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EVALUATION OF PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND *IN VITRO* CYTOTOXIC STUDIES OF AN ETHNO THERAPEUTICALLY IMPORTANT TREE, *GLIRICIDIA SEPIUM* (JACQ.) STEUD. (FABACEAE)

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ABSTRACT

Gliricidia sepium, a leguminous plant rich in alkaloids, phenols and flavonoids, is reported to be expectorant, sedative and suppurative. *Gliricidia sepium* is a multipurpose tree and the entire parts of tree- roots, leaves, bark and flowers have ethno medicinal properties. The present study is focused to investigate the phytochemical, antioxidant properties and cytotoxic studies of methanolic leaf extract of the plant. The preliminary phytochemical study was carried out using standard procedures. The quantitative analysis revealed the presence of alkaloids in high amounts followed by flavonoids and glycosides. The antioxidant activity of methanolic leaf extract of *G. sepium* was determined by following radical scavenging assay namely DPPH and ABTS radical scavenging assay. In DPPH assay, the percentage of inhibition was found to be 44.18 % at 60 mg/L. In ABTS radical scavenging activity, the percentage of inhibition was found to be 32.57 % at 60 mg/L. The cytotoxic studies of methanolic leaf extract against human breast cancer cell lines revealed that the plant is selectively cytotoxic and the IC_{50} values of extract against the human cancer cell line was calculated as 28 ± 0.5 µg/ml.

Keywords: *Gliricidia sepium*, phytochemical analysis, antioxidant, cytotoxic, MCF-7.

INTRODUCTION

From time immemorial plants have been used as source of medicine for treating human diseases. As per World Health Organization (WHO) estimates, nearly 80 percent of the population of developing countries depend on traditional medicines, mostly plant drugs for their primary health care needs (Srivastava *et al.*, 1995). Phytochemicals are biologically active substances produced by plants. On the basis of disease preventing function phytochemicals are of different classes such as antioxidants, detoxifying agents, dietary fiber, immunity potentiating agents anticancer, and neuropharmacological agents.The present study focus on the medicinal plant, *Gliricidia sepium* belongs to Fabaceae family. It is a medium sized multi-purpose legume tree, native to Central and South America. The study plant, *Gliricidia sepium* is reported to be insecticidal, rodenticidal, expectorant, sedative, suppurative and folk remedy for alopecia, cough, colds, boils,bruises,fever,fractures. The crude extracts have been shown to have antifungal and antibacterial activity. In the present study, methanolic extract of the plant is evaluated for phytochemical, antioxidant and cytotoxic activities.

MATERIALS AND METHODS

The fully matured fresh leaves of *G. sepium* were collected from santhigiri area in kannur district, Kerala. The leaves were washed thoroughly, shade dried and finely powdered. 10g of dried leaf powder were subjected to hydroalcoholic (methanol) extraction using Soxhlet apparatus at the temperature of 70°C for 24hrs.

PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of the leaf extract is carried out and bioactive compounds are determined by the following phytochemical test by the method of Harborne, 1984, wagner *et al.* 1984 and Sthal *et al*., 1965.

QUALITATIVE ANALYSIS

The plant extract was tested for phytochemicals using the following tests- Test for alkaloids, flavanoid, glycoside, polyphenol and tannin.

TEST FOR ALKALOIDS

2ml of extract was acidified with a few drops of dilute hydrochloric acid. Then 1ml of Dragendroff's reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

TEST FOR FLAVONOIDS

4ml of extract solution was treated with 1.5ml of methanol solution. The solution was warmed, and metal magnesium was added to this solution 5-6 drops of Con. HCl acid were added, and colour was observed for flavonoids and orange colour for flavones.

TEST FOR GLYCOSIDES

To 1ml of each extract a few drops of glacial acetic acid and ferric chloride and 3-4 drops of concentration sulphuric acid were added. The appearance of blue-green color indicates the presence of glycosides.

TEST FOR PHENOLS

1ml of plant extract with few drops of dilute iodine solution gives a transient red colour which indicates the presence of phenol content.

TEST FOR TANNINS

To 2ml of each extract a few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

QUANTITATIVE ANALYSIS

The plant extract was tested for phytochemicals using the following tests- Test for alkaloids, flavanoid, glycoside, polyphenol and tannin.

