Int J Pharm Bio Sci 2016 Jan; 7(1): (B) 546 - 551

Original Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

EVALUATION OF THE *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF THE ETHANOL EXTRACT FROM THE SEEDS OF *ENTADA PURSAETHA DC*

NARASIMHARAO BHOGIREDDY[1], PARDHASARADHI MATHI[2], VENUGOPAL GADDAGUTI[1], NAGAVAMSIKRISHNA AMBATIPUDI[3] AND VENKATA RAMAN BOKKA[1,4*]

[1]*Department of Biotechnology, K L University, Green fields, Vaddeswaram, Guntur Dist-522 502, AndhraPradesh, India.*
[2]*Upstream Process Development Team, Lupin Limited, Biotechnology R&D, 1156, Ghotawade Village, Mulshi Taluka, Pune-411042, India*
[3]*Department of Biochemistry, Acharya Nagarjuna University, Guntur Dist-522 510, AndhraPradesh, India.*
[4]*Department of Basic Sciences-Chemistry, Madanapalle institute of technology and science, Madanapalle, Chittoor Dist-517 325, AndhraPradesh, India.*

ABSTRACT

Inflammation mainly depends upon the disease clinically defined as a pathophysiological process characterized by edema and pain. Inflammation occurs in response to injury, lipid peroxidation or infection. The signs and symptoms of inflammation include redness, swelling, heat, pain and loss of function of the affected area. Cyclooxygenase (COX1&2) are the key enzymes involves in the conversion of arachidonic acid to prostaglandins which are playing main role in inflammation. *Entada pursaetha* seed extract was prepared and it was explored for its phytochemical constituents, scavenging and anti-inflammatory activity. This article explores the medicinal values of the *Entada pursaetha* which is having strong anti-inflammatory and analgesic properties. Diclofenac standard and *Entada pursaetha* ethanolic seed extract both exhibited equal absorbance at 500 µg/mL in BSA assay.

KEY WORDS: ***Cyclooxygenase, GC-MS, Entada pursaetha, anti-inflammation.***

VENKATA RAMAN BOKKA
Department of Biotechnology, K L University, Green fields, Vaddeswaram, Guntur Dist-522 502, AndhraPradesh, India.
Department of Basic Sciences-Chemistry, Madanapalle institute of technology and science, Madanapalle, Chittoor Dist-517 325, AndhraPradesh, India.

