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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF THE SEAGRASS - *Enhalus acoroides*

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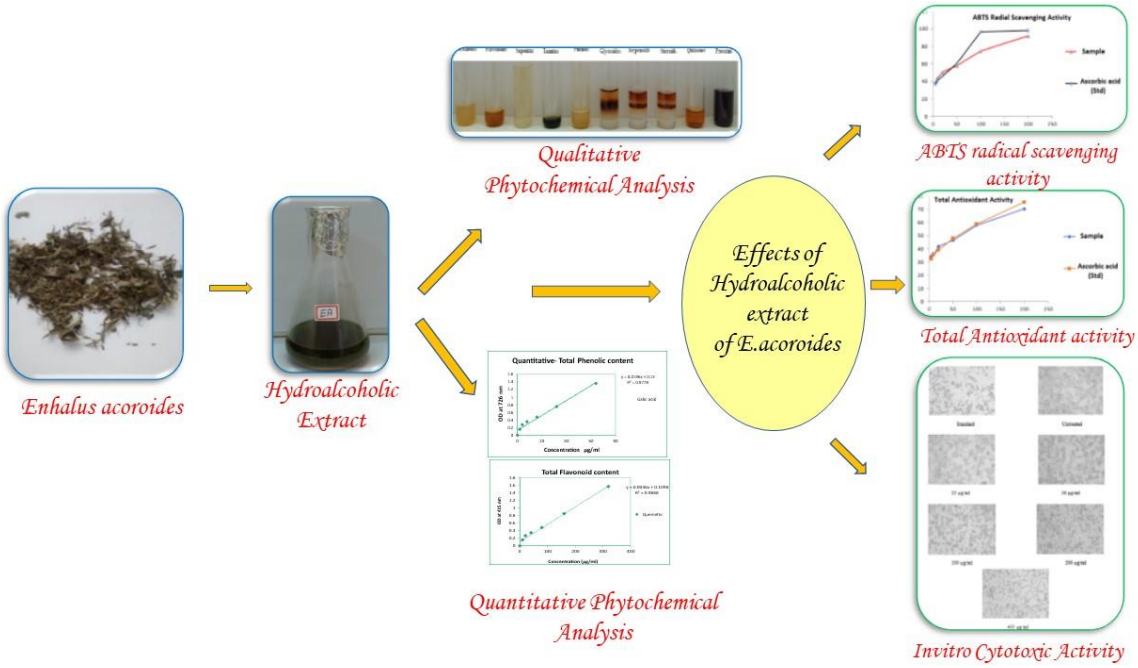
ABSTRACT:

Seagrasses are an excellent and potential bioresource to discover new natural bioactive compounds such as antioxidants that have beneficial effects on health. Natural antioxidants have many functions in biological systems, primarily for defense against oxidation which produces free radicals in food, chemicals, and living systems. The present study was conducted to determine the preliminary phytochemical analysis, antioxidant and cytotoxicity activity of hydroalcoholic extract of *Enhalus acoroides*. The hydroalcoholic extract were evaluated for total phenolic and total flavonoids contents. The antioxidant activity of hydroalcoholic extract of *Enhalus acoroides* was assessed by different scavenging assays. The present study revealed the presence of major phytochemicals like alkaloids, phenols, flavonoids, steroids, tannins and terpenoids. Quantitative analysis of the total phenols was found to be 45.95 mgGAE/g and the total flavonoids were found to be 33.42 mgQU/g in the aqueous methanolic extract. This study aimed to discover new natural antioxidant agents, *Enhalus acoroides* was evaluated for phytochemical constituents and the antioxidant activity was assessed. The information of secondary metabolites from *E. acoroides* will contribute to further research in determination of antioxidant, and anticancer of *E. acoroides*, as a promising marine drug candidate from India.

Keywords: *Enhalus acoroides*, Phytochemicals, Antioxidants, MTT, HepG2

GRAPHICAL ABSTRACT



HIGHLIGHTS

- Seagrasses are the only flowering plants to recolonising the beds of the sea.
- Seagrass possess several metabolites with multiple pharmacological activities.
- The hydroalcoholic extract of *Enhalus acoroides* possesses strong antioxidant and cytotoxic effects in MCF7 cancer cell line.
- *Enhalus acoroides* could be effectively used in nutraceutical industry.

