ejpmr, 2021,8(5), 417-422

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SCREENING OF SOLVENT EXTRACT OF *PSIDIUM CATTLEIANUM* LEAVES FOR ANTICANCER ACTIVITY AGAINST HEPATIC CANCER CELL LINE

Anusree K. V.[1]* and Dhivya R.[2]

[1]Student, PG and Research Department of Zoology, Nirmala College for Women, Coimbatore, Tamil Nadu.
[2]Assistant Professor, PG and Research Department of Zoology, Nirmala College for Women, Coimbatore, Tamil Nadu.

***Corresponding Author: Anusree K. V.**

Student, PG and Research Department of Zoology, Nirmala College for Women, Coimbatore, Tamil Nadu.

Article Received on 02/03/2021 **Article Revised on 21/03/2021** **Article Accepted on 11/04/2021**

ABSTRACT

Plants are used for medical functions since the start of human history and are the basis of modern medicine. From the dawn of ancient drugs, chemical compounds derived from plants are accustomed to treat human diseases. Natural sources have received increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents. In this view the present study was carried out to investigate the anticancer property of acetone extract from *Psidium cattleianum* leaves against Chang liver cell line by MTT assay, antioxidant activity by DPPH assay and phytochemical analysis was investigated by standard protocols. Results revealed that ethanol extract of *P. cattleianum* exhibited maximum anticancer activity against Chang liver cell line. A rapid evaluation for antioxidants using DPPH assay demonstrated that leaf extract had excellent free radical scavenging capacity. The phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins, tannins and quinones.

KEYWORDS: Anticancer activity; Antioxidant activity; DPPH Assay; Hepatic cancer cell line; MTT assay; Phytochemicals; *Psidium*; Solvent extracts.

INTRODUCTION

The International Agency for Research on Cancer estimates the incidence of mortality from major types of cancer, at national level. Within 5 years of diagnosis in 2012, worldwide 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer. By 2030, it was predicted that there will be 26 million new cancer cases and 17 million cancer deaths in a year.[1] Currently, cancer still remains an aggressive killer despite considerable efforts worldwide. Inspite of the development in synthetic chemotherapeutic agents that are in use we have not succeeded in fulfilling expectations, during the last decade. Therefore there is a constant demand to develop new, effective, and affordable anticancer drugs.[2]

The spreading of cancer is increasing worldwide, and the percentage of deaths caused by this fatal disease is rising, especially in the developing countries. Scientists and researchers are now giving more of their attention to the herbal medicine to provide treatment for more difficult diseases like cancer due to the fact that, the treatments of cancer patients with chemical therapy have serious side effects.[3] There are many studies that have proved the potential of plant-derived compounds as inhibitors of various stages of cancer and associated symptoms, underlining the importance of these products in cancer prevention and therapy. Nearly 60% of medicines recently used for cancer treatment have been isolated from plant based products as it has been the most significant source.[4]

Medicinal plants have been used from time immemorial in folk medicine as natural healing therapy with significant proven pharmaceutical effects in many areas including prevention of cardiovascular diseases and anticancer, anti-inflammatory and antimicrobial activity. In addition, the emergence of resistance to cancer chemotherapy has forced researchers to turn to natural products of plant and marine origin. Although many compounds isolated from plants are being rigorously tested for their anticancer properties, it is becoming increasingly recognized that the beneficial effects of plants are due to a complex interplay of the composite mixture of compounds present in the whole plant rather than constituent single agents alone.[5,6]

Phyto-constituents are the natural bioactive compounds found in plants. These phyto-constituents work with nutrients and fibres to form an integrated part of defence system against various diseases and stress conditions.[7] Antioxidants are the group of compounds with enormous interest for the biochemists and pharmaceutical industries. It is important for the innovation of the effectiveness of free radicals in a wide range of diseases,

which contributes a new way for health care systems.[8] Once the antioxidant defence becomes unstable by a sequence of factors physiological functions may deteriorate, as a result various ailments and aging can emerge. Though, in order to reduce the damage caused by oxidation, supply of antioxidants and antioxidants containing foods are required.[9] Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate.[10]