INTRODUCTION

Damage to the human body can inspire an arrangement of chemical changes in the harmed zone. In the first stage, provocative exudates develop because of upgraded vascular porousness and lead to edema. It is followed by diapedesis or extravasation causes the migration of leukocytes and phagocytes from blood vessels to vascular tissues. In the third phase, tissue degradation is followed by fibrosis[1]. Inflammation brings about the liberation of endogenous substances like histamine, serotonin, bradykinin, prostaglandins, and so forth. Prostaglandins are universal substances that show and regulate cell and tissue reactions included in inflammation. These substances even in little amounts can evoke torment reaction. The majority of the anti-inflammatory drugs now accessible are potential inhibitors of the cyclooxygenase (COX) pathways of arachidonic acid metabolism, which generates prostaglandins and leukotrienes. Prostaglandins are hyperanalgesic, pro-pain causing agents, intense vasodialators and help erythema, edema and agony. Subsequently for treating inflammatory pain relieving and anti-inflammatory drugs are in need. These pro-inflammatory agents contributing different types of rheumatic disorders such as rheumatic fever, rheumatoid arthritis, cancer and other diseases COX-1 & 2 play major role in proinflammatory activity are inhibited by both steroidal and non-steroidal anti-inflammatory drugs (NSAID's)[2]. Two COX isoforms are known: constitutive COX-1, which is considered to be involved in intercellular signaling and homeostasis maintenance, and COX-2, mostly induced during inflammation [3, 4]. Non steroidal anti-inflammatory medications can directly penetrate and initiate concerned genes through binding specifically or indirectly to atomic receptors. Because of the absence of cascading mechanism through action guided operation to limit direct activity of these drugs lead to the development of many undesirable reactions and unrelated responses. In this way it is of incredible enthusiasm to screen disconnect and mimic its system. When steroids are taken on multiple occasions, more serious side effects may occur. It is for these reasons that the dose and duration of systemic steroids should be minimized whenever possible. Some side effects can be decreased by taking systemic steroids every other day instead of daily, even if the total dose is the same. Many of the side effects are reversible if the steroids are stopped, while other side effects may be permanent. People taking long-standing systemic steroids should be directly checked for the signs, and should take precautions to forestall osteoporosis. These medications may incorporate supplemental calcium and vitamin D, along with medicines to prevent bone loss called bisphosphonates. Non-steroidal anti-inflammatory drugs (NSAIDs) are constantly utilized with rising frequency, because of their potent pain relieving impacts without having symptoms on the central neural system and furthermore because of the large number and varieties of these agents. The analgesic, anti-inflammatory properties and efficacy of NSAIDs in a wide variety of diseases have been already established. NSAIDs can restrain the inflammatory methodology and the structuring of fibrosis, due to their ability to suppress prostaglandin synthesis. It was exhibited in a rat model that breaking strength and collagen fixation at the injury site were less after use of NSAIDs than in the untreated wounds. Regardless of this, NSAIDs are generally utilized as perioperative analgesics. In spite of the wide utilization of NSAIDS throughout the most recent century, their mechanism of activity was not completely acknowledged until 1971, when Vane published his seminal observations proposing that the capability of NSAIDS to suppress inflammation rests principally on their capacity to inhibit the cyclooxygenase (COX) enzyme. This would confine the generation of proinflammatory prostaglandins (PGs) at a site of injury. Given this, Ns have been utilized by researchers throughout the previous 25 years to dissect the critical part that both the COX enzyme and the eicosanoids inferred from this pathway have in normal and abnormal physiologic states[5]. *Entada pursaetha* is an immense woody liana among legumes, which produces 90-150 cm long woody giant pods with 5-30 seeds. All parts of this species hold saponins and are accordingly utilized within the soap industry. This species is reported as tribal pulse[6]. Its semi ripe seeds are likewise utilized as a substitute for coffee. The plant material is used by the tribal's as a broad range compound. This species might be utilized as a narcotic or as a tonic, etc, or used in curing liver troubles, allaying body pains, in warding off cold, curing eye diseases, arthritis, and paralysis[7]. This species is reported as endangered [8,9,10]. In recent times, there has been a deeply felt concern for the preservation and conservation of *E. pursaetha* germplasm[11] owing to an increasing realization of its significance and usefulness. The present piece of work was carried out to investigate the tribal's knowledge and traditional uses of this species.

MATERIALS AND METHODS

Extraction of Entada pursaetha phytochemical compounds

Entada pursaetha seeds were collected from the reserve forest of Srisailam which is located at Western Ghats of Andhra Pradesh, India. Identification of plant was done by taxonomist and a voucher specimen was deposited in K L E F University, Guntur, India (Voucher number KLU 1108). The seeds of *E. pursaetha* were collected, dried and powdered in a blender to get fine powder. The extract was prepared by using ethanol in 1:3 ratios by using soxhlet apparatus and the filtrate was concentrated under reduced pressure on rotavapor under vacuum (BÜCHI, R-3000, Switzerland) at 40^oC temperature. The filtrate was used to measure the presence and absence of phytochemical compounds, anti-oxidant activity and for GC-MS studies.

Preliminary phytochemical studies

Preliminary phytochemical screening was performed to identify phytochemicals present in the ethanol. Several sophisticated techniques like thin layer chromatography, ultra violet spectroscopy, infrared spectroscopy, nuclear

magnetic resonance and HPLC have been used for identification of various groups of phytochemical compounds in plant extracts. In the present investigation, the phytochemical compounds were detected by simple color tests. These extracts were subjected to preliminary phytochemical tests as described by *Venugopal Gaddaguti et al & Mathi PS et al* [12,13], with slight modifications.

