Original Article

Phytochemical Screening, Antioxidant Potential, Isolation And Characterization Of Bioactive Compound From Enhalus Acoroides

Navaith Ahmed[1] , Vasantha kumar S[2] , John Alphonso[3] , Shobana Chandrasekar[4] , Usharani Boopathy[*5]

[1]Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai-600117, Tamil Nadu, India. E-mail address: naviahmed@yahoo.com

[2]Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai-600117, Tamil Nadu, India. E-mail address: vasanthkumarss97@gmail.com

[3]Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai-600117, Tamil Nadu, India. E-mail address: k.johnalphonso@gmail.com

[4]Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai-600117, Tamil Nadu, India. E-mail address: shobana.sls@velsuniv.ac.in

[5]Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai-600117, Tamil Nadu, India. E-mail address : raniushab1@gmail.com
DOI: 10.47750/pnr.2022.13.S09.511

Abstract

Background: Naturally derived bioactive compounds from marine sources such as seagrasses possess bio-pharmaceutical agents. The current study focusses on qualitative, quantitative analysis of phytochemical, in-vitro antioxidant activity of different extracts, isolation and identification of compound from Enhalus acoroides. The qualitative analysis reveals abundant presence of alkaloids, flavonoids, coumarin, glycosides, terpenoids, tannin, steroid, saponin and phenol in Hydroalcoholic extract. Quantitative analysis of total alkaloid, flavonoid, phenol, tannin and terpenoids were determined on Hydroalcoholic extract of Enhalus acoroides by spectrophotometric methods. The yield was flavonoid (121.26 ± 8.48 mg QE), phenol (206.08 ±14.42 mg GAE), tannin (88.22 ± 6.17 mg TAE) and Saponin (65.20 ± 4.56 mg QUE). Hydroalcoholic extract of Enhalus acoroides was evaluated for in-vitro antioxidant assays. IC_{50} value for DPPH radical scavenging activity, Total antioxidant activity, Hydroxyl radical scavenging activity, Superoxide anion scavenging activity and Nitric oxide scavenging activity were (40.81µg/ml), (41.82µg/ml), (40.54µg/ml), (41.34µg/ml), (39.42µg/ml) respectively. Hydroalcoholic extract showed good results in scavenging free radicals. Isolation and characterisation of Bioactive compounds were performed by Column Chromatography, TLC, UV, FT-IR, Mass spectrum and NMR study. The fractions obtained from column chromatography are Hexane, Chloroform and Methanol: Chloroform. On basis of R_f value in TLC separation the isolated compound was a flavonoid derivative. FT-IR analysis shows the presence of functional groups of isolated compound. ^{1}H-NMR Spectrum of Methyl gallate confirms the signals of protons. The ^{13}C -NMR Spectrum of Methyl gallate was inveterate by the 8 carbon signals. On basis of NMR study, the isolated compound was confirmed as Methyl gallate ($C_8H_8O_5$).

KEY WORDS: Column chromatography, Enhalus acoroides, Hydroalcoholic extract, Methyl gallate, NMR study, TLC.

INTRODUCTION

Plants are essential for survival of living beings by providing food and medicine [1]. Plant extracts are extremely potent and can be utilised for a wide range of applications. Around 80% of the world's population relies on traditional medicine for health care, and majority of therapies rely on plant extracts and bioactive compounds,

concluding that two-thirds of all plant species have medicinal potential. Natural antioxidants are being utilized in cosmetics, foods, and medicinal products due to its ability to scavenge free radicals. Reactive oxygen species (ROS) are produced in response to pollution, food xenobiotics, and radiation exposure, and these ROS cause oxidative stress. Antioxidants help to prevent the production of reactive oxygen species (ROS), neutralise them, and repairs the damages [2]. Medicinal herbs have been found to be only effective treatment for a number of serious disorders, including cancer and viral infections like hepatitis and AIDS [3]. Therapeutic plants are gaining prominence as a rich source of medicinal phytochemicals that could lead to the creation of new drugs [4]. The majority of seagrasses belongs from Enhalus genera tends to grow comparatively slow, mostly distributed and found in subtidal regions rather in intertidal and shallow subtidal regions. A tape seagrass named Enhalus acoroides was studied in order to understand the antioxidant efficacy for drug development. E. acoroides is the only species in the Enhalus genera that grows in the tropical shallow intertidal coastal regions of Western Pacific Ocean and Indian Ocean [5] Enhalus acoroides has been utilized in traditional medicine for a variety of therapeutic reasons, including the treatment of feverish and skin ailments, muscle cramps, bruises, stomach disorders, stings from many sorts of rays, and as sedation for babies. Antibacterial, antiviral, antifungal, antidiabetic, anti-inflammatory, and antioxidant properties have all been reported for E. acoroides [6].

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and solvents used for the study were purchased from Sigma Chemicals (St Louis, MO, USA).

Sample collection

Enhalus acoroides as a whole plant were collected at the shallow coastal regions of Devipattinam, near Ramanathapuram District, Tamil Nadu, India during the period of May 2019. The plant sample was authenticated in Plant Anatomy Research Centre by Dr. P. Jeyaraman, Ph.D., Director, Retd Professor, Presidency College. The fresh leaves were washed with water and dried in the shade at room temperature. An electronic blender was used to pulverise the dried leaves.

