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High performance thin layer chromatography profile of *Cassytha filiformis*

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ABSTRACT

Objective: To study the phenols, flavonoids, saponin profile of the medicinal plant *Cassytha filiformis* (*C. filiformis*) using high performance thin layer chromatography (HPTLC). **Methods:** The extracts were tested to determine the presence of various phytochmeicals like alkaloids, phenolic compounds, flavonoids, carbohydrates, glycosides, saponins, terpenoids, tannins, fixed oils, fats and protein and aminoacids (Harborne and Harborne, 1998). HPTLC studies were carried out by Harborne and Wagner et al method. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. **Results:** The results of the preliminary phytochemical studies confirm the presence of phenols, alkaloids, carbohydrates, saponins, flavanoids, terpenoids and tannins in the methanolic extracts of *C. filiformis*. The methanolic extracts of *C. filiformis* displayed the presence of 13 types of phenolic substances with 13 different *Rf* values ranging from 0.01 to 0.96. The results illustrated the presence of 9 different types of flavonoides with 9 different Rf values ranging from 0.01 to 0.97. The results of HPTLC analysis of saponins demonstrated the presence of 11 different types of saponins with 11 different Rf values ranging from 0.04 to 0.92. **Conclusions:** In the present study we observed the phenols, flavonoids, saponin profile of the medicinal plant *C. filiformis* using high performance thin layer chromatography (HPTLC). Hence it was concluded that the phenolic compounds present in the methonolic extract could be responsible for antioxidant activities. Plant derived antioxidants, especially phenols and flavonoids, have been described to have various properties like anticancer, antiaging and prevention of cardiovascular diseases. Furthur, separation and characterization of the bioactive compound from the plant is to be evaluated and reported in near future.

1. Introduction

Many bioactive compounds have been found in the past but, for varying reasons, their activity has not been persued. On passing through newer screening procedure, a number of these compounds with previously unsuspected mode of action were rediscovered (*e.g.* ginkgolides) and other activities become apparent[1].

The use of 35 plant species including Ranunculus hirtellus and Anemone rupicola by local people of a tribal dominated winter land in Western Himalayas, for curing a total of 21 diseases ranging from simple stomach ache to highly complicated male and female disorders. In most of the cases (45%) underground part of the plant was used for curing ailments followed by leaves and aerial plant parts. Stem and flowers were the least plant part used [2].

Research interest has focused on various herbs that possess hypolipidemic, antiplatlet, antitumour, immune-stimulating properties, anti-inflammatory, anti-viral etc. that may be useful adjuncts in reducing the risk of cardiovascular disease, cancer and other diseases. A wide variety of active phytochemicals,including flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, phthalides, tannins, gallic acid, quercetin, phytosterols, alcohols, aldehydes have been identified from medicinal plants [3].These phtochemicals are estimated by a variety of techniques such as spectroscopy and chromatography. High performance thin layer chromatography (HPTLC) chromatographic fingerprints can be applied for this kind of certification. Fingerprint analysis by HPTLC has developed into an effective and powerful tool for linking the chemical constituents profile of the plants with botanical identity and for estimation of chemical and biochemical markers [4-8].

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The saponins from plants are believed to be useful for the human diet in controlling cholesterol and in treatment of tuberculosis. Alkaloids can increase nutrient absorption and blood circulation, reduce pain and stimulate nerve system as it has necrotic effect [14]. The biochemical activity of the *Allium* sp., Labiate, Umbelliferae, Zingiberaceae families, as well as flax seed and green tea have been reported. They are found to contain potent antioxidant compound that provide significant protection against chronic diseases. These compounds may protect LDL cholesterol from oxidation, inhibit cyclooxygenase and lipooxygenase enzymes, inhibit lipid peroxidation and have antiviral activity [3].

A wide array of phenolic substances particularly those present in dietry and medicinal plants have been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of these naturally occurring phenolics retain antioxidative and chemoprotective activity [10]. The antibacterial, antioxidant, anti-inflammatory, neuroprotective and calcium inhibitory effect of Acorus calamus have been reported [11,12]. The antioxidant activity of Oroxulum indicum, a well known medicinal plant was analyzed [13].