Many synthetic chemicals and drugs are available in market and they used to show above common side effects and it was observed that many plant products play an important role in the treatment of cancer and they are available in many Ayurvedic preparations in market with lower risk of above side effects.[11] Hence in the present research work an attempt has been made to extract active constituents from plant *Psidium cattleianum* and to investigate it for its anticancer activity against Chang liver cell lines. In order to investigate the anticancer activity of extract *in vitro* methods such as MTT (Methyl- Thiazolyl Tetrazolium) assay has been adopted. Apart from it different solvent extracts of leaves of *P. cattleianum* were qualitatively screened for testing the presence of phytochemicals using standard tests and antioxidant potential was analysed using DPPH Assay.

MATERIALS AND METHODS

Collections of test materials for cytotoxicity evaluation

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/360) at the Botanical Survey of India, Southern Circle, Coimbatore.

Preparation of leaf powder and extracts

Fresh leaves of *Psidium 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 using an electronic balance (Denver XS-210) and made into packets using Zerohaze filter paper. The powder was subjected to extraction using ethanol extract.[12,13]

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. *In vitro* cytotoxicity determination by MTT assay, antioxidant activity and phytochemical analysis of the ethanol extract of *Psidium cattleianum* leaves were investigated by the following protocols.

In vitro cytotoxicity determination by MTT assay

Chang liver cell lines were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium Himedia). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (Galaxy® 170 Eppendorf, Germany). Following the MTT assay method, the viability of cells was evaluated by direct observation of cells by using the Inverted phase contrast microscope.

Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x104 cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of Plant Extract and Compound Stock

Plant extract (1 mg) was added to DMEM (1ml) and dissolved completely with the help of cyclomixer. Then the extract solution was filtered through 0.22 µm Millipore syringe filter to confirm the sterility.

Cytotoxicity Evaluation

After 24 hours the growth medium was removed, freshly prepared samples in 5% DMEM were five times serially diluted by two fold dilution (6.25µg, 12.5µg, 25µg, 50µg, 100µg in 100µl of 5% MEM). Each concentration of 100µl was then added in triplicates to the respective wells and was incubated at 37ºC in a humidified 5% CO_2 incubator.

Cytotoxicity effect by Direct Microscopic observation

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Labomed TCM-400 with MICAPSTM HD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity effect by MTT Method

15 mg of MTT (Himedia, M-5655) was mixed in 3 ml PBS until completely dissolved and was sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. When the incubation period was completed, the supernatant was removed and 100µl of MTT Solubilisation Solution was added and the wells were mixed gently by pipetting up and down in order to solubilise the formazan crystals. After that, absorbance values were measured using micro plate reader at a wavelength of 570 nm.[14]

The percentage of growth inhibition was calculated using the formula:
% viability = Mean of sample x 100/Mean OD of control group

Antioxidant activity of plant extracts
DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay
The antioxidant activity of different extracts was ascertained using DPPH assay according to the method of Chang *et al*.,[15] 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.

Reagent Preparation
0.1 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by dissolving 4 mg of DPPH in 100ml of methanol.

Procedure
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 secondary metabolites in leaf extract of the plant was carried out using the standard protocols.

Test for Alkaloids
Mayer's test[16]: 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[17]: 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[18]: The extract (50mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for theformation of dark green colour.

Lead acetate test[19,20]: 50 mg of the extract was mixed in 5 ml of distilled water and 3 ml of 10% lead acetate solution was also added and further it was observed for the formation of bulky white precipitate.

Test for Tannins
Ferric chloride test[21]: Nearly 0.5 g solvent extract was dissolved with about 10 ml of distilled water and was then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green orblue-green precipitate.

Test for Flavonoids
NaOH test[21]: 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. Yellow colour changes to colourless on addition of dilute hydrochloric acid and its an indication for the presence of flavonoids.

Lead acetate test[19,20]: 50 mg of test extract was stirred with few drops of lead acetate solution taken in a test tube and observed for yellow coloured precipitate.

