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IDENTIFICATION OF BIOACTIVE CONSTITUENTS OF *ALBIZIA AMARA* USING FT-IR, HPTLC AND GC-MS

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ABSTRACT

Phytochemicals play an important role in health of human and animals. They have natural medicinal property. Bioactive phytoconstituents have active compounds which can scavenge free radical damage. These bioactive constituents in plants when combined with nutrients and fibers form an integral part of defense mechanism. The present study investigates the bioactive constituent of Albizia amara leaves which is abundantly available southern area of India. The free radical scavenging activity and the active constituents of leaves were studied by FTIR, chromatographic technique using HPTLC and GC-MS.

Keywords *– Albizia amara*, phytochemical screening, HPTLC, GC-MS, FTIR and *in-vitro* antioxidant assay

1. INTRODUCTION

Plants serve as natural source for cleaning the system and the environment we live in. Exploiting the natural source of abundance like trees and their parts leaves, flowers and fruits are been widely accessed for environmental engineering and health. Identifying a green source of abundance and using it reduces the risk of pollution and environmental hazard. *Albizia amara* tree grows rapidly in dry conditions and exhibit abundant growth especially in southern part of India. It is a characteristic tree of the dry regions and is similar to the thorny species of plants like acacias[5].

Albizia amara belongs to the family Fabaceae (Mimosoideae Roxb.Boivin), is commonly called silk tree or oil cake tree, have small to moderate size dark green leaves, small yellow fragrant and globose flowers in clusters. The leaves of *Albizia amara* is pinnately compound and they give raise to flowers and fruits mostly in cold season. The plant *Albizia amara* constitutes an important source of active natural source which differ widely in their chemical properties[16].

In this study 50% ethanolic leaf extract of plant *Albizia amara* was taken up for the investigation. Secondary metabolites such as alkaloids, flavanoids, phenols, terpenoids, and saponins responsible for the free radical scavenging and antioxidant activities of plants were investigated. Many active components especially polyphenols such as flavanoids, phenol acids tannins possess therapeutic effects[2]. To ascertain the presence of secondary metabolites 50% ethanolic leaf extract of *Albizia amara* was subjected to HPTLC

analysis. Using GC-MS, the bioactive components of the leaves was identified. Antioxidant capacity was also determined. As a confirmation of these compounds spectral study was also done.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The leaves of *Albizia amara* was collected from the suburbs of Salem. This plant was then authenticated having an index of BSI/SRC/5/23/13-14/Tech-2055 by Botanical Survey of India- Coimbatore

2.2 Preparation of plant extract

Fresh leaves of *Albizia amara* collected were shade dried and powdered. 100g of the dried leaf powder was extracted with 50% ethanol using soxhlet apparatus and the extract was used for all investigations.

Preliminary phytochemical screening

The preliminary phytochemical screening was done with ethanolic leaf extract of *Albizia amara* to detect the secondary metabolites; alkaloids[21], flavanoids[10], tannins and phenols[20], glycosides[4] and saponins[13].

2.3 Determination of total phenol content

The total phenol content (TPC) was determined in crude extracts of plant leaf *Albizia amara* followed by the references[19]with small alterations. To 0.5 ml of test sample, 1.5 ml (1:10 v/v diluted with distilled water) of Folic ciocalteau reagent was added and allowed to stand for 5 min at 22ºC. After 5 min, 2ml of 7.5% sodium carbonate was added and this mixture was incubated for 90 min in the dark with intermittent shaking. After incubation, the development of blue color was observed. The absorbance of blue color developed in different samples was measured using colorimeter at 725nm. The result of the total phenol content in leaf extract *Albizia amara* obtained was measured as Gallic acid equivalents GAE/g on the basis of standard curve of Gallic acid. All the resolution was carried out six times.

2.4 Determination of total flavanoids content

Total flavanoids content was determined following method[18]. In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M) and 0.15 ml of AlCl3.6H2O (0.3 M) were mixed. After 5 min, 1 ml of Noah (1 M) was added. The solution of mixture was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavanoids was made using ruin standard solution (0 to 100 mg/l) under the same procedure as described earlier. The value obtained for total flavanoids content was exist ins milligrams of ruin equivalents per g of dried fraction.