1. INTRODUCTION

Seagrass are one of the groups of marine angiosperms widely distributed all over the coastal regions. The seagrass is very productive and traditionally used for a variety of therapeutic purposes (1). Marine natural products including seagrass, micro and macroalgae, sponges and corals are the important source which plays a major role in the discovery of the novel biologically active components (2). Seagrass are one of the groups of marine angiosperms which often live entirely submerged and can complete their life cycle in a coastal environment (3). Marine plants are distributed everywhere the plants and is a breeding, feeding for marine organism. Seagrass are the only submerged marine plants with an underground root and rhizome system. Seagrass below-ground biomass can equal that of above ground biomass, and is often much higher (4). Scientific concern for the health of our coastal marine environments against a background of anthropogenic pressures and global climate change is wide spread (5).

Seagrass are the sole flowering plants which grow in marine environments. There are about 60 species of fully marine seagrasses which belong to four families posidoniceae, Zosteraceae, Hydrocharcteritaceaceae. Seagrass are the only flowering plants which grow in marine environments. There are about 60 species of fully marine seagrass which belong to

four families, all in the order alismatales. They have roots, stems, leaves. They also form tiny flowers, fruits, and seeds.

Phytocomponents such as phenols, alkaloids, tannins, and flavonoids are examples of secondary metabolites produced by green plants, from which the plants are thought to get medicinal properties [6]. Phenolic compounds have been associated with antioxidant activity due to their free radical scavenging activities [7]. From plants, active components from stem, fruits, herbs, roots, and leaves have been studied extensively for their antioxidant properties [8]. Antioxidants are a group of substances that are useful to fight against carcinoma and other processes that potentially lead to disorders such as atherosclerosis, Alzheimer, Parkinson, diabetes, and cardiac disease [9].

This study aimed to screen the phytochemicals and to assess the antioxidant and cytotoxic activity from the hydroalcoholic extract of *Enhalus acoroides* against HepG2 cell lines.

2. MATERIALS AND METHODS

2.1 Collection of plant

The fully matured *E. acoroides* were taken from Devipattinam, Ramanathapuram District during the period of June 2022.

2.2 Preparation of Extract

The seagrass material was washed with distilled water for several times and was subjected to air drying under the shade. After drying they were ground by an electrical mixer until they became a powder. Then the sample was stored in a sterile place, and subjected to extraction method. Extraction of seagrass sample was done using aqueous ethanol. Aliquots of 10 g of the sample were soaked in 250 ml of the solvent for 72 h. Later the soaked sample was homogenized in an electric blender along with the solvent at room temperature, filtered, and concentrated under reduced pressure using a rotary evaporator and keep stored at -20° C.

2.3 ANALYSIS OF PHYTOCHEMICALS

2.3.1 Qualitative phytochemical analysis

10grams of *E. acoroides* leaves powder were used for extraction. Extraction was performed with cold extraction using maceration method in hydro-ethanol (ethanol and water (70:30)) for 24 hours using the “intermittent shaking” method to obtain extract. The extract were further filtered using Whatman filter No 1 paper and filtrate was used for phytochemical analysis. Preliminary phytochemical screening was carried out by using standard procedure followed by Trease and Evans (1989) [9] and Harborne (1984) [10].

2.3.2 Qualitative Analysis of Phytochemicals

Determination of total phenolic content [11]

The *Enhalus acoroides* (1000 µg/ml) was mixed with 0.5 ml of water and 0.2 ml of Folin-Ciocalteu's phenol reagent (1: 1). After 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 5 mL with distilled water. The reaction was kept in the dark for 30 min. and the absorbance of blue color from different samples was measured at 765 nm. The phenolic content was calculated as Gallic acid equivalents GAE/g of dry plant material based on a standard curve of Gallic acid (10-320 µg/ml), $y = 0.014x + 0.068$, $R^2 = 0.979$

Determination of total flavonoids [12]

Estimation of the total flavonoids in the test sample was carried out using the method of [12]. To 1000 µg/ml of *Enhalus acoroides*, a volume of 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420nm. A yellow colour indicated the presence of flavonoids. A calibration curve was constructed, using Quercetin (10–320 µg/ml) as standard. Total flavonoid contents were expressed as Quercetin (mg/g) using the following equation based on the calibration curve: y = 0.002x + 0.127, where y was the absorbance.

