

Antimicrobial and antioxidant properties of mistletoe (*viscum album*) growing on cola (*cola nitida*) tree in Akure North, Nigeria.

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ABSTRACT

The result from this research work showed that the percentage yield of the 60% methanolic-extract of *V. album* leaves was the highest (25.36 ± 1.58). The lowest yield was recorded for cold water extract (13.36 ± 0.50). The antioxidant assay of the *V. album* extracts was 194 mg/100g total phenol content and a dose-dependent (0.1 -1.0 mg/ml) relationship for the reducing ability, Free radical scavenging and the Fe²⁺ chelating tendency. The result of the antibacterial properties of the extracts recorded the highest inhibition zones (15.33 ± 0.33) for *Strep. faecium* and the least zone (7.33 ± 0.67) for *Enterococcus faecalis*. For the antifungal, the highest inhibition zone recorded was (9.17 ± 0.17) for *Aspergillus Niger* and the least zone was (2.00 ± 0.00) for *Fusarium oxysporium* and *Microsporum Sp*. The results from this work show that *V.album* leaves extract is a potential source of broad spectrum antimicrobial agent and a potent antioxidant.

Key words: Methanolic, *Viscum album*, Antimicrobial, Antioxidant, Broad spectrum

INTRODUCTION

Natural plant products have been used for therapeutic purposes since the time immemorial and their use is of a greater demand nowadays (Calixto, 2000). Majority of the users rely on herbal medicines for health care because the other treatment options available are more expensive and they are often thought to be more associated with serious side effects such as upper gastrointestinal complications, organ damages and blood cancer. Most of these plants have been ingested indiscriminately without minding if they could have any side effect. Research by scientists all over the globe suggests that plant – based drugs and additives are better and safer in addressing man's ever-growing health problems and that no plant is entirely useless to man.

Mistletoes are a polyphyletic group of flowering plants comprising over 1300 species from a broad range of habitats across all continents except Antarctica (Calder & Bernhardt 1983, Kuijt 1969, Watson & Dallwitz 1992, Nickrent, 2001). They share a common growth form – obligate hemi parasitism – such that all water and minerals are obtained from their host via a specialized vascular attachment (Ehleringer and Marshall 1995, Lamont 1985, Pate 1995). The group contains members of five families within the Santales (Nickrent and Soltis 1995), and the aerial parasitic life-form is thought to have evolved independently four or five times (Nickrent and Franchina 1990, Nickrent 2001). Mistletoe can grow on either edible or non-edible trees, while only those that grow on edible plants are used for medicinal purposes (Evans, 2005). The growth of Mistletoe on different kinds of plants, are of disease curing specificity, for example, mistletoe grown on Guava, Kolanuts and Citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia, while those grown on cocoa is best used for curing diabetes (Ekhaise et al., 2010).

The evidence of the presence of antimicrobial agents in plants stemmed from the noticeable resistance of such plant to pest attack. These natural products are the physiologically active substances which exert the curative properties on the plants (Ogundare and Onifade, 2009). Many of these bioactive compounds have been found to serve as lead

compounds for the development of new chemicals from the plethora of plants available to mankind. Ademiluyi and Oboh, (2008), reported that the medicinal effects of plants are often attributed to the antioxidant activities of the phytochemical constituent, mostly the phenolics. Many plant constituents have proven effective as remedy for some diseases and accounted for about seven thousand pharmaceutical important compounds in western pharmacopoeia and a number of important drugs, like taxol and artemisinin (Aderogba *et al.*, 2004).

Currently, there is also a strong demand from the food industry to replace synthetic additives including antioxidants, by natural ones (Dapkevicius *et al.*, 1998). However, there is still considerable controversy in the area of antioxidant research and many issues are yet to be clarified (Cai *et al.*, 2004). This studies therefore revealed the antimicrobial and antioxidant properties of mistletoes leaves that is widely used in Akure North Nigeria in the treatment of hypertension, diabetes and rheumatism.

