Phycochemical Analysis and Evaluation of Antifungal and Antioxidant Activities of *Rhizoclonium hieroglyphicum*

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ABSTRACT

A freshwater filamentous green alga *Rhizoclonium hieroglyphicum* was evaluated for phycochemical analysis, antifungal and antioxidant activities. Phytochemical screening reveals the presence of alkaloids, reducing sugar, tannins and terpenoids. The GC-MS analysis of sample revealed that fatty acids contents were present in *R. hieroglyphicum* done by Folch method. 9-Hexadecanoic acid was found to be the major component (46.57%). Other bioactive compounds including hydrocarbons, fatty acids and esters are also extracted from sample extract. Antifungal activity was done against three different pathogenic fungal strains (*Tricoderma harzianum, Pencillium notatum* and *Aspergillus niger*. The results indicate that the different organic solvent extracts of *R. hieroglyphicum* are an essential source of antifungal compounds that may provide various useful antifungal drugs. Antioxidant activity of the *R. hieroglyphicum* was estimated performing four antioxidant assays which are total phenolic content quantification (TPC), total antioxidant activity (TAA), ferric reducing antioxidant power assay (FRAP), and DPPH radicle scavenging action (DPPH). The findings of present study indicated that methanol and chloroform extract of *R. hieroglyphicum* showed the potential antioxidant properties. The presence of some of these bioactive constituents in the algal extract may provide the scientific evidences for many medicinal effects of this algae.

Keywords: Antifungal, Antioxidant, Fatty Acids, Fungal Pathogens, *R. hieroglyphicum.*

INTRODUCTION

Rhizoclonium hieroglyphicum, is edible algae and maximum polysaccharides, it exhibits maximum growth during the dry season (November-March), when the temperature and velocity of water are low. Some taxonomists described some species of *Rhizoclonium*, such as Kützing, 1843a; Blair, 1983 etc. About 7 freshwater species of *Rhizoclonium* species have been reported. *Rhizoclonium* possess unbranched filamentous. The cells have a single reticulated chloroplast with a number of pyrenoids, depending on the cell's age and size. The filaments are normally attached through rhizoids to soft or hard substrates or to other algae (Nienhuis, 1974). *R. hieroglyphicum*, is edible algae and rich in cell wall polysaccharides. During the dry season (November-March), it exhibits maximum growth. Many edible algae have been shown to have important nutritional and health benefits. It had proven to have a high nutritional value (containing protein and amino acids) and health benefits, including antifungal and antioxidant properties stemming from its fatty acids, phycocyanin, phycobiliprotein, and phenolics. It shows anti-inflammatory, analgesic, anti-gastric ulcer and antioxidant activities, offering the potent to play an essential role in pharmacological activities. A number of different fatty acids (saturated and unsaturated), sterols, sugars and terpenes have been isolated from them (Harvey, 1936; Jensen, 2003). Owing to their broad spectrum of antioxidant activities, these compounds have been recognized as having protective effects against many disease conditions, including cardiovascular diseases, atherosclerosis, diabetes, cancer, aging, and other degenerative diseases (Li *et al*., 2007; Cai *et al*., 2004).

OBJECTIVES

- To evaluate its phycochemical properties by GC-MS technique.
- To quantify the antifungal and antioxidant activity of *R. hieroglyphicum*.

METHODOLOGY

The algal collection was made from water bodies of Zoological Garden Lahore. Solvents used in extraction process included polar and non-polar solvents i.e. methanol, chloroform, *n*-hexane, and distilled water. On the basis of increasing polarity order, Algal sample was immersed in solvents successively.

Phytochemical Screening of *R. hieroglyphicum*

Phytochemical analysis was done for reducing sugar, tannins, alkaloids, tennins, and terpenoids using standard procedures according to Prabha *et al*., 2017.

Determination of Phycochemicals by Folch Method

Algal Extraction was done by using Folch method. It is extensively used method for better extraction (Folch *et al*., 1957).

Evaluation of Antifungal Activity

Antifungal activity is determined by agar well diffusion method against pathogenic fungal strains; *Tricoderma harzianum, Pencillium notatum* and *Aspergillus niger*.

Evaluation of Antioxidant Activity

Antioxidant potential of *R. hieroglyphicum* was determined by using following mentioned methods:

- Total phenolic content quantification (TPC)
- Total antioxidant activity (TAA)
- Ferric reducing antioxidant power assay (FRAP)
- DPPH radicle scavenging action (DPPH)

RESULTS AND DISCUSSION

Phytochemical Screening of *R. hieroglyphicum*

Qualitative phytochemical analysis of *Rhizoclonium hieroglyphicum* shows that different extracts of it contains reducing sugar, terpenoids, alkaloids, tannins and anthraquinones which are essential for different physiological functions in algae. F or better growth of plants, it can be used as fertilizer because of presence of important secondary metabolites.

