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Antioxidant activity of mangrove-derived marine thraustochytrids

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Abstract

Antioxidants are compounds that inhibit the reaction caused by free radicals thereby preventing or delaying damage to the cells and tissues. Protection against free radicals can be enhanced by taking sufficient amounts of exogenous antioxidants. The present study evaluated the antioxidant activity of the mangrove-derived thraustochytrids for their potential health benefits. Thirteen thraustochytrid strains were isolated from mangrove leaf litter, extracted them separately in methanol and tested for their antioxidant activity by using six different assays: DPPH radical scavenging, total phenol content, hydrogen peroxide radical inhibition, nitric oxide radical assay and total reducing power activity. The antioxidant activity was found significant between the thraustochytrid strains and between concentrations of their extracts ($P< 0.05$). The activity also increased with concentration of the extracts. The strain TSKK1 exhibited highest (78.95±1.29) antioxidant activity and TSSK5 the lowest antioxidant activity (72.52±2.03). This study proved the potent free radical scavenging and antioxidant activity of thraustochytrids.

Keywords – DPPH - free radicals – leaf litter - nitric oxide – total phenol

Introduction

Antioxidants play an important role to protect the human body against oxidative damage caused by free radicals. An antioxidant is a molecule, capable of inhibiting other molecular oxidation. Oxidative free radicals are highly reactive to attack molecules by capturing electrons and thus modifying chemical structures. Free radicals are harmful substances, produced during the normal metabolic process. The free radicals are generated through the oxidation of carbohydrates, fats and proteins through both aerobic and anaerobic processes. Over-production of the free radicals is responsible for tissue injury (Gomathi et al. 2013a). Cell membranes are made up of unsaturated lipids which are particularly susceptible to free radicals, which will breakdown or even harden the lipids of cell membrane. This interferes with the cells to get proper nutrients or intercellular signals and eventually leads to cell death (Khansari et al. 2009, Halliwell et al. 1987). In a normal healthy individual, there should be a well- maintained balance between free radical production and antioxidant defense mechanisms. However, in a diseased state, this balance shifts towards the overproduction of free radicals or deficit in antioxidant defense mechanisms and may lead to oxidative stress.

In the human body, there are various enzyme systems for free radical scavenging, but micronutrients such as vitamin E, beta-carotene and vitamin C are the major antioxidants. These must be provided in diet as the body cannot produce these nutrients. Protection against free radicals

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can be enhanced by taking sufficient amounts of exogenous antioxidants (Halliwell 1995). Lipid oxidation is one of the major causes for deterioration of many food products. It leads to changes in texture, flavor, odor and quality of foods. The lipid oxidation also causes some health hazards in human beings such as cardiovascular disease, cancers and neurological disorders as well as aging process (Gulcin 2011, 2012). Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipid or DNA and can initiate degenerative diseases. Reactive radicals play a very important role in signal transduction (Lee et al. 2004). However, excess free radicals can give rise to some diseases. Lipid peroxidation during processing and storage of foods can cause unacceptable flavor and taste, decrease consumer acceptability for foods. Numerous numbers of both natural and synthetic antioxidants are suggested for various human diseases (Cuzzocrea 2001). Some man-made antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole and tertiary butylhydroquinone are commonly used in processed foods. However, synthetic antioxidants have shown potential health risks and toxicity, most notably possible carcinogenicity. Therefore, it is of great importance to find new sources of safe and inexpensive antioxidants of natural origin in order to use them in foods and pharmaceutical preparations to replace synthetic antioxidants (Lee et al. 2004, Song 2010).

Mangrove-derived fungi and fungal-like organisms have been recognized as a repository of novel secondary metabolites, some of which have beneficial biological activities and recently numerous novel bioactive substances have been isolated from these microorganisms (Wagenaar & Clardy 2001, Brady et al. 2001, Shrestha et al. 2001, Kongsaeree et al. 2003, Sridhar 2004, Kathiresan & Qasim 2005, Kim 2013, Saravanakumar & Kathiresan 2014). There are however, only limited studies on antioxidant potential of thraustochytrids. Therefore, in the present study the antioxidant activity of the mangrove-derived thraustochytrids was evaluated for their potential health benefits.