MATERIALS AND METHODS

Collection and Preparation of Samples

The leaves of Mistletoe (*Viscum album*) were collected from Odo-orokuta camp at Ayede Ogbese, AKure North Local Government of Nigeria from cola (*Cola nitida*) host plant. The sample was identified as *V.album* and also *authenticated* by Dr. Akiyele B.O. of the Department of Crop, Soil and pest management of the Federal University of Technology, Akure. The leaves were destalked, washed and air dried at a room temperature of $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for six weeks until a constant weight was achieved. They were then pulverized and kept in an airtight container before analysis. Some of the leaves were grounded using electric blender and was stored in refrigerator in a well labeled air-tight container for analysis. 100g of the sample was weighed (using electric weighing balances) into 1000 ml of 60% ethanol, 60% methanol, 60% n-hexane and 1000 ml of water all in separate containers. Each solution was homogenized in a warring blender and was then filtered using Whatman no. 1 filter paper and the filtrate obtained were concentrated *in vacuo* using a rotary evaporator. It was reconstituted using dimethyl sulphoxide (DMSO) 1:50(w/v) for the analysis.

Antimicrobial assay

The microorganisms used for this assay were obtained from the stock cultures of the University Teaching Hospital, Ibadan, Oyo State, Nigeria. The identities of the organisms were confirmed using standard methods of the morphological and biochemical characteristics of each organism at the Microbiology Department Federal University of Technology, Akure, Nigeria. Antimicrobial activity of the ethanolic, methanolic and n-hexane extracts of the leaves was assayed using the paper disc diffusion method (Oluma *et al.*, 2004 , Doughari *et al.*, 2007). The concentrated leaf extracts were dissolved in 5% dimethyl sulfoxide (DMSO) and sterile discs (6mm, Hi-media, india) were each impregnated with 30 μ l of 30mg/ml of each extract. The discs were carefully and firmly placed on the Muller Hinton Agar (MHA) plates earlier seeded with standardized bacterial suspensions (approximately 1.5×10^6 cfu/ml). Paper discs impregnated with 30 μ l of a solution of 30 mg/ml of the following standard antibiotics; ciprofloxacin, and gentamicin were used as control for comparison. Filter paper discs dipped into sterile distilled water and allowed to dry were used as control. The plates were then incubated at 37 $^{\circ}\text{C}$ for 24 h. Antibacterial activity was determined by measurement of zone of inhibition around each paper disc. The MIC of the aqueous and methanolic extracts of the leaves extracts was determined as described by Akinpelu and Kolawole (2004). The MIC was taken as the lowest concentration that prevented bacterial growth.

Determination of total phenol

In the total phenol content of the extract determine by the method of Singleton *et al.*, (1999). 0.2 ml of the extract was mix with 2.5 ml of 10% Folin ciocalteau's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45 $^{\circ}\text{C}$ for 40mins, and the absorbance was measure at 700nm in the spectrophotometer, using garlic acid as standard phenol.

Determination of ferric reducing property

The reducing property of the extract was determined by Pulido *et al.*, (2000), 0.25 ml of the extract was mixed with 0.25 ml of 200mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50 $^{\circ}\text{C}$ for 20min,

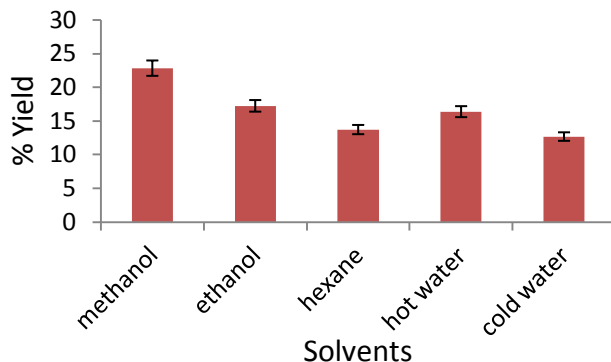


Figure 1. shows the percentage yield of *V.album* extracts from cola tree in different solvents.

thereafter 0.25ml of 10% TCA was also added and centrifuge at 2000rpm for 10min, 1 ml of the supernatant was mixed with 1ml of distilled water and 0.2 ml of FeCl_3 and the absorbance was measure at 700 nm.

Determination of free radical scavenging ability

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picrylhydrazyl) using Gyamfi *et al.*, (1999) method. 1 ml of the extract was mixed with 1ml of the 0.4mM methanolic solution of the DPPH the mixture was left in the dark for 30 min before measuring the absorbance at 516nm.

Determination Fe (ii) Chelation

The ability of the extract to chelate Fe_2^+ was determined using a modified method of Minotti & Aust (1987) by Puntel *et al.*, (2005). Briefly, 150 μl of freshly prepared 500 μM FeSO_4 added to a reaction mixture containing 168 μl of 0.1M Tris-HCl (pH 7.4), 218 μl saline and the methanolic leaf extracts (0 – 500 μl). The reaction mixture was incubated for 5min, before the additional of 13 μl of 0.25% 1, 10-phenantroline (w/v). The absorbance was then read at 510 nm in the spectrophotometer. Percentage Fe^{2+} chelation capacity = $\frac{\text{concentration of chelated } \text{Fe}_2^+ \times 100\%}{\text{concentration of total } \text{Fe}_2^+}$.

