

Antimicrobial and phytochemical potentials of arbuscular mycorrhizal fungi in Nigeria

S. O. Fapohunda¹, O. J. Olawuyi¹ and C. P. Okei¹

¹ Department of Biosciences and Biotechnology, Babcock University, Ilishan Remo, Nigeria.

Abstract

Studies were carried out on the antimicrobial effect of aqueous, ethanolic, methanolic and hexane extracts of mycorrhizal fungi; *Glomus clarum* and *Gigaspora gigantea* against selected pathogenic microorganisms using well-plate and disc-diffusion methods. The highest antibacterial inhibitory activities (10mm) were recorded for methanolic extract of *Glomus clarum* against *Proteus vulgaris* and *Klebsiella pneumoniae*. Hexane extract of *G. clarum* produced the highest antifungal activities (20mm) against *Aspergillus flavus*. Generally, the antimicrobial activity of *Glomus clarum* extracts was significantly higher than that of *Gigaspora gigantea*. The minimum inhibitory concentration (MIC) was 0.1-0.4mL for the extracts. *Shigella sonnei* was resistant to all extracts. The phytochemical screening carried out for the extracts showed that all extracts contained alkaloids and anthraquinone.

Key words: Mycorrhizal fungi, Extraction, Phytochemicals, Antimicrobial.

1 Introduction

Arbuscular mycorrhizal fungi (AMF) are biocontrol agents that protect plants from pathogens and their colonization increases pathogen resistance (Borowicz, 2001 and Bodker *et al.*, 2002). They excrete specific antibiotics which immobilize and kill disease causing micro organisms (Bonfante-Fasolo and Perotto, 1992). *Glomus clarum* and *Gigaspora gigantea* are both AMF of the division glomeromycota. AMF play important role in producing metabolites which have been linked to control of pathogen; *Aphanomyces enteiches* (Kjoller and Rosendahl, 1996).

They are known to confer disease resistance in some cereals. Colonization by *G.clarum* has increased plant height, diameter, shoot and root dry weights. Odebode *et al.* (1995) also reported that the potency of *Glomus clarum* on severity of *Sclerotium rolfsii* infection on *Lycopersicum esculentum* (Tomato) and *Capsicum annum* (pepper) seedlings could be compared to some extent with other fungi used.

Few higher fungi from Nigeria have been reported to possess important medicinal properties among the traditional doctors (Jonathan and Fasidi, 2003; Jonathan and Fasidi, 2005; Jonathan *et al.*, 2008). The diseases which mushrooms were reported to have therapeutic effect against in Nigerian ethnomedical practice includes; pneumonia, urinary tract infection, intestinal disorder and high blood pressure (Oso, 1977; Jonathan, 2002; Olawuyi *et al.*, 2010). Jonathan and Fasidi (2003) observed that alcoholic extracts of *Lycoperdon pusilum* and *L. giganteum* showed significant antimicrobial properties against some disease causing bacteria and fungi compared to their respective water extracts.

Information on *in vitro* antimicrobial activities of mycorrhizal fungi is scanty or not available in the literatures. This study therefore investigated the antimicrobial potentials of *Glomus clarum* and *Gigaspora gigantea* on selected pathogenic microorganisms in view of the limited scientific information on their medicinal values.

2 Materials and Methods

2.1 Collection of Samples

Two arbuscular fungal species; *Glomus clarum* and *Gigaspora gigantea* were collected from the units of the Department of Botany and Microbiology and Soil Microbiology of the Department of Agronomy, University of Ibadan Nigeria. All glasswares, forceps and spatula were sterilized in dry air oven for 30mins at 180°C. Test fungus used in this study was *Aspergillus flavus* IMI 500337, the test bacteria used were *Enterobacter cloaca* ATCC 13047, *Klebsiella pneumonia* KZN, *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922, *Streptococcus faecalis* ATCC 29212, *Staphylococcus aureus* OK2B, *Shigella sonnei* ATCC 29930, *Bacillus pumilus*, and *Proteus vulgaris* ATCC 6830. The bacterial isolates were collected from the standard bacteria strains from the University of Fort Hare South Africa, while the fungus was isolated from food spoilage samples in Babcock University, Ilishan remo. The extraction was carried out in the laboratory using three solvents and distilled water as a control, using constant concentration of the solvents and mycorrhizae.

