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Research Article

Preliminary Phytochemical Analysis of Methanolic Extract of the whole Plant of *Scleria Lithosperma*

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ABSTRACT

In this study, an attempt was made to examine a preliminary phytochemical analysis of *Scleria lithosperma* belonging to the sedge family and is a perennial plant that grows in shaded paths and fertile pine rocks, and is often found along streams, water tanks, hillsides and rocks found in the areas of Tirumala, Chittoor district, India, A.P. Preliminary phytochemical analysis was performed by the Harborn method and showed the presence of four secondary metabolites such as saponins, steroids, glycosides and flavonoids reported in the methanol extraction process. From the observations, it was concluded that the methanol extract of *Scleria lithosperma* shows the presence of many active secondary metabolites. Therefore, this report may lead to the isolation and characterization of these active secondary metabolites in terms of bioefficacy and bioactivity.

Keywords: Phytochemical, Alkaloids, *Scleria lithosperma*, Metabolites.

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CONTENTS

1. Introduction

Medicinal plants, also known as medicinal herbs, have been used in traditional medicine since prehistoric times, hundreds of compounds for a variety of functions can be synthesized from plants, including protection against insects, fungi, diseases and herbivorous mammals. The plant kingdom is a treasure house of potential drugs and in the recent years, there has been an increasing awareness about the importance of medicinal plants. Numerous

phytochemicals with potential or established biological activity have been identified. However, a single plant contains wide diverse phytochemicals. The effects of using a whole plant as medicine are uncertain.

Scleria lithosperma belonging to the family cyperaceae is a common perennial plant with short rhizomes, nodulose, aromatic when fresh.The genus *Scleria* is represented in India by 23 species and varieties[1]. It mainly contains flavonoids, steroids, saponins, and glycosides. The methanolic extract is traditionally used in the treatment of skin diseases, as abortifacient, in correcting the menstrual cycle[2]. In Van Rheede's "Hortus Malabaricus" the plant was mentioned as 'Kadan pullu' (wild grass) and the root was used to make antinephrenetic decoction[3].

2. Plant Profile

Scleria lithosperma grows in the forests of tropical and subtropical areas of India to a height of 40 cm (usually 15-20 cm) as a perennial mass herb, with reddish leaves forming alternating parallel veins on triangular stems up to 15 cm in length. The base of the leaf blade has triangular pilose extension[4].

2.1. Scleria Lithosperma Plant [5-6]

2.2.Taxonomical Classification [7-9]

Kingdom	**:**	Plantae-Plants
Subkingdom	**:**	Tracheobionta-Vascular plants
Superdivision	**:**	Spermatophyta-Seed plants
Division	**:**	Magnoliophyta- Flowering plants
Class	**:**	Liliopsida-Monocotyledons
Subclass	**:**	Commelinidae
Order	**:**	Cyperales
Family	**:**	Cyperaceae -Sedge family
Genus	**:**	*Scleria*
Species	**:**	*lithosperma*

2.3. Common Name[10]

English : Florida keys nutrush, slender nutrush, Scirpus lithosperma

Tamil : Kathipul, Kadan pullu (wild grass),Katinappul

Telugu : Kondashaka thunga, Vasanakundagaddi

Malayalam : Nakkupullu

2.4. SYNONYMS[11]

- Carex lithosperma(L.),
- Hypoporum lithospermum(L.)
- Nees, Schoenus Lithospermum (L.),
- Scirpus juncoides Roxb,
- Scirpus Lithospermum Linnaeus.

2.5. BINOMIAL NAME: *Scleria lithosperma.L*[12]

3. Ecology and Distribution

3.1. Geographical Distribution

Scleria lithosperma occurs on all island groupings in the Lucayan Archipelago and through old and new world tropical and subtropical region[13-14]. *Scleria lithosperma* is distributed throughout India, up to an altitude of 900m, except in arid areas in the west and is restricted to limestone soils. It is also available in the areas of Andaman, Assam, Bangladesh, Bolivia, Brazil, west central, Cambodia, China south east, Himalaya, Ethiopia, Florida, India, Mexico gulf, Mexico north east, Mexico south west, Thailand ,Tonga, Zambia, Zair, Philippines, Madagascar and Panama,West Indies, Central America, South America, tropical Asia, Africa. In India, it is found majorly in the areas of Japali Theertham, microwave station and papanasam in Tirumala, Kailasakona, Kambakkam hills. It is cultivated almost all year round[15].