Test for Glycosides
H_2SO_4 test[22]**:** 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[23]: 50 mg of the solvent extract of leaf was dissolved in ethanol. To this 1 ml of acetic anhydride was mixed followed by the addition of Conc. H_2SO_4. Pink to violet colour change showed the presence of terpenoids.

Test for Saponins
Foam Test: 50 mg of the test extract or test powder was dissolved with distilled water and made up to 20 ml. It was vigorously shaken in a graduated cylinder for 15 minutes and noted for the formation of 2 cm layer thick foam.

Test for Quinones
H_2SO_4 test[20]: In 1 ml of solvent extract add 1 ml of Conc. H_2SO_4 and observed for the formation of red colour.
HCl test[24,25]**:** To 1 ml of the extract 5 ml of HCl and observed for the presence of yellow colour precipitate.

RESULTS AND DICUSSION
Anticancer Activity of *Psidium cattleianum* Leaf Extracts
MTT assay is a rapid and high accuracy colorimetric approach that is widely used to determine cell growth and cell cytotoxicity, particularly in the development of new drug. In the present study ethanol extract of *Psidium cattleianum* leaves were tested for cytotoxicity against Chang (liver cancer cell line). Results showed that ethanol extract inhibited the viability of Chang liver cells (Table 1).

Table 1: Anticancer Activity of *Psidium cattleianum* Leaf Extracts.

Concentration (µg/ml)	Percentage viability	IC_{50} (µg/ml)
6.25	91.76	
12.5	78.82	
25	69.41	
50	45.88	
100	27.05	60.89

At the concentration of 100µg/ml cell viability percentage was noted as 27.05%. Cytotoxicity percentage at the concentrations of 12.5 µg/ml and 6.25 µg/ml was observed to be 78.82% and 91.76% cell viability. Percentage viability values of cells were found to reduce with increase in concentration. In parallel to this Azurah *et al*[26] investigated cytotoxicity of aqueous and ethanol extracts of *Ficus deltoidea* on Human Ovarian Carcinoma Cell Line. From this it was found that both ethanol and aqueous extract showed a significant reduction in the number of viable cells at the concentration higher than 250µg/ml.

Jayachandran *et al*[27] screened *Tecoma stans* plant for anticancer property. The results of MTT assay showed that the methanol extract of *T. stans* had high cytotoxic activity against the liver cancer cell line which was similar to that of present study. Branislav *et al*[28] studied on lichen for their anticancer property. The acetone extracts of studied lichen induced significant cytotoxic effect on the tested cancer cell lines. Bezivin *et al*[29] documented significant anticancer activity of *Parmelia caperata*, *Cladonia convoluta*, *Cladonia rangiformis, Platisma glauca* and *Ramalina cuspidata.* Manojlovic *et al*[30] reported anticancer potential of *Thamnolia vermicularis*. Triggiani *et al*[31] declared strong anticancer activity for *Xanthoria parietina.*

In the present study cytotoxicity percentage at 50 µg/ml and 25µg/ml of ethanol extract of *Psidium cattleianum* leaves were recorded as 45.88% and 69.41% respectively. In parallel to the present study, Jitendra and Vaibhavi[32] observed some plants anticancer property. Screening of methanol extracts of *Colocasia esculenta, Costus speciosus, Curcuma aromatic, Solanum nigrum, Swertia angustifolia* and *Rubia cordifolia* resulted in moderate anticancer activities against MCF-7 and HT-29 cell lines. Shridhar *et al*[33] reported that MTT assays revealed the methanol and ethanol extract of *L. indica* leaves was found to induce more cytotoxicity towards cancer cell lines DU-145 and PC-3.

Antioxidant activity of Psidium cattleianum Leaf Extracts

In the present work, highest radical scavenging activity was found in the leaves extract of *P. cattleianum*. The results of antioxidant activity of *P. cattleianum* leaf extracts were displayed in Table 2. Ethanol extract of *Psidium cattleianum* showed maximum percentage of radical scavenging at concentration of 250 µl i.e., 11.33% and minimum antioxidant activity was noted at the lowest test concentration of 50 µl (58.73%). Similar result was reported by Rohita and Saroj[34] by evaluating antioxidant potential of some common weeds of agriculture fields of Punjab plains. The result showed that highest radical scavenging activity was found in the leaves extract of *S. marianum*, *L. procumbens* and root extracts of *S. nigrum*.

