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Phytochemical screening of methanolic extract of mangrove *Avicennia marina (Forssk.) Vierh*

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ABSTRACT

Mangrove plants are widely used folk remedies and ethno botanical literatures have described the usage of plant extracts to treat human diseases since time immemorial. Avicennia marina (Forssk.) Vierh (A. marina) commonly known as grey mangrove has been used as traditional medicine for decades with multifunctional biological activity. We have identified 60 different compounds in the methanolic extract of A. marina by GC/MS and LC/MS analysis. The methanolic extract of A. marina shows higher content of pentanoic acid, decanoic acid, diethylhydroxylamine, pyrrolidine, 4-chlorophenyl, octadecylisocyanate, thiazolidinones and arabinopyranoside (flavonoid) which could be used as an alternative herbal medicine with wide range of biological properties such as anti -oxidant,- tumor,- microbial, -inflammatory, -allergic, -ageing and -artherosclerotic with minimum risk of adverse effects and with maximum potency that ensure better quality of life during treatment.

Key words: *Avicennia marina*; pentanoic acid, decanoic acid, arabinopyranoside, diethylhydroxylamide, pyrrolidine, herbal medicine.

INTRODUCTION

Mangroves are woody plants that grow at the interface between land and sea in tropical and sub-tropical latitudes. The term “mangrove” often refers to both the plants and the forest community. Mangroves are circumtropical distribution, occurring in 112 countries and territories. Global coverage has been variously estimated at 10 million hectares [1]. Mangrove forests are among the world’s most productive ecosystems. They enrich coastal waters, yield commercial forest products, protect coastlines, and support coastal fisheries. However, mangroves exist under conditions of high salinity, extreme tides, strong winds, high temperatures and muddy, anaerobic soils. There may be no other group of plants with such highly developed morphological, biological, ecological and physiological adaptation to extreme conditions. Mangroves are found in tropical and subtropical tidal areas, which have a high degree of salinity. Mangroves protect coastal areas from erosion, storm surge especially during hurricanes and tsunamis [2, 3].

Pichavaram Mangroves is situated in the southeast coast of India, located about 240 km south of Chennai City and about 45km south of Cuddalore. It is located between the Vellar in the north, the Coleroon in the south and the Uppanar in the west. Geographically it located 11°24'-11°27 N Latitudes and 79° 46'- 79° 48'E longitudes. Pichavaram forms a great wealth of biological diversity in mangrove ecosystem in India. The mangroves are distributed in varying degree of abundance, in which *Avicennia marina (Forssk.) Vierh* (*A.marina*) is the most common species followed by *Rhizphora apiculata, Rhizophora mucronata, Bruguiera cylindrica* and *Aegiceras corniculatum.* Extracts from mangrove plants and associates has been used worldwide for medicinal purposes, and having been recorded around 349 metabolites it turns out to be a rich source of steroids, diterpenes and triterpenes, saponins, flavonoids, alkaloids and tannins [4-8]. *A. marina* commonly known as grey mangrove belongs to the family Acanthaceae (Avicenniaceae). *A. marina* has been used as traditional medicine for years with variety of

biological activity such as anti-oxidant, -bacterial, -fungal, -ulcer, -cancer, -plasmodial and -tumor properties [9-13]. To verify the claim that *A. marina* has numerous medicinal properties, we have investigated the phytochemical composition of Methanolic extract of *A. marina* by GC/MS and LC/MS analysis.

MATERIALS AND METHODS

Plant collection

A. marina (Vernacular name - Venkandan in Tamil) whole plant was collected from Pichavaram mangrove forest which is located in Cuddalore District, Tamil Nadu in southeast coast of India. Geographically it is located 11°24'-11°27' North latitudes and 79° 46'-79° 48' East longitudes which is the second largest Mangrove forest in the world.

Methanolic Extract Preparation *A. marina*

The plant materials were taxonomically identified and authenticated by Department of Botany, M.E.S. Kalladi College, Mannarkkad, India and a voucher specimen has been deposited (Accession Number: AV-008). The whole plant material was washed in distilled water and dried at 45°C then powdered. Ten gram of the material was stirred overnight in 70% methanol (100 ml), and then centrifuged at 10,000 rpm for 10 min at 4°C for 10 min. The resultant supernatant was collected and the methanol removed by evaporation (yield of final product = 11% [w/w]).

