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ORIGINAL ARTICLE **OPEN ACCESS**

In-Vitro Anticancer activity of Phyto-Pharmacological and GC-MS Analysis of Bioactive Compounds presents in *Avicennia marina* Leaves Ethanolic Extract *a*gainst Hep-G2 Cell Line

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ABSTRACT
Mangroves are the compound manufacturing plants of nature and broad wellsprings of natural synthetic issue on this planet. The conventional methods are poor, time consuming as well as less systematic. The present study was carried out to A. marina and explores. This research may be useful for constituent(s)-based pharmacological activity. Plants are a rich wellspring of optional metabolites with intriguing organic exercises. The phyotochemical analysis of A. marina reveals the presence of tannin, flavonoids, terpenoids, alkaloids, cardiac glycosides, and steroids. .A. marina is a traditional medicinal plant and the leaves have tremendous medicinal values. In the current examination ethanolic leaf concentrate of A. marina was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) and the Major bioactive 12 compound structures were identified with help of National Institute of Standards and Technology (NIST) library. The different acetone extract of A. marina were prepared 40mg/ml, 80mg/ml, 120mg/ml, 160mg/ml, and Cyclophasphamide (Positive control) 180 µg/ml and their activity were determined by MTT Assay using Hep G2 Cell line (liver).Among these concentration the maximum anti-cancerous activity were observed at 120 mg/ml and it determined by using MTT Assay.
KEYWORDS - *Anticancer activity, Avicennia marina, GC-MS, Hep G2 Cell line, MTT assay.*

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INTRODUCTION

Mangrove plants are potential sources of biologically active chemicals that are discernible from their wide spread application in ethno pharmaceutical practices. One of these attributes is the secondary metabolites produced by the mangroves which have been used traditionally by medicinal local practitioners due to their proved medicinal values [1]. Nature has provided a complete storehouse of remedies to cure all ailments of mankind by providing our drugs in the form of herbs, plants and algae to cure the incurable diseases without any toxic effects. Nowadays allopathic system usage was decreased due to side effects, adverse reactions, so now a day's herbal drugs usage was increased due to fewer side effects and patience acceptance in these way herbal drugs usage was increased [2]. In recent decades, plants are being used as source of folk medicine for the treatment and prevention of various diseases because of infrequent side effects and significant results. Plant derived medicines are a cheap source of novel compounds and are in practice for the prevention and procurement of human, animal and plant diseases [3]. Alternative strategies of prime means are now emerging from bioactive compounds isolated from plants to limit the specific pathogens with no toxic effects on beneficial microbes, less environment retention and to rapidly degrade [4]. *Chenopodium quinoa* Wild a nutrition rich pseudo cereal crop belongs to family Chenopodiaceae, is widely cultivated in Andean highlands of South and North America. This crop is recently introduced in Pakistan because of its high commercial value and increasing demand all around the world. It is grown as a staple food with extreme tolerance to a biotic stresses, less water and fertilizers demand. All parts of the plant are utilized but the seeds are outstanding because of their protein contents (10-16%), lipids (6-10%), free sugars (9-16%), and minerals (0.6-1%). It is grown as a staple food with extreme tolerance to a biotic stresses, less water and fertilizers demand. All parts of the plant are utilized but the seeds are outstanding because of their protein contents (10-16%), lipids (6-10%), free sugars (9-16%), and minerals (0.6-1%) [5]. One function of these phytochemical contents can

protect against free radicals. Free radicals found in the environment could be tackled by antioxidant compounds. Antioxidant compounds exist in many plants such as sea grass, seaweed and mangrove. Antioxidant also exists in mangrove (*Avicennia marina*). Extracts and chemicals from mangroves are used mainly as folkloric medicine, insecticides, pesticides, and these practices continue until today [6]. Mangrove is used in traditional medicine for the treatment of skin disorders, boils and wounds, the against human and shrimp pathogens and to delineate bioactive constituents phyto-constituents of ethanolic extract of *Evolvulus alsinoides* whole part using gas chromatography-mass spectroscopy (GC-MS) [7]. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry [8]. Standardization of plant materials and using modern controlled technique and applying suitable standards [9]. Plants are integral part of human civilization. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs. Drugs based on the Plants are of prime importance for several remedies in traditional and conventional medicine throughout the world and serves as a substitute for drug supply in modern medicine. Tetracosane, also called tetrakosane, is an alkane hydrocarbon. Tetracosane showed some cytotoxic activity against AGS, MDA-MB-231, HT-29 and NIH 3T3 cells [10]. Medicinal plant extracts contain various types of bioactive compounds known as phytochemicals [23]. Traditional medicine can be used in treatment as anticancer, antimicrobial, antioxidant, anti-inflammatory agents. The studies show that these phytochemicals are safe, broadly effective and have less adverse effects [11]. The present study aims to investigate the presence of phytochemicals and anti-cancerous activity of *Coleus forskohlii* rhizome extract [12]. In the present study the crude methanol extracts of *Avicennia marina* were prepared by using soxhlet apparatus and to investigate the efficacy of the extract against HT-29 cell line. The anticancer activity of A. marina leaf extract on proliferation of HT-29 cancer cell line was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) micro culture tetrazolium viability assay [13,26].

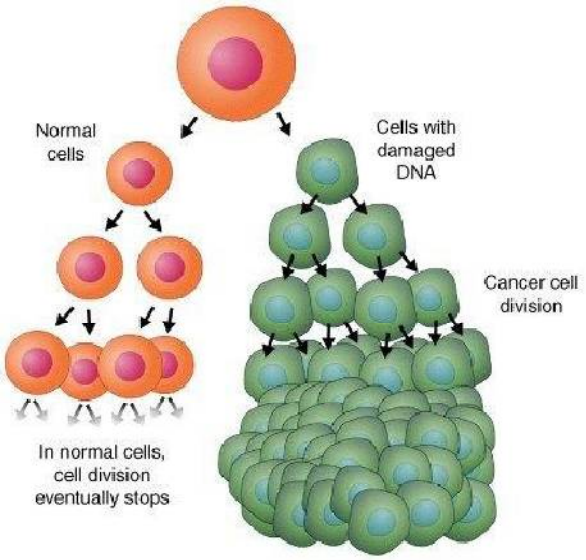


Fig: 1. Cancer cells and normal cell in multiplication

In organism, these exogenous antioxidants can manifest a good sort of actions, including inhibition of oxidizing enzymes, chelation of transition metals, transfer of hydrogen or one electron to radicals, singlet oxygen deactivation, or enzymatic detoxification of reactive oxygen species, cellular membrane stability and involves inhibition of radical production alongside enhancement of the body defence system [14,25]. In spite of good advancements for diagnosis and treatment, cancer is still a big threat to our society. In the last few decades, there has been an exponential growth in the field of herbal medicine. The aim of the present study is to evaluate the anticancer activity of Vitis vinifera against MCF 7 breast cell line.100gof Vitis vinifera pulp extract was mixed with 300 mL of ethanol [15]. Mangroves are the compound manufacturing plants of nature and broad wellsprings of natural synthetic issue on this planet and secondary metabolites of properties [16]. Recently, the bioactive compounds are gaining much importance for their ability in enhancing resistance to various diseases and to improve the health of people both by traditional and modern ways of administrations. *Avicennia marina* have high nutritive value and had potential bioactive substances that may be used as pharmaceutical ingredients for formulation of new or prospective potent drug to cure wide range of metabolic diseases.

MATERIALS AND METHOD

Plant Collection

Fresh and Healthy leaves of *Avicennia marina* were collected from their natural habitat of Muthupet mangrove in Thiruvarur district, Tamil Nadu, India.

Extraction of mangrove plant leaves

After washing with distilled water, the leaves were shade dried, powdered and extracted separately in ethanol. Plant powder (20 gm) was taken and absorbed 100 ml of dissolvable and kept in shaker for 24 hrs. After centrifugation at 5000 rpm, the solvent phase was separated and evaporated. The crude was stored at 40º C and used for further studies.

