Antifungal Activity of Two Plant Extracts against Fungi Isolated from Poultry Droppings in Owerri, Imo State, Nigeria

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Abstract: Several fungi have created havoc in the poultry industry and some of them cause direct harm to human health owing to their zoonotic implications. This research evaluated the phytochemical constituents and antimicrobial activity of Methanol extracts of *Ocimum gratissimum* (scent leaf) and *Azadirachta indica* (Neem leaf) against fungi strains isolated from poultry droppings and comparing them with commercial antifungal drugs. A total of 150 samples were collected from thirty different poultry farms in Owerri, Imo State using sterile spatula. Spread plate method was used to inoculate 0.1ml(using 10^{-2}dillution factor) of the samples unto Potato Dextrose Agar (PDA) and Saboraud Dextrose Agar (SDA) to which chloramphenicol (0.05 mg/ml) has been incorporated for primary isolation of fungi. The fungi were identified using cultural characteristics, microscopy and molecular analysis(using ITS region sequencing). Agar well diffusion method was used to test the antifungal activity of the plant extract after 48 and 72 hours time intervals. The Mean Diameter Zone Of Inhibition ranged from 7.50±0.00-12.30±1.53(after 48 hours) and 7.90±1.00-13.50±0.00(after 72 hours) for Neem leave and 6.00±1.00-10.00±0.00(after 48 hours) and 6.80±0.00 -10.50±1.00(after 72 hours) for Scent leave. Tube dilution technique using double-fold serial dilution method was employed for assaying Minimum Inhibitory Concentration (MIC).Total fungal counts ranged from 1.46×10^2 to 5.43×10^2.The study revealed that the extracts of the two plants showed a stronger inhibitory property against the isolates. This efficacy of the leave extracts of *Ocimum gratissimum* and *Azadirachta indica* as compared to synthetic antifungal shows promising results to be used as potent natural occurring antifungal agents.

Key words: Antifungal, Inhibition, phytochemical, Plant Extract, Microscopy.

INTRODUCTION

The term 'poultry' used in agriculture generally refers to all domesticated birds kept for egg laying or meat production (Rafloff, 2003). Poultry comes from the French word poul, which was derived from Latin word Pullus meaning small animals. Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat (Rafloff, 2003). In Nigeria, chickens are the most important of the poultry species in terms of number and development. The exotic breeds are managed intensively using either battery cages or deep litter system of management, while the local breeds are managed extensively and are allowed to scavenge food for survival (Aliyu *et. al.,* 2013). The major constraints in raising these chickens include shortage and cost of feeds and the substantial economic losses due to different diseases of which viral infections account for the highest percentage of the mortality in chickens because of their contagious nature (Adeboyega, 1999).

Livestock (poultry) get infected when pathogenic organism passes to the susceptible animal through feeding (Barnes *et al.,* 2003). To prevent pathogenic organisms from getting into the body of poultry, attention should be given to the factors that influence their infectious spread. First and foremost, they should have, clean range, proper feeding and quarantining new stock. Sanitation is very important in poultry management by cleaning of their water can, feeding troughs and finally disinfecting them to help reduce organic matter (Barnes *et. al.,* 2003).

Poultry droppings are waste products excreted by the poultry fowl. It can also be defined as by-product that resulted from the digestion of food intake by poultry birds. Droppings can be in form of solid, semi-solid or watery. The color of droppings varies among the species of birds. Some are whitish, ash and dark brown in color. The chemical composition of droppings consists

of water, nitrogen, phosphorus and potassium with some other minerals. The chemical composition supports the growth of billions of microorganisms which contaminate various products of poultry e.g. eggs and meat. The presence of these microorganisms causes various diseases in fowl and humans (Adegunloye, 2006).