Table 2: Antioxidant activity of *Psidium cattleianum* Leaf Extracts.

Test Concentrations (µl/ml)	Radical Scavenging Activity (% inhibition)	
	Standard	Ethanol
50	55.38	58.73
100	32.44	34.95
150	19.62	21.35
200	14.73	17.67
250	9.45	11.33

Quercetin Standard (1mg/ml)

Karnan and Subramani[35] studied antioxidant activity of some medicinal plants in South India. The DDPH method revealed that the scavenging of the free radicals was found to be highest for the ethanol extract of *Merremia gangetica* followed by *Cicca acida* and *Solanum nigrum* respectively. Sharareh *et al*[36] screened antioxidant properties of several medicinal plants such as, *Mentha piperita, Berberis integerrima, Berberis vulgaris, Melissa officinalis, Artemisia absinthium, Salvia officinalis,* and *Foenciculum vulgare.* Results showed that all samples had significant levels of radical scavenging activity in a dose dependent manner.

Phytochemical Analysis of *Psidium cattleianum* Leaf Extracts

The secondary metabolites that are present in the ethanol extracts of selected plant *Psidium cattleianum* were investigated. The results of phytochemical screening were summarized in Table 3.

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

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

"+" Presence, "-"Absence

Phytochemical screening of ethanol extracts of *P. cattleianum* leaves indicated the presence of phytochemicals like phenols, flavonoids, tannins, saponins, alkaloids, glycosides and quinones. The presence of these secondary metabolites showed the pharmacological potential of the selected *P. cattleianum* leaf extracts.

In accordance to the present study, Kannamba *et al*[37] has conducted a study on effect of extraction methods and solvent on phytochemical composition of medicinal plant extracts. The result of phytochemical screening of ethanol extracts of *Tridax procumbens*, *Murraya koenigii* and *Pongamia pinnata* leaves revealed the presence of alkaloids, phenolics, tannins, flavonoids and diterpenoids. In concordance to this in the present investigation seven secondary metabolites such as phenols, alkaloids, flavonoids, tannins, saponins, glycosides and quinones were found in the ethanol extract of *Psidium cattleianum* leaves. Kalaivani *et al*[38] studied phytochemical screening, HPTLC fingerprinting and *in vitro* antioxidant activity of root extract of *Asparagus racemosus*. The result showed the presence of Flavonoids, Phytosterols, Proteins, Tannins, Glycosides and Carbohydrates in the phytochemical screening of ethanol extract of *A. racemosus*.

Suhas *et al*[39] stated that phytochemical screening of the extracts of stem bark and root bark of *Zizyphus mauritiana* and revealed the presence of various phytochemicals of which alkaloids; flavonoids and tannins were the most prominent. Ismaili *et al.*,[40] reported many naturally occurring triterpenoids isolated from various parts of plants have been to be good anti-inflammatory compounds. Dhawale[41] screened eight plants viz, *Abutilon indicum*, *Euphrbia hirta*, *Ficus hispida*, *Melia azedarch*, *Phyllathus reticulatus*, *Psidium guajava*, *Vitex negundo* and *Vitex pinnata.* The result revealed presence of alkaloids, flavonoids, steroids, terpenoids.

CONCLUSION

Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory, and anticancer activities. Therefore, many plants have been examined to identify new and effective antioxidants and anticancer compounds, as well as to elucidate the mechanisms of cancer prevention and apoptosis. The present study suggests that the *P. cattleianum* leaf extracts most likely have anticancer properties. The findings also clearly indicated that ethanol extract of *P. cattleianum* leaf possessed significant antioxidant and a good source of various phytoconstituents. The qualitative analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, glycosides and quinines in *P. cattleianum* leaves, which may contribute to their medicinal properties. Further studies of this plant species should be directed to find out medicinally active components in order to prepare high valuable natural pharmaceutical.