Phytochemical Qualitative Analysis

The ethanolic leaves extracts were assessed for the existence of the phytochemical analysis by using the standard methods [17-18].

Gas Chromatography-Mass spectrometry (GC-MS) analysis

Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column was used for GCMS analysis. Initially, the instrument was set to temperature of 110°C, and then maintained at the same temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C per minute and maintained for 9 min. The temperature of injection port was ensured as 250°C and the flow rate of Helium as 1 ml/min. The ionization voltage was 70 eV. The samples were injected gradually in split mode as 10:1. The range of mass spectrum was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The discontinuity examples of mass spectra were contrasted and those put away in the spectrometer information base utilizing National Institute of Standards and Technology Mass Spectral information base (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds

Translation of mass range of GC-MS was directed utilizing the information base of National Institute Standard and Technology (NIST) having in excess of 62,000 examples. The unknown component's spectrum was compared with the spectrum of the known components stored in the NIST library. The structure, name and sub-atomic load of the parts of the test materials was learned.

Anticancer activity from leaves extracts of *A. marina*

Hep G2 cell line (Liver) was obtained from National centre for cell sciences Pune (NCCS). The cells were kept up in Minimal Essential Media enhanced with 10% FBS (Fetal Bovine serum), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified air of 50 µg/ml CO2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories, Foetal bovine serum (FBS) was purchased from Cistron laboratories. Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO).

In vitro assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on Hep G2 cell line (Liver) was determined by the MTT assay. Cells (1 × 105/well) were plate during 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 24 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 24h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide cells (MTT) phosphate -buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Estimations were performed and the focus required for a half restraint of feasibility (IC50) was resolved graphically. The absorbance at 570 nm was estimated with an UV-Spectrophotometer utilizing wells without test containing cells as spaces. The effect of the samples on the proliferation of Hep G2 cell line (Liver) was expressed as the % cell viability, using the following formula:

$$\% \text{ cell reasonability} = A570 \text{ of treated cells}/A570 \text{ of control cells} \times 100$$

Statistical analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were communicated as Mean ± Standard Deviation for p values <0.05 were considered significant for analysis of percent inhibition of cell growth.

RESULTS AND DISCUSSION

The preliminary antibiotic screening of *A. marina* revealed that there is a distinct difference between the antibiotic activities against test organisms with regards to the type of solvents used for the extraction of three solvents extract tested, crude extract of *A. marina* obtain from ethanol was important growth of every one test pathogens. The high rank of inhibitory action of the ethanolic extract might be due to the presence of higher concentration of the antibiotic constituents. However, antimicrobial activity observed in other solvent extracts was much inferior and therefore excluded in the further studies. As potent inhibitory activity was detected in ethanolic extract, it can be inferred the antibiotic compounds present.

Table: 1. Qualitative analysis of *A. marina leaves* extract

S. No	Analysed Phytochemicals factor	Ethanol	Methanol	Water
1.	Tannin	++	+	-
2.	Phlobatannins	-	+	+
3.	Saponin	-	+	-
4.	Flavonoids	++	-	-
5.	Steroids	+	-	+
6.	Terpenoids	+	+	+
7.	Triterpenoids	+	+	-
8.	Alkaloids	+	-	+
9.	Carbohydrate	+	-	-
10.	Protein	-	-	-
11.	Anthraquinone	+	-	+
12.	Polyphenol	++	+	+
13.	Glycoside	+	+	-

Indications: "+" means positive activity, "-" means negative activity

In concordance with our studies, purported that ethanolic leaf extract of *A. marina* showed the highest antibacterial activity [19]. Every constituent plays an important role and deficiency of anyone constituent may lead to abnormal developments in the body [20].

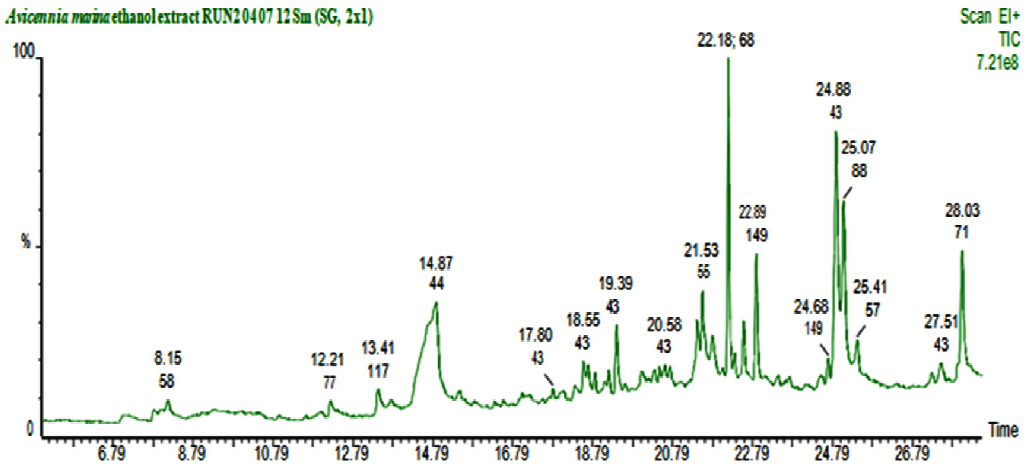
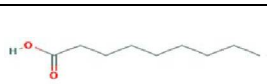
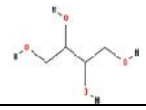
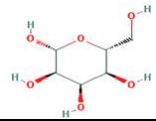
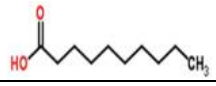
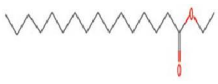
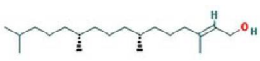


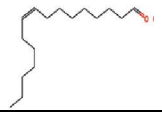
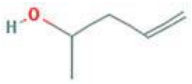
Figure 2: GC-MS CHROMATOGRAM OF *A. marina LEAVES* EXTRACT

Table 2: GCMS analysis - Bioactive compounds

S. No.	Name of the Compounds	Molecular weight g/mol	Molecular Formula	Retention time	Peak area
1	Nonanoic acid	**158**	$C9^{H}{}_{18}{}^{O}2$	13.75	1902114
2	2(R),3(S)-1,2,3,4-Butane Tetrol	**122**	$C_4H_{10}{}^{O}{}_4$	14.88	108862904
3	D-Allose	**180**	$C_6H_{12}O_6$	18.33	3060763
4	Dodecanoic acid	**200**	$C_{12}H_{24}O_2$	18.55	11052012
5	3,7,11,15-tetramethyl-2-hexadecen-1-ol	**296**	$C_{20}{}^{H}40^{O}$	22.18	34397904
6	N Hexadecanoic acid	**256**	$C_{16}H_{32}O_2$	24.88	56945332
7	Hexadecanoic acid, ethyl Ester	**284**	$C_{18}H_{36}O_2$	25.06	44265320
8	Phytol	**296**	$C_{20}H_{40}O$	28.03	21068804
9	(E)-9-Octadecenoic acid ethyl ester	**310**	$C_{20}H_{38}O_2$	29.04	23578426
10	(E-)Octadecanoic acid, 2-methyl-, methyl ester	**310**	$C_{20}H_{38}O_2$	29.50	23578426
11	cis-9 Hexadecenal	**238**	$C_{16}H_{30}O$	32.96	7569641
12	Squalene	**410**	$C_{30}H_{50}$	41.30	127456712

Fig 3: GC-MS Analysis of Activities/Uses of bioactive compounds of *A. marina* leaves