2.2 Extraction Procedure

Thirty grams each of *G. gigantea* and *G. clarum* were soaked separately in 100mL of methanol, ethanol, hexane, and distilled water respectively for 15minutes with constant agitation, the beakers containing the soaked samples were covered with filter paper. Following the extraction, all eight extracts were filtered through a Whatman No.1 filter paper. The residue was air dried on the filter paper, while the filtrate was concentrated into slurry using a rotary evaporator (Gallenhamp, England) to concentrate the extracts. Each of the concentrates was kept in well labeled and sterilized Petri-dishes. The concentrates were purified by adding 1mL of dimethylsulfoxide $\{(CH_3)_2.SO_4\}$. Potato Dextrose Agar (LABM, UK) with 0.25g of chloramphenicol (antibiotics) and nutrient agar (LABM, UK) were prepared in accordance with manufacturers' instructions. Screening of test organisms with extracts using well-plate method

Aspergillus flavus IMI 500337 was cultured on Potato Dextrose Agar, while *Enterobacter cloaca* ATCC 13047, *Klebsiella pneumonia* KZN, *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922, *Streptococcus faecalis* ATCC 29212, *Staphylococcus aureus* OK2B, *Shigella sonnei* ATCC 29930, *Bacillus pumilus*, and *Proteus vulgaris* ATCC 6830 samples were cultured on Nutrient Agar plates (one organism per plate). Well (5.3mm diameter) was made on each Petri dish using sterile cork borer. 0.1ml of the purified concentrates was inserted into the agar wells in each plate using sterile dropping pipette. The zones of inhibitions were measured in millimeters (mm) using a ruler and recorded after 24 hrs of incubation at 37°C for bacteria while the test fungus was incubated at 35°C for 5 days.

Screening of test organisms with extracts using disc diffusion method. The assay for antimicrobial activity in the tested organisms was determined by disk diffusion method described by Stoke and Ridgway (1980) as modified by Jonathan and Fasidi (2005). Paper disc of approximately 6.3mm were punched out from a Whatman No.1 filter paper. Precautions were taken to avoid overlapping. The disks were placed in labeled glass Petri-dish and sterilized in a hot air oven at 180°C for 30mins, and allowed to cool. Blank discs were soaked in known concentrations of extracts and allowed to dry in an oven at 30°C for 5mins. Each of the test organisms were emulsified in peptone water and poured on the surface of the agar in the plates, the excess was discarded into the sink and the disks were inserted in the labeled plates using a well flamed forceps. With the use of a ruler, the zones of inhibition were measured in millimeters (mm) after 24 hours of incubation at 37°C and recorded.

2.3 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was aimed at finding out the lowest concentration of the extracts that will inhibit the growth of the test microorganisms. Two concentrations of the extracts were prepared. The mixture was tested against the microorganisms using diffusion method with 0.1mL of the extracts. The lowest concentration (dilution) at which inhibitory zones was produced is regarded as the minimum inhibitory concentration (MIC) for each extract (Pelczar *et al.*, 1993). The standard procedures for phytochemical screening of mycorrhizal extracts were adopted as follows:

2.3.1 Tannins

To 1mL of extract of sample 0.5mL of iron II chloride was added. A blue-black, green or blue precipitate indicates the presence of tannins

2.3.2 Alkaloids

2mL of chloroform and a few drops of Wagner's reagent were added to 1mL of the extracts. A reddish brown precipitate indicates the presence of alkaloids.

