INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS

VOL.6 ISSUE 2, 2018; 173 – 179 ; http://ijpda.com; ISSN: 2348-8948

MEDFOOD'18 [1st February 2018]

National Conference on Phytochemicals in Medicinal Plants and Food

Department of Biochemistry, Bharathidasan University Constituent College for Women, Orathanadu - 614 625, Tamil Nadu, India

Research Article

Phytochemical analysis and Antioxidant effect of hexane extract of *Cassia fistula* using FT-IR and GC-MS analysis

J. Sujatha[1], S. Asokan[2]

1. PG and Research Department of Microbiology, Marudupandiyar College, Vallam, Thanjavur, TN, India - 613 403
2. Department of Microbiology, Annai college of Arts and Science, Kovilacheri, Kumbakonam-612 503

Date Received: 23rd January 2018; Date accepted: 29th January 2018; Date Published: 17th February 2018

Abstract

The aim of the present investigation is screening of phytochemical constituents for its free radical scavenging activity of hexane extract of *Cassia fistula* leaves. The preliminary screening was carried out by standard chemical method. It evidenced that the presence of phytochemicals such as alkaloids, anthraquinones, saponins, phenols, tannins, flavonoids and terpenoids in the hexane extract. Various functional groups like alcohols, phenols, carboxylic acid and ketones were associated with the extract were characterized using Fourier transform infrared spectroscopy (FTIR). A few common compounds available in the extract were identified by Gas chromatography – Mass spectrum (GC-MS) analysis. The DPPH free radical scavenging activity and 50 % inhibitory concentration (IC_{50}) was calculated and compared with the standard synthetic drug BHT. This study revealed that *C. fistula* hexane extract of leaves has alternative drugs for free radical it could be acted as a novel source of free radical scavengers.

Keywords: *Cassia fistula*, hexane extract, antioxidant, GC-MS, FT-IR

INTRODUCTION

Cassia fistula L. is a semi-wild Indian Laburnum commonly known as golden shower; it belongs to the family Fabaceae. This plant is commonly grown in Asia, South Africa, China and Brazil and each part of this plant is well known for its medicinal properties [1]. This plant is cultivated as ornamental tree due to its beautiful yellow flowers in tropical areas [2]. *C. fistula* is medium sized tree and grown up to 20 m height. The bark of this plant is a grey color. The leaves are dark green in color and are arranged in alternate. The fruit of *C. fistula* is dark colour with 60 cm long. The whole part of *Cassia fistula* tree has medicinal properties like astringent, cooling, purgative, tonic, laxative, antiperiodic [3], diuretic, anti-inflammatory, and antioxidant [4]. Moreover leaves are useful in treatment of skin diseases, burning sensation, dry cough and fever. Flowers are used in cardiac diseases and fever. The main chemical components of *C. fistula* are anthraquninone derivatives and tannins. Several investigations reported that *C. fistula* extract has therapeutical applications including anti-oxidant [5], anti-inflammatory [6,7] and antimicrobial [8,9], anticancer[10], anti-dermatophytic and wound healing properties.

Free radicals are unstable and as chemical mole-

cules independently having one or more unpaired electrons and they play role in metabolic activity. While exceeding the amount of free radicals in the body causes cell damage and tissues. This imbalance between free radical and antioxidant systems leads to the cause cardiovascular, diseases, cancer, aging etc [11]. Some of the common free radicals are nitric oxide, hydrogen peroxide, hydroxyl radical, superoxide anion radical etc. These are otherwise known as reactive oxygen species [12].

An antioxidant is defined as a molecule that prevents the oxidation of other molecule. Currently synthetic antioxidants like BHT (Butylated hydroxyl toluene) and BHA(Butylated hydroxyl anisole) are available to slowing or inhibit the free radicals formation and low effectiveness [13]. These drugs may cause negative health impact [14]. To overcome this problem plants are utilized in therapeutic applications. Plants are having natural antioxidants like phytochemicals such as phenols, flavonoids etc capable to scavenging harmful free radicals [15]. In this study hexane extract of *Cassia fistula* leaves was screened and characterized by FTIR, GC-MS and DPPH free radical scavenging activity.