Gas Chromatography and Mass Spectroscopy (GC-MS) separation conditions

The phytochemicals were analyzed by GC-MS Agilent 5975-C Series instrument employing the electron impact (EI) mode (ionizing potential -70eV) and a capillary column (DB-5ms Agilent)[16] (length 30 m × Diameter 0.25 mm, film thickness 0.25µm) packed with 5% phenyl dimethyl silicone) and the ion source temperature was monitored at 200°C. Further, the GC-MS settings were indicated as the initial column temperature was set at 70°C and kept hold for 2 min; the temperature was increased to 300°C at a rate of 10°C/min for 9 min, and placed in isothermal condition for 2 min. The column oven temperature was maintained at 70°C. Helium was used as carrier gas with 99.9995 % purity. Samples were injected at a temperature of about 250°C with a split ratio of 10: 1 with a flow rate of helium 1.51 ml/min. Mass scan (m/z): 45-1000, Total MS running time: 36 min. The constituents were identified after comparison with those available in the computer library (NIST ver. year 2005) attached to the instrument and reported.

In-vitro anti-inflammatory activity using bovine serum albumin (BSA) denaturation assay

To evaluate the anti-inflammatory activity of phytochemical compounds present *in E. pursaetha*, we used an anti-denaturation of BSA assay[13]. In brief the reaction mixture consists of 0.2 ml (10mg/ml) of bovine serum albumin (BSA), 2.8ml of phosphate buffered saline (PBS, pH -6.4), and 2 ml of varying concentrations of ethanol extracts of *E. pursaetha* 50, 100, 200,400, 800, 1200, 1600, 2000, and 5000 µg ml^{-1} to a final volume of 5 ml. PBS lacking BSA served as control. The samples were then incubated at 37±2^0C in an incubator for 15 min and then transferred to 70^0C water bath for 5 min. After cooling the sample, the turbidity was measured at 620 nm using a spectrophotometer. The anti-inflammatory activity of phytochemical compounds were determined by plotting the percentage of inhibition with respect to control against treatment condition. In our studies we used Diclofenac as a positive anti-inflammatory drug. The percentage inhibition of protein denaturation was calculated by using the following formula.

Percentage (%) of inhibition= 100 × (v_t/v_c -1),

Where Vt = Absorbance of test Sample, V_c = Absorbance of Control.

DPPH Free radical scavenging assay

In order to evaluate the free radical scavenging activity of the test samples, the change in optical density by DPPH radical was assessed[14]. The sample extracts were diluted with methanol to give different concentrations of the seed extracts (100, 200, 300, 400, 500, and 600 µg/mL). Then 0.2 mL of DPPH was added to 2.8 mL of the extracts at various concentrations and incubated at 37ºC for 30 min. Absorbance was measured at 517 nm. Ascorbic acid was used as reference standard. Percentage inhibition was calculated as:

DPPH Scavenged (%) = [(Abs control– Abs test) / Abs control] × 100

Where Abs control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the sample. This DPPH assay is established on the measurement of the scavenging capacity of antioxidants present. The odd electron of nitrogen atom in DPPH is cut down by receiving a hydrogen atom from antioxidants to the corresponding hydrazine.

RESULTS AND DISCUSSION

Identification of phytochemical constituents

Biochemical analysis showed the presence (+, ++) and absence (' –' indicates absence) of phytochemical excipients in ethanol extract represented in Table: 1. All the excipients present in the ethanol extract except steroids. Whereas steroids are the only phytoconstituent absent. Flavonoids and Glycosides are most abundantly present in the extract.

