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PHYTOCHEMICAL INVESTIGATION OF METHANOLIC EXTRACT OF *ARTEMISIA VULGARIS*, L. LEAVES

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ABSTRACT

The deleterious effect of oxidative stress can be counteracted by the presence of antioxidants. But due to the adverse side effects caused by synthetic antioxidants, the search for natural antioxidants has become important.In the present study, the methanolic extract of *Artemisia vulgaris*, L. was screened for the presence of phytochemicals by qualitative preliminary phytochemical screening, HPTLC, HPLC and GC-MS methods. Qualitative screening indicated the presence of phenolics and flavonoids. HPTLC results showed the presence of six phenols and four flavonoids. The HPLC spectrum showed 3 peaks, indicating the presence of three principle components. The GC results showed the presence of five major components at retention times 9.244, 10.174, 11.487, 13.388 and 13.568 respectively. Thus, the phytochemical analyses of methanolic extract of *Artemisia vulgaris*, L. leaves revealed the presence of phenolics, flavonoids and sesquiterpenoid type compounds.

KEYWORDS: **Antioxidants, Alkaloids, Phenols, Flavonoids, Sesquiterpenoids.**

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INTRODUCTION

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbits. To this group belongs reactive oxygen, nitrogen and chloride species[1]. Oxidative stress occurs due to the imbalance between the production and the elimination of a variety of oxygen species. These species have the ability to degrade macromolecules such as lipids, nucleic acids, proteins and pigments[2]. Growing research has given evidence that the deleterious effects of ROS are responsible for various chronic diseases including cancer and cardiovascular diseases[3]. In recent years, there is a tremendous interest in the possible role of nutrition in the prevention of diseases. In this context, antioxidants derived from natural sources such as medicinal plants and herbs require special attention. Antioxidants have many potential applications, especially in relation to human health, both in terms of prevention of disease and therapy[4]. Plants produce a higher number of naturally occurring secondary metabolites, many of them with unique pharmacologic activities. These metabolites include the flavonoids, phenols, phenolic glycosides, saponins, cyanogenic glycosides, unsaturated lactones and glucosinolates[5]. Natural products and related drugs are used to treat 87% of all categorized human diseases including cancer, bacterial infection and immunological disorders. About 25% of the prescribed drugs in the world originate from plants. About 80% of the population in developing countries relies on traditional plant-based medicines for their primary health care needs[6]. *Artemisia vulgaris* L. (mugwort) is a medicinal plant belonging to the family *Asteraceae* and is a tall aromatic perennial herb. In traditional medicine, this plant is used for the treatment of diabetes and the extracts of the whole plant are used for epilepsy and in combination of psychoneurosis, depression, irritability, insomnia, anxiety and stress[7]. This plant also showed antispasmodic, antiseptic, antibacterial, antimalarial, antitumour, antirheumatic and hepatoprotective properties[8]. *Artemisia vulgaris*, L has not only been used as an edible plant (spice) but also as a folk medicine resource[9]. Hence it was thought worthy to investigate the phytochemical constituents of the methanolic extract of *Artemisia vulgaris*, L. leaves. Preliminary phytochemical screening was carried out to find out the presence of active compounds; this was further analyzed by HPTLC, HPLC and GC-MS studies to find out the nature of phytochemicals present in the leaf extract.

MATERIALS AND METHODS

The plant sample was procured from Tamil Nadu Agricultural University, Coimbatore and grown as pot culture in Avinashilingam University Campus. The plant was authenticated by Botanical Survey of India, Coimbatore as *Artemisia vulgairs*, L. (Voucher number BSI/SC/5/23/08-09/Tech-1711).

Chemicals

All the chemicals used were of analytical grade obtained from Sigma and Merck.

Preparation of Methanolic Extract

The fresh leaves of *Artemisia vulgaris* (5g) were shade dried and coarsely powdered and then the plant material was packed in thimble and successively extracted with methanol (100ml) using soxhlet apparatus. After extraction, the solvent was evaporated to dryness using an evaporator.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was performed in the plant sample to find out the presence of alkaloids, phenolics and flavonoids[10].

