International Journal of Scientific Research in Biological Sciences **Research Paper**
Vol.7, Issue.2, pp.128-133, April (2020) **E-ISSN:** 2347-7520
DOI: https://doi.org/10.26438/ijsrbs/v7i2.128133

GC-MS Analysis and Antioxidant Activity of Organic Extracts of *Psidium cattleianum* Leaves

K. Sunisha[1] and R. Dhivya[2*]

[1]PG and Research Department of Zoology, Nirmala College for Women, Coimbatore, Tamilnadu – 641018
[2]PG and Research Department of Zoology, Nirmala College for Women, Coimbatore, Tamilnadu – 641018

[*]*Corresponding Author:* dhivya.adu@gmail.com, *Tel.: +91 9791229657*

Available online at: www.isroset.org

Received: 04/Apr /2020, Accepted: 23/Apr/ 2020, Online: 30/Apr/2020

***Abstract* -** Plants usually are reservoirs of organic compounds that provide definite physiological action on the body of human beings. Pharmacological activities of plants have been extensively investigated as a source of medicinal agents. For the discovery of lead compounds for use as therapeutic drugs, the active principle in medicinal plants needs to be identified. GC-MS method serves as an interesting tool for testing the amount of some active principles of herbs. Therefore, the present study was carried out to investigate the GC-MS analysis, antioxidant activity and phytochemical screening of ethanol and acetone extracts of *Psidium cattleianum* leaves. Compound identification of the extract was using Shimadzu GC-2010 gas chromatography coupled with QP2010 mass spectrometer. DPPH assay was applied using Q*uercetin* as a standard to test the antioxidant activity of acetone and ethanol extract of *P. cattleianum* leaves. Phytochemical analysis was done by following the standard procedures. The results of GC-MS analysis stated the presence of seven major compounds in ethanol extract and twelve major compounds in the acetone extract of *P. cattleianum* leaf extract. The results also revealed that ethanol extract exhibited more antioxidant activity than acetone extract. When screened for phytocompounds, ethanol and acetone extract recorded the presence of significant number of secondary metabolites viz., Phenols, Flavanoids, Alkaloids, Glycosides and Quinones that were present in both the extracts. The excellent pharmacological activities of acetone and ethanol extracts of *P. cattleianum* leaves might be due to the presence of various phytochemicals and compounds that were identified. It may be concluded that plant products can be considered as strong candidates that contribute for the development of new leads that can provide help in combating the emerging issues of drug resistance.

***Keywords*—** *Psidium*; Plants extracts; Solvents; GC MS Analysis; Antioxidant; Phytochemicals

I. INTRODUCTION

Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments [1]. They can be a source of chemical compounds of biological and pharmacological importance. From ancient times it is revealed that plants are sources of successful drugs, and will continuously be important for screening of new lead compounds [2]. An essential part in the investigation of plant is the identification of the biologically active compounds presents in plant leading to further biological and pharmacological studies [3], [4].

Each and every plant contains a certain specific type of chemical compounds, which are produced during the normal growth and development of the plant. These compounds are generally referred to as "phytochemicals" or sometimes also known as "secondary metabolites" [5]. These phytochemicals are synthesized by primary or rather secondary metabolism of living organisms. Phytocompounds are chemically and taxonomically extremely diverse compounds with obscure function. They are used widely in the human therapy, veterinary, agriculture, scientific research and countless other areas [6].

Antioxidants play an important role as protecting factor of health. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Natural sources of antioxidants are whole grains, fruits and vegetables. Disease risk can be reduced by plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids etc. that have been recognized as having the potential to reduce it [7]. Antioxidant compounds are mostly in a typical diet and are derived from plant sources and belong to various classes of compounds and have a wide variety of physical and chemical properties [8]. The use of the free radical is a rapid, simple and inexpensive method to measure antioxidant capacity of food. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [9].

