Research Article

Antioxidant Properties and Analysis of Bioactive Compounds Present in n-hexane Root Extract of *Zaleya decandra.*

Deivasigamani Malarvizhi[1], Palanirajan Anusooriya[1], Periasamy Meenakshi[3], Sundaram Sowmya[1], Palanisamy Chella Perumal[2], Enock Kiage Oirere[2], Velliyur Kanniappan Gopalakrishnan[1,2*]

[1]Department of Biochemistry, Karpagam University, Coimbatore, Tamil Nadu, India.
[2]Department of Bioinformatics, Karpagam University, Coimbatore, Tamil Nadu, India.
[3]Dept of Soil science and Agri chemistry, Anbil Dharmalingam Agricultural College and Research Institute, Trichy, Tamil Nadu, India.
***Corresponding author's E-mail:** vk.gopalakrishnan@karpagam.ac.in

Accepted on: 20-08-2015; **Finalized on:** 30-09-2015.

ABSTRACT

Many of the natural products in plants of medicinal value offer us new sources of drugs which have been used effectively in traditional medicine. The present study was designed to examine the radical scavenging activities and characterize the bioactive constituents of n-hexane root extract of *Zaleya decandra*. The result shows that the *Zaleya decandra* possesses good radical scavenging activities when compared with standard ascorbic acid. Maximum absorbance obtained 1.743 at 288nm and FTIR spectrum shows the presence of functional groups such as O–H, N–H C–H, C–H, C=O, C=O, C–H, C–H and C–N. The GC-MS chromatogram designates the presence of 24 bioactive compounds mainly, 6-Octadecenoic acid (66.97%), n-Hexadecanoic acid (14.63%) and Octadecane (2.19%). It can be concluded that n-hexane root extract of *Zaleya decandra* has more bioactive compounds and possesses good radical scavenging activities and it may serve as a good pharmacological source.

Keywords: *Zaleya decandra*, Radical scavenging, FTIR, GC-MS, Bioactive compounds.

INTRODUCTION

Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system, (Ayurveda) and proposed for their interesting multilevel activities.[1] Most of them have been identified as having potential antioxidant activities and their consumption recommended. Bioactive phenols, particularly bioflavonoids, are very interesting as antioxidants because of their natural origin and the ability to act as efficient free radical scavengers.[2] Antioxidants with free radical scavenging activities may have enormous significance in the prevention and therapeutics of diseases.[3] Approximately 25% of drugs prescribed in the United States are plant derived natural products and 74% of the 119 most important drugs contain ingredients from plants used in traditional medicine.[4] Almost, 70% of modern medicines in India are derived from natural products. Currently plant based drugs are researched and formulated in modern framework of medicine rather than in the form of galenical preparations or conventional dosage.[5]

Zaleya decandra is a prostrate herb distributed in the tropical and sub-tropical regions of the world, and also found abundantly in India. The root of this plant is used in the Indian systems of medicine for the treatment of hepatitis, asthma and orchitis and also the decoction of the root bark is credited with properties of aperients[6] and the juice of leaves (2 – 3 drops) dropped into the nostrils to relieve partial headache.[7] It has been known since ancient times for curative properties and has been utilized for the treatment of various ailments such as burns and wounds, fever, tooth ache, anti- inflammatory, antidiabetic and other skin disorders.[8] The present study was aimed to analyze the antioxidant properties and to identify the bioactive compounds present in the n-hexane root extract of *Zaleya decandra.*

MATERIALS AND METHODS

Plant collection and extraction

The roots of *Zaleya decandra* were collected from Pollachi, Tamil Nadu, India. The plant was identified and authenticated by Dr. G.V.S. Murthy from The Botanical Survey of India, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in the Botanical Survey of India (No.BSI/SC/5/23/08-09/Tech.1231).[6] The roots of *Zaleya decandra* were washed with distilled water, shade dried, powdered, and stored. The powdered roots of *Zaleya decandra* (100 g) were extracted with 500 ml n-hexane. Then the extract was concentrated under vacuum and it was stored in an airtight container for future use.