S. No.	Name of the Compounds	Nature of the compound	Structure	Activity / Uses
1	Nonanoic acid	Fatty acid		Anti-seizures
2	2(R),3(S)-1,2,3,4-Butane Tetrol	Polyol		Antioxidant, antihyperglycemic
3	D-Allose	Aldohexose sugar		Antioxidative activity
4	Dodecanoic acid	Saturated fatty acid		Antimicrobial, anti-inflammatory
5	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Terpene alcohol		Antimicrobial and anti-inflammatory
6	N Hexadecanoic acid	Palmitic acid		Anti-inflammatory, nematicide, pesticide, lubricant, antiandrogenic, flavor, haemolytic 5-alpha reductase inhibitor, antioxidant, hypocholesterolemic
7	Hexadecanoic acid, ethyl Ester	Fatty acid ester		Antioxidant, Hypocholesterolemic.
8	Phytol	Diterpene		Antimicrobial, anti-inflammatory, anticancer, diuretic
9	(E-) (E)-9-Octadecenoic acid ethyl ester	Fatty acid		Antioxidant, anti-inflammatory

10	Octadecanoic acid, 2-methyl-, methyl ester	Fatty acid		Potent antifungal, antimicrobial, antibacterial
11	cis-9 Hexadecenal	Aldehyde		antioxidant, antimicrobial and ant diarrheal activity
12	Squalene	Triterpene		Antibacterial, antioxidant, antitumor, cancer preventive immunostimulant, chemo preventive, lipoxygenaseinhibitor, pesticide

2,6-bis (1,1-dimethylethyl)-, Decane, 2,4,6-trimethyl-, 2,2-bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane. Above these compounds were identified based on the RT value, molecular weight, molecular formula, etc [21-24]. The ethyl acetate fraction was subjected to GC-MS analysis and nine compounds were identified, including undecane (**1**), benzene, nitro- (**2**), -ascorbic acid 2,6-dihexadecanoate (**3**), octadec-9-enoic acid (**4**), benzene,1,1'- (oxydi-2,1-ethanediyl) bis 3-ethyl- (**5**), cis-9-hexadecenal (**6**), 1,2-benzedicarboxylic acid, diisooctyl ester (**7**), 10- nonadecanone (**8**) and 1-triacontanol (**9**). Literature survey revealed that most of the identified compounds possess diverse biological activities [22].

IN VITRO Assays (Cytotoxic studies)

MTT Assay

The IC_{50} value for Hep G2 cell line (Liver) (120 mg/ml) ethanolic leaves extract was found to be effective, the reduction percentage of MTT at 24Hrs also estimated for Hep G2 cell line (Liver). When incubated with the extract, it induced cytotoxicity in a significant manner which implicit the damage to the member integrity of the cell when contributed with control. The cytotoxicity was minimize in extract treated cells and near normal level was attain at various concentrations (40mg/ml, 80 mg/ml, 120mg/ml and 160mg/ml) and maximum effect was found when treated at 120 mg/ml. From the above results, it was confirmed that *A. marina* Acetone rhizome extract at 120 mg/ml seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane and this active concentration was followed for further studies.

Tryphan Blue assay

Tryphan blue is one of the several stains optional used for dye exclusion process for viable cell counting. This assay is based on the principle that live cells do not take up blue, where as dead cells do and appear as blue under microscope - depicts the viability of cells by Tryphan Blue assay. The viability is measured in terms of percentage was found to decreased 98% in drug treated hepatic cell line. The cell treated with *A. marina* Acetone leaves extract at various concentrations (40mg/ml, 80 mg/ml, 120mg/ml and 160mg/ml) showed protective nature of the extract act against the deleterious effects and the maximum effect was observed at 120 mg/ml.

Table 3: MTT reduction % on Hep G2 cell line (Liver)

S. No	Concentration(µg)	MTT reduction (%)
1	Control	98.17
2	400	84.12
3	80	71.47
4	**120**	**46.03**
5	160	22.67
6	Positive control	11.13

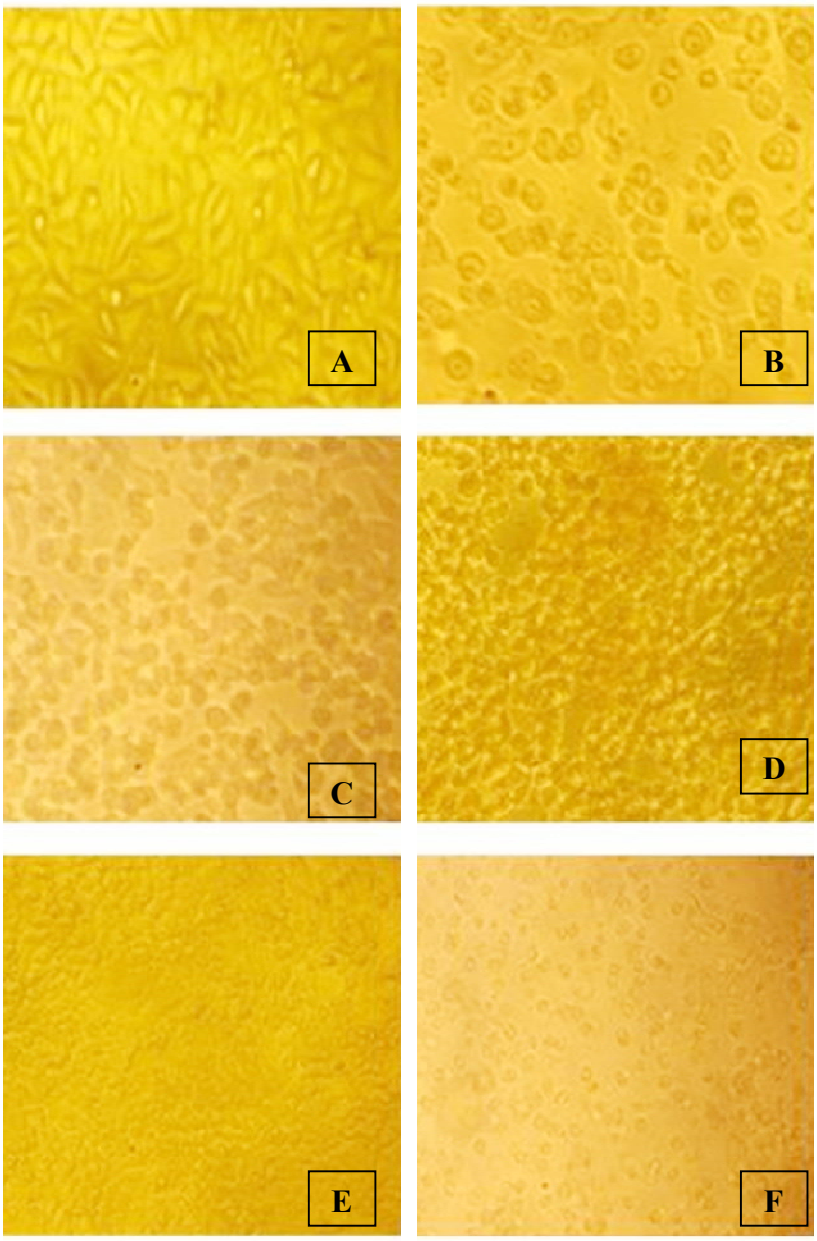
Table 4: Tryphan blue viability assay

S. No	Concentration(µg)	% Viability
1	Control	99.0
2	400	87.00
3	80	71.3
4	**120**	**51.04**
5	160	36.3
6	Positive control	10.67

Table 5: Showing the IC_{50} value of cell line

S. NO	Cell line	IC_{50} Value
	Hep G2 cell line (Liver) leaves extract of *A. marina*	120 mg/ml

Fig 3: Showing the effect *A. marina* leaves extract on Hep G2 cell line



a: Control cells (Untreated), **b:** *A. marina* leaves extract 40mg/ml, **c**: *A. marina* leaves extract 80 mg/ml, **d:** ***A. marina*** extract 120mg/ml, **e:** *A. marina* leaves extract 160 mg/ml, **f:** Cyclopha sphamide (Positive control) 180 µg/ml.