2.4 *In vitro* Anti-oxidant activity

2.4.1 ABTS radical scavenging assay [13]

The free radical scavenging activity was also determined by ABTS (2,2'azino bis (3-ethylbenzothiazoline-6-sulphonicacid) diammonium salt) radical cation decolourization assay. ABTS was generated by mixing 5 mL of 7 mM ABTS with 88 of 140 mM potassium persulfate under darkness at room temperature for 16 hours. The solution was diluted with 50% ethanol and the absorbance at 734 nm was measured. The ABTS radical cation scavenging activity was assessed by mixing 5ml ABTS solution (absorbance of 0.7µ, 0.05) with 0.1 mL extracts. The final absorbance was measured at 743 nm with spectrophotometer. The percentage of scavenging was calculated by the following formula,

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{AC - AS}{AC} \right) X\ 100$$

Where AC = control is the absorbance and AS = sample is the absorbance of reaction mixture (in the presence of sample).

2.4.2 Determination of Total Antioxidant Capacity [14]

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. [14].

Reagents:

- Sulfuric acid : 0.6M
- Sodium phosphate :28mM
- Ammonium molybdate :4mM

Procedure:

The assay is based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The extract (0.3ml) was combined with 3ml of reagent (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate in a tube and was incubated at 95○C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The scavenging activity was calculated according to the following equation: % Inhibition

$$\text{\% of Inhibition} = \frac{(A0-A1)}{A0} \times 100$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

2.5 *IN VITRO* ANTICANCER EFFECT OF *Enhalus acoroides*

2.5.1 Extract Preparation

The crude hydro-alcoholic extract of *Enhalus acoroides* was weighed (10mg/10ml) and dissolved in DMSO to prepare appropriate dilution to get required concentrations of around 25µg 50µg, 100µg, 200µg and 400 µg. The control cultures were treated with equivalent concentrations of DMSO alone. They were kept under refrigerated condition until they were utilized for the experiment.

2.5.2 Cell Line and Culture

The hepatocellular carcinoma (HepG2) cell line was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 5% CO2 at 37 °C. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.5.3 Reagents

Minimum Essential Medium (MEM) was purchased from Hi-Media Laboratories. FBS was purchased from Cistron laboratories. Trypsin, 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl- tetrazolium bromide (MTT) and Dimethyl Sulfoxide (DMSO) were purchased from Sisco research laboratory chemicals, Mumbai. All other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

2.5.4 Microculture Tetrazolium (MTT) Assay [15]

Cell Treatment Procedure

The cytotoxic assay was evaluated by the MTT reduction assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium]. The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediaminetetraacetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order to obtain the final density of 1x105 cells/ml. 96-well plates at a plating density of 10,000 cells/well were seeded with one hundred microliters per well of cell suspension and incubated for cell attachment at 37° C, 5% CO2, 95% air and 100% relative humidity. Aliquots of 100 µl of different concentrations of *Borreria hispida* (25, 50, 100, 200 and 400µg/ml) dissolved in DMSO (1%) were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations for 48h at 37°C, 5% CO2, 95% air and 100% relative humidity. After 48h of incubation, to each well 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) phosphate- buffered saline solution was added and incubated at 37°C for 4 h. Then, 100µl of 0.1% DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for inhibition Concentration (IC50) was determined graphically. The absorbance at 570nm was measured with a UV- Spectrophotometer. The medium without samples served as control and triplicate were maintained for all concentrations. The effect of

the samples on the anticancer activity of HepG2 cell line was expressed as the % Cytotoxicity using the following formulas:

% Cytotoxicity = 100 - [Abs (sample) /Abs (control)] x100.

% Cell Viability = [Abs (sample) /Abs (control)] x100.

2.6 Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals' concentration by 50% (IC_{50}), was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software. Results were expressed as graphically/mean±standard deviation.

3. RESULTS

3.1 Qualitative phytochemical analysis

The qualitative phytochemical screening of hydroalcoholic extract of *Enhalus acoroides* investigated and summarized in Table 1. Tannin, saponin, flavonoids, steroids, terpenoids, alkaloids, anthroquinone, polyphenol and coumarins present in hydro-alcoholic extracts.

Table 1: Qualitative phytochemical analysis of *Enhalus acoroides*

S. No	Phytochemicals	Hydroalcoholic Extract of *Enhalus acoroides*
1	Alkaloids	++
2	Flavonoids	+
3	Saponins	-
4	Tannins	++
5	Phenols	++
6	Cardiac glycosides	+
7	Steroids	+
8	Terpenoids	+
9	Quinones	+
10	Proteins	+

("+" indicates presence of the compounds; "-" indicates absence of the compounds, "++" indicates the high concentration).

