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PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL ACTIVITIES OF ETHYL ACETATE EXTRACT OF *SYRINGODIUM ISOETIFOLIUM*

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ABSTRACT

Syringodium isoetifolium belongs to the family of Cymodoceaceae and found throughout India. The present study has been carried out to evaluate the preliminary phytochemical screening and antioxidant activity of the ethyl acetate extract of *Syringodium isoetifolium*. The present investigation is to evaluate the antioxidant activities of ethyl acetate extract of whole plant of *S. isoetifolium* in different *invitro* methods. The antioxidant activity was evaluated by using DPPH scavenging assay, reducing power assay, Nitric oxide (NO) scavenging assay and ABTS cation decolorization assay. Further the study continued with the anticancer activity by MTT Assay. The viability of the cell decreases with the increasing concentration of the drug. So, the *invitro* study clearly indicates that *S. isoetifolium* has a strong antioxidant activity. This study revealed that ethyl acetate extract of whole plant of *S. isoetifolium* comprise effective potential source of natural antioxidant.

Keywords: *Syringodium isoetifolium*, ethyl acetate extract, antioxidant, anticancer, DPPH, ABTS

Introduction

Natural bioactive compounds especially from plant sources, including spices have been investigated for their characteristics and health effects. Plants are potential sources of natural bioactive compounds such as secondary metabolites and antioxidants. They absorb the sun light and produce high levels of oxygen and secondary metabolites by photosynthesis. Medicinal components produced are stored in plant leaves. Most of the secondary metabolites of herbs and spices are commercially important and find use in a number of pharmaceutical compounds. Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants [1]. They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer. Secondary metabolites are chemicals produced by plants; and their functions in growth, photosynthesis, reproduction and other primary processes are not known yet. Secondary chemicals are important in plant use by widely used especially in Asia. They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer. Secondary metabolites are chemicals produced by plants; and their functions in growth, photosynthesis, reproduction and other primary processes are not known yet. Secondary chemicals are important in plant use by widely used especially in Asia [2].

Syringodium isoetifolium is one of the major tropical seagrasses and belongs to the family Cymodoceaeceae. *Syringodium isoetifolium* is otherwise called as Noodle grasss. In both tropical and sub-tropical marine environment, the seagrass *S. isoetfolium* was found and in shallow intertidal areas it is rarely found. In Tamilnadu *S.isoetifolium* is commonly called as Oosi korai, Nool pasi, Neer pasi [3]. The phytocompounds present in the seagrass *S.isoetifolim* are flavonoids, terpenoids, alkaloids, carbohydrates, proteins, aminoacids, tannin, saponin, glycosides, sugars, coumarins, quinines, carboxylic acids, xanthoproteins and phytosteroids. Since it acts as a food source, the phytochemical study is essential. Some results shows that the *S.isoetifolium* has high phenol, flavonoid, tannin and vitamin E content [4].

Oxidative stress is considered to be substantial, if not crucial, in the initiation and development of many current conditions and diseases, including: inflammation, autoimmune diseases, cataract, cancer, parkinson's disease, arteriosclerosis and aging [5]. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process [6]. This theory is supported by increasing evidence suggesting/indicating that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower the risk of disease [7]. Antioxidants are substances that significantly delay or prevent the oxidation of an oxidizable substrate when present in low concentrations compared to the substrate [8]. Flavonoids are well known for their antioxidant activity. Antioxidants are specific compounds that protect human, animal and plant cells against the damaging effects of free radicals (reactive oxygen species, ROS). An imbalance between antioxidants and free radicals results in oxidative stress, will/may lead to cellular damage [9].

Breast cancer is the most common malignancy in women and the most life-threatening of the 100 distinct forms. Because of its influence on women, it is classified as a public health issue. Molecular research detailed its prognosis. Breast cancer accounts for over 14% of total

cancer fatalities. This field's research must overcome the psychological and economic challenges. Breast cancer causes many tumors to develop from the epithelial cells of the breast. Dr. Soule and colleagues at the Michigan Cancer Foundation first came up with the moniker for the cell line in 1973. At first, MCF-7 cell lines were obtained from a 69-year-old lady. Tamoxifen, an anti-estrogen, was shown in 1975 to reduce the development of MCF-7 cells, although this suppression may be restored by the hormone estrogen. This cell line provides an excellent model for the malignant development pathway. No other breast cell line can live for several months like the MCF-7 cell line. MCF-7 cancer cells are estrogen sensitive, with high amounts of ERα transcripts and low levels of ERβ [10]. Cell death is caused by two mechanisms: apoptosis and necrosis. The tumor-treating potency of naturally generated bioactive substances promotes apoptosis in tumor cells [11].