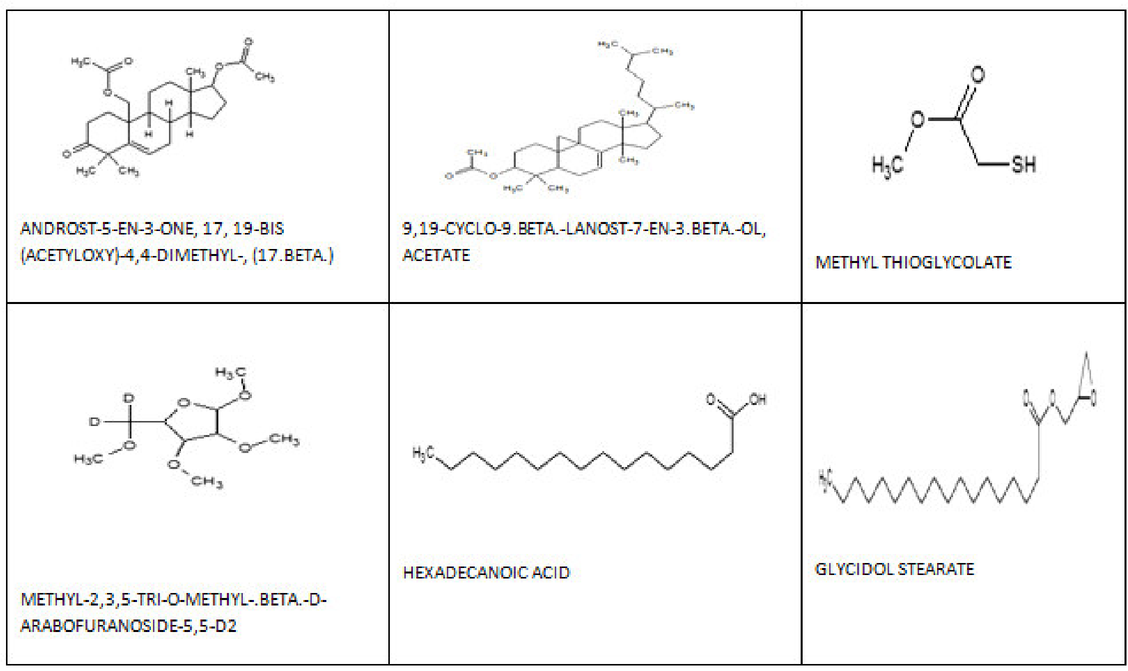
Table 1
List phytochemical constituents in Entada pursaetha

Biochemical tests	*E. pursaetha*
Alkaloids	+
Steroids	–
Flavonoids	+ +
Saponins	+
Terpenoids	+
Polyphenols	+
Tannins	+
Glycosides	+ +

GC-MS analysis

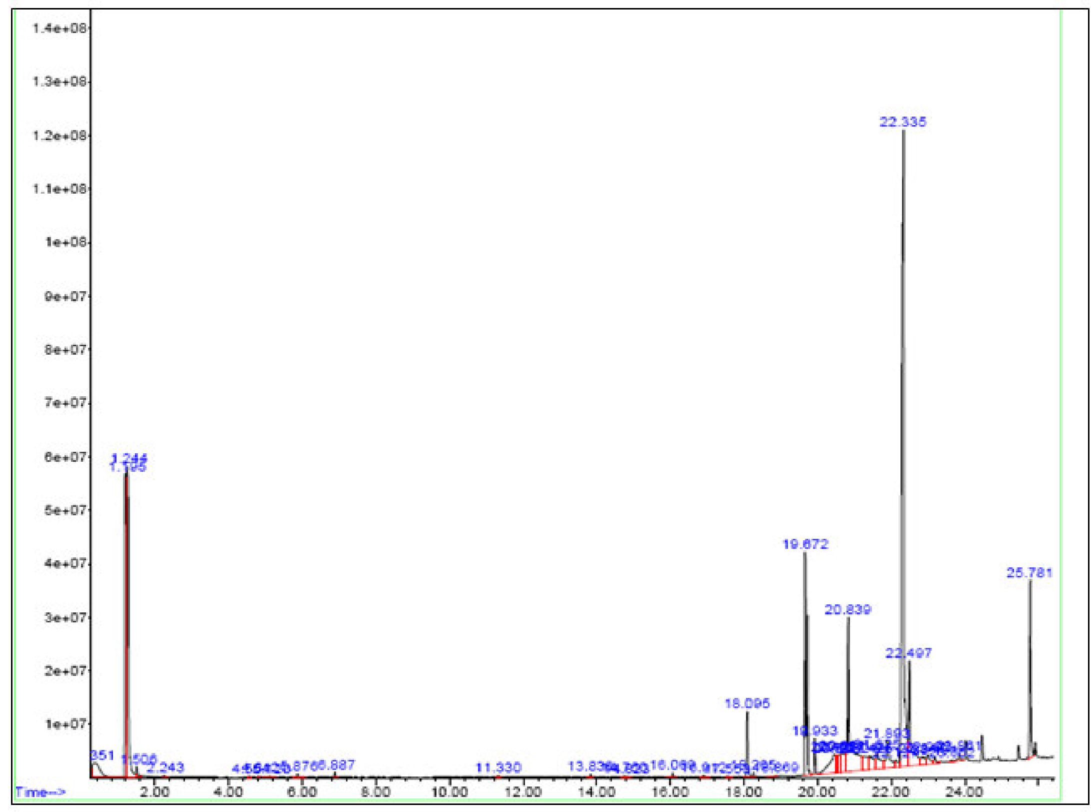
The spectrum profile of the GC-MS data of the *Entada* was compared with the known compounds stored in the NIST library (Fig: 1). Results showed six major peaks along with remaining other phytochemical constituents which was reported in Table: 2.

