



Fourier Transform Infra-Red Spectroscopy Analysis of *Erythrina variegata* L.

Subrahmanian Hemmalakshmi^a, Suriyamoorthy Priyanga^a and Kanakasabapathi Devaki^{a*}

^aDepartment of Biochemistry, Karpagam Academy of Higher Education,
Coimbatore-641 021, India.

Abstract

The purpose of the present study was to find out the functional groups present in the *Erythrina variegata* L. by using Fourier Transform Infra-Red (FTIR) Spectroscopy method. FTIR analysis were carried out by Shimadzu FTIR spectrometer 4000 series, the scan range between 4,000–400 cm⁻¹. FTIR spectroscopic investigation showed the presence of characteristic peak values with different useful mixtures of functional groups such as hydroxy group, aliphatic, metal carbonyl, alcohols, nitrile, phenols, alkynes, ketones, carboxylic acids, amide and aromatics. The FTIR results exhibited the *Erythrina variegata* L. leaves, flowers and barks have 17, 31 and 15 functional groups. An intense peak of 3348.42 cm⁻¹ in leaves, 1049.28 cm⁻¹ in flowers and 1583.56 cm⁻¹ in barks were observed in the FTIR spectra and it corresponded to the hydroxyl groups, phosphate ion and amide respectively. So, the present study concluded that the flower extract possessed strong functional groups, when compared with the leaf and bark extracts of *Erythrina variegata* L.

Key words: FTIR, phytoconstituents, Functional groups, *Erythrina variegata* L.

INTRODUCTION

Medicinal plants are the important bio resources of drugs for conventional system of medicine[1]. They comprise of various natural dynamic fixings, in this manner they are utilized for the treatment of an extensive number of irresistible sicknesses[2]. The identification of phytoconstituents from the medicinal plants using various techniques such as FTIR, HPTLC and GC-MS analysis [3]. Fourier Transform Infrared Spectroscopy is one of the extensively used method to categorize the chemical constituents and has been used as a necessary method to identify the medicines for pharmacopeia in several countries [4]. It is a nondestructive analytical technique that provides structural information on molecular features of a large range of compounds [5]. FTIR Spectroscopy has been recognized as a dependable and sensitive method for finding the functional groups present in the plant extracts and they were determined with the aid of IR region in the range of 400-4000cm⁻¹ [6]. It is possibly the major, authoritative technique used for identifying the types of chemical groups (functional groups) present in compounds. The wavelength of light fascinated is a characteristic of the chemical bond which might be seen in the annotated spectrum. The chemical bonds in the molecules have been predicted using FTIR [7].

Erythrina variegata Linn. (Fabaceae), ordinarily identified as Tiger's Claw, is a prickly deciduous tree developed in the tropical and subtropical districts of Eastern Africa, Southern Asia, and Northern Australia. In India, its leaves are generally utilized for diabetes[8]. Previous reports in the same species of plant has stated that the plant possesses hepatoprotective and anti-leprosy effects. Its extracts shows evidence of sedative, antidiuretic, antioxidant and antihyperlipidemic activities [9,10]. The aim of the present study is to identify the functional groups present in the *Erythrina variegata* L. leaves, flower and barks through FTIR spectroscopy.

MATERIALS AND METHODS

Plant collection and authentication

The fresh plant samples were collected in the month of August from in and around Kodaikannal, Dindigul district, Tamil Nadu, India. They were botanically authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, Southern Region, Coimbatore. The specimen was deposited in the herbarium for future reference (voucher number: BSI/SRC/5/23/2013-14/Tech/1500). The plant materials were dried under shade for couple of weeks. Then, the powdered plant material was stored in an airtight container and kept for further studies.

Preparation of extracts

50 g of powdered leaves, barks and flowers were weighed and extracted with 250 ml of ethanol and water. Then it is kept in an orbital shaker at 190-220 rpm for 48 hours. The supernatant was collected, concentrated and dried. The dried extracts were kept under -20°C for further use.

FTIR Spectrum Analysis

The extracts of *Erythrina variegata* L. leaves, barks and flowers were mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. The samples were loaded onto FTIR spectroscope and the spectroscopic results were recorded on a Shimadzu FTIR Spectrometer 8000 series, the scan range was between 4,000–400 cm⁻¹ [11].

