**EFFECT OF *Daniella oliveri* AQUEOUS EXTRACT ON ISOLATED FUNGI LOAD FROM *Clarias gariepinus* FINGERLINGS**

**CHAPTER ONE**

**1.0 INTRODUCTION**

Fish are abundant in most bodies of water. They are found in nearly all aquatic environments, from high mountain streams to the abyssal and even hadal depths of the deepest oceans with 33,100 described species, fish exhibit greater diversity than any other group of vertebrates (Gupta *et al*.,

2008).

Fungal infections are a significant cause of morbidity and mortality in humans. They are considered opportunistic as the etiologic agents cause mild illness or no illness in healthy people, but it may infect and cause serious diseases in immunosuppressed ones. A simple infection such as candidiasis may be severe and invasive, colonizing the esophagus, stomach and intestine in patients with immunodeficiency syndrome (Abbas *et al*., 2012; Coelho‑Castelo *et al*., 2009; Janeway *et al*., 2000). Some fungi have developed resistance to these chemicals. This necessitates higher dosage or the development of new chemicals to replace those to which fungi are resistant. Secondly, some fungicides are not readily biodegradable and tend to persist in the environment.

Even though synthetic fungicides are available and are used to control the disease, their indiscriminate use causes environmental hazards. Although the use of these chemicals are effective in controlling fungal diseases, there are however some major set back which tend to limit the usage. Hence, it becomes necessary to develop ecologically safe, effective and economically feasible method of disease management (Khoo, 2000). Aqueous extract of many allelopathic plants are known to exhibit antifungal properties. Allelochemicals reduce the germination of spores and mycelial growth of pathogenic fungi (Begum *et al*., 2008; Haikal, 2007; Sahayaraj *et al*., 2006; Bajwa *et al*., 2003). The use of plant extract to control fungal growth in freshwater fish is scarce in the literature (Rai *et al*., 2002). Herbal medicine is a common element in Ayurvedic, Homeopathic and Naturopathic treatments. Herbs or herbal products also have a role in aquaculture at present time (Direkbusarakom, 2000). At the moment, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants.

*Daniellia oliveri* is commonly known as Copaiba balsam, ‘Maje’ in Hausa, ‘iya’ in Yoruba, ‘Ozabwa’ in Igbo and belongs to the family of Fabaceae (subfamily Caesalpinioideae)*.* It is an evergreen plant that grows abundantly in bush fallows, secondary bushes and marginal lands in most of the savannah zones of Nigeria. Different parts of the plant are used for different purposes including mulching and fodder (leaves and twigs), firewood and ethno-medicine (stem and root) (Adekunle and Oyerinde, 2004; Hassan *et al*., 2008; El-Mahmood *et* *al.,* 2008). The seeds have very low preference as a human food value or in industrial use till now and could, therefore, form an alternative feed ingredient for livestock production. Some previous studies (Hassan *et al*., 2008; El-Mahmood *et al.,* 2008) concentrated on the nutrients and anti-nutrients of the seeds. Nutritionally, *D. oliveri* on dry matter basis have been reported to contain 57.84% carbohydrate, 0.60% crude fibre, 27.74 % crude protein, 9.67% lipid and 4.17% ash (Hassan *et al*., 2008). However, the presence of anti-nutritonal substances such as phytate, oxalate, hydrocyanide, tannin and nitrate in the seeds hinder animals from benefitting from it nutritionally (Hassan *et al*., 2008; El-Mahmood *et al.,* 2008).

**1.1 JUSTIFICATION**

Different traditional processing methods such as roasting, toasting, cooking and fermenting were pronounced that they reduce anti-nutritional factors and raise nutrients bioavailability (Ragab *et al*., 2010). The use of plant extract to control fungal growth in freshwater fish is scarce in the literature (Rai et al., 2002).Generally, there is a very limited number of documentation on the utilization of *D. oliveri leaves* as a therapeutic agent in the control of fungi disease in fish.

**1.2 OBJECTIVES**

1. To investigate the effects of aqueous extract of Daniella oliveri on fungi isolated from Clarias gariepinus fingerlings.
2. To determine alternative methods for treatments of fungal disease on fish.

**CHAPTER THREE**

**3.0 MATERIALS AND METHODS**

**3.1 COLLECTION OF PLANT SAMPLES**

The fresh leaves of Daniella oliveri will be collected within the Federal University of Agriculture, Abeokuta campus. The plant collected will be identified at the Department of Forestry and Wildlife Management of the Federal University of Agriculture, Abeokuta.

**3.2 PREPARATION OF PLANT MATERIALS**

The freshly harvested leaf will be washed thoroughly, rinsed with clean water and evenly spread to sun dry and thereafter dried in an oven at 65oC for 48 hours before being macerated.

**3.3 PHYTOCHEMICAL SCREENING**

The powdered plant parts were screened for secondary metabolites using standard procedures (Sofowora, 1993).

**3.4 PREPARATION OF EXTRACT**

The powdered leaves (500g) will be soaked 1000ml of water. After 96 hours, the soaked plant will be sieved using Whatman No 1 filter paper and the solution will be stored in a cool dry place for use.