DETERMINATION OF TOTAL ALKALOID CONTENT (Harborne method, 1973)

5g sample is treated with 30ml 10% Glacial Acetic acid is covered and allow to stand for 5 hours. Sample is filtered, concentrate on water bath to get 1/4 of its original volume. Then add 10ml concentrated Ammonium hydroxide dropwise with continues stirring until the precipitate was complete. All the solution can settle. Collect the precipitate and washed with diluted Ammonium hydroxide (5ml ammonium hydroxide + 5ml water) and the filtered through a pre-weighed filter paper. The residue was dried and weighed.

DETERMINATION OF TOTAL FLAVONOID CONTENT (Bohm and Kocipai-Abyazan method, 1994)

5g sample is treated with 30ml 80% methanol. Cover and allowed to stand for 2 hours. Whole solution was filtered through the Whatman filter paper No:42. The filtrate was transferred into a crucible(pre-weighed) and evaporated into dryness and weighed to a constant weight.

DETERMINATION OF TOTAL GLYCOSIDE CONTENT (Balget's test, 1981)

1ml of sample is treated with 1ml freshly prepared Balget's reagent (95ml 1% Picric acid + 5ml 10% NaOH). Incubated for one hour. After incubation diluted with 10ml distilled water. And the absorbance was read at 495nm.

DETERMINATION OF TOTAL POLYPHENOL CONTENT (Folin-Ciocalteu method, 1927)

20µl Sample is treated with 6.980ml distilled water, 2ml Sodium carbonate (Na_2CO_3) and 0.8ml Folin's reagent. And is allowed for incubation at 2 hours. Then the optical density is measured at 765nm.The control contains 7ml distilled water, 2ml Na_2CO_3 and 0.8ml Folin's reagent.

DETERMINATION OF TOTAL TANNIN CONTENT (Folin-Ciocalteu method, 1927)

20µl Sample is added with 980 µl of distilled water and 4.5ml Na_2CO_3. Then it can stand for 10 minutes. The add 0.5ml Folin's reagent and a 30 minutes incubation. The optical density is measured at 725nm. The control is prepared by treating 1ml distilled water with 4.5ml Na_2CO_3. Then it can stand for 10 minutes. The add 0.5ml Folin's reagent and a 30 minutes incubation.

ANTIOXIDANT ACTIVITY

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay (Blois – 1958)

The DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) assay have been widely used to determine the free radical-scavenging activity of various plants and pure compounds. DPPH is a stable free radical which dissolve in methanol or ethanol, and their colors show characteristic absorptions at 520 nm or 734 nm, respectively. Six varying concentrations (0, 5, 15, 30, 45 and 60 mg/L) of methanol solvent extract of *G.sepium* demonstrated different percentage of inhibition. Interestingly, scavenging activity of extract was increased in a concentration dependent manner. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Assay (Keesey – 1987)

ABTS is a stable free radical which dissolve in methanol or ethanol, and their colors show characteristic absorptions at 520 nm or 734 nm, respectively. The ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt)] assay have been widely used to determine the free radical-scavenging activity of various plants and pure compounds. Six varying concentrations (0, 5, 15, 30, 45 and 60 mg/L) of methanol solvent extract of *G.sepium* demonstrated different percentage of inhibition. The scavenging activity of extract was increased in a concentration dependent manner. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical.

CYTOTOXIC ACTIVITY AGAINST BREAST CANCER CELL LINE (MCF-7)

MTT Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Mosmann, 1983)

MCF-7 (Human Breast cancer) cell lines were cultured in 25 cm2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) 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% CO2 incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl 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% CO2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico *et al.*, 2004).

RESULTS AND DISCUSSION

Phytochemical study

The present study has been carried out to assess the phytochemical screening, antioxidant activity and cytotoxic activities of methanolic leaf extract of *Gliricidia sepium*. The preliminary phytochemical screening reveals the presence of alkaloids, flavonoids, glycosides, polyphenols and tannin (Table -1).The study revealed the quantitative details about the study plant. The quantitative analysis of the methalonic leaf extract of *G. sepium* reveals the presence of alkaloids in high amount followed by flavonoids and glycosides (Table – 2).