MATERIALS AND METHOD

Syringodium isoetifolium was gathered in Devipattinam, Ramanadhapuram District, as a sample on June 2019. The sample *Syringodium isoetifolium* was verified and authenticated in Plant anatomy Research Centre by Dr. P. Jeyaraman, PhD., Director, Retd Professor, Presidency College and also authenticated from the Regional Scientist, Botanical Survey of India, Coimbatore.

Extraction of *S. isoetifolium*

The sample seagrass *Syringodium isoetifolium* collected were washed with tap water. Then the seagrass was shadow dried and finally powdered in herbal grinder. The sample were homogenized and dried in order to reduce moisture content of the sample for more efficient extraction process. The powder was then soaked in n-Hexane to defat for 24 h. It was then soaked in ethyl acetate for 72 h to obtain ethyl acetate crude extract, which was concentrated using a rotatory evaporator at 40 °C.

PHYTOCHEMICAL SCREENING

The ethyl acetate extract of *S. isoetifolium* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973; Trease and Evans, 1983) [12].

TOTAL PHENOLIC CONTENT

The total phenolic content (TPC) of *S. isoetifolium* was determined using the method by Trease, G. E., & Evans, 1989) [13].

ESTIMATION OF FLAVANOID

A 1ml aliquot of each ethyl acetate extract of *S. isoetifolium* was mixed thoroughly with 1ml of 2% aluminium chloride and 0.5 ml of 33% acetic acid followed by the addition of 90% methanol and the content is thoroughly stirred and allowed to stand for 30 minutes (Delcour and de Varebeke, 1985). The absorbance was measured at 414 nm using a UV-Visible Spectrophotometer. Quercetin was used as a standard.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography of ethyl acetate extract of *S. isoetifolium* was performed using standard procedures [12]. The ethyl acetate extract of *S. isoetifolium* was placed carefully in precoated aluminum silica gel 60 F, Merck F 254 using a microcapillary tube. The spots were allowed to dry for few minutes and the TLC plate was placed in the solvent mixture, Toluene, acetone and Formic acid (6:6:1) or solvents of ethyl acetate-glacial acetic acid-formic acid-water (100:11:11:26 v/v/v/v). After drying, the TLC plates were observed under UV at 240nm and 360 nm in UV TLC viewer. The Rf value of the spots was calculated by using the standard formula,

$$\text{Retention factor Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

ABTS (2,2'-AZINO-BIS-3-ETHYL BENZTHIAZOLINE-6-SULPHONIC ACID) RADICAL SCAVENGING ASSAY

ABTS radical scavenging activity of ethyl acetate extract of *S. isoetifolium* was followed by Re *et al*., (1999) [14].

INHIBITION OF LIPID PEROXIDATION ACTIVITY

Lipid peroxidation induced by Fe^{2+} ascarbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al*., (1979) [15].

SUPEROXIDE RADICAL SCAVENGING ASSAY

This assay was based on the capacity of the ethyl acetate extract of *S. isoetifolium* inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of the riboflavin-light-NBT system (Tripathi and Pandey Ekta, 1999; Tripathi and Sharma, 1999) [16,17]

NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

Nitric oxide scavenging ability of ethyl acetate extract of *S. isoetifolium* was measured according to the method described by Olabinri *et al*., (2010) [18].

METAL CHELATING ACTIVITY

Metal chelating capacity of ethyl acetate extract of *S. isoetifolium* measured according to Iihami *et al*., (2003) [19].

PANCREATIC LIPASE INHIBITORY ACTIVITY

The lipase inhibition activity of ethyl acetate extract was determined as per the method proposed by Kim *et al*., (2010) [20].

MTT ASSAY

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-colored water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm [21].

RESULTS

PHYTOCHEMICAL SCREENING OF *S. isoetifolium*

The phytochemical screening of ethyl acetate extract of *S. isoetifolium* studied presently showed the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table -1).

