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IN VITRO CONSERVATION AND PROTECTIVE EFFECT OF *Premna Serratifolia* L. – AN IMPORTANT MEDICNAL TREE.

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ABSTRACT

An attempt for conservation of medicinal important shrub *Premna serratifolia* L. through i*n vitro* morphogenesis was achieved from callus tissue derived from leaf explants, production of Luteolin through callus culture using different explants and screening of callus derived Luteolin for anti inflammatory effect of *Premna serratifolia* L. The leaf explants excised from 8 years old *Premna serratifolia* L. and achieved highest frequency of callus mass from MS medium supplemented with 3% sucrose as carbon source, 0.8% agar and 5.0mg/l IAA. Regeneration of adventitious buds from calli and maximum number of shoots were achieved when calli were cultured on MS medium fortified with 3.0mg/l BAP in combination with 0.5mg/l KN. Regenerated shoots produced prominent roots when they were transferred on MS medium with 1.0mg/l of NAA. Rooted plantlets, thus developed were hardened and successfully established in the soil. In this search 89% of field survivals have been achieved. Among the various callus extracts root and root callus found to be the best biomass source for luteolin production and this root and root callus extracts of *Premna serratifolia* L. augments that it is having good anti-inflammatory activity against carrageenan induced paw edema.

Key Words: IAA: Indole-3- acetic acid, BAP: Benzyl amino purine, KN: Kinetin, paw edema.

1. INTRODUCTION

Medicinal plants are an important source of medicines and play a key role in world health [1,2]. In the past few decades there has been a notable interest in medicinal plants for its medicinal properties, approximately 85% of traditional medicine preparations involve the use of plants or plant extracts [3]. Many traditional

plant based remedies are back in use and find increasing application as, (i) source of direct therapeutic agent (ii) as a raw material base for the elaboration of more complete semi-synthetic chemical (iii) as model for new synthetic compounds for the production [4]. *Premna serratifolia* L. **[**syn. *Premna obtusifolia* R. Br., *Premna taitensis* Schauer., *Premna. integrifolia* Var.] Shrub or small tree about 10 m tall. Leaves opposite, petiolate, blades elliptic to oblong, up to 15 cm long and 9 cm wide, with base usually cordate, and the tip pointed. Flowers minute, white, 4-5 parted, borne in densely packed clusters. Fruit is a black globose drupe to 8 mm broad. Flowers and fruits are available throughout the year, belonging to the family Lamiaceae (previously: Verbenaceae) is a native of Konkan near the sea from Bombay to Malacca, widespread throughout the South Pacific, from India to Malaysia, South-East Asia, and Africa [5,6]. Root, leaf and stem bark of the plant produce medicinal substances which are found to possess anti-malarial, febrifuge, galactogenic and hypoglycemic activity [8-10]. Pepper along with leaf extract shown significant effects in patients of fever and cold, cough, constipation, Hypertension and cardiac insuffiency [10,11] Hepato protective activity [12-15] It is also used in the ayurvedic preparation of arishtam, Kvatham, ghritham [16,17]. Cardio tonic [18].The decoction of roots is also used to treat gonorrhea [19], Cancer [20], anti- parasitic agent [21] and used for infectious disease [22]. Traditionally it is used to promote menstruation, to treat shortness of breath and illness after childbirth, to remedy deep pains in bones, to treat bone fractures, appendicitis, rheumatic aches, swellings, headaches, diarrhoea, wounds, migraine and testicles swollen from hernia and also used for the treatment of eye injuries and inflammations [5].

Medicinal plant products have created a need for the development of solid scientific protocols for the phytochemical investigations and protections, and mass multiplication through controlled environments. Advances in tissue culture, combined with improvement in genetic engineering, specifically transformation technology, have opened new avenues for high volume production of pharmaceuticals, nutraceuticals, and other beneficial substances [23]. *Premna serratifolia* L. is dwindling at an alarming rate due to overexploitation by the pharmaceutical industry and low seed viability [24]. It is a vulnerable mangrove associates [25] and also this important species listed in the Red Data book of Andhra Pradesh, India [50]. Conventional breeding in some of the medicinal plants including *Premna serratifolia* is hampered due to poor seed viability and low percentage of germination through stem cutting [26]. Immediate measures have to be taken to save these species from extinction**.** Application of tissue culture methods used for conservation and large scale propagation of plant species [27,28]. This is our second scientific report for conservation of *Premna serratifolia* L. through *in vitro* technique. The present search was undertaken in order to contribute in developing a low cost and efficient protocol for the *in vitro* conservation and screening of anti inflammatory effect L. through callus extract of *Premna serratifolia* L..

