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IN-VITRO STUDIES ON ANTI DIABETIC AND WOUND HEALING ACTIVITY OF *CHLOROXYLON SWIETENIA* LINN (RUTACEAE) LEAF EXTRACT

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ABSTRACT

The plant *chloroxylon swietenia* linn popularly known as Ceylon Satin wood as branched perennial herb (rutaceae) and distributed in peninsular India, Srilanka. It shows potential herbal remedy in the treatment of chronic nebritis, cancer, diabetes, convulsions and jaundice. Though the plant is used for multiple purposes still proper pharmacognostic standardization as not been established for the leaves part. In microscopic studies it had revealed the presence of abundant xylem fibers and xylem vessels. The successive solvent extraction of leaves showed the phytoconstituents like flavanoids, saponins, glycosides, tannins and phenols. They were further confirmed by TLC analysis. The TLC studies showed the presence of chemical constituents with colored spots and Rf values. *In Ova* wound healing activity on fertilized egg was performed by making a window of $1cm^2$ on fertilized eggs and sterile cellulose discs loaded with aqueous. The plant extract had shown promising the results for anti-diabetic activity with glucose absorption study method at 1000 mg/ml, glucose oxidized method 100 mg/ml and wound healing activity by chorioallantoic membrane method at 600 mg/ml.

KEYWORDS: Diabetes, Wound healing, Flavonoids, Glucose absorption study, TLC.

INTRODUCTION

India possess a rich biodiversity of the medicinal plants that were still not explored completely. The need for the novel pharmaceutical products from the plant has attained a great interest in the present biomedical research due to potent source of natural antioxidant. *Chloroxylon swietenia* is a medicinal plant that shows different types of pharmacological

activities like antimicrobial, antioxidant, antidiarrheal, antidiabetic and wound healing. The aim of this study was to evaluate the wound healing activity of extract from the leaves in Chorioallantoic membrane (CAM) model was used as *in vitro* model and anti diabetic activity done by glucose absorption study *in vitro* method.

The beneficial multiple activities like manipulating carbohydrate mechanism by various mechanisms, preventing & restoring integrity, function of β-cells, insulin-releasing activity , improving glucose uptake utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them in to novel therapeutics. The multifactorial pathogenecity of diabetes demands multimodal therapeutic approach.

Thus future therapeutic strategies require the combination of various types of multiple agents. Thus plant based herbal drugs and botanicals with free radical scavenging activity are emerging as the primary components of holistic approaches to diabetes management.

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated.

There are three stages to the process of wound healing: inflammation, proliferation and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which possess major drawbacks and unwanted side effects.

MATERIALS AND METHODS

Collection and authentification

Chloroxylon swietenia L commonly called as Ceylon Satinwood is a hard wood tree native to South India and Srilanka. The plant was collected from the month of November from the forest region of Thallaverappagudem region of Nalgonda district, Telangana. The plant material was identified and authenticated by F.Shankara chary Professor, Department of Botony, Womens Government Degree college, Nalgonda.

Chemicals and reagent requirements

Petroleum ether, Chloroform, Ethylacetate, Iodine (Accord labs), Acetone (Universal labs), phloroglucinol (Sigma aldrich), Glucose kit, other reagents used were of analytical grades and obtained from various other commercial sources**.**

Collection of fertilized chick embryos

The fertilized eggs were provided by "Tirumala breeders (Pvt, Ltd)., Hanmakonda, Warangal Dist., Telangana, India" and collected at "Tirumala breeders (P) Ltd., Vadaigudem, Yadagirigutta, Nalgonda Dist., Telangana.

Equipment requirement

Soxhlet apparatus, heating mantle (Biotehnics), test tubes, china dishes, petri plate, TLC plates, waterbath and thermometer (Omsons Hygrometer).

Extraction of plant material

The leaf powdered material was subjected to soxhalation using petroleum ether, chloroform, ethyl acetate, ethanol and water by successive solvent extraction method based on the increasing order of polarity of solvent. The yield of the extract was obtained 5.6% w/w. The petroleum extract was screened for phytochemicals by using TLC. The obtained crude extract was evaluated for antidiabetic activity by *in vitro* methods and wound healing by *in vivo* method.