**3.5 MICROORGANISMS**

The fungal strains to be used for the study will be pure cultures obtained from laboratory stock. These will include: *Aspergillus niger, Candida albicans, Candida krusei, Rhizopus stolonifer, Epidermophyton floccosum, Trichophyton floccosum, Trichophyton interdigitale and Trichophyton rubrum.*

**3.6 IN VITRO ANTIFUNGAL ACTIVITY**

The antifungal activities of the plant extracts will be determined by agar plate method (Nwosu and Okafor, 1995). Plant extracts were added to PDA at 40oC to give the concentration of 50, 100, 200 and 400 mg/ml for the extract and then the PDA with extracts were poured (~10 ml/plate-1) each alone in petri plates (60mm in diameter). Seven-day-old agar discs (5mm in diameter) bearing the desired fungus growth was transferred in the petri plates. These fungus cultures were incubated at 25±20C for 7 days. Fungus growths will be recorded daily according to the methods of Onaran and Yilar, 2012. Commercial fungicide [Thiram 80% (Hektaş, group)] will be used as a positive control and 50% acetone was used as a negative control. Experiment set up 4 replications and repeated twice. The percentage of mycelial growth inhibition will be calculated using the formula proscribed by Pandey et al., 1982.

I=100× (dc˗dt)/dc

I; Mycelial growth inhibition

dc; Is the mycelial growth in control

dt; Is the mycelial growth in treatment

**3.6 STATISTICAL ANALYSIS**

Data obtained were subjected to analysis of variance (ANOVA) procedures and treatment means were compared by Duncan’s Multiple Range Test at 0.05 probability level (Duncan, 1955) using SPSS 10.0.

**REFERENCES**

Abbas, K.A., Andrew, H.H., Lichtman, A.H.H. and Pillai, S., 2012. *imunologia celular e molecular*. rio de janeiro: elsevier brasil. 560 p.

Adekunle, V. A. J. and O. V. Oyerinde. 2004. Food potentials and some indigenous wild fruits in low land rainforests ecosystem of south west Nigeria. J. Food Technol. 2: 125-130.

Bajwa R, Khalid A, Cheema TS (2003). Antifungal activity of allelopathic plant extracts III: Growth response of some pathenogenic fungi to aqueous extract of *Parthenium hysterophorus.* Pak. J. Plant Pathol. 2(3):145-156.

Begum J, Bhuiyan MDNI, Chowdhury JU (2008). Essential oil from inflorescence of *Spilanthes calva* D.C. Bangladesh J. Bot. 37(2): 217- 218.

Coelho-Castelo, A.A.M., Trombone, A.P.F., Rocha, C.D. and Lorenzi, J.C.C., 2009. Immune response to infectious diseases. *medicina*, vol. 42, no. 2, pp. 127-142.

D.K. Pandey, N.N. Tripathi, R.D. Tripathi, and S.N. Dixit, “Fungitoxic and phytotoxic properties of essential oil of *Hyptis suaveolens*,” *Z.* *Pflanzenk*, vol. 89 pp. 344–349. 1982.

Direkbusarakom, S. 2000. Application of herbs for aquaculture in Asia. *The AAHRI* *Newsletters*, 9 (2): 3 – 5

Duncan, D. E. 1955. Multiple range and multiple Ftests. Biometrics 11: 1- 42.

El-Mahmood, A. M.; J. H. Doughari and F. J. Chanji. 2008. *in vitro* antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri.* Scientific Res. Essay 3 (3): 102-105.

Gupta, C., Garg, A.P. and Uniyal, R.C. 2008. Antibacterial activity of Amchur (dried pulp of unripe *Mangifera indica*) extracts on some food borne bacteria. *Journal of* *Pharmacology Resources,* 1: 54-57

Haikal NZ (2007). Improving biological control of *Fusarium* root-rot in cucumber (*Cucumis sativus* L.) by allelopathic plant extracts. Int. J. Agric. Biol. 9(3): 459-461.

Hassan, L.G.; S. M. Dangoggo, K. J. Umar, I. Saidu and F. A. Folorunsho. 2008. Proximate, minerals and anti- nutritional factors in *Danellia oliveri* seed kernel. Nig. J. Basic Applied Sci. 18: 31-35.

Janeway, C.A., Travers, P., Walport, M. and Donald Capra, J., . 2000 *imunobiologia: o sistema imune na saúde e na doença*. 4th ed. porto alegre: artes médicas sul.

Khoo L (2000). Fungal diseases in fish. Seminars in Avian and exotic pet medicine, 9(2): 102-111.

M.O. Nwosu, and J.l. Okafor, “Preliminary studies of the antifungal activites of some medicinal plants against Basidiobolus and some other pathogenic fungi.,” *Mycoses*, vol. 38, pp. 191-195, May-June 1995. <http://dx.doi.org/10.1111/j.1439-0507.1995.tb00048.x>

Onaran, and M. Yılar, “Antifungal activity of *Trachystemon orientalis* L. aqueous extracts against plant pathogens,” *J. Food Agrıc. Environ.*, vol. 10, pp. 287-291, July-October 2012

Ragab, H. I.; C. Kijora, K. A. Abdel Ati and J. Danier. 2010. Effect of traditional processing on the nutritional value of some legumes seeds produced in Sudan for poultry feeding. Int’l. J. Poultry Sci. 9 (2): 198-204.

Rai MK, Kaushal SK, Acharya D (2002). *In vitro* effect of five Asteraceous essential oils against *Saprolegnia ferax,* a pathogenic fungus isolated from fish. The Antiseptic 99(4): 136-137.

Sahayaraj K, Namasivayam KR, Borgio JAF (2006). Influence of three plant extracts on *Fusarium oxysporum* F. sp. *Ciceris* mycelium growth. J. Plant Prot. Res. 46: 335-338.