RESULT

Figure 1 shows the percentage yield of the extract using different solvent. It was observed that 60% methanolic extract had the highest extract yield (22.81%) and the lowest (12.65%) was recorded for cold water. Figure 2 shows the reducing ability of the *V. album* extracts, Figure 3 shows its percentage iron (II) chelating tendency and Figure 4 shows the percentage free radical scavenging of the extracts. In figures 2, 3 and 4 the observed ability of the extract is directly proportional to the concentration used (mg/ml). Table 1 shows the antibacterial properties of the extracts. The zones of inhibition recorded revealed the potential of the extract in inhibiting microbes when compared with the standard antibiotics used. Table 2 shows the antifungal properties of the extracts. The extracts show potentials of being used as antifungal agent when compared with the controls (Ketoconazole and Fulcin).

Figures of the same row in the table with the same superscript are not significantly different at probability ($p \leq 0.05$) level.

DISCUSSION

The result in figure 1 showed that the percentage yield of the 60% methanolic extract of *V. album* was the highest (22.41 ± 0.32), while the lowest yield was recorded for cold water extract (12.65 ± 0.33). The relatively higher extracts yield with methanol may be due to the fact that methanol is not exotic and has high vapor pressure.

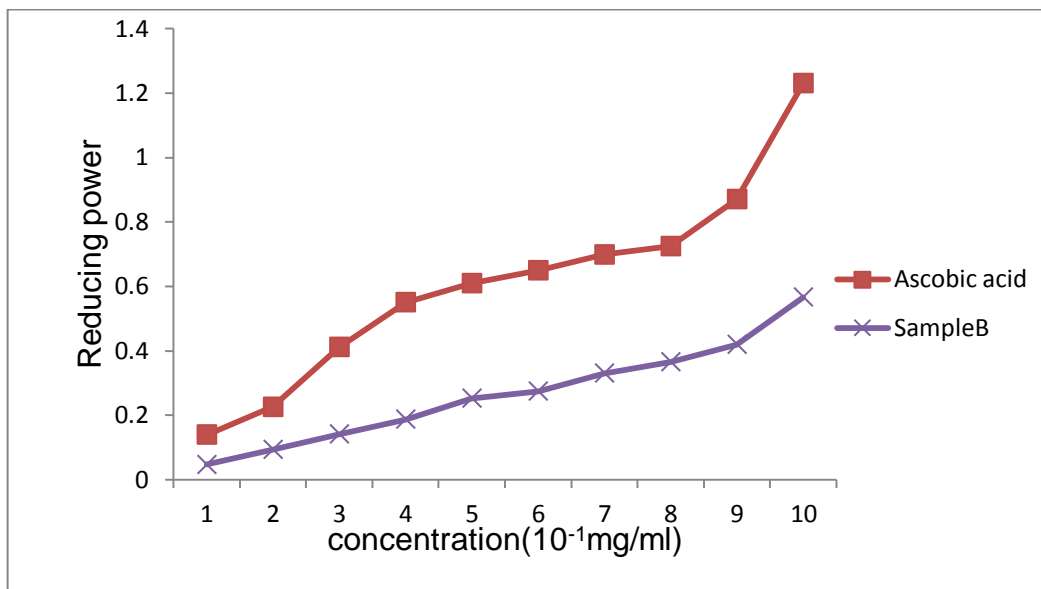


Figure 2. Shows the reducing power of *V.album* extracts from cola (sampleB). Control: Ascorbic acid.

