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Evaluation of Antioxidant activity and phytochemical studies of *Capparis divaricata* Lam fruit Extract

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Abstract : The occurrence of thousands of plant species on the planet is a gift to mankind as medicinal treasure to cure different human ailments. The present study was aimed to evaluate phytochemical profiling Antimicrobial activity antioxidant activities of *Capparis divaricata* dried fruit extract. *C. divaricata* is commonly known as spreading keeper belongs to the family Capparidaceae. The dried fruit extract showed the presence of alkaloids, flavonoids, phenols, terpenoids, steroids, lignins, tannins, anthocynidins, cardiac glycosides and reducing sugars. The major phytochemical constituents identified by GC-MS analysis are 1-Methyl-Pyrrolidine-2-Carboxylic Acid, Pentadecanoic Acid, 14-Methyl-, Methyl Ester, N-Hexadecanoic Acid, 11-Octadecenoic Acid, Methyl Ester, Heptadecanoic Acid, 16-Methyl-, Methyl Ester and Oleic Acid. The fruit extract showed the presence of significant amount of antioxidants correlated with Free radical scavenging activity. No significant antimicrobial activity was found.

IndexTerms – *Capparis divaricata*, Methyle esters, antioxidant potential, anticancer properties.

I. INTRODUCTION

The chemical compounds derived from natural sources i.e., from plants, microbes and animals are defined as natural products, which possess biological/pharmacological activities (Baker et al. 2007). Natural products include different classes of components such as terpenoids, phenolic compounds, amino acids, peptides, proteins, carbohydrates, lipids, nucleic acids etc., (Jarvis, 2000).These metabolites are synthesized by primary metabolism or rather secondary metabolism of living organisms. Secondary metabolites are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas (Vasu et al. 2009). Many phytochemicals have inhibitory effects on all types of microorganisms (Cowan, 1999).The genus *Capparis* consists of nearly 250 species. *Capparis* is distributed in tropical and subtropical zones of Asia, southern America, Australia, Africa, Europe, Madagascar and the Pacific Islands (Willis, 1988). *Capparis divaricata* Lam is commonly distributed in India especially in Deccan Peninsular region (The Wealth of India, 1992)

Capparis divaricata Lam is reported to be used as antiseptic, in asthma and post-delivery complaints in tribal medicine (Sandeep et al 2011). Leaves are used as anthelmintic, analgesic and aphrodisiac (Reddy et al. 2019). Bark paste is used to cure dysentery, stomach problems. Leaf juice with milk is used in infertility, stomach problems and as analgesic aphrodisiac, diuretic, antiulcer (Gunasekaran and Balasubramanian, 2012). Evaluation of the locomotor and diuretic activities of ethanolic extract of leaves of *C. divaricata* leaves reported for its significant locomotor and diuretic activity (Kondavar et al. 2011). Evaluation of analgesic activity of ethanolic leaf extract of *C. divaricata* showed significant activity at the dosage of 250 mg/kg (Rajamanya et al. 2012) and antipyretic activity (Khandare et al. 2012). A study of Anticancer Activity on MCF-7 cell lines of *C.divaricata* leaves revealed that Chloroform and ethyl acetate extracts showed significant cytotoxic activity (Hirave and Kondavar 2016). GC-MS studies of *C. divaricata* leaf (on which Part) revealed the presence of various bio-active compounds (Jacintha et al. 2017). Preliminary Phytochemical screening and in vitro antioxidant activities in all parts revealed the presence of phenols, flavonoids and tannins and antioxidant efficacy (Siva et al.). However, the present investigation has focused on phytochemical studies, quantification of total phenols and total antioxidants in addition to free radical scavenging studies of *C. divaricata* ethanolic and aqueous extracts of dried fruit.

Fig. 1: *Capparis divaricata* Lam. Fruit

II. MATERIALS AND METHODS

2.1. Collection and Identification of Plant material: *Capparis divaricata*, was collected from Ardhagiri hills of Chittoor District. The herbarium specimen (MHL 314) was prepared as per the method of Jain and Rao (1983) and deposited in the Department of Botany, S.V. University Tirupati. Provisional identification of voucher specimen was done by Prof. N. Yasodamma, Department of Botany, S. V. University, Tirupati with the help of Flora of the presidency of madras, Flora of Andhra Pradesh and Flora of Chittoor district. Identification is confirmed after comparing authentic specimens in Herbarium, Department of Botany, S. V. University, Tirupati.