Free Radical Scavenging Assays

The scavenging activity for DPPH free radicals of the n-hexane root extract of *Zaleya decandra* was restrained according to the procedure described.[9] The Nitric oxide was generated by sodium nitroprusside and measured by the Griess Illosvoy reaction by the method.[10] The superoxide scavenging activity of the n-hexane extract of *Zaleya decandra* was measured by reduction of nitroblue tetrazolium (NBT) method.[11] The hydroxyl radical scavenging activity was measured.[12] The ability of the n-hexane root extract of *Zaleya decandra* to scavenge hydrogen peroxide was determined according to the method.[13] The ABTS radical cation scavenging activity was

performed with slight modifications described.[14] The chelating of ferrous ions by the n-hexane root extract of *Zaleya decandra* was estimated by the method.[15] The Reducing power capacity was evaluated by the modified method.[16] And the FRAP assay was used to estimate the reducing capacity of root extract, according to the method.[17]

UV-VIS and FTIR Spectrum Analysis

100µl of samples were made up to 3 ml by using n-hexane and scanned in the range of 200nm to 800nm by using UV-Vis spectrophotometer instrument (Model – Shimadzu UV2450). For FTIR analysis the n-hexane root extract of *Zaleya decandra* was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded on a Shimadzu FTIR Spectrometer 8000 series, between 4000-400 cm^{-1}.

GC-MS analysis

GC-MS analysis of n-hexane root extract of *Zaleya decandra* was performed using the equipment Agilent technologies 7890 A. 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 flow rate of carrier gas (Helium) was 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.

RESULTS AND DISCUSSION

Free Radical Scavenging Assays

Naturally occurring herbs and spices have the natural antioxidants that are responsible for inhibiting or preventing the harmful effect of oxidative stress. The natural sources are much safer to use due to less toxicity and side effects. Antioxidant properties, particularly radical scavenging activity is very important, due to the deleterious role of free radicals in vegetation and in living systems. DPPH is relatively stable nitrogen centered free radical. The reduction capability of DPPH is determined by its decreased absorbance at 517nm as induced by natural antioxidants.[18] The DPPH radical scavenging (%) activity of *Zaleya decandra* was shown in Figure 1(A). The IC_{50} value of *Zaleya decandra* and ascorbic acid were found to be 480 ± 1.33 µg/ml and 400 ± 1.37 µg/ml respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH radical scavenging activity. Nitric oxide (NO) is one of the reactive nitrogen species present in the body that acts for several functions[19] and it's also known to be a ubiquitous free-radical moiety, which is distributed in tissues or organ systems and is supposed to have a vital role in neuromodulation or as a neurotransmitter in the central nervous system.[20] Figure 1(B) shows the nitric oxide radical scavenging activity of root extract of *Zaleya decandra*. The IC_{50} for the plant extract was 220 ± 1.83µg/ml and ascorbic acid was 145 ± 1.35 µg/ml respectively. *Zaleya decandra* inhibited nitric oxide in a dose dependent manner.

Superoxide anion radical is one of the strongest reactive oxygen species (ROS) among the free radicals and get converted to other harmful ROS such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases.[21] The superoxide scavenging activity of n-hexane root extract of the *Zaleya decandra* was increased markedly with the increase of concentrations (Figure 1(C)). The half inhibition concentration (IC_{50}) of root extract was 410 ± 2.98 µg/ml and ascorbic acid was 290 ± 1.83 µg/ml. The n-hexane root extract of the *Zaleya decandra* had notably superior superoxide radical scavenging effects. The hydroxyl radical is the most reactive of the reactive oxygen species. It induces severe damage in adjacent biomolecules and it has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity.[22] The hydroxyl radical scavenging ability of the extract was found to be effective with the IC_{50} of 175 ± 2.40 µg/ml, and the standard ascorbic acid was found to be 140 ± 2.16µg/ml (Figure 1(D)). Plant extract shows the almost similar hydroxyl radical scavenging activity compared to standard. This capability of the extract shows the hydroxyl radical quenching ability, which seems to be a good scavenger, of active oxygen species thus reducing the rate of chain reaction.

