

# GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L

Duraisamy Gomathi · Manokaran Kalaiselvi ·  
Ganesan Ravikumar · Kanakasabapathi Devaki ·  
Chandrasekar Uma

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**Abstract** Medicinal plants are at great interest to the researcher in the field of biotechnology, as most of the drug industries depend in medicinal plants for the production of pharmaceutical compounds. Plants are the traditional sources for many chemicals used as pharmaceutical biochemicals, fragrances, food colours and flavours in different countries especially in India. Most herbal medicines and their derivative products were often prepared from crude plant extracts, which comprise a complex mixture of different phytochemical constituents (plant secondary metabolites). The chemical features of these constituents differ considerably among different species. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry. The aim of this study was to carry out for identification of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of ethanolic extract was done by standard protocol using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The GC-MS analysis revealed the presence of various compounds like piperine, octodecanoic acids, hexadecanoic acid and squalene in the ethanolic extract of *Evolvulus alsinoides*. Hence, the *Evolvulus alsinoides* may have chemopreventive, anticancer, anti-microbial activity, antioxidant and antidiabetic activity due to the presence of secondary metabolites in the ethanolic extract. Due to the presence of esters which can be used as a flavoring agent in food industries. These findings support the traditional use of *Evolvulus alsinoides* in various

disorders. Further studies are needed to isolate active principle of the extract as well as to elucidate their exact mechanism of action in various disorders.

**Keywords** *Evolvulus alsinoides* · Secondary metabolites · GC-MS analysis · Whole plant

## Introduction

Free radicals play a crucial role in the development of tissue damage in pathological events. Antioxidants are chemical compounds which have the ability to quench the free radicals and thereby it prevents the human body against various diseases. Plants are the rich sources of antioxidants which contain secondary metabolites such as phenolic and flavonoid compounds commonly which act as antioxidants with redox and metal chelating properties (Karimi and Jaafar 2011). Antioxidants are chemical compounds extremely useful to humans, which has the ability to reduce free radicals and/or to decrease their rate of production and lipid peroxidation in human bodies that cause various human diseases and aging (Galati and Brien 2004). The active principles of many drugs found in plants are secondary metabolites (Cragg and Newman 2005). Plants provide us with rich sources of natural antioxidants (Biswas et al. 2005) and so the phytochemical investigation on the extract for their main phytocompounds is very vital. Extraction is the main step for the recovery and isolation of bioactive phytochemicals from plant materials, before component analysis (Karimi and Jaafar 2011). Hence the medicinal plants are important sources of medicines and presently about 25 % of pharmaceutical prescriptions (Uma et al. 2009). According to the world health organization, the traditional medicine system will continue to play an essential role in health care system since over 80 % of the population in the third world country relies on the use of traditional medicine. Medicinal plants are

D. Gomathi · M. Kalaiselvi · G. Ravikumar · K. Devaki  
Department of Biochemistry, Karpagam University,  
Coimbatore 641 021, India