Material and Methods

Microbial culture and extraction

Pure strains of *Thraustochytrids* isolated from decaying mangrove leaf litter in our laboratory were used for the present study. Fresh cultures of 13 thraustochytrids (TSKK1, TSKK2, TSKK3, TSKK4, TSKK5, TSKK6, TSKK7, TSKK8, TSKK9, TSKK10, TSKK11, TSKK12 and TSKK13) were cultured separately in one ml of medium composed of (50% seawater) glucose (10 $g.l^{-1}$), yeast (10 $g.l^{-1}$), peptone (1 $g.l^{-1}$), thiamine (0.5 $g.l^{-1}$) in 50% seawater, and then inoculated to 100 ml of the medium, maintained at different pH, temperature, salinity and source of carbon and nitrogen for 7 days (Gomathi 2009, 2011). Then biomass was air-dried and extracted in 80% methanol. The extract was filtered through Whatman No.1 filter paper, and the filtrate was evaporated to dryness under vacuum at 40^{o}C (Gomathi et al. 2013b).

Antioxidant assays

Based on the antimicrobial activity in the crude extracts, five potent thraustochytrids– (TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11) were selected for the antioxidant activity (Data not shown). The antioxidant activity of the extracts, prepared in different concentrations 50, 100, 200, 250, 500 $\mu g.ml^{-1}$ was tested for antioxidant property by using standard assay methods: Determination of total phenol content (Singleton et al. 1999), DPPH radical scavenging assay (Duan et al. 2006), Total antioxidant activity (Prieto et al. 1999), Measurement of reducing power (Oyaizu 1986), Nitric oxide radical inhibition assay (Govindarajan et al. 2003, Badami et al. 2005) and Hydrogen peroxide radical inhibition assay (Govindarajan et al. 2003, Gulcin et al. 2004). Each experiment was done in triplicate and mean values were taken.

The scavenging activity was calculated by using the following formula and is expressed in per cent.

% activity = [(control absorbance (Ac) - extract absorbance (As) / (control absorbance (Ac))] ×100

Where Ac = control, and As = sample

Statistical analysis

Statistical analysis was performed by using the SPSS (version-16). All *in vitro* results were calculated as mean ± SD and were analyzed by one-way analysis of ANOVA followed by Duncan's multiple range test. The P values less than 0.05 were considered statistically significant.

Results

Total antioxidant assay

The total antioxidant activity was significant between five thraustochytrids (TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11) and also between the concentrations of their extracts ($P<0.05$). The antioxidant activity increased with increasing concentrations of the extracts of thraustochytrids. The extract of the TSKK1 exhibited the highest (78.95±1.29) antioxidant activity and TSSK5 showed the lowest activity (72.52±2.03) (Table 1, Fig.1).