2. Materials and methods:

Leaf explants were collected from the 8 years old *Premna serratifolia* L. growing in Keelathaniyam, Pudukottai District, Tamilnadu, India. For the present investigation shoot tip, leaves, node, stem and root of *Premna serratifolia* were selected as the explants. The young leaf explants were collected and immersed in water immediately then they were thoroughly washed under running tap water for 30minutes. Teepol (2%) wash for 5minutes, leaf explants were sterilized with 80% ethanol for 30 seconds. Followed by treatment with 0.1% (w/v) mercuric chloride solution (Qualigens, India) for 2-3 minutes and then thorough washing with

sterilized double-distilled water using 5-10 fold volume rinses to completely remove the mercuric chloride. And inoculations of plant materials were carried out in clean laminar air flow chamber under aseptic condition.

2.1. Methods and culture condition:

MS [29] salts and vitamin medium supplemented with 30% sucrose (w/v) (Qualigens, India) was used in all the experiments. P^H of the medium was adjusted to 5.7-5.8 with 0.1N NaOH or HCL perior to adding agar 0.8% (w/v), (Qualigens, India). Culture tubes were plugged with non-adsorbent cotton wrapped in double layered gauze cloth. The media was steam sterilized for 20 minutes at 121°C. All the culture tubes were incubated at 16-h photoperiod under illumination of cool-white fluorescent tube with a light intensity of 3000 lux at 22±1°C. Cultures were examined regularly. All the experiments were repeated thrice. Data which showed advantageous effect were induced in the tables and presented in mean±SE of 80 explants per treatment. Mean values with the same superscript were not significantly different (p=0.05%) according to Duncan's Multiple Range Test (DMRT).

2.2. Luteolin production through callus culture:

Various explants (leaf, stem and root) of *Premna serratifolia* L were excised into 0.5 – 1.5 cm long segments and small incision was made on the surface of each explant using a surgical sterile blade. Stem and root explants were placed horizontally on culture medium. In the case of leaf, the abaxial side of leaflet was in contact with the medium. All the explants were cultured on MS basal medium supplemented with various concentrations of different auxins ranging from 1.0 – 7.0 mg/l of 2.4-D, NAA, IAA and IBA for callus initiation. Twenty-eight days old callus was sub cultured on fresh medium with same growth regulator combinations for further proliferation. Callus was subcultured twice with 4 week interval. All the culture were incubated at 25 ± 1^{o}C under a relative humidity of 50 to 60% and 12/12 – hour photoperiod, with irradiance of 30 µmol m^2 s^{-1} provided by cool white fluorescent tubes (Philips India Ltd. Mumbai). The callus growth was measured in terms of fresh and dry weight. Fresh weights of callus were taken after removing the excess of moisture on the surface using blotting paper. Dry weight of callus was determined by drying in a hot air oven at 60^{0}C for 24 h.

2.3. Plant materials preparation and Chromatographic separation of Luteolin

The *in vivo* plant parts (leaf, stem and root) were collected from 8 years old tree and washed in tap water and then chopped into small fragments. Then materials were dried under shade conditions for 30 days and the drying operation was carried out under controlled conditions to avoid chemical changes. The dried samples were powdered roughly with hands. The powdered samples were stored in polythene containers at room temperature. The *in vitro* derived callus materials obtained from leaf stem and roots were dried in hot air oven at 50^o C for 48 h. Then the dried callus materials were powdered using mortar and pestle and the powdered samples were stored in polythene containers at room temperature.

