The Pharma Innovation Journal 2018; 7(9): 348-354

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(9): 348-354

www.thepharmajournal.com
Received: 24-07-2018
Accepted: 26-08-2018

Gandham Sandeep Kumar
Division of Molecular Biology, Department of Zoology, S.V. University, Tirupati, Andhra Pradesh, India

Chintha Venkataramaiah
Division of Molecular Biology, Department of Zoology, S.V. University, Tirupati, Andhra Pradesh, India

Wudayagiri Rajendra
Division of Molecular Biology, Department of Zoology, S.V. University, Tirupati, Andhra Pradesh, India

Correspondence
Wudayagiri Rajendra
Division of Molecular Biology, Department of Zoology, S.V. University, Tirupati, Andhra Pradesh, India

Qualitative and quantitative phytochemical profile and *In vitro* anti-oxidant activity of methanolic extract of *Artemisia vulgaris*

Gandham Sandeep Kumar, Chintha Venkataramaiah and Wudayagiri Rajendra

Abstract
The present study is aimed to identification and quantification of phytoconstituents and to evaluate the *in vitro* antioxidant activity of methanolic extract of *Artemisia vulgaris.* Qualitative and quantitative analysis of phytoconstiuents was performed with methanolic extract of *Artemisia vulgaris*. Antioxidant activity of extracts was evaluated by estimating H_2O_2 scavenging activity, DPPH assay, Iron chelating activity and DNA protection assay. Active phytoconstiuents were identified in GC-MS and LC-MS. Physico-chemical properties of phytocostituents were predicted using computational tools. Qualitative analysis revealed the presence of phenols, flavanoids, tannins, steroids, carbohydrates and saponins. GC-MS and LC-MS analysis revealed the presence of 27 compounds. Crude extract contains 360 mg/gm and 110 mg/gm of phenols and flavonoids respectively. Whereas saponin and alkaloid contents were 2.8% and 1.5% W/V respectively. DPPH and H_2O_2 scavenging activities were 67% and 69% percent respectively. DNA protection assay of Plant extract showed maximum protection at 80µg of crude extract. From the results it is concluded that methanolic extract of *Artemisia vulgaris* is a good source of potential antioxidant compounds.

Keywords: Antioxidant activity, *Artemisia vulgaris*, DNA protection assay, phytochemical screening

1. Introduction
Indigenous medicinal plants and their derivatives have attained a greater importance in health of an individual [1]. Many worldwide organizations have recommended intake of plant derived foods to improve human health and prevent chronic diseases [2]. Recently, the World Health Organizations (WHO) estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. Plants have many essential phytochemicals such as alkaloids, phenols, flavonoids, essential oils, tannins and saponins, which can be used as therapeutically important compounds with lesser or no side effects. Based on the medicinal properties of the various phytoconstituents many attempts have been made using phytoconstituents as therapeutic drugs [3]. Natural bioactive compounds extracted from plant sources have been reported to exert potent antioxidant properties compared to the chemically synthesized compounds.

Flavonoids, one of the phytoconstituents, are important metabolites involved in protection against stress (temperature and oxidative) [4]. Phenolic acids are the secondary metabolites, a major bioactive phytoconstituents, contribute for greater antioxidant activity [5]. Saponins are known to show protective effect on blood cholesterol, anti-cancer activity and also known to show antiviral property [6]. Alkaloids are the compounds that show cytotoxic effect, and hence some of the alkaloids are used as anticancer agents [7].

Free radicals, Reactive Oxygen Species (ROS), are toxic byproducts produced by the living cells and excess production of ROS induces oxidative stress [8] causing damage to proteins, lipids and DNA. Natural antioxidants derived from plants overthrow the deleterious effects of ROS and capable of protecting body from oxidative damage [9].

Artemisia vulgaris is an aromatic herbaceous plant found all around the hilly regions of India. The whole plant is traditionally used in the treatment of various ailments such as anti-leishmanial activity, anti-malarial, anti-bacterial [10], antifungal [11], anti-diabetic, anti-epileptic, anti-helminthic, antiseptic, antispasmodic [12], anti-nociceptive [13] and anti-inflammatory activities.

Keeping in view of the importance of this medicinal plant, the present work is mainly focused

to carry out qualitative and quantitative analysis of the phytoconstituents and their antioxidant activity.

2. Method

2.1. Collection of plant

The *Artemisia vulgaris* plant is collected from the surrounding regions of Tirupati during the month of June. The plant material was identified and authenticated by taxonomists.

2.2. Preparation of plant extract

The plant material was dried under shade and made into coarse powder. The powdered plant material is soaked in three different solvents such as water, methanol and ethanol. The extract is filtered and concentrated using rotary evaporator and dried. Phytoconstituents present in the sample were identified using standard protocols as follows [14, 15].

A small portion of the dried extract was used to test phytoconstituents such as alkaloids, flavonoids, phenols, tannins, steroids, terpenoids, carbohydrates and saponins. The extract is treated with dilute HCl and filtered. The filtrate is treated with Mayer's reagent (potassium mercuric iodide) and formation of yellow colour indicates the presence of alkaloids. The extract is treated with a few drops of ferric chloride solution and formation of bluish black colour indicates the presence of phenols. A small portion of extract was mixed in 2ml of chloroform, and concentrated H_2S0_4 was added drop by drop to form a layer. Formation of reddish brown colour at the interface indicates the presence of steroids and appearance of bluish green interface indicates the presence of terpenoids. The extract was treated with a few drops of alcoholic α-napthol and a few drops of sulphuric acid are added drop by drop along the walls of the tube. Formation of violet ring indicates the presence of carbohydrates. Small amount of extract is dissolved in distilled water and shaken well. If foam persists for more than 10min, it indicates the presence of saponins.