RESULTS AND DISCUSSION

Restorative herbs are a vital wellspring of phytochemicals that offer conventional therapeutic treatment for different infirmities [12]. Plants are the major source of functional component for the improvement of new-fangled chemotherapeutic agents [13]. Fourier transform infrared (FTIR) spectroscopy is a vibration spectroscopic method that utilizes the infrared radiation to vibrate molecular bonds within the sample that absorbs it. Most of the examples have diverse sub-atomic bonds or distinctive setups of sub-atomic bonds, FTIR enables to get compound data on particles inside the specimen [14]. The FT-IR

spectrum is used to recognize the functional groups of the active components present in the extract based on the peak values in the region of IR radiation. At a point when the concentrate is passed into the FTIR, the practical gatherings of the parts are isolated which gives the clear view of its pinnacle proportion. The results of FT-IR spectroscopy confirm the presence of various chemical constituents such as alcohol, alkanes, aromatic carboxylic acid, halogen compound and alkyl halide in the ethanolic extract of *Erythrina variegata* L. leaves and flowers and aqueous extract of barks (Table 1,2,3 and Figure 1, 2 & 3).

The Table 1 and Figure 1 demonstrates the presence of 17 functional groups recognized from the ethanolic extract of *Erythrina variegata* L. leaves. The strong instance peaks are identified at 3348.42 and 1643.35 cm^{-1} which are assigned to the H-bonded and O-H stretching vibration.

The peaks at 2090.84, 1990.54 and 1851.66 cm^{-1} which are assigned to the carbonyl compound frequency vibration. It means that some carbonyl compounds are existed in the ethanolic extract of leaves. The peak at 2854.65 cm^{-1} attributes to symmetric stretching of -CH (CH₂) vibration (lipids) and some other groups such as carboxylic acids, nitriles, terminal alkynes, ketone compound, aromatic compound, phenol or tertiary alcohol, acid, phosphate ions are absorbed at 2522.89, 2422.59, 2345.44, 2276.00, 2137.13, 1643.35, 1481.33, 1381.03, 1226.73, 1041.56 cm^{-1} respectively. [15] Ragavendran has reported that the *Aerva lanata* plant has numerous functional groups such as carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons and halogens.

Table 1: FTIR Interpretation of compounds of ethanolic extract of *Erythrina variegata* L. leaves

S. No	Wave number cm^{-1} (Test sample)	Wave number cm^{-1} (Reference article)	Functional group assignment	Expected Phytocompounds Identified
1	3888.49	>3500	Non bonded, O-H stretch	Hydroxy group
2	3348.42	3570-3200	H-bonded, O-H stretch	Hydroxy compound, alcohols, phenols
3	2924.09	2935-2915	Asymmetric stretching of -CH (CH ₂) vibration	Saturated aliphatic compound-Lipids
4	2854.65	2865-2845	Symmetric stretching of -CH (CH ₂) vibration,	Fatty acids, Lipids, protein
5	2522.89	3500-2400	O-H stretch, Carboxylic group, Acidic	Carboxylic acids
6	2422.59	3500-2400	O-H stretch, Carboxylic group, Acidic	Carboxylic acids
7	2345.44	2248-2376	C=N (stretch)	Nitriles
8	2276.00	2300-1990	Multiple bonding	Nitrile compounds
9	2137.13	2260-2100	Carbon-Carbon triple bond	Terminal alkynes
10	2090.84	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
11	1990.54	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
12	1851.66	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
13	1643.35	1650-1600	C=O stretching	Ketone compound
14	1481.33	1510-1450	C=C-C, Aromatic ring	Aromatic compound
15	1381.03	1410-1310	O-H bend, Alcoholic group	Phenol or tertiary alcohol
16	1226.73	1210-1320	C-O stretch	Acid
17	1041.56	1100-1000	PO ₃ stretch	Phosphate ion