Thin Layer Chromatography (Tlc)

Thin layer chromatography is a separating technique used to separate the mixtures of chemical constituents. It is the most basic method of confirming the presence of phytochemical compounds. All the successive extracts (leaves of *Chloroxylon swietenia*) are subjected to TLC and their profiles were noted.

Adsorbent : Pre-coated Silica Gel GF

Detection : UV Chamber short wave length (254nm) and long wavelength (365nm).

Extract : ethanolic extract used.

TLC for glycosides

Adsorbent: Pre-coated Silica Gel GF254

Solvent system: Ethyl acetate: Methanol: Water (77: 15: 8)

Visualization: UV chamber

TLC for tannins

Solvent system: Chloroform: Ethyl acetate: Acetic acid (6: 2: 2)

Visualization: UV chamber

TLC for flavonoids

Solvent system: Ethyl Acetate: Formic acid: Glacial Acetic acid: Water (6: 1: 1: 2)

Visualization: UV chamber

TLC for saponnins

Solvent system: Ethyl acetate: Ethanol: Water: Ammonia (65: 25: 9: 1)

Visualization: UV chamber

***IN VITRO* ANTI DIABETIC ACTIVITY**

Procedure for glucose oxidase method

0.05 ml glucose solution of 20, 40, 60, 80, & 100 mg/dl was added with 0.05 ml of extract in to different test tubes and incubated in dark for 4 hrs. Then 5 ml of glucose oxidize enzyme was added to all the test tubes and kept for 30 minutes in dark room at 27^0C. Results were spectrophotometrically recorded at 546nm. Concentration of glucose was measured by following equation.

Concentration of glucose =Ak/Au X C

Where Ak= Absorbance of known (standard glucose),

Au = Absorbance of unknown (extracts of plants)

C = concentration of standard glucose.

Calculation of 50% Inhibitory Concentration (IC_{50})

% Inhibition = (Ac-As)/Ac X 100

Where Ac = Absorbance of the control

As = Absorbance of the sample

Glucose absorption study

Absorption is process by which a drug enters into the body fluids. Absorption involves passage of drugs across cell membrane consists of lipids and proteins. They are highly selective permeable barrier membrane lipids are relatively small molecules. They have both hydrophilic and hydrophobic moiety. Proteins serve as pumps, gates, receptor, energy transducer and enzymes.

There is need to sacrifice the laboratory animals for performing *in vitro* absorption study. However the goat ileum is a tissue that is easily available from slaughter house and no animal no need to be scarified for the experimental purpose.

Goat ileum as an alternative research tool from laboratory animals for absorption study of various pharmaceutical preparations.

Procedure

The fresh ileum of healthy, black, male goat was obtained from slaughter house keep in ringer solution. The tissues were transported were under ice to the laboratory. The intestinal contents were removed by washing with ringer solution and mesenteric residues were dissected out.

About 4-5 cm of goat ileum was mounted in a student organ bath containing ringer solution. Tissue was maintained at 30+/- 1 c, aerated with air and resting load 1% glucose solution was prepared and inserted in ileum of goat by using syringe.

In the present study one end of intestine tied with silk thread and then 1% of glucose solution is directly inserted into the intestine using syringe and other end tide with silk thread tightly. So the glucose solution remained in the inner side of the intestine containing mucosal layer (donor environment). The tissue was mounted in the inner organ bath (receiver environment) containing ringers solution. In order to the assure cell survivality both the donor and receiver environment are supplied by oxygen feed with help of aerator.

Immediate after placing the intestine bag containing glucose solution the initial zero reading was taken afterward sample was withdrawn in interval of 30 minutes from receiver environment in a fix (1 ml) volume and same volume was replaced with ringer solution to maintain the constant volume of ringer solution in inner organ bath.