2.4. Preparation of TLC plates (Harbone, 1998)

The glass plates to be used in TLC were cleaned carefully with ethanol in order to remove grease. Then the slurry of silica gel G in water (20 g of silica gel per plate) was shaken vigorously for 90 seconds and coated on the plates to a thickness of 0.5 mm using a commercial spreader. Then the plates were activated at 105° C for 30 minutes and then used. After spotting the TLC plates were kept in a chamber which contains the solvent, and the chromatogram was developed by ascending technique (the running solvent used was a tertiary mixture of chloroform, ethyl

acetate and benzene 4:1:5 ratios). After the development of chromatogram the resolved spots were revealed by spraying with copper sulphate, sodium citrate (copper sulphate 1.3 g, sodium citrate 17.3 g and sodium corbonate 10 g dissolved in 100 ml of distilled water). The eluted constituents were subjected to HPLC analysis.

2.5. HPLC analysis of different callus extracts:

Luteolin (62696) was purchased from Fluka (Buchs, Switzerland). The stock solution containing 780.0 µg/ml of luteolin was prepared in ethanol as standard reference solution and solution were stable for at least 2 months when stored at -20^{o} C. HPLC analysis of field grown plant materials and callus obtained from different explants (leaf, shoot and root) extracts was performed on the Agilent 1100 chromatographic system, consisting of G1314A isocratic pump, a thermostated column compartment, a variable-wavelength UV detector (VWD), and Agilent chemstation software. Chromatographic separation was achieved on the Agilent ODS C_{18} column (250 mm x 4.6 mm, 5 m), with a pre-column (Agilent ODS C_{18} , 10mm x 4.6 mm 5 m). Determination of luteolin was carried out with mobile phase composed of methanol and 0.2% phosphoric acid aqueous solution (55:45, U/V) at a flow-rate of 1.0 ml /min. The optimum separation of HPLC was achieved at 30º C and monitored at 350 nm.

2.6. Anti-inflammatory Activity of *Premna serratifolia* L.

Male wistar rats weighing 150-200 g were procured from National Institute of Nutrition, Hyderabad, India. They were acclimatized to laboratory conditions for a week prior to the initiation of the experiment. They were fed on standard rat feed and given free access to water. Twelve hours before the start of the experiment, rats were deprived of food, but given free access to water. The experiment was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional Animal Ethical Committee approved all the procedures.

2.7. Carrageenan Induced hind Paw edema

Different callus extract of *Premna serratifolia* L. screened for Luteolin production. The root and root derived callus were tested for Anti Inflammatory study. The root callus was determined by treating the animals having carrageenan induced hind Paw edema. Albino rats of either sex weighing 150-200 g were divided into four groups of six animals each. The dosage of the drugs administered to different groups was as follows. Group I served as Control, Group II and III received ethanolic extracts of root and root derived callus of *Premna serratifolia* (100 mg/kg) respectively and Group IV was treated with Indomethacin (10 mg/kg) which served as the standard reference drug. All the drugs were administered orally. All the above drugs were given to rats orally four hours before the commencement of the study. At the time of study a booster dose of each drug was given. 30 minutes after the administration of the booster dose 0.1 ml of 1% w/v carrageenan [Carrageenan is a mixture of polysaccharide composed of sulphated galactose units and is derived form Irish Sea moss *Chondrus crispus* [30]] solution in normal saline was injected into the subplantar tissue of the left hind paw of the rat to induce edema and right hind paw served as the control. The quantum of the swelling was measured by determining the thickness (volume) of the paw, its weight and the volume of water [31] or mercury [32 displayed in the plethysmograph. The volume displaced in the plethysmograph was measured at the end of 0 min., 60 min., 120 min., 180 min, 240 min., 360 min and 480 min. The % increase in paw edema of the treated group was compared with that of the control and the inhibitory effect of the drugs were studied.

The relative potency of the extracts under investigations was calculated based upon the percentage inhibition of the inflammation.

$$\% \text{ Inhibition of inflammation} = \frac{(\text{Volume of control} - \text{Volume of treated}) \times 100}{\text{Volume of control}}$$

2.8. Statistical analysis

All data were expressed are mean ± SE. The statistical analyses were performed using Student's't' test. $p<0.05$ was considered as significant.

