

Antifungal Properties and Phytochemical Screening of Crude Extract of *Lemna pauciscostata* (Helgelm) Against Fish Feed Spoilage Fungi

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Abstract

Aqueous and ethanolic extracts of duckweed (*Lemna pauciscostata*) meal was tested on fungal isolates from stored pelleted fish feeds to ascertain its efficacy as an antifungal agent against feed spoilage fungi. Test organisms used were *Fusarium oxysporium*, *Penicillium digitatum*, *A. niger*, *A. flavus*, *A. fumigatus*, *Rhizopus oryzae* and *.stolonifer*. Phytochemical analysis of the crude extract was also conducted to determine the active ingredients in duckweed meal. Proximate nutrient composition and amino acid analysis to determine the suitability or otherwise of duckweed meal as a feed additive was also carried out. Results showed that ethanolic extracts exhibited higher antifungal properties with total growth inhibition in some test organisms than the aqueous extract. However the efficacy of the extracts against fungal growth increased with increase in concentration. Result of the phytochemical analysis of duckweed meal revealed the presence of tannins and steroids. Determination of the proximate nutrient composition and amino acid analysis also showed that duckweed meal is rich in essential nutrients. [Life Science Journal. 2009; 6(3): 19 – 22] (ISSN: 1097 – 8135)

Keywords: duckweed meal; antifungal; extract; ethanolic; aqueous

1. Introduction

Antimicrobial agents, including food preservatives have been used to inhibit food borne fungi and extend shelf life of processed food for many centuries. Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as agents against food spoilage micro-organisms^[1,2]. Tannins and steroids have been shown to possess antimicrobial ability against several food spoilage fungi^[3,4].

Lemna, a group of tiny, free-floating vascular plants with worldwide distribution are found in small water bodies such as fishponds, ditches and lagoons, which are nutrient rich. Their ability to bloom within days after cultivation with high nutrient content has made them a rich source of food nutrients in the diet of fishes and animals alike. *Lemna* have been shown to exhibit antimicrobial activity^[5,6].

A major problem in fish feed production is associated with storage. A lot of losses occur in feedstuff during storage. Fungal attacks along with other kinds of storage problems are responsible for unreasonable losses occurring in feedstuff during storage. Such losses are loss in weight, loss in quality of feed and health risks to fish that feed on infected feed.

The addition of some fungicides could suppress the growth of fungi in feed, however as with all pesticides, these chemicals are likely to have side effects which may be hazardous to fish health. Therefore if a non-hazardous process could be found capable of suppressing or even eliminating fungal growth in stored compound feed, it would be of immense practical and economic benefits to the aquaculture industry in Nigeria.

Fungal isolates from stored pelleted fish feeds were therefore utilized as test organisms on extracts of *Lemna pauciscostata* used directly as fish feed.

2. Materials and Method

Samples of duckweed (*Lemna pauciscostata*) were harvested from the outdoor concrete tanks of National Institute of Freshwater Fisheries Research Hatchery Complex, New Bussa, Nigeria. They were thoroughly rinsed with clean water and evenly spread on a mosquito net-size mesh to dry and thereafter dried in a forced air oven at 65 °C for 48 hours before being grounded to powder with a milling machine^[7]. The powder was exhaustively extracted with 95% Ethanol and sterile distilled water at room temperature for 2 days.

Extracts were filtered and the solvent removed under reduced pressure at 40 °C^[8]. Preliminary antifungal assays were performed using seven test organisms and extracts at concentrations of 5% and 10% respectively. Control plates had 95% ethanol and sterile distilled water without extracts. Mycelial plugs of the test organisms measuring 5.0 mm in diameter were cut with sterile cork borer from the advancing margin of the fungal colonies and placed at the centre of Potato Dextrose Agar^[9]. All plates were incubated at 25 °C and radial mycelial growth recorded.

Dried duckweed was ground using Automatic weed Grinder (Scientific Instrument, Yoshida Seikusho Co. Ltd, Tokyo, Japan, No. 5678). Extracts for phytochemical analysis were concentrated to dryness in hot air oven at 45 °C^[10]. The dried extracts were tested for alkaloids, saponins, tannins, anthraquinones, flavonoids, steroids and phlobatannins^[11]. Proximate composition of the following nutrients was determined using standard procedures of AOAC^[12]: moisture, crude protein, lipid, crude fiber and Nitrogen free extract (NFE). Amino acid profile of duckweed meal was determined using the method of Abdullahi^[13].

3. Results and Discussion

Differential efficacy on the test organisms was noted between the aqueous and ethanolic extracts of *Lemna pauciscostata* (Table 1). Ethanol appeared a better

extractant judging from the wider activity spectrum and the effect of its extract on isolates. This observation perhaps suggests the possibility of the occurrence of bioactive substances that are not only soluble in water but also in organic solvent in the plant material.