Natural antioxidants are in high demand for biopharmaceuticals application in several fields of study. In fact, intensive research has been performed for the extraction, characterization, and utilization of natural products/ antioxidants, like essential oils (EOs) and plant extracts, which may serve as potent candidates in combating the oxidative-related pathologies [10]. The percentage of the chemical components in plant extracts varies among species and plants parts [11]. The present investigation was carried out to determine the possible chemical components from *Psidium cattlenium* leaf extracts by GC-MS analysis, that was carried out in PSG Arts and Science College, Coimbatore and its subsequent antioxidant potential and phytochemicals analysis was done in the Nirmala College for Women, Coimbatore.

II. METHODOLOGY

Leaves of *Psidium cattleianum L.* (Myrtaceae) were collected from Coonoor, Niligiris district, Tamil nadu. The specimen was identified, certified and deposited with voucher specimen number (BSI/SRC/5/23/2019/Tech/359) at the Botanical Survey of India, Southern Circle, Coimbatore.

Preparation of leaf powder and extracts

Fresh leaves of *P. cattleianum* were collected, and air dried under shade. Dried leaves were powdered using an electric pulveriser. Fine powder was obtained by sieving. 10g each of leaf powder was weighed and powders were subjected to extraction [12], [13]. Acetone extraction of the leaf power was followed by ethanol extraction.

The leaves extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use. Gas chromatography mass spectometry, antioxidant and phytochemical analysis activity of the leaves of *P. cattleianum* were investigated**.**

Gas Chromatography Mass Spectrometry Analysis of Plant Extracts

Acetone and ethanol leaf extracts of *P. cattleianum* obtained were used to investigate the bioactive compounds present in them with the help of Gas Chromatography Mass Spectrometry analysis. The component identification of extract was done using Shimadzu GC-2010 gas chromatography coupled with QP2010 mass spectrometer.

The sample was injected into the GC-MS on a 30 m glass capillary column with a film thickness of 0.25 µm (30 m × 0.25µm). Helium as carrier gas was used at 14.4 ml/min constant flow mode. Injector and detector temperatures were kept at 250 °C. The GC temperature programme was 70°C - 280°C at 15°C/min. Split ratio 1:30. The total GC running time is at 50 min. The MS was taken at 70 eV. The MS scan parameters included a mass range of m/z40-800, a scan interval of 0.5 s, a scan speed of 1666 amu s−1, and a detector voltage of +0.00 kV. The mass spectrum of the individual unknown compound was compared with the known compounds stored in the software database Libraries. The name, structure of the components and its molecular weight of the test materials were ascertained.

Antioxidant Activity of Plant Extracts

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay

The radical scavenging activity of acetone and ethanol extracts was determined by using DPPH assay according to Chang *et al*., [14]. Q*uercetin* was used as a standard to test the antioxidant activity. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 520 nm.

0.5 ml of 0.1mM DPPH solution in methanol was mixed with plant extract solution of varying concentrations (50, 100, 150, 200, 250 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (25-500µg/ml) was used as reference standard. Mixer of 0.5ml methanol and 0.5ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 520nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula. The radical scavenging activity was calculated as,

% RSA= {(Abs control – Abs sample)}/(Abs control)×100

Where, *RSA* is the Radical Scavenging Activity; *Abs control* is the absorbance of control; *Abs sample* is the absorbance of sample.

Phytochemical Analysis of Plant Extracts

Phytochemical analysis of leaf extract of *P. cattleianum* was carried out using the standard methods.

Test for Alkaloids

- **Mayer's test [15]:** A fraction of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test [16]:** A fraction of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.

Test for Phenols

- **Ferric chloride test [17]:** 50 mg of leaf extract (50mg) was stirred in 5 ml of distilled H_2O and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour.

- **Lead acetate test [18], [19]:** The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

Test for Tannins

- **Ferric chloride test [20]:** About 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

Test for Flavonoids

- **NaOH test [20]:** Few quantity of the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. On addition of dilute hydrochloric acid, change of colour from yellow to colourless is an indication for the presence of flavonoids.

- **Lead acetate test [18], [19]:** Test extract (50 mg) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

Test for Glycosides

- **H_2SO_4 test [21]:** To 100 µl of plant extract 2ml of concentrated H_2SO_4 was added. Formation of reddish brown colour indicates the presence of glycosides.