3. Result and discussion:

Plant tissue culture research has been considered as fruitful approach for conservation of medicinally important species. In this present study the callus initiation was observed from cut end of the explants after seven days of inoculation. By the fourth weeks, almost every explants turned into callus mass when leaf was cultured on MS medium with various concentrations of NAA, 2,4-D and IBA (Table-1). The highest frequency of callus induction mass (88.5%) was observed on MS medium fortified with 5.0mg/l of IAA. The texture of the callus was compact and green in colour. (Figure.1). IAA and NAA are found to be efficient hormones for callus induction [33]. The leaf derived calli were transferred to MS medium containing different concentrations of BAA and KIN (0.5-5.0mg/l) alone and incombination; BAP+KN (0.1-0.7mg/l) and KN+IAA (0.1-0.7mg/l) for shoot bud differentiation. The callus mass modified in to bud primordial and these buds developed in to normal shoots within 2 weeks of sub culture. High percentage (90%) and maximum number (9.4±0.1) of shoots obtained from the medium containing 3.0mg/l BAP with 0.5mg/l KN (Fig-1).

In this present investigation, it was found that BAP and KN combination were found to be the best growth promoters for shoot bud regeneration from leaf explants of *Premna serratifolia* L. Similar experience reported in *Elaeocarpus robustus* [34]. The use of mixture of two cytokinins for shoot differentiation has been reported in *Douglas* and *Calabrian* [35].

3.1 Root development:

Regenerated shoots carefully separated and aseptically transferred to the rooting medium without growth regulators did not promote root induction. Roots were formed on excised shoots grown in MS medium containing different auxins IAA, IBA and NAA(0.5-2.0mg/l) with 3%(w/v) sucrose (Table-3). About 89% of shoots, rooted in the medium supplemented with 1.0mg/l NAA. Root initials formed with 5days and developed in to a good rooting system after 11 days. The newly developed root were thin varied in number from 1.5-3.4 per shoot. Several authors reported that NAA, was found to be effective for root induction [36,37]

3.2. Acclimatization of rooted plants:

Rooted plantlets were transferred **into** pots containing soil: sand: organic fertilizer in the ratio of 1:2:1. The plants were irrigated regularly. 89% percentage of survival was observed. The plants grew normally without any morphological variations.

3.3. Effect of different auxins on biomass production from stem, root and leaf explants.

The evolving commercial importance of secondary metabolites in recent years resulted in a great interest in secondary metabolism, particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology. Plant growth regulators represent importance in many cases, especially for callus culture and secondary metabolites production [38]. Stem explants were cultured on MS medium supplemented with different concentrations of various auxins (IAA, IBA, NAA and 2, 4-D). The best biomass (140mg) was obtained from 5.0 mg/l IAA fortified culture. The growth regulator IAA showed best response (77 %) in this concentration. Least callusing response (80mg) was obtained from 2,4-D supplemented medium followed. The callusing response was also

minimum (33 % and 24 %) in this concentration of 2, 4-D and IBA. When the root explants were cultured on medium supplemented with various auxins (IAA, IBA, 2,4-D and NAA 1.0 – 7.0 mg/l each) the best biomass (80mg) was obtained from 5.0 mg/l of IAA and 60 % callusing response was observed. The least biomass production (22mg) was obtained from 7.0 mg/l of IBA.

But there was no callusing response observed in 1.0 to 3.0 mg/l of IBA and 1.0 mg/l of 2,4-D. Callus was observed when leaf explants were cultured on MS medium fortified with all the tested hormone concentrations. Among this highest biomass (178 mg/l) was observed in the medium supplemented with 5.0 mg/l IAA on 25th day.

The callus obtained from this supplementation condition was yellow and friable (Fig. 8). Relatively higher biomass (147mg) of green nodular callus was obtained in 7.0 mg/l of NAA, and least biomass (84 mg) white friable calli was obtained with 7.0 mg/l of 2, 4-D (Fig-2).

3.4.Thin layer chromatography analysis to determine the presence of luteolin

The presence of luteolin from leaf, stem and root of *Premna serratifolia* L. and their callus have been determined by the development of the light blue spot on a silica gel plate. The luteolin spot were separated using chloroform and methanol (19:1) solvent mixture.