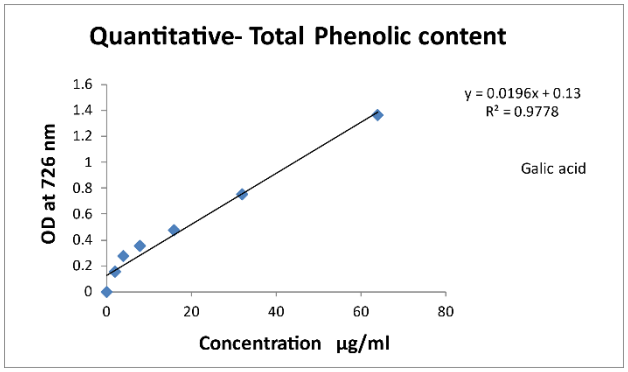
3.2 Quantitative Phytochemical Analysis

Total phenolic content and Total flavonoid content of the hydroalcoholic extract of *Enhalus acoroides*, using the calibration curve, was shown in the Table 2 and Graph (1&2) respectively. Total phenol content in *Enhalus acoroides* using the calibration curve, was found to be **45.95 mg** of Gallic acid equivalents/g dry weight of extract. Total flavonoid content in *Enhalus acoroides* using the calibration curve, was found to be **33.42 mg** of quercetin equivalents/g dry weight of the extract.

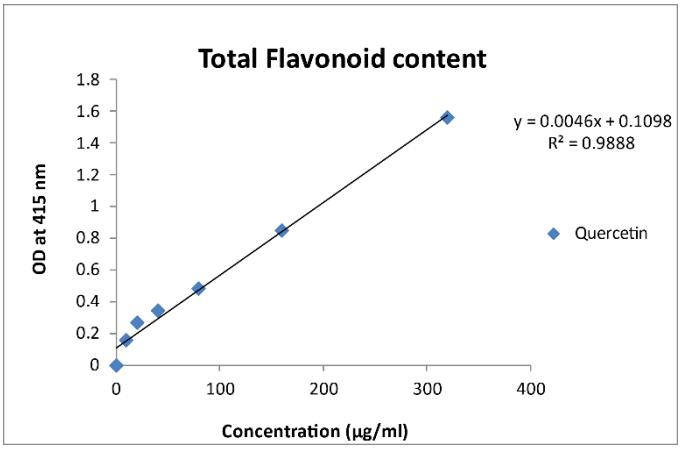
Table 2: Quantitative analysis of Total phenol and Total flavonoids of *Enhalus acoroides*

Name of Sample	Total phenol (Milligrams of Gallic acid (GAE) equivalents per gram)	Flavonoids (Milligrams of quercetin equivalents per gram)
Hydroalcoholic extract of ***Enhalus acoroides***	45.95 ±14.35	33.42 ± 8.49

Values are expressed as Mean ± SD for triplicates



Graph 1: Standard Curve for Phenol using Gallic acid



Graph 2: Standard Curve for Flavonoids using Quercetin

3.3 *IN VITRO* ANTIOXIDANT ACTIVITY

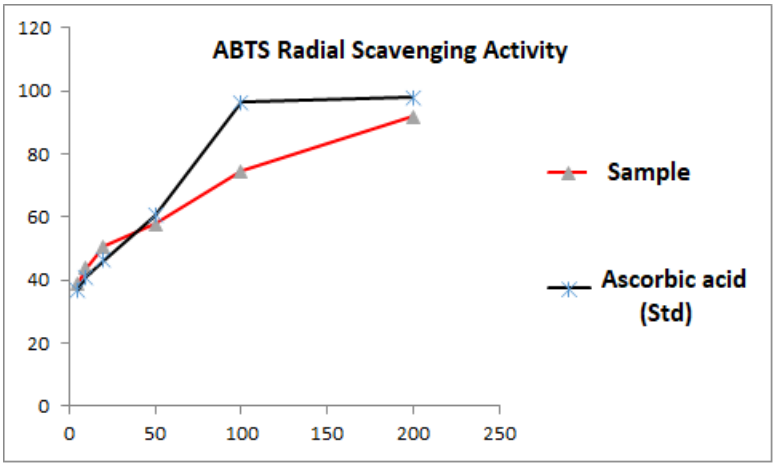
3.3.1 ABTS Free Radical Scavenging Assay:

The hydroalcoholic extracts of *Enhalus acoroides* registered better activity shown in (Table 3 & Graph 3). The scavenging effect increases with the concentration of standard and *Enhalus acoroides*.

