EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF AQUEOUS WHEATGRASS EXTRACT FOR FISH FEED FORMULATION

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ABSTRACT

The wheatgrass extract is well known for its medicinal properties. The present investigation was proposed to screen the phytochemical constituents and to study the antioxidant properties of aqueous extract of wheatgrass for proposed fish feed formulation. The phytochemical screening of the aqueous extract of wheatgrass showed the presence of various secondary metabolites but the absence of Quinone, Sterol and Steroids in general. As well wheatgrass was proved to be an effective in all the antioxidant assays. From the results obtained, it can be concluded that wheatgrass aqueous extract contains various bioactive compounds. It is potential source of natural antioxidants which indicated its probable efficacy in fish feed formulation.

Keywords: *Antioxidant, Aqueous extract, Phytochemical, Triticum aestivum, Wheatgrass*

Introduction

Plants are the good source of phenolic and polyphenolic compounds having potent antioxidant activities which can be exploited in preparation of food and pharmaceutical product (Li *et al.,* 2009). Being a rich source of secondary metabolites, medicinal plants are used by native people from ancient time and play a pivotal role as therapeutic and lifesaving drugs (Bewaji *et al.,* 1985). Herbal medicines are adventitious over synthetic medicines as they have varied properties and most of the world population is dependent on it (Olagunjua *et al.,* 2009). Use of phytomedicine is gaining importance as they have the capability to treat many diseases without any side effects and used in preparation of creams, decoctions, syrups and infusions.

Wheatgrass (*Triticum aestivum*) is the most commonly cultivated crop and herb in India. It refers to young grass of the common wheat plant, which belongs to Poaceae family (Padalia *et al.,* 2010; Mujoriya and Bodla, 2011). Wheatgrass culms are simple, hollow or pithy, glabrous and the leaves are approximately 1.2 m tall, flat, narrow, 20-38 cm long and 1.3 cm broad. The spikes are long, slender, dorsally compressed and somewhat flattened. Wheatgrass is a rich source of many minerals, vitamins and protein (Marawaha *et al.,* 2004; Mukhopadhyay *et al.,* 2009). Plant has been known to have antiinflammatory, antioxidant, anticarcinogenic, immunomodulatory, antidiabetic, laxative, astringent, diuretic, antibacterial, antihemolytic and antiaging properties as well improve the reproductive health (Kothari *et al.,* 2008; Alitheen *et al.,* 2012; Shakya *et al.,*2012). Its use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling has been shown to be most beneficial to human being (Kumar *et al.,* 2016; Sharma *et al.,* 2016; Islam *et al.,* 2017; Makode *et al.,* 2018, Uraiwan *et al.,* 2019).

Though, traditionally wheatgrass has been used for various disorders, but till date not much scientific literature is available on wheatgrass application in aquaculture. It clearly signifies that ample of scientific exploration need to be done on this herb which could give it lead as cheap natural additive in fish feed formulation. In this view, the present investigation deals with phytochemical screening and analysis of antioxidant potential of aqueous wheat grass extract. The obtained information would provide the scientific basis to justify its probable utility in fish feed formulation.

Materials and methods

Sample preparation: For the experiment, wheatgrass from conventional organic farm was used. A crop of mature wheatgrass (8 inches) trimmed to 1/2-inch above the soil. It took 6 to 9 days for wheatgrass to be mature. The harvested grass blades lay on a clean baking sheet. The oven temperature was set to 150^{O} F and inserted the baking sheet. The blades were allowed to dry out in oven. After two hours onward, the blades were dried and brittle. The wheatgrass grinded in a clean grinder once the blades of grass were dry and brittle. The developed powder was stored in dry airtight container for the experimental use. The 30 gm of dried powder was extracted with 300 ml water using Soxholet apparatus for 24 hrs. The aqueous extract was lyophilized and stored in 4^{O} C.

Phytochemical analysis: Standard protocols were use for the phytochemical analysis. Phytochemical screening for the presence of major types of compounds in the extract was done by Harbone (1973). Total polyphenol content of prepared aqueous extract was analyzed using Folin's Ciocalteau reagent according to the protocol designed by Singleton *et al.* (1965). The total flavonoid content was estimated by the method developed by Jia *et al.* (1999).