Test for Terpenoids

- **Liebermann-Burchard test [22]:** A little of extract (50 mg) was dissolved in ethanol and then acetic anhydride about 1 ml was added followed by the addition of Conc. H_2SO_4. Pink colour changes to violet colour which is an indicator for the presence of terpenoids.

Test for Saponins

- **Foam Test:** The extract (50 mg) was dissolved in distilled H_2O and made up to 20 ml. It was then vigorously shaken in a graduated cylinder for 15 minutes and noted for the appearance of 2 cm layer thick foam.

Test for Quinones

- **H_2SO_4 test [19]:** To 1 ml of extract adds 1 ml of Conc. H_2SO_4 and observed for the formation of red colour.
- **HCl test [23], [24]:** To 1 ml of the extract 5 ml of HCl and observed for the presence of yellow colour precipitate.

III. RESULTS AND DISCUSSION

GC MS Analysis of ethanol extract of *Psidium cattleianum*

The Gas chromatography – mass spectrometry is the analytical method used to identify different organic components and their mass within a sample. GC-MS analysis of solvent extract of *P. cattleianum* extract was analysed in the present study. The results of GC-MS analysis revealed the presence of Seven major compounds in ethanol extract of *P. cattleianum*. The compounds are identified based on their peak area, retention time, molecular weight and molecular formula. Gas Chromatogram of ethanol extract of *P. cattleianum* is shown in Figure 1. The major Retention times were 18.950, 19.775, 21.717, 28.633 and 49.217. The major compounds are listed in the Table 1.

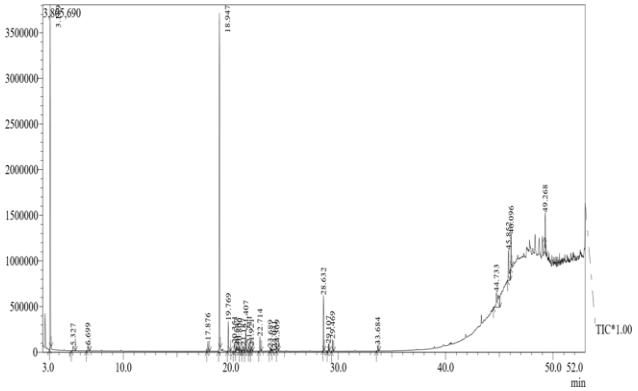

Fig 1: GC-MS Chromatogram of the Ethanol Extract of Psidium cattleianum

Table 1: Compounds of Ethanol Extract of *Psidium cattleianum* by GC-MS Analysis

Sl. No	Compounds	Molecular formula	Molecular weight	Retention time
1	Caryophyllene	$C_{15}H_{24}$	204	18.950
2	1,4,8-Cycloundectriene,2,6,6,9-tetramethyl-,(E,E,E)-	$C_{15}H_{24}$	204	19.775
3	Naphthalene,1,3,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$	204	21.717
4	3,7,11,15-Tetramethyl-2-hexandecen-1-ol	$C_{20}H_{40}O$	296	28.633
5	Neonaphytadiene	$C_{20}H_{38}$	278	28.633
6	9-Eicosyne	$C_{20}H_{38}$	278	28.633
7	Vitamin E	$C_{29}H_{50}O_2$	430	49.217

The compound are Caryophyllene, 1,4,8-Cycloundectriene, 2,6,6,9-tetramethyl-, (E,E,E), Naphthalene,1,3,3 1a,5,6,8a-hexahydro- 4,7-dimethyl- 1- (1-methylethyl)- ,3,7,11,15-Tetramethyl-2-hexandecen-1-ol, Neonaphytadiene, 9-Eicosyne and Vitamin E. Similar study was carried out by Karthishwaran *et al.*, [25] which reported twenty chemical compounds from ethanol extract of *Mussaenda frondosa* by GC-MS analysis. Similarly, Siva Krishnan *et al.*, [26] identified twelve compounds from ethanol extract of aerial

parts of *Albizia rocera.* Anand and Gokula Krishnan [27] identified the presence of thirteen chemicals from the ethanol extract of *H. enneaspermus.*

GC MS Analysis of acetone extract of *Psidium cattleianum*

The GC-MS analysis showed the presence of Twelve major compounds in the acetone extract of *P. cattleianum* extract by comparing their retention times. Gas Chromatogram of acetone extract of *P. cattleianum* is given in Figure 2. The important Retention times were noted as 3.192, 18.950, 28.633, 44.733 and 45.850. The major compounds are listed in Table 2.