GC/MS analysis of methanolic extract of *A. marina*

GC/MS analysis of methanol extracts of *A. marina* was performed using Thermo GC-Trace Ultra VER: 5.0 (Bremen, Germany). For MS detection, a Thermo MS DSQ II electron ionization mode with ionization energy of 70 eV and a mass range at m/z 50–650 was employed. A TR-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm) was used for the GC/MS. The column temperature was programmed from 80-250°C (rate = 8°C/min) with the lower and upper temperatures being held for 3 and 10 min, respectively. The GC injector and MS transfer line temperatures were set at 280 and 290°C, respectively. All analyses were done in the split-less mode. Helium was used as carrier gas (flow rate = 1.0 ml/min). An injection volume of 1 µl was used for analysis. Major and essential compounds were identified by their retention times and mass fragmentation patterns using data of standards from the National Institute of Standards and Technology (NIST) Wiley 9.0 library (Ringoes, NJ).

LC/MS analysis of methanolic extract of *A. marina*

LC/MS analysis of the methanolic extract of *A. marina* was carried out using Thermo/Finnigan Surveyor System consisting of a degasser, binary pump, auto sampler, and column heater. The column outlet was coupled to a Thermofleet (LCQ-Fleet) Ion Trap mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out in a personal computer with Data Analysis software (Qual Browser; Thermo Electron, San Jose, CA). For the chromatographic separation, a phenomenex luna 5-µm C8 column (250 × 4.6 mm) was used. The column was held at 95% Solvent A (0.1% acetic acid in water) and 5% Solvent B (0.1% acetic acid in acetonitrile) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, then 4 min with 100% B. Finally, elution was achieved with a linear gradient from 100% B to 5% B for 2 min. The flow rate was 200 µl / min and injection volume was 5 µl. The following parameters were used throughout the MS experiment: for electrospray ionization with positive ion polarity the capillary voltage was set to 20 V, the capillary temperature to 300°C, the nebulizer pressure to 40 psi, and the drying gas flow rate to 15 L/ min.

RESULTS

GC/MS analysis of methanol extracts from *A. marina*

GC/MS chromatogram of Methanolic extract of *A. marina* is presented in (Figure.1). The methanol extracts from *A. marina* was analysed by GC/MS. The relative retention times (Rt) and mass spectra of the extract components were compared with those of authentic samples and with mass spectra from a data library. As shown in (Table 1), GC/MS analysis of the *A. marina* extract at the Retention time (Rt) 17.16, 14.84 and 24.11 resulted in the identification of 52 different compounds, with more similarity with the standard mass spectra in the library, representing 12.54%, 13.22% and 4.94% of the relative area in the methanolic extract.

