

## Evaluation of Phytoconstituents of *Syzygium arnottianum* Leaves

M P Krishna, M Mohan\*

School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala- 686560

Available Online: 25<sup>th</sup> October, 2017

### ABSTRACT

**Aim:** The present study is carried out to explore the major phytoconstituents and functional groups present in the methanolic extract of the leaves of *Syzygium arnottianum* using FTIR and GC-MS. **Methods:** For the identification of the phytochemical constituents, Perkin – Elmer GC Clarus 500 system (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 µm DF of capillary column is used. The molecular weight and structure of the compounds were determined by analysis of the mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST) library data. For the analysis of functional groups, ATR- FTIR spectroscope (Shimadzu IR Prestige-21) is used. **Results:** The GC-MS analysis of the methanolic extract of *S. arnottianum* revealed the presence of 11 bioactive compounds with valuable biological activities. The FTIR analysis indicated the presence of alcohol, alkane, alkene, alkyl halide, alkyne, amine, aromatic, carbonyl, ether, acid, aldehyde, anhydride and ester. **Conclusion:** The phytochemical profile of the plant *S. arnottianum* leaf extract indicates the presence of various bioactive compounds which can be utilised further for medicinal purposes.

**Keywords:** FT-IR, GC-MS, plant extract, Western Ghats and medicine.

### INTRODUCTION

Phytochemical constituents are responsible for medicinal property of plant species. Plants are capable of producing a vast variety of secondary metabolites, which are low-molecular weight organic compounds, usually with unique and complex structures. Most of the herbal medicines and their derivatives were often prepared from crude plant extracts, which consist of a complex mixture of different phytochemical constituents. The chemical features of these constituents vary greatly among different species. The cell walls of different plant species have a variety of physically different polysaccharides and proteins. On the basis of the involvement in plant metabolism, phytochemicals are classified into two groups such as primary and secondary metabolites. Primary metabolites include carbohydrates, amino acids, proteins and chlorophylls whereas secondary metabolites include alkaloids, saponins, steroids, flavonoids, tannins and so on<sup>1</sup>. The phytochemical constituents play a major part in the identification of crude drugs<sup>2</sup>.

*Syzygium arnottianum* (Wight) Walp.) belongs to the family Myrtaceae (Fig.1) and the synonym is *Syzygium densiflorum*. It is endemic to southern Western Ghats and is coming under the category ‘vulnerable species’. It is a tall tree with grey bark and simple and opposite leaves<sup>3</sup>. The tree has been used as food and source of wood. Essential oil of leaves of *Syzygium arnottianum* showed high anti-oxidant capacity<sup>4</sup>. The fruit has been used for the treatment of toothache whereas the leaves have been used for the treatment of diabetes mellitus from earlier time itself. Trace elements present in this tree make a good daily

supplement for people suffering from bone and anaemic disorders<sup>5</sup>. The potential phytochemical compounds such as tannins, saponins, flavonoids, alkaloids, quinine, cardiac glycosides, terpenoids, phenols and carbohydrates were present in the leaves of *S. arnottianum*<sup>6</sup>.

Identification and determination of bioactive compounds can be done with the help of FT-IR and GC-MS technologies<sup>7,8,9,10,11</sup>. GC-MS is a unique method to recognise the bioactive components of long chain branched hydrocarbons, alcohols, acids, ester etc.<sup>12</sup>. The functional groups in plant samples can be identified by FT-IR Spectroscopy. The present study evaluated the functional groups and phytochemicals present in methanol extract of *S. arnottianum* leaf with the help of FT-IR and GC-MS.

### MATERIALS AND METHODS

#### Collection and processing of plant material

The leaves of *S. arnottianum* were collected from Pampadum Shola National Park, Kerala and identified with taxonomic keys<sup>13</sup>. Freshly fallen leaves of the plant were collected and thoroughly washed with distilled water. The leaves were then cut into small pieces and shade dried. The dried leaves were crushed into powder and preserved in air sealed polythene cover.