Table 3: ABTS radical scavenging activity of *Enhalus acoroides* and standard as ascorbic acid at different concentrations

Samples	Concentrations (µg/ml)					IC_{50} value (µg/ml)
	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
***Enhalus acoroides* (% of inhibitions)**	43.78	50.75	57.83	74.44	91.75	15.78
Std. Ascorbic acid (% of inhibitions)	41.11	46.34	60.51	96.28	97.79	15.46

Values are expressed as Mean± SD for triplicates



Graph 3: ABTS radical scavenging activity of *Enhalus acoroides* and standard as ascorbic acid at different concentrations

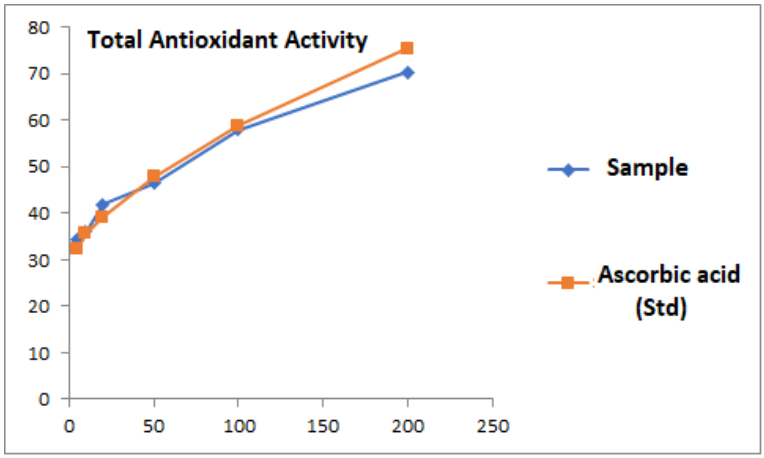
3.3.2 Total Antioxidant Activity

The hydroalcoholic extracts of *Enhalus acoroides* shows better antioxidant activity shown in (Table 4 & Graph 4). The scavenging effect increases with the concentration of standard and *Enhalus acoroides*.

Table 4: Total antioxidant activity of *Enhalus acoroides* and standard as ascorbic acid at different concentrations

Samples	Concentrations (µg/ml)					IC_{50} value (µg/ml)
	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
***Enhalus acoroides* (% of inhibitions)**	35.88	41.81	46.45	57.95	70.38	39.73
Std. (Ascorbic acid) (% of inhibitions)	35.54	39.25	47.85	58.88	75.37	37.11

Values are expressed as Mean± SD for triplicates



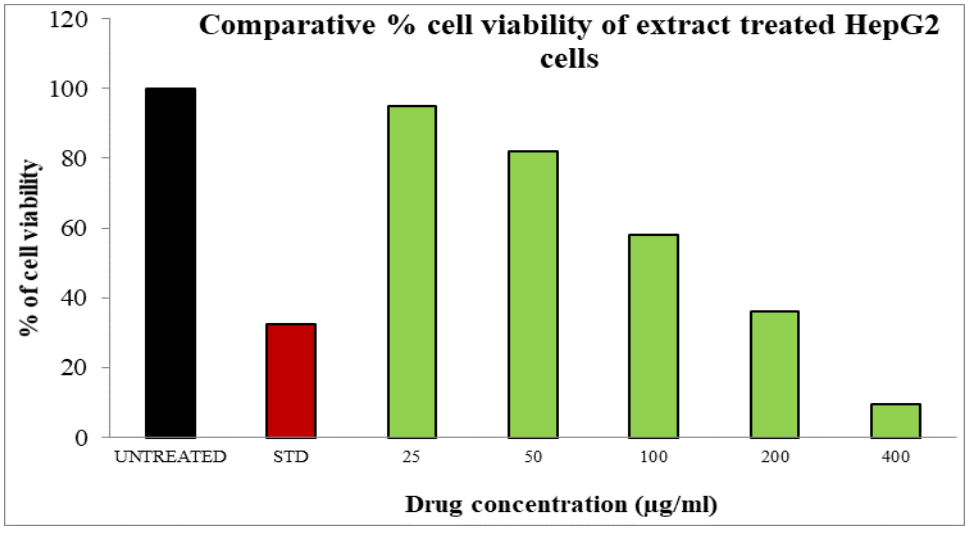
Graph 4: Total antioxidant activity of *Enhalus acoroides* and standard as ascorbic acid at different concentrations