3.5.Quantitative determination of Luteolin in field grown plant and their callus extracts by HPLC

Ethanolic exracts of natural plant material and their callus was collected for the quantification of luteolin content using 2 mg of extract as well as standard luteolin dissolved in 10 mg of solvent (ethanol). The solvent system acetonitrile : water (50 : 50) along with 0.1% ortho – phosphoric acid gave a good separation of luteolin. The retention time of luteolin in the extracts as well as for the authentic sample was 3.52 min. Further detection of this flavonoid was based on co-chromatography with the authentic luteolin sample. Based on this, luteolin flavonoid present in the various extracts of *Premna serratifolia* L.were separated identified and quantified using standard calibration curves of luteolin. The percentage of luteolin present in field grown leaf was 5.64%, stem was 0.51% and root was 10.75%. Luteolin content in leaf callus was 10.72%, stem callus was 1.57% and root callus was 17.57%. (Fig-3).

3.6. Anti-inflammatory Activity of *Premna serratifolia* L. (Carrageenan Induced hind Paw edema):

Ethanolic extracts of root and root callus of *Premna serratifolia* L. have been selected based on its maximum luteolin content. In the present investigation paw volume of carrageenan treated albino rats (Group I) were increased with increasing time (Table- 4). Rise in paw volume was retarded in group II (carrageenen + *Premna serratifolia* L. field root extract 10 mg / kg) animals. The reduction in paw volume was significant after 3 hours of treatment. The percent inhibition of inflammation in group II was 63% while group III (carrageenen + *Premna serratifolia* L. root callus extract 10 mg / kg) has showed 70% inhibition of inflammation in paw volume (table-4). The percent inhibition of inflammation in group IV treated with standard drug indomethacin (10mg/kg) was 76 %. Among the tested extracts, root derived callus extracts of *Premna serratifolia* L. showed the maximum present of inhibition (70%) which was significant at P>0.001 than the control by the student t test (Fig. 4).

Inflammation is generally considered as an essentially protective response to tissue injury caused by noxious, physical, chemical (or) microbiological stimulus. It is a complicated process involving various mediators, such as prostaglandins, leukotriens and platelet activity factor [39]. Inflammation induced by carrageenan involves three distinct phases *viz.*, the release of the meditor, including serotonin and histamine in

the first phase (0-2 h), kinins in the second phase (3 h) and prostaglandin in the third phase (>4 h).
In the first phase increase in vascular permeability occurs. Infiltration of leukocytes occurs in second phase and granuloma formation in third phase [40]. The major macrophage derived inflammatory mediators such as pro inflammatory cytokines, tumour necrosis factor – α (TNF – α) and the reactive free radical nitric oxide (No) synthesized by inducible No synthase (iNos), contribute to the development of inflammatory diseases [41] Exudate formation and leukocyte infiltration are important components of inflammation [42].
The anti-inflammatory activity of the alcoholic extract of the roots of *Premna serratifolia* L.can can be attributed to its luteolin content because, it has a variety of pharmacological effect including inhibition of 12-0-Tetradecamylphorbol β-acetate induced mouse ear edema [43,44]. Luteolin was compared with Indomethacin in inhibiting PGE_2 concentration and carrageenan induced flush cavity in rats. This PGE_2 inhibition is corrected with reduced Cox-2 protein synthesis, but not by enzymatic activity [45]. Luteolin also inhibits cyclooxygenase – 2 transcriptional activities [46] and Lipoxygenase activity [47] which are involved in inflammation reaction. The results of the present study is also supported by the findings of Narayanan et al. (2000) [48] who reported that the root extracts of *Premna herbacea* possessed anti-pyretic, antinociceptive and anti-inflammatory potential in rabbits Dimo et al. 2006 [49] who evaluated the acute and chronic anti-inflammatory properties of leaf extracts of *Kalanchoe crenata.*

CONCLUSION

In conclusion, an efficient protocol was developed for successful mass multiplication of *Premna serratifolia* L. through *in vitro* techniques. These techniques may help in the effective conservation and synthesis of active compounds from the cell biomass. No somatic variation has been observed throughout the study period. In this research 89 % of field survival has been achieved and still maintaining the *in vitro* regenerated plants in the medicinal garden.

Among the various callus extracts of *Premna serratifolia* L. root and root callus found to be the best biomass source for luteolin production. *In vitro* propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds and this root and root callus extracts of *Premna serratifolia* L. augments that it is having good anti-inflammatory activity against carrageenan induced paw edema. The results support the conservation through efficient and reproducible *in vitro* technique and proved the anti inflammatory effect of callus derived Luteolin through its biologically active components, which May worth for further investigation and elucidation.