HPTLC Analysis

The methanolic residue (100mg) of *Artemisia vulgaris* leaves was dissolved in methanol (1ml) and centrifuged at 3000rpm for 5 minutes. The supernatant was collected and used as a sample for HPTLC analysis. The test sample

(3µl) was loaded as an 8mm band in the 5 X 10 Silica gel 60 F_{254} plate using a Hamilton syringe and CAMAG INOMAT 5 instrument. After saturation with the solvent vapour, the TLC plate loaded with test and the reference was kept in a TLC twin trough developing chamber with the respective mobile phase and developed up to 90mm. The developed plates were dried in hot air to evaporate the solvents from the plates. The plates were kept in photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured in white light, UV 254nm and UV 366nm. After derivatization with the appropriate reagents, the plates were photo-documented at daylight for alkaloids, phenols and sesquipterpenoids, and at UV 366nm for flavonoids. Finally, the plates were fixed in the scanner stage and scanned at 500nm for alkaloid, phenolics and sesquiterpenoids, and 366nm for flavonoids. The peak table, peak display and peak densitogram of alkaloids, phenolics, flavonoids and sesquiterpenoids were noted.

HPTLC Profile of Alkaloids

The mobile phase used was ethylacetate : methanol : water (10:1.35:1). The developed plates were sprayed with Dragandroff's reagent followed by ethanolic sulphuric acid reagent. Then the plates were heated at 120°C for 5 minutes in a hot air oven. Nicotin was used as the reference and the presence of alkaloids was confirmed by the appearance of bright orange coloured zones in the daylight mode.

HPTLC Profile of Phenolics

The mobile phase used was toluene: chloroform : acetone (4: 2.5: 3.5). After development, the plate was sprayed with 25% aqueous Folin-Ciocalteu reagent and heated at 120°C for 5 minutes in a hot air oven. Quercetin was used as the reference standard for the analysis of phenolics. The presence of phenolics was confirmed by the appearance of blue or blue-grey coloured zones at daylight.

HPTLC Profile of Flavonoids

The mobile phase used was ethylacetate : butanone : formic acid in the ratio of 5:3:1. The plate was sprayed with 1% ethanolic aluminium chloride reagent and heated at 120°C for 5 minutes in a hot air oven. Rutin was used as the reference standard for flavonoid analysis. The presence of flavonoids was confirmed by the appearance of yellow and yellow-green fluorescence at 366nm.

HPTLC Profile of Sesquiterpenoids

The mobile phase used was n-hexane : ethyl acetate (7.2:2.9). After development, the plate was sprayed with 5% ethanolic molybdo-phosphoric acid reagent and heated at 120°C for 5 minutes in a hot air oven. Solanesol was used as the reference standard for sesquiterpenoid analysis. The presence of sesquiterpenoids was confirmed by the appearance of blue or grey-blue coloured zones at daylight.

HPLC Analysis

The residue of the methanolic extract of the *Artemisia vulgaris* leaves was dissolved in HPLC grade acetonitrile and 20µl of the sample was injected in to the HPLC apparatus (Shimadzu, Japan equipped with UV detector and a reverse phase C18 column). The sample analysis was performed at room temperature, in the wavelength range of 200-320nm at 1000psi. Acetonitrile and water in the ratio of 15:85 containing 1% acetic acid was used as the mobile phase, with a run time of 30 minutes at a flow rate of 1ml/minute.

GC-MS Analysis

The methanolic residue of *Artemisia vulgaris* leaves was analyzed using a Shimadzu gas chromatography apparatus (Model qp 5000 GC-MS) using a DB-S capillary column (30m) equipped with QP MS detector (EI, 70 ev) with helium as a carrier gas at a flow rate of 1ml/minute. The compounds were identified using the WILEY database available in the software provided.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Traditional medicinal plants have the ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. The useful major groups of phytochemicals can be divided into several categories that include alkaloids, flavonoids, flavonols, quinines, essential oils, lectins, polypeptides, phenolics, polyphenols, tannins and terpenoids[11]. The preliminary phytochemical screening indicated the absence of alkaloids and the presence of phenolics and flavonoids (Table 1).

