International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

RESEARCH ARTICLE

In-vitro antioxidant and GC-MS analysis ethanolic extract of poly herbal drug

Pattabiraman K[1*], Muthukumaran P[2] and Hazeenabegum.V[3]

[1*&2] Department of Siddha Medicine, Faculty of Science, Tamil University, Thanjavur- 613010.
[3]Professor, Department of Siddha Medicine, Faculty of Science, Tamil University, Thanjavur- 613010.
[2]Author to whom correspondence should be addressed/E-Mail: kumaran.bio14@gmail.com
Received: Feb 2017 / Accepted: Feb 2017/ Published: Mar 2017

ABSTRACT: Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics. *Poly herbal drugs* is used in Indian traditional medicine for a wide range of various ailments. To understand the mechanism of pharmacological actions, antioxidant properties of the *Poly herbal drugs* extract were tested using standard in vitro models. The ethanolic extract of *Poly herbal drugs* exhibited strong scavenging effect on superoxide, nitric oxide radical and reducing power radical scavenging assay. The free radical scavenging effect of *Poly herbal drugs* extract was comparable with that of the reference antioxidants. The data obtained in the present study suggests that the extract of *Poly herbal drugs* have potent Invitro antioxidant and Anti Diabetic activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage.
Keywords: Antioxidant, Free Radicals, GC-MS, Poly herbal Drug.

INTRODUCTION

Indian medicinal plants and many herbal formulations belonging to the traditional systems of medicine like Ayurveda have been investigated on various aspects of disease curing drugs. Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs which are without the side effects, especially those belonging to the traditional systems of medicine (Jose and Kuttan, 2000) Research emphasis has been directed towards herbal drugs either in single or in combination having specific diagnostic and therapeutic principles. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals (Kunchandy & Rado, 1990) currently, polyhedral formulations are employed for the treatment of various types of diseases, such as respiratory diseases, cancer, acquired immunodeficiency syndrome (AIDS), diabetics and ulcer in order to achieve enhanced therapeutic effects

In view of above information the present study has been undertaken to assess the invitro antioxidant and anti-diabetic activity of polyherbal formulation containing drugs from different herbs viz *Cassia auriculata , Mimosa pudica , Mangifera indica , Cyperus rotundus* and *Asparagus racemosus.*

MATERIALS AND METHODS

Plant collection

Cassia auriculata, Mimosa pudica, Mangifera indica, Cyperus rotundus and *Asparagus racemosus* were collected. The plants were dried in open air and then kept in an oven at 60°C for some time, then grinded into a fine powder by using electric blender and stored in clean labeled airtight bottles.

Preparation of the plant extract

Hundred grams of plant powder was subjected to hydroalcoholic extraction (Distilled water: Ethanol = 2:1) by hot percolation method through soxhlet apparatus. Thereafter extract was dried using rotary evaporator and dried extract was put through a process of standardization. The percentage yield of the extract was determined and was found to be 12%.

Antioxidative Activity:

Scavenging of DPPH radical

This assay (Yokozawa *et al.,* 1998) based on the measurement of the scavenging ability of antioxidant test extracts towards the stable radical. The free radical scavenging activity of methanol and aqueous extracts of *Poly herbs* were examined *invitro* using DPPH [1, 1 Diphenyl, 2-picryl-hydrazyl] radical. The test extracts were treated with different concentrations from a maximum of 250 µg/ml to minimum of 4µg/ml. The reaction mixture consisted of 1ml of 0.1mM DPPH in methanol, 0.95 ml of 0.05 M Tris-HCL buffer (pH-7.4),

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

1ml of methanol and 0.05 ml of herbal extract. The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding extracts. The experiment was performed in triplicate and percentage of scavenging activity was calculated using the formula 100-[100/blank absorbance X sample absorbance]. The blank was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid, which was used as a standard antioxidant.

ABTS radical cation decolourisation Assay

ABTS (54.8 mg) was dissolved in 50 ml of distilled water to 2 mM concentration and potassium persulphate (17 mM, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before use (Re and Pellegrini, 1999). To 0.2 ml of various concentrations of the extracts or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution was added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm. The assay was performed in triplicate.