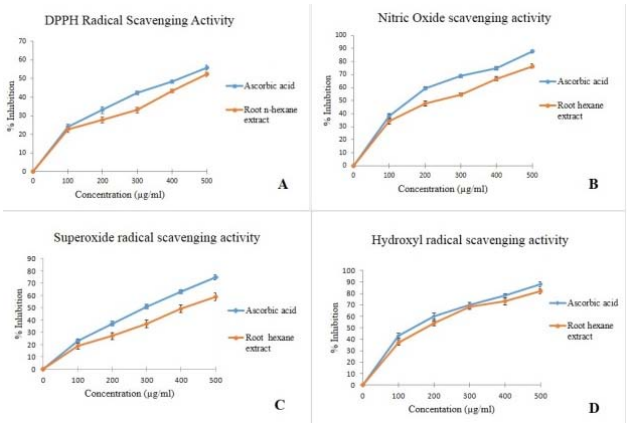

Figure 1: (A) DPPH radical scavenging assay; (B) Nitric oxide scavenging activity; (C) Superoxide radical scavenging activity; (D) Hydroxyl radical scavenging activity.

Hydrogen peroxide (H_2O_2) is a weak oxidizing agent that can inactivate a few enzymes directly, generally by oxidation of essential thiol (-SH) groups. H_2O_2 can cross cell membrane rapidly. Once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects.[23] Figure 2(A) shows the H_2O_2 radical scavenging activity of *Zaleya decandra* in a dose

dependent and significant manner in comparison with ascorbic acid standard. The IC_{50} value of *Zaleya decandra* and ascorbic acid were found to be 330 ± 2.84 µg/ml and 250 ± 2.26 µg/ml respectively. From the results, it appeared that the H_2O_2 scavenging activity of the plant extract is significant compared to that of the standard ascorbic acid. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen donating antioxidants is measured. ABTS radical scavenging assay involves a method that generates a blue/ green $ABTS^+$ chromophore via the reaction of ABTS and potassium persulfate.[24] The result of ABTS radical cation scavenging activity of *Zaleya decandra* is shown in Figure 2(B). The plant extract exhibited potent ABTS radical cation scavenging activity in a concentration dependent manner with the IC_{50} being 300 ± 2.24 µg/ml and the IC_{50} of the standard ascorbic acid was found to be 225 ± 2.09 µg/ml.

Most reactive oxygen species (ROS) are produced as byproducts during mitochondrial electron transport and other metabolic reactions. Reduction of the iron ion is an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. The presence of reductant (antioxidants) in the herbal extracts causes the reduction of Fe^{3+}/Ferric cyanide complex in the ferrous form.[25] Therefore, the reduction of the formation of reactive oxygen species can be attained by the chelation of metal ions with chelating agents. *Zaleya decandra* is the active extract restricted to the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The metal chelating activity is shown in Figure 2(C). The results are expressed as percentage metal chelating activity. Extract exhibited dose dependent metal chelating activity with an IC_{50} value of 280 ± 2.80 µg/ml and the IC_{50} of standard ascorbic acid was found to be 220 ± 1.97 µg/ml.

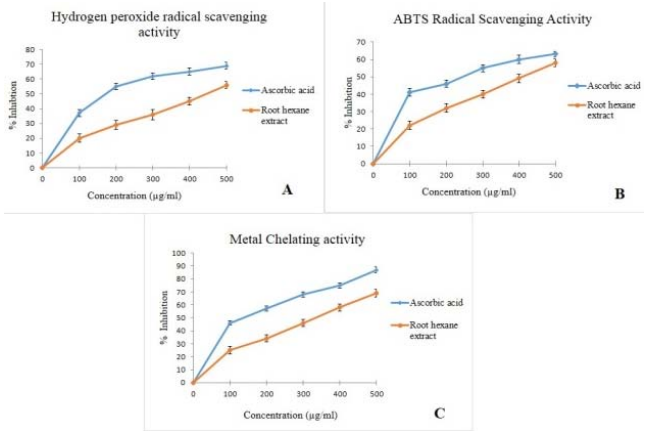

Figure 2: (A) Hydrogen peroxide radical scavenging activity; (B) ABTS radical scavenging activity; (C) Metal chelating activity.