Antioxidants Analysis: Standard protocols were use for the antioxidant analysis. DPPH Free Radical Scavenging Activity was determined by method of Esmaeili and Sonboli (2010). Hydrogen Peroxide Scavenging Activity was determined by the method of Rosen and Rauckman (1984). Hydroxyl Radical Scavenging Activity was determined according to the method described by Klein *et al*. (1981). Metal Chelating Activity was estimated by the protocol described by Chan *et al*. (2007). Nitric Oxide Radical Scavenging Activities of extract was examined by Royer *et al.* (2011). Superoxide Dismutase Assay was done by the method of Kakkar *et al.* (1984). Ferric Reducing Power Assay was determined according to Benzie and Strain (1996). Total antioxidant activity of the wheatgrass extracts was evaluated with procedure previously reported by Prieto *et al.* (1999).

Statistical Analysis: Data were collected, organized and analyzed by using Microsoft Office Excel, 2007. Results were recorded as Mean ± Standard Deviation and Coefficient of Variation of triplicate.

Results

The phytochemical screening of the aqueous extract of wheatgrass showed the presence of various secondary metabolites as well proved to be an effective in all antioxidant assays. The phytochemical potential of aqueous wheatgrass extract is represented in Table 1. It shows the presence of Alkaloids, Amino Acid and Protein, Carbohydrate, Cardioglycoside, Coumarin, Flavonoids, Phenols, Saponins, Tannins and Terpenoids but Quinone, Sterol and Steroids were absent in aqueous extract. The Total Phenolic content was found to be (219.27±2.47) µmol of GAE/g equivalent of wheatgrass. The Total Flavonoid content was found to be (167.93±2.69) µmol of quercetin equivalent of wheatgrass.

Table 1: Phytochemicals studies of aqueous extracts of wheatgrass.

Particulars	Aqueous extract	Particulars	Aqueous extract
Alkaloids	++	Phenols	+++
Amino acid and protein	++	Saponins	+
Carbohydrate	+	Tannins	++
Cardioglycoside	++	Terpenoids	++
Coumarin	++	Quinone	_
Flavonoids	+++	Sterol and steroids	_

+: Low concentration; ++: Moderate concentration; +++: High concentration; -Absent

Antioxidant potential of aqueous extracts of wheatgrass was observed as shown in Table 2. The result determined by DPPH Free Radical Scavenging Assay, Hydrogen Peroxide Scavenging Activity, Hydroxyl Radical Scavenging Activity, Metal Chelating Activity, Nitric Oxide Radical Scavenging Activity, Superoxide Dismutase Assay**,** Ferric Reducing Power Assay and Total Antioxidant Activity. The obtained results cleared that the antioxidant activities of aqueous wheatgrass extract increases with increased in concentration.

The Coefficient of Variation (CV) is defined as the ratio of the Standard Deviation to the Mean; it shows the extent of variability in relation to the Mean of the Result**.** The obtained results also showed that the highest value of CV of DPPH Free Radical Scavenging Assay (0.04) was found for 0.2 mg/ml concentration while the lowest (0.01) was found for 1.0 mg/ml concentration. The highest value of CV of Hydrogen Peroxide Scavenging Activity (0.03) was found for 1.0 mg/ml concentration while the lowest (0.01) was found for 0.2 mg/ml concentration. The value of CV of Hydroxyl Radical Scavenging Activity for all the studied concentrations was similar (0.01). The highest value of CV of Metal Chelating Activity (0.04) was found for 0.2 mg/ml concentration while the lowest (0.01) was found for 1.0 mg/ml concentration. The highest value of CV of Nitric Oxide Radical Scavenging Activity (0.03) was found for 0.2 mg/ml concentration while the lowest (0.01) was found for 0.4 mg/ml concentration. The highest value of CV of Superoxide Dismutase Assay (0.03) was found for 0.2 mg/ml concentration while the lowest (0.01) was found for 0.6 mg/ml concentration. The highest value of CV of Ferric Reducing Power Assay (0.03) was found for 0.8 mg/ml concentration while the lowest (0.01) was found for 0.6 mg/ml concentration. The highest value of CV of Total Antioxidant Activity (0.04) was found for 0.4 mg/ml concentration while the lowest (0.01) was found for 1.0 mg/ml concentration.