Brief description of plant: Small tree, with spreading branches. Bark is rough brown. Leaves are elliptic, lanceolate, Obtuse, mucronate 1-2 inch long, 0.25-1 inch wide, thick leathery. Flowers are light green, creamy yellow, 2-2.5 cm across, solitary or 2-3 in clusters, Sepals 4, ovate. Petals are oblong, rounded at the tip, velvety. Stamens are many, long and filamentous. gynophore 2 cm long, Fleshy dehiscent fruit, round 4x4 cm, 5-6 ribbed, tubercled, stalk of the fruit is beaked, Seeds many.

2.2 Preparation of the extract

The fruits of *C. divaricata* were collected from Ardhagiri Hills of Chittoor District, cut into small fragments, shade dried and made into coarse powder which was sieved with 40 mesh sieves to get uniform particle size. A weighed quantity (50 g) of selected parts of the plant powder was weighed and immersed in 500 ml of distilled ethanol and water respectively for 24 h and the extracts were filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness in a water bath carefully at 50°C. The extracts were stored in screw capped glass bottles in the refrigerator until further screening. The extracts were tested for Preliminary phytochemical screening of twelve groups of secondary metabolites, namely alkaloids, flavonoids, phenols, glycosides, tannins, steroids, lignins, saponins, terpenoids and anthocyanidins (Table 1) was done by the standard procedures prescribed by Harborne[17]

2.2 GC-MS analysis*:*

Five milligrams of each extract was taken and dissolved in methanol in a sterile clean test tube. The sample solution was filtered using 0.2 µm nylon membrane and the filtered sample solution was injected into the column for running GC-MS. The analysis was carried out on a Perkin-Elmer workstation, with model Clarus 680 GC coupled to a mass spectrometer (Perkin Elmer Technologies, Inc., Wilmington, DE). Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30m x 0.25 mm width film depth of 250 µm capillary tube was used under the following condition. The instrument has an oven with an initial temperature of 60 °C for 2 min and a ramp program which elevates from 10°C/min up to 300 °C, further 6 min isothermal hold. Helium (He) carrier gas was used, with flow rate split ratios of 10:1. One µl volume of sample was injected and the temperature of the injector was maintained to 260 °C. The mass detector conditions were as follows. Transfer line temperature was 240 °C and ion source temperature was 240°C. The ionization mode electron impact was at 70 eV and a scan time of 0.2 sec with a scan interval of 0.1 sec. The fragments analyzed were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library. An individual component was recognized with typical mass spectra from National Institute of Standards and Technology (NIST- 2008) libraries which is inbuilt by the software of the GC-MS system (Turbo Massver 5.4.2) and literature data. The individual phytochemicals present in the crude extract were separated by the gas chromatography column. An individual compound separated by GC enters the Mass Spectrum (MS) and gets ionized. The MS ionizing spectrum was recorded and compared to the MS spectrum of known compounds in the NIST library. Each compound was compared with a percentage score of reverse and forward spectrum. The MS spectrum displays the molecular weight of individual molecules accurately.

2.3. Quantification of Polyphenols

Total phenol content in the extracts was quantified using Folin - Ciocalteu reagent (Hagerman et al., 1998). 10 µl (100µg) of each extract was taken and added to 100 µl of Folin-Ciocalteu reagent and 300 µl of 20 % aqueous sodium carbonate solution, then the reaction mixture was made up to 1 ml. The reaction mixture was incubated in dark for 1hour and the absorbance was recorded at 725 nm. The total polyphenols in *C. divaricata* fruit extracts were calculated from the calibration curve prepared, in the same method with Gallic acid, a known polyphenol as a standard and expressed as mg standard was conducted equivalent/g of dry plant.

2.4 Antioxidant assays (Total Antioxidant Capacity):

For total antioxidant capacity assay, 0.1 ml of the extract (10 mg/ml) dissolved in water was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid,28mM sodium phosphate and 4mM ammonium molybdate). The tubes were

capped and incubated in a thermal block at 95 °C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid (Prieto et al., 1999).