***In ova* angiogenetic activity**

Chorioallantoic membrane (CAM) model was used as *in vitro* method. In this method embryonated chicken eggs (9 days old) were selected and a small window (1 cm^2) was made in the shell. Through the window, a sterile disc of cellulose treated with control, 200 mg/ ml, 400 mg/ml, 600 mg/ml, of the aqueous extract of *chloroxylon switenea* was placed inside triplicate sets of egg at the junction of two blood vessels. The window was resealed and the eggs were incubated at 37^0C in a well humidified chamber for 72 hrs. The window was then

opened and growth new capillary blood vessels were observed and finally compared with control eggs containing sterile discs without any extract of the plant.

RESULTS

Successive solvent extraction

Color, consistency and yield of successive extracts of leaf *Chloroxylon swietenea* Linn.

Extaracts	L		
	Color	**Consistency**	**Yield % w/w**
Petroleum ether	Green	Oily	5.6
Chloroform	Greenish black	Waxy	0.94
Ethyl acetate	Greenish brown	Waxy	0.36
Alcohol	Brown	Waxy	1.5
Water	Red	Dry	1.85

Thin layer chromatography (TLC)

Thin layer chromatography extract of leaf of *Chloroxylon swietenia* was found to be phytoconstitutents like flavanoids, tannins, glycosides and saponins.

Solvent system	**Phytoconstituents**	**Rf values**	**Color of spot**
Ethylacetate: Formic acid: Glacial acetic acid: water (6:1:1:2)	Flavanoids	Spot 1 : 0.35 Spot 2 : 0.73 Spot 3 : 0.85	White fluorescence
Chloroform: Ethyl acetate: Aceticacid (6 : 2 : 2)	Tannins	Spot 1: 0.15 Spot 2: 0.78 Spot 3: 0.86 Spot 4: 0.92	Bluish fluorescence
Ethylacetate: Methnol: Ethanol (77:15:8)	Glycosides	Spot 1: 0.31 Spot 2: 0.41 Spot 3: 0.84 Spot 4: 0.88	Whitish fluoresce
Ethyl acetate: Ethanol: Water: Ammonia (65:25:9:1)	Saponins	Spot 1: 0.37 Spot 2: 0.68 Spot 3: 0.82	Whitish blue fluorescence

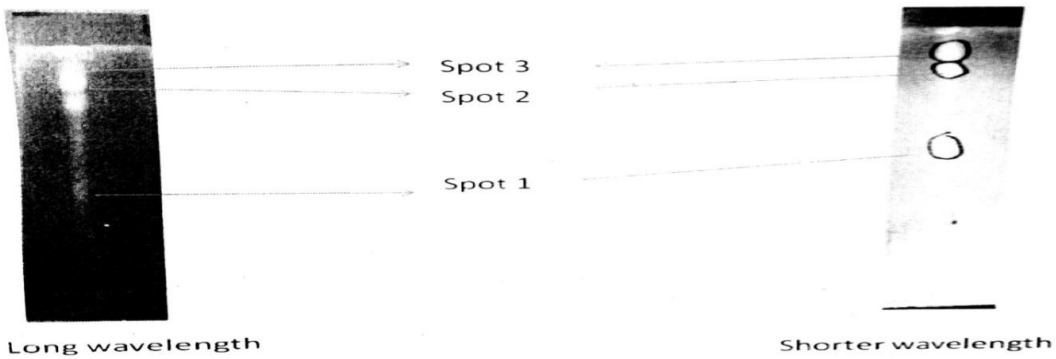

Fig. 1: Chromatograms for flavonoids (UV long and short wavelength).