The results similarly with the studies of Neethu and Neethu Simon, 2016. They have explained the phytochemical investigation of *Gliricidia sepium* leaves in water, alcohol and chloroform extracts. The analysis revealed the presence of alkaloids, flavonoids, glycosides, oils, saponins, phenolics, gums and mucilage. In another study, Sankar Narayan Sinha, 2013 has studied the phytochemical profiles of *G. sepium* leaf, revealed the presence of considerable amount of saponin, phenol, alkaloids and flavonoids.

The present study revealed that the methanolic leaf extract of *G. sepium* was rich in secondary metabolites like alkaloids, flavanoids, glycosides, phenols and tannins.

TABLE. 1: SHOWS THE QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *Gliricidia sepium*

Sl.No.	PHYTOCHEMICALS	METHANOLIC EXTRACT OF *Gliricidia sepium*
1	Alkaloid	+
2	Flavonoid	+
3	Glycoside	+
4	Polyphenols	+
5	Tannin	+

TABLE. 2: SHOWS THE QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *Gliricidia sepium*

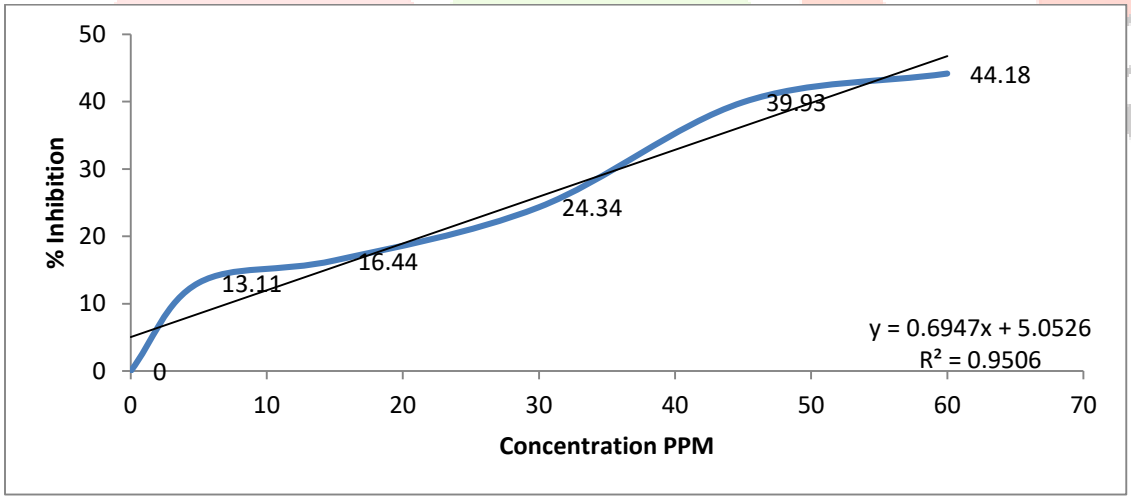
Sl.No.	PHYTOCHEMICALS	METHANOLIC EXTRACT (IN mg/ml)
1	Alkaloid	5.6
2	Flavonoid	2.4
3	Glycoside	1.726
4	Polyphenols	0.076
5	Tannin	0.441

Antioxidant activity

The antioxidant study reveals the free radical scavenging property of the methanolic extract of *Gliricidia sepium.* The DPPH and ABTS assays have been widely used for the assessment of free radical-scavenging activity of various natural products. Both DPPH and ABTS are stable free radicals which dissolve in methanol or ethanol, and their colors show characteristic absorptions at 520 nm or 734 nm, respectively.