REFERENCES

1. Thun MJ, Lancey JODe, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. Carcinogenesis, 2009; 31(1): 100–110.
2. Coseri S. Natural products and their analogues as efficient anticancer drugs. Mini-Reviews in Medicinal Chemistry, 2009; 9(5): 560–571.
3. Ahmad R, Ahmad N, Naqvi AA, Shehzad A, Al-Ghamdi MS. Role of traditional Islamic and Arabic plants in cancer therapy. J. Tradit Complement Med, 2017; 7(2): 195-204.
4. Gordaliza M. Natural products as leads to anticancer drugs. Clinical and Translational Oncology, 2007; 9(12): 767–776.
5. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. American Journal of Clinical Nutrition, 2003; 78(3): 517–520.
6. Karna P, Chagani S, Gundala SR. Benefits of whole ginger extract in prostate cancer. British Journal of Nutrition, 2012; 107(4): 473–484.
7. Amrit PS. Promising phytochemicals from Indian medicinal plants. Ethnobotonicals Leaflets, 2005; 9(1): 15-23, 2005.
8. Niki E, Shimaskiand H, Mino M. Antioxidantism-Free Radical and Biological Defence. Gakkai Syuppan Center, Tokyo, 1994.
9. Halliwell B, Gutteridge JM. Hydrogen peroxide scavenging, antioxidant and anti-radial activity of some phenolic acids. Oxford University Press USA, 2003.
10. Halliwell B. Biochemistry of oxidative stress. BiochemSoc Trans, 2007; 35: 1147-1150.
11. Nikita NK, Sambrekar SN, Suhas AP, Vishnu AK. Anticancer activity of whole plant of *Amaranthus tricolor* Linn. on breast cancer cell lines. Journal of Chemical and Pharmaceutical Research, 2018; 10(7): 63-69.
12. Harbourne JB. Phytochemical Methods. Chapman and Hall, Ltd, London, 1973; 49-188.
13. Vogel AI. Text book of practical organic chemistry. The English language book society and Longman, London, 1978; 1368.
14. Laura BT, Rosiane GMZ, Paula CSF, Luis AS, Maria ERD, Miguel DN, Carlos AP, Elsa BD. Anti-herpes simplex virus activity of sulphated galactans from the re seaweeds *Gymnogongrus griffithsiae* and *Cryptonemia crenulate*. International Journal of Biological Macromolecules, 2004; 34(1-2): 63-71.
15. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. Journal of Agricultural and Food Chemistry, 2011; 4(9): 3420–3424.
16. Evans WC. Trease and Evans Pharmacology. 14th Edn. Harcourt Brace and company. Asia. Pvt. Ltd. Singapore, 1997.
17. Wagner H. Pharmazeutische Biology 5th edn. AUFI. 15 BN 3-437-20 498-X. Gustav Fisher Vwelag. Stuttgart. Germany, 1993.
18. Mace ME. Histochemical localization of phenols in healthy and diseased tomato roots. Phytopathology, 1963; 16: 915-925.
19. Brain KR and Turner TD. Wright Scien Technica. 1st Edn. Bristol: Practical Evaluation of Phyto Pharmaceuticals, 1975; 144.