2.5 HPTLC analysis

HPTLC analysis is carried out using CAMAG LINOMAT 5 HPTLC instrument and CAMAG REPROSTAR3(photo documentation chamber).HPTLC is a chromatographic technique shows high resolution, reliability, simplicity, speed and accuracy[1].The ethanolic leaf extract of *Albizia amara* was subjected under vacuum, redissolved in methanol and centrifuged at 3000rpm for 5mins and diluted 2 times with methanol and used for analysis.5µl of the plant extract was loaded as 8mm band length in the 5X10 silica gel $60F_{254}$ TLC plate using Hamilton syringe. The sample loaded plate was kept in TLC twin trough developing chamber with respective mobile phases up to 90mm.The mobile phases or solvent used in this analysis for identification of flavanoids is 80% ethyl acetate,10% water,5% acetic acid

and 5% formic acid. The air dried plates were kept in a photo documentation chamber and the image captured in visible light at UV366 nm. The developed plate was dried in hot air oven to evaporate the solvents from the plate and thus detected by following method. Greenish or yellow fluorescence zone observed in the chromatogram confirms the presence of flavanoids.

2.6 GC-MS analysis

GC-MS is carried out using Claus 500 Perkin-Elmer(Auto system XL).Gas Chromatograph is equipped and coupled to a mass detector Turbo mass gold-Perkin Elmer Turbo mass 5.1 spectrometer with an Elite (100% Diethyl poly siloxane) and cross linked capillary column of 30m x 0.25 mm ID x 1µm. Using computer searches on a National institute standard and technology (NIST) Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS compounds present in the plants sample was identified.

2.7 FTIR analysis

FTIR analysis was carried out to determine the presence of functional group. For the study finely ground leaf sample was taken and mixed uniformly with 100 mg KBr (2% w/w) homogenized by using stir vortex and was used for the analysis[7].

2.8 Free radical scavenging assays

Free radical scavenging assay is carried out for DPPH (1,1- diphenyl 2-picryl hydrazyl), superoxide radical and nitric oxide scavenging activity with 50% ethanolic leaf extract of *Albizia amara*.

2.9 DPPH scavenging activity

The DPPH scavenging activity of leaf extract *Albizia amara* was assessed by following method using referances[17]. The 0.5ml of methanol solution and DPPH of 0.4mM was added to 1ml of plant extract at concentrations ranges from 125µg-1000µg and allowed to react in room temperature for 30minutes. Methanol served as the blank and DPPH in methanol without the extracts server as positive control. After 30 minutes the absorbance was measured at 517nm and converted to percentage radical scavenging activity as follows. Scavenging activity (%) = C-T/C*1. DPPH is a stable nitrogen-centered free radical compound change from violet colour to yellow upon reduction reaction due to hydrogen-donor.The substances which are able to perform this DPPH reaction can be considered as antioxidants and therefore radical scavengers[6].

2.10 Hydrogen peroxide scavenging activity

The percentage scavenging activity of hydrogen peroxide was determined by the method[12]. A solution of 40mM hydrogen peroxide was prepared in 50 mM phosphate buffer (pH 7.4).One ml of various concentration of extract and 0.6 ml of 40 mM hydrogen peroxide were mixed and incubated at room temperature for 10 minutes. After incubation, absorbance was measured at 230 nm against blank containing phosphate buffer without hydrogen peroxide. Ascorbic acid was taken as standard. Percentage scavenging activity was calculated.

2.11 Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity assay was based on a riboflavin light NBT system[14].The reaction mixture contain 0.5 ml of phosphate buffer (50 Mm, pH-7.6), 0.3 mL riboflavin (50 mom), 0.25 mL PMS (20 mM), 0.1 mL NBT (0.5 mM), prior to the addition of 1mL sample solution at varying concentrations (25-250 µg/ mL). The reaction was initiated by illuminating the reaction mixture with different concentrations of the methanol extracts using a fluorescent lamp. After 20 min of incubation, the absorbance was readed at

560 nm. The comparison is done for the standard reference control ascorbic acid. The percentage of inhibition of superoxide anion generation was calculated using the following formula

Scavenging activity (%) = 1- absorbance of sample x 100 absorbance of control

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis

Preliminary phytochemical screening of 50% ethanolic leaf extract *Albizia amara* depicted in table1 reveals the presence of flavanoids, tannins, terpenoids, phenols and saponins. Phytochemicals are otherwise known as primary antioxidants and has ability to protect the body from damage caused by free radicals inducing oxidative stress[17].