3.4 *In vitro* ANTI-CANCER ACTIVITIES OF *Enhalus acoroides* IN HepG2 CELL LINE

In the present study, hydroalcoholic extract was tested against HepG2 liver cancer cells at various concentrations shown in Table 5 and Graph 5. Based on the potentials of the *Enhalus acoroides* was used against HepG2 normal cell line for their cytotoxicity activity. The cell growth inhibition of the *Enhalus acoroides* tested against HepG2 cell line at different concentrations (25, 50, 100, 200 and 400µg/ml). The IC_{50} value 122.44 µg/ml shows the cell viability. Graph 6 shows the cell viability of HepG2 cancer cell population by various fractions of the hydro alcoholic extracts *Enhalus acoroides*. Inhibition of cell proliferation could be the result of the induction of apoptosis and/or inhibition of growth. *Enhalus acoroides* treatment caused morphological changes like detachment from the substratum, with rounding of cells and cell shrinkage. The above result affirms that the cytotoxicity of crude extract of *Enhalus acoroides* substantially increased with increase in concentration. The IC_{50} value of the *Enhalus acoroides* on HePG2 cell line was found to be 122. 44ug/ml (Figure 1).

Table 5: Table showing the % cell viability of the *Enhalus acoroides* treated HepG2 cells after the treatment period of 24hrs

S. No	Concentration (ug/ml)	% cell viability
1	**0**	100
2	**25**	94.98
3	**50**	81.97
4	**100**	58.12
5	**200**	36.64
6	**400**	9.65
7	**Aripiprazole-35Mm (Std)**	32.42
8	**IC 50**	122. 44ug/ml