Fungal/mycotic infections are common in all kinds of poultry birds but are less prevalent as compared to bacterial and viral infection (Jand *et al.,* 2005). Fungi are eukaryotic organisms, comprising of both yeast and molds. Fungi diseases of poultry includes aspergillosis, candidiasis, dactylariosis, cryptococcosis (Rai *et al.,* 2011). Out of these, the first two (Aspergillosis and Candidiasis) are having much importance and impact. In animals, Aspergillosis has been described in invertebrates (especially in bees and cnidariasis) (Rypien *et al.,* 2008) as well as in warm blooded vertebrates: mammals (like dogs, horses and cattle) and birds (a large number of species) (Tell,2005). Birds are much more susceptible to the infection than mammals. And the last two (histoplasmosis and cryptococcosis) have some zoonotic significance (Rai *et al.,* 2011).

Fungi produce disease in two ways viz. producing pathogenic signs and lesions of disease by invading, harming and destroying body tissues of the host (Singh *et al.,* 2012) and by producing secondary metabolites, like mycotoxins, in response to environmental changes. Mycotoxins may be pro-inflammatory, immunosuppressive, or carcinogenic (Bondy and Pestka 2000; Jarvis 2003). Fungi disease are assuming new importance due to the inappropriate use of antibacterials that eliminate the natural beneficial micro flora which otherwise suppress the growth of fungi (De lucca, 2007).

In the past few decades, a dramatic increase in fungal infections incidence has been noted across the world owning to the appearance of resistant fungi to different fungicidals used in medicinal practice (Singh, 2001).

Newer drug research targeting fungal species are the most neglected ones , this can be evident from the fact that “ gold standard drug” for antifungal therapy remains same since 1956. Very few antifungal agents are known till now, and their continuous and indiscriminate use have led to the development of resistance by fungal species, some shows ineffectiveness toward fungal disease (Tanwar*et al.,* 2014).

These drugs not only show ineffectiveness due to resistance by fungal species but also show undesirable side effects or are very toxic, shows recurrence or drug interactions causing severe problems (Sharanappa and Vidyasagar, 2013).

The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al.,* 2003, Moreillion *et al.,* 2005), and also with less severe adverse effects and relatively less economic burden, compared to chemical drugs.

The use of medicinal plants and herbs as medicine to treat various diseases could be traced as far back as the beginning of ancient human civilization (Hunny*et al.,* 2016). ‘Rigveda’, an ancient Hindu culture literature gives the earliest evidence of the use of medicinal plants to cure diseases of humankind. Medicinal plants are not only renewable in nature but also offers a wide variety of phytochemicals which are said to have significant antimicrobial and antifungal activities (Narasimhudu, 2012). Considering current trends in fungal diseases and the need to discover new plants and herbal products with potential medicinal properties; this in-vitro study was designed with the aim of investigating the antifungal efficacy of extracts of two medicinal plants, *Azadirachta indica* (Neem) and *Ocimum gratissimum* (scent leaf) against fungi

isolated from poultry droppings in Owerri, Imo State, and comparing it with commercial antifungal agents.

MATERIALS AND METHODS

Study area

The selected poultry farms were located in Owerri. Owerri is the capital of Imo State in Nigeria, set in the heart of Igbo land (Egbokhare *et al*., 2002). Owerri consists of three Local Government Area including Owerri Municipal, Owerri North and Owerri West, it has an estimated population of about 401873 as of 2006 (FRNOG, 2007), and is approximately 100 Square Kilometers(40 sq mi) in area. Owerri is bordered by the Otamiri River to the east and Nworie River to the south.

Collection of Poultry Droppings

A total of 150 samples were collected from thirty different poultry farms in Owerri, Imo State which included Chamsegus Farm (Obinze, Owerri), Livestock Specialities Ltd. (Tetlow Road, Off Wetheral Road, Owerri), Louren Farms(Naze Owerri) among others . Five samples of poultry droppings were collected from each farm. A sterile spatula was used to pick the samples at random from different locations in the farm into sterilized containers. The samples were then labeled accordingly and conveyed to the microbiology laboratory of Chukwu Emeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria where they were analysed within one hour from the time of collection.