2.3. Quantitative analysis

2.3.1. Determination of total phenol

The total phenol content in the methanol extract of *Artemisia vulgaris* (MEAV) was determined using Folin-Ciocalteu method slightly modified by Crop Research Institute Report [16]. 2.5ml of 10% folin-Ciocaulteu reagent and 2ml of 2% Na_2Co_3were added to 0.5ml of extract. The reaction mixture is incubated at 45ºC for 15min. The absorbance is measured at 765nm against reagent blank. The phenolic content was expressed in Gallic acid equivalents.

2.3.2. Determination of total flavonoids

Total flavonoid content in MEAV is determined using aluminium chloride by calorimetric method. 3ml of methanol, 0.2ml of 10% aluminium chloride and 0.2ml of 1M potassium acetate were added to 1ml of plant extract (1mg/ml). The final volume was made up to 10ml with distilled water and allowed to stand for 30min at room temperature. The absorbance was measured at 420nm against blank. The flavonoid content was expressed in Gallic acid equivalents.

2.3.3. Determination of Saponins

The total saponin content is determined using the method of Obadoni and Ochuko (2001) [17]. 20 gm of powdered sample was mixed with 100ml of 20% aqueous ethanol. The mixture was heated over a water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the reextracted with another 100 ml of 20% aqueous ethanol. The combined extracts were concentrated over water bath. The contents were transferred into a 250 ml separating funnel and 20ml of diethyl ether was added. The contents were gently shaken and the ether layer was discarded. 60 ml of n-butanol is added and washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was kept on a water bath and dried. The dried sample was weighed and calculated as percentage (w/v).

2.3.4. Determination of Alkaloids

Alkaloid content is measured using Harbone method [15]. 200 ml of 10% acetic acid in ethanol was added to 5gm of the powdered sample and allowed to stand for 4h. This was filtered and the extract was concentrated. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.4. Antioxidant Activity

2.4.1. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was determined by the method of Ruch *et al*. (1989) [18].Various concentrations of plant extract in 4ml was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was measured at 230 nm against blank solution.

2.4.2. Determination of reducing power

The reducing power of MEAV was evaluated according to the method of Oyaizu (1986) [18]. To 1ml of plant extract 2.5ml of 0.2M phosphate buffer (ph-6.6) and 2.5ml of 1% $K_3Fe\ (CN)_6$ were added. The reaction mixture was incubated at 50ºC for 20 min and 2.5ml of 10% TCA was added. The reaction mixture was centrifuged at 3000 rpm for 10min. The supernatant was collected and equal volume of 0.1% $FeCl_3$ was added. The absorbance was measured at 700nm against blank.

2.4.3. 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

The method of Kikuzaki *et al*. (1993) [19] was used for the determination of Scavenging activity of DPPH free radical. One ml of 0.135 mM DPPH prepared in methanol was mixed with 1.0 ml of extract ranging from 0.2-1.0 mg/ml. The reaction mixture was mixed well and kept in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm.

2.5. DNA Protection assay

DNA protection assay of plant extract was determined electrophoriticaly using DNA sodium salt from Salmon milt extract. To 5 µg of DNA, 10 µg of plant extract at varied concentrations (20-80 µg) is added followed by the addition of fenton's reagent (30mM H_2O_2, 50mM Ascorbic acid, 80mM $FeCl_3$). The reaction mixture is incubated for 15min at room temperature. The DNA is electrophoresed on 1% agarose gel and the band intensity is observed under UV light.

2.6. GC-MS and HR-LC/Q-TOF/MS analysis for the identification of antioxidant Compounds

The GC MS analysis of MEAV was performed at SAIF, IIT, Madras. Chromatographic method was performed using HP 5 MS Colum with an injecting temperature of 220ºC at 1ml/min

flow rate in pure helium. The oven temperature is gradually increased from 50 ºC to 230 ºC at the rate of 10ºC per min. The photon multiplier tube is used as detector and the chromatogram is recorded.

Phytoconstiuents (Non-polar and semi-polar) present in the MEAN were identified by Gas Chromatography JEOL GCMATE II GC–MS (Agilent Technologies 6890N Network GC system). The instrument is equipped with secondary electron multiplier, fused silica 50 m 9 0.25 mm I.D in the column and splitless injector. Different temperature conditions in column and time intervals were used to analyze volatilization of compounds of the extract. Total run time was 30 min. The photon multiplier tube is used as detector and the chromatogram is recorded. Chromatography peaks obtained from related compounds are coupled with Mass Spectroscopy. The mass and structure of the volatile phytochemicals of the extract were identified by comparing with National Institute of Standard and Technology (NIST) database, which constitutes of more than 62000 known compounds.