Extraction and screening of phytochemicals

About 20 grams of powder sample were placed separately into three conical flasks consisting 1000ml of various solvents (Aqueous, Ethanol, and Hydro-alcohol (70%) and vigorously shaken for one hour using rotatory shaker. The mixture was kept for 24 hours. The three separate extracts were filtered with Whatman No.1 filter paper after 24 hours. For phytochemical analysis, the filtrate was kept at 4°C.

Preliminary qualitative phytochemicals screening

Chemical tests were carried out using different extracts for identification of phytochemicals following the standard methodology of Sofowara [7], Trease and Evans [8] and Harborne [9]. The phytochemicals are qualitatively identified by adding chemical reagents to the extract.

Quantitative analysis of phytochemicals

Standard techniques were used to quantify the phytocompounds contained in Aqueous, Ethanol, and Hydroalcoholic extracts of Enhalus acoroides. The total amount of phenolic contents was assessed using a spectrophotometric technique, according to Kim et al [10]. The total flavonoids assay was carried out according to Katasani's procedures [11]. The content of total flavonoids was assessed using the aluminium chloride colorimetric technique. Colorimetric methods were used to determine the total saponin content of various extracts [12]. The Bajaj and Devsharma method were used to conduct the Total Tannins assay [13].

Invitro Antioxidant activity

Antioxidants are free radical scavengers that protect the body from diseases caused by free radicals [14]. Natural antioxidants including ascorbic acid, vitamin E, and phenolic compounds can prevent oxidative damage from number of disorders [15]. Shimada et al method was used to determine DPPH radical scavenging activity [16].

For total antioxidant activity, the phosphomolybdenum methodology according to Prieto et al., was performed [17]. Hydroxyl radical scavenging activity was performed accordingly to methodology of Husain et al [18]. Superoxide radical scavenging activity was determined by methodology of Noda et al [19]. Nitric oxide radical scavenging activity was performed according to Marcocci et al [20].

Isolation of Bioactive compounds

The Harman Institute of Science Education and Research in Thanjavur was used to isolate and characterize bioactive compounds from the Hydroalcoholic extract of Enhalus acoroides.

Column Chromatography

Isolation by column chromatography is proceeded by the methodology of Zhiguo Zhang et al., 2022) [21]. The detergent-washed, distilled-water-rinsed column, which measured around 15cm in length and 4cm in diameter, was left to dry. A small piece of glass wool was inserted in the bottom half of the column once it had completely cured, and the column was kept in place with a clap and retort stand. The open end of the column was linked to a funnel, and a little amount of clean white sand was poured on top of the formerly embedded glass wool, followed by a pour of the solvent down the column. The silica gel (40 micron) was activated in the oven for 2 hours at 120°C. A well-stirred silica gel solution (100–150 g in petroleum ether, 60°C–80°C) was injected into column (150 cm long and 50 mm in diameter). After the absorbent had settled, the excess petroleum ether was allowed to flow through the column. After passing through the silica gel in petroleum ether, the slurry was fed to the well-stirred column. The progressive setting was used to ensure homogenous packing by maintaining mild agitation while the solvent ran through the column. Different solvents were used to evaluate the hydroalcoholic extract of Enhalus acoroides.

Qualitative analysis of eluate

Biochemical tests using standard methodology described by Sofowara [7], Trease and Evans [8], and Harborne [9] were used to detect the presence of flavonoids.

Purification and characteristics analysis by Thin Layer Chromatography

The methodology of Adam et al., 2002, is used to perform thin layer chromatography [22]. On the fractions eluted from Hydroalcoholic extract of Enhalus acoroides by Column Chromatography, TLC characterization is performed. A thin-layered plate is created by applying an aqueous slurry of Silica gel G to the clean surface of a glass. Calcium carbonate or starch are added to the adsorbent to increase adhesion. Afterwards the, plate is activated by baking it at 105°C for around 30 minutes. And it is let to cool in the oven. Test samples were delivered in the shape of dots using a capillary tube. The characteristics of the chemical to be separated, as well as the adsorbent, define the type of solvent used. The solvent is placed in the chamber and carefully sealed before the chromatogram is conducted, and the chamber is saturated for few hours. Capillary tubes were used to draw the fraction, that was applied to a stationary phase (silica-gel coated plate) at a distance of 1 cm from the bottom. The plate was then immersed in a solvent solution of ethyl acetate, ethanol, and water (5:1:5). The plate is placed in a well-ventilated tank in a solvent-filled container. The solvent moves to the plate's edge. As the solvent rises through the thin layer, it separates distinct components of the mixture at different speeds, resulting in spots in the thin layer. Once the solvent has reached approximately the top edge of the plate is removed from the tank and dried briefly at moderate temperatures of 60-120°C. The presence of secondary metabolites in the extracts was determined using TLC.

STRUCTURAL CHARACTERIZATION OF BIOACTIVE COMPOUND

UV-Visible Spectrum

The UV-Visible spectra of the isolated compound were recorded at room temperature using (Shimadzu UV-2450) spectrometer. Spectra were obtained using around 1 mg of chemical diluted in 20 ml of methanol, in the wavelength range of (λ) 200 to 800 nm.

FT-IR Spectrum analysis

The infrared spectrum of compound was determined using an OMNI-sampler attenuated total reflectance (ATR) attachment on an FT-IR spectrophotometer (Perkin Elmer Spectrophotometer system, USA). IR spectra for the purified compounds were obtained using KBr pellets on a Bruker series FT-IR spectrometer.