The reducing capacity of the extract is another significant indicator of antioxidant activity. In the reducing power assay, the presence of antioxidants in the extract would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's blue at 700 nm.[26] The results show that there was increase in reducing power of the plant extract as the extract concentration increases. Figure 3(A) explains the reducing power potentials of the n-hexane root extract of *Zaleya decandra* in comparison with a standard ascorbic acid at 700 nm. It indicates the strong reducing power activity of *Zaleya decandra*. Increasing absorbance designates an increase in reducing ability. In FRAP assay, reduction of ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex to ferrous form which has an intense blue colour can be monitored by measuring the change in absorption at 593 nm[27] The tendency for ferric ion reducing activities of *Zaleya decandra* and ascorbic acid are shown in Figure 3(B). The absorbance of plant extract increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. It was found that the reducing power and ferric reducing activity of the extract increased with the increase of their concentrations.

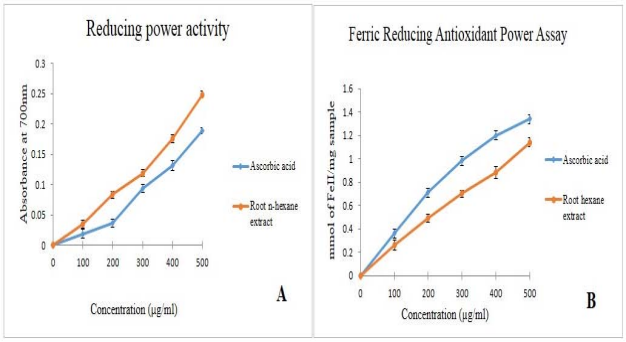

Figure 3: (A) Reducing power activity; (B) Ferric reducing antioxidant power assay.

UV-VIS and FTIR Spectrum Analysis

Ultraviolet-visible spectrophotometry (UV-Vis) related to the spectroscopy of photons in the UV-visible region. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. The color of the chemicals involved is directly affecting the absorption in the visible ranges.[28] The spectra of phenolic compounds (tannins) and flavonoids typically lie in the range of 230-290 nm.[29] The UV-VIS profile of *Zaleya decandra* was taken at the 200 to 800nm wavelength due to the sharpness of the peaks and proper baseline. The profile (Figure 4(A)) exposes the maximum absorption 1.743 at 288nm. The UV-VIS Spectrum result shows the presence of tannins and flavonoids in the n-hexane root extract of *Zaleya decandra.*

The FTIR spectrum was used to identify the functional groups of the bioactive components present in the plant extract based on the peak values in the region of IR radiation. The extract was passed into the FTIR; the functional groups of the components were separated based on its peak ratio. The FTIR analysis of n-hexane root extract of *Zaleya decandra* established the presence of alcohols, phenols (O–H stretch, H–bonded), 1°, 2° amines,

amides (N–H stretch), aromatics (C–H stretch), alkanes (C–H stretch), carboxylic acids (C=O stretch), α, β–unsaturated aldehydes, ketones (C=O stretch), alkanes(C–H bend), alkanes (C–H rock), aliphatic amines(C–N stretch) compounds which shows major peaks at 3410.15, 3394.72, 3076.46, 2924.94, 1708.93, 1666.50, 1446.61, 1367.53 and 1238.30 respectively and it also shows no absorbance in between the region 2220-2260 cm^{-1} indicates that there was no cyanide groups[30] present in n-hexane root extract of *Zaleya decandra* (Figure 4(B) and Table 1). In addition, some weak absorption bands were also recorded in the spectra.

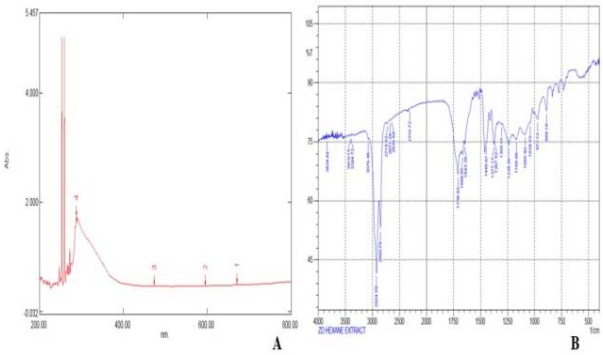

Figure 4: (A) UV-VIS Spectrum; (B) FTIR Spectrum.