Programmed 3 µL injection volume of the sample was injected by auto sampler into a C18 column (ZORBAX 2.1 9 50 mm 1.8 Micron). The auto sampler is capacitized with 100 µL/mL auxiliary draw and ejects speed of the sample. Desired mobile phase gradient with different fractions of solvents (Solvent A-Water and B-Acetonitrile) was delivered using two binary pumps (G4220B). Total run time was 30 min, in which the solvent A-95% and B-5% was started at 2 min, at 15min solvent A gradient ratio turned into 5%, finally from 25 to 30 min, the solvent A 95% was maintained. Binary pump pressure maintained at 1200 bar constantly and flow rate was 0.300 mL/min. Agilent G6550A Q-TOF Mass Spectrophotometer with Dual AJS ESI ion source connected with LC was used to find the profile of phytochemicals in the extract. The conditions were set in the Q-TOF as follows acquisition mode MS (minimum (m/z) 50, MS maximum (m/z) 1000), and MS/MS scan rate 1. Sourcre parameters, gas temperature 250 ^{0}C, gas flow 13 L/min, nebulizer 35 psig, sheath gas temperature 300 and sheath gas flow 11. Scan source parameters such as vcap 3500, nossil voltage 1000v, fragmentor 175, skimmer1 65 and octopole RF Peak 750. Mass hunter workstation software was used in the identification of accurate MS and MS/MS for LC profile of the extract.

2.7. Computational analysis of phytoconstiuents

Molecular properties like hydrogen bond donors, hydrogen bond acceptors, molecular weight, toxicity, mutagenic, tumorigenic, irritant, reproductive toxicity and druglikeness scores were analyzed using OSIRIS Data warrior tool. Bioactivity of the phytoconstiuents was also analyzed using Molinspiration server.

3. Results and discussion

3.1. Screening and Quantification of phytoconstituents

Preliminary qualitative analysis of MEAV extract revealed the presence of different phytoconstituents such as alkaloids, phenols, flavonoids, tannins, carbohydrates and saponins (Table-1). Further investigation was carried out to quantify the Total Phenols, Flavonoids, Steroids, Terpenoids, Saponins and Alkaloids in the methanolic extract. The total phenols, flavonoid content in crude methanolic extract were found to be 380 mg/gm (fig-1) and 110 mg/gm (Fig-2) respectively. The total alkaloid and saponins contents in the plant extract were 2.8 % and 1.5% w/v respectively.

Table 1: Qualitative analysis of Phytoconstituents in Methanol, Ethanol and Water extracts of *Artemisia vulgaris*

S. No	Test	Methanol	Ethanol	Water
I.	Alkaloids	+	+	+
II.	Flavanoids	+	+	+
III.	Tannins	+	+	+
IV.	Steroids	+	-	-
V.	Terpenoids	+	+	+
VI.	Saponins	-	-	+
VII.	Carbohydrates	+	+	-

+: Presence, -: Absence

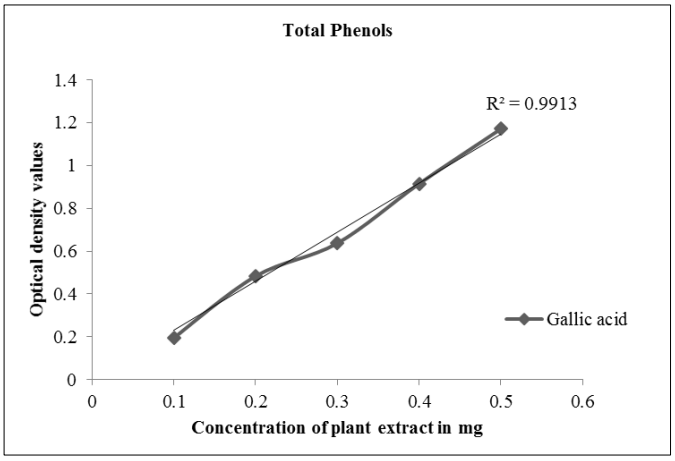

Fig I: Total Phenolic content in the methanolic extract of *Artemisia vulgaris* (MEAV)

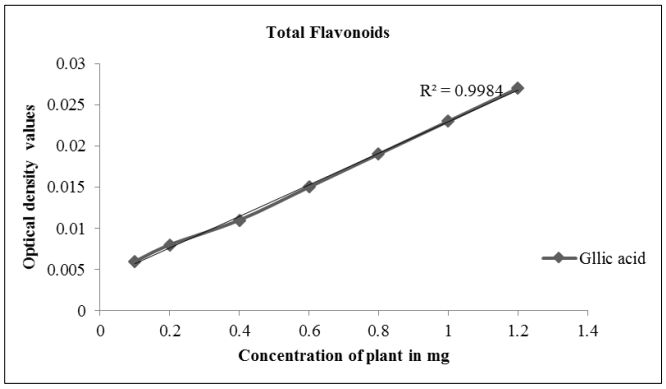

Fig 2: Total flavonoid content in MEAV

3.2. *In vitro* antioxidant assays

3.2.1. Hydrogen Peroxide scavenging activity

Hydrogen peroxide scavenging activity of methanolic extract showed relatively equal scavenging activity with ascorbic acid. From the results it is clear that the plant has potential phytoconstituents that are capable of scavenging the hydroxyl radical. Hydrogen peroxide is an important reactive oxygen species (ROS) having an ability to penetrate into biological membrane and capable of generating hydroxyl radicals. Hydrogen Peroxide scavenging activity of MEAV and Vitamin C was shown in Fig. 3. This scavenging activity may be due to the presence of Phenolic phytoconstituents in the extract which are capable of reducing hydrogen peroxide to water by donating an electron.

3.2.2. DPPH scavenging activity

DPPH is a stable synthetic free radical, which is most reliable and commonly used to investigate the free radical scavenging activity of natural compounds. The DPPH scavenging activity

results of the MEAVs were shown in Fig-4. Antioxidant activity of plant extract is compared with standard vitamin C. The IC_{50} (Half maximal inhibitory concentration) of MEAV and Vitamin C were found to be at 20mg/ml.