Mass Spectrometry

Mass spectrometry analysis was performed on samples of tiny molecular weight natural compounds using a JEOL GCmate II Benchtop double-focusing magnetic sector mass spectrometer in electron ionisation (EI) mode with TSS-20001 software on a JEOL GCmate II Benchtop double-focusing magnetic sector mass spectrometer in electron ionisation (EI) mode. Low-resolution mass spectra were acquired using a resolving power of 1000 (20 percent height definition) and scanning at 0.3 seconds per scan with a 0.2 second inter-scan delay between scans from m/z 80 to m/z 900. The magnet was scanned at 1 second intervals with a resolving power of 5000 to get high-resolution mass spectra.

Nuclear Magnetic Resonance Spectroscopy (NMR)

The NMR technique is used to quantify and identify individual components in complex mixtures. The NMR experiment was carried out in BRUKER-AMX400 MHz instrument with 5mg of purified compound in $DMSOd_6$ [23]. Tetra Methyl Silane is used as internal standard and chemical shifts are expressed in ppm. The mass spectrum of complex was obtained using JeolGcmate.

RESULTS AND DISCUSSION

Phytochemical analysis of Enhalus acoroides

Qualitative analysis

Phytochemicals like tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, polyphenol, coumarins and glycosides were present in Aqueous, Ethanol and Hydroalcoholic extracts. Phytocompounds like anthroquinone, emodins and anthocyanin were absent in all three extracts. Results of Phytochemical analysis are shown in Figure 1 and detailed in Table 1. Compounds in these extracts are polar in nature and solvent soluble. Flavonoids has various characteristics including enzyme inhibition, anti-inflammation, anti-allergy, antioxidant, antimicrobial activity, cytotoxicity, estrogenic activity. Flavonoids possess the ability to inhibit the bacterial growth by reducing cell wall permeability which enables them to act as antiviral and antimicrobial agents [24]. Alkaloids exhibit wide variety of pharmacological properties such as antibacterial, antimalarial, antihypertensive and anticancer activity [25]. Triterpenoids consists of steroids, glycosides and sterols possess properties like cytotoxicity, anti-inflammatory activity and used as sedative and insecticide. Steroids are used as a primary ingredient in the development of drugs (immunostimulants or immunomodulators) [26].

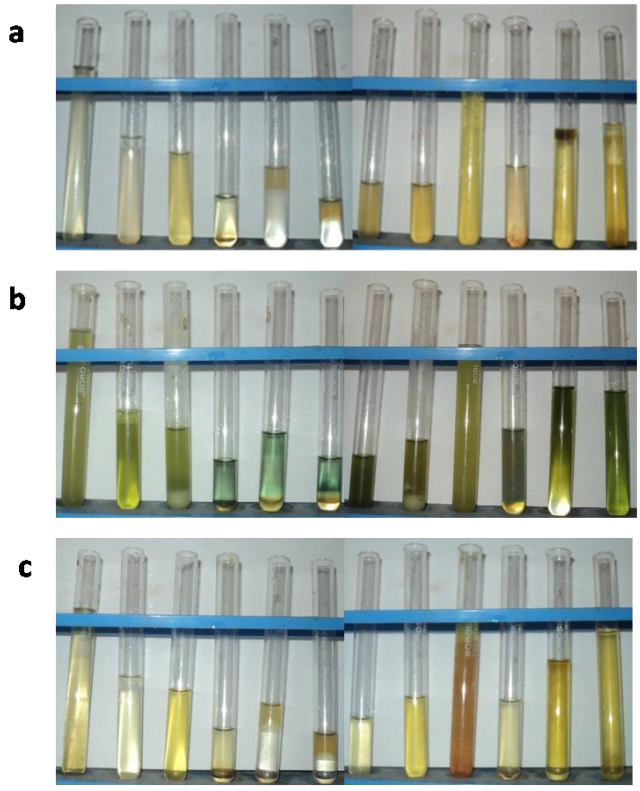


Fig. 1: Qualitative phytochemical analysis of Enhalus acoroides with Aqueous extract (a), Ethanol extract (b) and Hydro-alcoholic extract (c)

Table 1: Qualitative Phytochemical analysis of extracts of Enhalus acoroides

S.No	Phytochemicals	Aqueous extract (100%)	Ethanol extract (100%)	Hydro-alcoholic extract (70%)
1	Tannin	++	+	++
2	Saponin	++	++	++
3	Flavonoids	++	+	++
4	Steroid	++	++	++
5	Terpenoids	++	++	++
6	Triterpenoids	++	++	++
7	Alkaloids	+	++	++
8	Anthraquinone	-	-	-
9	Polyphenol	+	++	+
10	Glycosides	++	++	+
11	Coumarin	+	+	+
12	Emodins	-	-	-
13	Anthocyanins	-	-	-

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

Quantitative analysis of Phytochemicals

Quantitative analysis of total phenol, flavonoids, saponin and tannin of Hydroalcoholic extract of Enhalus acoroides are detailed in table 2.

Quantitative analysis of phenols, flavonoids, saponin and tannin shows good result in Hydroalcoholic extract of Enhalus acoroides. The ethanolic and aqueous extracts of Enhalus acoroides have a lower value in quantitative analysis. Enhalus acoroides phytoconstituents have been found to have a positive effect in various studies. Polyphenolic groups, such as tannin and phenols, have anticancer and antibacterial properties [27].

