Chemical Composition and Anti-Mosquito Potential of *Catharanthus roseus* Leaves Extract Against Larvae of *Aedes Aegypti*

V. Shoba[1*], Kavisa Ghosh[2], K.Krishnapriya[3],C.Elanchezhiyan[1]

Abstract— *The primary aim of this study was to screen non-toxic and easily available mosquito control biodegradable agent of botanical origin. Catharanthus roseus has been reported for various pharmaceutical properties for treating many ailments in Ayurveda. The ethanol leaf extract of the Catharanthus roseus possessed a significantly higher larvicidal activity against the 3rd instar larvae of Aedes aegypti than that of other extracts, with LC_{50} values of 157.8 ppm, respectively. The phytochemical screening and identification of phyto-compounds present in the ethanolic extract of Catharanthus roseus leaves using gas chromatography-mass spectrometry (GC-MS) analysis revealed various phytocompounds. The preliminary phytochemical screening of the ethanolic leaf extract of Catharanthus roseus showed the presence of alkaloids, flavonoids, terpenoids, saponins, tannin, protein and steroid. GC-MS chromatogram of the ethanolic leaf extract of Catharanthus roseus showed 15 peaks indicating the presence of 15 compounds. GC-MS analysis revealed that the presence of Dodecanedioic acid, Bis (Trimethylsilyl) ester (RT: 17.299), Methyl-19-methyl-Eicosanate (RT: 18.140), N-Hexadecanoic acid (RT: 18.575), (1S,15S)-Bicyclo (RT: 13.1.0) Hexadecan-2-one (RT: 19.600), Methyl 7,11,14 Eicosatrienoate (RT: 19.665), Alpha-Linolenic acid, trimethylsilyl ester (RT: 20.371), 1-Methylene, 2B-hydroxymerthyl-3, 3-Dimethyl-4B-(3-methylbut-2-ethyl)-cyclohexane (RT: 20.441), Alpha linolenic acid, trimethylsilyl ester (RT: 20.601), Alpha linolenic acid, trimethylsilyl ester (RT: 21.026), Hentriacontane (RT: 22.191), Sulfurous acid, octadecyl 2-Propyl ester (RT: 22.972), Sulfurous acid, butyl tridecyl ester (RT: 23.702), Vitamin E (RT: 26.783), Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (RT:30.360), Cyclotrisiloxane, Hexamethyl (RT: 30.665). Among all the identified compounds, Hentriacontane possesses insecticidal, anti-candidal, anti-fungal and anti-inflammatory activities. Thus, ethanol leaf extract of the Catharanthus roseus possesses various potent bioactive compounds and can be used as potent natural mosquito larvicidal. Further studies are required for exploring its pharmaceutical properties.*

Index Terms— *Catharanthus roseus*, *Aedes ageypti*, larvicidal activity, phytochemical screening, GC-MS.
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1 INTRODUCTION

MEDICINAL plants are valuable gift from nature to human. The approval of traditional medicine is an alternative form of health care and the development of microbial resistance to the existing antibiotics has encouraged the researchers to scrutinize the antimicrobial and other biological activities of compounds from various plant sources [Sumathi and Parvathi A, 2010]. Herbal medicines are safer than synthetic medicines because the phyto-compounds of the plant extract have no side effects. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries [Zaidan, 2005]. Plant-based natural constituent scan be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [Gordon, 2001]. The medicinal properties of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [Wink *et al*., 1999]. There is a growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [Prachayasittikul *et al*., 2008]. A variety of pharmacological functions of this plant like anti-inflammatory, diuretic, expectorant, hepatoprotective andnephroprotective activities were reported [Manokaran, 2008]. Plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties [De-Fatima *et al*., 2006].Dengue fever and dengue-dengue hemorrhagic fever (DHF) are considered to be the most important tropical infectious diseases, after malaria [Gubler, 1998b]. Ae. aegypti is considered to be the most efficient dengue vector due to its preference for human hosts and resides in densely populated locations. However, *Ae. albopictus* is considered to be the primary vector in some locations of the world in which *Ae. aegypti* populations are low. Ae. aegypti is mainly found in the sub-tropical zone of the Americas, and is a great threat to humans due to domestication and their diurnal habits. Additionally, Ae. species have been found to transmit various other diseases such as West Nile, LaCrosse encephalitis, and Yellow Fever [Watts, 1973],[WHO, 1997],[Nash *et al*., 2001] viruses.