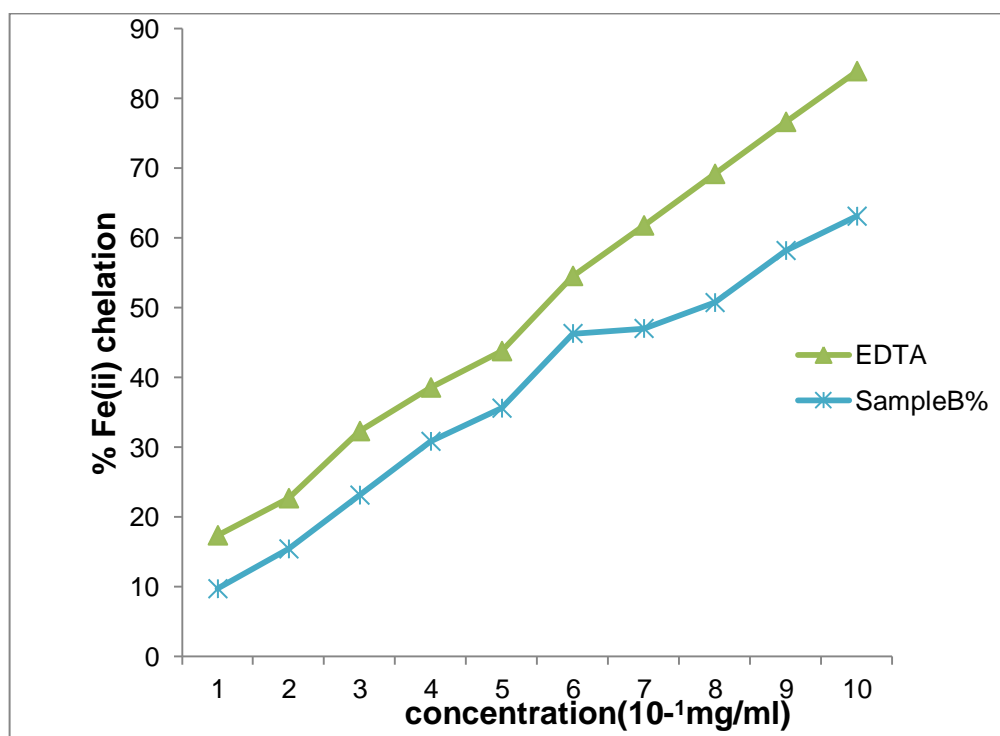


Figure 3. Shows percentage Fe (ii) chelation of *V.album* extracts from cola. Control: EDTA

Antioxidants are compounds that protect cell against the damaging effects of reactive oxygen species, such as singlet Oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxy nitrile. An imbalance between antioxidants and reactive Oxygen species results in oxidative stress leading to cellular damage (Burlon and Ingold, 1984). Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative disease (Palozza, 1998).