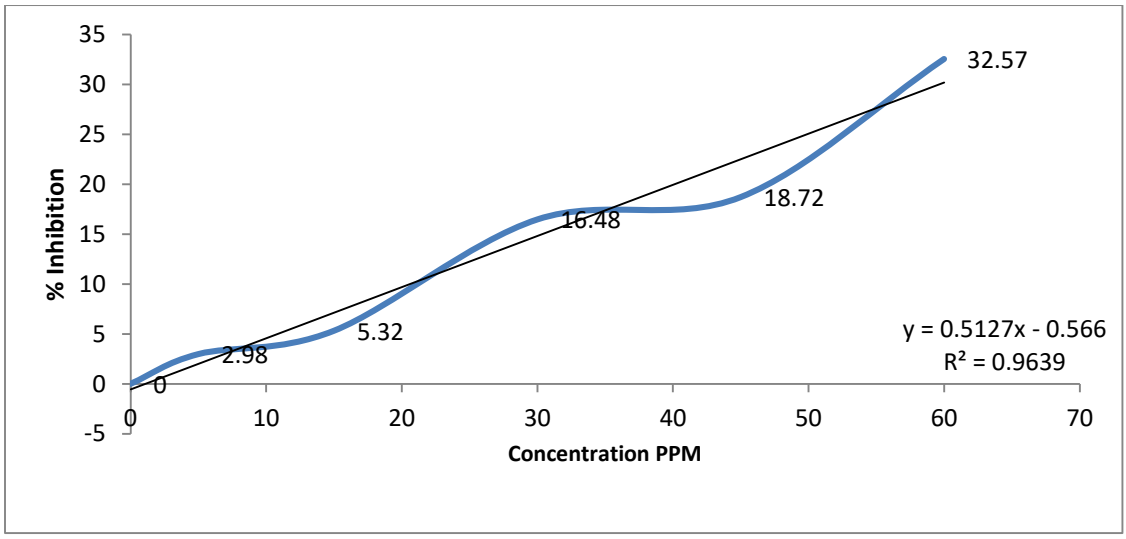
The methalonic plant extracts were most active and fully scavenged DPPH. The 60 mg/L extract showed 44.18% of inhibition in methanolic extract. The 5 mg/L extract showed lowest antioxidant activity and the scavenging activity. The concentration of the extract was increased with increase in activity. Linear regression equation is used for calculating the IC50 value of the free radical scavenging method, and the IC50 value was 64.70 mg/L (Fig -1).

Figure 1: Shows the radical scavenging activity of *Gliricidia sepium* represented by percentage of inhibition by DPPH



The methalonic plant extracts were most active and fully scavenged ABTS. The scavenging activity of extract was increased with increase in concentration. The plant extract showed high amount of ABTS radical scavenging activity in 98.63 mg/L concentration. The 5 mg/L extract showed lowest antioxidant activity. The 60 mg/L extract showed 32.57% of inhibition in methanolic extract. The results were expressed as trolox equivalence in mg/ L extract (Fig -2).

Figure 2: Shows the radical scavenging activity of *Gliricidia sepium* represented by percentage of inhibition by ABTS



The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. Thus, DPPH assay showed the highest antioxidant activity of the sample. The results gained for the antioxidant screening of plant exhibited that they had appreciable amount of bioactive components. The plant contained appreciable amount of metabolites like alkaloids, saponin, tannin, phenols and flavonoids. It also contained free radical scavenging and metal chelating activity which could have resulted to the inhibitory activity exhibited by plant extract.

The result similarly with the studies of Sankar Narayan Sinha, 2013 has explained the antioxidant activity of aqueous extract of *Gliricidia sepium.* The analysis revealed the free radical scavenging power and natural chelating property of the selected plant. In another study Kaisarun *et al*., 2016 analysed the activity of *Erythrina stricta* (Fabaceae) extract showed significant DPPH free radical scavenging activity. Jean *et al*., 2014 studied the antioxidant activities of acetone leaf extracts of nine under-investigated Fabaceae tree species such as *Baphia racemose, Crotalaria capensis, Dalbergia nitidula, Erythrina caffra, Indigofera cylindrica, Lonchocarpus nelsii, Podalyria calyptrata, Virgilia divaricata* and *Xylia torreana* using total phenolic content, ABTS radical assay and DPPH assay. From this study, they concluded that all the extracts had moderate to potent antioxidant activity.