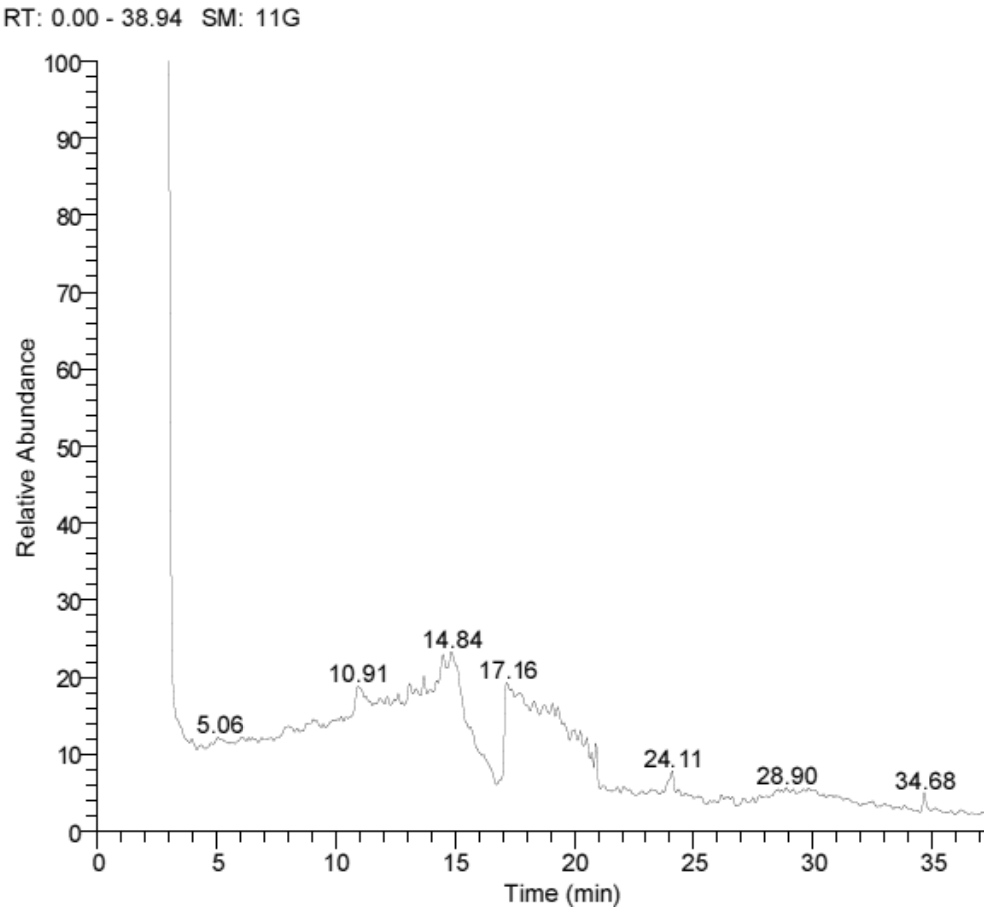

Figure 1. GC/MS chromatogram of methanolic extract of *A. marina*.

Table.1 GC/MS analysis of the methanolic extract of *A. marina* at the (Rt) 14.84, 17.16, and 24.11.

Library Search Results

SL No	Compounds	Molecular Formula	Molecular Weight	Area %
01	Pentyl glycolate	$C_7H_{14}O_3$	146	13.22
02	6-Chloro-2-methylquinoxaline	$C_9H_7C_lN_2$	178	13.22
03	á-l-Arabinopyranoside, methyl	$C_6H_{12}O_5$	164	13.22
04	Pyrrolidine	C_4H_9N	71	13.22
05	2-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130	13.22
06	Propanoic acid, 2-methyl-, 3-methylbutyl ester	$C_9H_{18}O_2$	158	13.22
07	3-[N-Aziridyl]-2-methylpropionitrile	$C_6H_{10}N_2$	110	13.22
08	2,2-Dimethylperhydro-1,3-oxazine	$C_6H_{13}NO$	115	13.22
09	Benzeneacetonitrile, à-(á-D-glucopyranosyloxy)-, (R)-	$C_{14}H_{17}NO_6$	295	13.22
10	3-Penten-1-yne, (z)	C_5H_6	66	13.22
11	3-Pentanone, 2,4-dimethyl-	$C_7H_{14}O$	114	13.22
12	1-Buten-3-yne, 2-methyl-	C_5H_6	66	13.22
13	Ethanethioic acid, S-(2-methylbutyl) ester	$C_7H_{14}OS$	146	13.22
14	Methoxyacetonitrile	C_3H_5NO	71	13.22
15	Isobutyrylurea	$C_5H_{10}N_2O_2$	130	13.22
16	2,5-Diamino-1,3,4-thiadiazole	$C_2H_4N_4S$	116	13.22
17	1-Propanamine, N-nitro-N-propyl-	$C_6H_{14}N_2O_2$	146	13.22
18	Pentane, 1-propoxy-	$C_8H_{18}O$	130	13.22
19	1,3-Diamino-2-propanol	$C_3H_{10}N_2O$	90	13.22
20	4-Amino-3-mercapto-1,2,4-triazole	$C_2H_4N_4S$	116	13.22
21	Pentanoic acid, 2-methyl-	$C_6H_{12}O_2$	116	12.54
22	Pentanoic acid, 2-methyl-, butyl ester	$C_{10}H_{20}O_2$	172	12.54
23	3-(Dioxolan-2-yl)-1,1-dimethyl-2-phenylpropylhydroxylamine	$C_{14}H_{21}NO_3$	251	12.54
24	Acetamide, N-methyl-2-(methylamino)-2-thioxo-	$C_4H_8N_2OS$	132	12.54