C. Uma (✉)  
Hawasaa University, Hawasaa, Ethiopia  
e-mail: umachandrasekaran29@gmail.com

**Table 1** GC-MS spectral analysis of ethanolic extract of *Evolvulus alsinoides*

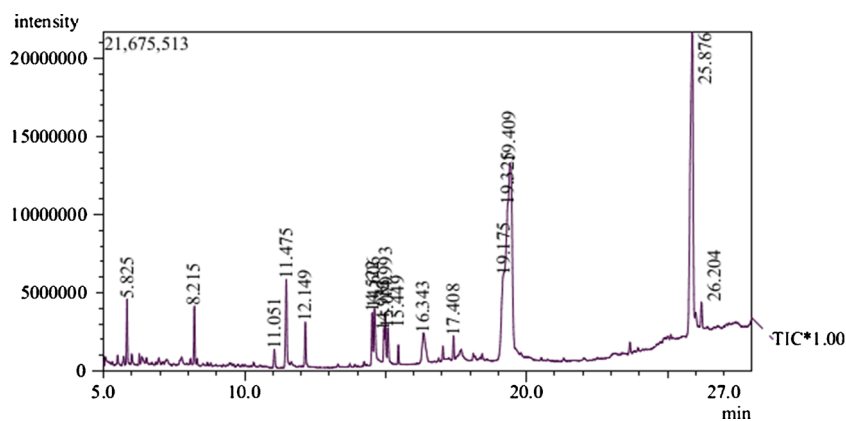
No.	RT (min)	Name of the compound	Molecular formula	Molecular weight	Peak Area %
1	5.825	4-(3-Hydroxybutyl) Phenol	C <sub>10</sub> H <sub>14</sub> O <sub>2</sub>	166	2.16
2	8.217	2-Methyl-5-(1,2,2-Trimethylcyclopentyl)Phenol	C <sub>15</sub> H <sub>22</sub> O	218	2.01
3	11.051	1,1-Dimethyldecahydronaphthalene	C <sub>12</sub> H <sub>22</sub>	166	0.89
4	11.475	Hexadecanoic acid (Palmitic acid)	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	3.98
5	12.149	Hexadecanoic acid, Ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	1.67
6	14.522	9,12-Octadecadienoic acid (Z,Z)	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	2.08
7	14.606	9-Octadecenoic acid, 1,2,3-propanetriyl ester	C <sub>57</sub> H <sub>104</sub> O <sub>6</sub>	884	2.91
8	14.933	Octadecanoic acid (Stearic acid)	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	1.29
9	14.993	Ethyl(9Z,12Z)-9,12 octadecadienoate	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	1.59
10	15.079	Ethyl oleate	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310	1.56
11	15.449	Methyl 17-methyl-octadecanoate	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	0.69
12	16.343	2,6,10,14,18,22-Tetracosahexaen (Squalene-triterpene)	C <sub>30</sub> H <sub>50</sub>	410	3.36
13	17.408	Methyl (2E) - 3-phenyl - 2-propeonate	C <sub>10</sub> H <sub>10</sub> O <sub>2</sub>	162	0.78
14	19.175	Cholesterol	C <sub>27</sub> H <sub>46</sub> O	386	10.38
15	19.325	Cholest-5-en-3-ol (3.β.)-	C <sub>27</sub> H <sub>46</sub> O	386	11.62
16	26.204	Piperine	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>	285	1.14

more prone to extinction. Hence, for the purpose of scientific validation of traditional medicinal plants or the discovery of lead compounds for use as therapeutic drugs, the active principals in medicinal plants needs to be identified (Vuorela et al. 2004).

*Evolvulus alsinoides* is an important popular Ayurvedic drug used to improve intelligence, memory and other higher mental functions. It is also used to treat bowel problems and to promote conception (Austin 2008; Sethiya et al. 2009). The entire plant was considered astringent, useful for treating hemorrhages and there are a variety of other medical applications including as an adaptogenic, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, stomachic, tonic, vermifuge, against asthma, bronchitis, scrofula, syphilis and to promote wound healing (Daniel 2008; Auddy et al. 2003). It is also used as an ingredient in formulations used in the management of diseases like psychosis, epilepsy and other conditions where brain activities are affected (Sharma 1983). It is used as best tonic for brain and nerves. It is included as a

Medhya drug in the treatises of Ayurveda like Carakasamhita, Susrutasamhita and Ashtanga Hridaya. The popular Ayurvedic formulations where *Evolvulus alsinoides* is included as one of the ingredients are Brahma rasayana, Jeevanyadi ghrita, Brahmi ghrita, Vachadi ghrita, Naladi ghrita and Agastya rasayana (Madhavan et al. 2008)

The extraction method presented is simple, rapid and inexpensive, with reduced solvent consumption. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry, environmental and forensic applications (Uma et al. 2009). It combines two analytical techniques to a single method of analyzing mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyzes each of the components separately. Chemical studies have shown that it mainly contains cardenolides, pregnane glycosides and volatile components (Deng et al. 1964; Hedeji