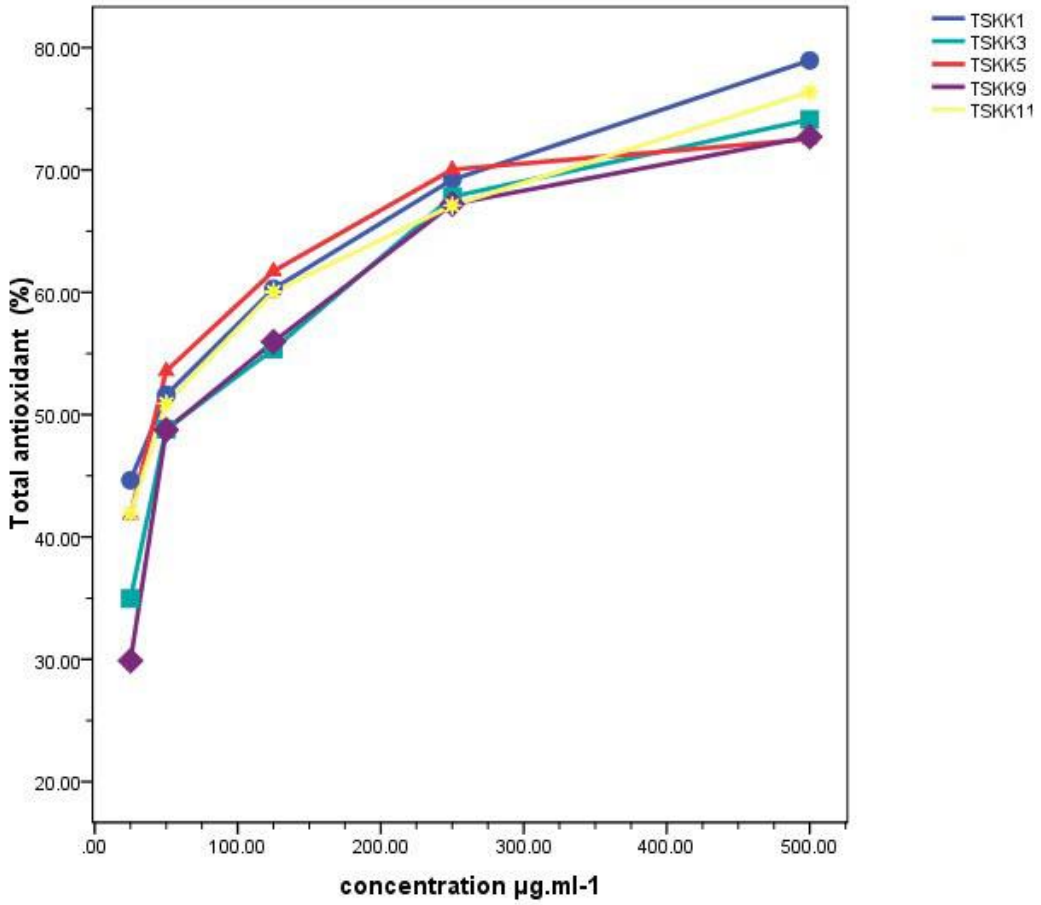

Fig. 1 – Total antioxidant (%) activity in extracts of mangrove-derived thraustochytrids, at different concentrations.

DPPH free-radicals scavenging assay

The DPPH Free-radical scavenging assay was significant between thraustochytrids and also between the concentrations of their extracts ($P< 0.05$) (Table 1). The decrease in absorbance of the radical was due to hydrogen donation. It was visually noticeable as the colour changed from purple to yellow. TSSK9 was found be the highest producer of free radical scavenging activity (81.20±3.01) as compared to other thraustochytrids extracts. TSSK1 was found to be the lowest in free radical scavenging activity (71.15±1. 90) (Table 1, Fig.2).

Table 1 Antioxidant activity in crude extracts of mangrove-derived thraustochytrids, at different concentrations.

Scavenging assay	Concentration µg/ml	% scavenging activity of different strains of thraustochytrids				
		TSKK1	TSKK3	TSKK5	TSKK9	TSKK11
Total phenol	25	39.20 ±0.83	28.82±2.09	41.77±1.36	42.79±2.34	31.86±1.46
	50	66.27±2.50	37.56±1.87	60.15±1.33	51.81±1.75	62.35±2.83
	125	75.41±2.00	43.85±1.66	63.04±2.60	57.38±1.58	69.64±0.80
	250	81.38±2.75	58.43±2.91	71.25±1.03	65.59±2.92	72.32±1.32
	500	87.15±2.25	68.84±2.79	76.54±1.39	75.26±2.39	80.20±1.49
Total antioxidant	25	44.63±2.05	34.96±1.86	41.80±2.24	29.88±1.81	41.93±2.34
	50	51.62±2.41	48.82±1.43	53.57±2.96	48.75±1.31	50.90±2.35
	125	60.29±1.45	55.35±2.05	61.73±1.85	55.95±2.46	60.04±2.87
	250	69.20±1.62	67.82±2.68	70.03±1.65	67.21±2.98	67.10±2.81
	500	78.95±1.29	74.12±2.15	72.52±2.03	72.71±2.23	76.37±1.42
DPPH radical scavenging activity	25	29.10±2.65	37.86±2.58	35.98±2.77	41.88±1.55	28.63±3.24
	50	43.13±2.73	46.52±2.87	41.30±2.77	51.26±2.37	47.22±2.61
	125	58.03±1.95	53.47±2.14	52.18±2.26	63.41±2.29	57.83±2.19
	250	62.10±2.66	66.01±2.88	69.44±2.76	73.21±2.43	63.42±2.36
	500	71.15±1.90	71.77±1.76	78.10±2.78	81.20±3.01	79.09±2.43
NO radical scavenging activity	25	38.30±2.81	43.46±2.70	42.47±2.17	31.46±1.51	34.92±2.42
	50	47.79±2.32	52.03±1.78	51.22±2.63	49.32±2.04	48.14±2.51
	125	54.02±2.75	60.37±1.97	62.18±1.64	57.01±1.65	58.26±1.53
	250	64.96±2.51	69.00±2.73	68.50±1.46	65.65±1.71	62.49±1.82
	500	75.28±2.92	72.90±2.47	74.77±2.40	71.33±2.89	73.44±1.68
H_2O_2 radical scavenging activity	25	33.79±2.06	40.31±2.71	31.22±2.96	42.16±1.19	36.20±2.46
	50	44.64±2.34	52.97±1.73	48.05±2.41	52.53±2.02	49.74±2.73
	125	49.48±2.63	61.06±1.70	55.38±2.43	60.66±2.10	57.68±2.30
	250	63.17±2.50	69.51±2.94	66.79±2.32	73.07±2.11	64.90±2.29
	500	70.88±2.92	75.30±1.84	76.24±2.55	81.27±2.49	77.03±1.19
Total reducing power	25	37.22±1.93	32.12±2.36	31.11±1.75	36.33±1.11	40.81±2.42
	50	41.61±2.10	44.78±2.86	42.95±2.71	42.94±2.50	49.23±2.75
	125	51.420±0.7	53.70±1.63	57.43±0.96	52.02±1.69	51.90±2.42
	250	59.86±2.45	61.08±1.31	65.48±2.52	63.47±2.12	64.98±2.57
	500	70.23±2.72	73.58±3.42	73.07±2.16	73.48±2.32	78.43±2.81