Table-1. Phytochemical screening of ethyl acetate extract of *S. isoetifolium*

S/No.	Constituents	Ethyl acetate extract *S. isoetifolium*
1.	**Alkaloids-Dragendroffs reagent**	+
	Alkaloids-Mayers Test	
2.	**Flavonoids- Alkali reagents**	+
3.	**Tannin- $Fecl_3$ test**	+
4.	**Saponins- Frothing test**	+
5.	**Terpenoids - Nollers test**	+
6.	**Glycosides- Keller-Killiani Test**	-
7.	**Polyphenols-Ferrozine**	+
8.	**Anthocynin- Ammonia Test**	+

+ indicate positive result; -- Indicate negative result

TOTAL PHENOLIC AND FLAVONOID CONTENT OF ETHYL ACETATE EXTRACT OF *S. isoetifolium*

In this context, the preliminary experiments revealed that ethyl acetate extract was the best solvent for the extraction of phenolics from the leaves of *S. isoetifolium* at 60 ℃ for 60 min since it afforded a maximum yield of phenolics. The yields dry plant of *S. isoetifolium* extracts ranged from 92.53% (w/w). Therefore, the total phenolic and flavonoid contents were reported as catechin and rutin equivalents respectively (Table-2).

Table-2. Yield of phenolic and flavonoid content ethyl acetate extract *S. isoetifolium*

Sample	**Yield of extract (g/100 g of defatted Content)**	**Total phenolic content (mg catechin equivalents per gram ethyl acetate extract)**	**Total flavonoid content (mg catechin equivalents per gram ethyl acetate extract)**
Ethyl acetate extract of *S. isoetifolium*	92.53±1.4	88.79±1.23	61.3±1.47

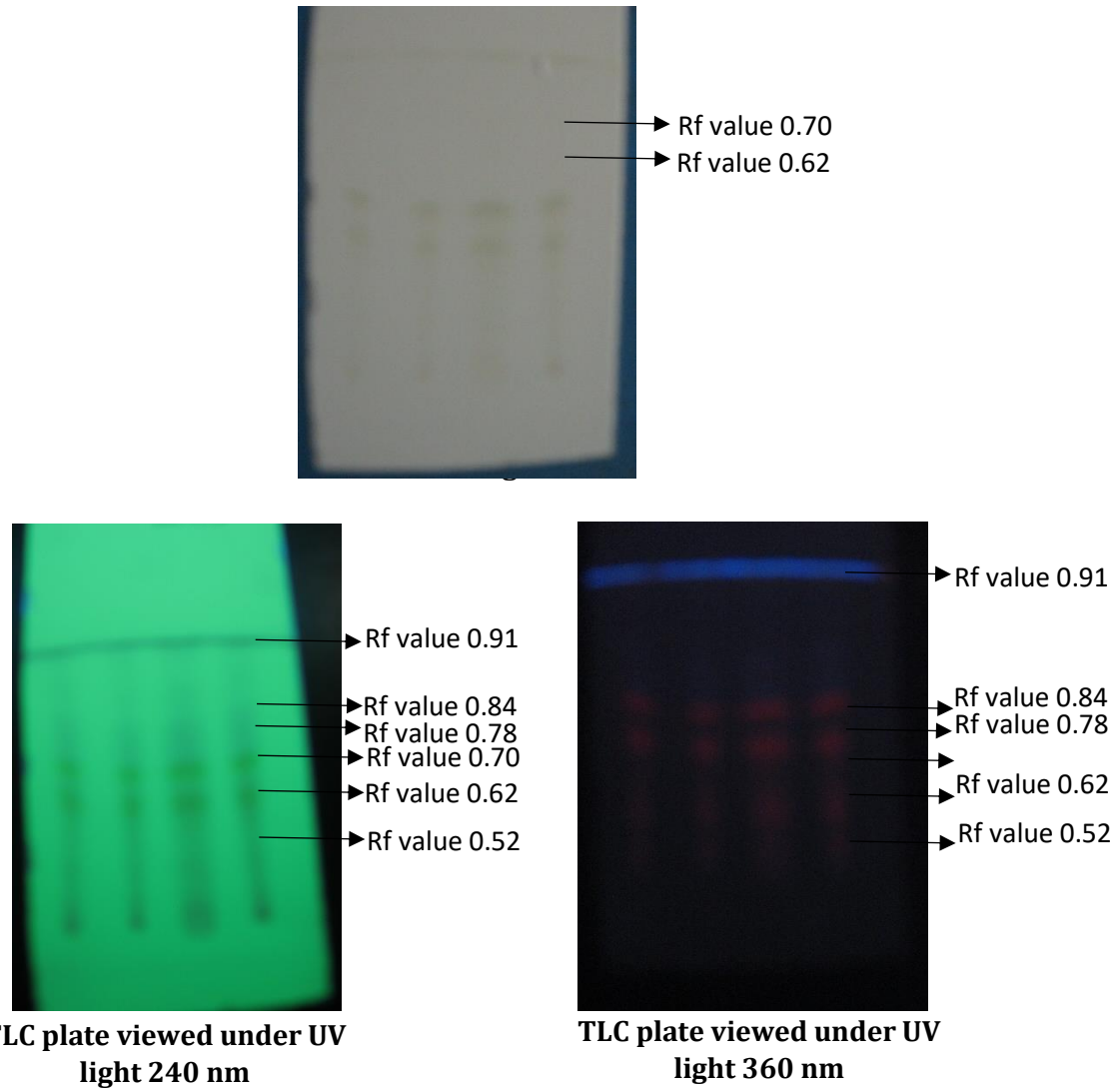
THE PARTIAL CHARACTERIZATION OF ETHYL ACETATE EXTRACT OF *S. isoetifolium* BY TLC