3.2 Quantitative analysis

The quantitative determination of phenols and flavanoids in 50% ethanolic leaf extract of *Albizia amara* was done. Levels of phenols were found to be 9.9mg catechol and flavanoids upto 4.9mg catechol. Quantitative analysis of plant *Albizia amara* holds antioxidant capacity. Phenols and flavanoids are the major secondary constituents present in plants with extensive phytopharmacological activity. Flavanoids are polyphenolic compounds and are antioxidants, they play a role in defense mechanism against reactive oxygen species beneficial to living beings[9].

3.3 HPTLC analysis for *Albizia amara*

A comparison has been done with the leaf sample and known reference standards. The result indicates the presence of flavanoids performing a light yellow colour fluorescence spot on the TLC plate with the Rf value 0.99. The developed chromatogram and Rf value will be specific with solvent system, and serve as a better tool for standardization of 50% ethanolic leaf extract of *Albizia amara.* This analysis helps to identify flavanoids and could be attributed to the synergistic effect of the plant which has the ability to scavenge free radical and be could be used as a new source of antioxidants.

Flavanoids are important plant pigments which are widely distributed in plants fulfilling many functions such as molecular messengers, physiological regulators, and cell cycle inhibitors and some flavanoids have inhibitory activity against organisms that cause plant diseases by oxidative stress[22].

3.4 Identification of active constituents by GC-MS

The results of GC-MS analysis reveal the presence of bioactive components present in 50% ethanolic leaf extract of *Albizia amara.* This is fast and efficient. The total mass of the unknown compound is normally indicated by the parent peak and is based on the retention time and a set of ions is characteristic of a certain compound[3]. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The components identified in 50% ethanolic leaf extract of *Albizia amara* is shown in fig2

The molecular compound 4-o-Methyl mannose shows at peak value 24.91RT peak, at 8.98RT n-Hexadecanoic acid, 14.64R, 9,12,5-Octa decanoic acid, 8.87RT n-Octadecanoic acid and 21.29RT 2,6,10,14,18,22-Tetra-cosahexaene. The above identified component has antioxidant activity, antibacterial activity, and anti-androgenic activity, anti inflammatory activity, anticancer activity, hepato protective and hypercholesteromic activity. Thus the identified active components in this leaf extract may help in various ailments used for many scientific activities.

3.5 FTIR analysis

The functional groups present in the leaf extract *Albizia amara* was identified by FTIR spectrum based on the peak values in the region of IR radiation. FTIR spectroscopy gives very accurate frequency in the spectrum and enables processing techniques such as spectral subtraction as well as it has a much shorter sampling time compared to wet chemical methods and even shorter than conventional spectroscopic techniques[11].

Figure: 3 reveals the spectral reflectance. A broad peak at 3379cm^{-1} could be due to the presence of alcohols and phenols. A sharp peak at 2924cm^{-1} may be due to the presence of primary amines and carboxylic acids. A band stretch at 2376cm^{-1}to 1627cm^{-1} is specific of plant due to nitocompounds and aromatic amino groups. At peak 1249cm^{-1} indicates the presence of alkenes and alkyl halides.

The FTIR analysis confirmed the presence of alcohols, phenols, alkanes, primary amines, aromatic amines, nitro compounds, alkenes and alkyl halides in the leaf extract of *Albizia amara*.

3.6 Free radical scavenging activity

Free radical scavenging activities in leaf extract *Albizia amara* is shown in fig4, fig4a depicts DPPH scavenging activity, and 4b shows H_2O_2 scavenging activity and 4c elicit Superoxide scavenging activity. All the three are non enzymic antioxidants and play a powerful role in maintaining the homeostasis of ROS.

For DPPH scavenging activity of leaf extract ascorbate standard was used as reference. The leaf extract shows DPPH scavenging has maximum of 83.71% scavenging activity at 1000µg/ml and for standard ascorbate at 1000µg/ml it was found to be 87%.Maximum inhibition activity shows at 1000mg/ml. It is a widely used method to evaluate the free radical scavenging ability of various samples[8].Similarly antioxidant scavenging activity of H_2O_2 and superoxide scavenging is carried out in leaf extract of *Albizia amara.* The radical scavenging activity in both H_2O_2 and superoxide, the maximum inhibition occurs at 73.98% and 98%. Thus explains that the plant leaf *Albizia amara* has good antioxidant potential.