2 MATERIALS AND METHODS

2.1 Laboratory maintenance of the mosquito larvae

The mosquitoe A. aegypti were reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. The larvae were divided in groups and maintained separately and fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at 28 ±

[1]Department of Zoology, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India
[2]Unit of Toxicology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India. E-mail id: kavisa9@gmail.com
[3]Maruthu pandiyar college, Vallam, Tanjavur-613 403. Tamil Nadu, India.
*Corresponding Author; E-mail id: shobav09@gmail.com

2°C, 70-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark. Third instar larvae of A. aegypti were used for the study.

2.2 Plant material collection and identification

Catharanthus roseus (pink variety) was taxonomically authenticated by the Department of Botany, Annamalai University, Chidambaram, and the voucher specimen was kept in the herbarium (Bot/Her/751) of our University. Fresh leaves of *C. roseus* (pink variety) were collected during September, from the Thiruchotruthurai, Tanjavur, and the leaves were shade dried and then grinded to fine powder.

2.3 Extraction

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant leaf powder (1.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with five different solvents namely ethanol, hexane, butanol, diethyl ether, acetone and aqueous extract individually. The solvents from the extracts were removed using a rotary vacuum evaporator (Buchi Labortechnic AG, Switzerland) to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassay.

2.4 Larvicidal Bioassay

The larvicidal activity of the plants crude extracts was evaluated as per the method recommended by WHO [2005]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage, the concentrations ranging from 100 to 300ppm. Five replicate were set up for each concentration and an equal number of control were set up simultaneously using tap water. To this 1 ml of appropriate solvent was added. The LC50 value was calculated after 24 h by Probit analysis [Finney, 2009].

2.5 Percentage yield of plant extracts

The percentage yields of the extracts were determined gravimetrically using the dry weight of the crude extract obtained (X) and dry weight of plant powder used for the extraction (Y) by using the following formula:
Percentage yield = X/Y * 100

2.6 Qualitative analysis

Phytochemical screening was carried out by using 1 gram of the dried ethanolic extract which was subjected to phytochemical test as described below [Harborne, 1973].

2.6.1 Detection of alkaloids (Mayer's Test)

The extracts was dissolved in dilute Hydrochloric acid and filtered. The filtrate was treated with Mayer's reagent (potassium mercuric iodide). Formation of yellow colored precipitate indicates the presence of alkaloids.

2.6.2 Detection of phenols (Ferric Chloride Test)

Extract was treated with 3-4 drops of 10% ferric chloride solution. Formation of green colour indicates the presence of phenols.

2.6.3 Detection of flavonoids (Alkaline Reagent Test)

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2.6.4 Detection of tannins (Gelatin Test)

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

2.6.5 Detection of saponins (Foam Test)

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

2.6.6 Detection of terpenoids (Salkowski test)

The extract was added 2 ml of chloroform. Concentrated H S0 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

2.6.7 Detection of carbohydrates (Molish's test)

To 2 ml of filtrate, two drops of alcoholic solution of α-naphthol are added, the mixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

2.6.8 Detection of Protein

To 1 mL of extract added few drop of mercuric chloride. Formation of yellow colour indicates the presence of protein.