Isolation and Identification of Fungi

Standard microbiological procedure was adopted. Spread plate method was used to inoculate 0.1ml (from 10^{-2}dillution factor) of the samples into Potato Dextrose Agar (PDA) and Saboraud Dextrose Agar (SDA) to which chloramphenicol (0.05 mg/ml) had been incorporated for primary isolation after a 10-fold serial dilution of 1.0 g of the poultry droppings using distilled water (Siva *et. al*., 2008) and incubated for 3-5 days at 25-28^{0}C. After incubation, fungal colonies were counted macroscopically using a colony counter. Colony forming units per gram (CFU/g) of sample was calculated.

A pure culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the SDA plates and incubated at 25-28^{0}c for 3-5 days.

Identification and characterization of the isolates was based on macroscopic (cultural characteristics) e.g. colour, shape of colony, surface and reverse pigmentation and texture of the colony. The technique of Oyeleke and Manga (2008) was also adopted for the identification of the isolated fungi using lactophenol cotton blue stain. The identification was achieved by placing a drop of the stain on clean slide using a sterile plastic pipette, with the aid of a mounting needle a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in the drop of lactophenol stain. The mycelia was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses to determine the microscopic structures (septate or nonseptatehyphae, structure of hyphae and conidia), (Forbes *et. al.,* 2002).

Collection of Plant Materials

Fresh and healthy leaves of *Azadirachta indica*(Neem) and *Ocimum gratissimum* (Scent leaf) were obtained from Obinze, in Owerri West L.G.A. of Imo State. They were identified and authenticated by Mr. Onwubuche B.C. a botanist in Imo State Polytechnic, Umuagwo.

Preparation of Plant Samples for Extraction

Fresh leaves of neem and scent leaf were collected and washed thoroughly using tap water and then sterile distilled water and kept at room temperature for 21 days to dry. The dried leaves were pulverized using a mechanical blender.

Extraction Procedure

The methanol extraction of the plant leaves was carried out using Maceration method (Ayange-kaa *et. al.,*2015). About 20.0 g of blended neem and scent leaf powder was introduced into separate flasks after which 200 ml of methanol was added to each of the flasks. The mixture was agitated and then allowed to stand for 3 days to allow for maximum extraction of the components after which the supernatants were separated from the residue by decanting and filtered using a funnel and Whatman no. 1 filter paper. The samples were then evaporated to dryness using a water bath (Ayange-kaa *et. al.,*2015). The weight of the dry mass of plant extract was determined to be 5.0 g this was then redissolved in 10 ml of methanol to give 500 mg/ml as stock which was used to calculate the concentration of the extracts in each solution in mg/ml.

Preparation of Standard Antibiotics

The standard antibiotic used for this work was fluconazole because fluconazole is effective for the treatment of fungal infections . A stock of 500 mg/ml of standard antibiotics was prepared by dissolving 5.0 g in 10 ml of distilled water. These was then used to prepare other concentration (250 mg/ml, 125 mg/ml, 62.5 mg/ml).

Determination of Antifungal Properties of the Plant Extracts

In order to investigate the antifungal activity of the plant extracts, agar well diffusion method of antimicrobial assay according to Ranjit *et. al.,* (2014) was used. The fungal spores were harvested using sterile saline after which conidial and hyphial suspension was adjusted to $1.5x10^{8}$ spores/ml using 0.5 McFarland standard (prepared by mixing 0.05 mL of 1% barium chloride dihydrate ($BaCl_2$•$2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4)).

Test organisms were aseptically inoculated into Sabouraud Dextrose Agar (Ranjit *et. al.,* 2014). A 5 mm cock borer was used to make wells on the solidified cultures. A 0.1 ml of each of the extracts and antibiotics were added in each of the wells for each organism taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow for proper diffusion of the extract into the media. The plates were then incubated at 25-28^{0}c for 3-5days. The diameter of the zone of inhibition was measured in mm.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms (Kumar, *et. al.,* 2007).