Table 1: Phytochemical analysis of *Albizia amara*

Sr.No.	Phytochemical constituents	Indication
1.	Alkaloids	**+++**
2.	Flavanoids	**+++**
3.	Tannins	**+**
4.	Terpenoids	**+**
5.	Phenols	**++**
6.	Saponins	**++**

(+ based on the color intensity)

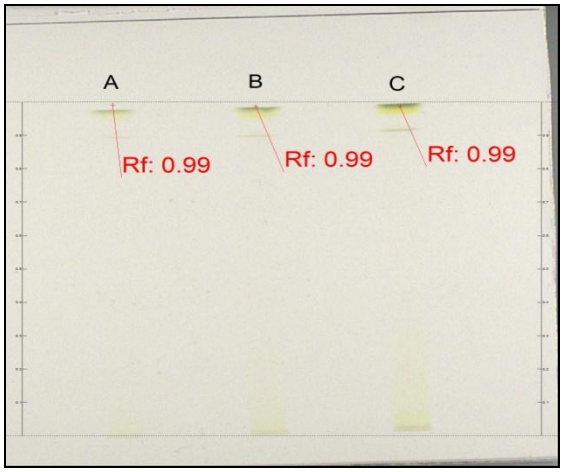

Figure 1 : HPTLC analysis of *Albizia amara*

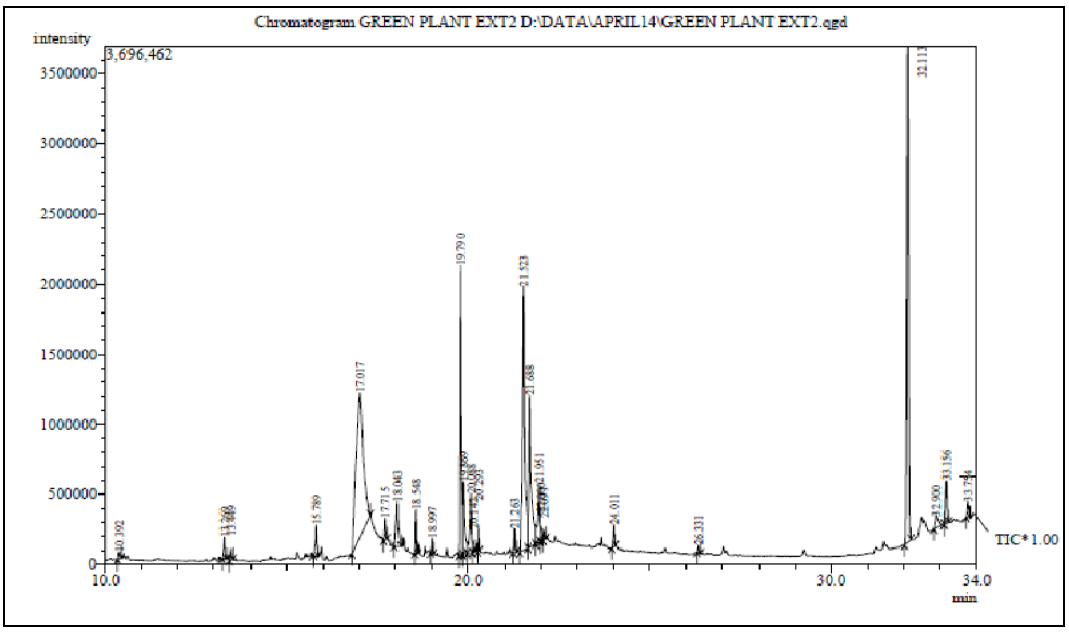

Figure 2: Results of GCMS analysis for active constituents of *Albizia amara*

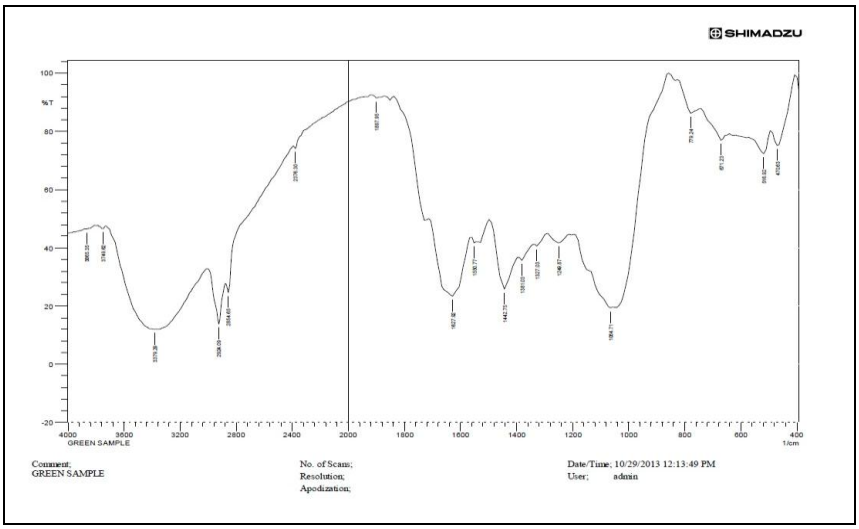

Figure 3: FT-IR analysis for *Albizia amara*

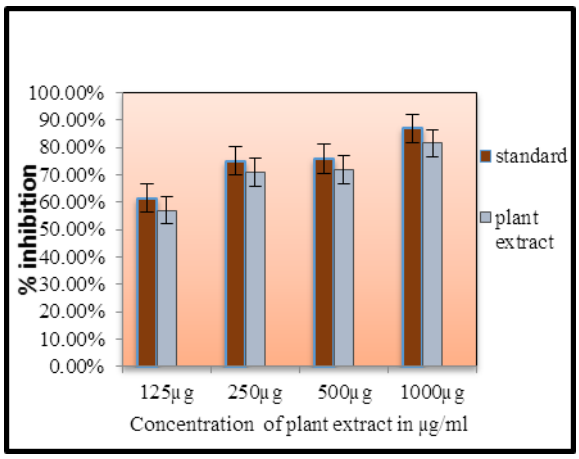

Fig. 4a: DPPH scavenging activity

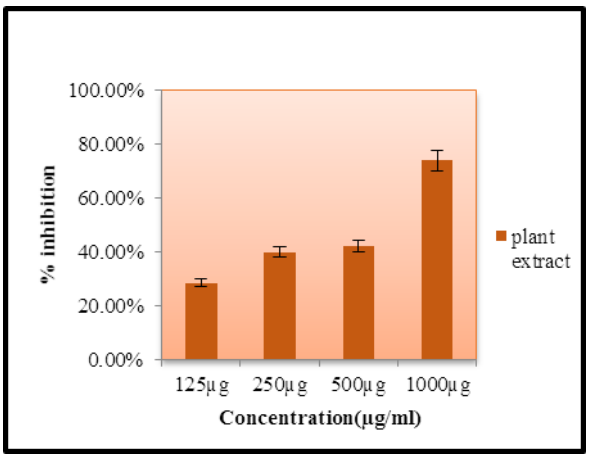

Fig. 4b:H_2O_2 scavenging activity

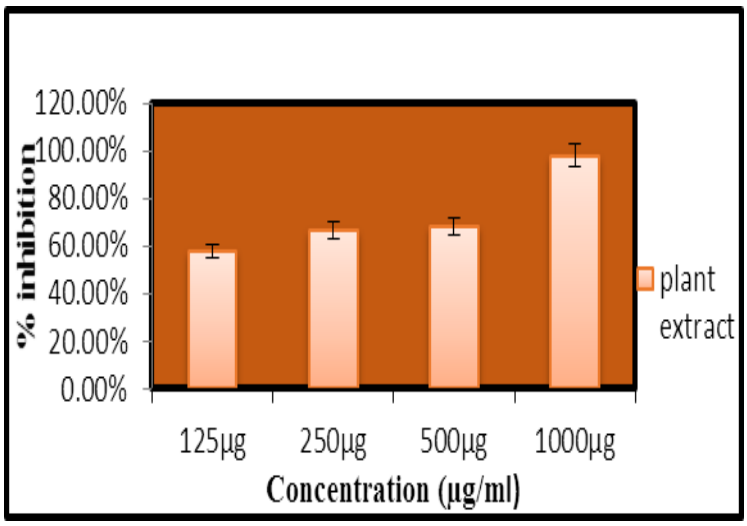

Fig. 4c: Superoxide scavenging activity

Figure 4: Free radical scavenging activity of *Albizia amara*

4. CONCLUSION

The phytopharmacological property of any plant is due to the presence of secondary metabolites and secretory products in it. The results of this study offer a platform for using this leaf extract *Albizia amara* for various research purposes. Six chemical components were signified with GC-MS. HPLC analysis shows the presence of flavanoids a potent antioxidant. FTIR analysis confirmed the existence of alcohols, phenols, alkanes, aromatic amines, primary amines, nitro compounds, alkenes and alkyl halides. The