Scavenging of Nitric Oxide

Sodium nitroprusside (5 µM) in standard phosphate buffer solution (Sreejayan Rao, 1997) was incubated with different concentration of the methanol and aqueous extracts dissolved in standard 0.025 M phosphate buffer (pH-7.4) and the tubes were incubated at 250 C for 5 hours. After 5hours, 0.5 ml of incubation solution was removed and diluted with0.5 ml of Griess reagent (prepared by mixing equal volume of 1%sulphanilamide in 2% phosphoric acid and 0.1% aphthylethylenediamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm.

Metal chelating activity

The chelation of ferrous ions by extracts was estimated by method of Dinis *et al.* (Dinis *et al.*, 1994). Briefly, 50 µl of 2 mM FeCl2 was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine–Fe 2+ complex formation was calculated as [(A0- As)/ As] × 100, where A0 was the absorbance of the control, and As was the absorbance of the extract/ standard. Na2EDTA was used as positive control.

GC –MS Analysis

Preparation of extract

Rhizome powder of *Curcuma caesia* Roxb were shade dried. 20 g of the powdered tubers were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatmann filter paper No.41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. 2 µl of these solutions was employed for GC/MS analysis (Merlin *et al.*, 2009)

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of Components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrum of unknown components was compared with the version, 2005, software, Turbo mass 5.2. This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extraction these compounds (Karthikeyan Ravichandran et al., 2015).

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

RESULTS AND DISCUSSION

DPPH assay is being used widely as a preliminary test which provides information on the reactivity of test compound. This method is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidant (AH) due to the formation of non-radical form DPPH-H. Antioxidant reacts with DPPH which is a stable free radical and is reduced to the DPPH-H and as a consequence the absorbance decreased from the DPPH˙ radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds present in the poly herbal extract in turns of hydrogen donating ability. (Burits, and Bucar, 2000)

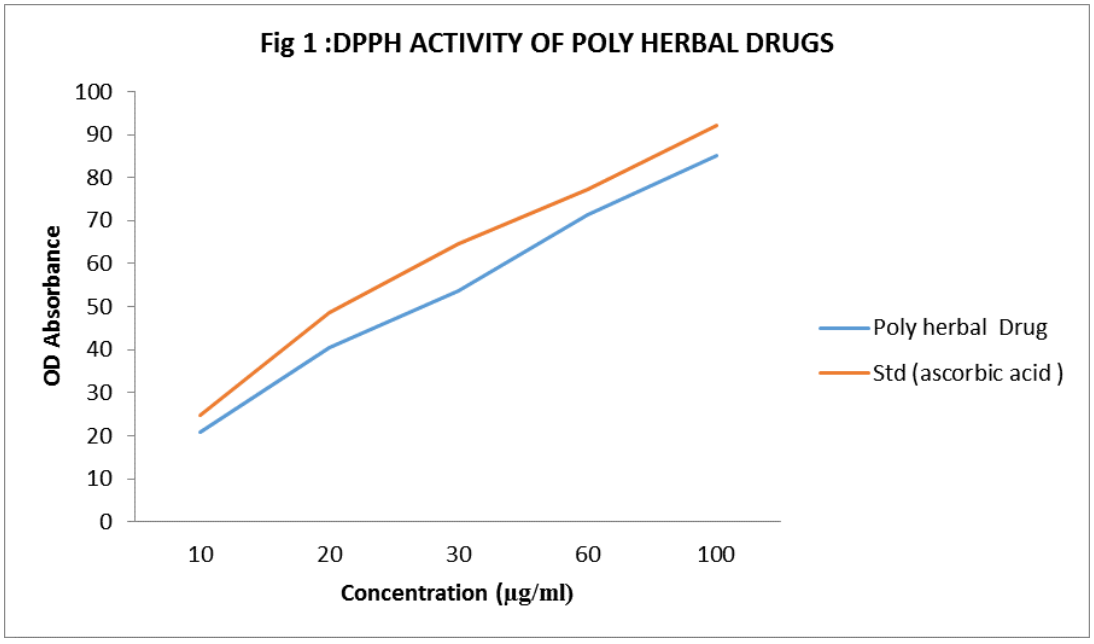
DPPH assay is a simple and acceptable method to evaluate the anti-oxidative activity of compounds (Chen and Ho, 1995). DPPH is a stable free radical, which when encounters proton donors such as antioxidants, the radicals get quenched, absorbance gets reduced and thus used to measure the antioxidant activity of specific compound or plant extract (Koleva *et al.*, 2002; Qureshi *et al.*, 2010).