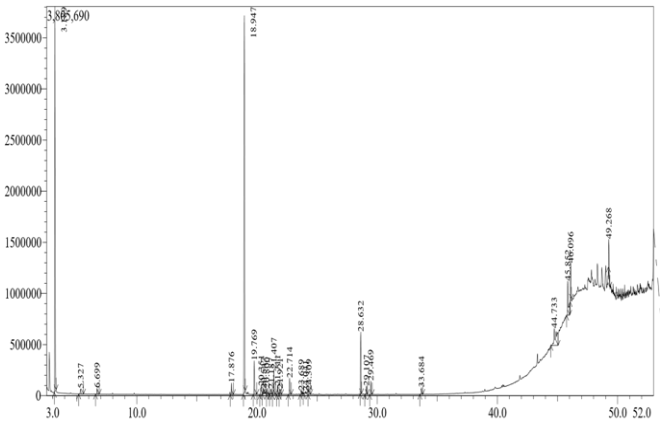

Fig 2: GC-MS Chromatogram of the Acetone Extract of Psidium cattleianum

Table 2: Compounds of Acetone Extract of *Psidium cattleianum* by GC-MS Analysis

Sl. No	Compounds	Molecular formula	Molecular weight	Retention time
1	4-Hydroxy-4-methyl-2-pentanone	$C_6H_{12}O_2$	116	3.192
2	Caryophyllene	$C_{15}H_{24}$	204	18.950
3	3,7,11,15-Tetramethylhexadec-2-en-ol	$C_{20}H_{40}O$	296	28.633
4	Neophytadiene	$C_{20}H_{38}$	278	28.633
5	9-Eicosyne	$C_{20}H_{38}$	278	28.633
6	Hexatriacontane	$C_{36}H_{74}$	506	44.733
7	Doctriacontane	$C_{32}H_{66}$	450	44.733
8	Tetrapentanecontane	$C_{54}H_{11}0$	758	44.733
9	1-Heptacosanol	$C_{27}H_{56}O$	396	45.850
10	1-Pentacosanol	$C_{25}H_{52}O$	368	45.850
11	n-Tetracosanol	$C_{24}H_{50}O$	354	45.850
12	1-Eicosanol	$C_{20}H_{42}O$	298	45.850

The compounds were identified as 4-Hydroxy-4-methyl-2-pentanone, Caryophyllene, 3, 7, 11, 15 Tetramethylhexadec-2-en-ol, Neophytadiene, 9-Eicosyne, Hexatriacontane, Doctriacontane, 1-Heptacosanol, 1 Pentacosanol, n-Tetracosanol and 1-Eicosanol. In parallel to the present study ethanol extract of *Acalypha indica* was subjected to GC-MS study by Chandra Mohan *et al*., [28] and result showed the presence of five compounds. Muthulakshmi *et al*., [29] conducted experiment in the ethanol extracts of leaves of *F. elephantum* and eighteen compounds were identified. Likewise, Selvamani and Balamurugan [30] in the acetone leaf extract of *Acalypha indica* reported five major compounds viz., 1,3-Dioxolane, 4-Ethyl-5-Octyl-2,2-Bis (Trifluoromethyl), Trans-Clotrisiloxane, Hexamathyl, Trimethyl [4-(1,1,3,3, Tetramethylbutyl) phenox] Silane, Phenol, 24 BIS (1,1-Dimethylethyl) and 2-Methyl-3 (3-Methyl-But-2 Enyl)-2-(4-Methul-Pent-3-Enyl)-Oxetane respectively along with other minor constituents.