DPPH radical scavenging activity depends on the ability of a compound to lose hydrogen [20, 21]. Table -2 shows the number of hydrogen donors in each compound of the extract, as the most possible evidence for the DPPH scavenging activity of the MEAV. Phytoconstituents present in the plant extract reduce DPPH radical to hydrazine by pairing the unpaired electrons [22]. MEAV showed a profound reducing activity against stable DPPH free radicals due to the presence of bioactive compounds.

3.2.3. Reducing power potential of the extract

The results of reducing power potential of MEAV compared with standard antioxidant Vitamin C were represented in fig-5. In this reducing power assay, the amount of Fe^{3+} ions reduced to Fe^{2+} by phytoconstituents present in the extract is higher than the amount of Fe^{3+}reduced by Vitamin C indicating that the antioxidant potential of plant extract is greater than the antioxidant potential of Vitamin C. This reducing potential may be due to the presence of potential antioxidant compounds capable of reducing Fe^{3+}ions to Fe^{2+}.

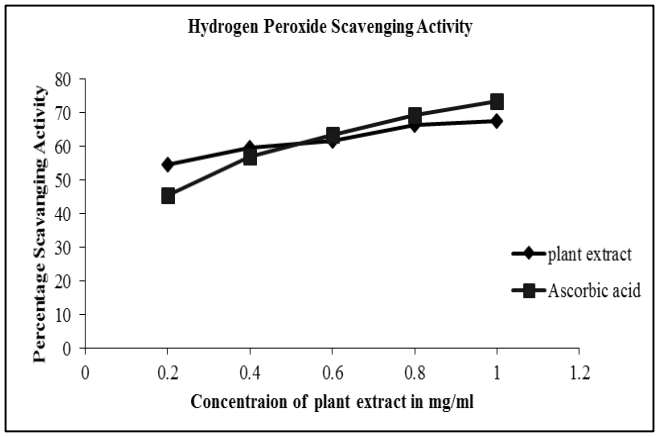

Fig 3: Hydrogen peroxide scavenging activity of MEAV

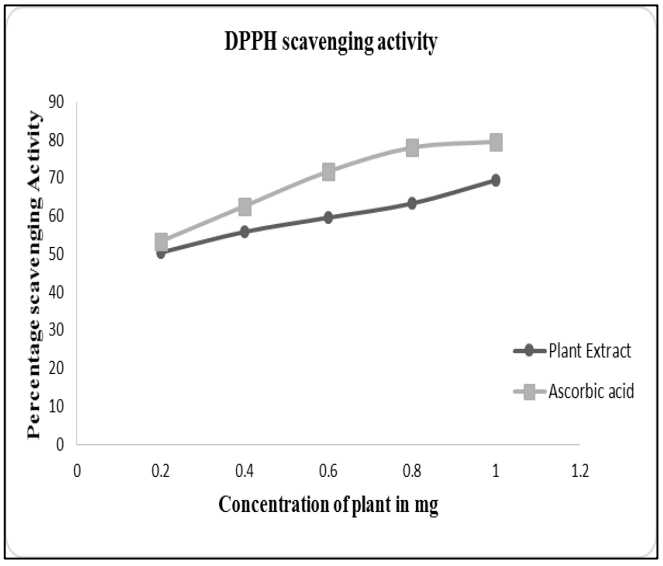

Fig 4: DPPH scavenging activity of MEAV

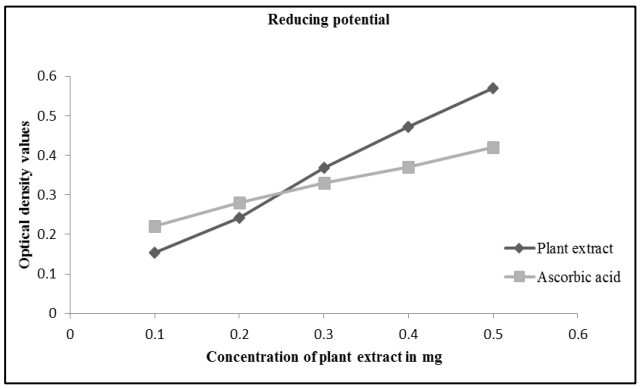

Fig 5: Reducing potential of MEAV

3.3. DNA Protection assay

Fenton's reagent generates hydroxyl radicals which causes breaks in DNA strands. DNA protection assay of MEAV at various concentrations is shown in the Fig-6. The results showed effective protection against DNA damage at 80µg of plant compared to the control. The protective effect of DNA might be due to the presence of phenolic and flavonoid compounds.

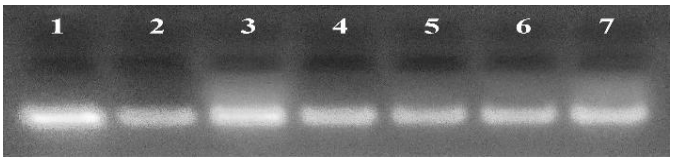

Fig 6: DNA protection assay of MEAV, Lane-1: DNA, Lane-2: DNA + fentons, Lane-3: DNA + plant extract, Lane-4: DNA + fentons + 20µg plant extract, Lane-5: DNA + fentons + 40µg plant extract, Lane-6: DNA + fentons + 60µg plant extract, Lane-7: DNA + fentons + 80µg plant extract.