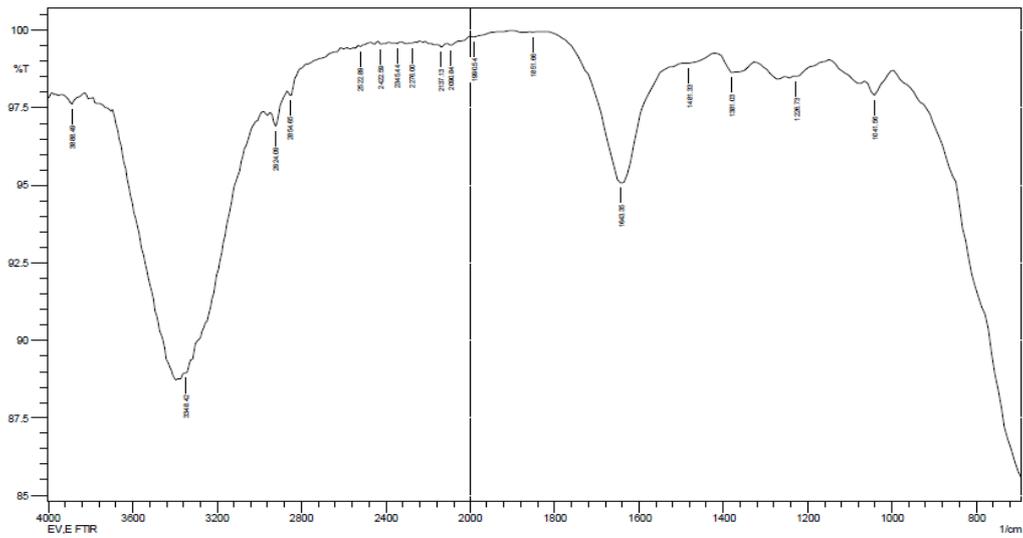


Fig. 1: FTIR Spectrum analysis of ethanolic extract of *Erythrina variegata* L. leaves

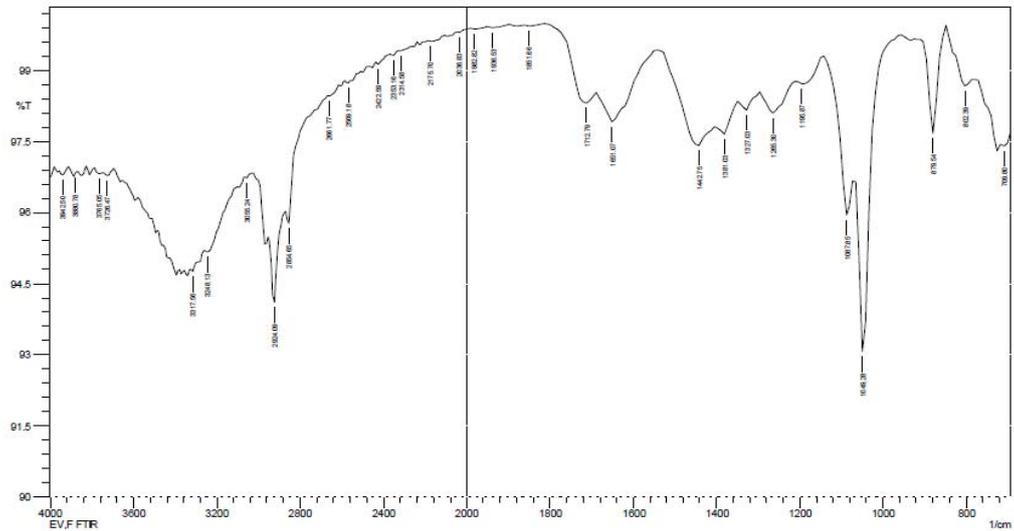


Fig. 2: FTIR Spectrum analysis of ethanolic extract of *Erythrina variegata* L. flowers

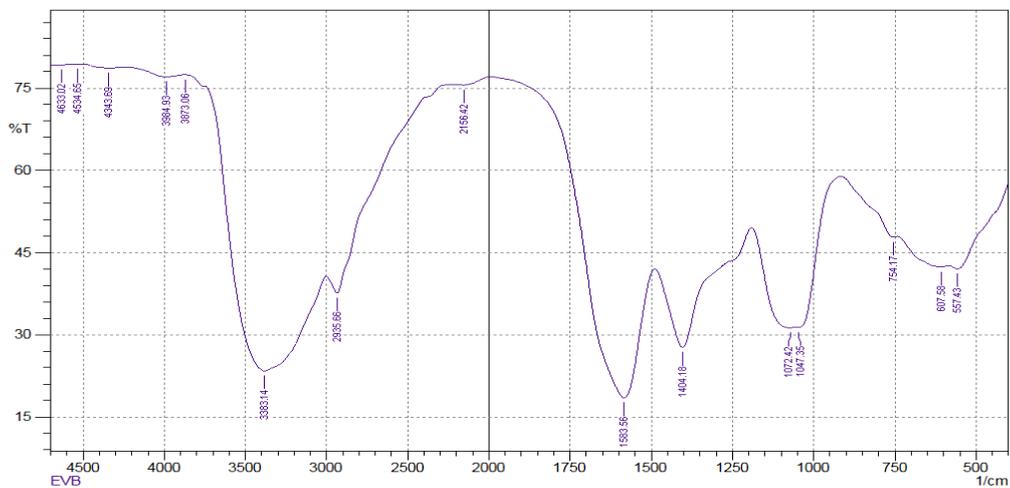


Fig. 3: FTIR Spectrum analysis of aqueous extract of *Erythrina variegata* L. barks Conclusion