Table 2
Compounds from GCMS analysis E.pursaetha ethanolic seed extract



ANDROST-5-EN-3-ONE, 17, 19-BIS (ACETYLOXY)-4,4-DIMETHYL-, (17.BETA.)	9,19-CYCLO-9.BETA.-LANOST-7-EN-3.BETA.-OL, ACETATE	METHYL THIOGLYCOLATE
METHYL-2,3,5-TRI-O-METHYL-.BETA.-D-ARABOFURANOSIDE-5,5-D2	HEXADECANOIC ACID	GLYCIDOL STEARATE

Figure 1
GCMS analysis of crude E.pursaetha ethanolic seed extract



Anti-inflammatory activity of EPEE

Anti-inflammatory effect of EPEE was evaluated by measuring percent inhibition of Bovine Serum Albumin denaturation (BSA). Our results confirm that EPEE inhibits the denaturation of BSA in a dose dependent manner throughout the concentration range of 50-1000

µg/mL. The percent inhibition of BSA denaturation is enhanced with increase in the concentration of the plant extract. Diclofenac (50-1000 µg/mL) was used as reference drug which also demonstrate concentration dependent inhibition of protein denaturation. At the concentration of 500 µg/mL diclofenac standard as exhibited equal absorbance with EPEE. However, at higher concentration, the effect of Diclofenac was found to be less as compared with EPEE (Fig: 2).