3.4. GC MS and LC-MS analysis

GC MS and LC-MS analysis of MEAV revealed the presences of 27 compounds with different retention times (Table-2; table-3: Fig-7). Bicyclo [2.2.1] heptan-2-one, 4-hydroxy-1, 7, 7-trimethyl (p-Oxycampher) is the potential anti-inflammatory compound that has been isolated and studied in many other medicinal plants. Caryophyllene oxide is a sesqueterpene compound which has been reported to exhibit various activities such as antifungal [23], anticancer [24], anti-inflammatory [25] and gastro protective [26] and also known to reduce a neuropathic pain. S-camphor is a terpenoid compound known for its strong aromatic odour. No specific reports have been documented on S-camphor, where as many reports have been found on camphor isomers representing as a therapeutic agent for neuroprotective [27], anti-inflammatory [28] and various ailments. Pentadecanoic acid is a fatty acid which has been reported for its anti-diabetic [29] and anti-inflammatory [30] activity.

Berberine is a benzoisoquinoline alkaloid which has been used in ancient Chinese medicine. Many scientific reports reveal various activities such as anti-diabetic [31], anticancer [32], neuroprotective [33] and prevent neonatal jaundice [34]. N-Desmethylnefopalm is a metabolite of nefopalm which is a potential analgesic than asprin [35]. Chepharenthine acts as an anti-inflammatory [36, 37] and anticancer [38] compound. Isoprene is used to treat respiratory syndrome [39]. Peruvoside has been reported for its anti-cancer activity [40, 41]. The compounds present in the extract show various activities such as anti-inflammatory, anti-cancer, anti-diabetic and anti-nociceptive activity. Most of the identified constituents of MEAV are known to show anti-inflammatory activity. These studies suggest that the plant shows potential anti-inflammatory activity.

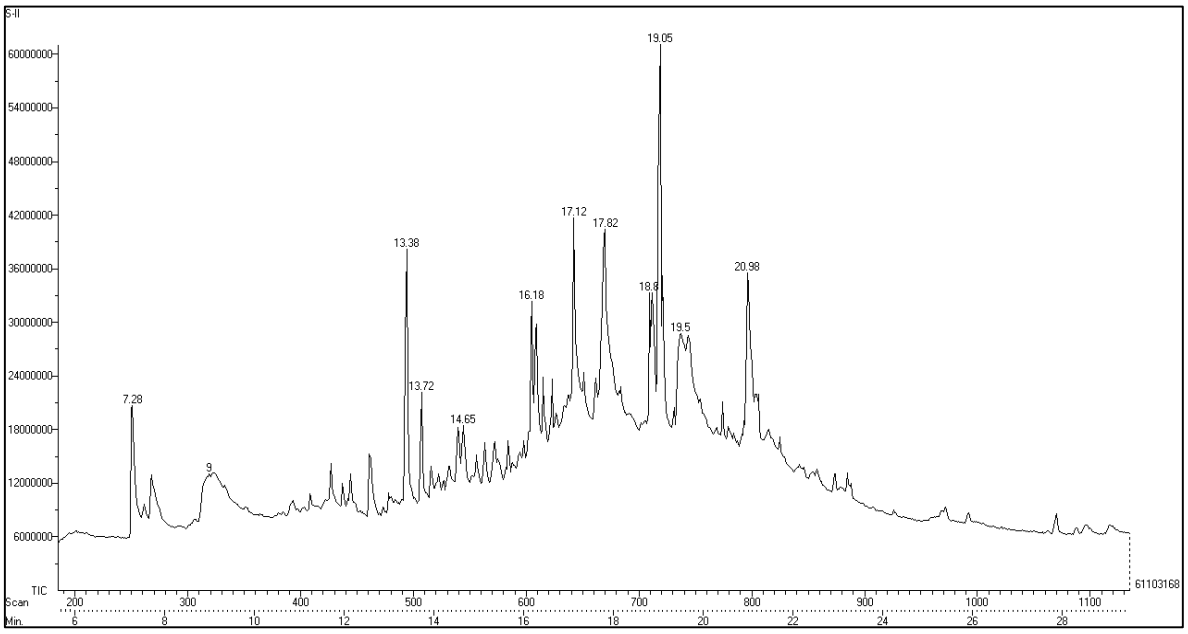

Fig 7: GC-MS chromatogram of MEAV Retention Time in min

3.5. Computational Analysis of Phytoconstituents

Molecular properties of the phytoconstiuents identified in GC-MS and LC-MS analysis were predicted in Osiris data warrior tool and the results were listed in table-2. Bioactivity of the Phytoconstituents was determined against G-Protein Coupled Receptor ligands (GPCR), ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets. Molecular properties and bioactivity results are useful to calculate the druglikeness of a compound. In simple words druglikeness is balance of various molecular properties to determine whether compound is similar to known drugs. Druglikeness include properties such as hydrophobicity, hydrogen bonding characteristics, electronic distribution, molecule size and flexibility, affinity to proteins, bioavailability, transport properties, reactivity, toxicity, metabolic stability and many others. Bioactivity of the compounds predicted using molinspiration shows that berberine, pravastatin, β-carryophyllene oxide, 24,24, difloro 1,25,26, trihydro vitamin D3 and 2-oxo 14,18 dihydroxy 9z, 13E, 15Z octadecanoic acid would act as a potential enzyme inhibitor. Dihyroceleseryl diacetate, 24, 24, difloro 1, 25, 26, trihydro vitamin D3, benzo chromen-3-one, prasvastatin and β –carryophyllene oxide act as nuclear receptor inhibitors.