Materials and methods

Preparation of hexane extract

The leaves were washed with tap water and distilled water. Washed leaves were air dried at room temperature for 3 days. The dried leaves were pulverized into fine powder. The hexane extract of the *C. fistula* leaf was prepared by soaking 10 g of fine powder in 100 ml of hexane solvent for 24 hours. Then, the extract was filtered using what man No.1 filter paper and collected the filtrate. The collected filtrate was packed in airtight container and stored in dark conditions. The extract was concentrated by vacuum rotary evaporator for the study of phytochemical screening and antioxidant studies.

Phytochemical screening of hexane extracts

Presence of bioactive phytochemicals like alkaloids, anthraquinones, glycosides, resins, quinines, saponins, phenols, tannins, flavonoids, steroids, terpenoids, proteins and sugars in hexane extract was carried out by following standard methodologies as described by Harborne[16, 17], Kokate [18], Trease and Evans [19] and Edeoga et al [20].

Finally the plant extracts were analysed using FTIR analysis, GC-MS analysis.

Antioxidant activity of hexane extract of *C. fistula* leaves

Antioxidant activity of hexane extract of leaf was analysed by Spectrophotometric method on the basis of determination of scavenging activity of DPPH free radical [10]. BHT was prepared at different concentrations (5, 10, 20, 30, 40, and 50 µg/ml) and considered as standard. Stock of test sample was prepared by dissolving 10 mg in 10 ml hexane at concentration of 1 mg/ml. From this stock solution, different concentration of 5, 10, 20, 30, 40, and 50 µg/ml was prepared. DPPH free radical was prepared by dissolving 1 mM DPPH in 3 ml methanol and kept in dark conditions to protect from sunlight by covering aluminium foil. A 3 ml of different concentrations of leaf extract and standard were mixed with 0.5 ml of DPPH solution and incubated in dark conditions for 30 min. After incubation, the absorbance at 517 nm is determined using UV-Vis Spectrophotometer. Methanol was used as blank. The percentage of free radical scavenging of leaf extract was calculated by following equation

$$\%\ of\ Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control} \times 100$$

The inhibition concentration to scavenge 50% free radical (IC_{50}) is determined by plotting a graph of concentration (µg/ml) against % of free radical inhibition.

Results and discussion

Phytochemicals analysis

Medicinally valuable plants are having large number of pharmaceutically important compounds which are considered for investigation of new herbal drugs for many harmful life threatening diseases like cancer, ulcer and tumors. Bioactive molecules from medicinal plants proved which inhibit microbial growth and radical scavenging activity. In this study preliminary phytochemical screening of hexane extract of *C. fistula* leaves was analysed for antioxidant activity. The results of phytochemical screening of hexane extract of *C. fistula* leaves are shown in Table 1. It concluded that the pres-

ence of phytochemicals such as carbohydrate, protein, phenols, saponins, flavonoids, alkaloids and terpenoids. Other phytochemicals such as, glycosides, steroids were not present in hexane leaf extract of *C.fistula*. Alkaloids, saponins and flavonoids are attributed to the medicinal properties of plants.

Table 1: Phytoconstituents screening of hexane extract of *C. fistula* leaves

Phytochemicals	Presence/Absence
Carbohydrates	+
Protein	+
Phenols	+
Saponin	+
Glycosides	-
Steroids	-
Flavonoids	+
Terpenoids	+
Alkaloids	+

FTIR

FTIR characterization studies are used to identify the functional molecules of the phytochemicals present in the plant extract or other materials. Figure 1 shows seven different absorption peaks at wave numbers which are corresponds to functional molecules of the hexane extract of *C. fistula* leaves. The strong and broad band was observed at 3268 cm^{-1} corresponds to H-bonded O-H stretch phenols and alcohols. The weak band at 2915cm^{-1} indicates the presence of H-bonded O-H stretch carboxylic acids. A very weak band was observed at 1625cm^{-1} corresponds C=O stretch ketones. The bands 1450 and 1010 cm^{-1} are assigned to C-C=C asymmetric stretch aromatic rings and C-O stretch ethers and esters, respectively. The narrow bands shown at absorption peak 1042 and 878 cm^{-1} are designated to C-N stretched aliphatic amines and N-H wag primary and secondary amines respectively. Hence this result concluded that the hexane extract of *C. fistula* has active functional groups like phenols, alcohols, carboxylic acid, ether, ester etc. These functional groups are associated with the bioactive phytochemicals in the leaf extract.