GC-MS analysis

GC-MS is one of the greatest techniques to detect the constituents of volatile substance, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The identification of the phytochemical compounds was confirmed based on the peak area, retention time, molecular weight and molecular formula. The GC-MS analysis of n-hexane root extract of *Zaleya decandra* revealed the presence of twenty five bioactive compounds that could contribute the medicinal quality of the plant. The percentage content of compounds are mainly 6-Octadecenoic acid (66.97%), n-Hexadecanoic acid (14.63%), Octacosane (2.19%), Octadecane (1.51%), 2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo- (1.13%), 11-Octadecenoic acid, methyl ester (1.01%), Eicosane (1.01%) and Hexadecane (1.00%). Other constituents were < 1%. The GC-MS chromatogram of the *Zaleya decandra* was shown in Figure 5. The active principles with their retention time (RT), molecular formula, molecular weight (MW), and peak area (%) are presented in Table 2. Among the identified 25 phytocompounds 6-Octadecenoic acid has highest % peak area (66.97) and it has cancer preventive and insectifuge property.[31] n-Hexadecanoic acid is known as anti inflammatory compound and also act as antioxidant, hypochloesterolemic, nematicide, pesticide, lubricant, antiandrogenic, haemolytic, 5-alpha reductase inhibitor.[31,32] Octacosane have been stated to improve the activity and parasitic efficacy of *T. chilonis*.[33] The Mosquitocidal activity of isolated octacosane from *Moschosma polystachyum* showed mortality against larvae of *Culex quinquefasciatus*.[34] These results show that *Zaleya decandra* has more biologically active compounds with anticancer, antioxidant and other pharmacological properties.

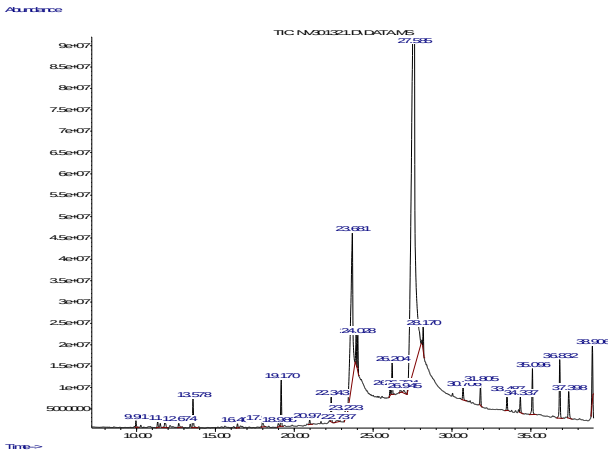

Figure 5: GC-MS Chromatogram of n-hexane root extract of *Zaleya decandra*.

Table 1: FTIR Peak Values of n-hexane root extract of *Zaleya decandra*.

Frequency, Cm–1	Bond	Functional Group
3410.15	O–H stretch, H–bonded	alcohols, phenols
3394.72	N–H stretch	1°, 2° amines, amides
3076.46	C–H stretch	Aromatics
2924.94	C–H stretch	Alkanes
1708.93	C=O stretch	carboxylic acids
1666.50	C=O stretch	α, β–unsaturated aldehydes, ketones
1446.61	C–H bend	Alkanes
1367.53	C–H rock	Alkanes
1238.30	C–N stretch	aliphatic amines

Table 2: Phytocompounds identified in the n-hexane root extract of *Zaleya decandra* by GC-MS analysis.

S.NO	RT	Peak Area %	Name	Molecular formula	Molecular weight (g/mol)
1.	9.962	0.19	Tetradecane	$CH_3(CH_2)_{12}CH_3$	198.39
2.	11.331	0.22	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202.3352
3.	11.788	0.35	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.3239
4.	12.674	0.21	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	208.2536
5.	13.578	1.00	Hexadecane	$C_{16}H_{34}$	226.44
6.	17.996	0.28	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-	$C_{15}H_{22}O$	218.3346
7.	18.986	0.15	1-Octadecene	$C_{18}H_{36}$	252.48
8.	19.170	1.51	Octadecane	$C_{18}H_{36}$	252.48
9.	20.977	0.19	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	418.609
10.	22.343	0.82	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507
11.	22.737	0.17	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709
12.	23.223	0.35	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34
13.	23.681	14.63	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241
14.	23.919	0.90	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772
15.	24.028	1.01	Eicosane	$C_{20}H_{42}$	282.5475
16.	26.204	1.01	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879
17.	26.945	0.24	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.45
18.	27.585	66.97	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614
19.	28.170	0. 61	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46
20.	30.706	0.48	1-Phenyl-1-hexyn-3-ol	$C_{12}H_{14}O$	174.24.
21.	31.805	0.54	Tetracosane	$C_{24}H_{50}$	338.65
22.	33.487	0.55	Heptadecane	$C_{17}H_{36}$	240.47
23.	34.337	0. 53	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	90.5561
24.	36.832	2.19	Octacosane	$C_{28}H_{58}$	394.761
25.	37.398	1.13	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-	$C_{14}H_{22}O$	222.3233

