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Research Article

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FTIR studies and spectrophotometric analysis of natural antioxidants, polyphenols and flavonoids in *Abutilon indicum* (Linn) Sweet leaf extract

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ABSTRACT

The present study was conducted to characterize various bioactive phytoconstituents in leaf aqueous extract of Abutilon indicum using FTIR and UV-Vis spectroscopy. Phytochemical profiling of leaf aqueous extract was carried out and the total phenolic compounds and flavonoids were determined spectrophotometrically following the standard methods. FTIR and UV-Vis profiles showed the presence of phenolic compound and flavonoids in leaf extract. The peaks at 304.0 nm and 278.0 nm in UV- spectrum confirmed the flavonoids and their derivatives. In addition to this, FTIR peaks obtained at 2171.7, 1636.2, 1407.1, 1347.6, 1130.4, 1254.5, 1047.1 and 904.3 cm^{-1} thereby confirming the presence of alcohol, phenol, alkanes, amino acids, aldehyde, aromatic compound, secondary alcohol, sulfur compounds, carboxylic acid, and amide groups in the extract. An intense peak observed at 3436.20 cm^{-1} in FTIR spectra correspond to the OH group. A spectrophotometric analysis of leaf extract showed 7.28 ± 0.15 µg of total phenolic compound (TAE per mg of dry leaf) and 9.13 ± 0.32 µg of flavonoids (QE per mg of dry leaf). The results obtained in the present study produced the FTIR and UV-Vis spectrum profile of medicinally important plant A. indicum which is helpful to screen and isolate various important phytoconstituents which are used for different kind of biological activities depending on their medicinal applications in pharmaceutical industries by herbal drugs manufacturers. Quantitative analysis indicates the presence of an appreciable amount of phenolic and flavonoids content in leaves thus, this plant can be used as a potent natural phenolic antioxidant in the treatment of various oxidative stress related diseases.

Keywords: *Abutilon indicum*, FTIR, flavonoids, total phenols, spectrophotometer, Antioxidant

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INTRODUCTION

Plants are the good sources for the discovery of pharmaceutical compounds and medicines which are used to cure ailments of human beings and other animals with no side effects compared with synthetic drugs. They have many secondary metabolites which confer specific characteristics and properties to plants [1]. Phytochemicals especially polyphenols constitute a major group of compounds that act as primary natural antioxidants [2] and thus an interest has been increased considerably among scientists, drugs and food manufacturers [3]. More than 4000 phenolic compounds (flavonoids, monophenols and poly phenols) are found in vascular plants. Phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents [4]. Medicinal plants are known to produce diverse substances possessing antioxidant properties having ability to protect the human body against cellular oxidation. Recent research suggested that diets rich in polyphenolic compounds and flavonoids are associated with longer life expectancy [5] and found effective in many health-related properties, such as anticancer, antiviral, anti-inflammatory activities, effects on capillary fragility and an ability to

inhibit human platelet aggregation [6]. Deshpande et al [7] have correlated natural phenolic antioxidants with reduced coronary heart disease.

Thus, detection of the chemical nature of phytochemical will provides information regarding different functional groups responsible for their medicinal properties. A variety of techniques are used to determine and analyze the presence of such in phytoconstituents in medicinal plants.

Chromatography and spectroscopic techniques are the most useful popular tools used for this purpose. The determination of phytoconstituents is mostly performed using relatively expensive and often laborious techniques such as LC-MS, GCMS, HPLC, HPTLC, NMR. However, simple, cost-effective and rapid tests for detecting phytocomponents are necessary. Spectroscopic methods like UV-Vis and Fourier Transform Infrared (FTIR) together or separate can be used to determine the phytoconstituents in plants. FTIR analysis is more sensitive, rapid, selective and authenticate method than spectrophotometry (UV-Vis), to characterize and identify functional groups of phytoconstituents [8][9]. Ultraviolet- visible spectrophotometry (UV-Vis) related to the spectroscopy of photons in the UV-visible region. The color of the chemicals is directly affects the absorption and the molecules present in colored solution undergo electronic transitions in visible ranges of spectrum [10]. In the present study, we recorded both UV-Vis and FTIR profiles of plant leaf extract to know the various phytoconstituents present in *A. indicum* plant leaf extract.