3.3. HABITAT

Scleria lithosperma grows as a perennial clumping herb to 40 cm in height (typically 15-20 cm). The reddish leaves are arranged alternatively, parallel veined, to 15 cm in length on a triangular shaped stem. At the base of the leaf blade, is a triangular pilose extension[16]. The incomplete, imperfect, zygomorphic, flowers are arranged terminally and in leaf axils. The individual flowers are subtended by 1 scale. The lower flowers in each spikelet are infertile. There is no calyx or corolla. It has 1 stamen. The ovary is superior with a single locule. The fruit is a white achene at maturity[17].

3.4. BIOLOGICAL SOURCE

Scleria lithosperma is the one among the species of lithosperma belonging to the family CYPERACEAE(Scleria sedge). It grows well in the tropical and subtropical forests of India[18].

4. MACROSCOPIC CHARACTERS

Plants are perennial, Rhizomes short, nodulose , aromatic when fresh. Culms in tufts, slender, 30-90cm, glabrous or slightly scabrous.

Colour: Leaves are green in colour

Odour: Characteristic

Taste: Bitter

5.MORPHOLOGY

5.1. GENERAL HABIT

Tall, slender, often rather hairy perennial 30–90 cm high, with short rhizome.

5.2.STEM

It is upto 1 to 2.5 mm wide, minutely scabrid, the stem bases are sometimes slightly swollen and forming a caespitose mass[19]. Tufted perennial with slender stems 40-60 cm or more tall.

5.3. LEAVES

The leaves are wingless, weakly ribbed, finely pilose or nearly glabrous; contra-ligules reddish, triangular, rigid , distinctly ciliate ,blades distinctly greyish green and

revolute when dry, linear , attenuate, keeled , 1-3mm wide, shorter than culms. Lower leaf sheaths brown, upper green the blades up to 20cm long, 2 to 5mm wide, scabrid on margin at least near tip, otherwise glabrous or hairy[20]. Sheaths purplish, leaves clustered about the middle of stem.

5.4. Inflorescence and Bracts

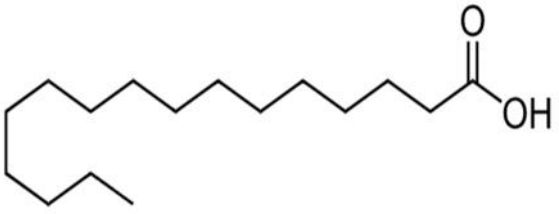
The inflorescence is arranged as axillary 1-3, terminal,quite lax; stalked spikes or panicles 2-4, terminal one 3-4.5 cm with 2-7 open fascicles 2-6(-9) mm wide of 1-4 spikelets; bracts are subtending and overtopping inflorescence leaflike, widely attenuated, rough. Spikelets arte bisexual(an occasional terminal staminate spikelet), few flowered 3-5 mm; staminate scales lanceolate, pistillate scales ovate-acuminate. Inflorescence of 1 terminal and 2 to 3 lateral panicles the latter arising singly from leafy bracts with cluster of 1 to 3 spikelets, each 4-5 mm long green or brownish, all androgynous. Glumes straw-coloured to pale green or light brown female often with green midrib,3-5 mm long, scabrid at least on midrib and margins, otherwise glabrous with prominent green keel[21]. Achenes whitish or grey between angles, obscurely trigonous, ovoid or globose, 2–2.5(–3) mm, smooth, base broadly attenuate, somewhat depressed between angles, trigonous, not porose, apex umbonate; hypogynium obsolete, reduced to distinct brown band at base of achene[22].

5.5. Fruits

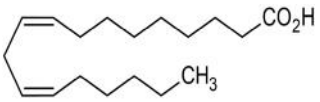
Fruits are spherical to egg-shaped, usually whitish, hard-covered, achene-type fruits normally perched atop a hardened pad known as the hypogynum. The white hypogynum is seen glistening beneath the achene. Nutlets olive-grey or olive-brown or pearly white ovoid or obovoid, 2.5 to 3mm long 1.5 to 2mm wide, Trigonous, smooth,apiculate with three depressions near the base, hypogonium reduced to unlobed disc and base of nut where it sits with a narrow annulus of chestnut brown[23].

6. Chemical Constituents [24-25]

- The major compounds were fatty acids (76.1%) with palmitic acid (43.3%), linoleic acid (14.0%) and oleic acid (7.8) being the major ones
- The two mono terpenoids were present in trace quantity.
- Sesquiterpinoids were present to the extent of (18.7%) with 9.8% sesquiterpene hydrocarbons and 8.9% oxygenated sesquiterpenoids.
- *Scleria lithosperma* contains glycosides, flavonoids, tannins, and saponins.