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REFERENCE:

1. Constabel, F., 1990. Medicinal plant biotechnology. Planta Med, Pp. 56:421–425.
2. Hu, Z., Yang, X., Ho, P.C., Sui, Y.C., Heng, P.W., Chan, E., Duan, W., Hwee, L.K., Zhou, S., Herb- drug interactions: a literature review. Drugs. 65, 1239-1282. 2005.
3. Vieira, R.F., Skorupa, L.A., 1993. Brazilian medicinal plants gene bank. Acta Hort. 330,51–58.
4. Dixon, R. A., 2001. Natural products and plant disease resistance. Nature. 411, 843- 847.

5. Han, S.T., 1998. Medicinal plants in the South Pacific. WHO Regional publications, Western Pacific series No.19, pp.159.
6. Cardenas, L.B., 1999. *Premna serratifolia* L. In de Padua, L.S., Bunyapraphatsara, N. & Lemmens, R.H.M.J. (Eds), Plant Resources of South-East Asia No. 12 (1). Medicinal and Poisonous Plants 1. Backhuys Publishers., Leiden, the Netherlands. pp. 416-417.
7. Ajitkar, B.K.; Choudry, B.K.; Bandyopadhyay, N.G., 2003. Comparitive evaluation of hypoglyceamic activity of some Indian medicinal plants in Alloxan diabetic rats. Journal of Ethnopharmocology. 84,105-108.
8. Husain, A., Virmani, O.P., Popli, S.P., Misra, L.N., Gupta, M.M., Srivastava, G.N., Abraham, Z., Singh, A.K., 1992. Dictionary of Indian Medicinal Plants. Central institute of Medicinal and Aromatic Plants, New Delhi, pp.375.
9. Chopra, R.N.,1969. Drug of India, orient Longman Ltd., Chennai, India.
10. Chopra, K. N., Nayar, S.L., Chopra, R.I.C., 1956. *Glossary of Indian medicinal plants*, publication and information Directorate, CSIR, New Delhi, India.
11. Will Mc Clatatchey., 1996. The ethanopharmacopoeia of Rotuma. J of Ethanopharmacology 50, 147-156.
12. Vadivul, R., Jerad Suresh, A., Girinath, K., Boopathi Kannan, P., Vimala, R., Sathish Kumar, N.M., 2009. Evaluation of Hepatoprotective and *In-vitro* Cytotoxic Activity of Leaves of *Premna serratifolia* Linn. J Sci. Res. 1 (1), 145-152.
13. Thamizh Selvam, N., Vengatakrishnan, V., Damodar Kumar, S., Murugesan, S., 2010. Evaluation of tissue level antioxidant activity of *Premna serratifolia* leaf in paracetamol intoxicated wistar albino rats, In. J of Pharmacy & Life Sciences 1(2),86-90.
14. Muthukumaran, P., Pattabiraman, K., 2010. Hepatoprotective Activity of *Premna serratifolia* Linn. On Experimental liver damage in rats. Asian journal of science and technology. 6,105-107.
15. Ravinder Singh, C., Nelson, R., Alagumanian, S., Siva Subramanian, S., Jayaraju, P., 2011. Seasonal variation in rooting response of stem cuttings of *Premna serratifolia* L. J B io s c i Res . 2(1), 21-23.
16. Deepti Yadav, Neeraja Tiwari, Madan, M. Guptu., 2011. Diterpinoid from *Premna integrgrifoliaI*. Phytochemistry Letters. 3,143-147.
17. Kasar Rahul, P., Laddha, K.S., Chaudhary, J., Shukla, A., 2007. Chyawanprash-Truth or Mythy. Pharmacognosy Reviews. 1,185-190.
18. Rekha, R., Suseela, L., Meenakshi Sundaram, R., Saleem Basha, N., 2008. Cardiac stimulant activity of bark and wood of *Premna serratifolia* L. Bangladesh J Pharmacol. 3,107-113.
19. Rao, B., Susheela, K., Rao, P.V.S., Krishna, P.G., Raju, G.V.S., 1984. Chemical examination of some Indian Medicinal plants. Indian J of Chem. 41, 787-788.
20. Suraj, K. G., Rajesh, R., Sunil Kumar, S., Sulekha, B., Prabha, B., 2008. A poly ayurvedic drug- *Indukantha Ghritha* as an adjuvant to cancer chemotherapy via immunomodulation. Immunobiology. 213, 641-649.
21. Julie, D., Jean, W., Pierre, C., Christine, H., Christian, B., Reynald, H., Alain, F., 2007. Antiparasitic activity of some New Caledonian medicinal plants. J of Ethanopharmacology. 112, 7-12.
22. Tatiana, S.T., Adriana, O. S., Tania U.N., Benedito, P. D., Celso, V. N., 2011. Recent advances in leishmaniasis treatment. I J of Infectious disease 15: e525-e532.
23. Hansen, G., Wright, M.S., 1999. Recent advances in the transformation of plants. Trends Plant Sci. 4,226-231.
24. Ravinder Singh, C., Nelson, R., Muthu Krishnan, P., Mahesh, K., 2011. Hepatoprotective and anti-oxidant effect of root and root Callus extract of *premna serratifolia* L. In paracetamol induced Liver damage in male albino rats. In. J. of Pharma and Bio Sciences. 2, 244-252.
25. Kathiresan, K. 2009. Mangroves and coral reefs of India, project report of Ministry of Environment and Forests, PP 42-50.
26. Ravinder Singh, C., Nelson, R., Siva Subramanian, S., 2011. Rapid *in vitro* propagation of *Premna serratifolia*, a medicinally important declining shrub, India. Conservation Evidence. 8, 66-73.
27. Litz, R.E., Moore, G.A., Srinivasan, C., 1985. *In vitro* systems for propagation and improvement of tropical fruit and plams. Hortic Rev. 7,157-198.
28. Negrutiu, I., Jacobs, N., Caboche, M., 1984. Theor Appl Genet 67, 289-304.
29. Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15,473-497.
30. Smith, C.J., Zhang, Y., Kobolt, C.M., 1998. Pharmacological analysis of cyclooxy-genase-1 in inflammation. Proc. Natl. Acad. Sci. USA. 95,13313- 13318.
31. Vineger, R., Truax, J.F., Salph, J.H., Johnston, P.R., Venable, A.L., Mckenzie, K.K., 1968. Pathway to carrageenan – induced inflammation in the hind limb of the rat. Fed. Proc. 46, 118- 26.
32. Winter, C.A., Risely, E.A., Nuss, G.R., (1962) Carrageenan induced edema in hind paw of assay