Toxicity prediction results revealed that no toxic effects have been exhibited by the compounds 1-napthyl glucoronide, acetoxykhivorin, 24, difloro 1,25,26, trihydro vitamin D3, berberine, bicycle (2,2,1) heptan-2-one,4,hydro-1,7,7,trimethyl, cepharanthine, ciaberic acid, dehydronifedipine, desmethylneopam, dihydrocelesteryl diacetate, pervuside, pravastatin, nonadecanoic acid and pentadecanoic acid. Whereas oxospiro (2, 5) octane, Benzo[f]chromen-3-one, oleic acid, palmetic acid, phenyl acetaldehyde and 11-Acetoxykhivorin are predicted to have irritant effect. Z-2-Acetoxy-12-tetradecenitrile, isoreserpine, linoloic acid, benzochromen-3-one were predicted to have reproductive effect. These result show that these compounds derived from *Artemisia Vulgaris* extract would possibly contribute to the antifertility activity. Since 11-Acetoxykhivorin and isoreserpine are shown to have greater number of hydrogen donors as per Lipinski rule of five, these two drugs are not suggested for oral administration. Computational analysis of the compounds 25, 26 and 27 in Table- 3 were not analyzed due to lack of structural information in Pub Chem database.

Table 2: Phytoconstituents of MEAV identified in GC-MS and LC-MS and their molecular properties

S. No	Compound name	C log p	C log s	H- acc	H-don	Drug likeness	M	T	R	I	DS
1	11-Acetoxykhivorin	2.765	-5.377	12	0	0.005s	-	-	-	-	0.288
2	1-Naphthyl glucuronide	0.169	-2.794	7	4	-0.799	-	-	-	-	0.588
3	1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)	3.619	-2.976	1	0	-13.50	++	++	-	++	0.090
4	24,24-Difluoro-1,25,26-trihydroxyvitamin D3	5.049	-5.024	4	4	-11.77	-	-	-	-	0.226
5	2-oxo-14,18-dihydroxy9Z,13E,15Z-octadecatrienoic acid	4.0683	-3.171	5	3	-16.40	-	-	-	-	0.377
6	5(E)-1beta, 25dihydroxyvitamin D2	5.937	-5.078	3	3	-4.126	-	-	-	++	0.123
7	7,10 Octadecadieonica acid	6.717	-4.551	2	1	-28.97	++	++	-	++	0.048
8	9,12 octadecadideonic acid	6.464	-4.323	2	1	-25.56	-	-	++	-	0.143
9	Benzene acetaldehyde	1.2801	-1.827	1	0	-4.346	-	-	-	++	0.292
10	Benzo[f]chromen-3-one, perhydro-2-acetyl-4a,7,7,10b-tetramethyl	3.137	-4.058	3	0	-9.277	-	-	++	++	0.137
11	Berberine	0.522	-4.669	5	0	-2.246	-	-	-	-	0.404
12	Beta Caryophyllene Oxide	4.055	-3.558	1	0	-4.768	-	+	+	-	0.246
13	Bicyclo[2.2.1]heptan-2-one, 4-hydroxy-1,7,7-trimethyl-	1.3559	-2.002	2	1	-1.897	-	-	-	-	0.539
14	Cepharanthine	6.752	-8.009	8	0	4.58	-	-	-	-	0.181
15	Dehydronifedipine	2.018	-4.385	8	0	-	-	-	-	-	0.375
16	Dihydrocelastryl Diacetate	6.3181	-7.066	6	1	-2.25	-	-	-	-	0.128
17	Hexadecanoic acid	0.602	-4.239	2	1	-25.21	-	++	-	++	0.092

18	Isoreserpine	3.575	-4.446	11	1	2.322	-	-	++	-	0.256
19	N-Desmethylnefopam	2.398	-2.91	2	1	0.717	-	-	-	-	0.746
20	n-nonadecanoic acid	7.425	-5.049	2	1	-25.216	-	-	-	-	0.192
21	Pentadecanoic acid	5.6081	-3.969	2	1	-25.216	-	-	-	-	0.287
22	Peruvoside	1.300	-4.311	9	3	-2.679	-	-	-	-	0.297
23	Pravastatin	2.454	-3.553	7	4	3.126	-	-	-	-	0.731
24	Z-2-Acetoxy-12-tetradecenitrile	5.3406	-4.425	3	0	-18.79	++	-	++	++	0.060

H-acc: Hydrogen acceptors, H-don: Hydrogen donors, M: Mutagenic, T: Tumorigenic, R: Reproductive toxicity, I: Irritant, DS: Drug score