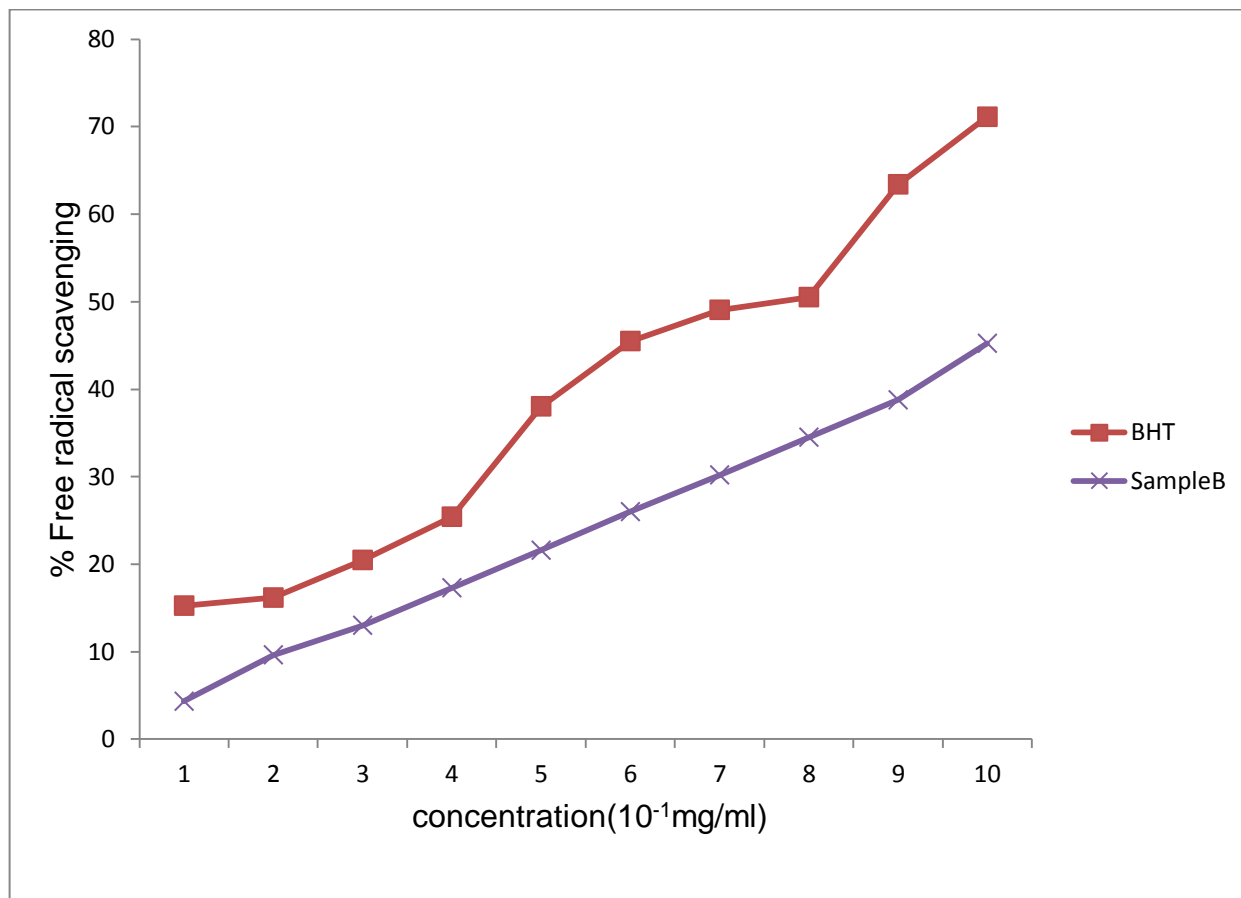


Figure 4: Shows percentage Fe (ii) chelation of the extracts (sample B). control BHT, Butylated hydroxytoluene.

The antioxidant assay of the *V. album* extracts in figures 2, 3 and 4 show a dose-dependent (0.1 -100mg/ml) relationship for the reducing ability, Free radical scavenging and the Fe²⁺ chelating tendency. The percentage scavenging ability of the *V. album* extracts (Figure 4) against stable DPPH was determined. It was observed that *V. album* extracts from cola tree compares favourably with the standards agents (Ascorbic acid, Ethylenediamine tetraacetic acid and Butylated hydroxytoluene used (figures 2- 4). The same pattern was noted for total phenol content and reducing property of their various extracts. Oboh, (2006); Oboh *et al.*, (2008). had established a correlation between the total phenol content of plant food and their antioxidant properties. The higher the doses used the higher the antioxidant tendency recorded for the *V. album* extract used. It can be inferred that methanolic extract of *V. album* will be a very good source of antioxidant polyphenols owing to its free radical scavenging tendency and strong Iron chelating ability. Oboh and Rocha (2007) reported that the dominating mechanism through which *Capsicum annum var aviculare* (Tepin) polyphenols protect brain and liver is through their Fe²⁺ chelating ability. The observed antioxidant properties of the samples (figures 2, 3 and 4) might be depending on their host plant. Onay-Ucar *et al.*, (2006) in their findings have reported that antioxidant capacity of extracts of *V. album* species is a factor of the harvest time and the nature of the host plant. Similar results were obtained by Ademiluyi and Oboh (2008), who evaluated the antioxidant activity of methanol extract of *V. album* leaves from two hosts (cocoa and cashew trees), showing that mistletoe from cocoa tree had higher total phenol content (182 mg/100g) than that from cashew tree (160 mg/100g), the main reason of their antioxidant capacity and that, the total phenolic content, more than carotenoids content can serve as a useful indicator for the antioxidant activities of mistletoe extracts. Carotenoids are less available also for extraction, being linked to proteins in the photosynthetic apparatus in leaves.

Since *V. album* is a parasitic plant might have absorbed pharmacological active compounds into their system through their haustoriums. The hydroxyl groups in phenol are thought to be responsible for its use as antimicrobial agents (Ogundare and Onifade, (2009), Ademiluyi and Oboh, (2008). High inhibition zones recorded for the extracts as compared with the

Table 1. shows the antibacterial assay(diameter zones of inhibition) of the *V. album* extract