Table 1
Preliminary phytochemical screening

Alkaloids		**Phenolics**		**Flavonoids**	
Name of the test	**Result**	**Name of the test**	**Result**	**Name of the test**	**Result**
Mayer's	**-**	**Ferric chloride**	**+**	**Aqueous NaOH**	**+**
Dragendroff's	**-**	**Lead acetate**	**+**	**Concentrated H_2SO_4**	**+**
Wagner's	**-**			**Aluminium chloride**	**+**

+ Presence - Absence

HPTLC analysis

The methanolic extract of *Artemisia vulgaris* leaves was subjected to HPTLC analysis for the presence of alkaloids, phenolics, flavonoids and sesquiterpenoids. The alkaloid profile of the methanolic extract was done with the reference standard nicotine and the developed plate was sprayed with Dragendroff's reagent. The absence of bright orange colour in plate after photo-documentation at day light indicated the absence of alkaloids (Plate 1). The peak table (Table 2) and peak densitogram (Figure 1) were recorded.

Table 2
Peak table for the alkaloids in the methanolic extract of Artemisia vulgaris leaves by HPTLC

Track	Peak	Rf	Height	Area	Assigned substance
AV	1	0.19	31	790.7	Unknown
AV	2	0.73	61.8	2588.2	Unknown
AV	3	0.80	273	10847.5	Unknown
R	1	0.1	220.4	5591.8	Nicotine

The phenolics present in the methanolic extract of *Artemisia vulgaris* leaves were analysed using quercetin as reference standard. The presence of blue, blue grey colour in day light showed the presence of phenolics (Plate 2). There were totally 6 phenols identified in the methanolic extract of *Artemisia vulgaris* leaves. Two unspecific spots were also obtained. The peak table (Table 3) and peak densitogram (Figure 2) were recorded after scanning at 500nm.

Table 3
Peak table for the phenolics in the methanolic extract of Artemisia vulgaris leaves by HPTLC

Track	Peak	Rf	Height	Area	Assigned substance
AV	1	0.04	95.8	1180.7	Phenolics 1
AV	2	0.08	37.9	481.6	Phenolics 2
AV	3	0.12	35.3	702.5	Phenolics 3
AV	4	0.24	57	1906	Phenolics 4
AV	5	0.33	27.7	810.8	Unknown
AV	6	0.61	195	7932.2	Phenolics 5
AV	7	0.66	174.7	6172.8	Unknown
AV	8	0.74	161.2	9556.2	Phenolics 6
R	1	0.27	202.8	9172.9	Quercetin

The flavonoids profile of the methanolic extract of *Artemisia vulgaris* leaves was analysed using rutin as a standard. Yellow and yellow green fluorescence zone at UV 366nm was observed from the chromatogram, which confirmed the presence of flavonoids (Plate 3). There were four flavonoids identified in the methanolic extract of *Artemisia vulgaris* leaves. There were also four unspecified spots in the chromatogram developed with the flavonoid specific spray reagent. The peak table (Table 4) and peak densitogram (Figure 3) were recorded.

Table 4
Peak table for the flavonoids in the methanolic extract of Artemisia vulgaris leaves by HPTLC

Track	Peak	Rf	Height	Area	Assigned substance
AV	1	0.1	35.8	674.9	Flavonoid 1
AV	2	0.18	43	1483.5	Unknown
AV	3	0.36	349.5	15599.2	Unknown
AV	4	0.44	176.4	5996.1	Flavonoid 2
AV	5	0.57	60.6	2061.9	Unknown
AV	6	0.7	53	2070.3	Flavonoid 3
AV	7	0.81	232.7	7269.9	Flavonoid 4
AV	8	0.85	76	1279.1	Unknown
R	1	0.29	81.3	1763	Rutin

The sesquiterpenoid profile of the methanolic extract of *Artemisia vulgaris* leaves was analysed using solanesol as a standard. The chromatogram showed distinct bands of sesquiterpenoids (Plate 4). The Rf values obtained for all the sesquiterpenoids are depicted in Table 5. The table shows the presence of eight sesquiterpenoids in the plant extract. The peak table (Table 5) and peak densitogram (Figure 4) were recorded.