2.7 GC-MS analysis of crude extract

GC-MS analysis was done at REFSYN BIOSCIENCES PVT. LTD., Puducherry, India. The GC–MS analysis of crude extract was performed using a PerkinElmer Clarus® 680 equipped with a mass spectrometer detector (Clarus 600 model) on an Elite-5MS (30.0 m, 0.25 mmID, 250 µm df) capillary column. The initial oven temperature was 60 ◦C for 2 min, ramped at 10◦C/min to 300◦C and held for 6 min. The carrier gas used was helium at a flow rate of 1 ml min-1. The injection was performed in split mode (10:1). The temperature of the injector was maintained at 250°C. The mass spectrometer was set to scan in the range of m/z 50–600. Mass transfer line and source temperature were set at 240°C and 240°C respectively. Total run time was 32.00 minutes.

The time at which each component eluted from the GC column was termed as Retention time (RT). The eluted component was detected in the Mass detector. Turbo Mass version 5.4.2 software was used for the spectral analysis. The name, molecular weight and structure of the unknown compounds of the test materials in GC-MS study was ascertained by comparing the spectrum of the unknown

compounds with the spectrum of the known compounds stored in the NIST (2008) library. The GC-MS information, acquisition parameters and Mass condition (EI) are summarized below.

GC-MS information
Make : Perkin Elmer
GC model : Clarus 680
Mass Spectrometer : Clarus 600 (EI)
Software : Turbo Mass version 5.4.2
Library version : NIST-2008
ACQUISITION PARAMETERS
Oven: Initial temp 60°C for 2 min,
ramp 10°C/min to 300°C, hold 6 min
Total Run Time: 32.00 mint
Injection =250°C, Volume=1 µL, Split=10:1,
Flow Rate: 1 mL/minute
Carrier Gas=Helium
Column=Elite-5MS (30.0m, 0.25mmID, 250µm df)
MASS CONDITION (EI)
Solvent Delay=2.00 min
Transfer Temp=240°C
Source Temp=240°C
Scan: 50 to 600Da

2.8 Statistical analysis

The data were expressed as mean ± SD (n = 3). Statistical analysis of the data was carried out by one-way analysis of variance (Anova) followed by Duncan's Multiple Range Test (DMRT) using a statistical package program (SPSS v11.5 for Windows) $p < 0.05$ were considered as statistically significant.

3 RESULTS

The results of the larvicidal activity are presented in Table 1. Among the different solvent ethanolic extract were found to be more susceptible followed by hexane, ethyl acetate, acetone, butanol, aqueous extract and control. The ethanolic extract was found to be the most effective at 157.8 ppm (LC50 value) against the larvae of *Aedes aegypti* at 24 hr. Almost negligible mortality was observed in control (non-treated).

Further the ethanolic leaves extract of *Catharanthus roseus* (Apocynaceae) was analyzed to determine the phyto-components. Table 2 shows the percentage yield of *Catharanthus roseus* leaves extract of different solvent and the ethanolic leaves extract showed more number of phyto-constituents (11.06) than the other solvent. The preliminary phytochemical screening of the ethanolic leaf extract of *Catharanthus roseus* showed the presence of alkaloids, flavonoids, terpenoids, Phenols, saponins, tannin, protein, carbohydrates, Protein and steroid Table 3.

The identification of the phyto-compounds was carried out based on the retention time and molecular formula through GC-MS analysis. The name of identified compounds in the ethanolic leaves extract of Catharanthus roseus with their retention time (RT), molecular formula (MF), molecular weight (MW) and peakarea percentage were represented in Tables 4 & 5. GC-MS chromatogram of the ethanolic leaf extract of Catharanthus roseus (Figure 1) showed 15 peaks indicating the presence of 15 compounds. GC-MS analysis revealed that the presence of Dodecanedioic acid, Bis (Trimethylsilyl) ester (17.299), Methyl-19-methyl-Eicosanate (18.140), N-Hexadecanoic acid (18.575), (1S,15S)-Bicyclo (13.1.0) Hexadecan-2-one (19.600), Methyl 7,11,14 Eicosatrienoate (19.665), Alpha-Linolenic acid, trimethylsilyl ester (20.371), 1-Methylene, 2B-hydroxymerthyl-3, 3-Dimethyl-4B-(3-methylbut-2-ethyl)-cyclohexane (20.441), Alpha linolenic acid, trimethylsilyl ester (20.601), Alpha linolenic acid, trimethylsilyl ester (21.026), Hentriacontane (22.191), Sulfurous acid, octadecyl 2-Propyl ester (22.972), Sulfurous acid, butyl tridecyl ester (23.702), Vitamin E (26.783), Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (30.360), Cyclotrisiloxane, Hexamethyl (30.665).