Once the nature of phytoconstituents presence in plants characterize, their quantification is necessary to justify its usefulness in various nutritional supplements, medicines and pharmaceutical formulations. As HPTLC, HPLC methods are very sophisticated and costly [11-13] the spectroscopic technique has becomes dominantly a powerful analytical techniques for the qualitative and quantitative analysis of biological materials of pharmaceutical importance [14][15]. Thus, in the present study we evaluated the total phenolic content and flavonoids - a biocompatible antioxidant with least side effects in plant leaf extract using spectrophotometer.

A. indicum is an herbaceous plant belongs to family Malvaceae and commonly known as 'Atibala/ Thuthi/ Kanghi'. It is a weed abundantly found in hotter part of India along road sides. This plant has been widely used in ayurvedic, herbal and folk medicine for the treatment of many diseases like diabetes, leprosy, ulcer, jaundice [16]. Plants of *A. indicum* possess many phytochemical with various bioactivities including antioxidant, anti-inflammatory and anticancer [17]. The plant is widely used as hepato-protective [18], anti-inflammatory, analgesic [19], antioxidative [20], hypoglycemic [21], antifungal [22], wounds healing [23], lipid lowering [24] and larvicidal [25] properties. The leaves are effective in ulcer [26], for the treatment of diabetes [27], diuretic infection and gingivitis [28].

A survey of literature revealed that FTIR and UV-Vis analysis of this medicinal plant was not done so far, thus, the present work has been aimed to produce UV-Vis and FTIR profiles of leaf extract to identify the phytoconstituents and further, to evaluate the natural antioxidants i.e. total phenolic compounds and flavonoids, present in leaf aqueous extract of *A. indicum*, using simple and authenticate method spectrophotometry, due to its enormous therapeutic uses.

EXPERIMENTAL SECTION

Collection and preparation of leaf aqueous extract

Plant leaves were collected from Green House, Department of Botany, The Institute of Science, Mumbai and authenticated in the Blatter Herbarium, Department of Botany, St. Xavier's College, Mumbai, where a Voucher specimen (no- K.V.S. 1888 of K.V. Shenoy) is deposited. Leaves were washed thoroughly in running tap water and followed by sterile distilled water to remove soil particles and adhered debris. Then dried under shed for three week and grounded. 5 gm of dried and powdered sample was mixed with 100 ml of distilled water and kept on shaker for 24 hours at room temperature. After incubation the solution was filtered through Whatmann No. 1 filter paper and volume was maintained up to 100 ml with distilled water. The filtrate was collected (crude extracts) into glass vials and kept at 4°C. All the studies were conducted with this extract within 15 days.

Spectroscopic analysis:

FTIR spectrum analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The aqueous extracts of *A. indicum* was mixed with KBr

salt, using a mortar and pestle, and compressed into a thin pellet and infrared spectra and peak values were recorded on a Perkin Elmer FTIR Spectrometer, between 4000–400 cm^{-1}.

UV-Vis analysis

For the UV- Vis profiling the leaf aqueous extract was diluted to 1:10 with the distilled water. The extracts were scanned in the wavelength ranging from 200-1100 nm using spectrophotometer (Shimadzu, UV-1800) and the characteristic peaks were detected.

Each and every analysis was repeated twice for the confirmation.

Quantitative spectrophotometric determination of phytochemicals present in leaf aqueous extract:

Estimation of the total phenolic content

Total phenolic content in leaf aqueous extract was determined by standard method of Makkar et al [29] with little modifications. Tannic acid was used as a standard phenolic compound. Standard calibration curve (Fig. 1) was plotted using known concentrations of tannic acid (10 – 100 µg/ml) at 760 nm. For analysis 250 µl of Tannic acid/ leaf aqueous extract was mixed with 1 ml of distilled water followed by the addition of equal amount of Folin-Ciocalteu reagent. The mixture was mixed and incubated at room temperature for 5 min before the addition of 7 % Na_2CO_3. Then final volume was made up to 6 ml with distilled water. Absorbance of blue colored mixture was observed spectrophotometrically at 760 nm. The total phenol content in the test samples was calculated from the standard curve and expressed as µg tannic acid equivalent (TAE) /mg of dry leaf. All the experiment was conducted in three replicates.