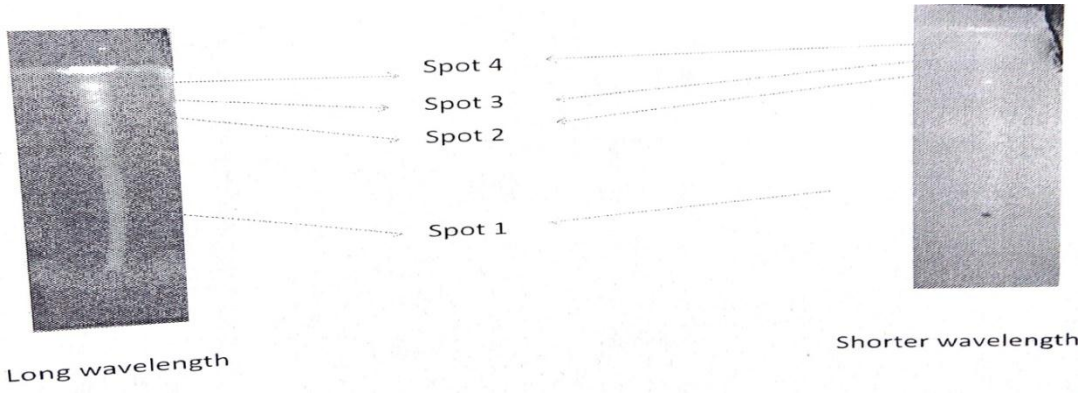

Fig. 2: Chromatograms for Glycosides (UV long and short wavelength).

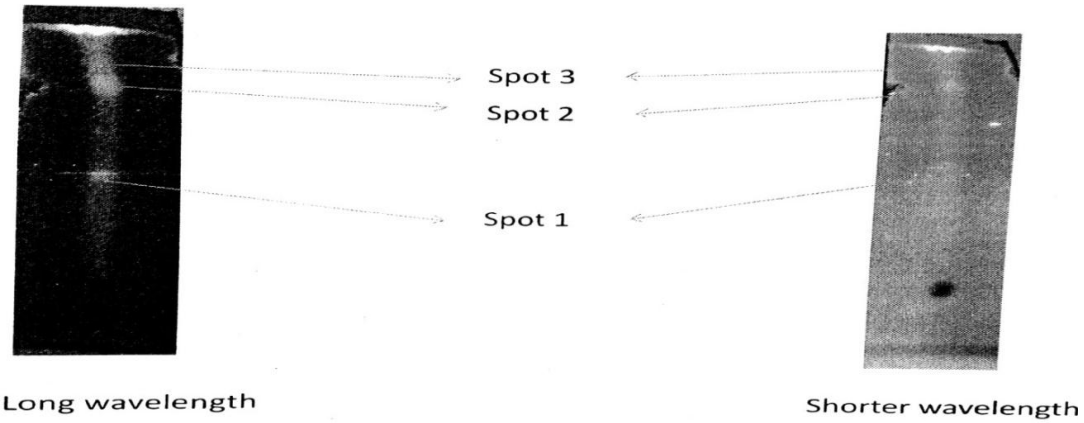

Fig. 3: Chromatograms for Saponins (UV long and short wavelength).

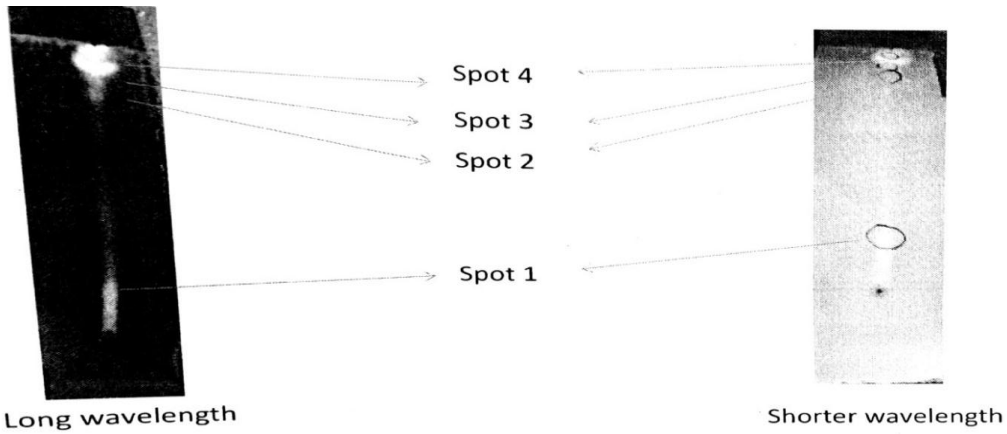

Fig. 4: Chromatograms for Tannins (UV long and short wavelength).