CONCLUSION

The presence of various bioactive compounds in the *A.marina* justifies the use of whole plant for various ailments by traditional practitioners. However the isolation of individual phytochemical compound and analyzing their biological activity will definitely yield productive results. From the results, it could be concluded that *A.marina* contains various bioactive compounds The GC-MS analysis of ethanolic extract of experimental plant showed the presence of pharmacologically major 12 bioactive compounds such as antioxidant and anti-Hyperlipidemic. This plant can be saved through biotechnological approaches and its

quality can be improved through secondary metabolites production and thus it can be used as a source for developing new drugs and commercialization. In the present study the phytochemical analysis of *A. marina* reveals, the presences of absences and MTT Assay, using Hep G2 Cell line (liver) at 120 mg/ml acetone leaves extract was found to be effective, the reduction percentage of MTT and cytotoxicity were also determined. *A. marina* is a good candidate for anticancer activity. It can be a new source as for antitumor medicine and efficiently used as a hepatoprotective agent after successful clinical trials.

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CONFLICT OF INTEREST
The authors stated that no conflicts of interest.

REFERENCES
1. S Poompozhil, D Kumarasamy. (2014).Studies on Phytochemical Constituents of Some Selected Mangroves, Journal of Academia and Industrial Research. 2(1): 590-592.
2. Mahadevi M.* and Azhagu Madhavan S. (2020). Pharmacognostical and Phytochemical Screening of Physico-Chemical Parameters and Fluorescence Analysis on Ethanolic Leaves Extract of *Ipomoea sepiaria.* KOENIG EX. ROXB. Waffen-Und Kostumkunde Journal, Volume XI, Issue VIII, ISSN NO: 0042-9945.
3. K Malik, M. Ahmad, G. Zhang, N. Rashid, M. Zafar, S. Sultana and S.N. Shah. (2018). Traditional plant based medicines used to treat musculoskeletal disorders in Northern Pakistan. *European Journal of Integrative Medicine*,19-17-64.
4. E Mitrani, E. Perdum, O.G. Iordache and I. Dumitrescu. (2018),Advantages and disadvantages of pesticide analysis methods used in agricultural samples. *Scientific Papers-Series B-Horticulture,* 62(2018)-709-714.
5. P.J Maughan, L. Chaney, D.J. Lightfoot, B.J. Cox, M. Tester, E.N. Jellen and D.E. Jarvis.(2019). Mitochondrial and chloroplast genomes provide insights into the evolutionary origins of quinoa (*Chenopodium quinoa* Willd.). *Scientific Reports*,9-185.
6. W Bandaranayake, M Wetl. Ecol. Manag..10,(2002)- 421.
7. B Alizadeh Behbahani F. Tabatabei Yazdi, F. Shahidi, F. Riazi, (2016). Antifungal effect of the aqueous and ethanolic Avicennia marina extracts on Alternaria citri and Penicillium digitatum, Zahedan J. Res. Med. Sci,. 18 (2) 1–6.
8. M Boominathan and V Ramamurthy,. (2009). Antimicrobial activity of *Heliotropium indicum* and *Coldeniaprocumbens*. J. Ecobiol.,; 24 (1). 11 – 15.
9. P Sharma, Kaushik S, Jain A, Sikarwar SM. (2019). Preliminary phytochemical screening and HPTLC fingerprinting of *Nicotianatabacum* leaf. J. Pharm. Res,; 3(5): 1144-1145.
10. J Shaikh Jamal, D Uddin, Darren Grice, T Evelin Tiralongo. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichumaureum.* Pharmaceutical Biology,; 50 (10): Pg No: 112-114.
11. M Mahadevi* and S Azhagu Madhavan. *(2010). In Vitro* Antioxidant Properties and Free Radical Scaveneing Activity of Aqueous Extract of Papaya Root. Alochana Chakra Journal, 2020 ;Volume IX, Issue V,231-240.
12. X Asbin Mary, L Robert Lotha. (2016). Phytochemical Screening And Anticancerous Activity of Rhizome,Extract *Coleus Forskholi* on Hep G2 Cell Line. National Conference on "Emerging Trends in Management of Infectious Diseases and Public Health,;4 – 5 Feb 16.
13. N Shanthi*, S Murugesan, S Janetta Nithia and M Kotteswari. (2019). *In Vitro* Anticancer Activity of Methanol Extracts of *Avicennia Marina* (Forssk) Vireh Against Ht-29 Colon Cancer Cell Line, Vol. 8, Issue 6, (2019). ISSN: 2319-5622. DOI: https://doi.org/10.5281/zenodo.3265336.
14. S Azhagu Madhavan, P Vinotha, V Uma. (2020). In Vitro Antioxidant Properties And Free Radical Scaveneing Activity of Ethanolic Extract of *Costus Spicatus (Jacq*-1788).; "International Conference on Cancer Research". Int J Life Sci Pharma Res. ISSN 2250 – 0480; SP-07.
15. S Dhasaradha. Bai1, A. Silpha Tenish, P.G.Geegi, And P.Anitha. (2020). Anticancer Activity of Ethanolic Extract of *Vitis Vinifera* L. Using Mcf7 Cell Lines. "International Conference on Cancer Research". Int J Life Sci Pharma Res. ISSN 2250 – 0480; SP-07.
16. M Padma, S Ganesan, T Jayaseelan, S Azhagumadhavan, P Sasikala, S Senthilkumar, P Mani. (2019). Phytochemical screening and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L). Journal of Drug Delivery & Therapeutics.; 9(1):85-89. DOI: http://dx.doi.org/10.22270 /jddt.v9i1.2174.
17. A Sofowora, (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria.
18. G. E Trease. and W. C. Evans, (1989). "Phenols and phenolic glycosides," in Textbook of Pharmacognosy,; vol. 12, pp.343–383, Balliese, Tindall and Co Publishers, London, UK.

19. M Aseer Manilal, T Tsegaye Tsalla, Z Zerihun Zerdo, A Gemechu Ameya1, M Behailu Merdekios, E Shiju Easo. John (2016). Evaluating the antibacterial and anticandidal potency of mangrove, *Avicennia marina* [*Asian Pac J Trop Dis*; 6(2): 136-140). doi: 10.1016/S2222-1808(15)60999-9.
20. S Andrews, S Azhagu Madhavan, S Ganesan, P Arjun, R Jeyaprakash. S Baskara Sanjeevi and M. Ramasamy. (2020). Different Bioactive Constituents and Biochemical Composition of Brown Seaweed *Spatoglossum marginatum.* Waffen-Und Kostumkunde Journal, 2020; Volume XI, Issue IV, ISSN NO: 0042-9945.
21. S. Azhagu Madhavan. GC–MS Analysis of Bioactive Compounds Present In Ethanolic Leaf Extract *Acalypha indica*. Asian Journal of Advances in Research. (2021). 6(4): 16-22.2021.
22. S. Azhagu Madhavan, Vinotha P2 and Uma V3. Pharmacological and Anti Cancer Activity of *Ipomoea sepiaria* Methanolic Extract against PC-3 Cell Line. Asian Journal of Advances in Medical Science. (2020);(3): 26-32.2020.
23. M. Mahadevi and S. Azhagu Madhavan S..(2020). Anticancer Activity of *Piper Nigrum* Methanolic Extract Against A549 Human Lung Cancer Cell Line. Vol-43, No.-4, (R) October-December 2020. Sambodhi Journal. ISSN NO: 2249-6661.
24. S. Azhagu Madhavan, P. Vinotha and V. Uma. (2020). Phytochemical Screening And Comparative GC–Ms Analysis of Bioactive Compounds Present In Methanolic Leaf and Latex Extract *Calotropis Gigantea* (L). Asian Journal of Advances in Medical Science.(2020); 2(2): 1-13.
25. P Sasikala, S Ganesan, T Jayaseelan, S Azhagumadhavan, M Padma, S Senthilkumar ,P Mani. (2019). Phytochemicals and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of Taraxacum officinale (L). Journal of DrugDelivery&Therapeutics.;9(1):90-94.
26. K Iqra Haider Khan, J Arshad Javaid, A Dildar Ahmed and K Uzman Khan. (2020). Identification of Volatile Constituents of Ethyl Acetate Fraction of *Chenopodium Quinoa* Roots Extract By GC-MS *INT. J. BIOL. BIOTECH.,*; 17 (1). 17-21.