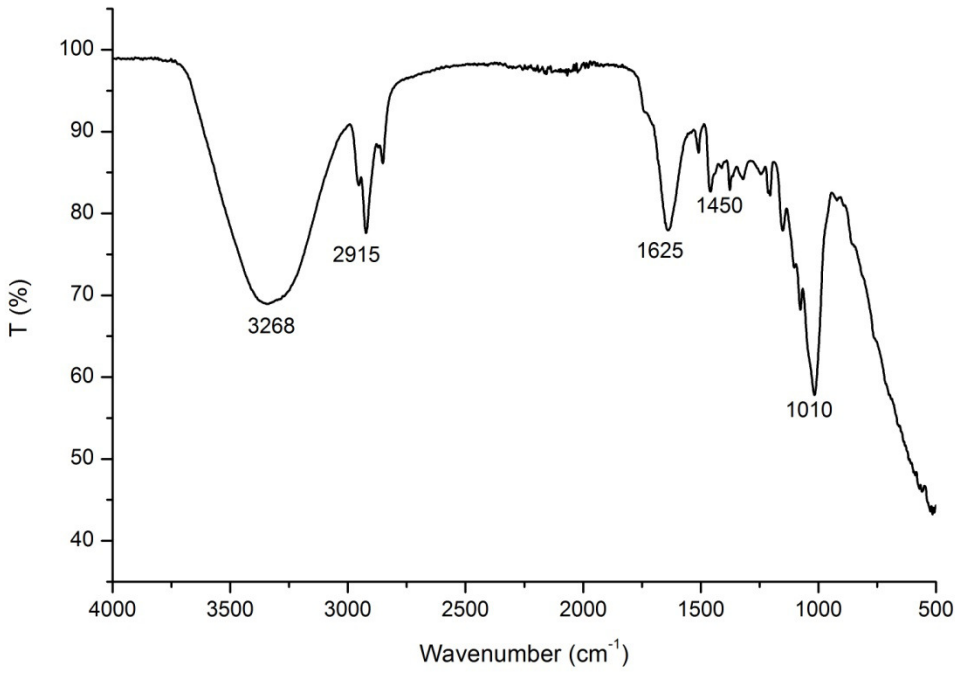

Figure 1: FTIR characterization shows the functional groups present in hexane extract of *Cassia fistula* leaves

Table 2: Functional groups of hexane extract of *Cassia fistula* leaves analysed by FTIR

S. No	Wave number (cm^{-1})	Functional groups
1	3268	H-bonded O-H stretch phenols and alcohols
2	2915	H-bonded O-H stretch carboxylic acids
3	1625	C=O stretch ketones
4	1450	C-C=C asymmetric stretch aromatic rings
5	1010	C-O stretch ethers and esters

GC-MS

GC-MS analysis of leaves of *C. fistula* hexane extract showed the presence of 6 components at the different retention time (Figure 2). 2-hydroxyethylhydrazine, phytol, n-hexadecanoic acid, oleic acid, cyclotrisiloxane, hexamethyl, di-n-decylsulfone. The molecular weight and formula of 6 main components is presented in Table 3.