2.5. Free Radical (1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity:

The free-radical scavenging activity of were measured by the decrease in absorbance of methanol solution of DPPH (Yaushisakono, 1978). A stock solution of DPPH (33 mg/L was prepared in methanol and 5 ml of this stock solution was added to 100µl of the *C. divaricata* fruit ethanol and aqueous solutions at concentrations in dose depending manner starting from 1 mg/ml. After 10 min, Absorbance was measured at 517 nm and compared with the ascorbic acid as a standard (1 mg/ml). Scavenging activity was expressed as the Percentage of inhibition. The percentage scavenging of DPPH, nitric oxide and hydrogen peroxide radicals with *C. divaricata* fruit ethanol and aqueous extracts and standard compounds were calculated using the following formula:

The inhibitory effect on the hydroxyl radicals was calculated as:

% DPPH scavenging capacity = (1 − absorbance of plant extract /Absorbance of DPPH solution) × 100

A C = absorbance of control (DPPH solution) and A S = absorbance of sample (Plant extract or Ascorbic acid) solution.

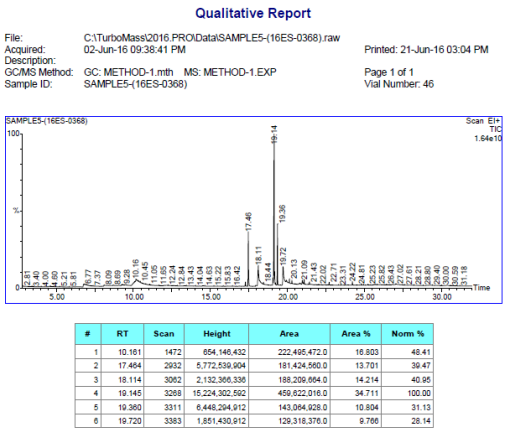
III RESULTS

3.1 Qualitative analysis of Phytochemicals of *C. divaricata* dried fruit ethanol and aqueous extracts

The preliminary screening of secondary metabolites with respect to the ***C. divaricata*** dried fruit ethanol and aqueous extracts resulted the presence of alkaloids, flavonoids, phenols, terpenoids, steroids, lignins, tannins, anthocynidins, cardiac glycosides and reducing sugars

Table 1: Preliminary phytochemical constituents of *C. divaricata* fruit extracts

S. No	Test	Aqueous extract	Ethanolic extract
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Phenols	+	+
4.	Terpenoids	+	+
5.	Steroids	+	+
6.	Anthocyanidins	+	+
7.	Anthraquinons	+	+
8.	Saponins	+	+
9.	Tannins	+	+
10.	Lignins	+	+
11.	Glycosides	+	+
12.	Reducing sugars	+	+
	Total	12	12



#	RT	Scan	Height	Area	Area %	Norm %
1	10.161	1472	654,146,432	222,495,472.0	16.803	48.41
2	17.464	2932	5,772,539,904	181,424,560.0	13.701	39.47
3	18.114	3062	2,132,366,336	188,209,664.0	14.214	40.95
4	19.145	3288	15,224,302,592	459,622,016.0	34.711	100.00
5	19.360	3311	6,448,294,912	143,064,928.0	10.804	31.13
6	19.720	3383	1,851,430,912	129,318,376.0	9.766	28.14

Fig. 2: Area % of GCMS analysis of ethanolic dried fruit extract of *C. divaricata*

3.2. GC-MS chromatogram of *C. divaricata* dried fruit extract revealed 6 peaks (Fig.2) indicating 6 phytochemical constituents. The major compounds identified with their retention time, molecular formula, molecular weight and peak area are presented are 1-Methyl-Pyrrolidine-2-Carboxylic Acid, Pentadecanoic Acid, 14-Methyl-, Methyl Ester, N-Hexadecanoic Acid, 11-Octadecenoic Acid, Methyl Ester, Heptadecanoic Acid, 16-Methyl-, Methyl Ester and Oleic Acid (Table.2). The biological activities of the identified compound were described in the Table 3. as per the Dr, Dukes ethno-botanical database.