CONCLUSION

Based on the above studies, it is concluded that the n-hexane root extract of *Zaleya decandra* has more bioactive compounds and possesses good radical scavenging activities.

Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation of techniques like purification, separation, and identification. The synergistic role of various components present in the plant extract might also attribute to the antioxidant nature of the extract and it may serve as a good pharmacological source.

Acknowledgement: We, the authors are thankful to our Chancellor, Chief Executive officer, Vice Chancellor and Registrar of Karpagam University for providing facilities and encouragement

REFERENCES

1. Syed MH, Yasmeen A, Hussain MS, Siva Subramanian N, Ramadevi M, Preliminary Phytochemical Screening and HPTLC Fingerprinting of Leaf Extracts of *Pisonea aculeate*. Journal of Pharmacognosy and Phytochemistry, 2, 2013, 36-42.
2. Langley-Evans SC, Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay, International Journal of Food and Nutritional Sciences, 51, 2000, 181–188.
3. Sowmya S, Perumal PC, Anusooriya P, Vidya B, Pratibha P, Gopalakrishnan VK, Quantitative analysis and *in vitro* free radical scavenging activity of *Cayratia trifolia* (L.), World Journal of Pharmaceutical Research, 3, 2014, 973-988.
4. Vaishali RM, Vinitha RP, Pratapchandra KH, Smitha H, Preliminary Phytochemical Screening of Members of Lamiaceae Family: *Leucas linifolia, Coleus aromaticus* and *Pogestemon patchouli*, International Journal of Pharmaceutical Sciences Review and Research, 21, 2013, 131-137.
5. Shadma W, Arshad H, Parwez A, Aleza R, Current Status of Herbal Drugs in the Development of Newer Therapeutics Agents, International Journal of Pharmacy and Pharmaceutical Sciences, 2, 2013, 1463-1473.
6. Meenakshi P, Bhuvaneshwari R, Rathi MA, Thirumoorthi L, Guravaiah DC, Jiji MJ, Gopalakrishnan VK, Antidiabetic Activity of Ethanolic Extract of *Zaleya decandra* in Alloxan-Induced Diabetic Rats, Applied Biochemistry and Biotechnology, 162, 2010, 1153–1159.
7. Rama Krishna N, Varma YNR, Saidulu Ch, Ethnobotanical Studies of Adilabad District, Andhra Pradesh, India, Journal of Pharmacognosy and Phytochemistry, 3, 2014, 18-36.
8. Geethalakshmi R, Sarada DVL, Ramasamy K, *Trianthema decandra* L: A review on its phytochemical and pharmacological profile,

International Journal of Engineering, Science and Technology, 2, 2010, 976-979.