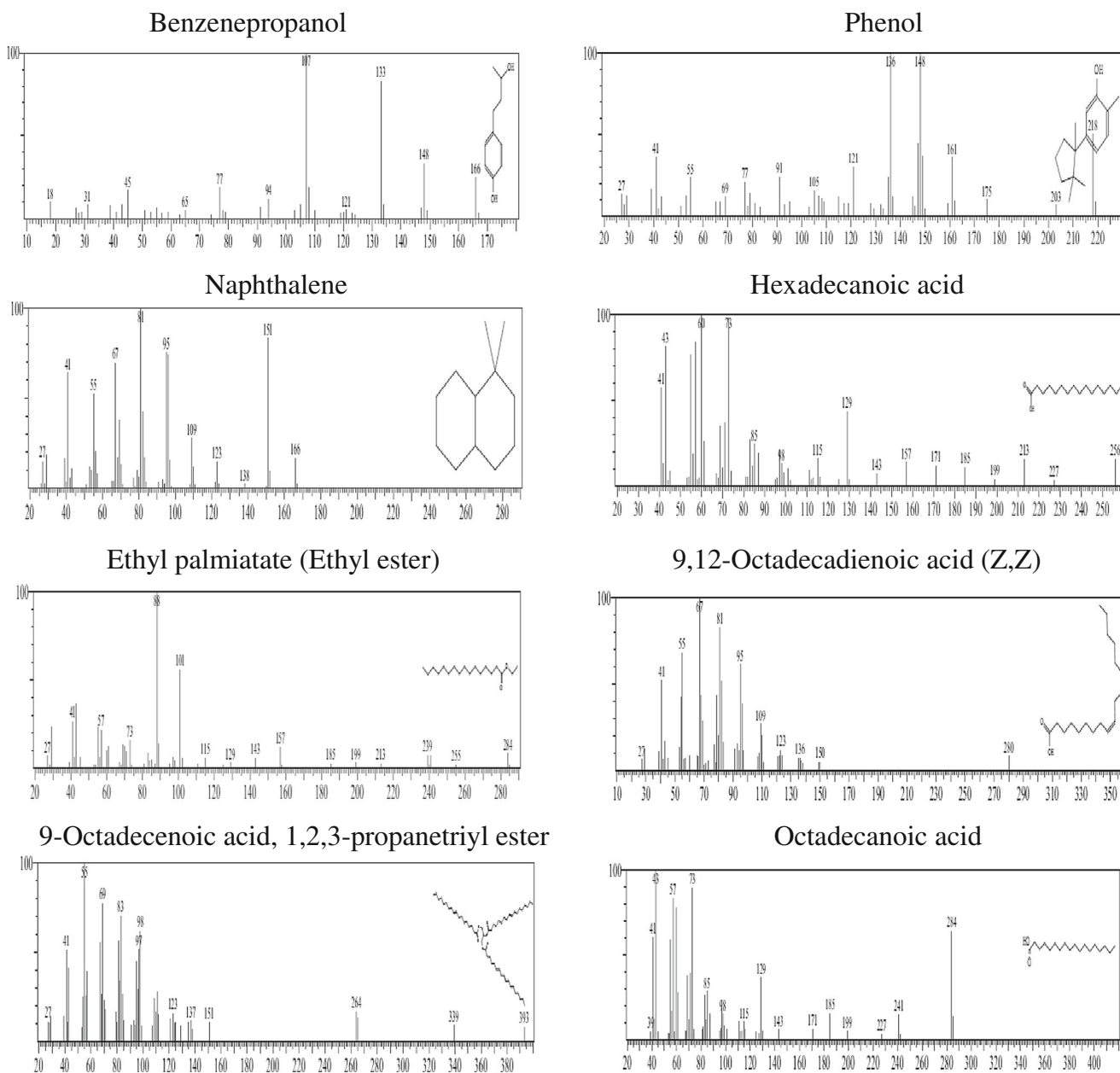
**Fig. 1** GC-MS chromatogram for whole plant ethanolic extract of *Evolvulus alsinoides*

and Xu 1988; Shi et al. 2005). Most of the volatile components are long chain unsaturated fatty acids which play very important roles in biological systems because they are structural elements of many valuable compounds as well as being important sources of energy (Mu et al. 2001). In recent years, increasing research has been carried out on fatty acids and the results obtained show that they possess significant sedative and hypnotic effects (Zhang et al. 1995). Hence, the present study was aimed to evaluate the chemical constituents in the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L.