The ethyl acetate extract of *S. isoetifolium* loaded on Pre-coated TLC plates (60F 254 Merck) and developed with a solvent system of hexane, ethyl acetate and acetic acid in the ratio of 10:5:0.5. The developed plate was viewed under UV 240nm and 360nm. The Rf value of compounds were shown in Table-3 and Fig-1.

Table-3. The partial characterization of ethyl acetate extract of *S. isoetifolium* by TLC

S.No.	**Ethyl acetate extract of *S. isoetifolium***		
	UV 240 nm Rf value	**UV 360 nm Rf value**	**Visible Rf value**
1.	0.91	0.91	-
2.	0.84	0.84	-
3	0.78	0.78	-
4	0.70	0.70	0.70
5	0.62	0.62	0.62
6	0.52	0.52	-

Fig-1. The partial characterization of ethyl acetate extract of *S. isoetifolium* by TLC



TLC plate viewed under UV light 240 nm

TLC plate viewed under UV light 360 nm

FREE RADICAL-SCAVENGING ABILITY USING ABTS ASSAY BY ETHYL ACETATE EXTRACT OF *BORRERIA HISPIDA*

The radical scavenging ability was measured by ABTS assay as per given in table 4. The inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found in ethyl acetate extract of *S. isoetifolium.* In ABTS assay, inhibition percentage was high in ethyl acetate extract of *Borreriahispida* 82.63% with EC_{50} value 72.26*µg*/ml. The pure ascorbic acid was lower activity (74.36 with EC50 value 89.63) (Table 4). Nevertheless, in present study, it is showed that these activities were mainly due to anthocyanin and flavonoids compounds. It is known that vitamin C (ascorbic acid) and carotenoids are chief source of discrepancy of antioxidant/ antiradical activities in plant materials.

Table-4. Free radical-scavenging ability using ABTS assay of ethyl acetate extract of *S. isoetifolium*

Different concentration of extract	ABTS radical activity	
	Ethyl acetate extract of *S. isoetifolium*	**ABTS radical activity Standard Vitamin-C**
25 μg/ml	28.00±1.54	22.63±1.20
50 μg/ml	45.00±1.49	39.19±1.19
75 μg/ml	59.33±2.36	51.49±2.18
100 μg/ml	82.63±1.24	74.36±2.1
IC_{50} value	72.26	89.63

Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean +SD of three experiments