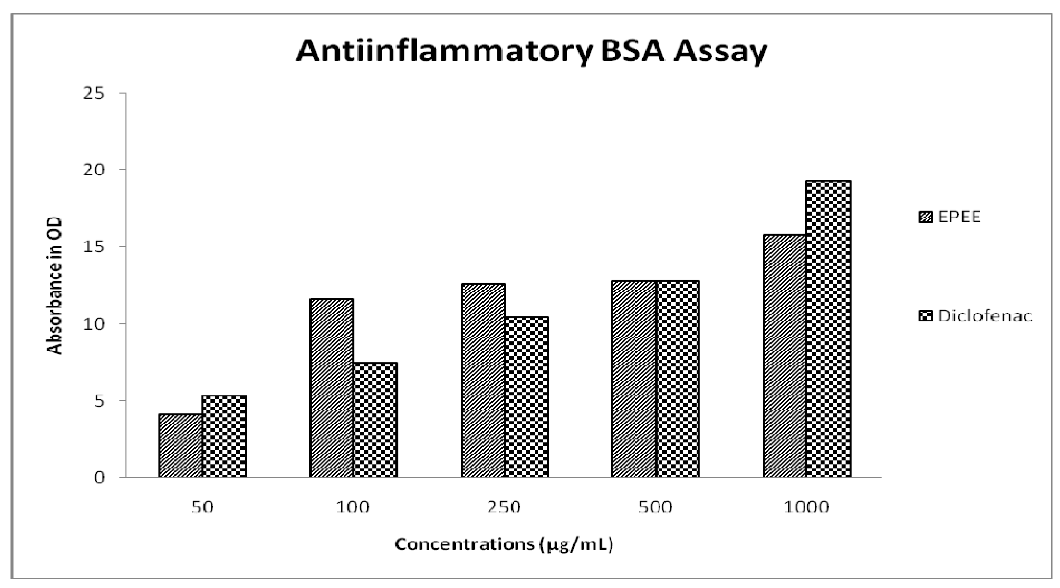
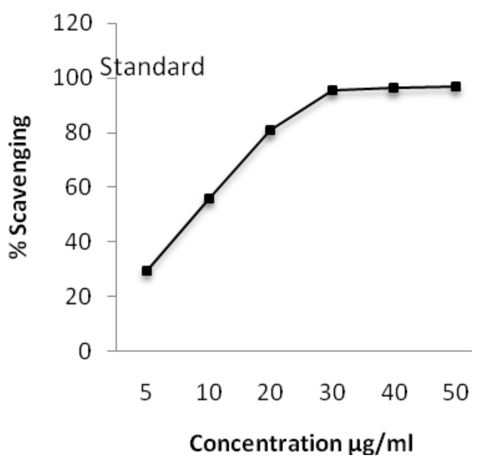


Figure 2
Invitro antiinflammatory assay of E.pursaetha ethanolic seed extract by BSA denaturation

DPPH Free radical scavenging assay
The radical scavenging activity of *E. pursaetha* and standard based on DPPH assay is depicted in Fig. 3(a). Ethanol seed extracts showed IC_{50} values of 350 µg/mL. IC_{50} value of ascorbic acid was found to be 8 µg/mL shown in Fig.3 (b). As lower IC_{50} values indicate higher scavenging activity. However, ascorbic acid displays significant (p <0.05) scavenging activity.



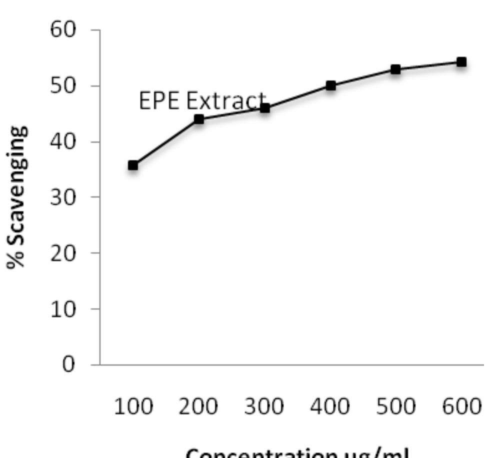


Figure 3(a&b)
Free radical scavenging assay of E. pursaetha by DPPH assay.

CONCLUSION

E. pursaetha ethanolic seed extract obtained from Entada seed was first tested for the availability of phytochemical constituents. GCMS proved the presence of different phytoconstituents. DPPH- free radical scavenging assay confirmed the presence of antioxidant properties in the extract. This was due to the presence of different phytoconstituents with different medicinal

properties. BSA denaturation assay which was performed to identify the anti-inflammatory activity of the extract concludes that the extract was very potent and has high degree of anti-inflammatory activity. It justifies the usage of this seed as a folklore medicine for preventing inflammation associated disorders.

ACKNOWLEDGEMENT

Authors thank K L University for providing necessary materials for carrying out this work.