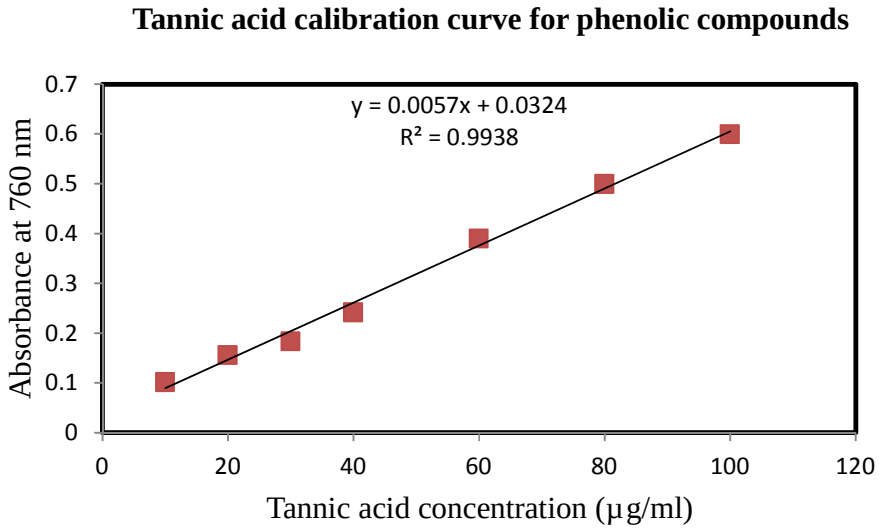

Figure 1: Calibration curve of tannic acid standard for total phenolic content

Total flavonoids content

Aluminum chloride colorimetric method was used for flavonoid determination based on Chang et al [30]. Reaction mixture containing 100 µl plant leaf extract in 2.0 ml of methanol followed by 0.1 ml of aluminum chloride, 0.1 ml of potassium acetate and 2.8 ml of distilled water was incubated at room temperature for 30 minutes. The absorbance of the colored reaction mixture was measured at 415 nm by spectrophotometer. Sample blank was prepared in similar way by replacing extract with distilled water. In this method quercetin was used as a standard to make calibration curve (Fig. 2). 10 mg of quercetin was dissolved in methanol and then diluted to make different concentrations (10 -50 µg / ml). Flavonoid content was estimated as quercetin equivalent µg / mg of dry leaf. All samples were run in triplicate.

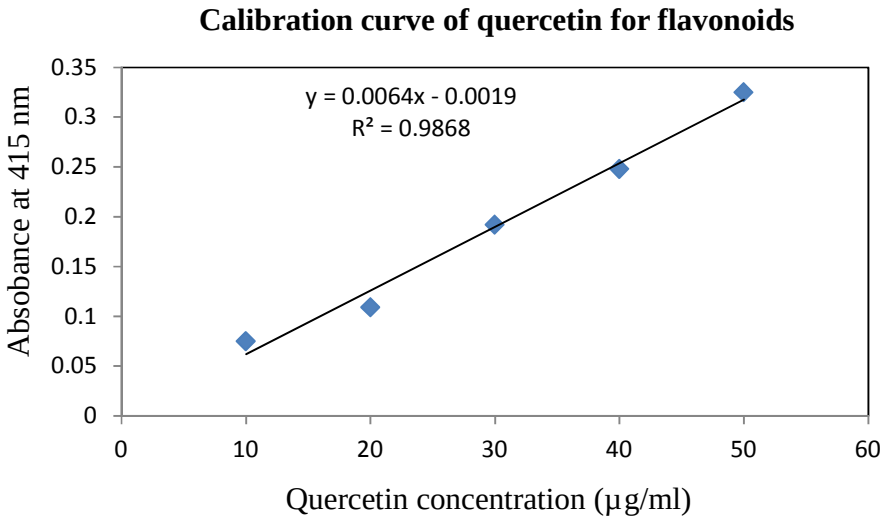

Figure 2: Calibration curve of quercetin standard for flavonoids

RESULTS AND DISCUSSION

FTIR fingerprinting analysis for functional groups identification:

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. Results of FTIR spectroscopic studies have revealed the presence of various chemical constituents in aqueous extract of *A. indicum* with various peaks values (Table 1 and Fig. 3) corresponds to 3436.2, 2171.7, 1636.2, 1407.1, 1347.6, 1254.5, 1130.4, 1047.1 and 904.3 cm^{-1} stretching frequency. The IR stretching frequency at 2171.7 cm^{-1} is due to the amino acids stretching frequency. A strong peak at 1636.2 cm^{-1} is to assign carbonyl C=O group which is primarily associated with amides. The band at 1407.1 and 1347.6 cm^{-1} was due to the -COOH and CH_2 groups, respectively. Nonappearance of any peak at 2260 cm^{-1} region indicates absence of cyanide group in the extract which shows nontoxic nature of the plants. A peak at 2171 and 1047 cm^{-1} confirms the presence of sulfur containing amino acids in proteins of *A. indicum* which are known to act as an antioxidant in plants under oxidative stress conditions [31]**.** The intense bands occurring at 1254.4 and 1130 stretching indicate the presence of esters and secondary alcohols. A strong peak at 3436.2 cm^{-1} stretching in FTIR is correspond to OH group which confirm the presence of phenolic compounds in leaf extract of *A. indicum*. Thus, The FTIR spectrum confirmed the presence of phenols, amino acids, amides, carboxylic acids, alkanes, aliphatic esters, secondary alcohols, sulfur compounds and mono substituted alkenes in leaf aqueous extract of *A. indicum.*