#### Preparation of plant extract

Soxhlet apparatus was employed to extract the dried leaves using methanol as a solvent. 20gm of plant material was extracted in 250ml of methanol, filtered and the extracts were concentrated using rotary evaporator at 100 rpm for



Figure 1: Plant of *Syzygium arnottianum*.

Table 1: Preliminary phytochemical analysis of methanol extract of *S. arnottianum*.

Phytochemical constituents	Methanol Extract
Proteins	+
Alkaloids	+
Phytosterols	-
Diterpenes	+
Phenols	+
Flavonoids	+
Glycosides	-
Saponins	+
Carbohydrates	+
Phenols/Tannins	+

30 minutes. This residue was collected, dried and was kept in refrigerator at 4°C for further phytochemical analysis.

#### GC-MS Analysis

The GC – MS analysis was carried out using Perkin – Elmer GC Clarus 500 system (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 µm DF of capillary column. The identification of components on the basis of comparing the relative retention time and mass spectra with NIST Library data<sup>14</sup>. The name, molecular weight and structure of the constituents of the sample material were also determined.

#### FT-IR Analysis

10 mg of the dried extract powder was condensed in 100 mg of KBr pellet. The powdered sample of each extract was loaded in ATR- FTIR spectroscope (Shimadzu IR Prestige-21 with ZnSe ATR crystal), with a scan range from 400 to 4000 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>. All the analysis was repeated thrice. The functional groups of the active compounds present were identified on the basis of the peak values in the region of IR radiation<sup>15</sup>.

## RESULTS AND DISCUSSION

The results showed that the leaves of *S. arnottianum* contains the foremost phytochemical constituents include alkaloids, proteins, phenols, tannins, carbohydrates, saponins, glycosides, flavonoids, diterpenes and phytosterols (Table 1).

The various phytochemical compounds detected are known to have beneficial importance in medical sciences. The secondary metabolites like phenolics and flavonoids from the plants are considered to be powerful free radical scavengers due to their inherent capacity to transform the body's response to allergies and virus; and they indicated their anti-allergic, anti-inflammatory, anti-microbial, anti-oxidant and anti-cancerous activities<sup>16</sup>. Flavonoids, a group of poly phenolic compounds, also can be used as an anti-inflammatory agent as it affects the radical scavenging, inhibition of hydrolytic and oxidative enzymes<sup>17</sup>.

Phenolic compounds perform as a cell supportive material; as they form an important part in the cell wall structure by polymeric phenolics<sup>18</sup>. Large quantity of phenolic content in any plant specifies its use in the treatment of inflammatory diseases and wound healing. Hence, pharmacologists are generally searching plants with high phenolic content. *S. arnottianum* leaves were already used indigenously for the treatment of inflammatory diseases.

Tannins are complex compounds of non-carbohydrate residue which have astringent, anti-inflammatory, antidiarrheal, antioxidant and antimicrobial activities like antiviral, antibacterial, antifungal and anti-tumorous activities<sup>19</sup>. It was also reported that certain tannins were able to prevent HIV replication selectively and was also used as diuretic. Hence, the leaves of *S. arnottianum* can also consider for such treatments as it exhibit these properties. The presence of saponins enhances the utilisation of *S. arnottianum* leaves for the treatment of hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss etc.