The DPPH radical has been widely used to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. The DPPH free radical scavenging activity of plant extract may be due to neutralization of DPPH radical either by transfer of hydrogen or of an electron (Shimada *et al.*, 1992).

The Poly herbal mixture exhibited a significant dose dependent inhibition of DPPH activity. In this study, at 100 µg /ml, the extract showed highest inhibition of DPPH activity shown in (Table1;Figure 1).The results of DPPH-free radical scavenging assay suggest that the Poly herbal mixture extract is more capable of scavenging free radicals.

Table 1: DPPH radical scavenging activity

Concentration (µg /ml)	Poly herbal Drug	Std (ascorbic acid)
10	20.83 ± 1.93	24.73 ± 1.84
20	40.46 ± 2.55	48.64 ± 3.39
30	53.54 ± 3.09	64.64 ± 3.80
60	71.34 ± 1.91	77.23 ± 5.12
100	85.02 ± 3.05	92.09 ± 4.36



ABTS^{+} Activity

ABTS^{+} is a blue chromophore produced by the reaction between ABTS and potassium per sulfate. Previous studies have suggested that the total polyphenolic content of the plant extracts are positively correlated to the scavenging activities and reducing potential of an extract. Our result shows that scavenges pre-generated ABTS+ radicals Addition of polyherbal extracts to this pre-formed

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

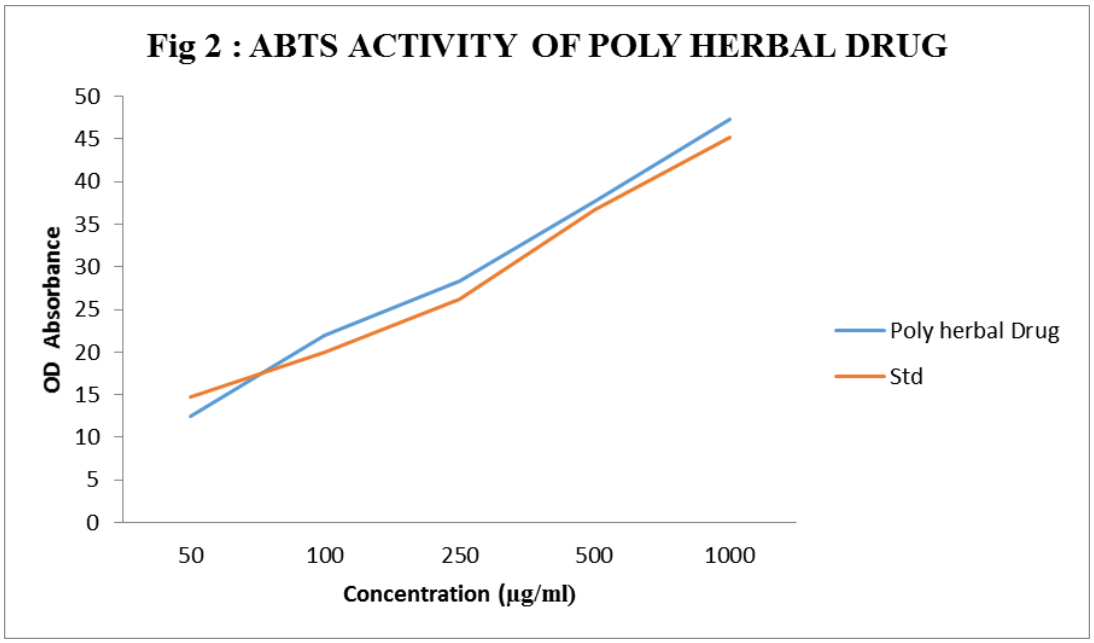
Available online at http://www.skyfox.co
ISSN: 2454-6127

radical cation reduced it to ABTS in a concentration dependent manner (Table 2;Figure 2). These results were compared with those obtained with ascorbic acid, which indicates that the extract is a potent antioxidant. This is measured in terms of inhibition of generation of O2. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per oxidation and by many other mechanisms and thus prevent disease. (Auudy *et al.,* 2003).