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In-Vitro Anticancer activity of Phyto-Pharmacological and GC-MS Analysis of Bioactive Compounds presents in *Avicennia marina* Leaves Ethanolic Extract *a*gainst Hep-G2 Cell Line

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ABSTRACT
Mangroves are the compound manufacturing plants of nature and broad wellsprings of natural synthetic issue on this planet. The conventional methods are poor, time consuming as well as less systematic. The present study was carried out to A. marina and explores. This research may be useful for constituent(s)-based pharmacological activity. Plants are a rich wellspring of optional metabolites with intriguing organic exercises. The phyotochemical analysis of A. marina reveals the presence of tannin, flavonoids, terpenoids, alkaloids, cardiac glycosides, and steroids. .A. marina is a traditional medicinal plant and the leaves have tremendous medicinal values. In the current examination ethanolic leaf concentrate of A. marina was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) and the Major bioactive 12 compound structures were identified with help of National Institute of Standards and Technology (NIST) library. The different acetone extract of A. marina were prepared 40mg/ml, 80mg/ml, 120mg/ml, 160mg/ml, and Cyclophasphamide (Positive control) 180 µg/ml and their activity were determined by MTT Assay using Hep G2 Cell line (liver).Among these concentration the maximum anti-cancerous activity were observed at 120 mg/ml and it determined by using MTT Assay.
KEYWORDS - *Anticancer activity, Avicennia marina, GC-MS, Hep G2 Cell line, MTT assay.*

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INTRODUCTION

Mangrove plants are potential sources of biologically active chemicals that are discernible from their wide spread application in ethno pharmaceutical practices. One of these attributes is the secondary metabolites produced by the mangroves which have been used traditionally by medicinal local practitioners due to their proved medicinal values [1]. Nature has provided a complete storehouse of remedies to cure all ailments of mankind by providing our drugs in the form of herbs, plants and algae to cure the incurable diseases without any toxic effects. Nowadays allopathic system usage was decreased due to side effects, adverse reactions, so now a day's herbal drugs usage was increased due to fewer side effects and patience acceptance in these way herbal drugs usage was increased [2]. In recent decades, plants are being used as source of folk medicine for the treatment and prevention of various diseases because of infrequent side effects and significant results. Plant derived medicines are a cheap source of novel compounds and are in practice for the prevention and procurement of human, animal and plant diseases [3]. Alternative strategies of prime means are now emerging from bioactive compounds isolated from plants to limit the specific pathogens with no toxic effects on beneficial microbes, less environment retention and to rapidly degrade [4]. *Chenopodium quinoa* Wild a nutrition rich pseudo cereal crop belongs to family Chenopodiaceae, is widely cultivated in Andean highlands of South and North America. This crop is recently introduced in Pakistan because of its high commercial value and increasing demand all around the world. It is grown as a staple food with extreme tolerance to a biotic stresses, less water and fertilizers demand. All parts of the plant are utilized but the seeds are outstanding because of their protein contents (10-16%), lipids (6-10%), free sugars (9-16%), and minerals (0.6-1%). It is grown as a staple food with extreme tolerance to a biotic stresses, less water and fertilizers demand. All parts of the plant are utilized but the seeds are outstanding because of their protein contents (10-16%), lipids (6-10%), free sugars (9-16%), and minerals (0.6-1%) [5]. One function of these phytochemical contents can

protect against free radicals. Free radicals found in the environment could be tackled by antioxidant compounds. Antioxidant compounds exist in many plants such as sea grass, seaweed and mangrove. Antioxidant also exists in mangrove (*Avicennia marina*). Extracts and chemicals from mangroves are used mainly as folkloric medicine, insecticides, pesticides, and these practices continue until today [6]. Mangrove is used in traditional medicine for the treatment of skin disorders, boils and wounds, the against human and shrimp pathogens and to delineate bioactive constituents phyto-constituents of ethanolic extract of *Evolvulus alsinoides* whole part using gas chromatography-mass spectroscopy (GC-MS) [7]. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry [8]. Standardization of plant materials and using modern controlled technique and applying suitable standards [9]. Plants are integral part of human civilization. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs. Drugs based on the Plants are of prime importance for several remedies in traditional and conventional medicine throughout the world and serves as a substitute for drug supply in modern medicine. Tetracosane, also called tetrakosane, is an alkane hydrocarbon. Tetracosane showed some cytotoxic activity against AGS, MDA-MB-231, HT-29 and NIH 3T3 cells [10]. Medicinal plant extracts contain various types of bioactive compounds known as phytochemicals [23]. Traditional medicine can be used in treatment as anticancer, antimicrobial, antioxidant, anti-inflammatory agents. The studies show that these phytochemicals are safe, broadly effective and have less adverse effects [11]. The present study aims to investigate the presence of phytochemicals and anti-cancerous activity of *Coleus forskohlii* rhizome extract [12]. In the present study the crude methanol extracts of *Avicennia marina* were prepared by using soxhlet apparatus and to investigate the efficacy of the extract against HT-29 cell line. The anticancer activity of A. marina leaf extract on proliferation of HT-29 cancer cell line was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) micro culture tetrazolium viability assay [13,26].

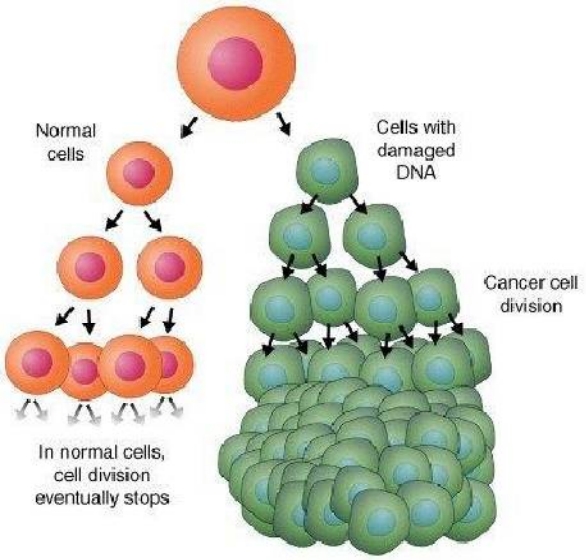


Fig: 1. Cancer cells and normal cell in multiplication

In organism, these exogenous antioxidants can manifest a good sort of actions, including inhibition of oxidizing enzymes, chelation of transition metals, transfer of hydrogen or one electron to radicals, singlet oxygen deactivation, or enzymatic detoxification of reactive oxygen species, cellular membrane stability and involves inhibition of radical production alongside enhancement of the body defence system [14,25]. In spite of good advancements for diagnosis and treatment, cancer is still a big threat to our society. In the last few decades, there has been an exponential growth in the field of herbal medicine. The aim of the present study is to evaluate the anticancer activity of Vitis vinifera against MCF 7 breast cell line.100gof Vitis vinifera pulp extract was mixed with 300 mL of ethanol [15]. Mangroves are the compound manufacturing plants of nature and broad wellsprings of natural synthetic issue on this planet and secondary metabolites of properties [16]. Recently, the bioactive compounds are gaining much importance for their ability in enhancing resistance to various diseases and to improve the health of people both by traditional and modern ways of administrations. *Avicennia marina* have high nutritive value and had potential bioactive substances that may be used as pharmaceutical ingredients for formulation of new or prospective potent drug to cure wide range of metabolic diseases.

MATERIALS AND METHOD

Plant Collection

Fresh and Healthy leaves of *Avicennia marina* were collected from their natural habitat of Muthupet mangrove in Thiruvarur district, Tamil Nadu, India.

Extraction of mangrove plant leaves

After washing with distilled water, the leaves were shade dried, powdered and extracted separately in ethanol. Plant powder (20 gm) was taken and absorbed 100 ml of dissolvable and kept in shaker for 24 hrs. After centrifugation at 5000 rpm, the solvent phase was separated and evaporated. The crude was stored at 40º C and used for further studies.