Antioxidant Activity of *Psidium cattleianum* Leaf Extracts

Leaf extracts of *P. cattleianum* extracted using different solvents exhibited varying degrees of antioxidant capacity. The standards and samples were tested in five different volumes 50, 100, 150, 200 and 250µl and the radical scavenging activity of standard was 55.38, 32.44, 19.62, 14.73 and 9.45 % respectively. The results of radical scavenging activity of ethanol and acetone extracts of *P. cattleianum* leaves are presented in Fig 3. Ethanol extract exhibited maximum antioxidant activity by recording radical scavenging activity as 58.73, 34.95, 21.35, 17.67and 11.33%. The antioxidant activity of ethanol extract was higher than that of acetone extract. In comparison to the results of the present study, Moure *et al.*, [31] suggested that ethanol offered the best results to extract phenolic compounds from *Gevuina avellana* compared to acetone. Similarly, Sun and Ho [32] discovered that extracting solvent significantly affected the yield of phenolic content of buckwheat extract.

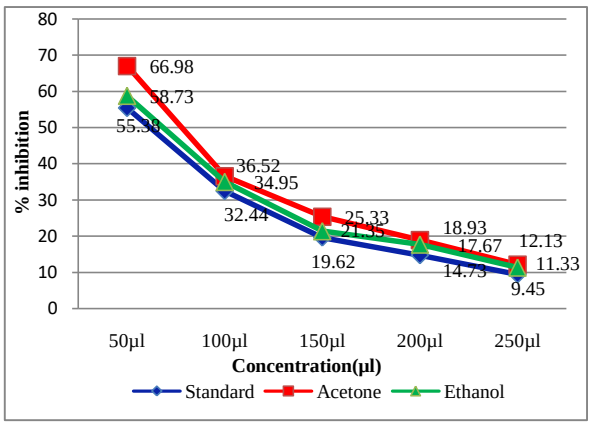

Fig 3: DPPH Radical Scavenging Assay of Psidium cattleianum Extract.

Activity of ethanol extract was followed by acetone extract that displayed an antioxidant activity percentage of 66.98, 36.52, 25.33, 18.93 and 12.13%. When the volume decreases the rate of scavenging percentage also increased. Antioxidant activity was concentration dependent. The results of this study are in agreement with the extraction yields of rice bran analysed by Chatha *et al.*, [33] and on some medicinal plants carried out by Sultana *et al*., [34]. Similar experiments done by Gurdip *et al.*, [35] showed that acetone extract of both pepper oil and linseed oil are

better antioxidants. Craciunescu *et al.,* [36] examined *Arnica montana* and *Artemisia absinthium* extract. According to the result *A. absinthium* extract revealed a higher antioxidant capacity than *A. montana* extract.

Phytochemical Analysis of *Psidium cattleianum* Leaf Extracts

The results of preliminary qualitative investigations of different bioactive components in different solvent extract of *P. cattleianum* are presented in the Table 3. Ethanol extract of *Psidium cattleianum* that exhibited highest antioxidant activity and seven major compounds in GC MS analysis, when screened for phytocompounds recorded the presence of Phenols, Flavanoids, Tannins, Saponins, Alkaloids, Glycosides and Quinones. Preliminary phytochemical analysis of aqueous extract of *S. grandiflora* conducted by Padmalochana and Dhana, [37] revealed the presence of similar bioactive compounds in different solvents extract.

Table 3: Phytochemical Analysis of *Psidium cattleianum* Leaf Extracts

Sl. No.	Tests	Ethanol extract	Acetone extract
1	Phenols	+	+
2	Flavonoids	+	+
3	Tannins	+	-
4	Saponins	+	-
5	Alkaloids	+	+
6	Glycosides	+	+
7	Terpenoids	-	-
8	Quinones	+	+

"+" Presence, "-"Absence

Mensah *et al.,* [38] reported alkaloids in 12 leafy vegetables studied. Pandit [39] conducted study on six native plants of Agra city i.e. *Achyranthus aspera, Acalypha indica, Euphorbia hirta, Lindenbergia indica, Parthenium hysterophorus* and *Peristrophe bicalyculata* were result showed the presence of active compounds like Alkaloids, Tannins, Saponins, Glycosides, Phenols, Flavonoids, Anthroquinone, Terpenoids and Steroid.