Table (1): **FTIR peak values of leaf aqueous extract of *Abutilon indicum***

Peak values	Functional groups
3436.2	Phenols
2171.7	Amino acids
1636.2	Amides
1407.1	Carboxylic acids
1347.6	Alkanes
1254.5	Aliphatic esters
1130.4	Secondary alcohols
1047.1	Sulfur compounds
904.3	Mono substituted alkenes

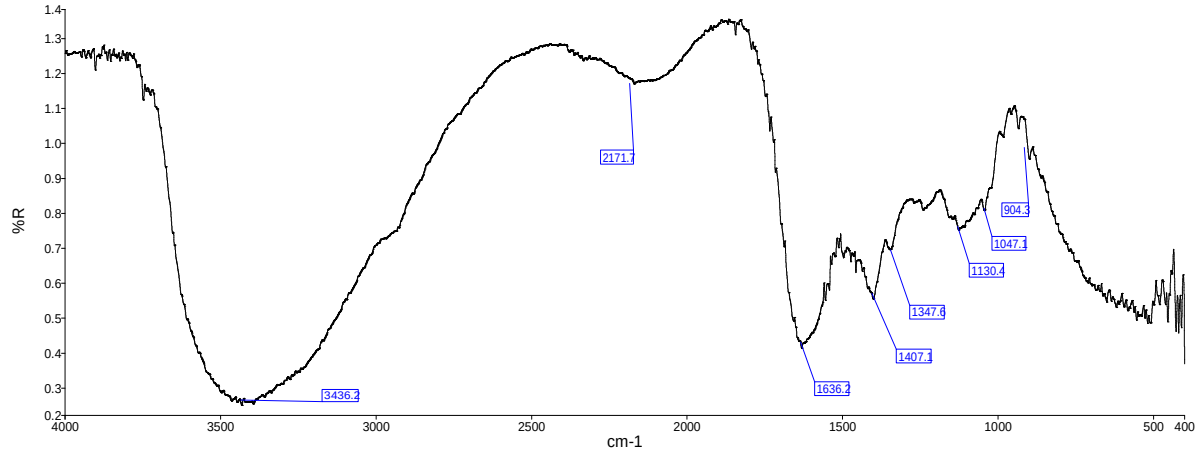

Figure 3: FTIR spectra of leaf aqueous extract of *Abutilon indicum*

UV-Vis profile:

The qualitative UV-Vis spectrum profile of leaf aqueous extract was taken at the 200 to 1100 nm wavelength. The profile showed two peaks at 321 nm and 270 nm with the absorption 1.975 and 1.487 respectively (Table-2 and Fig. 4). UV-Vis spectrum has absorption bands at 321 and 270 nm which are characteristic for flavonoids and its derivatives. The flavonoids spectra typically consist of two absorption maxima in the ranges 230-290 nm (band I) and 300-350 nm (band II). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoids. Our findings are in accordance with the previous studies [32][33].