INHIBITION OF LIPID PEROXIDATION ACTIVITY OF ETHYL ACETATE EXTRACT OF *S. isoetifolium*

The ethyl acetate extract of *S. isoetifolium* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in ethyl acetate extract of *S. isoetifolium* (77.12%) with IC_{50} value 93.12 µg/ml and lowest inhibition percentage ascorbic acid 72.50% with IC_{50} 98.52 (Table-5). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). In the present study, egg yolk was used as substrate for free radical mediated lipid peroxidation, which is a non-enzymatic method. Generally, the mechanism of phenolic compounds for antioxidant activity includes neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

Table-5. Inhibition of lipid peroxidation activity of ethyl acetate extract of *S. isoetifolium*

Different concentration of extract	Lipid peroxidation inhibition	
	Ethyl acetate extract of *S. isoetifolium*	**Lipid peroxidation activity of Standard Vitamin-C**
25 μg/ml	20.24±1.69	18.14±1.69
50 μg/ml	31.45±1.63	28.7±1.48
75 μg/ml	53.45±2.30	48.6±1.67
100 μg/ml	77.12±1.56	72.50±1.49
IC_{50} value	93.12	98.52

Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean +SD of three experiments.

SUPEROXIDE SCAVENGING ACTIVITYOF ETHYL ACETATE EXTRACT OF *S. isoetifolium*

Ethyl acetate extract of *S. isoetifolium* exhibited powerful scavenging activity for superoxide radicals in a concentration dependent process than positive control. Ethyl acetate extract of *S. isoetifolium* showed highest radical activity in the percentage of 71.10% with IC_{50} value 93.12µg/ml when compared to positive control 65.89%with IC_{50} Value 112.36µg/ml (Table-6). One of the standard methods to produce Superoxide radicals is through photochemical reduction of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system. These superoxide radicals are extremely toxic and may be produced either through xanthine activity or through mitochondrial reaction. Superoxide radicals are reasonably a weak oxidant may decompose to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals.

Table-6. Superoxide scavenging activity of ethyl acetate extract of *S. isoetifolium*

Different concentration of extract	Percentage of Superoxide scavenging activity	
	Ethyl acetate extract of *S. isoetifolium*	Superoxide scavenging activity of Standard Vitamin-C
25μg/ml	22.60±1.20	19.35±2.17
50μg/ml	34.13±2.16	30.14±1.29
75μg/ml	57.10±2.32	48.36±1.58
100μg/ml	71.10±2.15	65.89±1.32
IC_{50} value	93.12	112.36

Results are expressed as percentage of Superoxide scavenging activity with respect to control. Each value represents the mean +SD of three

METAL CHELATING ACTIVITYOF ETHYL ACETATE EXTRACT OF *S. isoetifolium*

The metal chelating property of ethyl acetate extract of *S. isoetifolium* was displayed in Table-7. The ethyl acetate extract of *S. isoetifolium* was evaluated for their ability to compete with ferrozine for ferrous iron in the solution. In this evaluation, the ethyl acetate extract of *S. isoetifolium* hindered the formation of ferrous and ferrozine complex, signifying that they have chelating activity and are capable of capturing ferrous iron before ferrozine. The ethyl acetate extract of *S. isoetifolium* reduced the greenish blue color complex immediately and showed the highest chelating activity 66.62% with EC_{50} Value 96.31µg/ml than positive control Vitamin-C 61.58% with EC_{50} value 102.36μg/ml.

Table-7. Metal chelating activity of ethyl acetate extract of *S. isoetifolium*

Different concentration of extract	Percentage of Metal chelating activity	
	Ethyl acetate extract of *S. isoetifolium*	**Standard Vitamin-C**
25*µg*/ml	23.38±1.47	20.78±2.45
50*µg*/ml	32.15±1.38	26.46±1.67
75*µg*/ml	46.10±1.56	39.21±1.79
100*µg*/ml	66.62±1.89	61.58±1.23
IC_{50} value	96.31	102.36

Results are expressed as percentage of Metal chelating activity with respect to control. Each value represents the mean +SD of three experiments.