Table 5
Peak table for the sesquiterpenoids in the methanolic extract of Artemisia vulgaris leaves by HPTLC

Track	Peak	Rf	Height	Area	Assigned substance
AV	1	0.32	106.2	7869.4	Sesquiterpenoid 1
AV	2	0.58	182.9	8002.5	Sesquiterpenoid 2
AV	3	0.71	111.7	3691.9	Sesquiterpenoid 3
AV	4	0.76	78.9	2243	Sesquiterpenoid 4
AV	5	0.82	26.7	571.7	Sesquiterpenoid 5
AV	6	0.88	18	294.1	Sesquiterpenoid 6
AV	7	0.92	76.3	1528.1	Sesquiterpenoid 7
AV	8	0.95	125	2898.5	Sesquiterpenoid 8
R	1	0.76	148.1	4793.2	Solanesol

The HPTLC analysis of *Annona squamosa* L. revealed the presence of linalol, borneol, eugerol, fernsol and geraniol[12]. In the HPTLC screening of *Strychnos potatorum* L., the ether fraction showed seven peaks, the unsaponifiable fraction showed five peaks and the alkaloid fraction showed seven peaks[13].

HPLC Analysis

The HPLC analysis of the methanolic extract of *Artemisia vulgaris* leaves was carried out using C18 RP column (Shimadzu equipped with UV detector). The results obtained are presented in Figure 5. The HPLC spectrum showed 3 peaks (2 major and 1 minor), indicating the presence of three principle components in the methanolic extract of *Artemisia vulgaris* leaves. The retention times of the major and minor peaks were 8.215, 14.972 and 12.054 minutes respectively. The retention time, height and peak area of all the three peaks are presented in Table 6.

Table 6
Peak table of the methanolic extract of Artemisia vulgaris leaves subjected to HPLC

Peak no.	Time (min)	Area	Height
1	8.215	36062145	513186
2	14.972	77832494	823738
3	12.054	4077992	56628

HPLC of the methanolic extracts of *Syzygium cummini* and *Terminalia bellerica* gave peaks at retention times 4.58 and 4.61 minutes respectively, which were similar to the retention time of gallic acid (4.6)[14]. Quercetin, a flavonoid identified from *Citrullus colocynthus*, showed a characteristic peak at a retention time of 3.475 minutes[15].

GC-MS Analysis

The GC-MS analysis of the methanolic extract of *Artemisia vulgaris* leaves was carried out to identify the nature of the components present. The GC results showed the presence of five major components at retention times 9.244, 10.174, 11.487, 13.388 and 13.568 respectively (Figure 6). In the mass spectrum of GC peak at retention time 9.244, molecular ion peak was observed at m/z 145.7 and the base peak was observed m/z at 118. The other significant m/z peaks are at 145.7, 118.0, 90, and 62.9 respectively (Figure 7). The fragmentation pattern of this compound exactly matched to that of coumarin from the WILEY database. The mass spectrum of the compound with the retention time 10.174 gave five major peaks at m/z 124, 95, 82, 68 and 57 (Figure 8). The fragmentation pattern showed the presence of (M-28) peak at m/z 96.2 which is characteristic of (M-CO) peak. Hence the compound may contain 'CO' group. Three (M-14) peaks were also indicating the presence of CH_2 groups.

This indicates that the fraction may contain an aliphatic compound with 'CO' function. The mass spectrum of the fraction at 13.388 gave peaks at m/z 138.4, 123.4, 96.6, 94.8, 81, 70.9, 55 and 52.9 (Figure 9). The fragmentation pattern of the compound eluted at retention time 13.388 showed (M-28) peak at m/z 96 and (M-44) peak at m/z 94, which is characteristic of M-CO and M-CO_2 groups. Hence the compound may contain 'CO' group and a carboxylic acid group. The mass spectrum of the compound with retention time 13.568 gave peaks at m/z 293.8, 262.5, 178.5, 163.3, 135.4, 123.3, 96.5, 94.8, 81, 67, 55, 53.9 and 52.9 (Figure 10). The fragmentation pattern of the compound eluted at retention time 13.568 showed (M-28) peak at m/z 219 and (M-44) peak at m/z 178, which is characteristic of M-CO and (M-CO_2) groups. Hence the compound may be a flavonoid type compound. The GC-MS analysis of *Gymnema sylvestre* R. Br. revealed the presence of terpenoids, glycosides, saturated and unsaturated fatty acids and alkaloids[16]. GC-MS analysis of the ethanolic extract of *Aloe vera* showed 26 bioactive phytochemical compounds[17]. GC-MS results of the chloroform extract of *Andrographis paniculata* revealed phenols, aromatic carboxylic acids and esters, which are the molecules that are implied to be responsible for their antimicrobial activity[18].