Table (2): UV- Vis peak values of leaf aqueous extract of *Abutilon indicum*

S.No.	Wave length (nm)	Absorbance
1.	270	1.975
2.	321	1.487

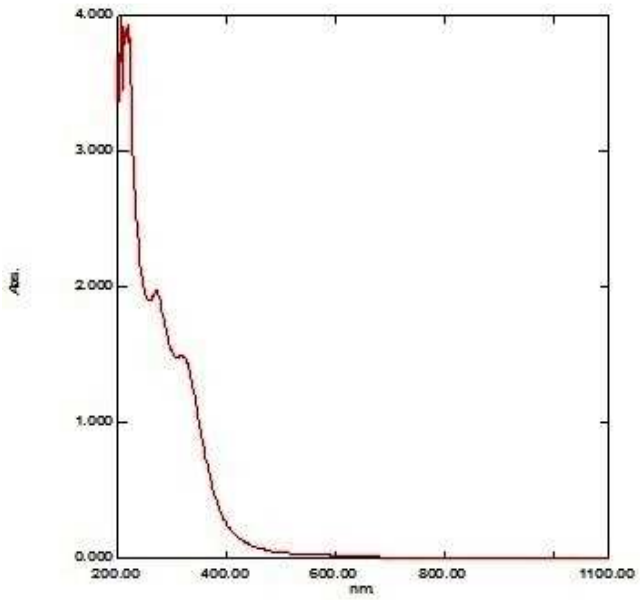

Figure 4: UV-Vis spectra of leaf aqueous extract of *Abutilon indicum*

UV-Vis and FTIR spectroscopy is proved to be a truthful and sensitive method for the detection of biomolecular composition. The FTIR and UV-Vis spectra of leaf extract confirmed the presence of phenols and flavonoids. As FTIR and UV –Vis profiles proved the presence of phenolic and flavonoids compounds

in leaf aqueous extract of *A. indicum*, the quantitative analysis is aimed to know the amount of these natural antioxidants in the leaf extract.

Quantitative spectrophotometric analysis for phenolic content and flavonoids:
Total phenolic content and flavonoids leaf aqueous extract were determined spectrophotometrically using the tannic acid and quercetin standard calibration curves, respectively, (Fig. 1 & 2). Both standard curves showed linearity with R^2 value 0.993 and 0.986. The total phenolic content was found to be 7.28 ± 0.18 µg of tannic acid equivalent (TAE) / mg of dry leaf. The flavonoids content was observed as 9.13 ± 0.32 µg of quercetin equivalent (QE) /mg of dry leaf (Table- 3)

Table (3): Total phenolic content and flavonoids content in leaf aqueous extract of *A. indicum*
Results are expressed as mean ± standard error (SE).

Phytochemicals	Amount (µg/mg dry leaf)
Total phenolic content (TAE)	7.28 ± 0.18
Flavonoids content (QE)	9.13 ± 0.32

Spectrophotometric analysis showed an appreciable amount of phenolic compounds (7.28 µg/mg dry leaf) as well as flavonoids (9.13 µg/mg dry leaf) in *A. indicum* leaf aqueous extract which make this plant suitable source of flavonoids and phenolic compounds. Various studies showed that flavonoids and polyphenols act as natural antioxidants. Antioxidant activity is directly related to the total amount of phenolics and flavonoids found in plant leaves extracts as reported by Dai et al [4], Dashpande et al [7] and Selvam et al [34]. The importance of antioxidant in plants materials for the treatment of various heart diseases and cancer is increasing interest among scientists, food manufactures and consumers. Tanaka et al [35] reported that polyphenol compounds had inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0g was ingested daily from a diet rich in fruits and vegetables.

CONCLUSION

Present study produced the UV-Vis and FTIR spectra for medicinally important plant *A. indicum* which can be used as an analytical tool for phytochemical biomarker to test not only the quality of the powder but also the presence of adulterants in pharmaceutical industry.

Further advanced spectroscopic studies are required for the structural elucidation and identification of compounds. Moreover, the leaf extracts of *A. indicum* is rich source of phenols and flavonoids, hence, it may be used as an antioxidants of natural origin in various drugs and pharmaceutical formulations against treatments of different oxidative stress related diseases.

SATISTICSL ANALYSIS

The results were recorded after repeating the experiments three times. The experimental results were expressed as mean ± standard error (SE) of (3n) measurements. The statistical analysis of the data were carried out using student's t-test and the results were considered significant when $p<0.005$.

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REFERENCES

[1] HPS Makkar; T Norvsambuu; S Lkhavatsere; K Becker, *Tropicultura*, **2009**, 27 (3), 159-167.
[2] P Anokwuru; I Esiaba; O Ajibaye; O Ayobami; O Adesuyi, *Research Journal of Medicinal Plant*, **2011**, 5, 557-566.
[3] J Loliger. Use of antioxidants in food, In: Aruoma OI and Halliwell B, Free Radials and Food Additives, Taylor and Francis, London, **1991**, 121-150.
[4] J Dai; R J Mumper, *Molecules*, **2010**, 15, 7313-7352.
[5] FB Hu, *Curr. Opin. Lipidol.* **2000**, 13, 3-9.
[6] O Benavente-García; J Castillo; FR Marin; A Ortuno; JA Del Río, *J. Agric. Food Chem*, **1997**, 45, 4505-4515.