PALMITIC ACID



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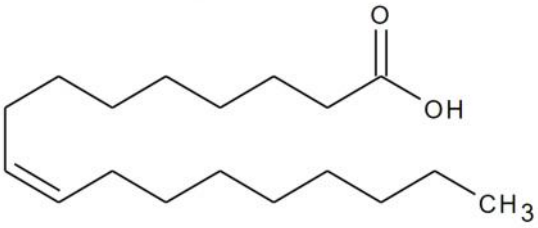
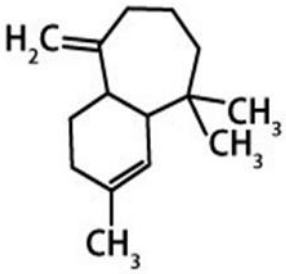


OLEIC ACID



SESQUITERPENOIDS

7. TRADITIONAL USES

Plants or herbs belonging to the family Sedges have exhibited properties such as anti-diabetic drugs, antioxidants, and hyperlipidemia[26]**.** Sebaceous cyst are common, non-cancerous cysts of the skin. Washing, baking, powdering of *Scleria lithosperma*(vakkathipullu) and applying to infections mixed with coconut oil alleviates the problem and thus the *Scleria lithosperma* used in the treatment of sebaceous cyst . It is also traditionally used as an anti-abortion drug during pregnancy and to correct the menstrual cycle. It is used to treat hypertrophy of the stomach in children, and also as a preservative. It is also used for urinary problems and the tea or decoction of the root is given to the mother after childbirth.

8. PHARMACOLOGICAL ACTIVITIES

8.1. ANTI-ULCER ACTIVITY

The methanol extract from Scleria lithosperma leaves has anti-ulcer activity in aspirin-induced, histamine-induced, and ethanol-induced acute ulcer models[27]. Aspirin is known to inhibit PG cyclooxygenase and reduce the production of PGE and endothelial PGI. This causes vasoconstriction, suppression of platelet aggregation (increased bleeding), and contributes to increased acid secretion[28]. From the extract, there was a decrease in gastric volume, total acidity, ulcer index, increased rate of protection, and increased prostaglandin levels. Methanol extracts from the leaves of Scleria lithosperma, which contain glycosides, steroids and saponins, have been observed to have antiseptic effects. The plant extract

showed better activity against histamine-induced ulcers than aspirin- and ethanol-induced ulcers[29].

8.2. CARDIOPROTECTIVE ACTIVITY

EEWSL's(Ehanolic extract of whole plant of *Scleria lithosperma)* phytochemical studies have revealed the presence of glycosides, tannins, phenols, saponins, alkaloids, and flavonoids that are responsible for their strong antioxidant properties. Animals treated with doxorubicin showed a hyperlipidemic effect by increasing serum levels of total cholesterol, TG, and LDL and reducing HDL compared to normal controls. The cardiotoxicity of doxorubicin may be due to disruption of lipid metabolism and increased membrane degradation. Pretreatment with EEWSL showed a significant decrease in serum levels of total cholesterol, TG, LDL, VLDL, and an increase in HDL, keeping the rat nearly normal, reducing cholesterol biosynthesis and increasing LDL uptake by the liver was shown[30].

8.3. HYPOLIPIDEMIC ACTIVITY

The hypolipidemic activity of *Scleria Lithosperma* extract was studied on high fat diet induced models of hyperlipidemia in rats. Hyperlipidemic condition was observed in rats by an enhancement in the levels of Cholesterols, Triglycerides, LDL and VLDL. Hypolipidemic effect was seen with Methanolic extract by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum Cholesterol, TG, LDL, VLDL and increase in HDL level which was similar to the standard drug Orlistat. Preliminary phytochemical analysis revealed the presence of phytoconstituents such as steroids, flavonoids, saponins and glycoside[31].