The functional group identification is made by FTIR analysis and the active components based on the peak value in the region of infrared radiation. The ethanolic flower extract of *Erythrina variegata* L. is passed into the FTIR spectroscopy and the functional groups of the components are separated based on the peak ratio. The results of FTIR analysis confirm the presence of functional groups such as non bonded, O-H stretch, carboxylic group, acidic, H-bonded, C-H stretch, asymmetric stretching of -CH (CH₂) vibration, C=N (stretch), carbon-carbon triple bond, multiple bonding, carbonyl compound frequency, C=O stretch, C=C stretch, O-H bend, alcoholic group, C-N stretch, C-O stretch, PO₃ stretch, =C-H bending and C-Cl. Which shows the major peak values of 3942.50, 3880.78, 3765.05, 3726.47, 3317.56, 3248.13, 3055.24, 2924.09, 2854.65, 2661.77, 2569.18, 2422.59, 2353.16, 2314.58, 2175.70, 2036.83, 1982.82, 1936.53, 1851.66, 1712.79, 1651.07, 1442.75, 1381.03, 1327.03, 1265.30, 1195.87, 1087.85, 1049.28, 879.54, 802.39 and 709.80 cm⁻¹

respectively in the Figure 2 and Table-2. An intense band occurs at 1049.28 cm⁻¹ corresponding to PO₃ stretch vibration indicates the presence of phosphate ion in the flower extract of *Erythrina variegata* L. The alkanes compounds are present in the wax of numerous species. They care for the plant against water loss, and protect against bacteria, prevent the leaching of important minerals by the rain, harmful insects, and fungi [16]. Carboxylic acids plays an important role in the formation of fat in the whole body. Aspirin is a carboxylic acid, and some people are sensitive to its acidity. The non-aspirin pain reliever ibuprofen is also a carboxylic acid [17]. Recent interest in these substances has been stimulated for the potential health benefits. Useful hydroxyl bunches in flavonoids intercede their cell reinforcement which impact by rummaging free radicals and by chelating metal particles [18,19].

Table 2: FTIR Interpretation of compounds of ethanolic extract of *Erythrina variegata* L. flowers

S.No	Wave number cm-1 (Test sample)	Wave number cm-1 (Reference article)	Functional group assignment	Expected Phyto compounds Identified
1	3942.50	>3500	Non bonded, O-H stretch	Hydroxy group
2	3880.78	>3500	Non bonded, O-H stretch	Hydroxy group
3	3765.05	>3500	Non bonded, O-H stretch	Hydroxy group
4	3726.47	>3500	Non bonded, O-H stretch	Hydroxy group
5	3317.56	3500-3000, 3570-3200	O-H stretch, Carboxylic group, Acidic, H-bonded, O-H stretch	Carboxylic acids, Hydroxy compound
6	3248.13	3500-3000, 3570-3200	O-H stretch, Carboxylic group, Acidic, H-bonded, O-H stretch	Carboxylic acids, Hydroxy compound
7	3055.24	3100-3000	C-H stretch	Aromatic
8	2924.09	2985-2915	Asymmetric stretching of -CH (CH ₂) vibration	Saturated aliphatic compound-Lipids
9	2854.65	3000-2850	C-H stretch	Alkane
10	2661.77	3300-2500	O-H stretch	Acid
11	2569.18	3300-2500	O-H stretch	Acid
12	2422.59	-	Unkown	Unkown
13	2353.16	2376-2348	C=N (stretch)	Nitriles
14	2314.58	2376-2348	C=N (stretch)	Nitriles
15	2175.70	2260-2100	Carbon-Carbon triple bond, Multiple bonding	Terminal alkynes, Nitrile compounds
16	2036.83	2100-1800, 2300-1900	Carbonyl compound frequency	Transition metal carbonyls, Nitrile compounds
17	1982.82	2100-1800	Carbonyl compound frequency	Transition metal carbonyls,
18	1936.53	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
19	1851.66	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
20	1712.79	1820-1670	C=O, stretch	Carbonyl
21	1651.07	1680-1620	C=C, stretch	Alkane
22	1442.75	1600-1400	C=C, stretch	Aromatic
23	1381.03	1410-1310	O-H bend, Alcoholic group	Phenol or tertiary alcohol
24	1327.03	1360-1210	C-N stretch	Amine
25	1265.30	1320-1210	C-O stretch, C-N stretch	Acid, Amine
26	1195.87	1360-1080 1300-1150(m)	C-N stretch, C-H wag (-CH ₂ , N)	Amine, alkyl halides
27	1087.85	1100-1000	PO ₃ stretch	Phosphate ion
28	1049.28	1100-1000	PO ₃ stretch	Phosphate ion
29	879.54	1000-675	=C-H bending	Alkane
30	802.39	1000-675	=C-H bending	Alkane
31	709.80	1000-675, 750-500	=C-H bending, C-Cl	Alkane, Halogen compound (Chloro compound)