In Taiwan *Cassytha filiformis* (*C. filiformis*) was reported as a beneficial medicine against the gonorrhoea, kidney ailments and as the diuretic. In Africa it was used to treat the cancer, African trypanosomiasis and other diseases. They possess several aporphine alkaloids that are often used in the African folk medicine to treat certain diseases such as a cancer, African trypanosomiasis and other diseases as mentioned above [14]. In the traditional ayurveda, *C. filiformis* is used as the major substitute for *Cuscuta reflexa*. The brown colour of the stem is used as the colouring agent and hence possesses a major application in the dyeing industries [15].

2. Materials and methods

C. filiformis was collected from Anaimalai forest. The plants were carefully washed with tap water, rinsed with distilled water, and air-dried for 1 h. Then it was cut into small pieces & dried at room temperature for one week. Then they were ground into powder and stored at room temperature. Direct extraction with hexane, petroleum ether, acetone, ethyl acetate, ethanol and methanol was followed [16]. In this method, finely ground plant material was extracted with hexane, petroleum ether, acetone, ethyl acetate, ethanol and methanol in the ratio of 1:10 in conical flask in shaking condition. The extract was decanted in to pre-weighed glass vials. The process was repeated 3 times with the same plant material but using fresh solvent. The solvent was removed by placing the extracts in front of a steam of air in a fume hood at room temperature. The extracted residues were weighed. The dried extract was dissolved in 10% DMSO and stored in refrigerator until used. The extract was quantitatively determined for phenols, flavonoids, saponin. The sample extract (50 mg) was dissolved in methanol and made up to 1ml with methanol .The solution was centrifuged at 3 000 rpm for 5 min and the supernatant was collected. This solution was used as test solutions. For HPTLC analysis, 2 μL of the test solution and 2 μL of standard solution was loaded in the 5 mm band length in the 3 × 10 silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (Flavonoid) and the plate was developed in the respective mobile phase up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at white light, UV 254 nm and UV 366 nm. The developed plate was sprayed with respective spray reagent (Flavonoid) and dried at 100 °C in hot air oven. The plate was photo-documented at daylight and UV 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 254 nm. The peak table, Peak display and Peak densitogram were noted.

3. Results

The results of the preliminary phytochemical studies confirm the presence of phenols, alkaloids, carbohydrates, saponins, flavanoids, terpenoids and tannins in the methanolic extracts of *C. filiformis*. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The mobile phase Toluene-Acetone-Formic acid (4.5: 4.5: 1) was used for the HPTLC analysis of phenolic compounds. The spray agent 20% sodium carbonate solution reagent sprayed and followed by Folin Cio-calteu reagent. The methanolic extract of *C. filiformis* displayed the presence of 13 types of phenolic substances with 13 different *Rf* values ranging from 0.01 to 0.96 (Table 1). Blue coloured zone at day light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the presence of phenolic in the given standard and sample (Figure 1). The Ethyl acetate-Butanone-Formic acid-Water (5: 3: 1: 1) was used as the mobile phase for the HPTLC analysis of flavonoids. The spray agent 1% ethanolic Aluiminium chloride was used. The results illustrated the presence of 9 different types of flavonoides with 9 different Rf values ranging from 0.01 to 0.97 (Table 2). Yellow coloured fluorescent zone at UV 366 nm mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the presence of flavonoid in the given standard and sample (Figure 2).