Phytochemical Qualitative Analysis

The ethanolic leaves extracts were assessed for the existence of the phytochemical analysis by using the standard methods [17-18].

Gas Chromatography-Mass spectrometry (GC-MS) analysis

Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column was used for GCMS analysis. Initially, the instrument was set to temperature of 110°C, and then maintained at the same temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C per minute and maintained for 9 min. The temperature of injection port was ensured as 250°C and the flow rate of Helium as 1 ml/min. The ionization voltage was 70 eV. The samples were injected gradually in split mode as 10:1. The range of mass spectrum was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The discontinuity examples of mass spectra were contrasted and those put away in the spectrometer information base utilizing National Institute of Standards and Technology Mass Spectral information base (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds

Translation of mass range of GC-MS was directed utilizing the information base of National Institute Standard and Technology (NIST) having in excess of 62,000 examples. The unknown component's spectrum was compared with the spectrum of the known components stored in the NIST library. The structure, name and sub-atomic load of the parts of the test materials was learned.

Anticancer activity from leaves extracts of *A. marina*

Hep G2 cell line (Liver) was obtained from National centre for cell sciences Pune (NCCS). The cells were kept up in Minimal Essential Media enhanced with 10% FBS (Fetal Bovine serum), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified air of 50 µg/ml CO2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories, Foetal bovine serum (FBS) was purchased from Cistron laboratories. Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO).

In vitro assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on Hep G2 cell line (Liver) was determined by the MTT assay. Cells (1 × 105/well) were plate during 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 24 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 24h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide cells (MTT) phosphate -buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Estimations were performed and the focus required for a half restraint of feasibility (IC50) was resolved graphically. The absorbance at 570 nm was estimated with an UV-Spectrophotometer utilizing wells without test containing cells as spaces. The effect of the samples on the proliferation of Hep G2 cell line (Liver) was expressed as the % cell viability, using the following formula:

$$\% \text{ cell reasonability} = A570 \text{ of treated cells}/A570 \text{ of control cells} \times 100$$

Statistical analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were communicated as Mean ± Standard Deviation for p values <0.05 were considered significant for analysis of percent inhibition of cell growth.

RESULTS AND DISCUSSION

The preliminary antibiotic screening of *A. marina* revealed that there is a distinct difference between the antibiotic activities against test organisms with regards to the type of solvents used for the extraction of three solvents extract tested, crude extract of *A. marina* obtain from ethanol was important growth of every one test pathogens. The high rank of inhibitory action of the ethanolic extract might be due to the presence of higher concentration of the antibiotic constituents. However, antimicrobial activity observed in other solvent extracts was much inferior and therefore excluded in the further studies. As potent inhibitory activity was detected in ethanolic extract, it can be inferred the antibiotic compounds present.

Table: 1. Qualitative analysis of *A. marina leaves* extract

S. No	Analysed Phytochemicals factor	Ethanol	Methanol	Water
1.	Tannin	++	+	-
2.	Phlobatannins	-	+	+
3.	Saponin	-	+	-
4.	Flavonoids	++	-	-
5.	Steroids	+	-	+
6.	Terpenoids	+	+	+
7.	Triterpenoids	+	+	-
8.	Alkaloids	+	-	+
9.	Carbohydrate	+	-	-
10.	Protein	-	-	-
11.	Anthraquinone	+	-	+
12.	Polyphenol	++	+	+
13.	Glycoside	+	+	-

Indications: "+" means positive activity, "-" means negative activity

In concordance with our studies, purported that ethanolic leaf extract of *A. marina* showed the highest antibacterial activity [19]. Every constituent plays an important role and deficiency of anyone constituent may lead to abnormal developments in the body [20].

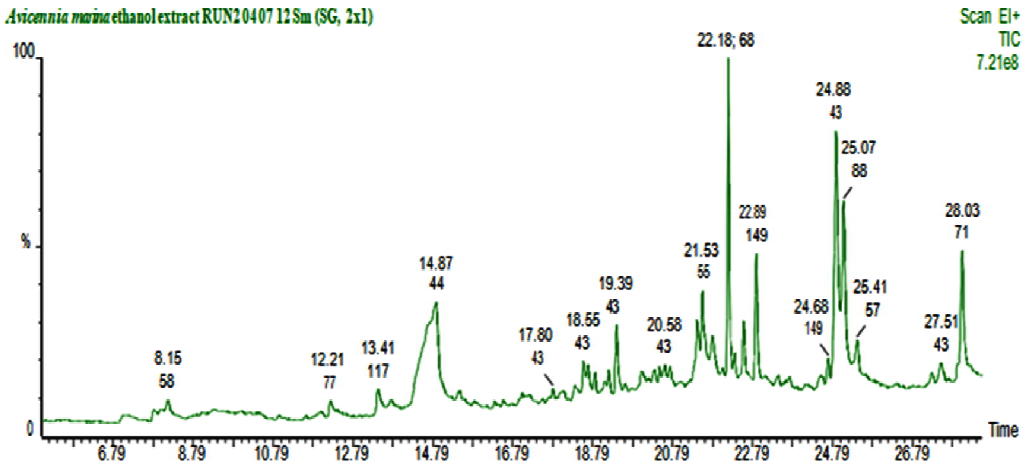
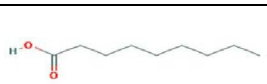
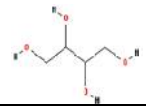
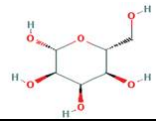
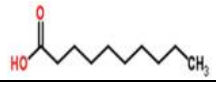
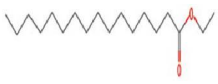
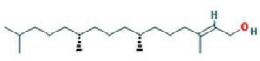


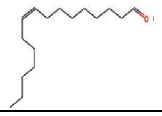
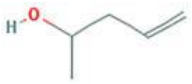
Figure 2: GC-MS CHROMATOGRAM OF *A. marina LEAVES* EXTRACT

Table 2: GCMS analysis - Bioactive compounds

S. No.	Name of the Compounds	Molecular weight g/mol	Molecular Formula	Retention time	Peak area
1	Nonanoic acid	**158**	$C9^{H}{}_{18}{}^{O}2$	13.75	1902114
2	2(R),3(S)-1,2,3,4-Butane Tetrol	**122**	$C_4H_{10}{}^{O}{}_4$	14.88	108862904
3	D-Allose	**180**	$C_6H_{12}O_6$	18.33	3060763
4	Dodecanoic acid	**200**	$C_{12}H_{24}O_2$	18.55	11052012
5	3,7,11,15-tetramethyl-2-hexadecen-1-ol	**296**	$C_{20}{}^{H}40^{O}$	22.18	34397904
6	N Hexadecanoic acid	**256**	$C_{16}H_{32}O_2$	24.88	56945332
7	Hexadecanoic acid, ethyl Ester	**284**	$C_{18}H_{36}O_2$	25.06	44265320
8	Phytol	**296**	$C_{20}H_{40}O$	28.03	21068804
9	(E)-9-Octadecenoic acid ethyl ester	**310**	$C_{20}H_{38}O_2$	29.04	23578426
10	(E-)Octadecanoic acid, 2-methyl-, methyl ester	**310**	$C_{20}H_{38}O_2$	29.50	23578426
11	cis-9 Hexadecenal	**238**	$C_{16}H_{30}O$	32.96	7569641
12	Squalene	**410**	$C_{30}H_{50}$	41.30	127456712

Fig 3: GC-MS Analysis of Activities/Uses of bioactive compounds of *A. marina* leaves