Table 3: FTIR Interpretation of compounds of aqueous extract of *Erythrina variegata* L. bark

S. No	Wave number cm-1 (Test sample)	Wave number cm-1 (Reference article)	Functional group assignment	Expected Phytochemicals Identified
1	4633.02	> 3500	Non bonded, O-H stretch	Hydroxy group
2	4534.65	> 3500	Non bonded, O-H stretch	Hydroxy group
3	4343.69	> 3500	Non bonded, O-H stretch	Hydroxy group
4	3984.93	> 3500	Non bonded, O-H stretch	Hydroxy group
5	3873.06	> 3500	Non bonded, O-H stretch	Hydroxy group
6	3383.14	3500-2400, 3570-3200	O-H stretch, Carboxylic group, Acidic, H-bonded,	Carboxylic acids, Hydroxy compound
7	2934.66	2935-2915	Asymmetric stretching of -CH (CH ₂) vibration	Saturated aliphatic compound-Lipids
8	2156.42	2300-1990, 2260-2100	Multiple bonding, Carbon-Carbon triple bond	Nitrile compounds, Terminal alkynes
9	1583.56	1640-1550	N-H bending	Amide
10	1404.18	1410-1310	O-H bend, Alcoholic group	Phenol or tertiary alcohol
11	1072.42	1100-1000	PO ₃ stretch	Phosphate ion
12	1047.35	1100-1000	PO ₃ stretch	Phosphate ion
13	754.17	1000-675	-C-H, bending	Alkene
14	607.58	620-490, 730-500	C-I, C-Cl	Halogen compound (Chloro compound)
15	557.43	620-490, 730-500	C-I, C-Cl	Halogen compound (Chloro compound, Iodo compound), alkyl halides

The absorption spectra of the samples are done and the associated functional groups are presented in the Table 3 and Figure 3. Presence of 15 functional groups and the expected phytochemicals are identified in the aqueous bark extract of *E. variegata* L. The strong instance peaks are identified at 3383.14, 1583.56, 1404.18, and 1072.42 cm⁻¹ which are assigned to the carboxylic acids, hydroxy compound, amide and phenol or tertiary alcohol, phosphate ion vibration respectively. The peaks at 4633.02, 4534.65, 4343.69, 3984.93, and 3873.06 cm⁻¹ which are assigned to the carbonyl compound frequency vibration and it means that some carbonyl compounds has existed in the aqueous bark extract of *E. variegata* L. The peak at 2854.65 cm⁻¹ attributes at symmetric stretching of -CH (CH₂) vibration (lipids). Some other groups such as carboxylic acids, nitriles, terminal alkynes, ketone compound, aromatic compound, phenol or tertiary alcohol, acid and phosphate ions are absorbed at 2522.89, 2422.59, 2345.44, 2276.00, 2137.13, 1643.35, 1481.33, 1381.03, 1226.73, and 1041.56 cm⁻¹ respectively. Based on the functional group analysis, It is evident that *E. variegata* L. plant does not consist of any toxic compounds.

The present study concludes that the FTIR analysis reveals clear differentiation between the leaves, flowers and barks extract of *E. variegata* L. The comparative account of leaves, barks and flowers extract of *E. variegata* L. depicted large variation and it can be used to determine the part of the plant that recorded more phytoconstituents which can be used as plant remedies for many diseases. The results of the study enlightens the fact that this plant is medicinally important and can be further taken for study for locating bioactive compounds to find its significance in the pharmaceutical industries. In future advanced spectroscopic investigations are needed for the identification and structural elucidation of compounds present in the *E. variegata* L.

Acknowledgement

We, the authors are thankful to the Chancellor, Chief Executive Officer, Vice Chancellor and Registrar of Karpagam Academy of Higher Education for providing facilities and for their kind support and encouragement.

Conflict of Interest Statement

The authors declare no conflict of interest.