Table 2: Quantitative analysis of phenol, flavonoids, saponin and tannin of Hydroalcoholic extract of Enhalus acoroides

Name of sample	Total phenols (Milligrams of Gallic acid equivalents per gram)	Total flavonoids (Milligrams of Quercetin equivalents per gram)	Total Saponin (Milligrams of Quillaja saponin equivalents per gram)	Total tannins (Milligrams of Tannic acid equivalents per gram)
Aqueous extract	179.25 ±12.53	95.28 ± 6.65	45.84 ± 3.15	55.05± 3.85
Ethanol extract	190.00 ±14.63	101.44 ± 7.07	51.56 ± 3.57	61.44 ± 4.30
Hydroalcoholic extract	206.08±14.42	121.26±8.48	65.20±4.56	88.22±6.17

Values were expressed as mean ± Standard deviation for triplicates

Invitro antioxidant activity

Free radical reactions in the body have been associated to a wide variety of illnesses and health problems, including cardiovascular, neurological, cancer, and pulmonary problems. Free radicals play an important function in the ageing process. A major health concern is the imbalance between antioxidant enzymes and reactive oxygen species. DPPH is scavenged by ascorbic acid. Plant extracts were tested for their ability to scavenge different forms of reactive oxygen species using different methods. The best IC50 value is the one with the highest antioxidant activity. Increasing total antioxidant levels has the potential to treat neurological illnesses as well as mellitus, malignancy, and respiratory ailments [28]. Phytoconstituents like tannin, phenol, flavonoids and fatty acids possess antioxidant potential. Phytoconstituents ability to scavenge radicals were tested separately for their potential to scavenge different forms of reactive oxygen species [15]. Hydroalcoholic extract of Enhalus acoroides shows excellent antioxidant activity. Free radical scavenging capacity of Hydroalcoholic extract of Enhalus acoroides is detailed in table 3.

Table 3: In vitro Antioxidant activity of Hydroalcoholic extract of Enhalus acoroides

Tests	Samples	Concentration (µg/ml)				IC_{50} value (µg/ml)
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	
DPPH radical scavenging activity	**Hydroalcoholic extract**	23.51±1.64	37.05±2.59	63.46±4.44	84.31±5.90	48.01
	Std. (Ascorbic acid)	25.84±1.80	46.61±3.26	75.32±5.27	96.43±6.75	40.81
Total antioxidant activity	**Hydroalcoholic extract of**	22. 18±1.55	38.41±2.68	66.71±4.66	81.05±5.67	47.96
	Std. (Ascorbic acid)	26.09±1.82	45.32±3.17	72.45±5.07	93.74±6.56	41.82

Hydroxyl radical scavenging activity	**Hydroalcoholic extract**	21.86±1.53	33.51±2.34	62.48±4.37	82.19±5.75	49.99
	Std. (Ascorbic acid)	25.04±0.35	49.86±3.49	75.71±5.29	92.63±6.48	40.54
Superoxide anion scavenging activity	**Hydroalcoholic extract**	20.45±1.43	32.24±2.25	61.46±4.30	83.98±5.87	50.42
	Std. (Ascorbic acid)	24.46±1.71	47.31±3.31	74.21±5.19	95.59±6.69	41.34
Nitric oxide scavenging activity	**Hydroalcoholic extract**	23.07±1.61	35.78±2.50	68.94±4.82	86.12±6.02	46.86
	Std. (Ascorbic acid)	26.32±1.84	49.89±3.49	77.46±5.42	97.01±6.79	39.42

Isolation by Column Chromatography

On separation of Hydroalcoholic extract of Enhalus acoroides by Column Chromatography yields Hexane, Chloroform and Methanol+Chloroform (3:1) fractions. The Hexane fraction is colourless, Choloroform fraction was light green and Methanol+Chloroform (3:1) fraction was golden yellow in colour. At 40° C, the resultant fractions were dried in a rotary evaporator. Elution of Column chromatography are shown in Figure 2 and the details are depicted in table 4.

Hexane elution **Chloroform elution** **Methanol+Chloroform elution**

Figure 2: Chromatographic separation of Hydroalcoholic extract of Enhalus acoroides

Table 4 : Chromatographic Separation

S. No.	Eluents	Number of fraction(s)	Nature of fractions

1	Hexane	01	Colour less
2	Chloroform	01	Light Green
3	Methanol + Chlorofoprm (3:1)	01	Dark Yellow

Qualitative analysis of fractions

Fractions obtained from Chromatographic separation of Hydroalcoholic extract of Enhalus acoroides are subjected to qualitative analysis of flavonoids. Results of qualitative analysis are detailed in figure 3 and table 5. The presence of flavonoid in eluted fraction was determined by the formation of a yellow colour on exposure to ammonia and confirmed by comparison to Quercetin (Standard of flavonoid). Derivatives of flavonoid were present in Methanol+Chloroform fraction and absent in fractions of Hexane and Chloroform.

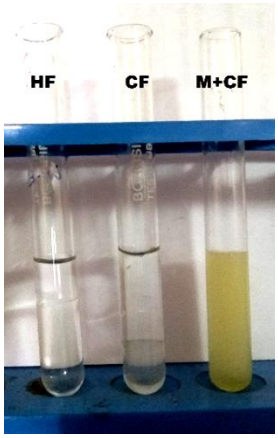


Figure 3: Qualitative analysis of flavonoid of fractions obtained from Chromatographic separation