NITRIC OXIDE RADICAL SCAVENGING ASSAY ETHYL ACETATE EXTRACT OF *S. isoetifolium*

Nitric oxide radical quenching activity of ethyl acetate extract of *S. isoetifolium* were identified and compared with the standard ascorbic acid. The ethyl acetate extract of *S. isoetifolium* displayed the maximum inhibition of 74.12% at a concentration of 100µg/ml, in a concentration-dependent process when compared to ascorbic acid with inhibition percentage 67.12% (Table-8). In the current study, nitrite was produced by incubation of sodium nitroprusside in standard phosphate saline buffer at 25°C was reduced by ethyl acetate extract of *S. isoetifolium* Significant scavenging activity may be due to the antioxidant property of flavonoid, which compete with oxygen to react with nitric oxide, leading to less production of nitric oxide.

Table-8. Nitric oxide radical scavenging assay of the ethyl acetate extract of *S. isoetifolium*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Ethyl acetate extract of *S. isoetifolium*	**Standard Vitamin-C**
25 *µg*/ml	22.13±1.24	18.23±1.52
50 *µg*/ml	36.43±3.21	31.56±2.47
75 *µg*/ml	45.00±1.45	40.23±2.15
100 *µg*/ml	74.12±1.12	67.21±1.25

IC_{50} value	98.23	110.23

Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean+SD of three experiments.

INHIBITION OF PANCREATIC LIPASE ACTIVITY OF ETHYL ACETATE EXTRACT OF *S. isoetifolium*

In the present study, ethyl acetate extract of *S. isoetifolium* have been evaluated for lipid lowering activity through percentage inhibition of pancreatic lipase. Table-9 shows the results of pancreatic Lipase inhibition of the ethyl acetate extract of selected medicinal plants at various concentrations. From the data of the results obtained, maximum percentage of lipase inhibition was shown by ethyl acetate extract of *S. isoetifolium* (78.92 %). The ethyl acetate extract of *S. isoetifolium* has shown minimum activity (69.93 %) (Table-9 and Fig-10). Pancreatic lipase inhibition is the most widely studied mechanism for the identification of potential anti-obesity agents. Discovery of orlistat was done from the naturally occurring molecule lipstatin. The success of naturally occurring compounds for treatment of obesity has influenced the research for the identification of newer pancreatic lipase inhibitors that lack unpleasant side effects.

Table-9. Inhibition of pancreatic lipase activity of ethyl acetate extract of *S. isoetifolium*

Different concentration of extract	**Percentage of Inhibition of pancreatic lipase activity**	
	Ethyl acetate extract of *S. isoetifolium*	**Standard Orlistat**
25μg/ml	20.26±1.47	17.87±1.52
50μg/ml	34.78±1.29	31.67±2.47
75μg/ml	53.78±1.19	49.47±2.32
100μg/ml	76.92±2.47	70.14±1.19
IC_{50} value	79.63	84.56

Results are expressed as percentage of Inhibition of pancreatic lipase with respect to control. Each value represents the mean+SD of three experiments.

MTT ASSAY:

Ethyl acetate extract of *S. isoetifolium* shows more cytotoxic effect on Human Breast Cancer Cells (MCF-7). The observations in statistical data of cell cytotoxicity study suggesting us that against MCF-7 cells treated with extract shows significant cytotoxic potency with IC_{50} (50% cell viability) concentration compared to the standard drug, The percentage of cell viability was shown in the Table 10 after the treatment period of 24 hrs.

Table 10: % of cell viability of MCF-7 cells treated with Ethyl acetate extract with different concentrations and observed IC50 value after the treatment period of 24hrs

Culture condition	% cell viability	IC50 conc (µg/ml)
Untreated	100	**87**
Std control	48.18	
EAE of *S. isoetifolium*-12.5ug	96.43	
EAE of *S. isoetifolium* -25ug	85.11	
EAE of *S. isoetifolium* -50ug	75.14	
EAE of *S. isoetifolium* -100ug	57.04	
EAE of *S. isoetifolium* -200ug	25.87	

Graph 1: % cell viability of Ethyl acetate extract treated MCF-7 cells after the incubation period of 24hrs

DISCUSSION

At present, there is a growing trend in the use of natural antioxidants from plants sources, because of their efficiency and negligible toxicity. Thus, antioxidant and radical scavenging activities of medicinal plants have been extensively studied. Plant phenolics have shown to possess antioxidant, hypocholesterolemic, hypolipidimic, antihypertensive, antidiabetic and anti-cancerous properties. Phenolics are powerful chain breaking antioxidants; contributing directly to the antioxidant action. The great antioxidant potential of phenolics may be due to the presence of hydroxyl group. Another group of polyphenol flavonoids are also important for human health and act through scavenging or chelating the metals ions [22]. Phenolic compounds are responsible for the antioxidant activity of vegetables and medicinal plants. Although in recent years the antioxidant analysis of medicinal plants has been extensively researched worldwide, very few studies have been carried out to assess medicinal plants and vegetables grown in India and consumed locally or exported to several countries [23].