The minimum inhibitory concentration (MIC) was carried out using tube dilution technique according to Vallekobia *et. al.,* (2001). Different concentrations of the extracts (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml) were obtained using double-fold serial dilution method.

Pure culture was incubated at 28^{0}c for 7-10 days to allow for proper sporulation then it was harvested using sterile saline after which conidial and hyphial suspension was adjusted to $5x10^{8}$/ml using 0.5 McFarland standard (prepared by mixing 0.05 mL of 1% barium chloride dihydrate ($BaCl_2$•$2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4)).After harvesting and adjusting the conidial and hyphial suspension 1.0 ml of each of the test organisms was added into the test tubes containing Saubourad broth and incubated at 25-28^{0}c for 3-5days. The MIC was recorded as the lowest concentration of the extract that inhibited the growth of the test organism (Kumar, *et al.,* 2007).

RESULTS

Result of Isolation of Fungi from the Poultry Droppings

This study was carried out on 150samples of poultry droppings collected from 30 different poultry farms in Owerri, Imo State. A total of 14 fungi was isolated in these study as shown in Table 1 and 2. Table 1 shows the Cultural Morphology and Microscopy of Fungi Isolates.

Table 2 shows the Fungi isolates and their percentage of occurrence. *Candida tropicalis*80(53.30 %) showed the highest occurrence followed by *Aspergillus niger* 35(23.30 %). *Aspergillus fumigatus, Penicillum* spp. and *Rhizopus azygosporus* showed the least occurrence with 5(3.33%).

Results of Phytochemical Analysis

Phytochemicals are naturally occurring constituents of plants. The phytochemical determination of the leave extracts are shown in table 3. The analysis revealed the presence of alkaloids, tannins, flavonoid, saponins, phenolics, glycosides and steroid in the two plants. The *Azadirachta indica* (Neem) showed high activity due to an abundance of phenolics, glycosides and tannins.

Table 4 and 5shows the Mean And Standard Deviation Of Diameter Zone Of Inhibition of the plant extracts after 48and 72 hours respectively.

The Minimum Inhibitory Concentration (MIC) of the two studied plants against the tested isolates are shown in Table 6.

Table 1: Cultural Morphology and Microscopy of Fungi Isolates

Cultural morphology	Microscopy	Isolate
White to cream coloured Smooth, glarous, yeast-like Colonies.	Spherical to sub-spherical budding yeast-like cells or blastoconidia, 3.5-7 x 5.5-10 µm	*Candida tropicalis*
Flat, compact colonies, slightly white at first, then becoming black.	Very rough conidia, glubose in shape	*Aspergilus niger*
Flat, rapid growing colony. White at first, then developing dark green central portion.	Numerous small spores held together in a clump.	*Gliocladium cibotti*
Cells are ovoidal to elongate, Growth is white to faintly yellowish-white in colour	Shows pseudohyphae	*Millerozyma farinose*
Surface growth is velvety, downy or powdery, showing various shades of green	Hyphae are septate with smooth walled conidiophores.	*Aspergillus fumigatus*
Colonies rapidly growing, green to cream-buff.	Conidia round, smooth to rugulose, (3-4 µm dia.) in short chains.	*Aspergillus nindulans*
Dull to deep green to a grayish turquoise, with yellow to orange areas.	Hyphae are septate and hyaline.	*Aspergillus glaucus*
Colonies are cream coloured, raised, entire, smooth and butyrous.	Round to oval cells about 4 to 8 µm.	*Candida dubliniensis*
Colonies are cream coloured, raised, entire, smooth & butyrous.	Round to oval cells about 4 to 8 µm.	*Candida albicans*
Surface texture is velutinous (soft, velvety surface) to floccose	Produces septate, hyaline (clear, not pigmented) hyphae.	*Penicillium citrinum*
A woolly growth resembling cotton.	Broad hyphae which are non- septate	*Mucor zygomycetes*