Table 5 : Qualitative analysis of flavonoid

S. No.	Eluents	Result
1	Hexane	-
2	Chloroform	-
3	Methanol + Chloroform (3:1)	++

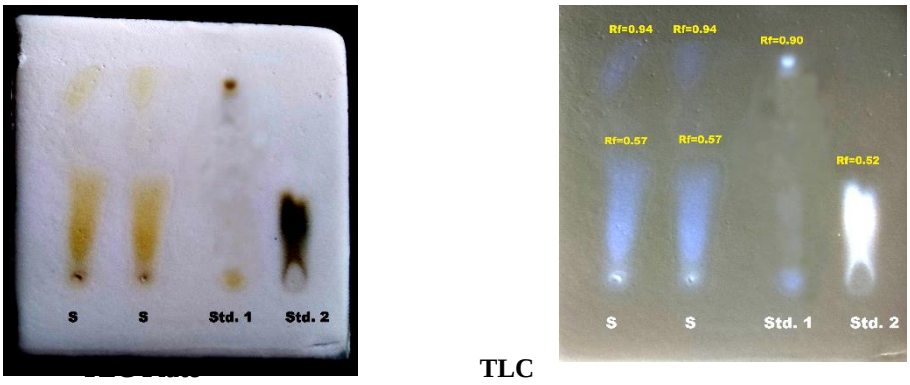
(-) Absent, (+) present and (++) High concentration

Thin layer chromatography

The compound with the highest mobile phase attraction follows it, while the compound with the highest stationary phase attraction is adsorbed on it. Various compounds appear on the TLC plate as a band, each with a different Rf value, while performing this technique. Methanol+Chloroform (3:1) fraction were subjected for TLC separation and compared to standard Quercetin. R_f value of Methanol+Chloroform (3:1) fraction is 0.94 and R_f of Standard Quercetin is 0.90. On conclusion, the flavonoid derivatives in the methanol+Chloroform fraction are higher. Results of Thin layer Chromatography are interpreted in Table 6 and Figure 4.

Table 6: Analysis of flavonoid by TLC

Sample	R_f value
Methanol+Chloroform (3:1) fraction	0.94
Std (Quercetin)	0.90



TLC

Band Lumination

Figure 4: TLC of the Methanol+Chloroform (3:1) fraction eluted from Hydroalcoholic extract of Enhalus acoroides

Characterization of Bioactive Compound

UV-Visible Spectrum

The UV λ_{max} value of isolated compound shows strong absorption band at 275 nm and suggesting the presence of aromatic moiety having auxochromes. The isolated compound is similar to UV spectrum of methyl gallate from Accacia nilotica reported by Ali Eltayeb et al [29]. UV spectrum of isolated compound is shown in Figure 5.

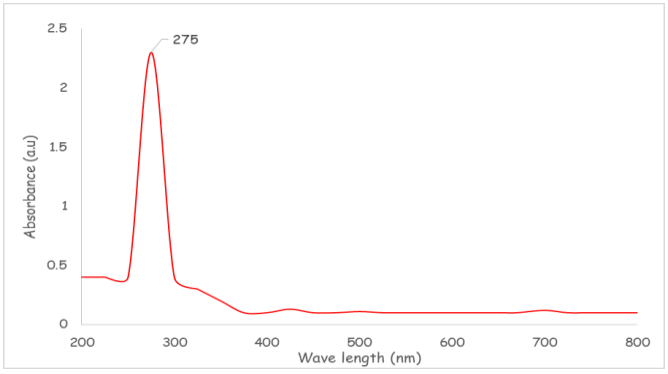


Figure 5: UV Spectrum of Methyl gallate

FT-IR Spectrum analysis

The FT-IR is used to identify and characterise compounds or functional groups in plant extracts or fractions extracted using the Chromatographic technique. The IR Spectrum of isolated compound is shown in Figure 6, indicates the absorption band at 3402.22cm^{-1} characteristic to hydroxyl functionalities with intra hydrogen bonding. A(-C-H) absorption band at 2975.72cm^{-1} was observed. Band at 1718.06cm^{-1} indicates the presence of -C=O group. Presence of aromatic C=C group shows band at 1648.94cm^{-1}. Structure of isolated compound possess methyl (-CH_3) group, having characteristic band at 1450-1400cm^{-1}. Multiple absorption frequencies ranging from

1300-1000cm^{-1} shows presence of -C-O bonds in varies chemical environment. Presence of methine moiety (C-H) shows sharp peak at 703.84cm^{-1}. Ali Eltayeb et al previously reported the IR spectra of Methyl gallate from Accacia nilotica [30].