Table 2: Chemical constituents of ethanolic dried fruit extract of *C. divaricata*

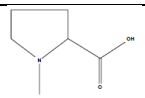
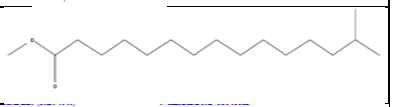
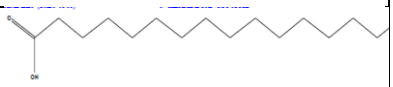
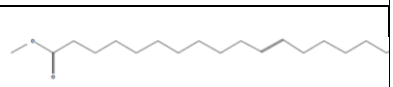
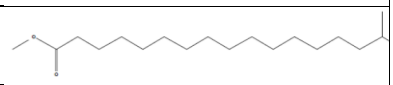
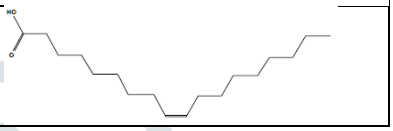
S. No	RT	Area%	Name of the compound	Probable Structure
1	10.161	16.803	Name of the compound	
2	17.464	13.701	1-Methyl-Pyrrolidine-2-Carboxylic Acid	
3	18.114	14.214	Pentadecanoic Acid, 14-Methyl-, Methyl Ester	
4	19.145	34.711	N- Hexadecanoic Acid	
5	19.360	10.804	11- Octadecanoic Acid, Methyl Ester	
6	19.720	9.766	Heptadecanoic Acid, 16-Methyl-, Methyl Ester	

Table 3: Assumed Biological activities of constituents of ethanolic extract of *C. divaricata* dried fruit

S. No	RT	Name of the compound	Biological Activity
1	10.161	1-Methyl-Pyrrolidine-2-Carboxylic Acid	Used in the formulation of drugs by both oral and transdermal delivery routes
2	17.464	Pentadecanoic Acid, 14-Methyl-, Methyl Ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, antiandrogenic, Hemolytic, 5-alpha reductase inhibitor which has antifngal and antimicrobial activities (Vijisaral and Arumugam 2014), (Akpuaka et al. 2013),
3	18.114	N- Hexadecanoic Acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-α reductase inhibitor, Cancer prevention agent, hypocholesterolemic, nematicide, pesticide, hostile to androgenic flavor, hemolytic, 5-α reductase inhibitor (Sivalingam 2021).
4	9.145	11- Octadecanoic Acid, Methyl Ester	
5	19.360	Heptadecanoic Acid, 16-Methyl-, Methyl Ester	Antimicrobial, antioxidant, cancer preventive, anemeagenic, antiandrogenic
6	19.720	Oleic Acid	Antioxidant, hypocholestrerolemic, Antimicrobial, cancer preventive, anemiagenic and anti-androgenic activities. Insectifuge, Dermatitigenic

3.3. Total phenolic contents

The alcohol and aqueous dried fruit extracts of *C. divaricata* contain considerable amount of Phenols. The aqueous extract shown high total phenols which were estimated using gallic acid as a standard compound. The alcoholic and aqueous extracts showed 35 and 165 mg/g dry weight respectively. (Fig. 3)

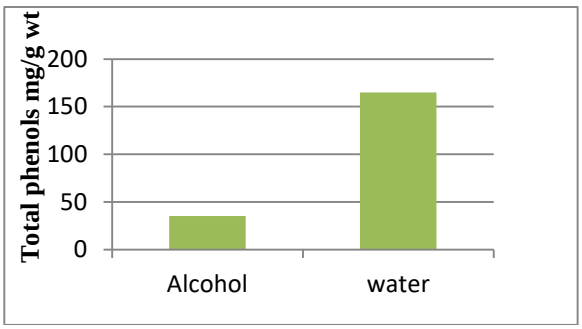


Fig 3: Total Phenols in dried fruit extract of *C. divaricate*

3.4 Antioxidant studies

The alcohol and aqueous dried fruit extracts of *C. divaricata* tested for the presence of total antioxidant activity. The aqueous extract shown high total phenols which were estimated using Ascorbic acid / Vitamin C as a standard. The alcoholic and aqueous extracts possessed quantity of 80 and 59 mg/gram dry weight respectively in ammonium molybdate reduction assay. (Fig. 4: Table 4.)