GC-MS Analysis of Crude Extract from *R. hieroglyphicum*

GC-MS analysis of *R. hieroglyphicum* was done by Folch method. Nine phycochemicals were identified. These compounds comprise hydrocarbon, fatty acids and fatty acid esters. 9-Hexadecanoic acid is present in high concentration with peak area 46.57% constituting the bulk of oil followed by benzene (15.56%). The other compounds identified include 9,12-Octadecadienoic acid (13.41%), Hexadecanoic acid (5.55%), 9-Octadecenoic acid (5.13%), Octane (4.51%), Ethylbenzene (3.76%), 1,2-Benzenedicarboxylic acid (3.12%) and 3-Trifluoroacetoxydodecane (2.39%). The GC-MS analysis of sample revealed that low fatty acids contents were present in sample done by Folch method.

Determination of Antifungal Activity

In the present investigation, it seems that the *n*-hexane extract of *R. hieroglyphicum* expressed with maximum zone of inhibition almost in all concentration used against *Tricoderma harzianum* strain. The chloroform extract showed maximum zone of inhibition against *Aspergillius niger* in 1/100 g/ml. Methanolic extract showed maximum zone of inhibition against *Pencillium notatum* in 1/100 g/ml.

Table 1. Antifungal activity of *Rhizoclonium hieroglyphicum* with different test organisms.

Test sample	Concentration g/ml	Diameter of zone of inhibition(mm)		
		Th	***Pn***	***An***
Methanol	1/10	5±0.8	7±0.6	7±0.5
	1/100	8±0.5	12±0.5	-
	1/1000	8±0.8	10±0.5	-
Chloroform	1/10	5±0.5	7±0.8	13±0.8
	1/100	8±0.4	9±0.5	15±0.8
	1/1000	3±0.8	6±0.3	8±0.3
***n*-hexane**	1/10	16±0.3	-	-
	1/100	17±0.6	-	-
	1/1000	13±0.6	-	-

Th= Trichoderma harzianum, Pn= Pencillium notatum, An=Aspergillus niger.

Determination of Antifungal Activity

Total Phenolic Content Quantification (TPC)

Total phenolic contents of *R. hieroglyphicum* extracted using different solvents and conditions are presented in table 2. The chloroform extract has exhibited significantly higher phenolic content (25.22 µg GAE/mg) compared to the other solvent extracts. The lowest phenolic content was registered with the methanolic extract (15.05 µg GAE/mg).

Table 2. Total phenolic content (µg GAE per mg extract) of algae in different solvents.

Algal extract	Methanol	Chloroform	*n*-Hexane	Aq. extract
R. hieroglyphicum	15.05 ± 0.87^{a}	25.22 ± 0.33^{c}	17.0 ± 0.67^{a}	21.33 ± 0.16^{b}

Data shown are mean ± standard error (SD) of three replicates. $^{a, b}$ and ^{c} are statistical comparison between groups using ANOVA post hoc Tukey's b Test ($p<0.05$).

Total Antioxidant Activity (TAA)

Total antioxidant activity of algal sample was determination by phosphomolybdenum complex formation method. The methanolic extract exhibited the significantly highest value (13.94 µg AscAE/ mg). The lowest activity was registered by aqueous extract.

Table 3. Total antioxidant activity (µg AscAE per mg extract) of algae in different solvents.

Algal extract	Methanol	Chloroform	*n*-Hexane	Aq. extract
R. hieroglyphicum	13.94 ± 0.67^{c}	9.87 ± 0.23^{a}	10.93 ± 0.23^{b}	9.3 ± 0.07^{a}

Ferric Reducing Antioxidant Power assay (FRAP)

Ferric reducing antioxidant power of *R. hieroglyphicum* extracts is presented in Table 5. The highest ferric reducing antioxidant power was noted with the *n*- Hexane extract (257.83 µM Trolox per mg extract). The lowest activity was observed with aqueous extract.

Table 4. Ferric reducing antioxidant power assay (µM Trolox per mg extract) of algae in different solvents.

Algal extract	Methanol	Chloroform	*n*-Hexane	Aq. extract
R. hieroglyphicum	234.17±0.86^{c}	174.33±0.92^{b}	257.83±0.72^{d}	114.67±0.88^{a}

DPPH radicle scavenging action

The radicle scavenging activity of different extracts is illustrated in table no. 6. It is clearly observed that the chloroform extract of this algal sample has exhibited highest % of RSA (77.6 %) followed by n- Hexane extract (49.6%) and methanolic extract (29.8%). The lowest inhibition was found in aqueous extract (23.7%).

Table 5. Relative radical scavenging activity (RSA) of algae by the use of stable DPPH radical expressed as % inhibition.

Algal extract	Methanol	Chloroform	*n*-Hexane	Aq. extract
R. hieroglyphicum	29.8±0.29a	77.6±0.04c	49.6±0.66d	23.7±0.14b

ACKNOWLEDGEMENT

In the light of previous findings, it can be concluded that *R. hieroglyphicum* contains alkaloids, terpenoids and phenolic compounds that exhibits high antioxidant potential activities and has high radicle scavenging activity. It possesses antifungal activity and was potent source of active compounds against different pathogens and can be used as natural non-toxic preservative and may be more acceptable to consumers. Green algae have potential to return pharmaceutically useful, which can be harnessed for the drug development.

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