4 DISCUSSION

In the present study the phytochemical composition and larvicidal (3rd instar larvae of *Aedes aegypti*) potential of leaf extracts of *Catharanthus roseus* were tested. Among all the extracts, the ethanolic leaf extract of the *Catharanthus roseus* possessed a significantly higher larvicidal activity against the 3rd instar larvae of *Aedes aegypti*, with LC_{50} values of 157.8 ppm, respectively.

The findings of present study are quite comparable with previous reports of [Vinayaka *et al*., 2009] who have reported the larvicidal activities of different solvent leaf extracts of *Elaeagnus kologa* in which methanol, ethyl acetate and acetone extracts showed 100% in 15 and 20 mg/ml concentrations against *Ae. aegypti.* Ansari *et al.* [Ansari *et al*., 2005] was observed the larvicidal activity of *Pinus longifolia* oil against *An. stephensi* (LC_{50} 112.6 ppm), *Ae. aegypti* (82.1 ppm) and *Cu. quinquefasciatus* (85.7 ppm). The toxicity to the late third instar larvae of *A. aegypti* by the hexane leaf extracts of *Abutilon indicum* and *Cx. quinquefasciatus* by dichloromethane whole plant extracts of *Citrullus colocynthis* and hexane extracts of aerial parts of *Hyptis suaveolens* was reported by Arivoli and Samuel [Arivoli and Samuel, 2011a; Arivoli and Samuel, 2011b; Arivoli and Samuel, 2011c]. The LC_{50} values of aqueous extracts of *Catharanthus roseus* were 8.79, 55.26, 90.92, 272.36 and 4.25 ppm, respectively, against *A. aegypti* [Rodrigues, 2005]. Previous studies showed that ethanol extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica*, two members of the family Meliaceae, were found to have lethal effects on *A. aegypti* larvae, with LC_{50} values ranging from 0.017 to 0.034 g % [Wandscheer *et al*., 2004].

In 2005 [Hadjiakhoondi *et al*., 2005] reported that the LC_{50} and LC_{90} values of the methanolic extract from *Tagetes minuta* L. against *An. stephensi* larvae were 2.5 mg/l and 11 mg/l respectively. The methanol extract of dried root powder of *Rhinacanthus nasutus* was tested against the larvae of *A. aegypti* and *C. quinquefasciatus* [Debella *et al*., 2007]. [Nathan, 2007] reported that the larvicidal activity of essential oil from *Eucalyptus tereticornis* with LC_{50} and LC_{90} values were 23.8 and 63.9 ppm respectively against *An. stephensi* larvae. [Sedaghat *et al*., 2011] studied oils from *Heracleum persicum*, *Foeniculum vulgare* and *Coriandrum sativum* at much lower concentrations and reported LC_{50} values equivalent to 104.8, 20.1 and 120.95 mg/l, respectively. [Mathivanan *et al*., 2010], reported that the highest larval mortality was found in benzene extract of *Ervatamia coronaria* against the larvae of *C. quinquefasciatus*, with LC_{50} and LC_{90} values of 96.15 and 174.10 ppm. The

corresponding LC_{50} value of leaf acetone, absolute alcohol, petroleum ether, chloroform/methanol (1:1, v/v), benzene and ethyl acetate extracts of *Solanum nigrum* were 72.91, 59.81, 54.11, 32.69, 27.95 and 17.04 ppm, respectively, after 24 h of exposure period against *C. quinquefasciatus* [Rahuman *et al*., 2008]. The Neem oil formulation was found effective in controlling mosquito larvae in different breeding sites under natural field conditions [Virendra *et al*., 2009]. The methanol extract of *Ocimum canum* and the acetone extract of *Ocimum sanctum* were reported to have a toxic effect against the larvae of *Spodoptera litura, Aedes aegypti* and *Culex quinquefasciatus* [Kamaraj *et al*., 2010; Shahi *et al*., 2010].