S. No.	Name of the Compounds	Nature of the compound	Structure	Activity / Uses
1	Nonanoic acid	Fatty acid		Anti-seizures
2	2(R),3(S)-1,2,3,4-Butane Tetrol	Polyol		Antioxidant, antihyperglycemic
3	D-Allose	Aldohexose sugar		Antioxidative activity
4	Dodecanoic acid	Saturated fatty acid		Antimicrobial, anti-inflammatory
5	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Terpene alcohol		Antimicrobial and anti-inflammatory
6	N Hexadecanoic acid	Palmitic acid		Anti-inflammatory, nematicide, pesticide, lubricant, antiandrogenic, flavor, haemolytic 5-alpha reductase inhibitor, antioxidant, hypocholesterolemic
7	Hexadecanoic acid, ethyl Ester	Fatty acid ester		Antioxidant, Hypocholesterolemic.
8	Phytol	Diterpene		Antimicrobial, anti-inflammatory, anticancer, diuretic
9	(E-) (E)-9-Octadecenoic acid ethyl ester	Fatty acid		Antioxidant, anti-inflammatory

10	Octadecanoic acid, 2-methyl-, methyl ester	Fatty acid		Potent antifungal, antimicrobial, antibacterial
11	cis-9 Hexadecenal	Aldehyde		antioxidant, antimicrobial and ant diarrheal activity
12	Squalene	Triterpene		Antibacterial, antioxidant, antitumor, cancer preventive immunostimulant, chemo preventive, lipoxygenaseinhibitor, pesticide

2,6-bis (1,1-dimethylethyl)-, Decane, 2,4,6-trimethyl-, 2,2-bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane. Above these compounds were identified based on the RT value, molecular weight, molecular formula, etc [21-24]. The ethyl acetate fraction was subjected to GC-MS analysis and nine compounds were identified, including undecane (**1**), benzene, nitro- (**2**), -ascorbic acid 2,6-dihexadecanoate (**3**), octadec-9-enoic acid (**4**), benzene,1,1'- (oxydi-2,1-ethanediyl) bis 3-ethyl- (**5**), cis-9-hexadecenal (**6**), 1,2-benzedicarboxylic acid, diisooctyl ester (**7**), 10- nonadecanone (**8**) and 1-triacontanol (**9**). Literature survey revealed that most of the identified compounds possess diverse biological activities [22].

IN VITRO Assays (Cytotoxic studies)

MTT Assay

The IC_{50} value for Hep G2 cell line (Liver) (120 mg/ml) ethanolic leaves extract was found to be effective, the reduction percentage of MTT at 24Hrs also estimated for Hep G2 cell line (Liver). When incubated with the extract, it induced cytotoxicity in a significant manner which implicit the damage to the member integrity of the cell when contributed with control. The cytotoxicity was minimize in extract treated cells and near normal level was attain at various concentrations (40mg/ml, 80 mg/ml, 120mg/ml and 160mg/ml) and maximum effect was found when treated at 120 mg/ml. From the above results, it was confirmed that *A. marina* Acetone rhizome extract at 120 mg/ml seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane and this active concentration was followed for further studies.

Tryphan Blue assay

Tryphan blue is one of the several stains optional used for dye exclusion process for viable cell counting. This assay is based on the principle that live cells do not take up blue, where as dead cells do and appear as blue under microscope - depicts the viability of cells by Tryphan Blue assay. The viability is measured in terms of percentage was found to decreased 98% in drug treated hepatic cell line. The cell treated with *A. marina* Acetone leaves extract at various concentrations (40mg/ml, 80 mg/ml, 120mg/ml and 160mg/ml) showed protective nature of the extract act against the deleterious effects and the maximum effect was observed at 120 mg/ml.

Table 3: MTT reduction % on Hep G2 cell line (Liver)

S. No	Concentration(µg)	MTT reduction (%)
1	Control	98.17
2	400	84.12
3	80	71.47
4	**120**	**46.03**
5	160	22.67
6	Positive control	11.13

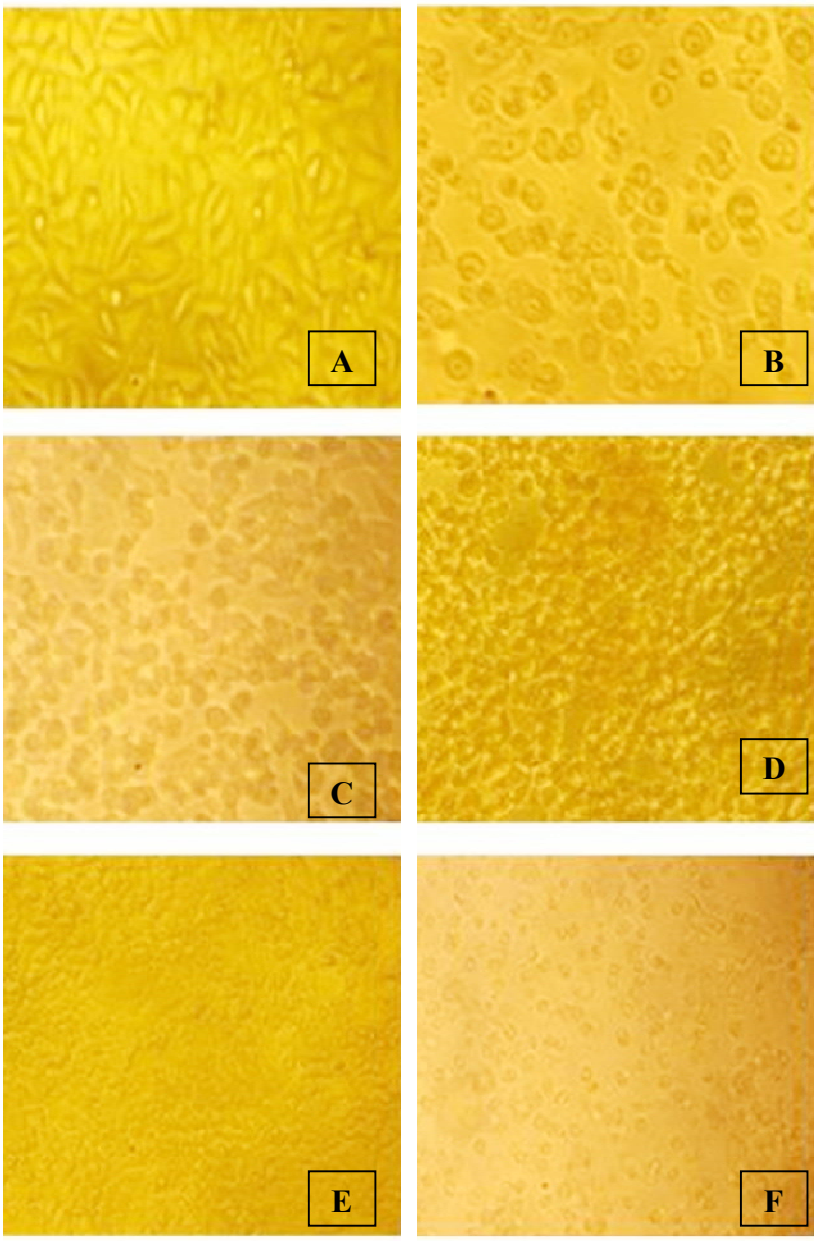
Table 4: Tryphan blue viability assay

S. No	Concentration(µg)	% Viability
1	Control	99.0
2	400	87.00
3	80	71.3
4	**120**	**51.04**
5	160	36.3
6	Positive control	10.67

Table 5: Showing the IC_{50} value of cell line

S. NO	Cell line	IC_{50} Value
	Hep G2 cell line (Liver) leaves extract of *A. marina*	120 mg/ml

Fig 3: Showing the effect *A. marina* leaves extract on Hep G2 cell line



a: Control cells (Untreated), **b:** *A. marina* leaves extract 40mg/ml, **c**: *A. marina* leaves extract 80 mg/ml, **d:** ***A. marina*** extract 120mg/ml, **e:** *A. marina* leaves extract 160 mg/ml, **f:** Cyclopha sphamide (Positive control) 180 µg/ml.