Natural phenolics exert their beneficial health effects mainly through their antioxidant activity by decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non-radical species, and breaking chains to prevent continued hydrogen abstraction from substances.(Sharma *et al.*,2012).

Table 2: ABTS Assay

Concentration (ug/ml)	Poly herbal extract	Std
50	12.49 ± 1.14	14.69 ± 1.14
100	21.96 ± 1.14	19.95 ± 1.14
250	28.31 ± 1.6	26.22 ± 1.6
500	37.66 ± 1.27	36.65 ± 1.27
1000	47.29 ± 0.84	45.20 ± 0.84



No Activity

Nitric oxide, a gaseous free radical and is relatively less reactive. But its metabolic product peroxynitrite, formed after reacting with oxygen is extremely reactive and directly induce toxic reactions such as SH group oxidation, protein - tyrosine nitration, lipid peroxidation and DNA modification (Moncada *et al.*, 1991; Yermilov *et al.*, 1995). Nitric oxide has been demonstrated to participate in the beta cell damage during STZ- induced diabetes (Duran Reges *et al.,* 2004). Nitric oxide plays an important role in various inflammatory processes. Sustained level of production of this radical is directly toxic to tissues and contribute to vascular collapse associated with septic shock, whereas chronic exposure of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Tylor *et al.*, 1997).

The toxicity of NO increases greatly when it reacts with superoxide radical, producing the highly reactive peroxynitrite (ONOO-) anion (Huie and Padmaja, 1993). Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, alzheimer's and arthritis. Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals. (Hashem *et al.*, 2007).

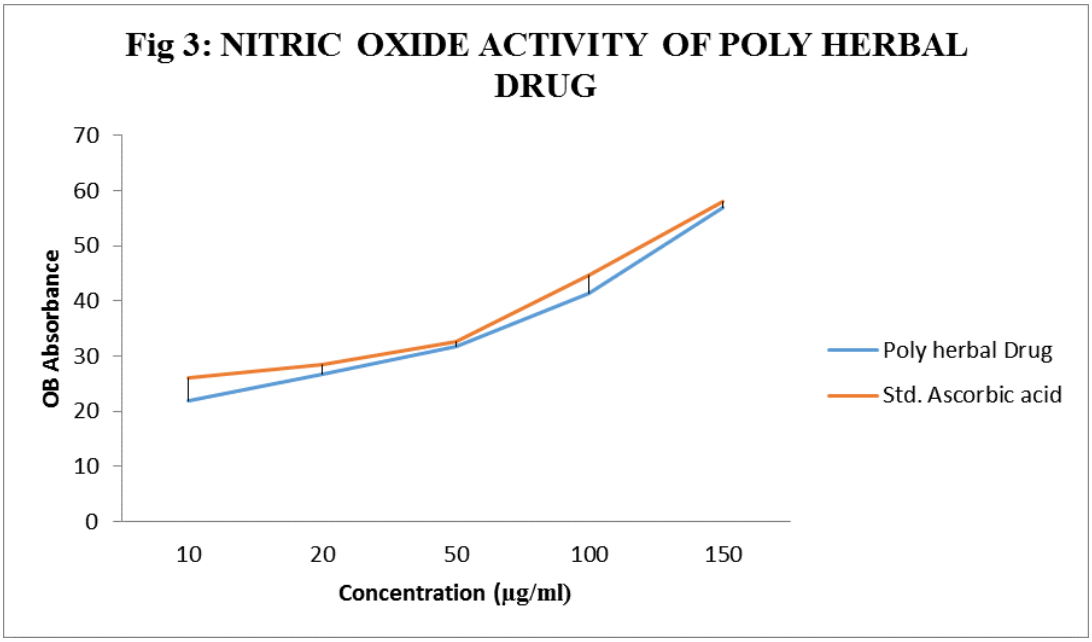
International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

From results of Nitric oxide method, it proved that poly herbal drug extract is equally effective as anti-oxidant compared to the ascorbic acid (Table 3; Figure3). These compounds compete with oxygen to react with NO and thus inhibit the generation of the nitrite and peroxy nitrite anions. This results indicates that, the extract might contain compounds capable of inhibiting nitric oxide and offers scientific evidence for the use of the drug in the indigenous system in treatment of various diseases.