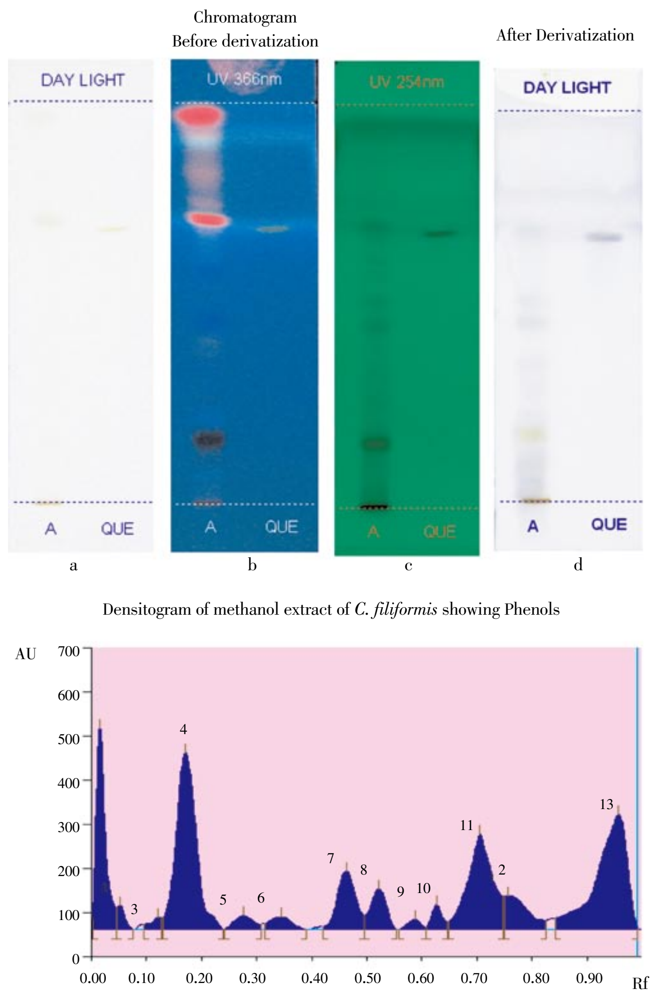

Figure 1. HPTLC Chromatogram of Phenols.
a-HPTLC plate exposed to day light; b- HPTLC plate exposed to UV 366 nm; c- HPTLC plate exposed to UV 254 nm; d- HPTLC plate showing phenol after adding the spray agent.

Table 1

Rf values of different phenols obtained using HPTLC analysis.

Track	Peak	*Rf*	Height	Area	Assigned substance
Sample A	1	0.01	456.0	7154.6	Unknown
Sample A	2	0.05	54.3	692.3	Phenolic 1
Sample A	3	0.12	27.8	511.6	Phenolic 2
Sample A	4	0.17	400.2	13510.3	Unknown
Sample A	5	0.28	30.6	994.4	Phenolic 3
Sample A	6	0.34	27.9	1024.9	Phenolic 4
Sample A	7	0.46	131.8	4103.9	Phenolic 5
Sample A	8	0.52	91.9	2432.3	Phenolic 6
Sample A	9	0.59	23.3	487.3	Phenolic 7
Sample A	10	0.63	55.7	992.8	Unknown
Sample A	11	0.70	214.8	8948.2	Phenolic 8
Sample A	12	0.76	76.1	3002.7	Unknown
Sample A	13	0.96	259.9	13093.0	Unknown
QUE	1	0.69	506.8	12455.6	Quercetin standard

The Chloroform-Glacial acetic acid-Methanol-Water (6.4: 3.2: 1.2: 0.8) was used as the mobile phase for the HPTLC analysis of saponins. The spray agent anisaldehyde sulphuric acid was used. The results demonstrated the presence of 11 different types of saponins with 11 different *Rf* values ranging from 0.04 to 0.92 (Table 3). Blue, brown and yellow coloured zones at day light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the presence of saponin in the given standard and sample (Figure 3).