2.3.3 Steroids

0.5mL of acetic anhydride was mixed with 1mL of the extract and a few drops of concentrated H₂SO₄ were added. A bluish-green precipitate indicates the presence of steroids.

2.3.4 Saponins

1mL of distilled water was added to 1mL of the extracts persistence of frothing indicates the presence of saponin.

2.3.5 Phlobatannins

A few drops of 1% hydrochloric acid were added to 1mL of extract. A reddish-brown precipitate indicates the presence of phlobatannins.

2.3.6 Flavonoids

2mL of dilute sodium hydroxide was added to 2mL of extract. The presence of flavonoid is indicated by a yellow color.

2.3.7 Anthraquinones

10mL of chloroform was added to 1mL of extract, an appearance of greenish yellow precipitate indicates the presence of anthraquinone.

2.3.8 Terpenoids (Salkowski test)

5mL of each extract was mixed in 2mL of chloroform, and concentrated H₂SO₄ (3mL) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive result for the presence of terpenoids (Sofowora, 1993; Edeoga *et al.*, 2005).

3 Results and Discussion

The extracts of the mycorrhizal fungi used for this investigation possessed varying degrees of antibacterial and antifungal properties against the tested organisms using both well-plate method and disc diffusion methods (Tables 1-6).

Table 1. Antimicrobial potentials (mm) of aqueous and solvent extracts of *G. clarum*.

Extracts	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>E. coli</i> ATCC 8739	<i>S. faecalis</i>	<i>E. coli</i> ATCC 2922	<i>S. sonnei</i>	<i>P. vulgaris</i>	<i>S. aureus</i>	<i>B. pumilus</i>	<i>A. flavus</i>
Distilled water	-	-	-	-	-	-	-	-	2	-
Ethanol	-	-	4	-	-	-	-	-	-	-
Methanol	4	-	2	4	-	-	-	-	-	-
Hexane	-	-	-	-	-	1	-	-	-	18
- No zone of inhibition										

Methanolic extract of *G. clarum* produced the widest zone of inhibition (10mm) in *P. vulgaris* and *K. pneumoniae*, followed by ethanolic and distilled water extracts. While hexane extract of *G. clarum* produced the widest zone of inhibition for *A. flavus* (20mm), its antibacterial activity on the test bacteria was low.

Table 2. Antimicrobial potentials (mm) of aqueous and solvent extracts of *G. gigantea* using well-plate method.

Extracts	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>E. coli</i> ATCC 8739	<i>S. faecalis</i>	<i>E. coli</i> ATCC 2922	<i>S. sonnei</i>	<i>P. vulgaris</i>	<i>S. aureus</i>	<i>B. pumilus</i>	<i>A. flavus</i>
Distilled water	-	-	-	-	-	-	1	-	-	-
Ethanol	-	-	-	-	-	-	-	4	-	-
Methanol	-	6	2	-	2	4	-	-	2	-
Hexane	-	-	-	-	-	-	-	-	-	-

- No zone of inhibition

Table 3. Antimicrobial potentials (mm) of aqueous and solvent extracts of *G. gigantea* @0.4ml per filter paper disc.

Extracts	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>E. coli</i> ATCC 8739	<i>S. faecalis</i>	<i>E. coli</i> ATCC 2922	<i>S. sonnei</i>	<i>P. vulgaris</i>	<i>S. aureus</i>	<i>B. pumilus</i>	<i>A. flavus</i>
Distilled water	-	8	2	-	2	-	6	10	6	-
Ethanol	4	1	1	1	-	-	-	4	6	2
Methanol	8	6	2	-	-	-	10	2	6	6
Hexane	2	-	-	-	-	-	-	-	-	18

- No zone of inhibition

Table 4. Antimicrobial potentials (mm) of aqueous and solvent extracts of *G. clarum* @ 0.4ml per filter paper disc.