Table 2: Antioxidant potential of aqueous extracts of wheatgrass

Antioxidant Activity	**Concentration (mg/ml)**					
		0.2	**0.4**	**0.6**	**0.8**	**1.0**
DPPH Free Radical Scavenging Assay (Inhibition %)	Mean	18.886	21.084	24.214	25.678	27.830
	±SD	0.730	0.633	0.485	0.569	0.276
	CV	0.04	0.03	0.02	0.02	0.01
Hydrogen Peroxide Scavenging Activity (Inhibition %)	Mean	35.030	42.316	48.861	57.320	61.548
	±SD	0.406	0.729	0.753	0.863	2.115
	CV	0.01	0.02	0.02	0.02	0.03
Hydroxyl Radical Scavenging Activity (Inhibition %)	Mean	63.546	72.660	81.653	89.785	94.641
	±SD	0.507	0.557	0.634	0.623	0.521
	CV	0.01	0.01	0.01	0.01	0.01
Metal Chelating Activity (Inhibition %)	Mean	19.263	24.510	42.146	49.150	55.796
	±SD	0.773	0.480	0.818	0.851	0.296
	CV	0.04	0.02	0.02	0.02	0.01
Nitric Oxide Radical Scavenging Activity (Inhibition %)	Mean	39.422	49.693	54.579	58.755	60.654
	±SD	0.986	0.354	0.843	0.889	0.969
	CV	0.03	0.01	0.02	0.02	0.02
Superoxide Dismutase Assay (Inhibition %)	Mean	20.854	36.155	37.538	39.710	40.553
	±SD	0.602	0.746	0.434	0.832	0.983
	CV	0.03	0.02	0.01	0.02	0.02
Ferric Reducing Power Assay (OD at 700 NM)	Mean	0.829	1.930	2.378	4.414	3.892
	±SD	0.013	0.035	0.019	0.127	0.082
	CV	0.02	0.02	0.01	0.03	0.02
Total Antioxidant Activity (OD at 695 NM)	Mean	0.195	0.252	0.339	0.426	0.545
	±SD	0.004	0.009	0.006	0.009	0.005
	\CV	0.02	0.04	0.02	0.02	0.01

Discussion

The herbs with medicinal potential can be a source of many phytochemical compounds mainly polyphenols having biological activity (Nowak *et al.,* 2016). Such herbs when supplemented in diet of fish, it can influence the overall productive performance of the fish by increasing the growth performance, improving nutrient profile, immunity and breeding. The farmed fish are continuously exposed to the antigenic pressure by microbial and environmental agents, which may lead to a condition of chronic inflammation. Recently, a positive correlation has been established between dietary supplementation with antioxidants and the reduction of detrimental effects such as reduced growth rates, alterations in the physical condition, health status and the activation of stress responses in fish under stocking density (Bhosale, 2010; Guz *et al.,* 2011).

In view of the notion, present experiment was aimed to screen the phytochemical constituents and to study the antioxidant properties of aqueous extract of wheatgrass. The aqueous extract of wheatgrass was screened for the presence of bioactive compounds. The results showed the presence of Alkaloids, Amino Acid and Protein, Carbohydrate, Cardioglycoside, Coumarin, Flavonoids, Phenols, Saponins, Tannins And Terpenoids But Quinone, Sterol And Steroids were absent in aqueous extract (Table 1). Aqueous extract of wheatgrass was evaluated quantitatively for the percentage composition of Total Phenolic and Flavonoids. The Total Phenolic content was found to be (219.27 +2.47) µmol of GAE/g equivalent of wheatgrass. The Total Flavonoid content was found to be (167.93+2.69) µmol of quercetin equivalent of wheatgrass. According to Joan *et al* (2012) and Suriyavathana *et al.,* (2016), the presence of very high concentration of Flavonoids and Phenols indicates rich antioxidant property of extract. Magrone *et al.,* (2016) and Joshi and Gulhane (2017) suggested that presence of theses secondary metabolites assist to enrich the medicinal properties of the plant and indicate its suitability as additive to fish feed.

In the present investigation, antioxidant potential of aqueous wheatgrass extract was determined by DPPH Free Radical Scavenging Assay, Hydrogen Peroxide Scavenging Activity, Hydroxyl Radical Scavenging Activity, Metal Chelating Activity, Nitric Oxide Radical Scavenging Activity, Superoxide Dismutase Assay**,** Ferric Reducing Power Assay and Total Antioxidant Activity (Table 2). The obtained results cleared that the antioxidant activities of aqueous wheatgrass extract increase with increase in concentration. Elham *et al.* (2016), Islam *et al.,* (2017) Makode *et al.,* (2018) and Uraiwan *et al.,* (2019) had suggested that the herbs with rich bioactive phytochemical content and high antioxidant potential can be the effective additive in fish feed formulation.

Conclusion

The obtained results of the present study indicate that aqueous wheatgrass extract is rich with active phytochemical compounds and having the strong antioxidant potential. These properties of the wheatgrass supported its possible use as additive in formulated feed by which fish can grow rapidly and attains maximum weight in shortest possible time.

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