Plate 1

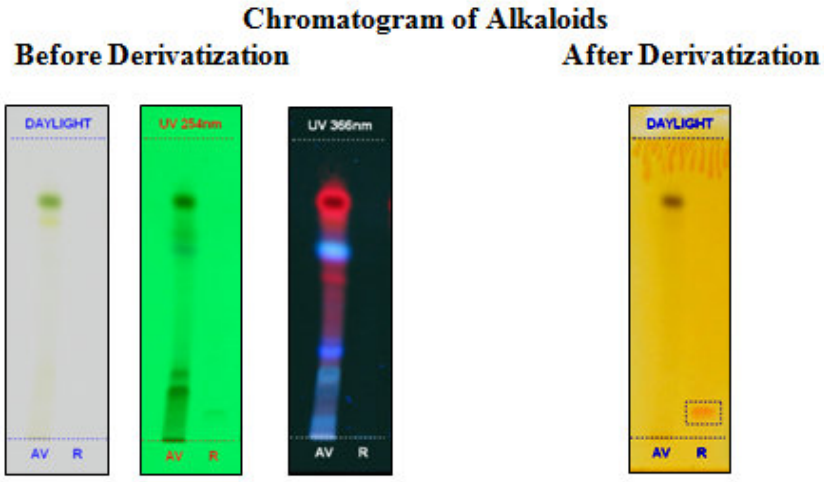


Figure 1
Peak densitogram of alkaloids in the methanolic extract of Artemisia vulgaris leaves by HPTLC

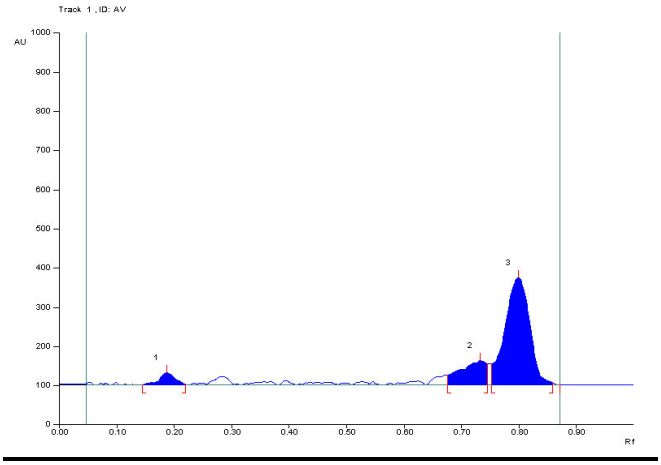


Plate 2

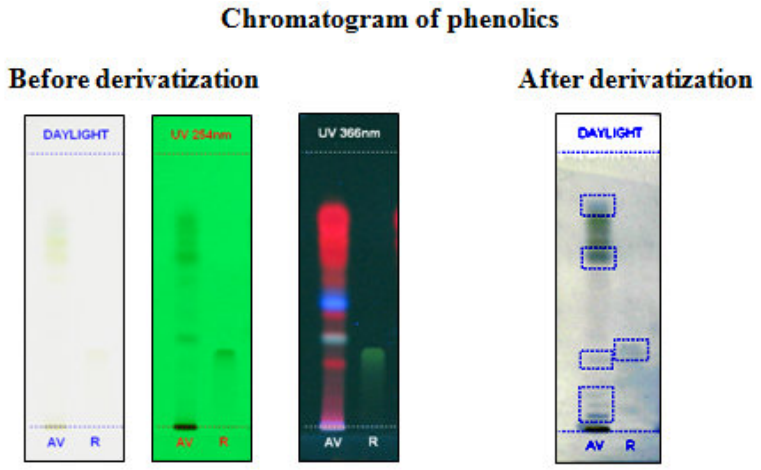


Figure 2
Peak densitogram of phenolics in the methanolic extract of Artemisia vulgaris leaves by HPTLC