9. Blois MS, Antioxidant determinations by the use of stable free radical, Nature, 81, 1958, 1199-2000.
10. Green LC, Wagner DA, Glogowski J, Analysis of nitrate, nitrite and nitrate in biological fluids, Analytical Biochemistry, 126, 1982, 131-138.
11. Fontana M, Mosca L, Rosei MA, Interaction of Enkephalines with oxyradicals. Biochemical Pharmacology, 61, 2001, 1253-1257.
12. Elizabeth K, Rao MNA, Oxygen radical scavenging activity of curcumin, International Journal of Pharmaceutics, 58, 1990, 237-240.
13. Ruch RJ, Cheng SJ, Klaunig JE, Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10, 1989, 1003-1008.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evance C. Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radical Biology and Medicine, 26, 1999, 231-237.
15. Dinis TCP, Madeira VMC, Almeida LM, Action of phenolic derivatives (Acetoaminophen, Salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Archives of Biochemistry and Biophysics, 315, 1994, 161-169.
16. Oyaizu M, Studies on product of browning reaction prepared from glucose amine, Japanese Journal of Nutrition, 44, 1986, 307-315.
17. Benzie IFF, Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power" The FRAP assay, Analytical Biochemistry, 239, 1996, 70-76.
18. Kalaiselvi M, Narmadha R, Ragavendran P, RaviKumar G, Gomathi D, Uma C, *In vitro* scavenging activity of *Jasminum sambac* (L.) Ait Oleaceae, Asian Journal of Pharmaceutical and Biological Research, 1, 2011, 370-375.
19. Hofseth LJ, Saito S, Hussain SP, Espey MG, Miranda KM, Araki Y, Jhappan C, Higashimoto Y, He Pn, Linke SP, Quezado MM, Zurer I, Rotter V, Wink DA, Appella E and Harris CC, Nitric oxide-induced cellular stress and p53 activation in chronic inflammation, Proceedings of the National Academy of Sciences USA, 100, 2003, 143-148.
20. Gulati K, Ray A, Masood A, Vijayan VK, Involvement of nitric oxide (NO) in the regulation of stress susceptibility and adaptation in rats, Indian Journal of Experimental Biology, 44, 2006, 809-815.
21. Fulya UA, Ceren A, Oya U, Tulay B, Ataman BS, Antioxidant and proliferative effects of aqueous and ethanolic extracts of *Symphytum officinale* on 3T3 Swiss albino mouse fibroblast cell line, Asian Journal of Plant Science and Research, 4, 2014, 62-68.
22. Arul Raj C, Ragavendran P, Sophia D, Rathi MA, Gopalakrishnan VK, Evaluation of *in vitro* antioxidant and anticancer activity of *Alpinia purpurata*. Chinese Journal of Natural Medicines, 10, 2012, 0263–0268.
23. Kasthuri B, Chitra M, *In vitro* studies of antioxidant and antiinflammation activity of *Argemone maxicana* L. flower extract, International journal of medicinal chemistry and analysis, 4, 2014, 79-82.
24. Arockia J, Veerabahu RM, *In vitro* antioxidant potential of leaf extracts of the endemic plant *Becolepsis nervosa* Decne. ex. Moq. (Periplocaceae), Journal of Advanced Pharmacy Education & Research, 4, 2014, 206-215.
25. Rahmat AK, Muhammad RK, Sumaira S, Mushtaq A, Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill, Chemistry Central Journal, 6, 2012, 12.
26. Olayinka A, Aiyegoro, Anthony IO, Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC, BMC Complementary and Alternative Medicine, 10, 2010, 21.
27. Rasleen S, Madhulika B, Sahil G, Jasvinder S, Anupurna K, Iron (FeII) Chelation, Ferric Reducing Antioxidant Power, and Immune Modulating Potential of *Arisaema jacquemontii* (Himalayan Cobra Lily), BioMed Research International, 2014, 2014, 1-7.
28. Gunasekaran S, UV-VIS spectroscopic analysis of blood serum, Asian Journal of Microbiology, Biotechnology & Environmental, 5, 2003, 581-582.
29. Liu HX, Sun SQ, Lv GH, Chan KKC, Study on *Angelica* and its different extracts by Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy, Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy, 64, 2006, 321-326.
30. Ragavendran P, Sophia D, Arul Raj C, Gopalakrishnan VK, Functional group analysis of various extracts of *Aerva lanata* (L.,) by FTIR spectrum, Pharmacologyonline, 1, 2011, 358-364.
31. Elezabeth DV, Arumugam S, GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves, Journal of Chemical and Pharmaceutical Research, 6, 2014, 294-300.
32. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M, Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment, Chemical Biology & Drug Design, 80, 2012, 434-439.
33. Paul AVN, Singh S, Singh AK, Kairomonal effect of some saturated hydrocarbons on the egg parasitoids, *Trichogramma brasiliensis* (Ashmead) and *Trichogramma exiguum*, Pinto, Platner and Oatman (Hym. Trichogrammatidae), Journal of Applied Entomology, 126, 2002, 409–416.
34. Rajkumar S, Jebanesan A, Mosquitocidal activities of octasane from *Moschosma polystachyum* Linn. (Lamiaceae), Journal of Ethnopharmacology, 90, 2004, 87–89.

Source of Support: Nil, **Conflict of Interest:** None.