IN VITRO ANTI DIABETIC ACTIVITY OF EHANOLIC EXTRACT OF *CHLOROXYLON SWIETENIA*

In vitro glucose oxidase method

Wavelength	Concentration of extract	% of inhibition	Standard
546	20	42.4 ± 0.54	17.2 ± 0.5
546	40	54.2 ± 0.69	19.3 ± 0.123
546	60	58.6 ± 0.96	25.3 ± 0.241
546	80	62.5 ± 0.79	30.2 ± 0.325
546	100	75.2 ± 0.98	38.3 ± 0.942

In vitro glucose absorption method

Ethanolic extract at 250 mcg/ml

Wavelength	Concentration of extract	Time duration	Percentage of inhibition	Standard
640nm	250	30	62.34 ± 0.69	18.6 ±0.123
640nm	250	60	64.77 ± 0.132	28.6 ±0.305
640nm	250	90	72.53 ±0.353	36.4 ±0.432

Ethanolic extract at 500 mcg/ml

Wavelength	Concentration of extract	Time duration	Percentage of inhibition	Standard
640nm	500	30	62.45 ± 0.71	18.6 ±0.123
640nm	500	60	70.56 ± 0.413	28.6 ±0.305
640nm	500	90	84.35±0.924	36.4 ±0.432

Ethanolic extract at 1000 mcg/ml

Wavelength	Concentration of extract	Time duration	Percentage of inhibition	Standard
640nm	1000	30	71.1± 0.515	18.6 ±0.123
640nm	1000	60	90.03 ± 1.32	28.6 ±0.305
640nm	1000	90	94.78 ±0.565	36.4 ±0.432

WOUND HEALING ACTIVITY

Chorioallantoic membrane assay

In CAM model, the aqueous extract of *C.swieteneia* shown angiogenetic activity from slight to marked which was dose dependent. Increase in the size of blood vessel at a dose of 200 mcg/ml, was slight as compared to the control on the same day, whereas at a dose of 400 mcg/ml, caused a marked increase in the size and number of the blood vessel. The 600 mcg/ml, concentration shows a slight increase in the size and number of blood vessel.

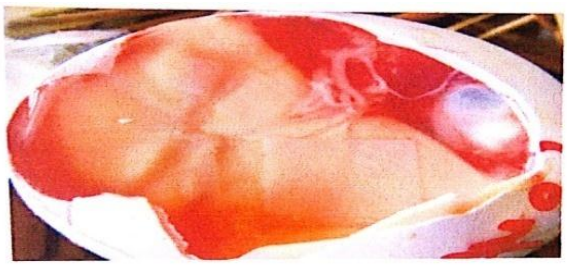

Fig no 1: control
Embryo inserted with sterile disc with out plant extract

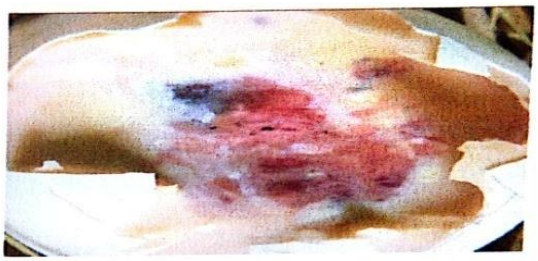

Fig no2: 200 mcg/ml
Embryo inserted with sterile disc contain 200 mcg/ml plant extract

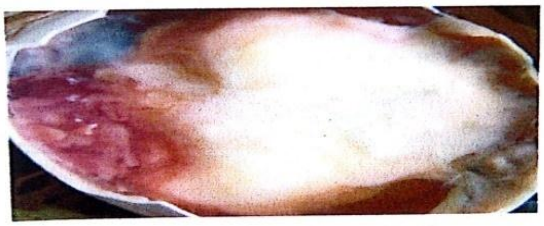

Fig no3: 400 mcg/ml
Embryo inserted with sterile disc contain 400 mcg/ml plant extract

Fig no4: 600 mcg/ml
Embryo inserted with sterile disc contain 600 mcg/ml plant extract