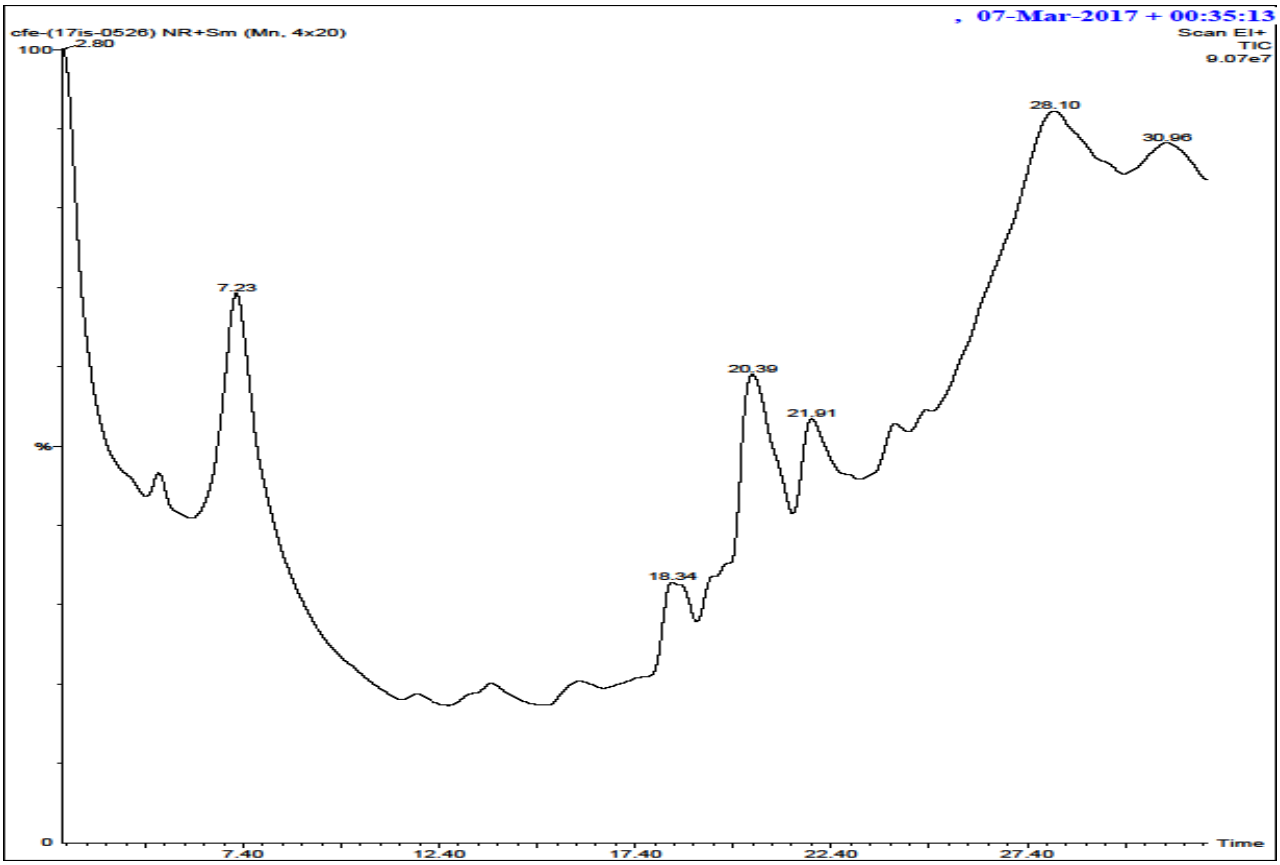

Figure 2: GC-MS analysis of hexane extract of *Cassia fistula* leaves

Antioxidant activity of hexane extract of leaves of *C. fistula*

Hexane leaf extract of *C. fistula* exhibited greater antioxidant activity compared to standard BHT at different concentration (10, 20, 40, 80 and 100 µg /ml). In a dose dependant manner, percentage of the antioxidant activity of leaf extract was increased as increasing the concentration (Figure 4). The leaf extract at a concentration of 10µg/ml showed a percentage inhibition was found to be

20.15 and for 50 µg/ml it was 91.12. The BHT at a concentration of 10µg/ml exhibited a percentage inhibition was found to be 20.15 and for 50 µg/ml it was noted as 59.23±1.23 (Table 4). The 50% inhibition concentration (IC_{50}) value of leaf extract and BHT was found to be 16.32µg/ml and 55.28 µg/ml, respectively. Regression analysis shows the good linear relation in plant extract towards concentration and inhibition activity.

Antioxidant activity is determined on the basis of the stable DPPH free radical accepting an electron from molecules. It is visually identified by changing colour from purple to yellow. In this study, flavonoids, tannins, alkaloids phenols were may responsible antioxidant activity which they donate an electron to DPPH and neutralizes the free radicals. Similarly,

Bhalodia *et al.*, [13] reported that antioxidant activity of flower extract of *C. fistula* plant. Proletariat [21] stated that the flavonoids, phenols and tannins may responsible for the free radical scavenging effects and they act as free radical scavengers. Some other reports demonstrated that phenol [22-24], flavonoids [25], saponins [26] and tannins [27] have been pounds have been found to possess potent antioxidants, antimicrobial and anti-inflammatory activity.