[7] SS Deshpande, US Deshpande, DK Salunkhe. Nutritional and health aspects of food antioxidants. In DL Madhavi, SS Deshpande & DK Saunkhe (Eds.), Food antioxidants, New York, USA, Marcel Dekker, **1996**, 361-469.
[8] P Aysal; AD Ambrus; SJ Lehotay; A Cannavan, *J. Environ. Sci. Heal*, **2007**, 42, 481-490.
[9] M Grube; O Muter; S Strikauska; M Gavare; B Limane, *Journal of Indian Microbiology and Biotechnology*, **2008,** 35, 1545–1549.
[10] S Gunasekaran, *Asian Journal of Microbiology Biotech and Environmental Science*, **2003**, 5 (4), 581-582.
[11] V D Mendhulkar; S N Kharat, *Int J Pharm Bio Sci.*, **2015**, 6 (2) (B), 36 – 42.
[12] S N Kharat; R Singh; V D Mendhulkar, *Der Pharmacia Lettre* , **2015,** 7 (5), 236-244.
[13] PV Rajalakshmi; K Kalaiselvi Senthi, *J Pharmaceutical Sci Technol*, **2009**, 1 (2), 80-83.
[14] A Kale; S Gaikwad; K Mundhe; I Deshpande; J Salvekar, *International Journal of Pharma and Bio Sciences*, **2010**, 1 (3), 1-4.
[15] T Samatha; R Shyam Sundarachary; P Srinivas; N R Swamy, *Asian J Pharm Clin Res*, **2012**, 5 (4), 177-179.
[16] RN Chopra; SL Nayer; IC Chopra, *National Institute of science and communication*, CSIR publications, New Delhi, India.
[17] A Sharma; RA Sharma; H Singh, *Int. J. Pharm. Sci. Rev. Res*., **2013**, 20 (1) , 120-127.
[18] E Porchezhian; SH Ansari, *Phytomedicine*, **2005**, 1, 253-262.
[19] M Ahmad; S Amin; M Islam; M Takahashmi; E Okuyama, *Pharmazine*, **2000**, 55, 314-316.
[20] J Ahmad; I Khan, *J Plant Pathol and Microb*, **2012**, 3 (3), 124.
[21] Y Seetharam; G Chalageri; Ramachandra Setty; Sheemachar, *Fitoterapia*, **2002**, 73, 156-159.
[22] SK Prabhuji; DK Singh; AK Srivastava; R Sinha, *Medicinal Plants*, **2010**, 2, 215- 218.
[23] G Suresh; R Ganesana; M Dharmalingam; S Baskar; PK Senthil, *Int J Biol Med Res.*, **2011**, 2 (4), 908 – 911.
[24] RK Giri; SK Kanungo; VJ Patro; S Das; DC Sahoo, *J Pharma Res*, **2009**, 2 (11), 1725-1727.
[25] Abdul Rahuman; G Gopalakrishnan; P Venkatesan; K Geetha, *Parasitology Research.* **2008,** 102, 981-988.
[26] NL Dashputre; NS Naikwade, *Int J Pharma Sci Drug Res*, **2011**, 3 (2), 97- 100.
[27] B Lipinski, *J Diabetes Complications*, **2001**; 15, 203-210.
[28] C Krisanapun; P Peungvicha; R Temsiririrkkul; Y Wongkrajang; *Nutrition Research*, **2009,** 29 (8), 579-587.
[29] HPS Makkar; M Bluemmel; NK Borowy; K Becker, *J. Sci. Food Agric.*, **1993**, 61, 161–165.
[30] C Chang; Y Mang-Hua; W Hwei-mei; Jing- chuan, *Journal of food and Drugs Analysis*, **2002,** 10 (3), 178-182.
[31] R Singh**;** PK Srivastava; VK Singh; G Dubey; SM Prasad, *Acta Physiologiae Plantarum*, **2012**, 34 (3), 1119-1131.
[32] JK Kumar; AG Devi Prasad , *J. Biophys*. **2011**, 21 (1), 63-71.
[33] M Saxena; J Saxena, *Int J. Biol & Pharma Res*. **2012**, 3 (3), 498-501.
[34] K Selvam; S Arunprakash; TS Kumar; M Govarthanan; A Sengottaiyan, *Int J Pharma Sci Res*, **2012**; 3, 2011-2017.
[35] M Tanaka; CW Kuie; Y Nagashima; T Taguchi, Nippon Suisan Gakkaishi, 54, **1988**, 1409-1414.