3. Materials and Methods

Collection and Authentication of Plant Materials: The plant material used in the present study is *Scleria lithosperma* belonging to the family Cyperaceae. The plants of *Scleria lithosperma* was collected from the forests of Chittoor district of Andhra Pradesh and authenticated by Dr.Madhava Shetty who is a professor in the department of Botany.[32]

MICROSCOPIC CHARACTERISTICS

PHYTOCHEMICAL ANALYSIS

It refers to the extraction, screening and identification of the medicinally active substances found in plants. Different chemical tests were performed on the extracts. These tests were performed to determine the presence of compounds like Carbohydrates, Proteins, Amino acids, Fats, Steroids, Glycosides, Alkaloids, Tannins and Phenolic compounds, Enzymes etc[33]**.**

PREPARATION OF EXTRACT

For the preparation of *Scleria lithosperma* extract, the plant specimens was washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender and then sieved by using sieve#44. The powdered samples were then stored in the refrigerator for further use. 30g of this coarse powder was successively extracted using Soxhlet extractor with methanol successively. Finally the marc was macerated with distilled water (chloroform was used as a preservative while drying) for 24 hrs to obtain the extract. Powdered samples were packed in Soxhlet apparatus and extracted with methanol for 8 hours [34].

TESTS FOR CARBOHYDRATES

Molisch test:

To 2-3ml of extract added a few drops of α-naphthol solution in alcohol, shaken well and added conc. sulphuric acid from the sides of the test tube. Violet ring is not formed at the junction of two liquids.Indicates the absence of carbohydrates.

Fehling's test:

1ml Fehling A and 1ml Fehling B solution was mixed and boiled for 1 min. Equal volumes of test solutions were added. Heat in a boiling water bath for 5-10 mins. First yellow then brick red precipitate was not observed. Indicates the absence of carbohydrates.

Benedict's test:

Mixed equal volumes of Benedict's reagent and test solution in the test tube. Heat in a water bath for 5 mins. Solution does not appear green, yellow or red depending on reducing sugar present in the solution. Indicates the absence of carbohydrates.

TESTS FOR PROTEINS

Biuret test: To 3ml test solution add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour are not appear. Indicates the absence of proteins.

Millon's test: Mix 3ml.test solution with 5ml Millon's reagent. White precipitate not appears. Warm precipitate does not turn into brick red or the precipitate dissolves does not gives a red colored solution. Indicates the absence of proteins.

Xanthoprotein test

Mix 3ml. test solution with 1ml.conc. sulphuric acid. White precipitate is not formed. Boil precipitate does not turn yellow;added ammonium hydroxide precipitate does not turn orange. Solution does not turn black or brownish due to PbS formation.Indicates the absence of proteins.

TESTS FOR AMINO ACIDS

Ninhydrin test:

Heat 3ml test solution and 3 drops of 5% Ninhydrin solution in boiling water bath for 10 mins. Purplish or Bluish colour does not appear. Indicates the absence of amino acids.

TESTS FOR STEROIDS

1ml extract was added with 2ml of chloroform and 1ml of sulphuric acid. The formation of a reddish brown ring indicates the presence of steroids.

Salkowski reaction:

To 2ml chloroform and 2ml conc.H_2SO_4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence indicates the presence of steroids.

Liebermann-Burchard reaction:

Mix 2ml extract with chloroform. Add 1-2ml acetic anhydride and 2 drops conc.H_2SO_4 from the sides of test tube. First red, then blue and finally green color appears indicates the presence of steroids.

TEST FOR GLYCOSIDES: 2ml of 50% H_2SO_4 was added to the 2ml of extract in a boiling tube. The mixture was heated in a boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A brick red precipitate indicates the presence of glycosides.

Legal's test (test for Cardenolides)

To aqueous or alcoholic extract, add 1ml pyridine and 1ml sodium nitroprusside. Pink to red colour appears,indicates the presence of glycosides..

Keller-Killiani test (test for Deoxy sugars)

To 2ml extract add glacial acetic acid, 1 drop 5% $FeCl_3$. and conc.H_2SO_4. Reddish brown colour appears at the junction of two liquid layers and the upper layer appears bluish green indicates the presence of glycosides.

Test for Saponin Glycoside

2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed indicates the presence of saponins.

Test for Flavonoids

A few drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. A yellow coloration indicates the presence of flavonoids.

Shinoda test

To dry powder or extract, add 5ml 95% ethanol, few drops conc. Hcl and 0.5gms magnesium turnings. Pink colour observed. indicates the presence of flavonoids.

TEST FOR ALKALOIDS

Dragendroff's test

To 2-3 ml of extract, add a few drops of dragendorff's reagent. Orange brown precipitate is not formed. Indicates the absence of alkaloids.

Mayer's test: 2-3ml filtrate with reagent not precipitate. Indicates the absence of alkaloids.

Hager's test: 2-3ml filtrate with Hager's reagent not give yellow precipitate. Indicates the absence of alkaloids.