Table 3: GC-MS analysis of hexane extract of *Cassia fistula* leaves

Retention time	Name of the compound	Molecular weight	Molecular formula
7.23	2-HYDROXYETHYLHYDRAZINE	76	$C_2H_8ON_2$
18.34	PHYTOL	296	$C_{20}H_{40}O$
20.31	N-HEXADECANOIC ACID	256	$C_{16}H_{32}O_2$
21.91	OLEIC ACID	282	$C_{18}H_{34}O_2$
28.10	CYCLOTRISILOXANE, HEXAMETHYL	222	$C_6H_{18}O_3Si_3$
30.90	DI-N-DECYLSULFONE	346	$C_{20}H_{42}O_2S$

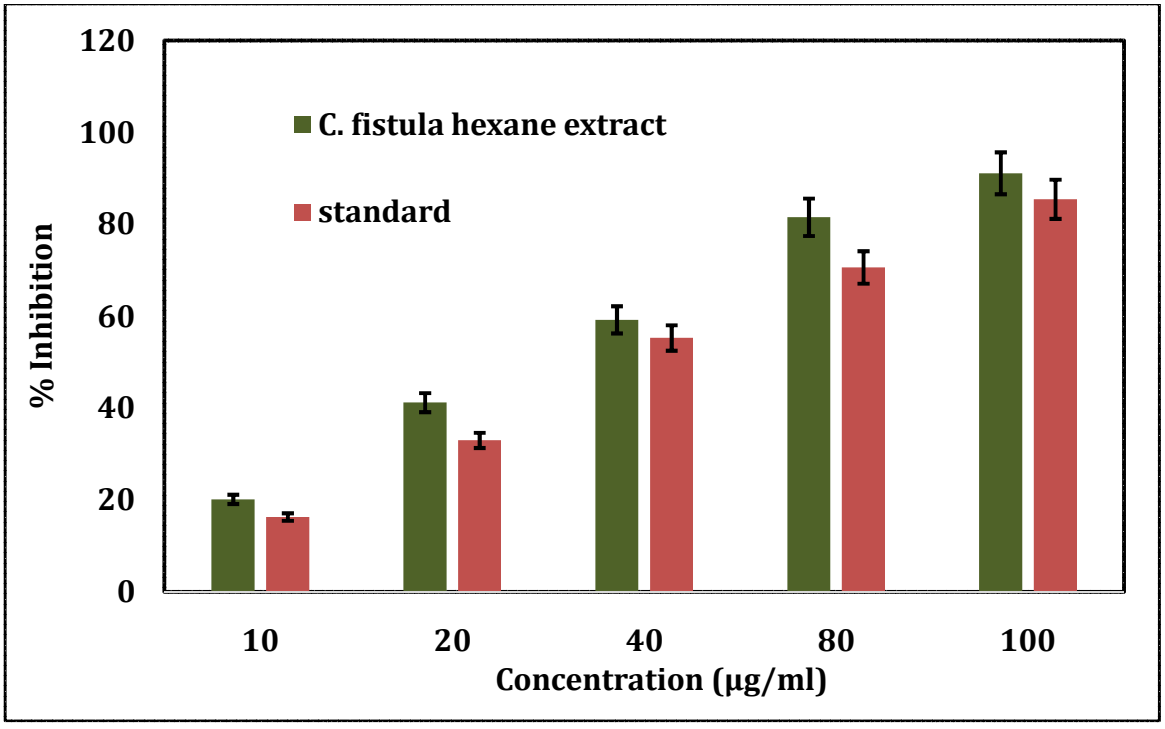

Figure 3: DPPH free radical scavenging activity of hexane extract of *Cassia fistula* leaves

Table 4: DPPH free radical scavenging activity of hexane extract of *Cassia fistula* leaves

Concentration of hexane *C. fistula* leaf extract (µg/ml)	% Inhibition of DPPH free radical	
	Plant extract	Standard
10	20.15	16.32
20	41.21	32.98
40	59.23	55.28
80	81.54	70.63
100	91.12	85.45

Conclusion

The qualitative preliminary screening shows the presence of alkaloids, flavonoids, phenols, terpenoids and anthraquinone were established. FTIR shows the available functional bioactive molecules in the hexane extract of *C. fistula* leaves. The 6 major components present in *Cassia fistula* hexane extract of leaves were identified by GC-MS. The potential antioxidant activity of *Cassia fistula* hexane extract of leaves was established by measuring DPPH radical scavenging at different concentrations. The activity was compared with synthetic drug shows greater percentage of inhibition of free radical.