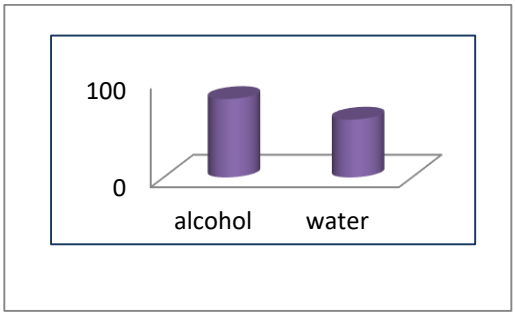


Fig 4: Total Antioxidants in dried fruit extract of *C. divaricata*

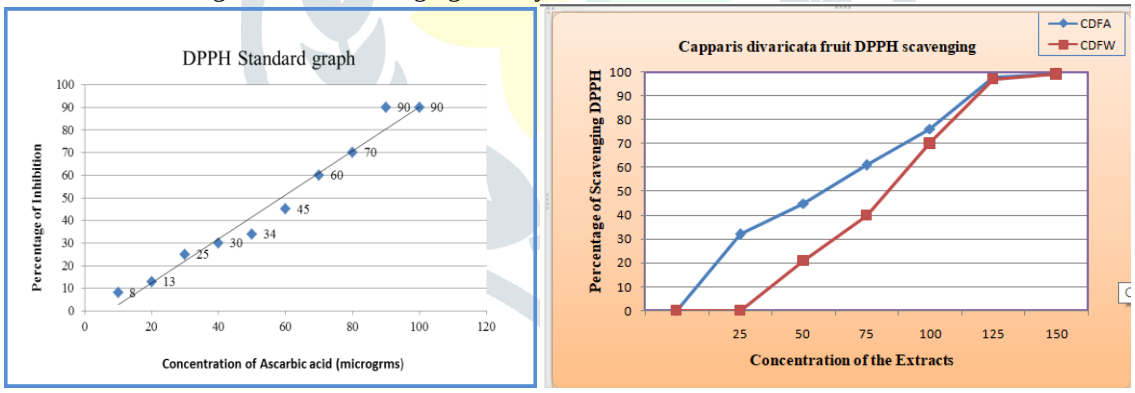
Table 4: Total Phenols, antioxidants and IC $_{50}$ values of DPPH Activity in dried fruit extract of *C. divaricata*

Name of the Plant	Plant part	Extract	Total Phenols of gallic acid equivalents in mg/ gram dry wt	Total antioxidants Ascorbic acid equivalents in mg/ gram dry wt	DPPH Scavenging Activity(IC50 in µg/ml)
Capparis divaricata	Fruit	Alcohol	35	80	60
		Water	165	59	82

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

The alcohol and aqueous dried fruit extracts of *C. divaricata* subjected to the scavenging capacity of DPPH a free radical commonly used in the testing of antioxidant activity. The tested extracts were exhibited strong scavenging activity based on the concentration and the IC 50 (Inhibition concentration of extracts) values were values were calculated as 60 and 82 µg/ml of alcohol and aqueous extracts respectively. Alcohol fruit extract showed DPPH scavenging which is equal to ascorbic acid standard. The amount of total phenols and total antioxidants are correlated with antioxidant potential (Table 4, fig. 5)

Fig 5. DPPH scavenging activity of dried fruit extract of C. divaricata



IV DISCUSSION

Antioxidants have been a topic of interest in the field of health due to their potential benefits in preventing various diseases. These compounds are known to neutralize harmful free radicals in the body, which are responsible for causing oxidative stress and damaging cells. This damage has been linked to chronic diseases such as cancer, heart disease and Alzheimer's besides the organ damage. Antioxidants can be found in many foods, such as fruits, vegetables, nuts, and grains, as well as in supplements. Studies have shown that a diet rich in antioxidants can help reduce the risk of these diseases and promote overall health. Additionally, antioxidants have been found to have anti-inflammatory properties, which can aid in reducing inflammation and preventing related diseases. However, more research is needed to fully understand the specific effects and benefits of antioxidants in different health conditions. Overall, incorporating antioxidants into one's diet and lifestyle may have a positive impact on overall health and wellbeing. In the present study the preliminary phytochemical screening of *C. divaricata* dried fruit extracts revealed the presence of the significant phyto-constituents like Alkaloids, steroids, lignins, flavonoids, phenols, glycosides, tannins, cardiac glycosides and reducing sugars. Methyl-esters and their derivatives cause Apoptosis and acts as anti-proliferative agents (Yu *et al*. 2005).