Table 3: Nitric oxide radical scavenging activity

Concentration (ug/ml)	Poly herbal extract	Std. Ascorbic acid
10	22.02± 0.18	26.02 ±1.1
20	26.78 ± 0.86	28.51 ±1.40
50	31.73 ± 1.76	32.67 ±2.51
100	41.4 ± 1.86	44.61± 1.80
150	56.98 ± 0.55	58.16 ± 1.68



Iron chelating activity

Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by decomposing lipid hydro peroxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Thus, the chelating effect of the coexisting chelators can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe2+ complex is interrupted in the presence of aqueous extract of poly herbal extract indicating that have chelating activity (Table 4; Figure 4).

Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydro peroxides into peroxyl and alkoxy radicals (Hashem *et al.,* 2007). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that forms bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion (Cao G *et al.*,1996).Thus, *poly herbal extract* demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

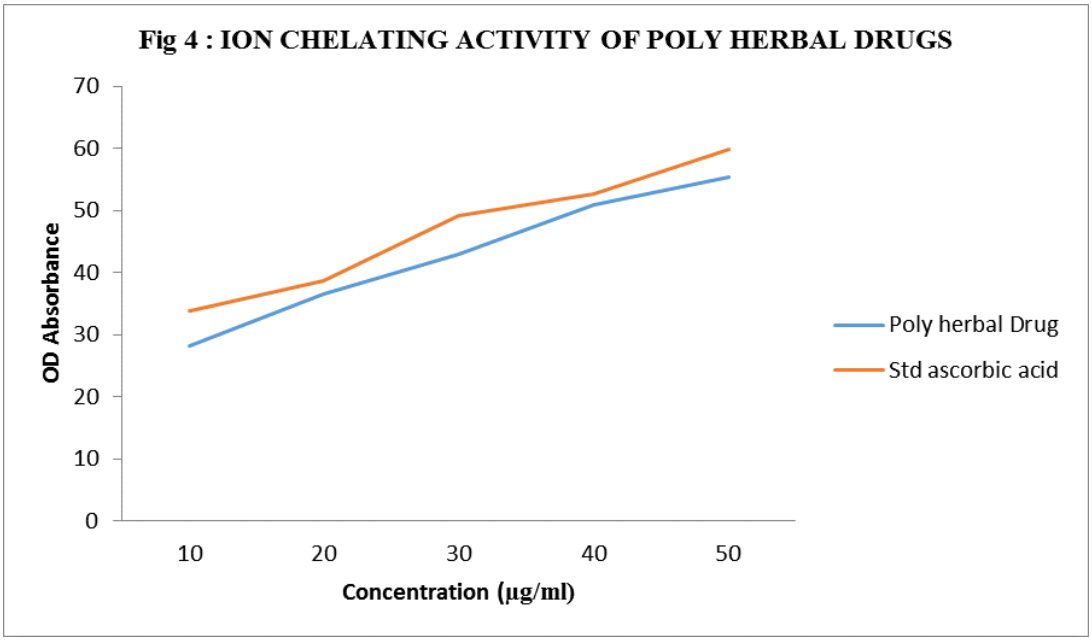
Table 4: Iron Chelating Activity

Concentration (ug/ml)	Poly herbal extract	Std ascorbic acid
10	28.12 ± 1.73	33.85 ± 1.94
20	36.52 ± 1.17	38.70 ± 1.58

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

30	43.04 ± 1.79	49.07 ± 2.97
40	50.91 ± 2.86	52.72 ± 2.25
50	55.29 ± 3.20	59.93 ± 2.30



Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS is a method that combines the features of Gas-Liquid Chromatography (GLC) and Mass Spectrometry (MS) to identify different substances within a test sample.