Extracts	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>E. coli</i> ATCC 8739	<i>S. faecalis</i>	<i>E. coli</i> ATCC 2922	<i>S. sonnei</i>	<i>P. vulgaris</i>	<i>S. aureus</i>	<i>B. pumilus</i>	<i>A. flavus</i>
Distilled water	-	2	2	-	2	-	6	1	6	-
Ethanol	2	6	-	1	-	-	-	4	6	5
Methanol	2	1	4	-	-	-	10	2	6	6
Hexane	-	-	-	-	-	-	-	-	-	18

- No zone of inhibition

Aqueous and methanolic extracts of *G. gigantea* had the lowest antimicrobial activity against test organisms (Plate 1: A and B). The potent antibacterial activity exhibited by *G. clarum* against *P. vulgaris* (Plate 2) is a confirmation of the earlier report of Oso (1981), Thomson *et al.* (1986), Gianinazzi-Pearson *et al.* (1996), Dar *et al.* (1997), Matsubara *et al.* (2000), Karagiannidis *et al.* (2002), Kasiamdari *et al.* (2002) and Pozo *et al.* (2002) in host-pathogen interactions of plants. Likewise, the result of antifungal property of *G. clarum* against *A. flavus* which showed similar inhibitory effect against *C. albicans*

was observed by Jonathan, (2002) for *C. occidentalis* and *D. concentrica*. Similar result was obtained for some mushrooms against *A. niger* and *A. flavus* (Jonathan *et al.*, 2007). Kenji *et al.* (1993) and Eun-Jeon *et al.* (1997) also reported similar observation with *Hericium erinaceum* and *Ganoderma lucidum* respectively.

Table 5. Antimicrobial potentials (mm) of aqueous and solvent extracts of *G. gigantea* @0.3ml per filter paper disc.

Extracts	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>E. coli</i> ATCC 8739	<i>S. faecalis</i>	<i>E. coli</i> ATCC 2922	<i>S. sonnei</i>	<i>P. vulgaris</i>	<i>S. aureus</i>	<i>B. pumilus</i>	<i>A. flavus</i>
Distilled water	2	4	2	-	-	-	2	4	4	-
Ethanol	2	4	-	2	-	-	6	2	6	-
Methanol	4	-	-	4	-	-	-	-	-	-
Hexane	-	-	2	-	-	-	-	-	-	2

- No zone of inhibition

Table 6. Antimicrobial potentials (mm) of aqueous and solvent extracts of *G. gigantea* @0.4ml per filter paper disc.

Extracts	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>E. coli</i> ATCC 8739	<i>S. faecalis</i>	<i>E. coli</i> ATCC 2922	<i>S. sonnei</i>	<i>P. vulgaris</i>	<i>S. aureus</i>	<i>B. pumilus</i>	<i>A. flavus</i>
Distilled water	2	10	2	5	10	-	2	4	4	-
Ethanol	2	4	2	2	8	-	2	4	8	-
Methanol	4	-	-	4	-	-	-	-	-	-
Hexane	1	-	-	-	-	-	-	-	2	2

- No zone of inhibition



A

B

Plate 1. Inhibitions of growth of *E. coli* by *G. gigantea* extract (A) methanol using well-plate method, (B) aqueous using disc-diffusion method.

Many AMF have germination-inducing factor in their solvent extracts (Bjurman and Fries, 1984). Promotion of plant growth may be a resulting inhibitory effect on plant pathogens in the soil.

The test organisms were sensitive to one or more of the extracts except *Shigella sonnei* which was resistant to all

the extracts. The result of the phytochemical screening carried out on extracts showed that all extracts contained alkaloids and anthraquinone, while saponin and phlobatannin was completely absent in all extracts. While all *G. clarum* extracts were negative for the presence of terpenoids, *G. gigantea* hexane extract showed a positive result. Also, the methanolic extracts of both mycorrhizal fungi was positive for the presence of tannins. Also, only ethanolic extract of *G. clarum* was negative for the presence of steroids, while methanolic and distilled water extract of *G. clarum*, and distilled water extract of *G. gigantea* were positive for the presence of flavonoids. The occurrence of flavonoids is a confirmation of the results of Soares *et al.* (2005) and Edeoga *et al.* (2005) which indicates the medicinal and bioactive properties of *G. clarum* and *G. gigaspora*



Plate 2. Inhibition of growth of *P. vulgaris* by methanolic extract of *G. clarum* using disc-diffusion method.