Plant alkaloids resulted in a significant loss in fecundity and fertility in the adult species of mosquitoes [Saxena, 1992]. These compounds jointly or independently contribute to produce larvicidal and adult emergence inhibition activity against mosquitoes [Harborne, 1998]. The biological activity of the plant extracts might be due to the present of various phytochemical compounds [Amer and Mehlhorn, 2006; Kalaivani et al., 2012].Phytochemical screening of the ethanolic extract of *Catharanthus roseus* leaves revealed that the leaf extract contains alkaloids, Terpenoids, flavonoids, tannins, saponins, protein and carbohydrate. Phytochemical compounds such as alkaloids are commonly implicated in the antiplasmodial activity of many plants [Atta-ur-Rahman, 1995; Okokon, 2006]. Terpenes or terpenoids have been identified as active antiprotozal and antimalarial agents in many pharmacological studies [Philipson, 1991; Asase *et al*., 2010]. Flavonoids revealed significant anti-parasitic activities against different parasite strains of malaria, trypanosome and leishmania [Kim *et al*., 2004; Tasdemir *et al*., 2006].

Earlier studies observed that phytochemicals have a major role in mosquito control programme [Hag *et al*., 1999; Palsso *et al*., 1999]. [Gopieshkhanna, 2007] have observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extract having mosquito larvicidal activity. [Pelah, 2002] reported the use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal against *Aedes aegypti* and *Culex pipens.*

Govindasamy [Govindasamy *et al*., 2012] reported the phytochemical analyses of *Catharanthus roesus* were showed the presence of soluble sugar, reducing sugar, protein, amino acids, lipids, total chlorophyll, phenol and ortho-dihydroxyphenols in the ethanolic extract. [Hussain *et al*., 2011] reported that the phytochemical analysis of *Ranunculus arvensis, Equisetum ravens, Carathamus lanatus and Fagonia critica* showed more phytochemical constituents. [Thenmozhi *et al*., 2011] reported the phytochemical screening with the *Catharanthus roseus* was showed that presence of tannin, flavonoids, alkaloids, saponins and terpenoids. In the Present investigation the ethanolic extract of *Catharanthus roseus* showed more number of phyto-constituents than the other solvent.

Ethanolic leaves extract of *Catharanthus roseus* showed that the presence of fifteen different phytocompounds. Among these, the four compounds such as N-Hexadecanoic acid (5.948%), Methyl 7, 11, 14 Eicosatrienoate (8.302%), Hentriacontane (2.881%), and Vitamin E (6.102) possess pharmacological activities. Octanoic acid ethyl ester possesses insecticidal, anticandidal and antifungal activities (Table 5). Similarly, [Prabhadevi *et al*., 2012] reported that the presence of octanoic acidethyl ester in the ethanolic extract of stem of *Allamanda cathartica* by GC-MS analysis. [Jasim Uddin Chowdhury *et al*., 2007] reported that sixty two compounds were identified in the fresh matured leaves of *Lantana camara* by GC-MS technique. The researchers were reported that the presence of Phytol and 6, 9, 12-Octadecatrienoicacid in the ethanol extract of leaf of *Aloe vera* [Arunkumar *et al*., 2009] and the compound 6,9,12-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, in the ethanol extract of *Caesalpini asappan* [Sarumathy *et al*., 2011].

N-Hexadecanoic acid-Palmitic acid can be an antioxidant, hypo-cholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. 9,12-Octadecanoic acid and squalene were identified in the ethanol leaf extract of *Aloe vera* [Yamunadev *et al*., 2013] and *Vitex negundo* [Merlin et al., 2009] Hexadecanoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* [Grace *et al*., 2002] and *Melissa officinalis* [harafzadeh *et al*., 2011]. [Parasuraman *et al*., 2009] identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus.* GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid [Siddiq Ibraham *et al*., 2009]. Squalene is used in cosmetics as a natural moisturizer**.** [Devi *et al*., 2009] reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and 9, 12-Octadecadienoic acid. These reports are in accordance with the result of this study.

