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 R.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 2010;7(3):1-4]. (ISSN: 1097-8135).

Key Words: Duckweed meal, antifungal, extract, ethanolic, aqueous.

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 posses' antimicrobial functions and could serve as agents against food spoilage micro-organisms (Deans and Ritche, 1987; Janseen et al., 1985). Tannins and steroids have been shown to possess antimicrobial ability against several food spoilage fungi (Lucia et al., 2002; Costa et al., 2000).

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 (Skilicorn et al., 1993; Mbagwu, 2001).

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.

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 65^0C for 48 hours before being grounded to powder with a milling machine (Mbagwu and Adeniyi, 1988). 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 40^0C (Souza et al; 2002). 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 5.0mm 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 (Adedayo, 1994).

All plates were incubated at 25^0C 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 45^0C (Odebiyi and Sofowara, 1978). The dried extracts were tested for alkaloids, saponins, tannins, anthraquinones, flavonoids,

steroids and phlobatannins (Harbone, 1984). Proximate composition of the following nutrients was determined using standard procedures of AOAC (2000): moisture, crude protein, lipid, crude fiber and Nitrogen free extract (NFE). Amino acid profile of duckweed meal was determined using the method of Abdullahi (2001).

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., (1996) and Martinez et al., (1996) reported that a higher activity of extractable natural products was obtained in ethanol compared with aqueous extracts. Ahmed et al., (1998) 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., (2005) 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., (2002) on the antifungal properties of Brazilian cerrado plants. They stated that ethanolic extracts of the plants showed higher antifungal activity.

Silva et al., (2001) and Costa et al., (2000) also reported the antifungal activity of extracts of *Eugenia dysentrica* 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., (2002) reported the presence of tannins and phytic acid in duckweed meal. Baba Moussa et al., (1999) 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.

Adekunle and Ikunimapayi (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 (Onadapo and Owonubi, 1993; Barnabars and Nagarajan, 1988; Adekunle et al., 2003; Subhisha and Subramoriam, 2005; Adio et al., 2004).

Barapedjo and Bunchoo (1995) 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 (Fasakin and Balogun, 1998; Fasakin et al., 2001; Fasakin et al., 1999; Edwards, 1980; Robinette et al., 1980).

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.

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

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

Table 4:Amino acid analysis of duckweed meal.

AMINO ACID	AMOUNT
LYSINE	5.30
HISTIDINE	2.03
ARGININE	4.25
ASPARTIC ACID	6.87
THREONINE	4.81
SERINE	3.89
GLUTAMIC ACID	10.11
PROLINE	3.08
GLYCINE	4.87
ALANINE	1.91
CYSTEINE	1.21
VALINE	4.24
METHIONINE	1.01
ISOLEUCINE	4.81
LEUCINE	6.30
TYROSINE	3.04
PHENYLALANINE	4.26