Table 7. Phytochemical screening for *G. clarum* extracts.

Extracts	Tannin	Alkaloid	Saponin	Phlobatannins	Steroid	Flavonoids	Anthraquinone	Terpenoids
Hexane	-	+	-	-	+	-	+	-
Ethanol	-	+	-	-	-	-	+	-
Methanol	+	+	-	-	+	+	+	-
Distilled water	-	+	-	-	+	+	+	-

Table 8. Phytochemical screening for *G. gigantea* extracts.

Extracts	Tannin	Alkaloid	Saponin	Phlobatannins	Steroid	Flavonoids	Anthraquinone	Terpenoids
Hexane	-	+	-	-	+	-	+	+
Ethanol	-	+	-	-	+	-	+	-
Methanol	+	+	-	-	+	-	+	-
Distilled water	-	+	-	-	+	+	+	-

+ Presence, - Absence of constituent

It can thus be concluded that the tested mycorrhizal extracts especially methanolic fractions of *G. clarum* can

serve as a potential therapeutic agent against some of the medically important bacteria. The result confirms that mycorrhizal fungi possess antimicrobial activities, capable of attracting industrial investment in sustainable agriculture.

References

- Bodker, L., Kjoller, R., Kristensen, K., and Rosendahl, S. 2002. Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. *Mycorrhiza* **12**, 7-12.
- Bonfante-Fasolo, P., and Perotto, S. 1992. Plant and endomycorrhizal fungi: the cellular and molecular basis of their interaction. In 'Molecular Signals in Plant-Microbe Communication' 445-470 pp.
- Borowicz, V.A. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology* **82**, 3057-3068.
- Björman, J. and Fries, N. 1984. Purification and properties of germination-inducing factor in the ectomycorrhizal fungus, *Leccinum aurantiacum* (Boletaceae). *Physiologia plantarum* **62**, 465-471.
- Brundrett, M. 1991. Mycorrhizas in natural ecosystems. *Advances in Ecological Research* **21**, 171-313.
- Burggraaf, A., and Beringer, J.E. 1989. Absence of nuclear DNA synthesis in vesicular-arbuscular mycorrhizal fungi during in-vitro development. *New Phytologist* **111**, 25-33.
- Dar, G.H., Zargar, M.Y., Beigh, G.M. 1997. Biocontrol of Fusarium root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. *Microbial Ecology* **34**, 74-80.
- Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4**, 685-688.
- Eun-Jeon, P., Geonil, R., Jaebae, K. and Dong, H.C. 1997. Antifibrotic effects of polysaccharides extracts from *Ganoderma lucidum*. *Biology Pharmaceutical Bulletin* **20**, 417-420.
- Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Tahiri-Alaouia, A., Gianinazzi, S. 1996. Cellular and molecular defense related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytologist* **133**, 45-57.
- Jonathan, S.G. 2002. Vegetative growth requirements and antimicrobial activities of some higher fungi in Nigeria. Ph.D thesis. University of Ibadan, Nigeria.
- Jonathan, S.G. and Fasidi, I.O. 2003. Antimicrobial activities of two Nigerian edible macrofungi- *Lycoperdon pusillum* (Bat. Ex) and *Lycoperdon giganteum* (Pers) *African Journal of Biomedical Research* **6**, 85-90.
- Jonathan, S.G. and Fasidi, I.O. 2005. Antimicrobial activities of some selected Nigerian mushrooms. *African Journal of Biomedical Research* **8**, 83-87.
- Jonathan, S.G., Kigigha, L.T. and Ohimain, E. 2008. Evaluation of the inhibitory potentials of eight higher Nigerian fungi against pathogenic microorganisms. *African Journal of Biomedical Research* **11**, 192-202.