free radical scavenging of DPPH, SOD and H_2O_2 explains the leaf extract shows maximum scavenging potential at 1000µmg/ml. The results of the present study conclude that *Albizia amara* leaves have identifiable bioactive compounds which have antioxidant potential and can be used as plant therapeutics.

5. ACKNOWLEDGEMENT

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REFERENCES

1. Agarwal A A, Conner J. K and Stinch combe J. R. Ecology Letters(2004), 7: 1199-1208.

2. Ajam, S.M.S., Salleh, B., Al-khalil, S. and Sulaiman, S.F. International Conference on Environment, Chemistry and Biology (2012), 49: 150-155.
3. Amirav, A; Gordin, A,Poliak, M. Alon, T and Fialkov, AB, Poliak Marina F. *Journal of Mass Spectrometry* (2008),43 (2): 141–163.
4. Ayoola G.A, Coker H.A.B, Adesegun S.A, Adepoju-Bello A. Trop. J. Pharm. Res (2008), (7), 1019-1024.
5. Azhar I., Hasan M.M., Mazhar F. and Ali, M.S. Pakistan Journal of Pharmacology (2009) 26: 47-53.
6. Dehpour, A.A., Ebrahimzadeh, M.A., Navabi, S.F. and Nabavi, S.M. *Grasas Aceites*, (2009), 60(4): 405-412.
7. Derkacheva, O. Proceedings of first international workshop on physiochemical properties of lignins (2005), 255-257.
8. Ebrahimzadeh, M.A, Navabi, S.M., Navabi, S.F., Bahramian F, Bekhradnia., A.R Pak. J. Pharm. Sci.(2008),23,29-34.
9. Halliwell B, *Nutr. Rev.* (2012), 70(5)**:** 257-265.