Table 3: Phytoconstituents of MEAV and their bioactivity Scores

S. No	Compound name	RT	Pub Chem ID	GPCR	ICM	KIN	NR	PI	ENZ
1	11-Acetoxykhivorin	9.965	76311434	-0.09	-0.72	-0.75	-0.13	0.00	-0.03
2	1-Naphthoic acid glucuronide	6.173	114851	0.21	0.06	-0.17	0.33	0.14	0.43
3	1-Oxaspiro[2,5] octane,5,5 dimethyl-[-3-methyl-1,3,butadienyl]	14.65	5362888	-0.24	0.13	-1.08	0.49	-0.21	0.28
4	24,24-Difluoro-1,25,26trihydroxyvitamin D3	14.061	24779638	0.39	0.29	-0.15	1.13	0.36	0.75
5	2-oxo-14,18-dihydroxy9Z,13E,15Z-octadecatrienoic acid	15.68	6438613	0.22	-0.01	-0.21	0.34	0.26	0.50
6	5(E)-1beta, 25dihydroxyvitamin D2	15.072	9547243	0.29	0.25	-0.19	1.35	0.09	0.82
7	7,10 Octadecadieonica acid	18.82	445639	0.17	0.07	-0.22	0.23	0.07	0.27
8	9,12 Octadecadideonic acid	19.50	5280450	0.29	0.17	-0.16	0.31	0.12	0.38
9	Benzeneacetaldehyde	9.02	998	-2.16	-1.41	-2.39	-2.18	-1.82	-1.57
10	Benzo(f)chromen-3-one,Perhydr 2-acetyl 4a,7,7,10b tetra methyl	20.98	540296	-0.08	0.14	-0.67	0.50	-0.06	0.24
11	Berberine	7.73	2353	-0.11	0.71	-0.27	-0.78	-0.35	0.82
12	Beta Caryophyllene	13.38	1742210	-0.08	0.14	-0.86	0.62	0.00	0.57
13	Bicyclol[2.2.1]heptan-2-one,1,7,7 tirmethyl	7.28	557958	-0.73	-0.32	-1.40	-0.42	-0.81	-0.12
14	Cepharanthine	16.027	10206	-0.04	-0.67	-0.59	-0.64	-0.11	-0.37
15	Dehydronifedipine	10.457	128753	-0.19	-0.04	-0.17	-0.29	-0.30	0.16
16	Dihydrocelastryl Diacetate	12.699	9828620	0.15	-0.19	-0.33	0.53	0.03	0.42
17	Hexadecanoic acid	17.82	985	0.02	0.06	-0.33	0.08	-0.04	0.18
18	ISORESERPINE	16.88	5701996	0.10	-0.36	-0.39	-0.36	-0.02	-0.21
19	N-Desmethylnefopam	9.952	601526	0.04	0.05	-0.35	0.44	-0.34	0.10
20	n-nonadecanoic acid	19.05	12591	0.14	0.05	-0.16	0.21	0.10	0.19
21	Pentadecanoica acid, 13-methyl	17.12	13849	-0.04	0.05	-0.42	0.01	-0.11	0.16
22	Peruvoside	22.366	12314120	0.09	-0.09	-0.33	0.21	0.13	0.71
23	Pravastatin	15.646	54687	0.45	0.27	-0.24	0.75	0.21	0.81
24	z-2-acetoxy 12-tetra decentrile	16.18	5363096	-0.02	0.04	-0.39	0.03	0.06	0.19

GPCR: G-protein coupled receptor, ICM: ion channel modulator, KIN: Kinases inhibitor, NR: Nuclear receptor ligand, PI: Protease inhibitor, EI: Enzyme inhibitor.

4. Conclusion

Present study demonstrate that the methanolic extract of *Artemisia vulgaris* (MEAV) exhibits potential antioxidant activity which might be due to the presence of Flavonoids, Phenols, Alkaloids and Saponins, having relatively equal activity with vitamin-C. In addition, different bioactive compounds in the extract may also exhibit significant therapeutic properties against various ailments.

5. Acknowledgment

Mr. G. Sandeep Kumar is grateful to the University Grants Commission (UGC), New Delhi for financial assistance through the award of a CSIR-UGC-NET-JRF to complete the above Research work. We also thank the SAIF, IIT, Chennai for GC-MS data acquisition and SAIF IIT, Mumbai for LC-MS data acquisition.

6. References

1. Edeoga HO, Okwu DE, Mbaebie BE. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4(7):685-688.
2. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A *et al*. Critical review: vegetables and fruit in the prevention of chronic diseases. Eur J Nutr. 2012; 51(6):637-63.
3. Dev S. Impact of natural products in modern drug development. Indian Journal of Experimental Biology. 2010; 48:191-198.
4. Mierziak J, Kostyn K, Kulma A. Flavonoids as important molecules of plant interactions with the environment. 2014; 19(10):16240-65.
5. Aneta W, Jan O, Renata C. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry. 2007; 105:940-949.
6. Vickers A, Zollman C, Lee R. Herbal medicine, Western Journal of Medicine. 2001; 175:125-128.
7. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* D C. BMC Complementary and Alternative Medicine. 2010; 10:21-28.
8. Saikat S, Raja C, Sridhar C, Reddy YSR, Biplab D. Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research. 2010; 3(1):91-100.
9. Oluwaseun AA, Ganiyu O. Antioxidant properties of methanolic extracts of mistletoes (*Viscum album*) from cocoa and cashew trees in Nigeria. African Journal of Biotechnology. 2008; 7:3138-3142.
10. Ahameethunisa AR, Hopper W. Antibacterial activity of *Artemisia nilagirica* leaf extracts, 2010.
11. Earl R, Stadtman. Role of Oxidant Species in Aging. Current Medicinal Chemistry. 2004; 11:1105-1112.
12. Duke JA, Godwin MJB, Du CJ, Duke PNK. Handbook of medicinal herbs (2nd Ed.). Washington, DC: CRC