REFERENCES

1. Abdullahi, S.A. (2001). Investigation of Nutritional status of *Chrysichthys nigrodigitatus*, *Barus filamentous* and *Auchenoglanis occidentalis*: Family Barigdae. Journal of Arid Zone Fisheries 1:39-50
2. Adedayo, O. and Kolawole, D. O. (1994). Resistance of mouse-virulent encapsulated nasal isolates of *Staphylococcus aureus* to disinfectant and antiseptics. Biomedical letters 50: 151 -156.
3. Adekunle, A. A. Duru, C. and Odufuwa, O. M. (2003). Antifungal activity and phytochemical screening of the crude extracts of *Khaya ivorensis* Juss (meliaceae) and *Tetracera potabria* L. (Dileniaceae) South African Journal of Botany 69: 568-571
4. Adio, A. M; Paul C; Kloth, P; and Konig, W.A. (2004). Sesquisterpenes of the liverwort, *Scapania undulata*. Phytochemistry 65: 199-206.
5. Ahmad, I; Mechmood, Z. and Mohammed F. (1998). Screening of some Indian Medicinal plants for antimicrobial properties. Journal of Ethano Pharmacology 62(2); 183-193.
6. AOAC (2000)."Official Methods of Analysis" 17th Ed;Association of of official Analytical chemists, Washington, D.C.
7. Baba Moussa, F; Akpagana, K; and Bouchet, P (1999). Antifungal activities of seven West African combretaceae used in traditional medicine.Journal of Ethno pharmacology.Vol. 66(3) 335 – 338.
8. Bairagi, A; SarkarGhosh, K; Sen, S. K. and Ray, A. K. (2002). Duckweed (*Lemna polyrrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita* Ham) fingerlings after formulation with a fish intestinal bacterium. Bio resour-Technol. 85(1); 17-23
9. Barnabas, C. G. and Nagarajan, S. (1988). Antimicrobial flavonoids of some medicinal plants.Fitoterapia 3: 508-510.
10. Burapedjo, S. and Bunchoo, A. (1995). Antimicrobial activity of tannins from *Terminalia citrina.* Plant medica 61:365-366
11. Costa, T. R; Fernandes, O. F. L; Santos, S. C.; Oliveira, C. M. A; Liao, L. M. Ferri, P. H; Paula, J. R. P., Ferreira, H. D.; Sales, H. N. and Silva, M. R. R. (2000). Antifungal activity of volatile constituents of *Euglenia dysentrica* leaf oil J. Ethno Pharmacol. 72: 111-117
12. Deans, S.G; Ritchie, G.A (1987). Antimicrobial properties of plants essential oils. Int. J. Food Microbio.l (5) 165 – 180.
13. Edwards, P. (1980). Food potential of Aquatic Macrophytes, ICLARM Studies and Reviews. The International Centre for Living Resources Management, Makati, Metro Manila, Philippines.Pp261-265.
14. Fasakin, E. A. and Balogun, A. M. (1998). Evaluation of dried water fern (*Azolla-pinnata*) as a replacer for soybean dietary components for *Clarias gariepinus* fingerlings. Journal of Aquaculture in the Tropics 13(1):57-64.
15. Fasakin, E. A. and Balogun, A. M. and Fagbenro, O. A. (2001). Evaluation of sun dried water fern *Azolla africana*, and Duckweed, *Spirodela polyrrhiza*, in practical diets for Nile Tilapia, Orechromis niloticus fingerlings. Journal of Applied Aquaculture Vol. 11(4):83-92.
16. Fasakin, E. A.; Balogun, A. M. and Fasuru (1999). Use of Duckweed, Spirodela polyrrhiza L.Schleiden, as a protein feedstuff in practical diets for Tilapia, *Orechromis niloticus* L. Aquaculture Research 30(5):313-318.
17. Harbone, J. B. (1984). Phytochemical methods: A guide to modern techniques of plant analysis. 2nd Edition. Chapman and Hall. Pp 4-18, 288.
18. Janssen, A. M; Scheffer, J. J. C; Svendsen, A; Aynehchi, Y.B. (1985). Composition and antimicrobial activity of essential oil of Ducrosia anethifolia. In Essential oils and Aromatic plants; Svendsen, A. B, Scheffer, J .J.C; Eds; Martinus Nijloff Publishers: Dordrecht. The Netherlands Pp. 213 – 216
19. Lucia, K.H.S. Cecilia, A. D. O.; Pedro, N. F. Suzana, C. S; Juldsio, G. D. O; Andre, T. B. M; Luciano, M.L. and Maria, R. R. Silva (2002). Antifungal properties of Brazillian carrado plants. Braz. J. Microbiology. Vol. 33(3) 102-107.
20. Majekodunmi, O. F; Zany, L; Ohayaga, I. E; Shi Le and Mclanghin, I. L (1996). Selective cytotoxic diterpene from Euphorbia poisonic. Journal of Medicinal Chemistry 39(4) 1005-1008.
21. Martinez, M. J; Betancant, I; Alonso-Gonzalez, N; and Jauregai, A (1996). Screening of some Cuban medicinal plants for antimicrobial activity. Journal of Ethano-Pharmacology 52: 171-174.
22. Mbagwu, I. G. (2001). The effect of long-term storage on the nutrient characteristics of duckweed (*Lemna pauciscostata* Helgelm) J.Arid Agric. Vol 11; 147-149
23. Mbagwu, I. G. and Adeniyi H.A. (1988). The nutritional content of Duckweed (*Lemna pauciscostata* helgelm) in the Kainji Lake Area - Nigeria. Aquatic Botany 375-366
24. Natajaran, D; Srinivasan, K; Mohanasoundari, C; Perumal G; Dheen, M. A. N; Ganapathi, G. A. and Rajarajan T (2005). Antifungal properties of three medicinal plant extracts against *Cercospora arachicola*. Advances in plant Sciences. Vol. 18(1) 45-47.
25. Odebiyi, O. O. and Sofowora, E. A. (1978). Phytochemical screening of Nigerian Medicinal plants II. Lloydia. 41(3): 234-236.
26. Onadapo, A. and Owonubi, C. A. (1993). The antimicrobial properties of Tremaguineensis. In: 1st NAAP Proceedings. Faculty of Pharmaceutical Science ABU, Zaria. 139-144.
27. Robinette, H. R.; Brunson, M. W. and Day, E. J. (1980). Use of duckweed in diets of channel catfish. Mississippi Agricultural Experiment Station. Publication No. 4532. Mississippi State University, Mississippi State, Miss. 13Pp.
28. Silva, M. V. Costa, T. R.; Costa, M. R.; Ferreira, E. C; Fernandes, O.F.L. Santos, S.C. Liao, L.M; Ferri P.H. Paula, J.R., Ferreira, H.D. and Silva, M.R.R. (2001). Growth inhibition effect of Brazillian cerrado plant extracts on candida species. Pharm. Biol. 39: 138-141
29. Skilicorn, P; Spirar, W. and Journey, W (1993). Duckweed Aquaculture: A new Aquatic farming system for Developing countries. A World Bank Publication 76P. National Agricultural Research Project (NARP), Nigeria. 309P
30. Souza, L.K.H; Oliveira, C. M. A; Ferri, P.H; Santos, S.C; Oliveira, J.G. Miranda, A.T.B; Liao, L.M. and Silver, M.R.R.(2002). Antifungal properties of Brazilian cerrado plants. Braz. J. Microbiology. 33(3) 121-124.
31. Subhisha, S. and Subramoniam, A. (2005). Steroid screened from Pallavicinia Iyelli against four test organisms. Indian Journal of Pharmaceutics. 37(5) 304-208

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