DISCUSSION

The successive solvent extraction of leaf was done according to increasing polarity of solvent. The preliminary phytochemical screening is used to know the presence of various chemical constituents present in the plant extracts. Then the extracts were subjected to the preliminary tests in which the ethanolic extract had shown the presence of various constituents like flavonoids, steroids, cardiac glycosides, tannins, alkaloids and saponins. Hence the ethanol was selected as the suitable solvent for the major extraction. The information obtained from preliminary phytochemical screening will be useful in finding out in the genuity of the drug. The TLC analysis of leaf showed spots for flavonoids, tannins, glycosides, in two different solvent systems and the Rf values were obtained and tabulated.

Reported pharmalogical properties *Chloroxylon swietenia* include anthelmintic, anti-inflammatory, antibacterial, anti-diarrheal, larvicidal, insecticidal and wound healing activities. It is powerful astringent and is given in many forms of chronic diarrhea. Seeds have anthelmintic activity especially for roundworms and tapeworms. Such herbal medicines may provide potential effect as compared to the conventional available synthetic drugs, with less or no side effects.

ANTIDIABETIC ACTIVITY BY GLUCOSE ABSORPTION STUDY

In this method ethanolic extract shows the concentration of dose and time dependent manner. The activity shows the compared to the concentration of 500 mcg/ml, extract and 1000 mcg/ml, shows the good percentage of inhibition.

WOUND HEALING ACTIVITY BY CAM ASSAY

In CAM model the ethanolic extract of *Chloroxylon swietenian* shown the angiogenetic activity from slight to marked which was dose dependent. Increase in the size of blood vessel at a dose of 200 mcg/ml, was slight compared to the control on the same day, whereas the dose of 400 mcg/ml, caused a marked increase in the size and number of the blood vessels. The 600 mcg/ml, concentration shows slight increase the number of blood vessel.

CONCLUSION

Based on literature review *Chloroxylon swietenia* Linn belongs to the family Rutaeae, leaf of this plant was traditionally used in the treatment of diabetes and wound healing, it is commonly called as billudu in Telugu. Different activities are already reported in different parts of plant. Preliminary phytochemical screening of ethanolic extract revealed the presence of flavonoids, proteins, cardiac glycosides, tannins and Carbohydrates. The presence of these active constituents confirmed by thin layer chromatography (TLC).

The ethanolic extract *Chloroxylon swietenia* of leaf powder were screened for in *vitro* anti diabetic activity and wound healing activity. These studies were carried out by Glucose absorption method on goat ileum showed potent antidiabetic activity and wound healing activity on chick embryos showed effective results have been obtained by dose dependent manner.

Further more research has to be conducted on identification, isolation and characterization of the specific phytochemical responsible for the antidiabetic activity.

REFERENCES

1. Anand RM., Nandakuma N., Karunakara L., Ragunathan M., Murugan V, A Survey of medicinal plants in Kollimalai hill tracts. Tamil Nadu, Natural Product Radiance, 2006; 5(2): 139-143.
2. Asian Regional Workshop (Conservation and Sustainable Management of Trees, Viet Nam, August 1996) 1998. *Chloroxylon swietenia*. In: IUCN 2012. IUCN Red List of Threatened Species. Version, 2012; 2.
3. Ahmed F and U Asna. Pharmacognostical studies on Ficus racmosa stem bark. Phrarmacognosy J, 2011; 3(19): 19-24.
4. Bartram T: Encyclopedia of herbal medicine, Grace, Dorset, 1995; 1-25.