25	Butanedioic acid	$C_4H_6O_4$	118	12.54
26	Urea, heptyl-	$C_8H_{18}N_{2O}$	158	12.54
27	2-Acetyl-2,3,5,6-tetrahydro-1,4-thiazine	$C_6H_{11}NOS$	145	12.54
28	Decanoic acid, 2-methyl-	$C_{11}H_{22}O_2$	186	12.54
29	Acetamide, N-acetyl-N-methyl-	$C_5H_9NO_2$	115	12.54
30	N,N'-Methylenebis(formamide)	$C_3H_6N_2O_2$	102	12.54
31	Butanoic acid, 3-methyl-3-nitroso-, methyl ester	$C_6H_{11}NO_3$	145	12.54
32	3-Octanamine	$C_8H_{19}N$	129	12.54
33	1-Methylcyclopropanemethanol	$C_5H_{10}O$	86	12.54
34	4,5-Dihydro-5-methyl-4,4-pentamethylene-2-phenyl-1,3-oxazin-6-one	$C_{16}H_{19}NO_2$	257	12.54
35	1-Methylcyclopropanemethanol	$C_5H_{10}O$	86	12.54
36	Diethylhydroxylamine	$C_4H_{11}NO$	89	12.54
37	Benzenemethanol, à-[1-(methylamino)ethyl]-, (R*,S*)-(ñ)-	$C_{10}H_{15}NO$	165	12.54
38	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	$C_{10}H_{22}O_3S_2$	254	4.94
39	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (ñ)-	$C_6H_{10}O_3$	130	4.94
40	Oxirane, butyl-	$C_6H_{12}O$	100	4.94
41	Pantolactone	$C_6H_{10}O_3$	130	4.94
42	Butane, 1-bromo-2-methyl-	$C_5H_{11}Br$	150	4.94
43	Oxirane, propyl-	$C_5H_{10}O$	86	4.94
44	Butane, 1-bromo-2-methyl-, (S)-	$C_5H_{11}Br$	150	4.94
45	S-(Neopentyloxythiocarbonyl)thiohydroxylamine	$C_6H_{13}NOS_2$	179	4.94
46	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate	$C_{10}H_{22}OS_2$	222	4.94
47	Butane, 1-bromo-2-methyl-, (S)-	$C_5H_{11}Br$	150	4.94
48	Butane, 2,2-dimethyl-	C_6H_{14}	86	4.94
49	Oxalic acid, butyl cyclobutyl ester	$C_{10}H_{16}O_4$	200	4.94
50	1-Heptene, 3-methoxy-	$C_8H_{16}O$	128	4.94
51	s(+)-1-Cyano-2-methyl-azetidine	$C_5H_8N_2$	96	4.94
52	Propane, 2-methyl-1-nitro-	$C_4H_9NO_2$	103	4.94

LC/MS analysis of methanol extracts from *A. marina*

The LC/MS chromatogram of Methanolic extract of *A. marina* extract at 254 nm is shown in (Fig.2). The (Table.2) shows the retention times, the mode (+/ -), λ max, molecular weight and their respective components. In the analysis of LC/MS of the *A. marina*, a negative molecular ion at $[MS-H]^+$ at an *m/z* of 341.25 corresponding to 3-({5-[(3-bromophenyl)amino]-1,3,4-thiadiazol-2-yl}sulfanyl)propanenitrile and (*RS*)-6-Fluoro-1-methyl-7- [4- (5-methyl-2-oxo-1,3- dioxolen-4-yl) methyl-1-piperazinyl]-4-oxo-4*H*-[1,3] thiazeto [3,2-*a*] quinoline-3-carboxylic acid at negative molecular ion at $[MS-H]^+$ at *m/z* of 461.46 was observed. 5-bromo-2-chloro- N- { [4-(2,5-dimethoxyphenyl)-5- ({ [(1,3-thiazol-2-yl) carbamo, 4-(4-chlorophenyl) - 3-{ [(2,6-dichlorophenyl)methyl] sulfanyl}- 5- phenyl-4H-1,2 and octadecylisocyanate were observed in a negative molecular ion at $[MS-H]^+$ at an *m/z* of 623.93, 446.78 and 295.50 respectively.