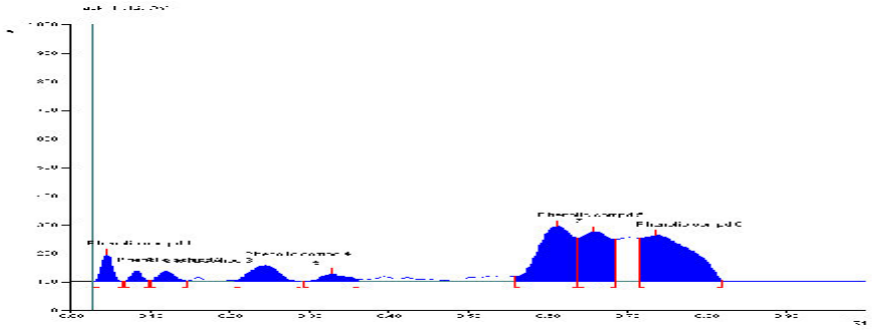


Plate 3

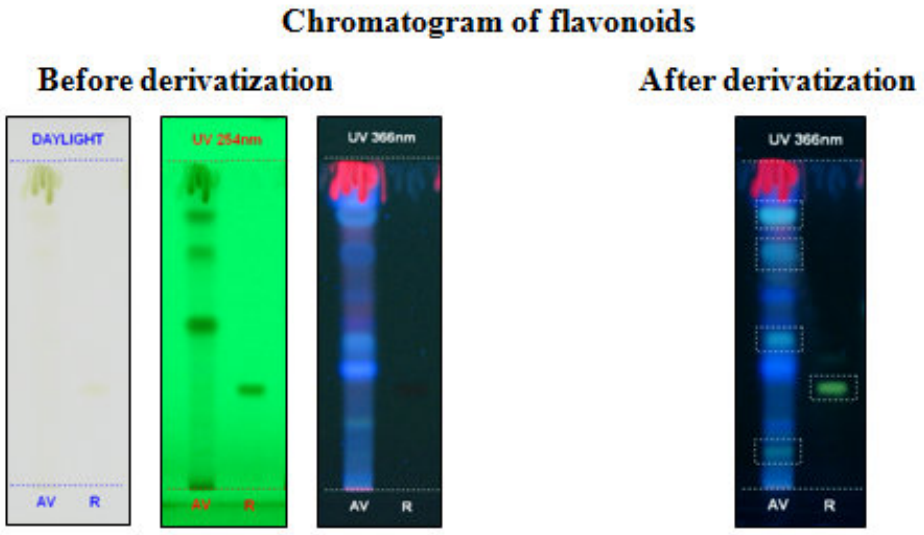


Figure 3
Peak densitogram of flavonoids in the methanolic extract of Artemisia vulgaris leaves by HPTLC