Free radicals play a crucial role in the development of tissue damage in pathological events. 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. The acidic fractions were silylated and subjected to GC-MS investigation. It is evident from the Table 7 that all fractions have a complex chemical composition. Some of the GC-MS peaks remained unidentified, because of lack of authentic samples and library data of corresponding compounds.

The phytochemical compounds present in the selected poly herbal combined extract were identified by GC-MS analysis. GC-MS chromatogram of the ethanolic extract of combined poly herbal showed the presence of 20 phytochemical constituents.

Table 5: GC-MS analysis of ethanolic extract of *poly herbal combined drug*

S. No.	Compounds	Retention Time (Min.)	Peak Area %	Molecular Formula	MW
1.	Butane1,1-diethoxy-3-methyl-	3.37	0.43	$C_9H_{20}O_2$	160
2.	Diglycerol	3.73	4.11	$C_6H_{14}O_5$	166
3.	benzeneacetaldehyde	4.44	0.55	C_8H_8O	120
4.	Propane,1,1,3-triethoxy -	4.76	1.15	$C_9H_{20}O_3$	176
5.	4h-pyran-4-one,2,3-di-hydro-3,5-dihydrooxy6-methyl	5.94	0.83	C6H8O4	144
6.	Benzofuran,2,3-dihydro -(coumaran)	6.97	3.97	C_8H_8O	120
7.	resorcional	8.17	5.26	$C_6H_6O_2$	110

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

8.	1,2,3-benzenetriol(pyrogallol)	9.52	4.17	$C_6H_6O_3$	126
9..	1,6-anhydro-a-d-glucopyranose(synonym:levoglucosan)	10.59	1.16	$C6H_{10}O_5$	162
10.	Naphthalene 1,2,3,4-tetrahydro-1,6dimethyl-4(1-methylethyl)-,(1s-cis)-(synonym:calamenene)	10.97	0.11	$C_{15}H_{22}$	202
11.	Dodecanoic acid	11.21	0.27	$C_{12}H_{24}O_2$	200
12.	3-o-methyl-d glucose	13.74	41.09	$C_7H_{14}O_6$	194
13.	n-hexadecannoic acid	16.66	3.82	$C_{16}H_{32}O_2$	256
14.	Hexodeconoic acid ethl ester	16.98	0.23	$C_{18}H_{36}O_2$	284
15.	phytol	18.98	0.16	$C_{20}H_{40}O$	296
16.	9,12-octadecadienoic acid (z,z)-	19.28	2.32	$C_{18}H_{32}O_2$	280
17.	Octadecanoic acid	19.68	0.71	$C_{18}H_{36}O_2$	284
18.	1,3-benzodioxole,5,5(tetrahydro-1h,3h-furoi[3,4-c]furan-1,4-diyl)bis-,[1s-(1a3aa,4a,6aa)]-(synonym:d-asarinin)	22.01	1.74	$C_{20}H_{18}O_6$	354
19.	Ergosterol	26.55	18.39	$C_{28}H_{44}O$	396
20.	Ercosta-7,22-dien-3ol,(3a,22e)-	27.17	8.96	$C_{28}H_{46}O$	398

The major phytochemical compounds identified were 3-o-methyl-d glucose, ergosterol, Ercosta-7,22-dien-3ol,(3a,22e)-, resorcinol, n-hexadecannoic acid.

Phenolic compounds have also been known as antioxidant agents, which act as free radical terminators and have shown medicinal activity as well as exhibiting physiological functions. It was reported that compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of this extract.