Tests for Tannins and Phenolic Compounds

To 2-3ml of aqueous or alcoholic extract, add few drops of following reagents:

5%$FeCl_3$ solution Deep blue-black colour (not formed indicates the absence of tannins and phenols)

Acetic acid solution

Red colour solution (not formed indicates the absence of tannins and phenols).

4. Results and Discussion

Table: Indicating the different phytoconstituents in the methanolic extract of *Scleria lithosperma*

S.NO	CHEMICAL CONSTITUENTS	NAME OF THE TEST	METHANOLIC EXTRACT
1	CARBOHYDRATES	Molisch test Fehling's test Benedict's test	- - -
2	PROTEINS	Biuret test Millon's test Xanthoprotein test	- - -
3	AMINO ACIDS	Ninhydrin test	-
4	STEROIDS	Salkowski reaction Liebermann-Burchard reaction	+ + +
5	GLYCOSIDES	Legal's test (test for Cardenolides) Keller-Killiani test (test for Deoxy sugars)	+ +
6	SAPONIN GLYCOSIDES	Foam test	+
7	FLAVONOIDS	Shinoda test	+
8	ALKALOIDS	Dragendroff's test Mayer's test Hager's test	- - -
9	TANNINS AND PHENOLIC COMPOUNDS	5%FeCl3 solution Acetic acid solution	- -

(+) Indicates the presence of the component

(-) Indicates the absence of the component

The phytochemical tests were performed for the identification of different chemical constituents' present in the *Scleria lithosperma plant* extract.

DISCUSSION

Scleria lithosperma is a perennial plant grows in shady places most commonly along the roadside areas. It is widely distributed in the tropical and subtropical areas of India. It is tall, slender plant with short rhizome. Leaves are wingless, weakly ribbed. The stem grows upto 1 to 2.5 mm wide. It consists of essential fixed oils. Major compounds were fatty acids like Palmictic acid,linoleic acid and oleic acid. The most common traditional use of *Scleria lithosperma* is Sebaceous cyst which is non-cancerous cysts of the skin and also it is used as an anti-abortion drug during pregnancy and to correct the menstrual cycle.

In the preliminary phytochemical analysis of *Scleria lithosperma*, four different types of secondary metabolites (steroids, saponins, flavonoids and glycosides) were found to be present in the methanolic extract of whole plant of *Scleria lithosperma*. These tests helps in identifying the constituents as well as to analyze the drug for its activity. There is evidence that saponins, flavonoids, steroids and glycosides have anti-oxidant properties and thus are useful for cardioprotective, anti-ulcer, anti-cancer, lipid-lowering effects and can also be used against hepatic disorders[35-36].

5. Conclusion

The identification tests were performed on the extract of Scleria lithosperma L. The methanolic extract showed the presence of glycosides, flavonoids, saponins and steroids. It was also observed that methanol gave the highest percentage yield and thus methanolic extract of the plant *Scleria lithosperma L*. can be used for further studies.

6. References

[1] D.M.Verma and V.Chandra, Bull. Bot. Surv. India 32,57-79

[2] Daniels SR, Greer FR; Committee on Nutrition. Lipid screening and cardiovascular health in childhood. Pediatrics. 2008; 122198-208. [PubMed: 18596007].

[3] K.S. Manial, Van Rheede's Hortus Malabaricus, vol.12, English edition, p.161(2003) University of Kerala, Thiruvananthapuram.

[4] Takagi K., Hee P.E., and Histoshi K., Chem.Bull., 28, 1183 (1980)

[5] Zehavi U.M.L. and Segel R.J., Phytopathol.,116, 338-343 (1986)

[6] Sofowora A., Medicinal plants and Traditional medicine in Africa: Spectrum Books Ltd, Ibadan, Ibadan, Nigeria, 289 (1993).

[7] Ayitey-Smith E. and Addae-Mensah I., W. Afr. J. Pharmacol. Drug Res.,4, 7-8 (1977).

[8] Sati O.P., Chandra C.D., and Usha R., Planta Medica, 5, 981-983 (1987)

[9] Gill L.S., Ethnobotanical Uses of Plants in Nigeria: University of Benin Press, Benin City, 350 (1992)

[10] Banso A. and Adeyemo S.O., Afr. J. Biotechnol., 6, 1785-1787 (2007)

[11] Daniels SR, Greer FR; Committee on Nutrition. Lipid screening and cardiovascular health in childhood. Pediatrics. 2008; 122198-208. [PubMed: 18596007].