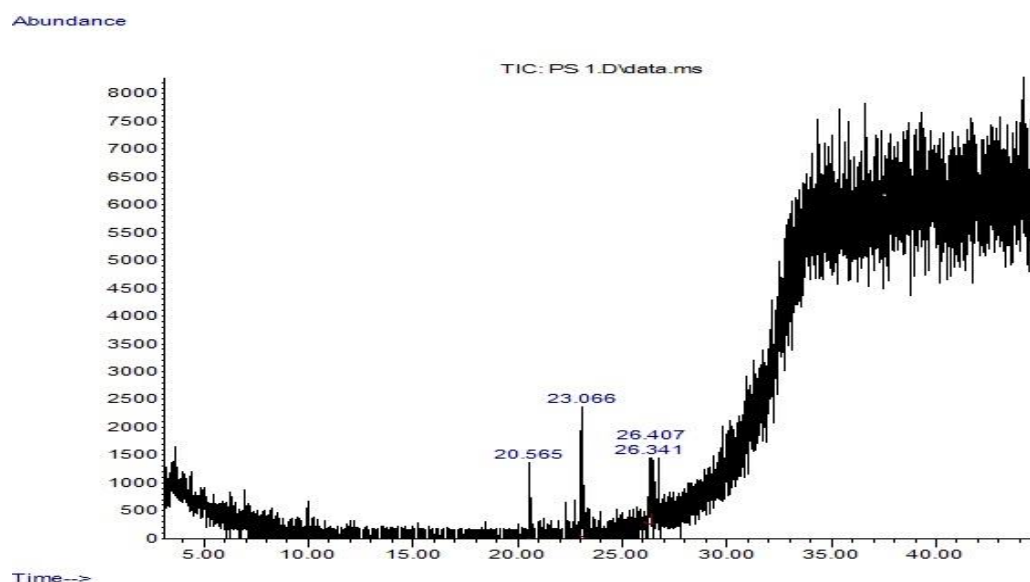
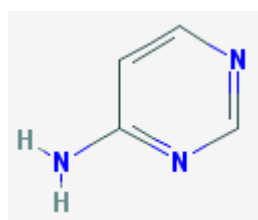
The GC-MS chromatogram was represented in fig. 2 and the constituents obtained from GC-MS analysis are given in table 2.

The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula (Table 2). Eleven phytochemical compounds are present in the leaves of *S. arnottianum*. These compounds are 4-Aminopyrimidine (RT=20.567), 5-methyl-1-Methyl-1H-1,2,4-triazole (RT=20.567), Oxazole (RT=23.068), 4,5-dihydro-2-methyl- Dichloro acetic acid (RT=23.068), hexyl ester Hydrazine (RT=23.068), [(1,2,3-.eta.)-2-butenyl].eta.8-1,3,5,7-cyclooctatetraene)-Cyclopentane (RT=26.341), 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediy] bis-Propanedinitrile (RT=26.341), Cyclohexane (RT=26.410), 1,2,4,5-tetraethyl-2-Thiopheneacetic acid (RT=26.410), Oct-3-en-2-yl ester Cyclopentanone (RT=26.410), [(1,2,3-.eta.)-2-butenyl].eta.8-1,3,5,7-cyclooctatetraene)-Cyclopentane). The identified compounds have several biological properties<sup>20</sup>.

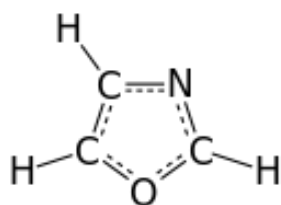
The results obtained from GC-MS analysis shows that most of the phytochemical groups in the leaves of *S. arnottianum* have pharmaceutical activities and some of are discussed here. 4-Aminopyrimidine has been used as a drug for curing multiple sclerosis<sup>21</sup>. This is used as a hair growth stimulant and potent convulsant<sup>22</sup>. Oxazole have anti-cancerous, anti-viral, anti-diabetic and antibiotic activity. Cyclopentanone, another compound obtained, has

Table 2: GC-MS spectral analysis of methanol extract of *Syzygium arnottianum*.