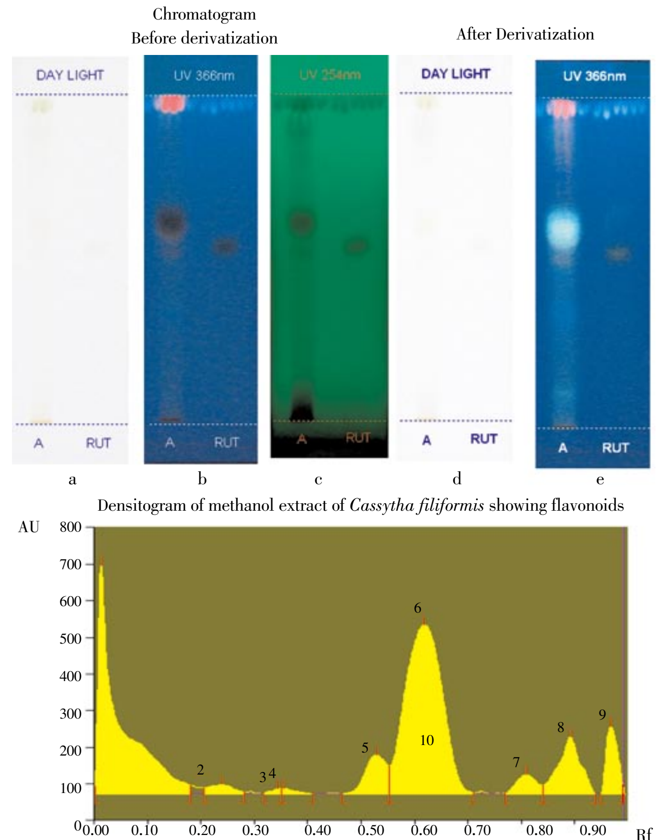

Figure 2. HPTLC Chromatogram of flavonoids.
a-HPTLC plate exposed to day light; b- HPTLC plate exposed to UV 366 nm; c- HPTLC plate exposed to UV 254 nm; d- HPTLC plate showing flavonoids after adding the spray agent exposed to day Light; e- HPTLC plate showing flavonoids after adding the spray agent exposed to UV 366 nm.

Table 2

Rf values of different flavonoids obtained using HPTLC analysis.

Track	Peak	*Rf*	Height	Area	Assigned substance
Sample A	1	0.01	624.9	22094.5	Unknown
Sample A	2	0.24	26.7	1113.9	Unknown
Sample A	3	0.34	15.4	254.6	Unknown
Sample A	4	0.35	15.0	354.7	Unknown
Sample A	5	0.53	106.2	3863.9	Unknown
Sample A	6	0.62	462.0	29836.0	Flavonoid 1
Sample A	7	0.81	53.1	1870.3	Unknown
Sample A	8	0.89	156.4	5938.4	Unknown
Sample A	9	0.97	184.2	3693.1	Unknown
RUT	1	0.54	399.4	17447.4	Rutin

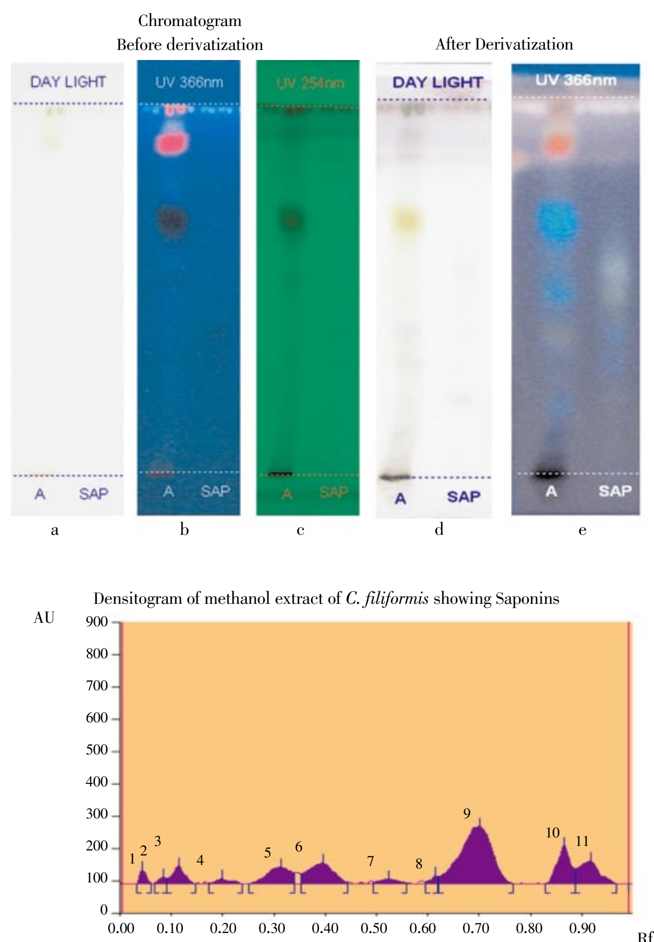

Figure 3. HPTLC Chromatogram of Saponins.

a-HPTLC plate exposed to day light; b- HPTLC plate exposed to UV 366 nm; c- HPTLC plate exposed to UV 254 nm; d- HPTLC plate showing Saponins after adding the spray agent exposed to day light; e-HPTLC plate showing Saponins after adding the spray agent exposed to UV 366 nm.