Values between concentrations or strains significant at 0.05% level

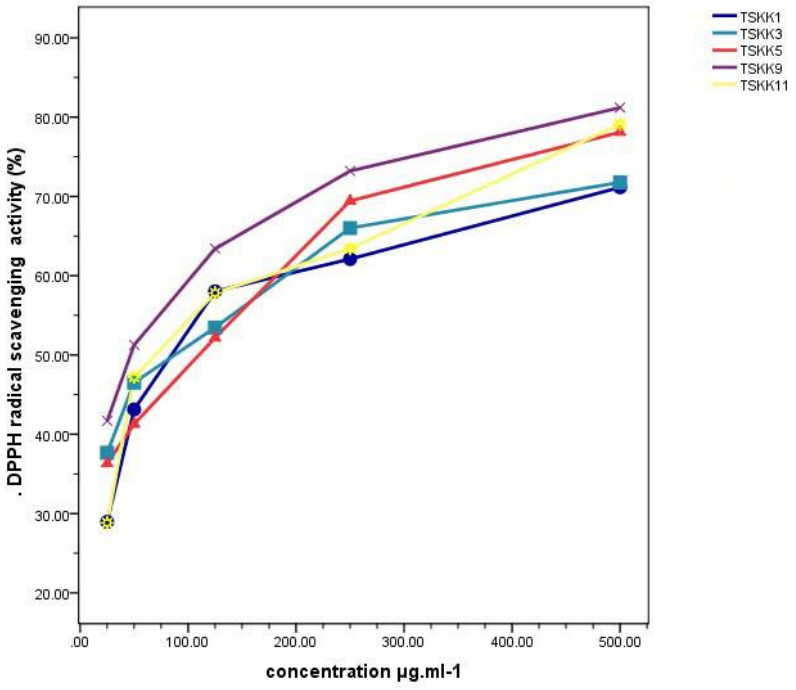

Fig. 2 – DPPH free-radicals scavenging (%) activity in extracts of mangrove-derived thraustochytrids, at different concentrations.

Total phenol assay

The total phenolic content was significant between five thraustochytrids (TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11) and also between concentrations of their extracts (P< 0.05) (Table 1). TSKK1 was found to be the highest in total phenol content (87.15±2.25) and TSSK3 was found to be the lowest (68.84±2.79) and TSKK11 (Table 1, Fig.3).