Table 2: Fungi isolates and their percentage of occurrence

Organisms	Number of Samples	Percentage of Occurrence (%)
Candida tropicalis	80	53.30
Aspergillus niger	35	23.30
Gliocladium cibotti	15	10.00
Millerozyma farinosa	20	13.30
Aspergillus fumigatus	5	3.33
Aspergillus flavus	10	6.66
Aspergillus nindulans	15	10.00
Aspergillus glaucus	13	8.66
Candida dubliniensis	15	10.00
Penicillum spp.	5	3.33
Mucor zygomycetes	7	4.66
Rhizopus azygosporus	5	3.33
Candida albicans	15	10.00
Candida glabrata	17	11.33

Table 3 Phytochemical screening of methanol extract of *Azadirachta indica (Neem) and Ocimum gratissimum*(scent leaf).

S/ No.	Phytochemicals	scent leaf	neem
1	Alkaloids	+++	+++
2	Flavonoids	+++	+++
3	Glycosides	++	+++
4	Phenolics	++	+++
5	Saponins	++	++
6	Steroids	++	++
7	Tannins	++	+++

Key: – (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance)

Table 4: Mean And Standard Deviation of Diameter zone of inhibition (mm) of *Azadirachta indica (Neem) and Ocimum gratissimum*(scent leaf) extracts against the test organisms after48 hrs

	ZONE DIAMETER (mm)					
Organisms	**Neem**	**Scent leaf**	**Fluc.**	**Casp.**	**Vor.**	**Meth.**
Candida tropicalis	11.30±1.53	8.00±1.00	18.30±0.58	6.30±0.58	7.30±1.52	-
Aspergillus niger	10.30±0.58	6.30±1.00	15.70±1.15	6.00±0.00	6.00±0.00	-
Gliocladium cibotti	10.00±1.00	6.00±1.00	15.70±1.00	6.30±1.00	6.70±1.15	-
Millerozyma farinosa	12.30±1.53	10.00±1.00	15.00±0.00	6.70±0.58	6.30±1.15	-
Aspergillus fumigatus	11.30±1.53	6.30±1.00	15.70±1.00	6.00±0.00	6.00±1.00	-
Aspergillus flavus	11.00±1.53	6.00±1.00	15.70±0.00	6.00±1.00	6.00±1.15	-
Aspergillus nindulans	10.30±0.57	6.30±0.00	15.70±1.00	6.00±0.00	6.00±1.00	-
Aspergillus glaucus	9.50±1.53	6.70±1.15	14.00±1.00	6.00±0.00	6.00±1.00	-
Candida dubliniensis	11.30±1.53	8.00±1.00	18.30±1.15	6.30±1.00	7.30±0.00	-
Penicillum spp.	12.30±1.53	10.00±0.00	15.00±0.00	6.70±0.00	6.30±1.00	-
Mucor zygomycetes	10.70±0.57	6.70±1.53	15.80±1.00	6.00±1.00	6.00±0.00	-
Rhizopus azygosporus	7.50±0.00	6.50±1.00	16.00±0.00	6.50±0.00	6.00±1.00	-
Candida albicans	11.30±1.53	8.00±0.00	18.30±1.00	6.30±1.53	7.30±0.00	-
Candida glabrata	11.30±1.53	9.00±1.00	17.30±0.00	6.30±0.00	6.30±1.00	-

Table 5: Mean And Standard Deviation of Diameter zone of inhibition (mm) of *Azadirachta indica (Neem) and Ocimum gratissimum*(scent leaf) extracts against the test organisms after72 hrs