ABTS radical scavenging assay is the most common assay to determine the antioxidant activity. Ethyl acetate extract of *S. isoetifolium* scavenged ABTS significantly greater than the Vitamin-C. -OH radical is the most reactive species that can damage and implicate DNA, proteins and lipids. Therefore, removal of -OH is important to defense from various disorders [24]. It was obvious that Ethyl acetate extract of *S. isoetifolium* had the greatest antioxidant activity against ABTS and showed protective capacity from ·OH radical than Vitamin-C. These results indicated that extract contained rich phenolic compounds in the Ethyl acetate extract of *S. isoetifolium* would play the protective role from free radical-induced damage. The major polyphenolic constituents of brassicaceae family plants, flavonols such as querxxxxxxxcetin and kaempferol, and anthocyanidins, show a greater efficacy as antioxidants on a mole for mole basis than the antioxidant nutrients vitamin C, vitamin E and carotenoids [25]. As it is known that lipid peroxidation is the net result of any free radical attack on membrane and other lipid constituents present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). Present study has been used egg yolk as a substrate for free

radical mediated lipid peroxidation, which is a non-enzymatic method. Ethyl acetate extract of *S. isoetifolium* significantly inhibited the degree of lipid peroxidation. This inhibition of lipid peroxidation may be either due to chelation of Fe or by trapping of the free radicals. To test this situation, further investigated the role of ethyl acetate extract of *S. isoetifolium* on metal chelation and other free radical species.

The antioxidant effect of ethyl acetate extract of *S. isoetifolium* may be due to its compounds, like flavonoids and iridoids. Three flavonol glycosides (quercetin 3-O-sambubioside, kaempferol-3-O-[2-O-(E-6-O-feruloyl) -β-d-glucopyranosyl]-β-d galactopyranoside and quercetin 3-O-sophoroside) and six known iridoid glycosides (asperuloside, asperulosidic acid methyl ester, (E)-6-O-p-methoxy cinnamoylscandoside methyl ester, (E)-6-O-feruloyl scandoside methyl ester and (E)-6-O-coumaroyl scandoside methyl ester) were determined for their antioxidant effects on xanthine oxidase inhibition, xanthine-xanthine oxidase cytochrome c and TBA-MDA systems [26]. The present study was generated superoxide radicals by photochemical reduction of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system, which is one of the standard methods. These superoxide radicals ($O-_2$) are highly toxic and may be generated either through xanthine activity or through mitochondrial reaction. Although it is a relatively weak oxidant, it may decompose to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals [27]. Removal of superoxide in a concentration dependent manner by ethyl acetate extract of *S. isoetifolium* may be attributed to the direct reaction of its phytomolecules with these radicals or inhibition of the enzymes. Since here, SO radicals are being generated through the non-enzymatic method, the action of ethyl acetate extract of *S. isoetifolium* were a clear indication of its direct reaction with this radical species.

Iron is essential for life system because it is required for oxygen transport, respiration and activity of many enzymes. In complex systems such as food and food preparation many different mechanisms may contribute to oxidative process such as Fenton reaction, where transition metal ions play a vital role. Different reactive oxygen species might be generated and due to which various target structures like lipids, proteins, carbohydrates etc. can be affected. Therefore, it is important to characterize the extracts by a variety of antioxidant assays [28]. The chelating effect on ferrous ions by ethyl acetate extract of *S. isoetifolium* is presented in Table 8. Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation. It was already reported that chelating agents which form ó bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, there by stabilizing the oxidized form of the metal ion. Antioxidants inhibit interaction between metal and lipid through the formation of insoluble metal complexes with ferrous ion. Hence, the data obtained for study species reveals that the extracts of both leaf and stem bark demonstrate an effective capacity for iron binding and hence the antioxidant property [27].