- Gbolagade, J.S., Ajayi, A., Oku, I. and Wankasi, D. 2007. Nutritive value of common wild edible mushrooms from southern Nigeria. *Global Journal of Biotechnology and Biochemistry* (In Press).
- Kasiamdari, R.S., Smith, S.E., Smith, F.A., and Scott, E.S. 2002. Influence of the mycorrhizal fungus, *Glomus coronatum*, and soil phosphorus on infection and disease caused by binucleate. *Rhizoctonia* and *Rhizoctonia solani* on mung bean (*Vigna radiata*). *Plant Soil* **238**, 235-244.
- Karagiannidis, N., Bletsos, F., Stavropoulous, N. 2002. Effect of *Verticillium* wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, Growth and nutrient uptake in tomato and egg plant seedlings. *Science Horticultural* **94**,145-156.
- Kenji, O., Atsushis., R.S., Hideki, S., Satoshi, Y., Eumihiro, O., Yukio, I., Takuo, S and Hirokazu 1993. Antimicrobial chlorinated orcinol derivations from mycelia of *Hericium erinaceum*. *Phytochemistry* **34**, 1445-1446.
- Kjoller, R., and Rosendahl, S. 1996. The presence of the arbuscular mycorrhizal fungus *Glomus intraradices* influences enzymatic activities of the root pathogen *Aphanomyces euteiches* in pea roots. *Mycorrhiza* **6**, 487-491.
- Matsubara, Y., Kayukawa, Y., Yano, M. and Fukui, H., 2000. Tolerance of asparagus seedlings infected with arbuscular mycorrhizal fungus to violet root rot caused by *Helicobasidium mompa*. *Journal of Japan Society of Horticultural Sciences* **69**, 552-556.
- Odebode, A.C., Ladoye, A.O. and Osonubi, O. 1995. Influence of arbuscular mycorrhizal fungi on disease severity of pepper and tomato caused by *Sclerotium rolfsii*. *Journal of Science Research* **2**, 49-52.
- Olawuyi, O.J., Jonathan, S.G., Aina, D.A and Baysah, G.I. 2010. Studies on anti-bacterial activities of *Fomes lignosus*- A Nigerian Mushroom. *Nigerian Journal of Microbiology* **24**, 2153-2159.
- Oso, B.A. 1977. Mushrooms in Yoruba mythology and medicinal practices. *Economy Botany* **31**, 367-71
- Oso, B.A. 1981. Fungi and Mankind. University of Ibadan, Ibadan (Nigeria) Inaugural lecture, 40p.
- Pozo, M.J., Cordier, C., Dumas-Gaudot, E., Gianinazzi, S., Barea, J.M. and Azcon- Aguilar, C. 2002. Localised versus systemic effect of arbuscular mycorrhizal fungi on defense responses to *Phytophthora* infection in tomato plants. *Journal of Experimental Botany* **53**, 525-534.
- Pelczar, M.J., Chan, E.C.S and Krieg, N.R. 1993. *Microbiology: Concepts and Application*. MacGraw-Hill Inc. New York, 967p.
- Soares, A.C., Martins, M.A., Mathias, L and Frietas, M.S 2005. Arbuscular mycorrhizal fungi and the occurrence of flavonoid in roots of pension fruit seedlings. *Scientia Africola* **62**, 331-336.
- Stoke, J.E and Ridgway, G.L. 1980. *Clinical Bacteriology*. 2nd Editon, Edward Arnold Ltd. London.
- Thomson, B.D., Robson, A.D., and Abbott, L.K. 1986. Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytopathology* **103**, 751-765.

Correspondence to: S. O. Fapohunda
E. mail: oystak@yahoo.co.uk