20. Evans WC. Pharmacognosy 14th Edn., WB Saunders Co. Ltd. Singapore, 1996; 9: 713-734.
21. Trease GE, Evans WC, Pharmacognosy 15th Ed., London*:* Saunders Publishers, 2002.
22. Prakash M, Avetik M, Venkata Sai PC, Mary C, Rafael SM, Siddhartha P, Marquita J. Methods for purifying steviol glycosides and uses of the same. US Patent 9, 2015; 169: 285.
23. Sofowora A. Medicinal plants and traditional medicinal in Africa. Screening Plants for bioactive Agents, 2nd ed., Sunshine House, Spectrum Books Ltd. Nigeria, Ibadan, 1993; 134–156.
24. Kokate CK, Practical Pharmacognosy, Vallabh Prakashan, Delhi, 2000; 107-111.
25. Harborne JB, Phytochemical methods, London. Chapman and Hall, Ltd, 1973; 49-188.
26. Azurah MAN, Lee SC, Fadzilah AAM, Mohamad RS. Cytotoxicity of aqueous and ethanolic extracts of *Ficus deltoidea* on human ovarian carcinoma cell line. British Journal of Medicine and Medical Research, 2011; 1(4): 397-409.
27. Jayachandran PR, Kumaresan S, Ramasamy S, Ponnusamy P. Antioxidant and cytotoxic activity of *Tecoma stans* against lung cancer cell line (A549). Brazilian Journal of Pharmaceutical Sciences, 2017; 53(3): e00204.
28. Branislav RR, Marijana MK, Tatjana PS. Antioxidant, antimicrobial and anticancer activity of the lichens *Cladonia furcata*, *Lecano raatra* and *Lecano ramuralis.* BMC Complementary and Alternative Medicine, 2011; 11: 97.
29. Bezivin C, Tomasi S, Lohezicand LDF, Boustie C. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. Phytomed, 2003; 10: 499-503.
30. Manojlovic N, Vasiljevic P, Juskovic M, Najman S, Jankovic S, Milenkovic AA. HPLC analysis and cytotoxic potential of extracts from the lichen, *Thamnolia vermicularis* var. Subuliformis. J Med Plants Res, 2010; 4(9): 817-823.
31. Triggiani D, Ceccarelli D, Tiezzi A, Pisani T, Munzi S, Gaggi C, Loppi S. Antiproliferative activity of lichen extracts on murine myeloma cells. Biologia, 2009; 64: 59-6.
32. Jitendra M, Vaibhavi J. Anticancer activity evaluation and some Indian medicinal plants from Amarkantak Mekal Plateau Madhya Pradesh, India. Int. J. Curr. Microbiol. App. Sci, 2016; 11(5): 478-483.
33. Shridhar CG, Sridevi IP, Vijay MK, Rajendra BN, Sunil SJ, Murigendra B, Hirematha, Shivayogeeswar, Neelagund, Ravindranath A. *In vitro* antioxidant and anticancer activity of *Leea indica* leaf extracts on human prostate cancer cell lines. Integrative Medicine Research, 2017; 6(1): 79-87.
34. Rohita S and Saroj KP. Antioxidant potential of some common weeds of agriculture fields of Punjab plains. *Journal of Pharmacognosy and Phytochemistry*, 2019; 8(2): 06-09.
35. Karnan R, Subramani K. Antioxidant properties of certain South Indian medicinal plants, Journal of Medicinal Plants Studies, 2015; 3(2): 58-60.
36. Sharareh R, Hamid RP, Javad J, Antioxidant properties of several medicinal plants growing wild in northeastern Iran. Asian Journal of Plant Science and Research, 2015; 5(2): 63-68.
37. Kannamba B, Winnie TD, Surekha M, Lavanya B. Effect of extraction methods and solvent on phytochemical composition of medicinal plant extracts. Der Pharma Chemica, 2017; 9(9): 152-156.
38. Kalaivani S, Girija S, Aruthra AP, Vishnu PV, Srinivasa RB, Geetha RV, Gayathri R, Joel PJ, Janardhana PB. Phytochemical screening, HPTLC fingerprinting and *in vitro* antioxidant activity of root extract of *Asparagus racemosus*. Pharmacogn J, 2019; 11(4): 818-823.
39. Suhas AT, Arti MB, Mandakini BP. Phytochemical analysis of stem bark and root bark of *Zizyphus mauritiana*. International Journal of Innovative Science, Engineering & Technology, 2014; 1(4): 526-535.
40. Ismaili H, Sosa S, Brkic D, Fkih TS, Ilidrissi A, Touati D, Aquino RP, Tubaro A. Topical anti–inflammatory activity of extracts and compounds from *Thymus broussonettii*. J Pharm Pharmacol, 2002; 5(4): 1137– 1140.
41. Dhawale PG, Phytochemical analysis of eight medicinal plants from Amravati district (MS) India. International Journal of Scientific and Research Publications, 2013; 3(1): 1-3.