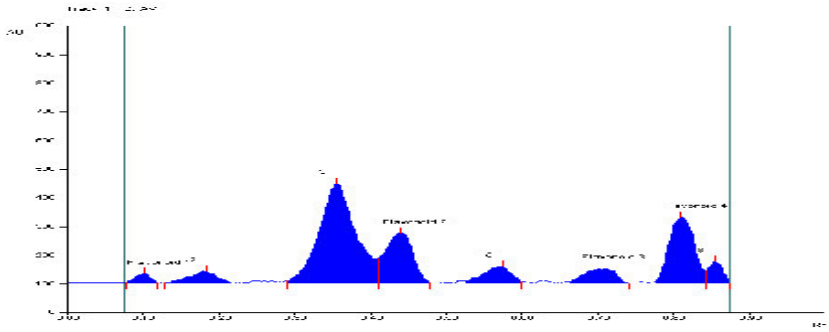


Plate 4

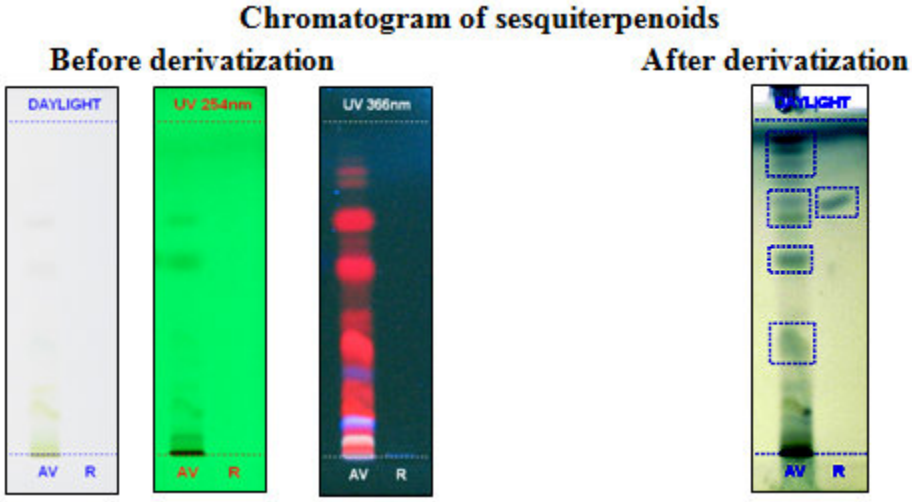


Figure 4
Peak densitogram of sesquiterpenoids in the methanolic extract of Artemisia vulgaris leaves by HPTLC

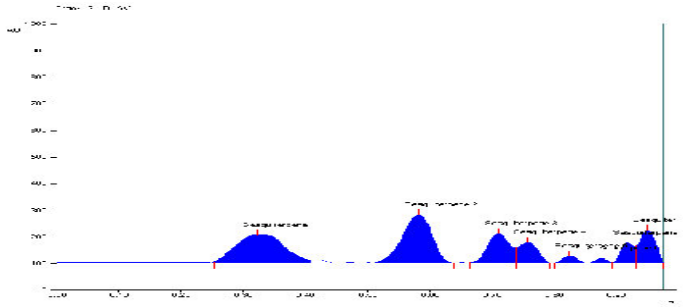


Figure 5
HPLC profile of the methanolic extract of Artemisia vulgaris leaves

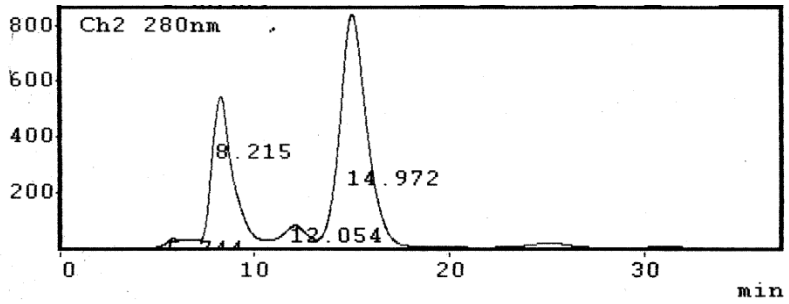


Figure 6
GC-MS profile of the methanolic extract of Artemisia vulgaris leaves

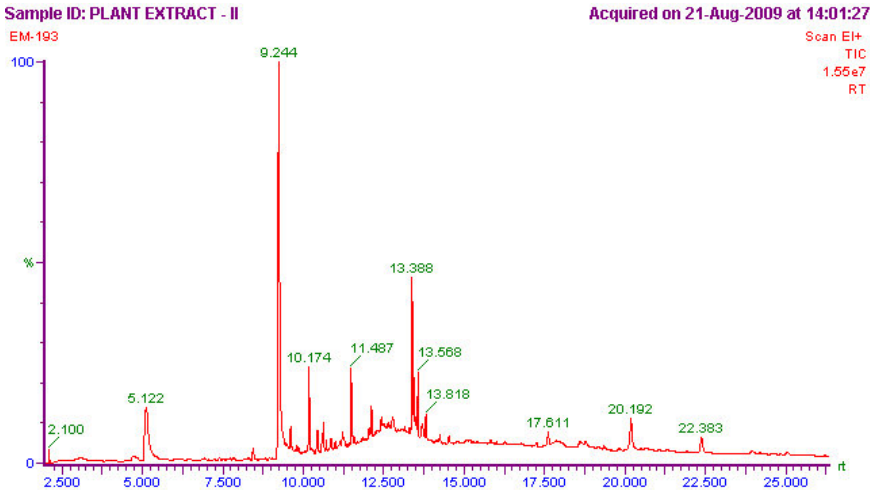


Figure 7
Peak fragmentation of GC-MS spectrum (9.244)