Majekodunmi *et al.*^[14] and Martinez *et al.*^[15] reported that a higher activity of extractable natural products was obtained in ethanol compared with aqueous extracts. Ahmed *et al.*^[16] also observed that alcoholic extracts showed greater activity than the aqueous and hexane extract of some Indian medicinal plants with antimicrobial properties. While ethanolic extracts showed total growth inhibition on some organisms even at 5% concentration, aqueous extract showed none although; growth rate was slower at the 10% aqueous than 5% aqueous extract. The most susceptible isolates to both the aqueous and ethanolic extracts were *Aspergillus fumigatus* and *Fusarium oxysporium* where total growth inhibition was observed.

Several authors have reported on the antimicrobial activity of various plant extracts using different means of extraction on various plants materials. Natarajan *et al.*^[17] reported the antifungal properties of three medicinal plant extracts against *Cercospora arachidicola*. They reported that fungal growth was gradually suppressed with increasing extract concentration. Similar findings have been reported by Lucia *et al.*^[3] on the antifungal properties of Brazilian cerrado plants. They stated that ethanolic extracts of the plants showed higher antifungal activity.

Silva *et al.*^[18] and Costa *et al.*^[4] also reported the antifungal activity of extracts of *Eugenia dysenterica* and *Annora crassiflora* against some pathogenic fungi. The findings from this study are similar to the report of these authors.

Adekunle and Ikunmapayi (2006) working on the antifungal properties and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius* reported varying degrees of antifungal activity of the plant extracts on some test organisms including *Aspergillus flavus* and *Penicillium* species. The same authors reported that the ethanolic extracts of the plants showed higher antifungal activity on the test organisms than the aqueous extracts.

The result of the phytochemical screening of the *Lemna pauciscostata* extracts (Table 2) revealed the presence of tannins and steroids. Research findings from several authors have shown that both tannins and steroids possess antimicrobial ability. Bairagi *et al.*^[19] reported the presence of tannins and phytic acid in duckweed meal. Baba Moussa *et al.*^[20] reported antifungal activities of seven West African combretaceae extracts used in traditional medicine against several fungal species. The result of the phytochemical screening of these plant extracts showed that they were rich in tannins and saponins.

Table 1: Efficacy of duckweed extracts on the mycelial growth of fungal isolates after 72 Hours.

TEST ORGANISMS	MYCELIAL GROWTH IN MM					
	AQUEOUS EXTRACT			ETHANOLIC EXTRACTS		
	0%	5%	10%	0%	5%	10%
<i>Fusarium oxysporium</i>	46	21	10	10	-	-
<i>Penicillium digitatum</i>	50	35	24	9	5	-
<i>Aspergillus niger</i>	47	27	18	16	7	2
<i>Aspergillus fumigatus</i>	38	18	12	4	-	-
<i>Aspergillus flavus</i>	50	38	20	16	-	-
<i>Rhizopus oryzae</i>	36	29	16	14	-	-
<i>Rhizopus stolonifer</i>	42	21	13	22	10	4

Table 2: Phytochemical analysis of duckweed meal.

Test	Result
Alkaloids	-
Saponins	-
Tannins	+
Anthraquinones	-
Flavonoids	-
Phlobatannins	-
Steroids	+

Table 3: Proximate composition of duckweed (*Lemna pauciscostata*) meal

Sample	% Crude Protein	% Ether Extract	% Ash Content	% Moisture Content	% Crude Fiber
Duckweed	34.18	5.3	13.55	2.8	14.28

Adekunle and Ikunmapayi (2006) reported the presence of tannins, saponins and steroids among other substances from the extracts of *Funtumia elastica* and *Mallotus oppositifolius* which they inferred were likely to be responsible for the antifungal activity exhibited by these plants. Other authors have also reported similar findings^[21-25]. Burapedjo and Bunchoo^[26] implicated these phytochemicals to inhibit cell wall formation in fungi leading to the death of the organisms. The findings of this experiment are similar to the report of these authors.

The results of the proximate composition of nutrients as well as that of amino acid profile in Tables 3 and 4 respectively showed that duckweed meal is rich in essential nutrients. Therefore, incorporating it into fish feed formulation will not cause any negative effect to fish growth and survival. Several authors have reported the use of duckweed as fish feed ingredient^[27-31].

Table 4: Amino acid analysis of duckweed meal.

AMINO ACID	AMOUNT
LYSINE	5.30
HISTIDINE	2.03

4. Conclusion

From the findings of this experiment, there are indications that duckweed meal could be incorporated into formulated fish feeds to serve as antifungal agent against feed spoilage fungi. This will be of immense benefit to the local fish farmers and therefore improvement in the fisheries aquaculture practice in Nigeria.

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