Organisms	methanolic extract(30mg/ml)	ethanolic extract(30mg/ml)	hexane extract(30mg/ml)	cyprofloxacin	gentamycin
<i>E. coli</i>	13.67 ± 0.67 ^d	10.00 ± 0.56 ^b	7.33 ± 0.88 ^a	11.33 ± 0.33 ^{bc}	12.17 ± 0.17 ^{cd}
<i>K. pneumoniae</i>	6.33 ± 0.33 ^b	5.00 ± 0.00 ^a	4.00 ± 0.58 ^a	7.67 ± 0.33 ^c	11.00 ± 0.50 ^d
<i>P. aeruginosa</i>	5.00 ± 0.58 ^b	6.00 ± 0.58 ^b	2.67 ± 0.67 ^a	9.33 ± 0.67 ^c	10.33 ± 0.33 ^c
<i>Proteus mirabilis</i>	5.83 ± 0.73 ^b	6.67 ± 0.58 ^b	4.00 ± 0.00 ^a	9.03 ± 0.26 ^c	11.33 ± 0.17 ^d
<i>S. typhi</i>	9.00 ± 0.00 ^c	5.67 ± 0.67 ^b	2.00 ± 0.00 ^a	9.00 ± 0.58 ^c	12.17 ± 0.73 ^d
<i>Shigella dysenteriae</i>	8.00 ± 1.15 ^b	5.83 ± 0.72 ^b	1.00 ± 1.15 ^a	8.00 ± 0.50 ^b	10.67 ± 0.75 ^d
<i>Bacillus cereus</i>	12.00 ± 0.58 ^b	10.00 ± 1.15 ^a	8.00 ± 0.00 ^a	11.00 ± 0.58 ^b	13.33 ± 0.88 ^c
<i>E. faecalis</i>	5.67 ± 0.33 ^a	5.33 ± 0.33 ^a	5.00 ± 0.58 ^a	7.33 ± 0.67 ^b	9.67 ± 0.33 ^c
<i>Staph aureus</i>	13.33 ± 0.88 ^c	10.33 ± 0.33 ^{ab}	9.00 ± 0.58 ^a	10.50 ± 1.04 ^{ab}	12.17 ± 0.44 ^{bc}
<i>Staph. faecium</i>	20.67 ± 0.66 ^d	13.67 ± 0.33 ^b	7.66 ± 1.20 ^a	15.33 ± 0.33 ^b	18.00 ± 0.99 ^c
<i>Strep. pyogenes</i>	12.67 ± 1.20 ^c	9.67 ± 0.33 ^b	7.00 ± 0.00 ^a	14.33 ± 0.88 ^c	14.50 ± 0.86 ^c
<i>Clostridium pefringens.</i>	10.67 ± 0.67 ^b	10.33 ± 0.33 ^{ab}	8.67 ± 0.67 ^a	10.67 ± 0.33 ^b	8.67 ± 0.00 ^a

Figures of the same row in the table with the same superscript are not significantly differently at probability ($p \leq 0.05$) level.

Table 2. shows the antifungal assay of the *V. album* extracts

Organisms	methanolic extract(30mg/ml)	ethanolic extract(30mg/ml)	hexane extract(30mg/ml)	Ketoconazole (30mg/ml)	Fulcin (30mg/ml)
<i>Aspergillus flavus</i>	14.00±0.58 ^d	9.67±0.33 ^c	7.00±0.00 ^b	7.17±0.60 ^b	9.17±0.73 ^b
<i>Aspergillus niger</i>	10.66±1.45 ^{cd}	12.00±1.15 ^d	7.00±0.58 ^{ab}	9.17±0.17 ^{bc}	17.33±0.33 ^e
<i>Fusarium oxysporium</i>	1.67±0.88 ^{bc}	0.33±0.33 ^{ab}	0.00±0.00 ^a	2.00± 0.00 ^c	0.33±0.33 ^{ab}
<i>Microsporium gypseum</i>	2.0±0.00 ^b	1.67±0.33 ^b	0.00±0.00 ^a	8.83±0.44 ^d	10.17±0.88 ^d
<i>Penicillium oxalium</i>	3.00±0.58 ^c	2.00±0.00 ^b	0.00±0.00 ^a	2.00±0.00 ^b	1.67±0.33 ^b
<i>Tricophyton canis</i>	1.67±0.33 ^b	0.33±0.33 ^a	0.00±0.00 ^a	5.66±0.37 ^c	7.33±0.67 ^d

the standards antimicrobial agents used (tables 1-2) is an indication that mistletoe extract is a promising source of a broad spectrum antimicrobial agent. However, the antimicrobial properties was generally found to be more pronounced in the bacterial used than in the fungi except for the *Aspergillus* species used. Fungi had been known to possess more complex structure than bacteria and this might confer resistance on them, or provide permeability barrier for the extract to get to the fungi. Musa et al.,(2000) when screening *Acalypha recemosa* for antimicrobial activities, noted that the fungi were more resistant than the bacteria used. Yusuf, (2013) reported that *V.album* extracts from cola contained phenol, alkaloid, saponins, flavonoids, terpenoid and phytate. These phytochemicals have been found to confer antimicrobial properties on the plant.

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