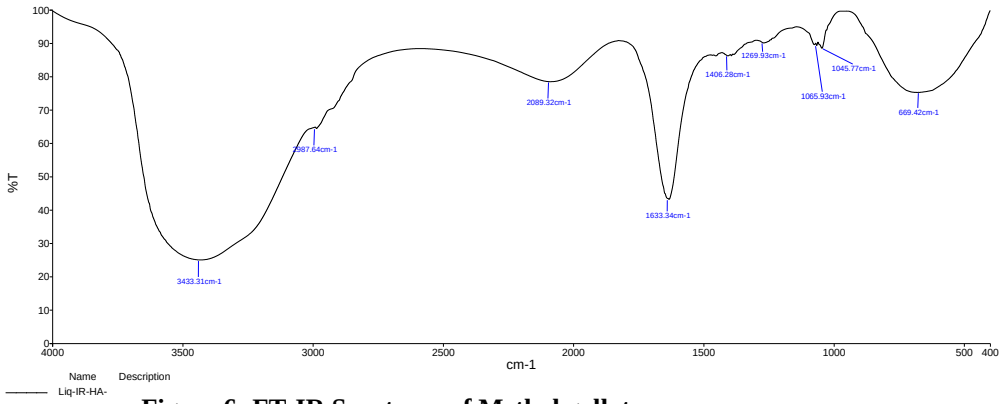


Figure 6: FT-IR Spectrum of Methyl gallate

Mass Spectrometry

The electrospray ionization spectra (ESI-MS) were obtained on an Aglient 1260 series single Quadrupole LC/MS system. The methanol fraction of Hydroalcoholic extract of Enhalus acoroides subjected to LCMS. Mass spectrometric analysis by (ESI-MS) showed the m/z $[M^+H]^+$ of isolated compound as 185.06. The molecular formula of compound is $C_8H_8O_5$. Methyl gallate isolated from Archidendron jiringa has a mass spectrum peak at 185.35, according to Misri Yanty Lubis et al characterization of the compound's spectrum [30]. The chromatogram of isolated compound is shown in Figure 7.

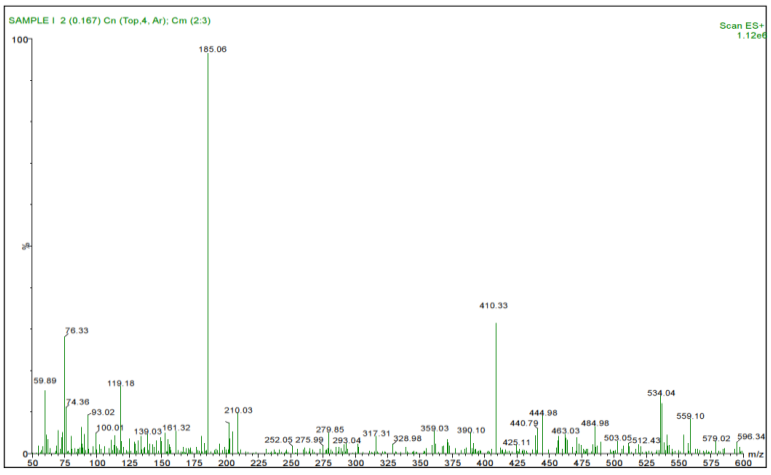


Figure 7: Mass spectrum of Methyl gallate

^{1}H-NMR Spectrum of Methyl gallate

NMR data of proton H in compound is characetized and shown in Figure 8. The peak between δ6.5 to 7.03 shows two protons at position 2 and 6 and δ3.77 along the data NMR of proton H bounded with OCH_3. The NMR data of proton H was compared with NMR data of methyl gallate isolated from mushroom (Pholiota adiposa). The peaks of proton H methyl gallate from mushroom (P.adiposa) were at δ7.04 with two protons (H) at position 2 and 6, δ3.79 shows H bounded with OCH_3. Methyl gallate isolated by Kamatham et al from seed coats of

Givotiarottleriformis Griff shows NMR data as proton peak δ7.22 with two protons at position 2 and 6 [31]. The compound was characterized as Methyl gallate with ^{1}H-NMR results.

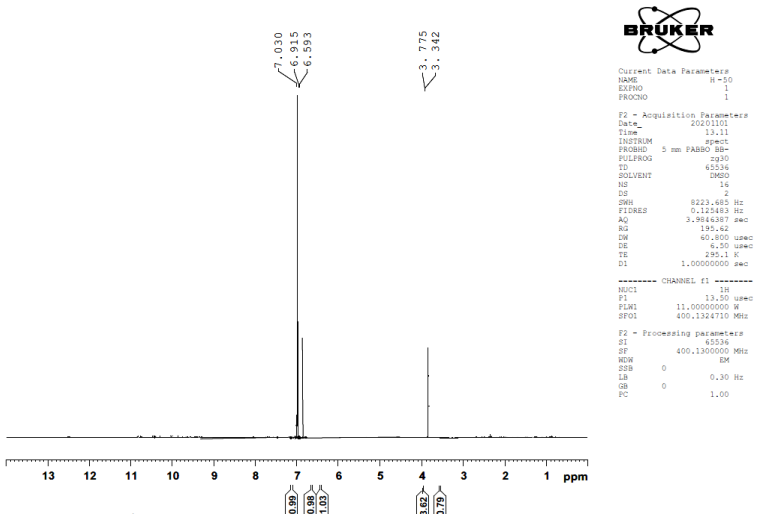


Figure 8: ^{1}H-NMR Spectrum of Methyl gallate

^{13}C -NMR Spectrum of Methyl gallate

The structure of Methyl gallate was characterized by ^{13}C -NMR analysis and NMR spectrum is shown in figure 9. The peaks at δ166.85 identified carbon at position -COOH, δ146.10 and δ146.63 of positions of carbon at 3 and 5, δ138.76 position of carbon at 4, δ119.17 (C-1), δ109.08 and δ109.17 of C2, C6 and δ52.12(-OCH_3) confirms the compound as Methyl gallate by comparing with NMR data of carbons of Methyl gallate from mushroom (P.adiposa). Data NMR of carbon methyl gallate isolated from Pholiota adiposa by Wang et al., shows peaks at δ168.99, carbon position at -COOH, δ146.34(C3, C5), δ139.66(C4), δ121.38(C1), δ110.00 (C2, C6) and δ52(-OCH_3) [32].