No.	RT (min)	Name of the compound	Library/ID	Ref#	CAS#	Peak Area %
1	20.567	4-Aminopyrimidine, 5-methyl-1-Methyl-1H- 1,2,4-triazole	C:\Database\NIST11.L	2651 1287 1282	000591-54-8 005765-44-6 006086-21-1	14.32
2	23.068	Oxazole, 4,5-dihydro-2-methyl- Dichloroacetic acid, hexyl ester Hydrazine	C:\Database\NIST11.L	1564 71578 277	001120-64-5 037079-04-2 000057-14-7	41.34
3	26.341	[(1,2,3-.eta.)-2- butenyl].eta.8-1,3,5,7- cyclooctatetraene)- Cyclopentane, 1,1'-[3-(2- cyclopentylethyl)- 1,5pentanediyl]bis- Propanedinitrile,	C:\Database\NIST11.L	66801 148323 1015	039015-02-6 055255-85-1 000922-64-5	20.16
4	26.410	Cyclohexane, 1,2,4,5- tetraethyl-2- Thiopheneacetic acid, oct-3-en-2-yl ester Cyclopentanone	C:\Database\NIST11.L	58317 103739 3234	061142-00-5 1000299-38-6 001120-72-5	24.18

Figure 2: GC-MS Chromatogram of Methanol Extract of *syzygium arnottianum*.

4-Aminopyrimidine



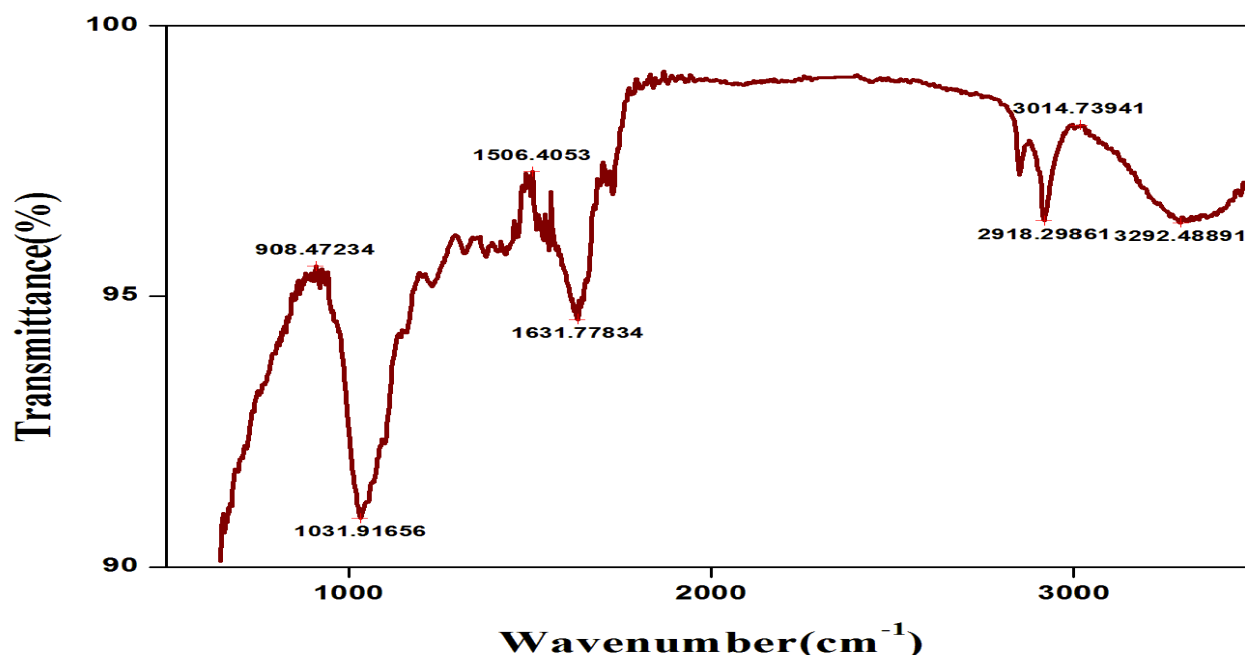
Oxazole

Figure 3: The structure of various phytochemicals contribute to the medicinal activity.

been used as an intermediate for the production of medicines and perfumes. The structure of the various phytochemicals (Fig -3) which contribute to the medicinal activity of the plant methanol extract of *S. arnottianum*.