Press, 2002.
13. Pires JM, Mendes FR, Negri G, Duarte-Almeida JM, Carlini EA. Antinociceptive peripheral effect of Achillea millefolium L. and *Artemisia vulgaris* L. both plants known popularly by brand names of analgesic drugs. Phytother Res. 2009; 23(2):212-9.
14. Sofowara A. Medicinal plants and traditional medicine in Africa. Spectrum books ltd. Ibadan, Nigeria, 1993, 298-300.
15. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. London, Chapman and Hall, Ltd. 1973; 2:49-188.
16. Spanos GA, Wrolstad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agriculture and Food Chem. 1990; 38:1565-1571.
17. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci. 2001; 8:203-208.
18. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10:1003-1008.
19. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese J Nutrition. 1986; 44:144-158.
20. Kikuzani H, Nakatani N. Antioxidant effect of some ginger constituents. J Food Sci. 1993; 58:1470-1410.
21. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J Agr. Food Chem. 1992; 40:945-948.
22. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J Agr. Food Chem. 1992; 40:945-948.
23. Sanchez-MC. Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Tech Int. 2002; 8:121-37.
24. Depo Y, Lara M, Jean P, Chaumont, Joelle MC. Use of Caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of onycomycosis. Mycopatholalogia. 2000; 148:79-82.
25. Kyung-RP, Dongwoo N, Hyung MY, Seok GL, Hyeung JJ, Gautam S *et al*. β-Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. Cancer Letters. 2011; 312:178-188.
26. Saad SD, Yasser MT, Mohamed B, Khadeer A, Amin MS, Abdul M. *In vivo* anti-inflammatory activity of β-caryophyllene, evaluated by molecular imaging. Molecules & Medicinal Chemistry. 2015; 1:e1001.
27. Hikari K, Soh K, Takaaki K, Hitoshi N, Maria TC, Giacinto B *et al*. Local Peripheral effects of β-Caryophyllene through CB2 Receptors in Neuropathic Pain in Mice. Pharmacology & Pharmacy. 2012; 3:397-403.
28. Sun LN, Shen J, Su F, Wang Q, Zhu YJ, Lou XE, *et al*. Bicyclol attenuates oxidative stress and neuronal damage following transient forebrain ischemia in mouse cortex and hippocampus. Neurosci Lett. 2009; 459(2):84-7.
29. Zhang YW, Guo YS, Bao XQ, Sun H, Zhang DJ. Bicyclol promotes Toll-like 2 receptor recruiting inosine 5'-monophosphate dehydrogenase II to exert its anti-inflammatory effect. Journal of Asian Nat Prod Res. 2016; 18(5):475-485.
30. Gao D, Zhang YL, Xu P, Lin YX, Yang FQ, Liu JH, *et al*. *In vitro* evaluation of dual agonists for PPARγ/β from the flower of *Edgeworthia gardneri* (wall.) Meisn. J Ethnopharmacol. 2015; 162:14-19.
31. Ringbom T, Huss U, Stenholm A, Flock S, Skattebol L, Perera P *et al*. Cox-2 inhibitory effects of naturally occurring and modified fatty acids. J Nat Prod. 2001; 64(6):745-749.
32. Kim C, Cho SK, Kapoor S, Kumar A, Vali S, Abbasi T *et al*. Beta Carryophyllene oxide inhibits constructive and inducible STAT 3 signalling pathway through induction of the SHP-1 protein tyrosine phosphate. Mol carcinog. 2014; 53(10):793-806.
33. Kim HS, Kim MJ, Kim EJ, Yang Y, Lee MS, Lim JS. Berberine-induced AMPK activation inhibits the metastatic potential of melanoma cells via reduction of ERK activity and COX-2 protein expression. Biochem Pharmacol. 2012; 83(3):385-94.
34. Brunhofer G, Fallarero A, Karlsson D, Batista GA, Shinde P, Gopi MC *et al*. Exploration of natural compounds as sources of new bifunctional scaffolds targeting cholinesterases and beta amyloid aggregation: the case of chelerythrine. Bioorg Med Chem. 2012; 20(22):6669-79.
35. Chan MY. The effect of berberine on bilirubin excretion in the rat. Comp Med East West. 1997; 5(2):161-168.
36. Brayfeild AE. (2011) Nefopam hydrochloride. Medicines complete London, UK. Pharmaceutical press.
37. Kubota R, Kubota K, Yamada S. Enhancement of the effects of anticancer agents on B16 melanoma cells by combination with cepharanthine-II. Antimetabolite, alkylating agent, nitrosourea. Gan to Kagaku Ryoho 1992; 19(13):2169-2174.
38. Kobuchi H, Li MJ, Matsuno T, Yasuda T, Utsumi K. Inhibition of neutrophil priming and tyrosyl phosphorylation by cepharanthine, a nonsteroidal antiinflammatory drug. Cell Struct Funct. 1992; 17(6):385-93.
39. Wu CY, Jan JT, Ma SH, Kuo CJ, Juan HF, Cheng YS, *et al*. Small molecules targeting severe acute respiratory syndrome human coronavirus. Proc Natl Acad Sci U S A. 2004; 101(27):10012-7.
40. Simpson CD, Mawji IA, Anyiwe K, Williams MA, Wang X, Venugopal AL *et al*. Inhibition of the sodium potassium adenosine triphosphatase pump sensitizes cancer cells to anoikis and prevents distant tumor formation. Cancer Res. 2009; 69(7):2739-47.
41. Hairi L, Hongyan Z, Dong W, Jinsong Q, Yu Z, Xiangqiang L *et al*. Versatile pathway-centric approach based on high-throughput sequencing to anticancer drug discovery. 2012; 109(12):4609-4614.