5. Bhattacharya S and MK Zaman. Pharmacognostic evaluation of Zanthoxylum nitidum bark. Intl. J. Pharm. Tech. Res., 2009; 1: 292-298.
6. Evans WC: Trease and Evans Pharmacognosy, W.B saunders, Newyork, 15th edition, 2002; 3-7.
7. Jana GK, Gupta A, Das A, Tripathy A and P Sahoo. Herbal Treatment to Skin Diseases: A Global Approach. Drug Invention Today, 2010; 2(8): 381-384.
8. Khandelwal KR. Practical Pharmacognosy, 18th Ed. Nirali Prakashan, Pune, 2007; 31-40 and 100-101.
9. Khasim SM. Botanical Microtechniques: Principle and Practice. Capital Publishing Company, New Delhi, 2002; 41-52 and 85-88.
10. Kiran SR., Bhavani K., Devi PS., Rajeswara Rao BR., Reddy JK., Composition and larvicidal activity of leaves and stem essential oils of Chloroxylon swietenia DC against Aedes aegypti and Anopheles stephensi, Bioresource Technology, 2006; 97: 2481–2484.
11. Kokate CK. Preliminary phytochemical screening. In 4th Ed. Practical Pharmacognosy. Nirali Prakashan, Pune, 2000; 107-111.
12. Kokashi CJ, Kokashi RJ and M Sharma. Fluorescence of powdered vegetable drugs in ultra-violet radiation. J American Pharma. Assoc, 1958; 47: 715-717.
13. Mandeep S, Hayat MM and V Deepak. Standardization of stem bark of Ficus bengalensis. Int J of research in Pharnacy and Chemistry, 2012; 2(3): 790-793.
14. Pramod VP and M Jayaraj. Paharmacognostic and phytochemical investigation of Sida cordifolia L. - A threatened medicinal herb. Int J of Pharmacy and Pharmaceutical Sciences, 2012; 4(1): 114-117.
15. Palani S., Raja S., Kumar SB., Hepatoprotective and antioxidant potential of Chloroxylon swietenia (Rutaceae) on acetaminophen induced toxicity in male albino rats. International Journal of Pharmtech Research, 2010; 2: 162.
16. Parotta AJ. Healing plants of Peninsular India. CABI Publishing Company, USA, 2001; 636-637.
17. Reddy CS., Reddy KN., Rao KT., Pattanaik C., Ethnobotanical Studies on Medicinal Plants Used by the Chenchus of Nallamalais in Kurnool District, Andhra. Pradesh, India. Research Journal of Medicinal Plant, 2007; 1: 128-133.
18. Reddy KN., Trimurthilu G., Reddy SC., Medicinal plants used by ethnic people of Medak district Andhrapradesh, Indian Journal of Traditional Knowledge, 2010; 9: 184.

19. Reddy VB., Use of Various Bio-Fencing Plants in the Control of Human Diseases by the Lambada Tribe Inhabiting Nalgonda District, Andhra Pradesh, India, Ethnobotanical Leaflets, 2008; 12: 520-23.
20. Sivakumar T., Kanagasabai R., Sampathkumar R., Perumal P., Gupta PM., Mazumder UK., 11th Napreca Symposium Book of Proceedings. Antananarivo, Madagascar, 2008; 201-213.
21. Survase SA., Raut SD., Ethnobotanical Study of some Tree Medicinal Plants in Marathwada, Maharashtra, Journal of Ecobiotechnology, 2011; 3(2): 17-21.
22. Venkata Subbaiah KP., Savithramma N., Bio- Prospecting And Documentation of Traditional Medicinal Plants Used To Treat Itching, Psoriasis And Wounds By Ethnic Groups of Kurnool District, Andhra Pradesh, India, Journal of Medicinal Plants Research, 2011; 5(17): 4087-4093.
23. Venkataswam R., Mohamad MH., Doss A., Ravi TK., Sukumar M., Ethnobotanical Study of Medicinal plants used by Malasar tribals in Coimbatore District of Tamil Nadu (South India). Asian Journal of Experimental Biological Sciences, 2010; 1(2): 387.
24. Venkata Ratnam K., Venkata Raju RR., Folk remedies for Insect bites from Gundlabrahmeswaram wild Life sanctuary, Andhra Pradesh, Indian, Journal of Traditional. Knowledge, 2008; 7: 436.
25. Yamini K., Anto Shering M., Reddy PK., Reddy LN., Pharmacognostical and Preliminary Phytochemical Screening on Leaves of Trianthema decandra Linn, International Journal of Pharmaceutical & Biological Archives, 2011; 2(3): 960-962.