FTIR spectroscopy is used to be an essential and sensitive technique for find out the bio molecular composition<sup>23</sup>. The results of FTIR spectroscopic analysis in the methanol extract of leaf litter of *Syzygium arnottianum* have revealed the presence of numerous chemical compounds (Fig. 4). The peak formation in the FTIR spectrum represents the functional groups (Table 3).

The absorption at  $3363.86\text{ cm}^{-1}$  assigned to O-H of alcohols and phenol groups. The band at  $3307.92\text{ cm}^{-1}$ ,  $3292.49\text{ cm}^{-1}$ ,  $3263.56\text{ cm}^{-1}$ ,  $3203.76\text{ cm}^{-1}$ ,  $1627.92\text{ cm}^{-1}$ ,  $1614.42\text{ cm}^{-1}$  is due to the N-H stretching that present in the extract. The band at  $2918.30\text{ cm}^{-1}$ ,  $2850.79\text{ cm}^{-1}$  is due to C-H stretching of methylene asym. /sym. The IR finger prints of protein are featured by a set of absorption regions represented as the amide region and the C-H region. The band at  $1379.10\text{ cm}^{-1}$  showed aromatic C-H in plane bend.

Figure 4: FT- IR spectrum of methanol extract of *Syzygium arnottianum*.Table 3: FTIR Frequency range and functional groups present in the leaf extracts of *Syzygium arnottianum*.

IR Frequency range	Functional Groups
3363.86	O-H
3307.92	N-H
3292.49	N-H
3263.56	N-H
3203.76	N-H
2918.30	C-H
2850.79	C-H
1728.22	C=O
1627.92	N-H
1614.42	N-H
1571.99	C=C
1537.27	C=C
1517.98	C=C
1433.11	C=C
1379.10	C-H
1317.38	C-F
1228.66	C-F
1220.94	C-F
1201.65	C-F
1153.43	C-F
1031.92	C-O
696.30	C-Cl

The band at 1571.99  $\text{cm}^{-1}$ , 1537.27  $\text{cm}^{-1}$ , 1517.98  $\text{cm}^{-1}$ , 1433.11  $\text{cm}^{-1}$  showed alkenyl C=C stretch. The band at 1728.22  $\text{cm}^{-1}$  showed C=O stretching vibration of the peptide group means that some carbonyl compounds existed in the leaves of *S. arnottianum*. The band at 1317.38  $\text{cm}^{-1}$ , 1228.66  $\text{cm}^{-1}$ , 1220.94  $\text{cm}^{-1}$ , 1201.65  $\text{cm}^{-1}$ , 1153.43  $\text{cm}^{-1}$  showed C-F stretching present in the extract. The band at 1031.92  $\text{cm}^{-1}$  showed the stretching vibration of C-O. The band at 696.30  $\text{cm}^{-1}$  showed the presence of carbohydrates present in the leaves. Bands in 1220  $\text{cm}^{-1}$  represents C-O stretching of phenolics and asymmetric C-

C-O stretching of esters. The major components are identified based on the fingerprint characters of the peak positions, shapes and intensities<sup>24</sup>. Based on the analysis of functional groups, it can be proved that carboxylic acids, aldehydes, aromatics, alkenes, phenols or tertiary alcohols, alkanes, aliphatic bromo compounds and alkynes might be accountable for numerous pharmaceutical properties of *S. arnottianum*<sup>25, 26</sup>.