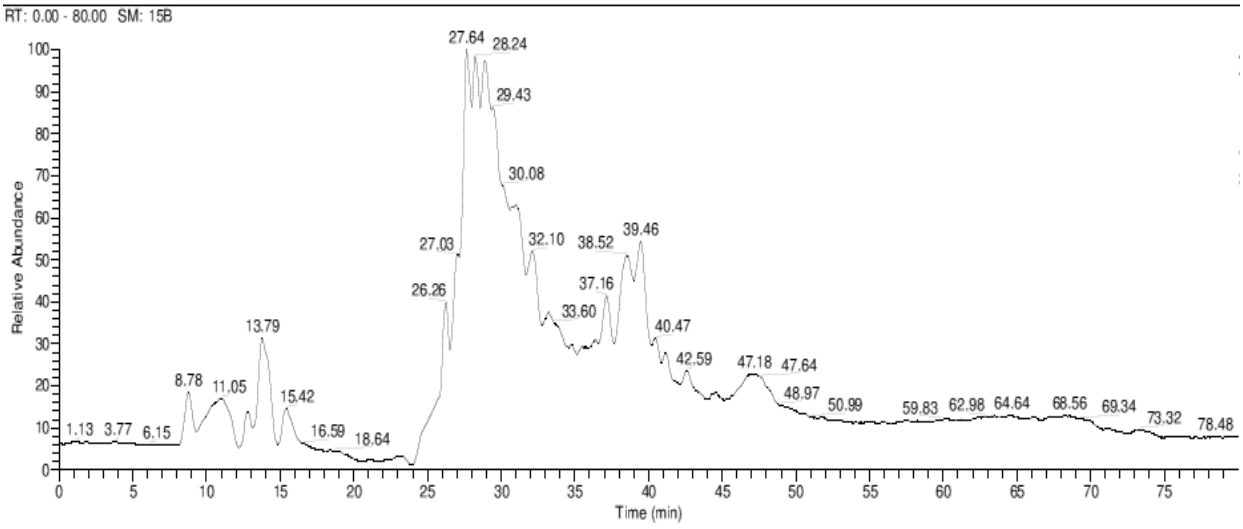

Figure 2. LC/MS chromatogram of *A. marina* methanolic extract at 254 nm.

Table 2. Identification of major compounds of methanolic extract of *A. marina* using their retention times by LC/MS analysis

SlNo	Rt (min)	λmax (min)	Molecular Weight	Mode (+/ -)	Compounds	Molecular Formula
1	13.79	254	341.25	-	3-({5-[(3-bromophenyl)amino]-1,3,4-thiadiazol-2-yl}sulfanyl)propanenitrile	$C_{11}H_9BrN_4S_2$
2	26.26	254	461.46	-	(RS)-6-Fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl-1-piperazinyl]-4-oxo-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid	$C_{21}H_{20}FN_3O_6S$
3	27.61	254	623.93	-	5-bromo-2-chloro-N-{[4-(2,5-dimethoxyphenyl)-5-({[(1,3-thiazol-2-yl)carbamo	$C_{23}H_{20}BrClN_6O_4S_2$
4	28.24	254	623.86	-	Unknown	-
5	29.43	254	446.78	-	4-(4-chlorophenyl)-3-{[(2,6-dichlorophenyl)methyl]sulfanyl}-5-phenyl-4H-1,2.	$C_{21}H_{14}C_{l3}N_3S$
6	32.21	254	529.60	-	Unknown	-
7	37.16	254	295.50	-	Octadecyl isocyanate	$CH_3(CH_2)_{17}NCO$
8	39.46	254	951.46	-	Unknown	-

Retention time (Rt) refers to LC/MS chromatogram in Figure. 2

DISCUSSION

Previous literature has reported that *A. marina* contains tannin, phenolic group, alkaloids, xanthoproteins, resins and coumarin [14]. The TDC identification has reported that *A.marina* also contains compounds such as erythro-guaiacylglycerol-β-ferulic acid ether, marinnone A, marinnone B, threo-guaiacylglycerol-β-ferolic acid ether, eleutheroside E_2, (+) - lirioresinol S, dihydroxymethyl-bis(3,5-dimethoxy-4-hydroxyphenyltetrahydrofuran-9-*O-β*-lucopyranoside, (+)-lyoniresinol 3 a –*O-β* -D- glucopyranoside, (-) - lyoniresinol 3 a –*O-β*-D - glucopyranoside, epi-pinoresinol, leucoseceptoside A, Jionoside C, Salsaside C, ilicifolioside A, acteoside, isoacteoside, ethyl ferulate, avicennone D, avicenone E, avicennol C, and stenocarpoquinone [15]. The aerial part of *A. marina* and leaves contains flavonoids such as luteolin 7-o-methylether 3'-O-β-D-glucoside, galactoside analogue, chrysoeriol 7-O-glucoside, Isorhamnetin 3-O-rutinoside, 5-hydroxy-4, 7-dimethoxyflavone, quercetin, kaempferol, 4'5-dihydroxy-3'-5,7-trimethoxyflavone and 4',5,7-trihydroxyflavone,3',4',5-trihydroxy-7-methoxyflavone [16]. The presence of flavonoids has important effects in plant biochemistry and physiology as antioxidants, enzyme inhibitor, precursors of toxic substances and they are recognized to possess anti-inflammatory, antioxidant, anti-allergic, anti-carcinogenic activities [16,17].