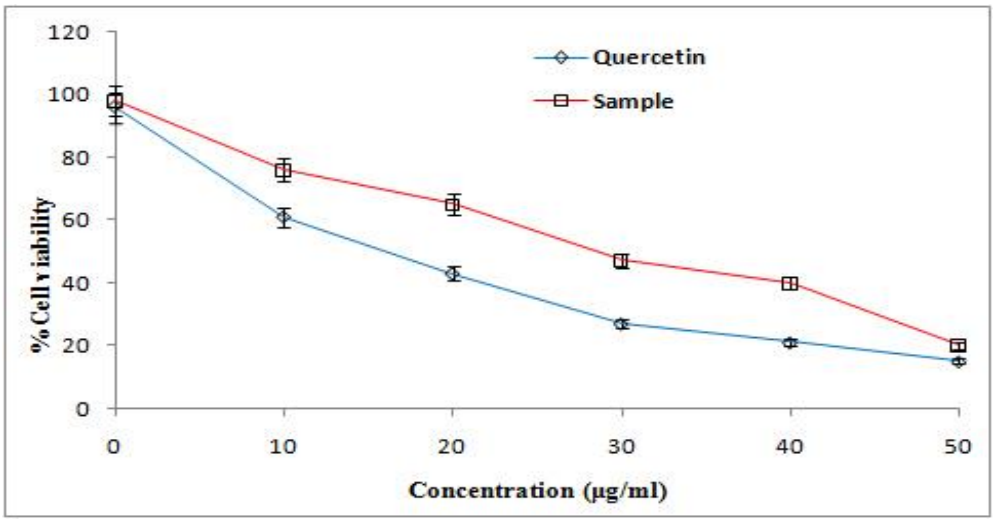
Stanley *et al*., 2013 investigated the antioxidant studies on roots of *Senna italica* (Fabaceae). They extracted the plant with various solvents using DPPH radical scavenging assay to determine its antioxidant activity. The result showed that, all the extracts showed significant antioxidant activity and methanolic extract had the highest antioxidant activity. Similarly, Senthil *et al*., 2005 studied the *in vitro* antioxidant activities of *Mucuna pruriens* seeds. From the result they concluded that, the methanolic extract of *M. pruriens* seeds showed strong antioxidant activity. Adetuyi *et al.*, 2012 has explained the antioxidant activities of the leaf extracts of *Gliricidia sepium*. The analysis revealed the significant antioxidant potential of DPPH scavenging activity in 48.7 mg/ml concentration.

The present study revealed the significant antioxidant activities of *G.sepium*. They evaluated both DPPH and ABTS assays to confirm the free radical scavenging activity.

Cytotoxic activity

The methalonic leaf extract of *Gliricidia sepium* has selectively cytotoxic to the Human breast cancer cell line (MCF-7). The experimental results demonstrate that various concentration of extract has the ability to inhibited cell proliferation in a dose dependent manner. The IC_{50} values of extract against human breast cancer cells were calculated as 28 ± 0.5 µg/ml. The control cells did not show any remarkable changes on their morphology. However, in the presence of extract the cells shows the improved cell shrinkage, membrane blebbing and forms floating cells in a dose-dependent manner. It is well accepted that cytological investigations elucidate the antiproliferative effect routed through membrane blebbing, membrane instability and distressing the cytoskeleton of the cells by the extract. The results showed that the methalonic extract of *G. sepium* has significant anticancer activity (Fig -3).

Fig 3: Shows cytotoxicity of methanolic extract of the Gliricidia sepium determined through MTT assay



Natalizia *et al.,* 2016 studied the role of flavonoid rich fraction in cytotoxic activities of *Bauhinia forficata* (Fabaceae) leaf extract. The study validate better potential of *B. forficata* on the development of anti-tumour drugs. The cytotoxic activity of ethanolic leaf extracts of *Gliricidia sepium* was considered. The result showed the presence of flavonoids, phenolics, tannins and terpenoids. The results indicate that the detected phytochemicals may account for the exhibited biological activities of *G. sepium.* The study concluded that the plant extract were potentially cytotoxic and may exhibit a potential anti - tumour activity (Emma *et al*., 2019).

Gomathi *et al*., 2020 reported that different phytochemicals in fabaceae family such as flavonoids, lectins, saponins and phenolic compounds have significant anticancer potential. Therefore, these phytomolecules are suitable ingredients for the development of new anticancer agents. Some of these compounds are excellent lead molecules and by making suitable pharmaceutical interventions such as structural modification, alternative formulation and effective delivery systems, the pharmacological potential can be increased.

CONCLUSION

The medicinal properties of the plant mainly depend on phytochemical constituents that have great pharmacological significance. It has great potential to be developed as a drug by pharmaceutical industries. The present study was undertaken to find out the phytochemical screening, antioxidant activity and cytotoxic activities of the plant *Gliricidia sepium* in methanolic extract.