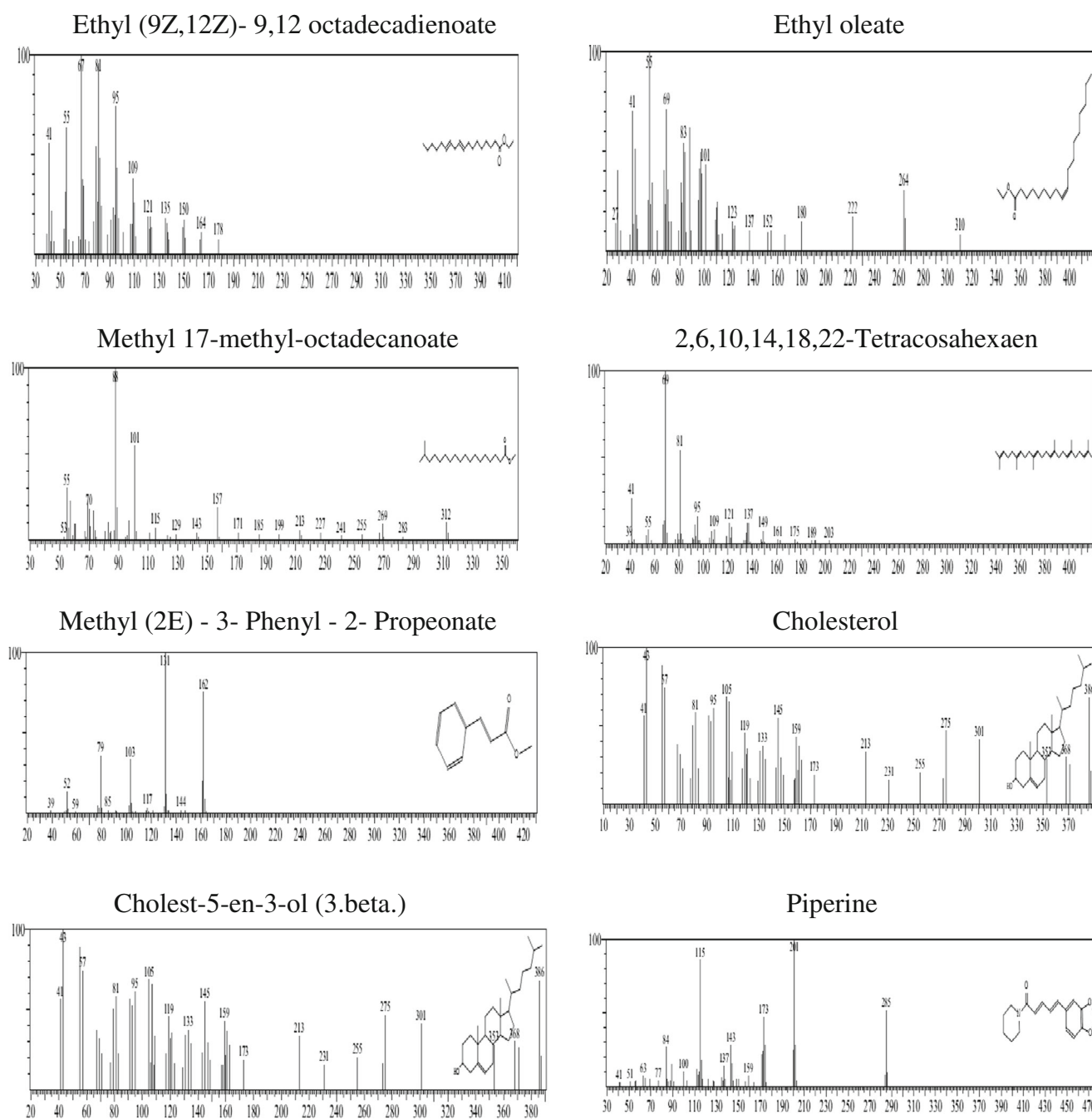
## Materials and methods

### Collection and identification of plant material

*Evolvulus alsinoides* (L.) L. used for the investigation was obtained from Coimbatore District, Tamilnadu, India. The plant was authenticated by Dr. P. Satyanarayana, Botanical Survey of India, Tamil Nadu Agricultural University (TNAU) Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2011-12/Tech.-514. Fresh whole plant material of *Evolvulus*



**Fig. 2** Mass spectra of identified compound from ethanolic extract of *Evolvulus alsinoides*

**Fig. 2** (continued)

*alsinoides* was washed under running tap water, air dried and powdered in electric blender.