10. Harborne J. B. Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis. (1998),1stedn: Chapman and Hall; London, UK.
11. Nakanishi, K and P.H. Solomon (1977). Infrared Absorption Spectroscopy, 2nd Edn. Holden-Day, Boca Raton, Fl, pp. 100 – 235.
12. Kaser S., Celik S., Turkoglu S., Yilmaz O., Turkoglu I.Chemistry Journal, (2012),2(1):9-12.
13. Kokate C.K. A Textbook for Practical Pharma-cognosy (2005), 5th Ed edn.
14. Kokwaro J.O.. Medicinal plants of East Africa. East African Literature Bureau Nairobi, (1976),pp.127.
15. Mensor L.L., Meneze F.S., Leitao, G G., Reis A S., Dos santor J C., Coube C S and Leitao S G. Phytother.Res. (2001) 15: 127-130.
16. Orwa C., A. Mutua, R. Kindt, R. Jamnadass, A. Simons. Botanical information, Agroforestery Database: a tree reference and selection guide version 4.0; (2009) p2.
17. Ozsoy N, Can A, Yanardag R, Akev N. Food Chem. (2008), 110: 571-583.
18. Park YS, Jung ST, Kang SG, Heo BK, Arancibia A P, Toledo F, Drzewiecki J, Namiesnik J, Gorinstein S.Food Chem, (2008),107:640–648.
19. Singleton VL, Orthofer R, Lamuela-Raventos RM, Methods Enzymol (1999), 299:152-178.
20. Trease, GE and WC Evans. A textbook of pharmacognosy, (1996),14th Ed. Bailliere Tindall Ltd. London
21. Wagner H, Baldt S, Zgainski EM. Plant drug analaysis. Berlin: Springer; 1996.
22. Zaveri M and Jain S. J Global Pharma Technol (2010)2:79–87.