The FT-IR spectrum at 1101.28- 1152.10  $\text{cm}^{-1}$  is because of the vibration stretching for (C-H) bond of aromatic compound which contains carboxylic acids, aldehydes, aromatics, alkenes, phenols, alkanes, aliphatic bromo compounds, alkynes, carbonyl and ether group<sup>27</sup>. Alkenes are used as anaesthetics. Carboxylic acids help in maintaining the cell membrane and control nutrient use along with metabolism. Phenol is used as an antiseptic and is also used as a preservative in some vaccines. Alkynes have antifungal and antitumor activities. Phenol spray is used medically to help sore throat. The peak at 2923.95-2926.37  $\text{cm}^{-1}$  is allocated to the C-H stretching means that some alkane compounds were present in rare medicinal plants<sup>28</sup>. Hence, the FT-IR results showed that the leaves of *S. arnottianum* have antiseptic, anaesthetic, antimicrobial and antitumor activities. The functional groups appeared in FT-IR was related to qualitative phytochemical screening of methanol extracts and these studies pave a method for active separation of different phytochemical compounds with the help of GC-MS.

The plants species can be used for antifungal agents as it is confirmed that phenolic compounds from natural resources have antifungal activity<sup>29</sup>. Carboxylic acids, present in various plant metabolites, were linked with numerous antimicrobial and antifungal activities<sup>30</sup>. Hence the leaf extract of the studied species can be used as antibacterial agent.

Every organism is physiologically controlled by enzymes and hormones, which are basically proteins<sup>31</sup>. The leaves

of *S. arnottianum* contain rich content of proteins as observed in this study. Hence the present study substantiates the use of leaves as food supplement during the past.

## CONCLUSION

The present study concluded that the leaves of *S. arnottianum* have numerous medicinal properties and isolation of individual phytochemical constituents may proceed to find out novel drugs.

## ACKNOWLEDGEMENT

The first author acknowledges the Junior Research Fellowship from the Department of Environment and Climate Change (DoECC/E3/1296/2014 Dated 09.03.2015), Government of Kerala. The authors acknowledge the Kerala Forest Department for supporting the field work. Also acknowledges DST-PURSE and KSCSTE-SARD for instrumental support.