#### Sample extraction

A 100 g sample of dried plant powder was extracted in 500 ml of ethanol in an orbital shaker for 72 h. Repeated extraction was done with the same solvent until a clear colorless solvent was obtained. Obtained extract was evaporated to dryness and stored at 4 °C in an airtight container for further use.

#### Gas chromatography-mass spectroscopic analysis of *Evolvulus alsinoides*

GC-MS analysis of the whole plant extract of *Evolvulus alsinoides* was performed using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm×0.25 mm ID×0.25 μm film. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was

programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated.

## Result and discussion

Medicinal plants are the resources of new drugs. Many of the modern medicines are produced indirectly from the medicinal plants. They have contributed many ingredients to fight against various diseases and illness. The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations. Studying of medicinal plants also facilitates to comprehend plant toxicity and also helps to protect human and animals from natural poisons. Hence the present study was undertaken to find out the bioactive compounds present in the ethanolic extract of *Evolvulus alsinoides* by using Gas chromatography and Mass spectroscopy. The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area %) are presented in Table 1 and Fig. 1 which shows the presence of 16 bioactive phytochemical compounds in the ethanolic extract of *Evolvulus alsinoides*. The mass spectra of identified compounds from *Evolvulus alsinoides* were presented in Fig. 2.

Among the identified phytocompounds, tetradecanoic acid, squalene, piperine and n Hexadecanoic acid have the property of antioxidant, 5- $\alpha$ -reductase inhibitor, antifibrinolytic, hemolytic and antimicrobial activities (Bodoprost and Rosemeyer 2007; Abirami and Rajendran 2011; Kala et al. 2011). Octadecadienoic acid (Z, Z) have the property of anti-inflammatory, hypocholesterolemic and antiarthritic activity which was reported by the earlier workers (Rani et al. 2009; Ponnammam and Manjunath 2012; Uma et al. 2009). Naphthalene also having good antimicrobial activity (Uma et al. 2009).

The squalene (triterpine) is a phenolic compound and that the terpenes are found in latex and resins of some plants and physiological function of these compounds are generally believed to be a chemical in defense against certain pathogens causing human and animal diseases (Scortichini and Rossi 1991). Their activity is a function of the lipophilic properties of the constituent terpenes, the properties of their functional groups and their aqueous solubility (Mahato and Sen 1997; Ezhilan and Neelamegam 2012). It possesses anti microbial activity, chemopreventive activity against colon carcinogenesis (Rao et al. 1998). In addition to antimicrobial activity, the squalene was also reported to have anticancer, antioxidant, chemopreventive, gastroprotective and hepatoprotective

effects, pesticide, anti-tumor and sunscreen properties (Sunitha et al. 2001; Ukiva et al. 2002; Katerere et al. 2003).

Piperine is used all over the world for various illnesses. Some health benefits that can be gained from using piperine may be helpful in reducing inflammation, improving digestion, the reduction of pain and the relief of Asthma. It has also been extensively evaluated antidepressant (Li et al. 2007), anticonvulsant (Wang et al. 1999), antioxidant (Rauscher et al. 2000; Prakash and Srinivasan 2010), antimutagenic (El Hamass et al. 2003), hepatoprotective (Matsuda et al. 2008) endocrine and several other activities (Vijayakumar and Nalini 2006; Atal et al. 2012).

Esters are important organic compounds with increasing number of commercial applications (Foresti et al. 2005). These compounds are largely used in fragrances, cosmetics detergents, flavors and pharmaceuticals. Esters (ethyl oleates) may also be used as plasticizers and lubricants; biological additives and hydraulic fluids (Hazarika et al. 2002). The use of ethyl oleate in commercial applications has been hampered due to the low amounts that can be recovered from natural sources (Radzi et al. 2006; Martiinez-Ruiz et al. 2008). Due to the presence of above mentioned compounds in the whole plant ethanolic extract of *Evolvulus alsinoides* may be used in various pharmaceutical and industrial applications.

## Conclusion

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. Thus, the identification of bioactive compound in *Evolvulus alsinoides* was done by GC-MS analysis which shows the presence of 16 compounds. Among the identified compounds, octadecanoic acid, n-hexadecanoic acid, squalene and piperine have the role in antioxidant, antimicrobial, anticancer, antidiabetic and antiinflammatory effects. From this study it can be concluded that the *Evolvulus alsinoides* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds.

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