REFERENCES:

- Saxena M, Saxena J, Evaluation of phytoconstituents of *Acorus Calamus* by FTIR and UV-Vis spectroscopic analysis. International Journal of Biological & Pharmaceutical Research, 3(3), 2012, 498-501.
- Maobe MGA, Nyarango RM. Fourier Transformer Infra-Red Spectrophotometer Analysis of *Urtica dioica* Medicinal Herb Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. World Applied Sciences Journal, 21(8), 2013, 1128-1135.
- Grube M, Muter O, Strikauska S, Gavare M, Limane B, Application of FT-IR spectroscopy for control of the medium composition during the biodegradation of nitro aromatic compounds. Journal of Indian Microbiology and Biotechnology, 35, 2008,1545–1549.
- Subashini MS, Rajendran P, Ashok G, Kanthesh BM, TLC, FTIR and GCMS analysis of leaves of *Gymnema sylvestre R.Br* from Kolli Hills, Tamil Nadu, India. International Journal of Current Microbiology and Applied Sciences, 4(7), 2015, 757-764.
- Agatonovic-Kustrin QS, Morton DW, Yusof AP, The Use of Fourier Transform Infrared (FTIR) Spectroscopy and Artificial Neural Networks (ANNs) to Assess Wine. Modern Chemistry & Applications, 2013, 1: 110. doi:10.4172/2329-6798.1000110.
- Florence AR and Jeeva S, FTIR and GC-MS spectral analysis of *Gmelina asiatica* L. Leaves. Science Research Reporter, 5(2), 2015, 125-136.
- Sahayaraja PA, Gowri J, Dharmalingama V, Shobanaa R, Angelin Premab AA, Phytochemical screening by FTIR spectroscopic analysis of leaf and stem Extracts of *Wedelia biflora*. International Journal of Nano Corrosion Science and Engineering, 2(5), 2015, 322-334.
- Kumar A, Lingadurai S, Shrivastava TP, Bhattacharya S, Haldar PK, Hypoglycemic activity of *Erythrina variegata* leaf in streptozotocin-induced diabetic rats. Pharmaceutical Biology,49(6), 2011, 577–582.
- Javed SB, Anis M, Cobalt induced augmentation of *in vitro* morphogenic potential in *Erythrina variegata* L.: a multipurpose tree legume. Plant Cell Tissue Organ Culture, 120, 2015,463-74.
- Hemmalakshmi S, Priyanga S, Devaki K, Phytochemical screening and HPTLC fingerprinting analysis of ethanolic extract of *Erythrina variegata* L. Flowers. International Journal of Pharmacy and Pharmaceutical science, 8, 2016, 210-217.
- Cakmak G, Togan I, Severcan F, 17 β -estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FTIR spectroscopy: a comparative study with nonylphenol. Aquatic Toxicology, 77, 2006, 53-63.
- Priyanga S, Hemmalakshmi S, Devaki K, Comparative chromatographic fingerprint profiles of ethanolic extract of *Macrotyloma uniflorum* L. leaves and stem, International Journal of Pharmaceutical and Clinical Research, 6, 2014, 299.
- Starlin T, Raj AC, Ragavendran P, Gopalakrishnan VK, Phytochemical screening , functional groups and element analysis of *Tylophora pauciflora* weight and arm. International Research Journal of Pharmacy,3(6), 2012, 180-183.
- Baker MJ, Gazi E, Brown MD, Shanks JH, Gardner P, Clarke NW, FTIR-based spectroscopic analysis in the identification of clinically aggressive prostate cancer. British Journal of Cancer, 99, 2008, 1859 – 1866.
- Ragavendran P, Sophia D, Arul Raj C, Gopalakrishnan VK, Functional group analysis of various extracts of *Aerva lanata* (L.) by FTIR spectrum. Pharmacologyonline, 1, 2011, 358-364.
- Baker EA. Chemistry and morphology of plant epicuticular waxes. In: Cutler DF, Alvin KL, Price CE. eds. The Plant Cuticle, London: Academic Press; 1982. p. 139-165.
- Janakiraman N, Sathish SS, Johnson M, UV VIS and FTIR Spectroscopic studies on *Peristrophe bicalyculata* (Retz.) Nees. Asian Journal of Pharmaceutical and Clinical Research, 4(4), 2011, 125-129.
- Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal, 2013, 1-16.
- Dyson PJ, McIndoe JS, Transition Metal Carbonyl Cluster Chemistry, 2000 by CRC Press, Textbook - 180 Pages.