## REFERENCES

- Kumar A, Ilavarasan R, Jayachandran T. Phytochemical investigation on tropical plants. Pakistan Journal of Nutrition 2009; 8: 83-85.
- Savithamma N, Linga RM, Prabha B. Phytochemical studies of *Dysophylla myosuroides* (Roth.) Benth. In wall and *Talinum cuneifolium* (Vahl.) Willd. Phytotherapy Research 2011; 5: 163-169.
- Ramasubbu R, Divya C, Anjana S. A note on the taxonomy, field status and threats to three endemic species of *Syzygium* (Myrtaceae) from the southern Western Ghats, India. Journal of threatened taxa 2016; 8: 9384-9390.
- Saranya J, Eganathan P, Sujanapal P. Influence of altitudinal variation on the antioxidant capacity of essential oil of *Syzygium densiflorum* from southern Western Ghats, India. International Journal of Green Pharmacy 2013; 7: 287-300.
- Subramanian S, Woolford CA, Desai JV, Lanni F, Mitchell AP. Cis- and trans-acting localization determinants of pH response regulator Rim13 in *Saccharomyces cerevisiae*. Eukaryotic Cell 2012; 11(10):1201-1209.
- Nasrin SB and Pandian R. Qualitative and quantitative analysis of medical trees belonging to Myrtaceae family. 31<sup>st</sup> IRF International Conference 2015; 54-59.
- Roberts JKM, Xia JH. High-Resolution NMR Methods for study of higher plants. Methods in Cell Biology 1995; 49: 245-258.
- Alagammal M, Tresina SP, Mohan V. GC-MS determination of bioactive compounds of *Curculigo orchoides* Gaertn. Journal of Scientific Research and Reports 2011; 1: 49-52.
- Bharathy V, Sumathy BM, Uthayakumari F. Determination of phytochemicals by GC-MS in leaves of *Jatropha gossypifolia* L. Journal of Scientific Research and Reports 2012; 2: 286-290.
- Asha V, Jeeva S, Paulraj K. Phytochemical and FT-IR spectral analysis of *Caralluma geniculata* Grev. et Myur- An endemic medicinal plant. Journal of Chemical and Pharmaceutical Research 2014; 6(7): 2083-2088.
- Nithyadevi J and Sivakumar R. Phytochemical screening and GC-MS, FT-IR analysis of methanolic extract leaves of *Solanum torvum* Sw. International Journal of Research Studies in Biosciences 2015; 3: 61-66.
- Nishaa S, Vishnupriya M, Sasikumar JM. Phytochemical screening and GC-MS analysis of ethanolic extract of rhizomes of *Maranta arundinacea* L. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2013; 4: 52.
- Gamble JS and Fischer CE. Flora of Presidency of Madras. 1935; 1: 1-2017.
- Kalimuthu K and Prabakaran R. Preliminary phytochemical screening and GC-MS analysis of methanol extract of *Ceropegia pusilla*. International Journal of Research in Applied, Natural and Social Sciences 2013; 1: 49-58.
- Manfred H, Meier H, Zeeh B. Spectroscopic Methods in Organic Chemistry. 1997; 365.
- Asha KR, Priyanga S, Hemmalakshmi S, Devaki K. GC-MS Analysis of the Ethanolic Extract of the whole Plant *Drosera indica* L. International Journal of Pharmacognosy and Phytochemical Research 2017; 9(5): 685-688.
- Frankel E. Nutritional benefits of flavonoids, International conference on food factors: chemistry and cancer prevention. Abstracts C-2 1995.
- Gupta VK, Singh GD, Singh S. Medicinal Plants: Phytochemistry, Pharmacology and Therapeutics. 2010.
- Killedar SG and More HN. Estimation of tannins in different parts of *Memecylonum bellatum* Burm. Journal of Pharmaceutical Research 2010; 3: 554-556.
- Duke J. Duke's Phytochemical and Ethnobotanical Databases. 1998.
- Solari A, Uitdehaag B, Giuliani G. Aminopyridines for symptomatic treatment in multiple sclerosis. Cochrane Database System Reviews 2001.
- Yamaguchi S and Rogawski MA. Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. Epilepsy Research 1992; 11 (1): 9-16.
- Kumar JK and Prasad AGD. Identification and composition of biomolecules in medicinal plants of *Tephrosia tinctoria* and *Aloisia alibicans* by using FTIR. Romanian Journal of Biophysics 2011; 21: 63-71.
- Chen RJ, Xu GY, Wang HY. Study on isolation and structure of polysaccharide XC-1 from *Angelica sinensis* (oliv) diels. Chemical Bulletin 2001; 64 (6): 372-374.
- Dukor RK. Vibrational spectroscopy in the detection of cancer. HVS 2002; 3335-3360
- Sohrabi MR, Davallo M, Tadayyon F. Simultaneous determination of acetyl salicylic acid and acetaminophen in A.C.A Tablets by FT/IR/ATR spectrometry with multivariate calibration data treatment. Asian Journal of Chemistry 2005; 17(1):541-547.

27. Silverstein RM and Webster FX. Spectrometric Identification of Organic Compounds. John Wiley & Sons 1997; 12-20.
28. Starlin T, Raj CA, Ragavendran P. Phytochemical screening, functional groups and element analysis of *Tylophora pauciflora* Wight and Arn. International Research Journal of Pharmacy 2012; 3: 180-183.
29. Soundararajan V, Zuraini Z, Yeng C. The Antimicrobial efficacy of *Elaeis guineensis*: Characterization, *in vitro* and *in vivo* studies. Molecules 2012; 17:4860-4877.
30. Sultana T, Rashid MA, Ali MA. Hepatoprotective and antibacterial activity of ursolic acid extracted from *Hedyotis corymbosa* L. Journal of Scientific and Industrial Research 2010; 4:27-34.
31. Movasaghi Z, Rehman S, Rehman IU. Fourier transform infrared spectroscopy of biological tissues. Applied Spectroscopic Reviews 2008; 43: 134-179.