In the present study, the nitric oxide radical quenching activity of the ethyl acetate extract of *S. isoetifolium* was detected and compared with the standard ascorbic acid. The ethyl acetate extract of *S. isoetifolium* exhibited the maximum per cent inhibition of 53.8% at a concentration of 100 μg/mL, in a concentration-dependent manner. The decrease in the concentration of the nitric oxide radical was more significant than ascorbic acid, which is due to the antioxidant activity of the ethyl acetate extract of *S. isoetifolium.* Nitric oxide is a potent

pleiotropic mediator of physiological processes, such as smooth muscle relaxation, neuronal signalling and inhibition of platelet aggregation and regulation of cell-mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems, including neuronal communication, vasodilatation and antimicrobial and antitumor activities. Moreover, in pathological conditions, nitric oxide reacts with superoxide anion and forms potentially cytotoxicmolecules, such as peroxynitrite [29].

From the data of the present study results obtained, maximum percentage of lipase inhibition was shown by ethyl acetate extract of *S. isoetifolium* (78.92%). The ethyl acetate extract of *S. isoetifolium* has shown minimum activity (69.23 %). Hydrolysis of triglyceride into free fatty acids and monoglyceride is accomplished majorly by pancreatic lipase, a water-soluble enzyme. It is a lipolytic enzyme which catalyzes the hydrolysis of the ester bonds present in the triacylglycerols. The enzyme works to remove the fatty acids located at position 1 and position 3 of the triglyceride, hence leaving a 2-monoglyceride and two free fatty acids. The enzyme is present in the gastrointestinal tract inside the adipocytes. Pancreatic lipase on entering the pancreatic duct mixes with bile salts and liquids following which it reaches the duodenal lumen in order to complete fat digestion. The enzyme is substrate specific. It gives preference to triglycerides over phospholipids. It does not act on water soluble substrates and prefers water insoluble substrates. Pancreatic lipase activity increases when it encounters a water-oil interface. This property is called interfacial activation. Even though lipase is secreted into the duodenum, it is inhibited by bile salts and hence requires a pancreatic protein called colipase for its activity. The property of hydrolyzing water-insoluble substrates and interfacial activation distinguishes pancreatic triglyceride lipase from the rest of the lipases [30].

The MTT experiment revealed that the cytotoxicity of extract arose dramatically as the concentration increased. Succinate dehydrogenase is employed to split the tetrazolium ring and convert MTT to an insoluble purple formazan, with the amount of formazan provided proportional to the number of live cells [31]. This method is used to identify live cells. Vishal Gulecha., & Thangave Sivakumar [32] report that the treated cells exhibit apoptotic characteristics such as separation from the culture plate, cytoplasmic condensation, cell shrinkage, nuclear chromatin condensation and accumulation, and loss of contact with nearby cells. The IC_{50} values for *Enhalus acoroides* extract in HepG2 and MCF-7 cell lines were 112.20 g/ml and 101.60 g/ml, respectively [33]. In comparison, after treatment with *S. isoetifolium* ethyl acetate extract, the number of viable cells reduced dramatically at all dosage levels. The percentage of cell viability reduces as the concentration increases. Cell viability reached 96.43% at 12.5 µg/ml and decreased to 25.87% at 200 µg/ml. The results indicate that the ethyl extract extract of *S. isoetifoelium* may have strong cytotoxic activity against the MCF-7 cell line.

CONCLUSION

Polyphenols are valuable plant constituents for the scavenging of free radicals because of their phenolic hydroxyl groups. This, together with the obtained results, suggests that as the number of polyphenolic compounds increases, the antioxidant and anti-obesity, activity also increases. In conclusion, the present study demonstrates that the ethyl acetate extract of *S. isoetifolium* can protect the body from oxidative stress from ROS, which may be due to the phytochemicals in the form of polyphenols that occur in the plant. Thus, the results of this study indicate that the commonly used green leafy of, *S. isoetifolium* significantly inhibit the activity

of pancreatic lipase which can be attributed to the presence of saponins, phenols, flavonoids or alkaloids which is comparable to orlistat. These may be used in nutraceuticals and the food industry. However, additional studies are necessary to develop a method for the fractionation and identification of polyphenols and to determine the most active antioxidant compounds in the *S. isoetifolium*

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