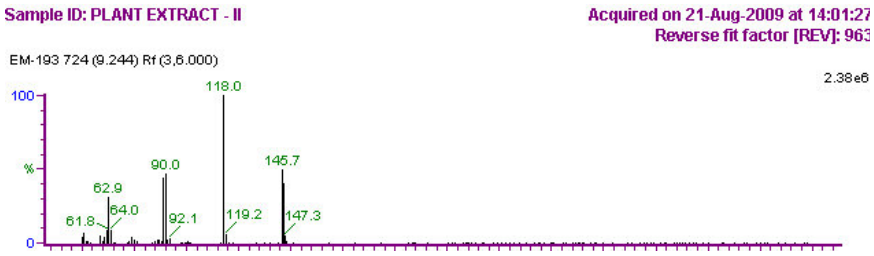


Figure 8
Peak fragmentation of GC-MS spectrum (10.174)

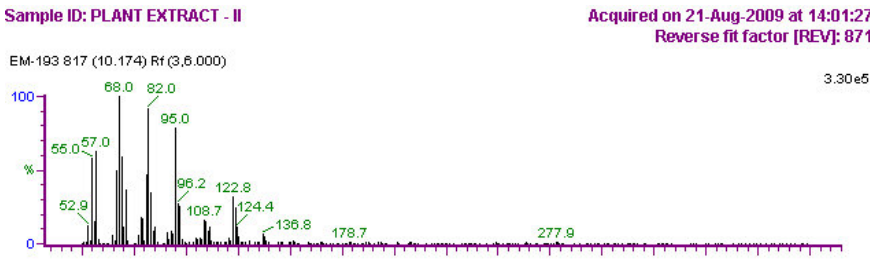


Figure 9
Peak fragmentation of GC-MS spectrum (11.487)

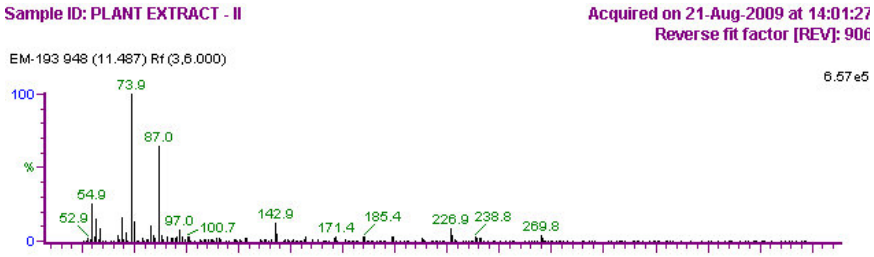


Figure 10
Peak fragmentation of GC-MS spectrum (13.388)

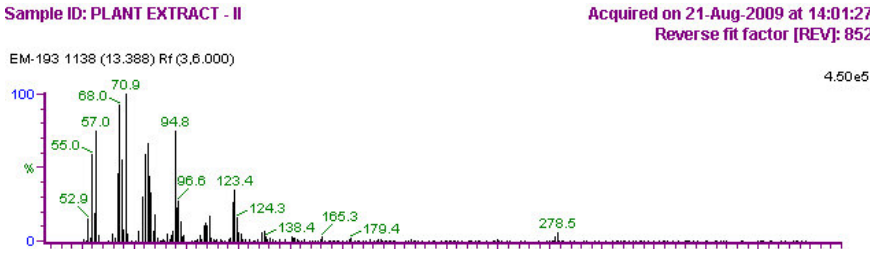
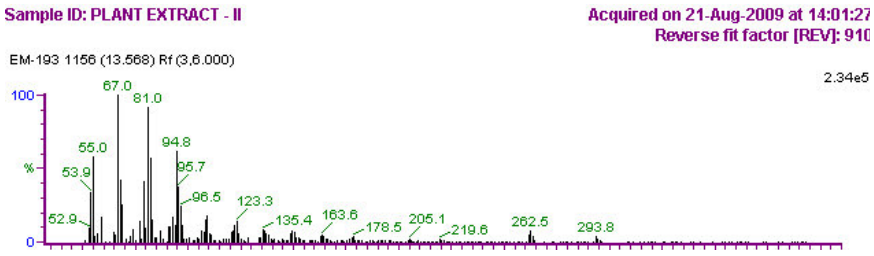


Figure 11
Peak fragmentation of GC-MS spectrum (13.568)



CONCLUSION

In the present study, preliminary phytochemical screening revealed the presence of phenols and flavonoids. This result was further supported by the results of spectral studies like HPLC. The HPTLC results revealed that, in addition to phenols and flavonoids, sesquiterpenoids were also present. The GC-MS spectra identified coumarin as one of the major compounds.

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