Table 3

Rf values of different saponins obtained using HPTLC analysis

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.04	41.9	521.3	Unknown
Sample A	2	0.08	20.7	259.0	Unknown
Sample A	3	0.12	55.5	1225.1	Saponin 1
Sample A	4	0.20	17.6	438.7	Saponin 2
Sample A	5	0.31	52.8	2213.9	Saponin 3
Sample A	6	0.40	64.9	2979.8	Saponin 4
Sample A	7	0.52	15.7	515.0	Saponin 5
Sample A	8	0.61	25.2	316.0	Unknown
Sample A	9	0.70	177.1	10282.3	Saponin 6
Sample A	10	0.86	118.4	2813.6	Unknown
Sample A	11	0.92	70.8	2579.7	Unknown
SAP	1	0.20	84.5	2693.9	Saponin standard 1
SAP	2	0.28	21.8	556.9	Saponin standard 2
SAP	3	0.38	23.7	627.2	Saponin standard 3
SAP	4	0.55	38.0	1079.9	Saponin standard 4

4. Discussion

Plant derived antioxidants, especially polyphenols and flavonoids have been ascribed to various properties like anticancer, antidiabetic, antiaging and prevention of cardiovascular diseases [12, 13]. The action of polyphenols is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [19]. Polyphenolic compounds like flavonoids have been labelled as "high level" natural antioxidants based on their abilities to scavenge free radicals and active oxygen species [20]. They contain conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants in vitro or cell free system by scavenging superoxide anion, singlet oxygen, lipid peroxyradicals and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species [21]. Some reports also indicated that the *Cassytha* sp. contains variety of bioactive components such as Vanillic acid, alkaloids, isoquinoline alkaloid, triterpenoids, flavonoids, sesquiterpenelactones, sesquiterpenoids, phytosterols, steroids, fatty oil, essential oil, liganans, saponins, vitamins, minerals, sugars, organic acids and trace elements [22].There is now a strong consensus that flavonoids and related polyphenols are responsible for much of anti oxidant activity of fruits and vegetables [23-25]. Many fruits and vegetables are rich in flavonoid content, consuming flavonoid regularly increases longevity by reducing inflammation and contributing to the amelioration of atherosclerosis from CHD [24]. Green tea is the commonly used beverage in Asian countries is a significant source of polyphenols. These polyphenols have recently attracted the medicinal attention as bioactive agents with anticancer, antidiabetic, antiviral, antimalarial, hepatoprotective, neuroprotective and cardioprotective effects. Ficus flavonoid leucopelargonin and its derivative isolated from *Ficus bengalensis* are proved as good hypoglycemic agents and antioxidants. *Ficus racemosa* also showed significant anticancer and antihelminthic activities [26]. The preliminary HPTLC analysis of methanol extract of *C. filiformis* shows the presence of various phytochemicals such as phenols, flavonoids, saponins. This can be used in the pharmaceutical industry as a pharmacognostical tool to identify this medicinally important plant. In addition it can be adopted as a chemo-taxonomical tool in the plant systematic. Furthur, the separation and characterization of the bioactive compound from the plants is to be evaluated and reported in near future.

References

[1] Anton R, Bereyz A, Hang-Berrurier H. New properties of old compound,Biologically Active Natural Products,Clarendon

Press.1991:117–125.Oxford.

[2] Uniyal S.K, Singh,K.N, Jamwal P, Lal.B Traditional use of medicinal plants among the tribal communities of Chhota Bengal, Western Himalaya. *J. Ethnobiol. Ethnomed*. 2006; **2**(14): 90–95.

[3] Craig. W, Beck.L Phytochemicals:Health protective effects. *Can J Diet Pract Res.* 1999; **60**(2): 78–84.

[4] Patil AG, Koli SP, Patil DA, Chandra N, Pharmacogonostical standardization and HPTLC fingerprint of Crataeva tapia Linn.SSP. Odora(Jacob.) Almeida leaves. *Int.j.pharm.Biosci.*2010; **1**(2): 1–14.

[5] Ramya V, Dheena Dhayalan V, Umamaheswari S. In vitro studies on antibacterial activity and separation of active compounds of selected flower extracts by HPTLC. *J.Chem.Pharm.Res.*, 2010; **2**(6): 86–91.

[6] Manikandan A,Victor Arokia Doss A.Evaluation of biochemical bontents, nutritional value,trace elements,SDS–PAGE and HPTLC profiling in the leaves of Ruellia tuberose L.and Dipteracanthus patulus (Jacq.).*J.Chem.Pharm.Res.*2010; **2**(3): 295–303.

[7] Yamunadevi M, Wesely EG, Johnson M. Chromotographic fingerprint analysis of steroids in Aerva lanata L.by HPTLC technique.Asian Pac.*J.Trop.Biomed.*2011; **1**: 428–433.