	ZONE DIAMETER (mm)					
Organisms	**Neem**	**Scent leaf**	**Fluc.**	**Casp.**	**Vor.**	**Meth.**
Candida tropicalis	12.30±1.00	8.50±1.53	18.30±0.00	6.30±0.57	7.30±0.00	-
Aspergillus niger	11.40±1.53	6.00±0.00	15.70±1.00	6.00±0.00	7.00±0.00	-
Gliocladium cibotti	10.50±0.58	6.50±1.00	15.70±1.53	5.30±0.57	6.70±1.00	-
Millerozyma farinosa	13.50±0.00	11.00±0.00	15.00±0.00	6.70±1.00	6.30±0.53	-
Aspergillus fumigatus	12.30±0.57	6.30±1.53	15.70±0.58	6.00±0.57	6.00±1.53	-
Aspergillus flavus	11.50±0.00	6.50±1.53	15.70±0.00	6.00±1.00	6.00±0.00	-
Aspergillus nindulans	11.30±1.53	6.30±0.00	15.70±1.00	6.00±0.58	6.00±0.00	-
Aspergillus glaucus	10.50±0.00	6.80±0.00	14.00±1.00	6.00±0.00	6.00±1.00	-
Candida dubliniensis	11.70±0.58	8.00±1.53	18.30±0.00	6.30±1.53	7.30±0.57	-
Penicillum spp.	13.30±0.00	10.50±1.00	15.00±0.57	6.70±0.00	6.30±1.00	-
Mucor zygomycetes	11.70±0.00	6.90±1.53	15.80±0.00	6.30±0.00	6.00±0.57	-
Rhizopus azygosporus	7.90±1.00	6.50±0.00	16.00±1.00	6.00±1.53	6.00±1.53	-
Candida albicans	12.30±0.00	8.00±0.00	18.30±1.53	6.30±0.00	7.30±0.00	-
Candida glabrata	12.30±1.00	9.50±1.53	17.30±0.00	6.30±1.00	6.30±1.57	-

Table 6: Minimum Inhibitory Concentration (MIC) of the inhibitory substances in (mg/ml)

Organisms	Neem	Scent leaf	Fluconazole
Candida tropicalis	125	250	62.5
Aspergillus niger	250	500	125
Gliocladium cibotti	125	500	62.5
Millerozyma farinosa	125	250	125
Aspergillus fumigatus	125	500	62.5
Aspergillus flavus	250	500	62.5
Aspergillus nindulans	250	250	62.5
Aspergillus glaucus	250	500	125
Candida dubliniensis	250	500	62.5
Penicillum spp.	125	250	125
Mucor zygomycetes	250	500	62.5
Rhizopus azygosporus	500	500	125
Candida albicans	250	250	125
Candida glabrata	250	250	62.5

The result of this study revealed that the methanolic extract of the Neem and Scent leaf showed pronounced activity against the tested organisms, and the inhibitory effect among the leaves extracts differed significantly from the control (fluconazole). The study further revealed that the plant extracts exhibited most activity after 72hrs. The result showed that the methanolic activity of the Neem leaf showed more activity against the isolates compared to the Scent leaf.

The MIC for *R. azygosporus* was very high (500mg/ml). This is because the organism was the least susceptible to the plant extracts. The *Azadirachta indica (Neem)* showed highest inhibitory activity against *M. farinose* after 72 hours(13.50±0.00).

DISCUSSION

The present study, Antifungal Activity Pattern Of Two Plant Extracts Against Fungi Isolated From Poultry Dropings in Owerri, Imo State, deals with possibility of presence of yeasts and other filamentous fungi in poultry droppings in Owerri, and also the effect of methanolic extract of *Ocimum gratissimum*(scent leaf) and *Azadirachta indica* (Neem) on the fungi isolates. The following fungi strains was isolated from the poultry droppings as shown in table 2;*C. Tropicalis* 80(53.3%), *A. Niger* 35(23.3%), *G. Cibotti* 15(10%), *M. Farinose* 20(13.3%), *Aspergillus fumigatus* 5(3.33%), *Aspergillus flavus* 10(6.66%), *Aspergillus nindulans* 15(10%), *Aspergillus glaucus* 13(8.66%),*Candida dubliniensis* 15(10%), *Penicillum* spp. 5(3.33%), *Mucor zygomycetes* 7(4.66), *Rhizopus azygosporus* 5(3.33%), *Candida albicans* 15(10%) *and Candida glabrata* 17(11.33%).