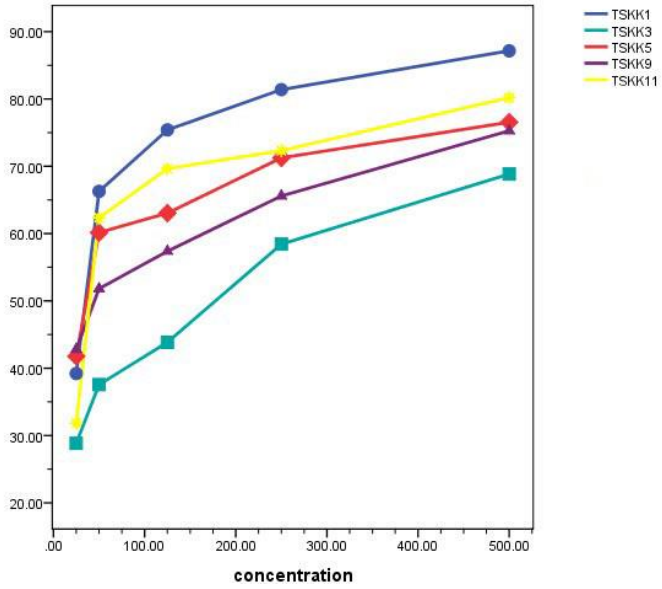

Fig. 3 – Total phenol (%) content in extracts of mangrove-derived thraustochytrids, at different concentrations.

Hydrogen peroxide radical inhibition assay

The hydrogen peroxide radical inhibition activity was significant between five thraustochytrids (TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11) and also between concentrations of their extracts (P< 0.05) (Table.1). Hydrogen peroxide radical scavenging potential of TSKK9 was found to be 81.27±2.49 µg/ml and this value was higher than that of other extracts (TSKK1, TSKK3, TSKK5 and TSKK11), whereas the TSSK1 was found be lowest in radical scavenging activity (70.88±2.92) (Table 1, Fig.4).

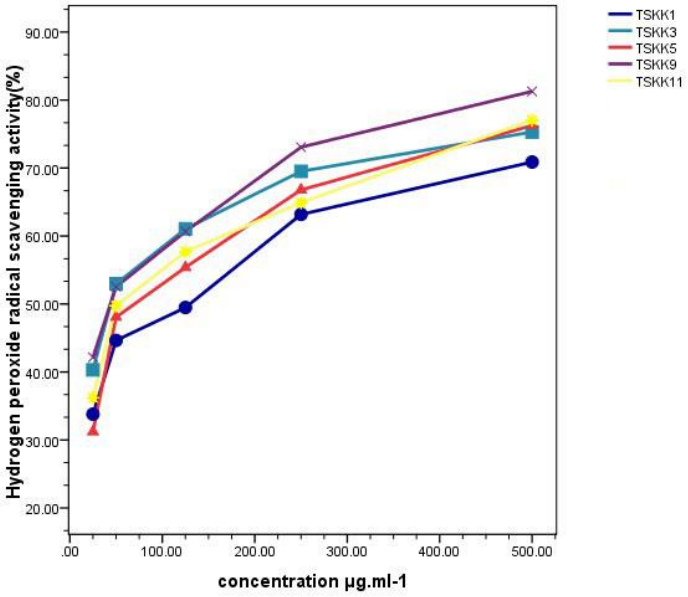

Fig. 4 – Hydrogen peroxide radical inhibition (%) activity in extracts of mangrove-derived thraustochytrids, at different concentrations.

Nitric oxide radical inhibition assay

The nitric oxide radical activity was significant between five thraustochytrids (TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11) and also between concentrations of their extracts (P< 0.05) (Table.1). TSKK1 was found be highest in nitric oxide radical content (75.28±2.92) and TSKK9 was the lowest (71.33±2.89) (Table 1, Fig.5).