The identified phytochemical compounds in the Methanolic extract of *A. marina* by GC/MS analysis reported to exhibit multifunctional biological activity. The identified pentanoic acid which is alkyl carboxylic acid which is also found in perennial flowering plant Valerian (*Veleriana officinalis*) used as food additive in semi-hard goat milk cheese preparation [18]. The authors disclose that acetamide an organic compound and its derivatives have anti-oxidant and anti-atherosclerotic activity. Evidences shown that acetamide exhibits anti-atherosclerotic activity by inhibition of acyl-COA: cholesterol acyl transferase (ACAT) an intracellular enzyme catalysing cholesterol etherification [19, 20]. The 4-thiazine derivatives reported to have anti-tubercular activity and an efficient inhibitor of IL-8 production by HGF (Human gingival fibroblast) and nitric acid production by activated RAW 2647 cells [21,22]. Decanoic acid or Capric acid is a saturated fatty acid used as food additives and in pharmaceutical drug production. The identified diethylhydroxylamine (DEHA) involves in anti-ageing mechanism by decreasing, reduce lipid peroxidation level and lipofuscin concentration in animal model involving in ageing process [23]. DEHA also attributes to have anti-tumor property which is supported by induction of glutathione 5- transferase activity and it also increases life expectancy of the experimental animals [23-25]. Pyrrolidine also known as tetrahydropyrrole an organic compound found naturally in the plants used as pharmaceutical drugs such as procyclidine and bepridil (anti-cholinergic) for the treatment of Parkinson diseases, acute dystonia and akathisia syndrome. Propanoic acid a naturally occurring carboxylic acid has anti-microbial property and also used as food additives [26]. The 4-thiadiazole derivatives, ethylene oxide and 2-dimethyl-propanesulfinylsulfone compound has excellent anti-microbial, anti-inflammatory and analgesic activity [27, 28, 42]. The author reveals that arabinopyranoside a plant derived flavonoids has the property of inhibiting respiratory syncytial virus (RVS) which shows that their efficient anti-viral activity [29].

The identified compounds in the methanolic extract of *A. marina* by LC/MS were reported to have numerous biological activities. 3({5-[(3-bromophenyl) amino derivatives was reported to have effective adenosine kinase inhibitor with analegestic and anti-inflammatory property [30]. The compound of 4-chlorophenyl derivative and octadecylisocyanate has the property such as anti-fungal, -tuberculosis, -convulsant and -tumor activity by inhibiting

colon cancer cell lines [31-35]. 2,6-dichlorophenyl derivative was reported to be most prominent diuretic activity without any toxicity to liver and also reported to have effective anti-bacterial activity and 3-thiazole-2yl (thiazolidinones) derivatives was reported to be an estrogens related receptor alpha antagonist to treat breast cancer and also inhibit HT29 (human breast cancer) and H460 (human lung cancer) which has an effective anti-tumor agent [29, 35-41]. 2-6-dimethoxy phenyl derivatives are a novel sortase inhibitor reported to have effective anti-bacterial activity [43, 44]. In conclusion the present study shows methanolic extract of *A. marina* (whole plant parts) contains variety of medicinal compounds that have wide range of biological properties such as anti-oxidant,-tumor,-inflammatory,-allergic,-microbial,-ageing,-cholinergic,-convulsant,-artherosclerotic and -tuberculin activities. The identified compounds of *A. marina* extract could be used as herbal medicine for treating variety of biological dysfunctions with minimum risk of adverse effects with maximum potency for better quality of life during treatment.

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