Graph 5: % cell viability of HepG-2 cells treated with the *Enhalus acoroides*

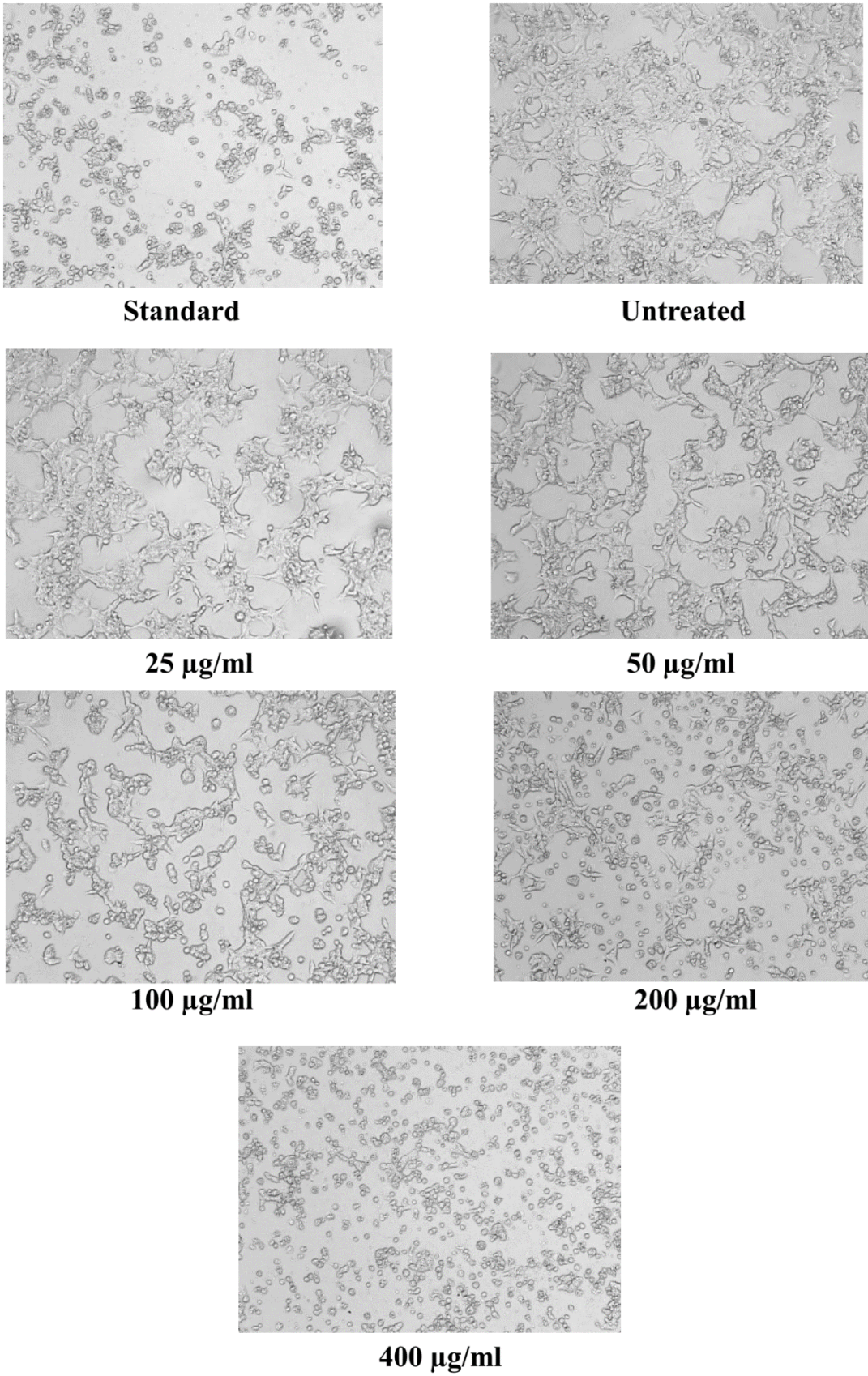


Figure 1: Morphological Changes of HepG2 Cell Line on *Enhalus acoroides* after treatment of various Concentrations

4. DISCUSSION

At present, there is a growing trend in the use of natural antioxidants from plant 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 anticancerous 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 (16). 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 (17).

Many studies have shown the benefits of *Enhalus acoroides.* It has long been recognized as critical coastal nursery habitat for estuarine fisheries and wildlife. It functions as direct food sources for fish, waterfowl, dugongs, manatees, and sea turtles. It also participants in nutrient cycling processes and acts as a stabilizing agent in coastal sedimentation and erosion processes (18-20). In folk medicine, seagrass have been used for a variety of remedial purposes, for example the treatment of fever and skin diseases, muscle pains, wounds, stomach problem, a remedy against stings of different kinds of rays and tranquillizers for babies (21). In India, seagrass is used as medicine example: Treatment of heart conditions, seasickness, food (nutritious seeds), fertilizer (nutrient- rich biomass) and livestock feed (goats and sheep) (22). Seeds of *Enhalus acoroides* are thought to have aphrodisiac and contraceptive properties (23). In Thailand, only rhizome of *Enhalus acoroides* was used as a medicine for treatment of carminative and to increase blood and lymph circulation (24, 25).

ABTS radical scavenging assay is the most common assay to determine the antioxidant activity. Hydroalcoholic extract of *Enhalus acoroides* 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 (26). It was obvious that yydroalcoholic extract of *Enhalus acoroides* 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 hydroalcoholic extract of *Enhalus acoroides* would play the protective role from free radical-induced damage. The major polyphenolic constituents of brassicaceae family plants, flavonols such as quercetin 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 (27).

The reduction of the 2,2’azinobis [3-ethylbenzothiazoline sulphonate] radical cation [ABTS•+] has been widely used to measure the antioxidant capacity of natural extracts [28]. ABTS•+, a stable free radical with the characteristic absorbance at 734 nm, was used to study

the radicals scavenging effect of hydro alcoholic extract of Hydroalcoholic extract of *Enhalus acoroides*. The presence of bioactive chemical compounds in the tested extracts that inhibit the potassium persulfate activity may reduce the production of ABTS•+. This study reveals that the hydro alcoholic extract of Hydroalcoholic extract of *Enhalus acoroides* exhibited higher ABTS•+ radical scavenging activity which has IC_{50} value is 15.78 when compared to the standard Ascorbic acid 15.46.