This study highlights the presence of many secondary metabolites in the poly herbal combined extract provide an overview of the different classes of molecules present that have led to their pharmacological activities. This study confirmed that the plant extract could be used for the treatment of various diseases. The GC-MS analysis of poly herbal combined extract showed the presence of various types of compounds in present. Compounds like 3-O-methyl-d glucose ($C_7H_{14}O_6$), to possess antitumor, oncogenic, and diabeto genic properties (Michael M *et al.*,1977) Ergosterol ($C_{28}H_{44}O$) to act a Anticomplementary activity, Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective (Dr.Duke's *et al.*,2011) Ercosta-7,22-dien-3ol,(3a,22e)-($C_{28}H_{46}O$) used to treat Cancer prevention Anti-inflammatory Hypocholesterolemic, anti-hyper glycemic,(Gowdhami *et al.*,2015) Resorcional ($C_6H_6O_2$) having liver hepatopathology enzyme activity, anti-microbial activity (Dr.Duke's Phytochemical and Ethnobotanical Databases) n- hexadecanoic acid ($C_{16}H_{32}O_2$) act as nematicide, pesticide, hemolytic, 5-alpha reductase inhibitor; Insectifuge, Hypatoprotective, Antihistaminic, Antieczemic, Antiacen, 5- Alpha reductase inhibitor, Antiarthritic, Anticoronary activities. (Lalitha Rani *et al.,* 2009) phytol ($C_{20}H_{40}O$) to use in Antimicrobial, Anti-cancer, Anti-inflammatory Hypocholesterolemic, Nematicide, Anticoronary, Antiarthritic, Hepatoprotective, Anti –androgenic (Gowdhami *et al.,* 2015) d-asarinin ($C_{20}H_{18}O_6$) Cyto toxic activity (Gowdhami *et al.*,2015 Octadecanoic acid ($C_{18}H_{36}O_2$) Antifungal, Antitumour, Antibacterial(Lalitha Rani *et al.,* 2009) .

The plants sample on subjecting to GC-MS provides the result of different peaks determining the presence of seven different compounds. The molecular weight of these compounds is also known. By interpreting these compounds, it is found that this plant possess various therapeutically uses. This typical gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The numbers at various peaks are the retention time in minutes. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios (Kalimuthu *et al.*, 2013).

These observations may be due to the nature of biological active components and the stronger extraction capacity of hydro ethanol could have been produced number of active constituents responsible for medicinal activity (Komal Kumar *et al.*, 2011). The biological activities based on Dr. Duke's Phytochemical and Ethnobotanical Databases.

Our investigation through the present study revealed that the poly herbal combined extract *is* a reliable source of bioactive compounds like fatty acid esters, alcohols, hydrocarbons, alkanes, amines, terpenes, and sugars that justify the traditional usage of this plants As GC-MS is the first step towards understanding the nature of active principles (Ghorbani, *et al.,* 2011), further investigation in this species is suggested for the development of novel drugs.

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many in curable diseases.