REFERENCES

[1] Akpuaka A, Ekwenchi MM, Dashak DA, Ahmed D. 2013. Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* (Neem) leaves. New York Sci J. 11:141-147

[2] Baker DD, Chu M, Oza U, Rajgarhia V. 2007. The value of natural products to future pharmaceutical discovery. Nat Prod Rep 24:1225–1244

[3] Cowan MM. 1999. Plant products as antimicrobial agents, Clinical Microbiology Reviews, 12(4):564–582.

[4] Gunasekaran, Balasubramanian. 2012. Ethnomedicinal uses of Sthalavrikshas (temple trees) in Tamil Nadu, Southern India, www.ethnobotanyjournal.org/vol.10: ISSN 1547-3465-10-253.

[5] Hagerman AE, Riedl KM, Jones, GA, Sovik KN. Ritchard NT, Hartzfeld PW, and Riechel TL. 1998. High molecular weight plant polyphenols (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry, 46(5):1887–1892.

[6] Harborne JB, Baxter, Herbert, Moss, Gerard P. 1999. eds., General Introduction Phytochemical dictionary a handbook of bioactive compounds from plants, 2nd ed. London: Taylor & Francis., p. vii. ISBN 9780203483756

[7] Hirave RV, Kondawar MS. 2016. Anticancer activity of *Capparis divaricate* Lam leaves extract. IRJP: 7(8):13-15.

[8] Jacintha TM, Sarvana G and Uma M. 2017. Phytochemical screening of leaf extracts of of *Capparis divaricata* Lam by GC MS analysis. IJAPSA, 132-138.

[9] Jain SK, Rao RR. 1976. A Hand Book of Field and Herbarium Methods. Today and Tomorrow's, Printers and Publishers, New Delhi.

[10] Jarvis B B. 2000. The role of Natural products in evolution. Recent Adv. Phytochem. 34:1-24.

[11] Kondavar MS. Kamble KG, Kandare MM, Maharshi KH and Avale VB. 2011. Evaluation of the Locomotor and Diuretic Activities of Ethanolic Extract of Leaves of *Capparis divaricata* Lam. IJPPS,3(4):265-267

[12] Mahendra K, Manish K, Sandeep P. 2012. Analgesic and antipyretic properties of *Capparis divaricata* Lam: May 2012, Journal of Pharmacy Research:, 5(5):2584-2586

[13] Prieto P, Pineda M and Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry, 269(2):337–341.

[14] Rajamanya TM, Kondawar MS, Mali NN. 2012. Analgesic activity of *Capparis divaricata* Lam. and Evaluation of Synergistic Activity of *Capparis divaricata* Lam. and *Caesalpinia bonducella* Linn, Journal of Pharmacy Research,5 (8):4259-4262.

[15] Reddy AM, Babu MVS. Rao. RR, 2019. Ethnobotanical study of traditional herbal plants used by local people of Seshachalam Biosphere Reserve in Eastern Ghats, India. Herba Polinica, 65(1):40-54.

[16] Sandeep BP, Nilofar SN, Chandrakanth SM and Vikas B. 2011. Some medicinal plants used by people of Sangli District, Maharashtra. A.J.P.Res,1(2):53-54

[17] Siva PK, Satish, Parvathi T, Armugam, 2021. A study on preliminary Phytochemical screening and antioxidant activities of *Capparis divaricata* L, IJBS, 6(4):235-238

[18] Sivalingam AM. 2021. Phytochemical Screening and GC-MS Analysis Of Bioactive Compounds Present In Ethanolic Leaf Extract *Murraya koenigii*. Bulletin of Engineering Geology and the Environment. 10:158-164.

[19] The Wealth of India: 1992, A dictionary of Indian Raw Materials and Industrial Products, Revised publication, New Delhi, 3:214-215.

[20] Vasu K, Goud J, Suryam A, Charya M, 2009. Biomolecular and phytochemical analyses of three aquatic angiosperms. African Journal of Microbiology Research. 3:418-421.

[21] Vijisaral E D, and Arumugam S. 2014. GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves. 6(8):294-300.

[22] Willis JC. 1988. A Dictionary of the Flowering Plants and Ferns. Eighth Edition. Cambridge Univ. Press, Cambridge.

[23] Yu FR., Lian XZ, Guo HY, Mc Guire PM, Li RD, Wang R, Yu FH. 2005. Isolation and characterisation of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the Human SGC-7901 cells. J. Pharm. Pharm. Sci. 8:528-535.