The major phytochemicals in the acetone extract of the *Psidium cattleianum* that showed moderate antioxidant activity and presence of Twelve major compounds in GC MS analysis are Phenols, Flavanoids, Alkaloids, Glycosides and Quinones. As compared to the polarity of the solvents ethanol extract is comparatively more polar than acetone. So, Ethanol extract showed more secondary metabolites than acetone extract. Similar to the present study, Vaghasiya *et al.,* [40] also conducted study on medicinal plants and showed the presence of various secondary metabolites in the acetone extract of different medicinal plants tested.

In accordance to the results of the present study Mital and Sumitra [41] assessed the acetone extract of *P. guajava* and reported that it contains maximum amount of alkaloids and saponins while triterpenes was present in moderate amount. Similarly, Yadav and Munin [42] analysed the plants *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves), *Tinospora cordifolia* (Stem) and *Xanthium strumarium* (Leaves) respectively. The result showed the presence of Proteins, Carbohydrates, Phenols and Tannins, Flavonoids and Saponins in all the plants.

IV. Conclusion and Future Scope

The compounds present in plant extracts can be identified from the peak pattern of the chromatograms obtained. The plotting of chromatographic patterns aims at evaluating the quality of the plant extract. Not only that, it also helps to know the organic compounds and their uses in the future study of the plant in herbal medicine. Therefore, GC-MS method is a direct and fast approach for the identification of terpenoids and steroids and it needs only very few grams of plant materials. It was evident from the present study that different solvent extracts of *P. cattleianum* leaves exhibited prominent antioxidant activity. Many evidences gathered in earlier studies confirmed that identified phytochemicals are much bioactive. This study is important because of presence of these compounds terpenoids, alkaloids, flavanoids and steroids. In the present study *P. cattleianum* leaf extract contains these compounds and it suggested that this plant contribute on the pharmacological activity that should be evaluated. Although, further investigations is needed that may lead to the development of new drugs formulation.

References

[1] Z.Y. Bachrach ZY, "Contribution of selected medicinal plants for cancer prevention and therapy. *Acta Fac Medicae Naissensis*, Vol. **29,** Issue. **3,** pp. **117-23, 2012.**

[2] A.G. Atanasov, B. Waltenberger, E.M. Pferschy-Wenzig, T. Linder, C. Wawrosch and P. Uhrin, "Discovery and resupply of pharmacologically active plant-derived natural products: a review," *Biotechnol Adv,* Vol. **3,** Issue. **8**, pp. **1582-614, 2015.**

[3] M.A. Momin, S.F. Bellah, S.M. Rahman, A.A. Rahman, G.M. Murshid and T.B. Emran, "Phytopharmacological evaluation of ethanol extract of *Sida cordifolia L*. roots," *Asian Pac J Trop Biomed,* Vol. **4,** Issue. **1,** pp. **18-24, 2014.**

[4] M.M. Farid, S.R. Hussein, L.F. Ibrahim, M.A. Desouky, A.M. Elsayed and A.A. Oqlah, "Cytotoxic activity and phytochemical analysis of *Arum palaestinum Boiss*," *Asian Pac J Trop Biomed,* Vol. **5,** Issue. **11,** pp. **944-7, 2015.**

[5] Sonia, D. Anju, S. Govind and S. Saurabh, "Gas chromatography-mass spectroscopy analysis of bioactive compounds in the whole plant parts of ethanolic extract of *Asclepias Curassavica* L," *International Journal of Green Pharmacy*, pp. **107-114, 2018.**

[6] G.E. Trease and W.C. Evans, "Pharmacology 11th Ed.," *Bailliere Tindall Ltd,* London**,** pp. **60-75, 1978.**

[7] Annonymous, "Indian herbal pharmacopoeia, revised new edn," *Indian drug Manufacturers Association,* Mumbai, pp. **79-87**, **2002.**