REFERENCES

1. Auudy, B., Ferreira, F., Blasina, L., Lafon, F., Arredondo, F., Dajas, R. and Tripathi, P.C. (2003). Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J. Ethnopharmacol. 84: 131-138.
2. Burits M, Bucar F(2000). Antioxidant activity of Nigella sativa essential oil. Phytother Res. 14(5):323-8.
3. Cao, G., E. Sofic, and R.L. Prior. 1996. Antioxidant capacity of tea and common vegetables. J. Agr. Food Chem. 44:3426–3431.
4. Chen, C.W. and Ho, C.T. (1995). Antioxidant properties of polyphenols extracted from green and black tea. J. Food lipids. 2: 35-46.
5. Dinis TCP, Madeira VMC, Almeida MLM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169
6. Duran Reges G., Pascoe Lira D., Vilar Rojas C., Medina Navarro R(2004). Diabetogenic effect of STZ diminishes with loss of Nitric oxide role of ultra violet and carboxy PT10 pharmacology, 71(1);17-24.
7. Ghorbani, A.; Rakhshandeh, H (2012) The most effective herbs for diabetes. Mashhad: Mashhad University of Medical Sciences Publisher, p.21-127.
8. Gowdhami T, Rajalakshmi AK , Sugumar N , Valliappan REvaluation of antimicrobial activity of different solvent extracts of aromatic plant: Jasminum sambac linn. Int J Res Pharm Sci 2015, 5(4) ; 18 –23.
9. Hashem, F.A. (2007) Investigation of free radical scavenging activity by ESR for coumarins isolated from Tecoma radicans. J.Med.Sci. 7:1027-1032.
10. Huie RE, Padmaja S. (1993).The reaction of no with superoxide. Free Radic Res Commun. 18(4):195-9.
11. Jose J.K. and Kuttan R (2000) Hepatoprotective activity of Emblica officinalis and Chyavanprash. J Ethnopharmacol. 72, p.135.
12. Kalimuthu K. & Prabakaran R. (2013). Preliminary Phytochemical Screening and Gc-Ms Analysis Of Methanol Extract Of Ceropegia Pusilla.International Journal Of Research In Applied, Natural And Social Sciences (Impact: Ijranss) Vol. 1, Issue 3, 49-58.
13. Karthikeyan R., Srinivasan K., Muthukumaran P., Prithiviraj P., Sadhana R & Kumaravel S (2015). Organochlorine and Organophosphorus Pesticide Residues analysis by GC-MS/MS in Raw Cow Milk. In International Journal of Agricultural and Life sciences 1 (4), pp. 7–11. Available online at 10.9379/sfjal-122059-002-0081-x.
14. Koleva, I.I, Van Beek, T.A., Linssen, J.P.H., De Groot, A., Evstatieva, L.N., (2002): Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis 13: 8-17.
15. Komal Kumar J and Devi Prasad AG (2011). Identification and comparison of biomolecules in medicinal plants of Tephrosia tinctoria and Atylosia albicans by using FTIR. Romanian J. Biophysics; 21(1):63-71.
16. Kunchandy. E & Rado M.N.A (1990).Oxygen radical scavenging activity of curcuminoid. Int J Pharmacognosy, 58, p. 237.
17. Lalitharani S., Mohan V.R., Regini G.S., Kalidass C (2009) GC-MS analysis of ethanolic extract of Pothos scandens L. leaf. J. Herb. Medi. Toxicology. 3: 159-160.
18. Merlin N. J., Parthasarathy V., Manavalan R and Kumaravel S.(2009)Investigation of Aerial Parts of Gmelina asiatica Linn by GC-MS. Pharmacognosy Research., 1(3), 152-156.
19. Moncada S., Palmer R.M. and Higgs E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews 43: 109–142.
20. Qureshi, R. G.R. Bhatti and R. A. Memon (2010). Ethnomedicinal uses of herbs from Northern part of Nara Desert, Pakistan. Pak. J. Bot., 42(2): 839-851.
21. Re R, Pellegrini N., Proteggente, A., Pannala, A., Yang, M, Rice_Evans, C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic Biol Med., 26: 1231-7.
22. Sharma S, Mustafiz A, Singla-Pareek S, Shankar Srivastava P, Sopory S *(2012) Characterization of stress and methylglyoxal inducible triose phosphate isomerase (OscTPI) from rice.* Plant Signal Behav , *7(10):1337-45.*
23. Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.
24. Sreejayan Rao M. N. A.(1997) *J. Pharm. Pharmacol.,* **49**, 105.
25. Taylor G, Bagby R, Parker J. (1997) Disorders of affect regulation. Cambridge: Cambridge University Press.

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (1) pp.106-114
http://dx.doi.org/10.22573/spg.ijals.017.s12200074

Available online at http://www.skyfox.co
ISSN: 2454-6127

26. Yermilov V., Rubio J., Becchi M., Friesen M.D., Pignatelli B. and Ohshima H. (1995). Formation of 8- nitroguanine by the reaction of guanine with peroxynitrite in vitro. Carcinogenesis 16: 2045–2050.
27. Yokozawa, T, Chen C. P, Dong E, Tanaka T, Nonaka G. I, Nishioka I., (1998) *Biochemical Pharmacology.,* **56**, 213.

How to cite this article
Pattabiraman, K., Muthukumaran, P., & Hazeenabegum, V. (2017). In-vitro antioxidant and GC-MS analysis ethanolic extract of poly herbal drug. Int. J. Agr. Life. Sci, 3(1), 106-114. doi: 10.22573/spg.ijals.017.s12200074.

CONFLICTS OF INTEREST
"The authors declare no conflict of interest".