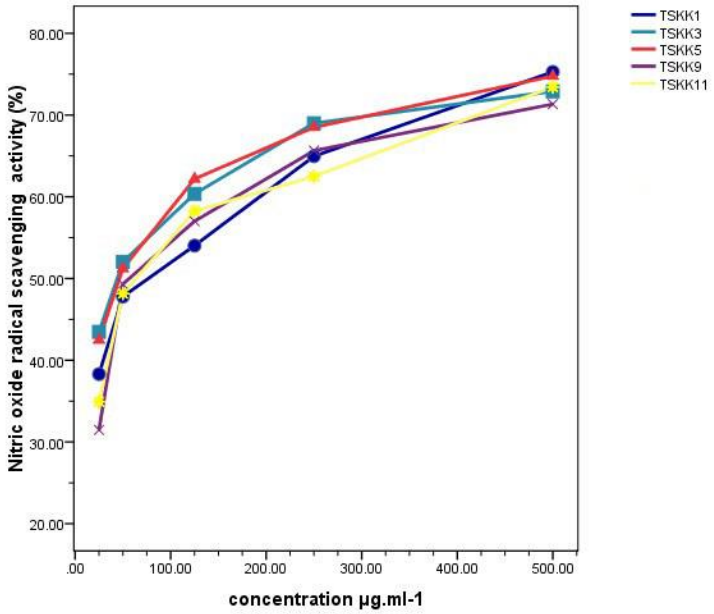

Fig. 5 – Nitric oxide radical inhibition (%) activity in extracts of mangrove-derived thraustochytrids, at different concentrations.

Total reducing power assay

The total reducing power was significant between five thraustochytrids (TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11) and also between concentrations of their extracts (P< 0.05) (Table.1). TSKK11 was found be highest in total reducing power (78.43±2.81) and TSKK9 was the lowest (70.23±2.72) (Table 1, Fig.6).

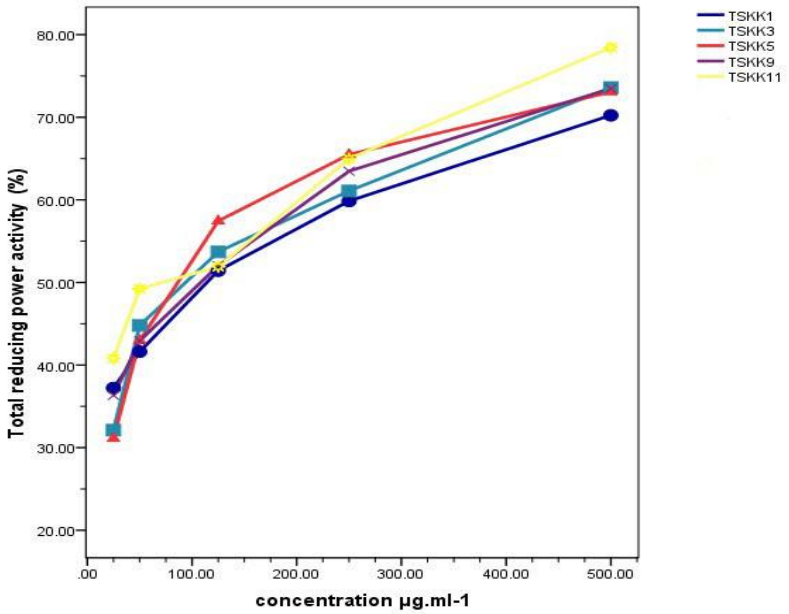

Fig. 6 – Total reducing power (%) activity in extracts of mangrove-derived thraustochytrids, at different concentrations.

Discussion

Marine thraustochytrids are becoming a potential source of polyunsaturated fatty acids (PUFA) (Gomathi 2011, Gomathi et al. 2013b). Docasahexaenoic acids and eicosapendaenoic acids are predominantly found in the marine thraustochytrids, which have recently attracted considerable research interest because of their diverse biological activities. The present study investigated the antioxidant activity of five marine thraustochytrids (TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11) isolated from mangrove decaying leaf litter. The antioxidant properties were evaluated by using six different assays.

The potent strains of *thraustochytrids* species - TSKK1, TSKK3, TSKK5, TSKK9 and TSKK11 showed significat antioxidant activity with increasing concentrations of extracts (Figs 1-6). This is in accordance with previous antioxidant studies in bacteria, fungi, plants (Lu & Foo 2000, Kim et al. 2002, Gomathi et al. 2013a, Saravanakumar & Kathiresan 2014). Antioxidant activity has also been reported in *Aplanochytrium* sp., (KGA2512) (Gomathi et al. 2013a) and endophytic fungal extract of *Trichoderma* (EMFCAS8) (Saravanakumar & Kathiresan 2014). The present study inferred that marine thraustochytrids were rich source of natural antioxidants (Table 1, Figs 1-6). In general, polyunsaturated fatty acid derivatives are reportedly responsible for such antioxidant activity in removal of toxic free radicals (Plaza et al. 2009). Omega-3 fatty acids are known for their important dietary roles as antioxidant and chemo-protective agents (Tlili et al. 2010). The omega-3 fatty acids are reportedly found commonly in plants, fungi, microalgae having several biological activities including antimicrobial, antioxidant, anti-apoptotic, anti-aging, anti-carcinogenic properties (Huang & Ebersole 2010, Guedes et al. 2011, Schmitz & Ecker 2008, Mozaffarian & Wu 2011).