[8] T. Sravani and P.M. Paarakh, "Antioxidant activity of *Hedychium spicatum Buch. Ham* Rhizomes," *Indian journal of*

natural products & resources, Vol. **3,** Issue. **3,** pp. **354-358**, **2012.**

[9] K.R. Kirtikar and B.D. Basu, "Indian medicinal plants," *International book distributors*, Dehradun**,** pp. **993-994, 2006.**

[10] T. Ozen, I. Demirtas and H. Aksit, "Determination of antioxidant activities of various extracts and essential oil compositions of Thymus praecox subsp. *skorpilii var. skorpilii*," *Food Chem*, Vol. **12,** Issue. **4,** pp. **58–64**, **2011.**

[11] F.S. Santos and M.G.M. Novales, "Essential oils from aromatic herbs as antimicrobial agents," *Current Opinion in Biotechnology*, Vol. **23,** Issue. **2,** pp. **136-141, 2012.**

[12] J.B. Harbourne, "Phytochemical Methods". Chapman and Hall, Ltd, London, p. **49-188**, **1973**.

[13] A.I. Vogel. "Text book of practical organic chemistry". *The English language book society and Longman*, London. p. **1368, 1978.**

[14] S.T. Chang, J.H. Wu, S.Y. Wang, P.L. Kang, N.S. Yang, L.F. Shyur. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *Journal of Agricultural and Food Chemistry*. **Vol**. 4(9), pp. **3420–3424, 2011**.

[15] W.C. Evans, "Trease and Evans Pharmacology. 14th Edn.," *Harcourt Brace and company. Asia. Pvt. Ltd.* Singapore, **1997.**

[16] H. Wagner, "Pharmazeutische Biology 5th edn.," *AUFI. 15 BN 3-437-20 498-X. Gustav Fisher Vwelag. Stuttgart.* Germany, **1993.**

[17] M.E. Mace, "Histochemical localization of phenols in healthy and diseased tomato roots. *Phytopathology*," Vol. **16,** pp. **915-925, 1963.**

[18] K.R. Brain and T.D. Turner, "Wright Scien Technica. 1st Edn," *Bristol: Practical Evaluation of Phyto Pharmaceuticals*, pp.**144, 1975.**

[19] W.C. Evans, "Pharmacognosy. 14th Edn.," *WB Saunders Co. Ltd.* Singapore, Vol. **9,** pp. **713-734**, **1996.**

[20] G.E. Trease and W.C. Evans, "Pharmacognosy 15th Ed.," London*: Saunders Publishers*, **2002.**

[21] M. Prakash, M. Avetik, P.C. Venkata Sai, C. Mary, S.M. Rafael, P. Siddhartha and J. Marquita, "Methods for purifying steviol glycosides and uses of the same," US Patent 9, Vol. **169,** pp. **285**, **2015.**

[22] A. Sofowora, "Medicinal plants and traditional medicinal in Africa. Screening Plants for bioactive Agents, 2nd ed.," *Sunshine House, Spectrum Books Ltd.* Nigeria, Ibadan, pp. **134–156, 1993.**

[23] C.K. Kokate, "Practical Pharmacognosy," *Vallabh Prakashan*, Delhi, pp. **107-111**, **2000.**

[24] J.B. Harborne, "Phytochemical methods," London. *Chapman and Hall, Ltd*, pp. **49-188, 1973.**

[25] K. Karthishwaran, S. Muthukkumarasamy and M. Sankaran, "GC-MS analysis of methanolic extract of aerial parts of *Pergularia daemia* (Forsk.)," *E-Journal of Life Science*, Vol. **1,** Issue. **1,** pp. **50-55, 2012.**

[26] S. Siva Krishnan, M.A. Kottai and J. Kavitha, "GC-MS analysis of ethanolic extract of *Albizia rocera* (Roxb.) Benth," *Int. J. Pharmacy Pharm. Sc.*, Vol. **5,** Issue. **3,** pp.**702-704, 2013.**