[8] Yamunadevi M, Wesely EG, Johnson M.Chemical profile studies on the alkaloids of medicinally important plant Aerva lanata L using HPTLC.*J.Nat.Conscientia*.2011; **2**(2): 341–349.

[9] Ong, H.C. Tumbuhan liar khasiat dan kegunaan lain,Kulala Lumpur,Utusan publication. 2004: 6–9.

[10]Surh, Y. Molecular mechanism of chemoprotective effect of selected dietry and medicinal phenolic substances. *Mutat. Res.* 1999; **428**(1): 305–327.

[11]Bhuvaneswari, R, Balasundaram.C Antibacterial activity of Acorus calamus and some of its derevatives against fish pathogen Aeromonas hydrophia. *J. Med. Plants Res*. 2009; **3**(7): 538–547.

[12]Muthukumaran, A, Singh.N Atteneuting effect of Acorus calamus extract in chronic constriction induced neuropathic pain in rats;an evidence of anti–oxidative,anti–inflammatory,neuroprotective and calcium inhibitory effects, BMC complement.*Altern.Med.* 2011; **11**(24):1–14.

[13]Gupta, R.C, Sharma,V Sharma,N Kumar ,N Singh. B In vitro antioxidant activity from leaves of Oroxylum indicum– A North Indian highly threatened and vulnerable medicinal plant. *J. Pharma. Res.* 2008; **1**(1): 65–72.

[14]Abubacker, M, Prince,N.M Hariharan.Y Histochemical and biochemical studies of parasite host interaction of *Cassytha filiformis* Linn. and Zizyphus jujube Lamk. *Current science.* 2005; **89**: 2156–2159.

[15]Sakshy Sharma, K.K. Hullatti,K.K Prasanna ,S.M, Paras Sharma. Comparative morpho–anatomical and preliminary phytochemical studies of Cuscuta reflexa and *Cassytha filiformis*. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2010 ; **2**: 59–64.

[16]Eloff, J.N. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica,* 1998; **64**: 711–713.

[17]Rice–Evans, C.A, Miller,N.J Paganga.G Structure–antioxidant releationship of flavonoides and phenolic acids. *Free Radic. Biol. Med.*2004; **20**(7): 933–956.

[18]Dixion, R. A. Natural products and plant disease resistance, *Nature*, 2005; **411**: 843–847.

[19]Itagaki, S, Kurokawa,T Nakata,C Saito,Y Oikawa,S Kobayashi,M Hirano ,T Iseki.K In vitro and In vivo antioxidant properties of ferulic acid: A Comparatives study with other natural oxidation inhibitors. *Food Chem*. 2009; **114**: 466–471.

[20]Birt, D. S. Hendrich , W. Wang. Dietary agents in cancer prevention:flavonoids and isoflavonoids. *Pharmacology and Therapeutics,* 2001 ; **90**: 157–177.

[21]Klahorst, S. Exploring antioxidants.Wd.Food Ingred.2002:54–59.

[22]Sharma, M. The isoquinoline Alkaloids. Academic Press, Newyork, 1972 : 245.

[23]Frankel, E.N. J. Kanner, J.B. German, E. Parks, J.E. Kinsella. Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. *The Lancet*, 1993; 341: 454 – 457.

[24]Pietta, P.G. Flavonoids as antioxidants, *Journal of Natural Products,* 2000; **63**:1035–1042.

[25]Vinson J, Jang J, Yang J, Dabbagh Y, Liang X, Serry M, et al. Vitamins and especially flavonoids in common beverages are powerful invitro antioxidants which enrich low density lipoproteins and increase their oxidative resistance after exvivo spiking in human plasma. *Journal of Agricultural and Food Chemistry* 1999; **47**: 2502–2504.

[26]Manian R, Anusuya,N Siddhuraju ,P Manian. S The antioxidant activity and free radical scavenging potential of two different solvent extracts of Camellia sinensis (L.) O. Kuntz, Ficus bengalensis L. and Ficus racemose L. *Food Chem* . 2008; **107**(3):1000–1007.

[27]Harborne, J, A.Harborne. Phytochemical methods: a guide to modern techniques of plant analysis. Springer, 1998.

[28]Wagner H,Baldt S,Zgainski E,M. Plant drug analysis .Berlin: Springer, 1996.