In conclusion, it is apparent that the pharmacological activities of *Gliricidia sepium* groups alkaloids and flavonoids content and its antioxidant activity. The plant showed significant antioxidant and cytotoxicity activities with very low toxic effects. Consequently, the isolation of bioactive compounds from this plant might be our future research.

REFERENCE

Abdulaziz A A and Dapar M L G (2019). Qualitative evaluation of the antimicrobial, antioxidant, and medically important phytochemical constituents of the ethanolic extracts of the leaves of *Gliricidia sepium* (Jacq.) Walp. *Pharmacophore,* 10(4): 72-83.

Akharaiyi, Fred, Bola and Adetuyi F (2012). Antibacterial, Phytochemical and Antioxidant Activities of the Leaf Extracts of *Gliricidia sepium* and *Spathodea campanulata. World Applied Sciences Journal.* 16: 155-163.

Aslam and Ahmad M S (2016). Worldwide Importance of Medicinal Plants: Current and Historical Perspectives. *Recent Advances in Biology and Medicine.* 2: 88-93.

Gbolade, Adebayo A, Oluwasegun and Bello A (2019). Cytotoxic and growth inhibitory activities of *Gliricidia sepium* (jacq.) Kunth ex walp. (fabaceae) and *Hymenocardia acida* tul. (phyllanthaceae) stem bark. *Nigerian Journal of Pharmaceutical Sciences,* 18(2): 1-10.

Ghoshal, G. (2018). Emerging food processing technologies. *Food processing for increased quality and consumption*, pp. 29-65.

Gnanaraja R, Prakash V, Peter, S and Mahendraverman M (2014). Qualitative and quantitative phytochemicals analysis of selected fabaceae medicinal plants from Allahabad region. *The Pharma Innovation Journal*, *3*(7): 53-56.

Kumar M and Kumar P (2013). Cultivation of *Gliricidia sepium* and its use for improving soil fertility. *Journal of The Kalash Science,* 1(1): 131-133.

Lee J J, Saiful L, Kassim N K, Che Abdullah C A, Esa N, Lim P C and Tan D C (2020). Cytotoxic activity of *Christia vespertilionis* root and leaf extracts and fractions against breast cancer cell lines. *Molecules*, 25(11): 2610.

Neethu S K and Neethu S (2016). In vitro antibacterial activity and phytochemical analysis of *Gliricidia sepium* (L.) leaf extracts. *Journal of Pharmacognosy and Phytochemistry*,5(2): 131-133.

Pagare S, Bhatia M, Tripathi N and Bansal Y K (2015). Secondary metabolites of plants and their role: Overview. *Current Trends in Biotechnology and Pharmacy,* 9: 293-304.

Pullaiah T, Bahadur B and Krishnamurthi K (2015). Plant Biology and Biotechnology. *Plant Diversity, Organization, Function and Improvement,* 1(2): 177-195.

Rawat S J and Bahukhandi A K(2016). Anti-oxidant and anti-microbial properties of some ethno- therapeutically important medicinal plants of Indian Himalayan Region. *US National Library of Medicine* , 6 (2): 154.

Saxena M, Jyoti S, Nema R, Dharmendra S and Abhishek G (2013). Phytochemistry of Medicinal Plants. *J Pharm Phytochem*, 1: 168-182.

Sinha S N (2013). Phytochemical profiles and antioxidant activities of The leaf extracts of *Gliricidia sepium*. *International Journal of Innovation in Biosciences Research 3*(3): 87- 91.

Sumitra C and Nagani K (2013). *In vitro* and *in vivo* Methods for Anticancer Activity Evaluation and Some Indian Medicinal Plants Possessing Anticancer Properties: An Overview. *Journal of Pharmacology and Phytochemistry* 2(2): 140-152.

Tom C and Mity T (2019). Phytochemical investigation of the leaves of *Gliricidia sepium* and its antimicrobial properties. *The Pharma Innovation Journal*, 8(2): 594-596.

Zak B, Dickenman R C, White E G, Burnett H and Cherdey P J (1954). Rapid estimation of free and total cholesterol. *American journal of clinical pathology*, 24: 1307-1315.