[27] T. Anand and K. Gokula Krishnan, "GC-MS analysis and anti-microbial activity of bio active components of *Hybanthus enneaspermus*," *Int. J. Pharmacy Pharm. Sci*, Vol. **43**, pp. **646-650**, **2012.**

[28] S. Chandra Mohan, S. Dinakar, T. Anand, R. Elayaraja, B. Sathiya Priya, "Phytochemical, GC-MS analysis and Antibacterial activity of a Medicinal Plant *Acalypha indica*," *Int. J. PharmTech Res*, Vol. **4,** Issue. **3,** pp**. 1050 – 1054, 2012.**

[29] M. Muthulakshmi, R. Jothibai, V.R. Mohan, "GC-MS Analysis of Bioactive Components of *Feronia elephantum* Correa (Rutaceae)," *Journal of Applied Pharmaceutical Science*, Vol. **02,** Issue. **2, pp**. **69-74**, **2012.**

[30] S. Selvamani and S. Balamurugan, "GC-MS Analysis of Acetone Leaf Extract of *Acalypha indica* (Linn.)," *International Journal of Research Studies in Biosciences*, Vol. **3,** Issue. **5,** pp. **229-232, 2015.**

[31] J.M. Moure, D. Cruz, J.M. Franco, J. Dominguez, H. Sineiro, M. Dominguez, J. Nunez and J.C. Parajo, "Natural antioxidants from residual sources," *Food Chemistry*, Vol. **72,** Issue. **2,** pp. **145-171, 2001.**

[32] T. Sun and C.T. Ho, "Antioxidant activities of buckwheat extracts," *Food Chemistry*, Vol. **90** Issue. **I4,** pp. **743-749**, **2005.**

[33] S.A.S. Chatha, F. Anwar and M. Manzoor, "Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays," *Grasas Aceites*, Vol. **57,** pp. **328-335, 2006**.

[34] B. Sultana, F. Anwar and M. Ashraf, "Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts," *Molecules*, Vol. **14,** pp. **2167-80, 2009.**

[35] S. Gurdip, P. Marimuthu, C. Catalan and M.P. DeLampasona, "Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract," *J Sci Food Agric*, Vol. **84,** pp. **1878–1882, 2004.**

[36] O. Craciunescu, D. Constantin, A. Gaspar, L. Toma, E. Utoiu, L. Moldovan, "Evaluation of antioxidant and cytoprotective activities of *Arnica montana L.* and *Artemisia absinthium L.* ethanolic extracts," *Chemistry Central Journal*, Vol. **6,** Issue. **7,** pp. **1-11**, **2012.**

[37] K. Padmalochana and R.M.S. Dhana, "Antimicrobial activity of Aqueous, Ethanol and Acetone extracts of Sesbania grandiflora leaves and its phytochemical characterization," *International Journal of Pharma Sciences and Research*, Vol. **5,** pp. **975-962**, **2014.**

[38] J.K. Mensah, R.I. Okoli, J.O. Ohaju-Obodo and K. Eifediyi, "Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria", *Afr. J. Biotechnology*, Vol. **7**, Issue. **14**, pp. **2304-2309, 2008.**

[39] J.I. Pandit, "Phytochemical screening of certain plant species of Agra city," *Journal of Drug Delivery & Therapeutics***,** Vol. **2,** Issue. **4,** pp. **135-138, 2012.**

[40] Y. Vaghasiya, D. Priya, K. Rajaram, K.P. Suresh. "Phytochemical studies and GC-MS analysis of *Caralluma fimbriata* Wall," *International Journal of Pharmaceutical Research and Development*, Vol. **3,** Issue. **10,** pp.**105-110, 2011.**

[41] K. Mital and C. Sumitra, "Phytochemical and Pharmacognostic Evaluation of Leaves of *Psidium guajava* L. (Myrtaceae)," *Pharmacognosy Journal*, Vol. **3,** Issue. **23,** pp. **41-45, 2011.**

[42] R.N.S. Yadav and A. Munin, "Phytochemical analysis of some medicinal plants," *Journal of Phytology*, Vol. **3,** Issue. **12,** pp. **10-14**, **2011.**