References

1. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial antifungal agents from selected Indian medicinal plants, J Ethnopharmacol, 2006; 107: 182-188.
2. Bahorun T, Neergheen VS, Aruoma OI. Phytochemical constituents of *Cassia fistula*, African Journal of Biotechnology, 2005; 4(13): 1530-1540
3. Kirtikar KR, Basu BD. In: B. Singh and M. Pal Singh (Eds), Indian Medicinal Plants, Dehradun. 1975; 2: 858.
4. Satyavati GV, Sharma M. 1989. In Medicinal plant in India. ICMR, New Delhi.
5. Siddhuraju P, Mohan P S, Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. J. Agric. Food Chem. 2002; 79: 61-67.
6. Rajeswari R, Thejomoorthy P, Mathuram LN, Narayana-Raju KVS. Anti-inflammatory activity of *Cassia fistula* linn, bark extracts in sub-acute models of inflammation in rats. Tamilnadu J Vet Anim Sci, 2006; 5:193-199.
7. Kulkarni A, Govindappa M, Channabasava, Chandrappa CP, Ramachandra YL, Prasad SK. Phytochemical analysis of *Cassia fistula* and it's in vitro antimicrobial, antioxidant and anti-inflammatory activities, Advancement in Medicinal Plant Research, 2015; 3(1): 8-17
8. Irshad M, Singh M, Zafaryab M, Rizvi MMA. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. Int J Med Chem, 2012: 1-6
9. Irshad M, Aijaz A, Zafaryab M, Ahmad F, Manzoor N, Singh M, Rizvi MMA. Composition of *Cassia fistula* oil and its antifungal activity by disrupting ergosterol biosynthesis. Nat Prod Commun, 2013; 8:261-264.
10. Irshad M, Mehdi SJ, Al-Fatlawi AA, Zafaryab M, Ali A, Ahmad I, Singh M, Rizvi MMA. Phytochemical composition of *Cassia fistula* fruit extracts and its anticancer activity against human cancer cell lines. J Biol Active Prod Nat, 2014; 4 :158-170.
11. Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr, 1996; 6:33-50.
12. Joyce DA. Oxygen radicals in disease. Adv Drug React Bull, 1987);127: 476–79.
13. Berahou AA, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA. Antibacterial activity of *Quercus ilex* bark's extracts. J Ethnopharm, 2007; 112:426-429.
14. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. Evaluation of in vitro Antioxidant activity of Flowers of *Cassia fistula* Linn. International Journal of PharmTech Research, 2011; 3(1): 589-599
15. Ozgova S, Hermánek J, Gut I. Different antioxidant effects of polyphenols on lipid peroxidation and hydroxyl radicals in the NAPH-,

Feascorbate-, Fe-microsomal systems. Biochem Pharmacol., 2003; 66: 1127–1137.

16. Harbone JB. 1973 Phytochemical Methods, Chapman and Hall, Ltd., London, 49-188.
17. Harbone JB . 1998 Phytochemical Methods 3rd Edn., Chapman and Hall, London, 1-302.
18. Kokate CK . 2000 Practical Pharmacognosy, New Gyan Offset Printers, Delhi, 107-109.
19. Trease GE , Evans WC. 2002 Pharmacology. 15thEdn, Saunders publishers, London, pp.42-44.
20. Edeoga HO, Okwu DE, Mbabie BO. Phytochemical constituents of some Nigerian medicinal plants, African Journal of Biotechnology, 2005; 4:685- 688.
21. Polterait O. Antioxidants and free radical scavengers of natural orgin. Current Org Chem. 1997; 415-440
22. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC Compl Altern Med, 2009; 9: 30.
23. Lai HY, Yau YY, Kim KH. *Blechnum orientale* Linn - a fern with potential as antioxidant, anticancer and antibacterial agent. BMC Complem Altern Med, 2010; 10:15.
24. Garg VKR, Jain M, Sharma PKR, Garg G. Anti-inflammatory activity of *Spinacia oleracea*. Int J Pharma Prof Res, 2010; 1(1):1-4.
25. Lopez-Lazaro M. Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem, 2009; 9:31-59.
26. Mandal P, Babu SSP, Mandal NC. Antimicrobial activity of saponins from *Acacia auriculiformis*. Fitoterapia, 2005; 76:462-465.
27. Fawole OA, Amoo SO, Ndhlala AR, Light ME, Finnie JF, Van Staden J. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. J Ethnopharmacol, 2010; 127:235-241.