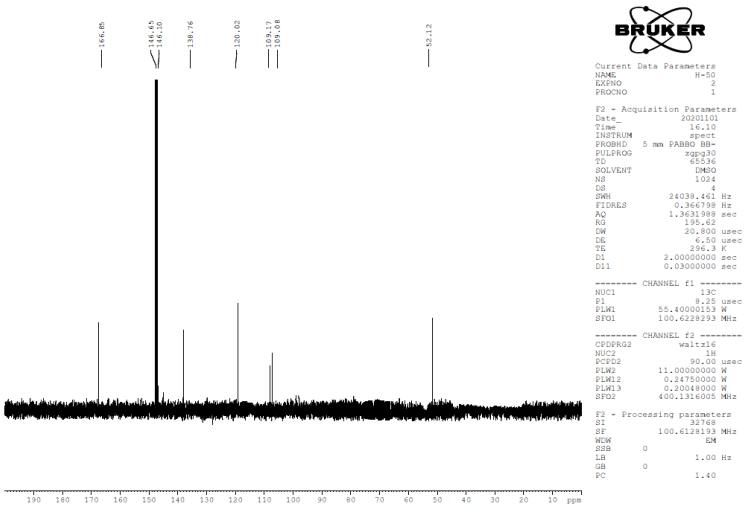


Figure 9: ^{13}C -NMR Spectrum of Methyl gallate

Isolated Compound

Based of spectral and chemical analysis, the isolated compound has been characterized as Methyl gallate (Methyl 3,4,5-trihydroxybenzoate) as shown in Figure 10.

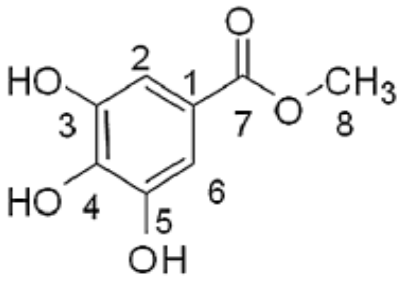


Figure 10: Isolated compound - Methyl gallate (Methyl 3,4,5-trihydroxybenzoate)

CONCLUSION

The goal of current study is phytochemical investigation, in-vitro antioxidant study, Isolation and Characterization of bioactive compound. The preliminary phytochemical screening shows the presence of tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, polyphenol, coumarins and glycosides in Aqueous, Ethanol and Hydroalcoholic extracts. Phytocompounds like anthroquinone, emodins and anthocyanin were absent in all three extracts. The total phenols, flavonoids, saponin and tannin are determined in Hydrolacoholic extract. The antioxidant activity of the Hydroalcoholic extract of Enhalus acoroides was determined in-vitro, revealing that it scavenges free radicals due to presence of phytoconstituents. We can conclude that Enhalus acoroides are high in antioxidants and may help to treat diseases caused by oxidative stress. Isolation and characterization of bioactive compound on Hydroalcoholic extract of Enhalus acoroides results in yield of Hexane, Chloroform and Methanol+Chloroform (3:1) fractions. The Hexane fraction is colourless, Choloroform fraction was light green and Methanol+Chloroform (3:1) fraction was golden yellow in colour. The qualitative analysis performed on fractions revealed the presence of flavonoid in Methanol+Chloroform (3:1) fraction on comparison to standard (Quercetin). The Rf value of the Methanol fraction from Thin Layer Chromatography demonstrates that the fraction contains flavonoid on comparison to the Rf value of Quercetin (a flavonoid standard). Spectra obtained from FT-IR analysis shows the presence of functional groups of isolated compound. The m/z $[M^+H]^+$ of the isolated compound was 185.06g/mol according to mass spectrometric analysis (ESI-MS). The chemical formula of compound is $C_8H_8O_5$.The ^{1}H-NMR study shows the positions and peaks of protons bounded with OCH_3 and analysis of position of carbon atoms in compound by ^{13}C NMR confirms the compound as Methyl gallate. The isolated compound was confirmed as Methyl gallate (Methyl 3,4,5-trihydroxybenzoate) with Molecular formula $C_8H_8O_5$ on basis of FT-IR, Mass spectrum and NMR study. Futher study of Methyl gallate deals with in-vitro cell line study and in-silico docking studies.

Acknowledgments

The authors would like to acknowledge Dr. S.Velavan, Ph.D., Managing Director, Harman Institute of science education and research, Thanjavur for providing facilities and assisting during the research work.

References

1. Gomathy G, Vijay T, Sarumathy K, Gunasekaran S, Palani S. Phytochemical screening and GC-MS analysis of Mukia maderaspatana (L.) leaves. Journal of Pharmaceutical Science. 2012; 2(12): 104-106.

2. Made Pharmawati, Luh Putu Wrasiati. Phytochemical screening and FTIR spectroscopy on crude extract from Enhalus acoroides Leaves. Malaysian Journal of Analytical Sciences. 2020; 24(1): 70-77.

3. Rafaz Ahmad Dar, Mohd Shahwaz, Parvaiz Hasan Qazi. General overview of medicinal plants: A review. The journal of Phytopharmacology. 2017; 6(6): 349-351.

4. Azwanida NN. A Review on the extraction methods use in medicinal plants, Principle, Strength and Limitation. Medicinal & Aromatic Plants. 2015; 4(3): 1-6

5. Amudha P, Vanitha V, Pushpa Bharathi N, Jayalakshmi M. Phytochemical analysis and invitro antioxidant screening of seagrass Enhalus acoroides. Health & Environmental Research Online (HERO). 2017; 8(2): 251-258.

6. Febry SI Menajang, Mohammad Mahmudi, Uun Yanuhar, Endang Yuli Herawati. Evaluation of phytochemical and superoxide dismutase activities of Enhalus acoroides (L.f.) royle from coastal waters of North Sulawesi, Indonesia. Veterinary World. 2020; 13(4): 676-680.

7. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993; 289.

8. Trease GE & Evans WC. Phenols and Phenolic glycosides. In: Textbook of Pharmacognosy. (12th edition.). Balliese, Tindall and Co Publishers, London. 1989; 343-383.

9. Harborne, J. B. Phytochemical methods, London. Chapman and Hall, Ltd. 1973; 49-188.

10. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 2003; 81: 321-326.

11. Katasani D. Phytochemical screening, quantitative estimation of total phenolic, flavonoids and antimicrobial evaluation of Trachyspermumammi, Journal of Atoms and molecules. 2011; 1: 1-8.

12. Hiai S, Oura H, Nakajima T. Color reactions of some sapogenins and saponins with vanillin and sulphuric acid. Planta Medica. 1976; 29 : 116-122.

13. Bajaj KL and Devsharma AK. A Calorimetric method for the determination of tannins in Tea. Mikrochimica Acta (Wien)1997; 2: 249-253.

14. Moyeenudin HM, Vijayalakshmi S. The Antihypertensive Effect from Aqueous Extract of Oxalis corniculata by In Vitro Antihypertensive Activity Assay. Research Journal of Pharmacy and Technology. 2019; 12(6): 2981-2986.

15. Vaghela JS, Sisodia SS. In vitro antioxidant activity of Terminalia chebula Fruit extracts. Research Journal of Pharmacy and Technology. 2011; 4(12): 1835-1843.

16. Shimada K, Fujikawa K, Yahara K and Nakamura T. Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992; 40: 945–948.

17. Prieto P, Pineda M and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex Specie application to the determination of vitamin E. Analytical Biochemistry. 1999; 269: 337-341.

18. Rafat Hussain S, Josiane Cillard, Pierre collard. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry. 1987; 26(9): 2489-2491.

19. Yasuko Noda, Kazunori Anzai, Akitane Mori. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR Spectrometer system. Biochemistry and Molecular biology international. 1997; 42(1): 35-44.

20. Marcocci L, Maguire J J, Droylefaix MT, Packer L. The Nitric Oxide-Scavenging Properties of Ginkgo Biloba Extract EGb 761. Biochemical and Biophysical Research Communications. 1994; 201(2): 748-755.

21. Zhiguo Zhang, Meilin Wang, Shu Xing, Chen Zhang. Flavonoids of Rosa rugosa Thumb. Inhibit tumor proliferation and metastasis in human hepatocellular carcinoma HepG2 cells. Food Science and Human Wellness. 2022. 11, 374-382.

22. Adam JH, Omar R, and Wilcock CC. Phytochemical screening of flavonoid in three hybrids of Napenthes (Napenthaceae) and their putative parental species from Sarawak and Sabah, Journal of Biological Sciences. 2002; 2(9): 623-625.

23. Verpoorte R, Choi YH, Kim HK. NMR-based metabolomics at work in phytochemistry. Phytochem Rev. 2007; 6: 3-14.

24. Kurmukov. Medicinal plants of central Asia: Uzbekistan and Kyrgystan. Phytochemistry of Medicinal Plants. 2012; 13-14.

25. Jeffrey B Harborne, Christine A Williams. Advances in flavonoid research since 1992. Phytochemistry. 2000; 55(6): 481-504.

26. Liza Meutia Sari, Rachmi Fanani Hakim, Zaki Mubarak, Andriyanto Andriyanto. Analysis of phenolic compounds and immunomodulatory activity of areca nut extract from Aceh, Indonesia, against Staphylococcus aureus infection in Sprague-Dawley rats. Veterinary World. 2020; 13(1): 134-140.

27. Kavitha M, Dr. Srinivasan PT, Lakshmi Priya S, Jayakumar LV. Evaluation of Nutrient Composition and Antibacterial potential of leaf and stem of Mukia maderaspatana I. International journal of Pharmaceutical Research & Allied Sciences. 2013; 2(3): 68-76.

28. Kalaivani P, Kavitha D, Amutha P. In vitro Antioxidant activity and phytochemical composition of Syringodium isoetifoelium. Research journal of pharmacy and Technology. 2021; 14(12): 6201-6206.

29. Ali Eltayeb, Eyman Mohamed Eltayi, Malik Suliman Mohamed. Tilal Elsaman, Elrasheed Ahmed Gadkariem, Magdi Awadalla Mohamed. Bioactivity guided isolation of methyl gallate as an active antileishmanial agent from Accacia nilotica. Pharmacology online. 2021; 1: 298-305.

30. Misri Yanty Lubis, Rikson Siburian, Lamek Marpaung, Partomuan Simanjuntak, Muhammad Pandapotan Nasution. Methyl gallate from Jiringa Archidendron jiringa and antioxidant activity. Asian journal of Pharmaceutical and Clinical Research. 2018; 11(1): 346-350.

31. Kamatham S, Kumar N, Gudipalli P. Isolation and characterization of gallic acid and methyl gallate from the seed coats of Givotia rottleriformis griff. And their anti-proliferative effect on human epidermis carcinoma A431 cells. Toxicol Rep. 2015; 2: 520-529.

32. Wang CR, Zhou R, Ng TB, Wong JH, Qaio WT, Liu F. First report on isolation of methyl gallate with antioxidant, anti-HIV-1 and HIV-1 enzyme inhibitory activities from a mushroom (Pholiota adiposa). Environ Toxicol Pharmacol. 2014; 37: 626-637.