Hepatocellular carcinoma (HCC) is a tumor of significant epidemiologic, clinical and pathological interest. Liver cancer is the sixth most common cancer worldwide and the third most common cause of death from cancer. It is comparatively more commonly found in Japan, Saudi Arabia and predominantly in Africa. HCC is usually rapidly progressive and carries a dismal prognosis in both the children and the adults. The most important risk factors for liver cancer are infectivity with the hepatitis B and C viruses, both of which amplify the risk of liver cancer. Malignant tumors of hepatoma usually existing as multifocal lesions in patients with pre-existing cirrhosis, though solitary tumors and those not related with cirrhosis may also are encountered. Cytologically, the cells generally bear a similarity to normal hepatocytes in that they have large eosinophilic cytoplasm with a central nucleus (29).

The cytotoxic effect of natural products is based on antitumor metabolites present in them. Cytotoxic activity of the phytocompounds could be related to the occurrence of diterpenes, phlorotannins and sulfated polysaccharides. For *in vitro* study, HepG2 cell lines are broadly used to investigate metabolic and toxic effects of xenobiotics on liver. The HepG2 cell lines were made with H_2O_2 as toxicant to prepare the cell line model. H_2O_2 could produce reactive oxygen species (ROS), damage to DNA, homeostasis alterations and DNA formation adducts which leads to tumourigenesis. It oncogenic cells, there has been seen comparatively high level of ROS and promotes signalling pathways like NF-kB (Nuclear factor-kappa B) and AKT (serine/threonine-specific protein kinase) in carcinogenesis. The intracellular ROS scavenge the free radicals which can trigger apoptosis and enhance cell cycle arrest (30). Various studies have proved the importance of the role of antioxidants in cancer degeneration, utilization of diet rich in antioxidants decreases many types of cancer. In our study, *In vitro* cytotoxicity test was performed by MTT assay method against the HepG2 cells. Cells were cultured for 48 hrs in the presence of different concentrations (25, 50, 100, 200 and 400µg/ml) of *Enhalus acoroides* and percentage of cell viability was calculated. The concentration of drug causing lethality to 50% of the cells (IC_{50}) was calculated. The IC_{50} value of *Enhalus acoroides* on HepG2 cell line was found to be 122.44µg/ml.

The present report show that the cytotoxic effect fortifies with the increase in the concentration of the compound. The mechanism of viable cells recognized by cleaves the tetrazolium ring and changes over the MTT to frame an insoluble purple formazan by succinate dehydrogenase and the amount of formazan delivered is specified corresponding to the number of viable cells (31). Similar to our results, Mohd Saufee *et al.,* (2012) stated that cytotoxic activities that could be used to reduce the growth of tumour cells in cancer patients. Polyphenol compounds may restrain the malignancy cells by xenobiotic processing enzymes that change metabolic enactment of potential cancer-causing agents, while some flavonoids could also alter hormone generation and inhibit aromatase to prevent the improvement of cancer cells (32).

The main objective of this assay is to check the cytotoxicity achieved by the *Enhalus acoroides* and to discover the lethality levels in terms of IC_{50} measurement when live and dead cell rates are equivalent, which is considered as the ideal dosage for the different examines. It has been demonstrated that the *Enhalus acoroides* has anticancer activity at higher concentration. *Enhalus acoroides* has cytotoxic activity to the HepG2 cell line, which was clearly seen under an inverted microscope. MTT assay was usually used to anticipate the cytotoxicity built upon the metabolic reduction of MTT. Along these lines, the *Enhalus acoroides* is non-toxic to the normal cells and furthermore has anticancer activity against the cancerous cells.

5. CONCLUSION:

It can be concluded that hydro alcoholic extract of *Enhalus acoroides* has effective in scavenging free radicals and has the potential source of natural antioxidants and this justified its uses in folkloric medicines. Based on response in terms of scavenging radicals and reducing power activity, it is concluded that the species, *Enhalus acoroides* possessed potential antioxidant activity. It may be due to the presence of respective secondary metabolites such as phenolics, flavonoids, tannins etc. in the species. The IC_{50} value of *Enhalus acoroides* against HepG2 cell lines showed potent anticancer activity in a dose dependent manner because of the presence of many anticancer compounds which are anti-proliferative and apoptotic nature. The enhanced anticancer activity might be because of combination of multiple anti-tumor phytochemicals present in *Enhalus acoroides*. Therefore, this species can be attempted to derive the drugs of antioxidant properties. It further reflects a hope for the development of many more novel anticancer agents or templates from *Enhalus acoroides* which in future may serve to produce biologically improved therapeutic agents.

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