CONCLUSION

The presence of various bioactive compounds in the *A.marina* justifies the use of whole plant for various ailments by traditional practitioners. However the isolation of individual phytochemical compound and analyzing their biological activity will definitely yield productive results. From the results, it could be concluded that *A.marina* contains various bioactive compounds The GC-MS analysis of ethanolic extract of experimental plant showed the presence of pharmacologically major 12 bioactive compounds such as antioxidant and anti-Hyperlipidemic. This plant can be saved through biotechnological approaches and its

quality can be improved through secondary metabolites production and thus it can be used as a source for developing new drugs and commercialization. In the present study the phytochemical analysis of *A. marina* reveals, the presences of absences and MTT Assay, using Hep G2 Cell line (liver) at 120 mg/ml acetone leaves extract was found to be effective, the reduction percentage of MTT and cytotoxicity were also determined. *A. marina* is a good candidate for anticancer activity. It can be a new source as for antitumor medicine and efficiently used as a hepatoprotective agent after successful clinical trials.

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CONFLICT OF INTEREST
The authors stated that no conflicts of interest.

REFERENCES
1. S Poompozhil, D Kumarasamy. (2014).Studies on Phytochemical Constituents of Some Selected Mangroves, Journal of Academia and Industrial Research. 2(1): 590-592.
2. Mahadevi M.* and Azhagu Madhavan S. (2020). Pharmacognostical and Phytochemical Screening of Physico-Chemical Parameters and Fluorescence Analysis on Ethanolic Leaves Extract of *Ipomoea sepiaria.* KOENIG EX. ROXB. Waffen-Und Kostumkunde Journal, Volume XI, Issue VIII, ISSN NO: 0042-9945.
3. K Malik, M. Ahmad, G. Zhang, N. Rashid, M. Zafar, S. Sultana and S.N. Shah. (2018). Traditional plant based medicines used to treat musculoskeletal disorders in Northern Pakistan. *European Journal of Integrative Medicine*,19-17-64.
4. E Mitrani, E. Perdum, O.G. Iordache and I. Dumitrescu. (2018),Advantages and disadvantages of pesticide analysis methods used in agricultural samples. *Scientific Papers-Series B-Horticulture,* 62(2018)-709-714.
5. P.J Maughan, L. Chaney, D.J. Lightfoot, B.J. Cox, M. Tester, E.N. Jellen and D.E. Jarvis.(2019). Mitochondrial and chloroplast genomes provide insights into the evolutionary origins of quinoa (*Chenopodium quinoa* Willd.). *Scientific Reports*,9-185.
6. W Bandaranayake, M Wetl. Ecol. Manag..10,(2002)- 421.
7. B Alizadeh Behbahani F. Tabatabei Yazdi, F. Shahidi, F. Riazi, (2016). Antifungal effect of the aqueous and ethanolic Avicennia marina extracts on Alternaria citri and Penicillium digitatum, Zahedan J. Res. Med. Sci,. 18 (2) 1–6.
8. M Boominathan and V Ramamurthy,. (2009). Antimicrobial activity of *Heliotropium indicum* and *Coldeniaprocumbens*. J. Ecobiol.,; 24 (1). 11 – 15.
9. P Sharma, Kaushik S, Jain A, Sikarwar SM. (2019). Preliminary phytochemical screening and HPTLC fingerprinting of *Nicotianatabacum* leaf. J. Pharm. Res,; 3(5): 1144-1145.
10. J Shaikh Jamal, D Uddin, Darren Grice, T Evelin Tiralongo. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichumaureum.* Pharmaceutical Biology,; 50 (10): Pg No: 112-114.
11. M Mahadevi* and S Azhagu Madhavan. *(2010). In Vitro* Antioxidant Properties and Free Radical Scaveneing Activity of Aqueous Extract of Papaya Root. Alochana Chakra Journal, 2020 ;Volume IX, Issue V,231-240.
12. X Asbin Mary, L Robert Lotha. (2016). Phytochemical Screening And Anticancerous Activity of Rhizome,Extract *Coleus Forskholi* on Hep G2 Cell Line. National Conference on "Emerging Trends in Management of Infectious Diseases and Public Health,;4 – 5 Feb 16.
13. N Shanthi*, S Murugesan, S Janetta Nithia and M Kotteswari. (2019). *In Vitro* Anticancer Activity of Methanol Extracts of *Avicennia Marina* (Forssk) Vireh Against Ht-29 Colon Cancer Cell Line, Vol. 8, Issue 6, (2019). ISSN: 2319-5622. DOI: https://doi.org/10.5281/zenodo.3265336.
14. S Azhagu Madhavan, P Vinotha, V Uma. (2020). In Vitro Antioxidant Properties And Free Radical Scaveneing Activity of Ethanolic Extract of *Costus Spicatus (Jacq*-1788).; "International Conference on Cancer Research". Int J Life Sci Pharma Res. ISSN 2250 – 0480; SP-07.
15. S Dhasaradha. Bai1, A. Silpha Tenish, P.G.Geegi, And P.Anitha. (2020). Anticancer Activity of Ethanolic Extract of *Vitis Vinifera* L. Using Mcf7 Cell Lines. "International Conference on Cancer Research". Int J Life Sci Pharma Res. ISSN 2250 – 0480; SP-07.
16. M Padma, S Ganesan, T Jayaseelan, S Azhagumadhavan, P Sasikala, S Senthilkumar, P Mani. (2019). Phytochemical screening and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L). Journal of Drug Delivery & Therapeutics.; 9(1):85-89. DOI: http://dx.doi.org/10.22270 /jddt.v9i1.2174.
17. A Sofowora, (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria.
18. G. E Trease. and W. C. Evans, (1989). "Phenols and phenolic glycosides," in Textbook of Pharmacognosy,; vol. 12, pp.343–383, Balliese, Tindall and Co Publishers, London, UK.

19. M Aseer Manilal, T Tsegaye Tsalla, Z Zerihun Zerdo, A Gemechu Ameya1, M Behailu Merdekios, E Shiju Easo. John (2016). Evaluating the antibacterial and anticandidal potency of mangrove, *Avicennia marina* [*Asian Pac J Trop Dis*; 6(2): 136-140). doi: 10.1016/S2222-1808(15)60999-9.
20. S Andrews, S Azhagu Madhavan, S Ganesan, P Arjun, R Jeyaprakash. S Baskara Sanjeevi and M. Ramasamy. (2020). Different Bioactive Constituents and Biochemical Composition of Brown Seaweed *Spatoglossum marginatum.* Waffen-Und Kostumkunde Journal, 2020; Volume XI, Issue IV, ISSN NO: 0042-9945.
21. S. Azhagu Madhavan. GC–MS Analysis of Bioactive Compounds Present In Ethanolic Leaf Extract *Acalypha indica*. Asian Journal of Advances in Research. (2021). 6(4): 16-22.2021.
22. S. Azhagu Madhavan, Vinotha P2 and Uma V3. Pharmacological and Anti Cancer Activity of *Ipomoea sepiaria* Methanolic Extract against PC-3 Cell Line. Asian Journal of Advances in Medical Science. (2020);(3): 26-32.2020.
23. M. Mahadevi and S. Azhagu Madhavan S..(2020). Anticancer Activity of *Piper Nigrum* Methanolic Extract Against A549 Human Lung Cancer Cell Line. Vol-43, No.-4, (R) October-December 2020. Sambodhi Journal. ISSN NO: 2249-6661.
24. S. Azhagu Madhavan, P. Vinotha and V. Uma. (2020). Phytochemical Screening And Comparative GC–Ms Analysis of Bioactive Compounds Present In Methanolic Leaf and Latex Extract *Calotropis Gigantea* (L). Asian Journal of Advances in Medical Science.(2020); 2(2): 1-13.
25. P Sasikala, S Ganesan, T Jayaseelan, S Azhagumadhavan, M Padma, S Senthilkumar ,P Mani. (2019). Phytochemicals and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of Taraxacum officinale (L). Journal of DrugDelivery&Therapeutics.;9(1):90-94.
26. K Iqra Haider Khan, J Arshad Javaid, A Dildar Ahmed and K Uzman Khan. (2020). Identification of Volatile Constituents of Ethyl Acetate Fraction of *Chenopodium Quinoa* Roots Extract By GC-MS *INT. J. BIOL. BIOTECH.,*; 17 (1). 17-21.

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