Among different assays used, DPPH free radical scavenging assay is widely used as the most accurate screening method used to evaluate the antioxidant activity. The DPPH test provides information on the reactivity of test compounds with a stable free radical. DPPH is not affected by metals and enzyme inhibition. Because of its odd electron, 2, 2- diphenyl-picryl-hydrazyl radical (DPPH) gives a strong absorption band at 517 nm in visible spectroscopy (Brand-Williams 1995). The electron becomes paired off in the presence of a free radical scavenger, the absorption varnishes, thus the resulting decolourization is stoichiometric with respect to the number of electrons taken up. The scavenging properties of antioxidants are often associated with their ability to form stable radicals. The present study observed a considerable DPPH radical scavenging activity with thraustochytrids, in particular, TSKK9 which showed maximum activity (81.20±3.01). This can be attributed to the abundant presence of omega-3 fatty acids in thraustochytrids as potential source for antioxidant and free-radical scavengers (Plaza et al. 2009, Gomathi et al. 2013a).

Natural polyphenols are important groups of metabolites which play a vital role in natural medicine in radical scavenging activity which increases with increasing concentration of polyphenols (Lu & Foo 2000, Kim et al. 2002, Saravanakumar & Kathiresan 2014). The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity (Meir et al. 1995). The presence of reducing compounds causes reduction of the Fe^{3+}/ferricyanide complex to ferrous ion (Fe^{2+}). In the reducing power assay, reducing ability of a compound depends on the electron donor and free radical quenching capacity (Singh & Rajini 2004). Reducing agents hinder lipid peroxidation as they donate a hydrogen atom and stop the chain reaction which causes membrane lipid damage (Xing 2005). There is a positive relationship between total phenol content and antioxidant activity (Lu & Foo 2000).

Hydrogen peroxide is a normal cellular metabolite that is continuously generated and maintained at low concentrations. It is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Miller et al. 1993). Hydrogen peroxide itself is not very reactive but sometimes it rapidly decomposes into oxygen and water producing hydroxyl

radicals (OH^-) that causes DNA damage (Halliwell 1991). The hydroxyl scavenging activity was found highly dependent on concentrations of the crude extracts of thraustochytrids (Table 1, Fig.4).

The total antioxidant capacity is used as an important screening tool for the identification of cancer risk patients and as a monitoring tool for the effect of drug treatments (Duan et al. 2006). Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxy nitrite, which can be decomposed to form hydroxyl radical. The toxicity of NO increases greatly when it reacts with super oxide radical, forming the highly reactive peroxy nitrite anion (NOOO-) which leads to serious toxic reactions with biomolecules, such as protein, lipids and nucleic acids (Moncada et al. 1991). In the present study, the thraustochytrid extracts inhibited nitrite formation by directly competing with oxygen in the reaction with NO (Table 1, Fig.5).

This study proved that thraustochytrid species have potent free radical scavenging and antioxidant activity. The antioxidant mediated mechanism of thraustochytrids is hypothesized as a contributing factor for enhanced enzyme activity and reduced lipid peroxides as the antioxidant properties which can be correlated with oxidative stress defense in different human diseases. Antioxidants present in the diet can delay lipid peroxidation by inhibiting the initiation or propagation phase of oxidizing chain reactions by scavenging free radicals. The compounds present in the crude extracts may enhance the potency of the antioxidative property by additive or synergistic positive activity, while other compounds may neutralize or inhibit the antioxidation (Kulkarni 1997). In this regard, further purification of the crude extracts of thraustochytrids and evaluation of antioxidative activity is necessary to discover the antioxidant compounds present in the thraustochytrids. Thraustochytrid biomass and thraustochytrid-derived antioxidant compounds will have a very wide range of potential applications, from animal feed in aquaculture to human nutrition and health products.

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