GC-MS analysis of *Mentha arvensis* was compared by [Sharma et al., 2009]. The water distilled essential oils extracted from leaves, stems and roots of *Chrysanthemum parthenium* were analyzed by GC-MS methods [Ali Shafaghat *et al*., 2009]. [Govindarajan, 2010] reported phytochemical contents from the essential oil extract from the leaf of *Clausena anisata* by GC-MS and larvicidal activity. [Parasuraman *et al*., 2009] identified number of constituents present in *Cleistanthus collinus* leaves and they were quantified by GC-MS method. [Sarumathy *et al*., 2011] reported anti-inflammatory activity and nature of compounds present in *Caesalpinia sappan* by GC-MS. [] Maria Jancy Rani *et al*., 2011] identified possible chemical components present in *Lantana camara* leaves by GC-MS method. Similar work and same method were carried out by [Sriram Sridharan *et al*., 2011] in the methanol extract of *Mimosa pudica.* [Hema *et al*., 2011] evaluated the bioactive components of *Murraya koenigii* leaves using GC-MS.

CONCLUSION

The present study, revealed the presence of the chemical constituents from *Catharanthus roseus* leaf extract by GC-MS, responsible for the synergic mosquito larvicidal action. Further, the contribution of these compounds on the pharmacological activity should be further evaluated individually for their long term effect and larvicidal activities. There is no doubt that plants are reservoir of potentially useful chemical compounds which serve as drugs, provide newer leads and clues for modern drug design. Due to its many medicinal properties there is enormous

scope of future research on *Catharanthus roseus*. Further investigation of pharmacological study should be conducted to explore potential lead compounds from this plant to develop potent larvicidal products.

TABLE 1: PROBIT ANALYSIS OF LARVICIDAL POTENTIAL OF LEAF EXTRACTS OF *CATHARANTHUS ROSEUS* AGAINST *AEDES AEGYPTI*

Solvent	LC_{50} (ppm)	95% Confidence interval		LC_{90} (ppm)	95% Confidence interval		Chi-square
		Lower limit	Upper limit		Lower limit	Upper limit	
Ethanol	157.8	137.3	174.2	332.3	302.0	379.0	0.209
Aqueous	172.1	154.7	187.2	337.8	308.1	382.6	2.104
Ethyl acetate	206.4	189.6	223.9	392.1	351.9	456.3	0.700
Butanol	176.4	160.5	190.7	332.1	304.9	372.1	0.979
Acetone	192.8	154.1	228.6	357.4	298.8	512.2	5.433
Hexane	202.0	188.6	215.5	345.8	318.9	384.5	1.513

TABLE 2: PERCENTAGE YIELD OF *CATHARANTHUS ROSEUS* LEAVES EXTRACTS FOR DIFFERENT SOLVENT

Solvent	Method	Weight of crude extract (g)	% yield
Acetone	Soxhlet extraction	08.10	0.81
Hexane	Soxhlet extraction	08.76	0.876
Diethyl ether	Soxhlet extraction	07.98	0.798
Ethanol	Soxhlet extraction	11.06	1.106
Butanol	Soxhlet extraction	08.05	0.805
Aqueous	Soxhlet extraction	10.03	1.003

TABLE 3: PRELIMINARY PHYTOCHEMICAL CONSTITUENTS OF DIFFERENT SOLVENT EXTRACTS OF *CATHARANTHUS ROSEUS*

Phytochemical Constituents	Leaves Extract					
	Acetone	Hexane	Diethyl ether	Ethanol	Butanol	Aqueous
Alkaloids	-	+	+	+ +	-	-
Phenols	+	-	+	+	+	-
Flavonoids	-	+	+	+ +	-	+
Carbohydrate	-	-	-	+	-	-
Protein	-	-	-	+	-	-
Tannins	+	+	-	+	+	+
Saponins	-	+	-	+	-	+
Terpenoids	-	+	+	+	-	+
Steroid	-	-	+	+	-	-

