrooms. Environmental Toxicology and Pharmacology, 26: 187–191.

Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, Leitao SG. 2001. Screening of brazillian plant extracts for the antioxidant activity by the use of DPPH free radical method. Phytotherapy Research, 15:127-130.

Mizuno T. 1999. Antitumor and hypoglycemic activities of polysaccharides from the sclerotia and mycelia of Inonotus obliquus. International Journal of Medicinal Mushrooms, 1 (1): 301–16.

Moradali MF, Mostafavi H, Ghods S, Hedjaroude GA. 2007. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macro fungi). International Immunopharmacology, 7: 701–724.

Ooi VE and Liu F. 2000. Immunomodulation and anticancer activity of polysaccharide-protein complexes. Current Medical Chemistry, 7: 715–729.

Sies H. 1997. Oxidative stress: Oxidative and antioxidants. Experimental physiology, 82(2):291-295.

Singleton VL, Rosi JA, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16,144-158.

Sofowora. A. 1991. Medicinal plants and traditional medicine. John Wiley and Sons: 66-79.

Stamets P. 1993. Growing Gourmet and Medicinal Fungi. Ten Speed. Berkeley.CA.