[12] Gennest J, Libby P. Lipoprotein disorders and cardiovascular disease. In: Bonow RO, Mann DL, Zipes DP, Libby P, eds. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 9th ed. Philadelphia, Pa: Saunders Elsevier; 2011: chap 47.

[13] Espejo Serna, A. & López-Ferrari, A.R. (1997). Las Monocotiledóneas Mexicanas una Sinopsis Florística 5: 1-98. Consejo Nacional de la Flora de México, México D.F.

[14] Figueiredo, E. & Smith, G.F. (2008). Plants of Angola Strelitzia 22: 1-279. National Botanical Institute, Pretoria.

[15] Govaerts, R. & Simpson, D.A. (2007). World Checklist of Cyperaceae. Sedges: 1-765. The Board of Trustees of the Royal Botanic Gardens, Kew.

[16] Govaerts, R. (2004). World Checklist of Monocotyledons Database in ACCESS: 1-54382. The Board of Trustees of the Royal Botanic Gardens, Kew.

[17] Hammel, B.E. & al. (2003). Manual de Plantas de Costa Rica 2: 1-694. Missouri Botanical Garden Press, St. Louis.

[18] Hoenselaar, K., Verdcourt, B. & Beentje, H. (2010). Cyperaceae Flora of Tropical East Africa: 1-466.

[19] J.G.S.Maia,M.H.L.da Silva,E.H.A.Andrade and N.A.Rosa,Fl.Fr.J.20,472-473 (2005)

[20] Acevedo-Rodríguez, P. & Strong, M.T. (2012). Catalogue of seed plants of the West Indies Smithsonian Contributions to Botany 98: 1-1192.

[21] Akoègninou, A., van der Burg, W.J. & van der Maesen, L.J.G. (eds.) (2006). Flore Analytique du Bénin: 1-1034. Backhuys Publishers.

[22] Browning, J. & al. (2020). Flora Zambesiaca 14: 1-455. Royal Botanic Gardens, Kew.

[23] Brunel, J.F., Hiepo, P. & Scholz, H. (eds.) (1984). Flore Analytique du Togo Phanérogames: 1-751. GTZ, Eschborn.

[24] S.Hoet, C.Stevigny, M.F.Herent and J.Q.Leclercq,Planta Med.72,480-482 (2006)

[25] M.khan, S.K.Srivastava, K.V Syamasunder and A.K. Yadav, Chem.Nat.Compds, 44,807-809(2008).

[26] Nagaraju D & Mukherjee A, Ethnomedicine in Bankura district, West Bengal,Indian J Appl Pure Biol, 3(2) (1998) 53-55.

[27] Hafiza M.A., Parveen B., Ahmad R. and Hamid K., Online J. of Biol. Sci., 2, 130-132 (2002)

[28] Edeoga H.O., Okwu D.E. and Mbaebie B.O.,Afr. J. Biotechnol., 4, 685-688 (2005)

[29] Banso A. and Adeyemo S.O., Afr. J. Biotechnol., 6, 1785-1787 (2007)

[30] Wu S, Ko YS, Teng MS, Ko YL, Hsu LA, Hseuh C, Chou YY, Liew CC, Lee YS. Adriamycin-induced cardiomyocyte and endothelial cell apoptosis: In vitro and in vivo studies. J. Mol. Cell. Cardiol. 2002;34(12): 1595-1607.

[31] Madhava Chetty K, et al. Flowering plants of Chittoor District, Andhra Pradesh, India. 1 st ed. Student offset printers, Tirupati, Andhra Pradesh, India; 2008.

[32] Harborne JB. Photochemical methods - A guide to modern techniques of plant analysis, Chapman and Hall, London, 1998.

[33] Hafiza MA, Parveen B, Ahmad R, Hamid K. Phyto-chemical and antifungal screening of Medicago sativa and Zinnia elegans. Online J. of Biol. Sci., 2(2), 2002, 130-132.

[34] Feroz M, Ahmad R, Sindhu STAK, Shahbaz AM. Antifungal activities of saponins from indigenous Medicago sativa roots. Pak. Vet. J., 13, 1993, 14.

[35] Bone K. Clinical Applications of Ayurvedic and Chinese Herbs. Queensland, Australia: Phytotherapy Press, 1996.

[36] Duke JA. Handbook of Phytochemical Constituents of GRAS Herbs and Other Economical Plants. CRC Press, 1994.McGuffin M, et al.(eds.) AHPA Botanical Safety Handbook. CRC Press, 1997