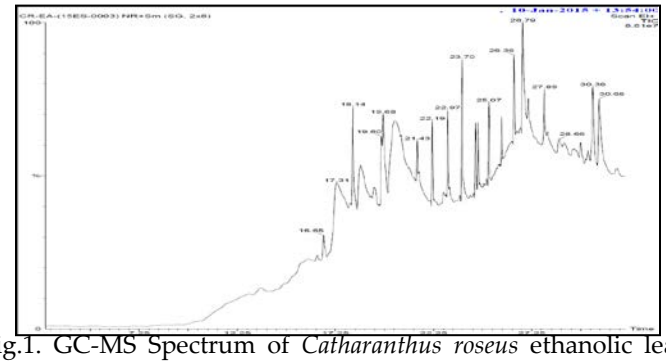

Fig.1. GC-MS Spectrum of *Catharanthus roseus* ethanolic leaf extract

TABLE 4: COMPOUNDS DETECTED IN *CATHARANTHUS ROSEUS* ETHANOLIC LEAF EXTRACT

S.No.	RT	Name of the compound	Molecular formula	Molecular Weight	Peak area %
1	17.299	Dodecanedioic acid, Bis (Trimethylsilyl) ester	$C_{18}H_{38}O_4Si_2$	374	14.676
2	18.140	Methyl-19-methyl-Eicosanate	$C_{22}H_{44}O_2$	340	5.974
3	18.575	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.948
4	19.600	(1S,15S)-Bicyclo (13.1.0) Hexadecan-2-one	$C_{16}H_{28}O$	236	2.660
5	19.665	Methyl 7,11,14 Eicosatrienoate	$C_{21}H_{36}O_2$	320	8.302
6	20.371	Alpha-Linolenic acid, trimethylsilyl ester	$C_{21}H_{28}O_2Si$	350	18.425
7	20.441	1-Methylene, 2B-hydroxymerthyl-3, 3-Dimethyl-4B-(3-methylbut-2-ethyl)-cyclohexane	$C_{15}H_{26}O$	222	5.663
8	20.601	Alpha Linolenic acid, trimethylsilyl ester	$C_{21}H_{38}O_4Si$	350	9.473
9	21.026	Alpha Linolenic acid, trimethylsilyl ester	$C_{21}H_{38}O_4Si$	350	3.389
10	22.191	Hentriacontane	$C_{31}H_{64}$	436	2.881
11	22.972	Sulfurous acid, octadecyl 2-Propyl ester	$C_{21}H_{44}O_3S$	376	3.615
12	23.702	Sulfurous acid, butyl tridecyl ester	$C_{17}H_{36}O_3S$	320	4.867
13	26.783	Vitamin E	$C_{29}H_{50}O_2$	430	6.102
14	30.360	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{50}O_7$	578	3.092
15	30.665	Cyclotrisiloxane, Hexamethyl	$C_6H_{18}O_3Si_3$	222	4.933

TABLE 5: ACTIVITY OF COMPOUNDS IDENTIFIED IN THE GC-MS STUDY OF *CATHARANTHUS ROSEUS* ETHANOLIC LEAF EXTRACT

S.No.	RT	Name of the compound	M.Formula	M. Wt	Peak area %	Activity
1	18.575	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.948	Anti-inflammator, Rheumatic symptoms
2	19.665	Methyl 7,11,14 Eicosatrienoate	$C_{21}H_{36}O_2$	320	8.302	Anti-inflammatory
3	22.191	Hentriacontane	$C_{31}H_{64}$	436	2.881	Potent insecticidal, good antifungal, Anti-inflammatory activity
4	26.783	Vitamin E	$C_{29}H_{50}O_2$	430	6.102	Antioxidant, Anti-inflammatory

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