REFERENCES

1. Matsumuraa Y., Saikaida I., Uchidaa K., Kimuraa T., Ishiharab T.,Okitaa K. Prolyl 4-hydroxylase inhibitor (HOE 077) inhibits pig serum-induced rat liver fibrosis by preventing stellate cell activation. *J. hepatology*, 27(1): 185-192, (1997).
2. Hoff T., DeWitt D., Kaever V., Resch K., Goppelt-Struebe M. Differentiation-associated expression of prostaglandin G/H synthase in monocytic cells. *FEBS Lett*, 320: 38-42, (1993).
3. Garcia-nieto R., Perez C., Checa A.,Gago F. Molecular model of interaction between nimsulide and human Cyclooxygenase-2. *Rheumatology*, 38(1): 14-18, (1999).
4. Emanuela R., FitzGerald G.A. Prostaglandins and Inflammation. *Arterioscler Thromb Vasc Biol*, 31(5): 986-1000, (2011).
5. Krishna P.S., Vani K., Ram Prasad M., Samatha B., Laxmi Hima Bindu N.S.V.S.S.S., Singara Charya M.A., Prakasham Reddy Shetty. In –silico molecular docking analysis of prodigiosin and cycloprodigiosin as COX-2 inhibitors. *SpringerPlus*, 2: 172, (2013).
6. Sai Vishnu Priya K.,Srinivasa Rao J.V. Exploration of Tribal Knowledge of Entada pursaetha DC: An Endangered Gigantic Medicinal Legume in Eastern Ghats. *Ethnobotanical Leaflets*, 12: 36-43, (2008).
7. Ganga Rao B., Madhukiran P., Vijaya Raju A.D. In Vitro Evaluation for Free Radical Scavenging Activity of Methanolic Leaf Extract of *Entada Pursaetha*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3): 324-327,(2012).
8. Janardhanan K., Vadivelu V., Pugalenthi E. Biotechnology for improvement of legumes. In: *Jaiwal, P. K. and Singh, R. P. Biotechnology in Indian tribal/under exploited pulses*. 18-21, (2001).
9. Varak V.D., Suryanarayana M.C. Enumeration of wild edible plants from Susala Island, Mulshi reservoir, Pune district. *Journal of Economic & Taxonomic Botany*, 19: 555-569, (1995).
10. Jadhav S.N., Ved D.K., Reddy K.N., Reddy Ch.S. Proceedings of the Workshop on Conservation Assessment and Management Planning for medicinal plants of Andhra Pradesh. Medicinal Plants Conservation Centre (MPCC), EPTRI, Hyderabad.. 4, (2001).
11. Das C.R. Rare & beautiful crawling climbers of special interest in India. *Journal of Living World*, 1: 85-88, (1994).
12. Pardhasaradhi M., Kumar N., Nagavamsikrishna A., Partha R., Venkata Raman B., Mahendran B. In-Vitro and In-Silico characterization of *Sophora interrupta* plant extract as an anticancer activity. *Bioinformation*, 10(3): 144-151, (2014).
13. Venugopal Gaddaguti., Srinivasa Reddy Ch., Narasimha Rao Bhogireddy, Botla Venkat Rao., Venkateswara Rao Talluri., Allu Prasada Rao. Pharmacognostic and Preliminary Phytochemical Evaluation of *Ocimum basilicum L. var. pilosum (Willd.) Benth*. and *O.tenuiflorum var. CIM-AYU, International Journal of Pharmacognosy and Phytochemical Research,* 7(3): 519-526, (2015).
14. Menon D.B., Sasikumar J.M., Latha K. Anti inflammtory and cytotoxic activity of methanolic extract of *Plectranthus hadiensis* stem. *Pharmacologyonline*, 3: 275-282, (2011).
15. Chandra S., Chatterjee P., Dey P., Bhattacharya S. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pacific J Trop Biomed*, 2(1) S178-S180, (2012).
16. Kanthal L.K, Dey A., Satyavathi K., Bhojaraju P. GC-MS analysis of bio-active compounds in methanolic extract of Lactuca runcinata DC. Pharmacognosy Res. Jan;6(1):58-61 (2014).