The highest percentage of fungi isolated was *Candida tropicalis* 80(53.3%), this result is in agreement with that reported in surveys of bird dropping associated yeasts and filamentous fungi in several other countries (Hanka *et. al.*,2010;Imran and Rusu,2014).

Antifungal activities of methanolic extract of *Ocimum gratissimum* (scent leaf) and *Azadirachta indica* (Neem) leaf was also evaluated against the fungi isolates and compared with commercial antifungal agents . The phytochemical compounds present in the leave extracts were determined. The following phytochemicals were found to be present in the leaves; steroids, glycosides, phenolics, saponins, flavonoids, tannins and alkaloids. The phytochemical composition of methanolic extracts of *Ocimum gratissimum* (scent leaf) and *Azadirachta indica* (Neem leave) showed inhibitory effects on the fungi isolates. These interesting results may be explained by the presence of tannins, which are known antimicrobial agents that could inhibit the growth of microorganisms by precipitating out the microbial protein and thus depriving them of nutritional proteins needed for their growth and development (Xu *et. al.,* 1996). The presence of tannins in plants could be considered to be of homoeopathic value for its potential antiviral, antibacterial, antifungal and anti-parasitic effects (Xu *et. al.,* 1996). Saponins are commonly known as an anti-nutrient, but it is also hypothetical to be useful in human nutrition for the regulation of cholesterols. Its presence in the leavestherefore could suggests that the plant is of therapeutic value (Asland Hosseinzadeh, 2008).

Plant extracts are quite efficient in the control of several diseases caused by microorganisms. Significant (35%) growth reduction of mycelia of *Phytophthora* spp. with neem extracts has been recorded (Ramos *et. al.,* 2007). Interesting results were also observed on the study of Koba *et. al*., (2009)showing inhibitory effects of *O. gratissimum* where it elicited various dermatophytes, imperfect filamentous fungi and pathogenic yeasts.

The result of these study showed that the methanolic extract of *Ocimum gratissimum* (scent leaf) and *Azadirachta indica* (Neem) leaf have inhibitory effects on the growth of the test organisms. With *Azadirachta indica* (Neem) leaf showing the highest inhibitory property against most of the fungi isolates (table 4 and 5) this could be as a result of high abundance of tannin, Glycosides and phenolics in the neem plant. However, further studies are required to gain more clarity as to the specificity and biochemical mechanisms responsible for the antifungal properties of these two plants in different concentration and on other fungi isolates. New milestone in the development of pharmaceutical products can be achieved by discovering bioactive natural products from these medicinal plants that address unfulfilled therapeutic needs against fungi infections.

CONCLUSION

Fungi/mycotic infections are common in all kinds of poultry birds but are less prevalent as compared to bacterial and viral infections. Both birds dropping and floor of cages conceders as a suitable media for fungal growth due to presence of many essential materials to growth such as cellulose, Nitrogen, carbohydrates...etc.

The presence of the isolated fungi strains in the poultry droppings in Owerri, can cause contamination of poultry products which can lead to different types of infection. The present study revealed that the extracts of the two plants showed a stronger inhibitory property against the isolates when compared to synthetic antifungal agents such as Caspofungin and Voriconazole, but lesser efficacy when compared to Fluconazole. *C. tropicalis, M. farinose, A. fumigates, C. dubliniensis, Penicillum* spp. And *C. albicans* showed to be more susceptible to the Neem extract after 48 and 72 hours, while *R. azygosporus* was least affected by the neem extract(table 4 and 5). The scent leaf extract showed more activity against *M. farinose* and *Penicillum spp*. After 48 and 72 hours with *R. azygosporus* having low susceptibility to the scent leaf extract. This efficacy of the leave extracts of *Ocimum gratissimum* (scent leaf) and *Azadirachta indica* (Neem) as compared to synthetic antifungal shows promising results to be used as potent natural occurring antifungal agents.

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