for anti-inflammatory drugs. Pro. Soc. Exp. Biol. Med. 111,544-547.
33. Das, P., Sammtaray, S., Rout, G.R., 1996. *In vitro* propagation of *Acaccia catechu*, a xerophilous tree. Plant tissue culture. 6(2),117-126.
34. Rahman, M.M., Amin, N.M., Ahmed, R., Azad, M.A.K., Begum, F., 2004. *In vitro* plantlet regeneration from internode explant of native – olive (*Elaeocarpus robustus*). J F Biosciences. 4(5),676-680.
35. Chang, T.Y., 1997. Factors affecting adventitious bud formation of cotyledon culture of *Douglas fir*-plant sci. Lett. 9,179-187.
36. Meng-ling, H.E., Shu-yuan, Q.I., Lan-juan, H.U., 2005. Rapid *in vitro* propogation of medicinally important *Aquilaria agallocha*. J Zhejiang Univ sci 6B (8),849-852.
37. Dodds, J.H., 1983. Tissue culture of trees. American edition, the Avi publishing company, Inc. Wesport. Connecticut. Pp . 71-74.
38. Siatka, T., 1998. Effect of auxins on Bellis perennis L. callus culture growth and flavonoids production. Ces. Slov. Farm. Pp. 47.
39. Gryglewski, R.J., 1981. Molecular mechanism of inflammation. Eur. J. Rheumatol inflamm. 4,153-159.
40. Singh, S., Manjumdar, D.K., Rehan, H.M.S., 1996. Evaluation of Anti-inflammatory potential of *Ocimum sanctum* and its possible mechanism of action J.Ethanopharmacol.. 54, 19 – 26.
41. Freeman, B.D., Natanson, C., 2000. Anti-inflammatory therapies in sepsis and septic shock. Expert Opin Investig. Drugs. 9,1651 – 1663.
42. Mahat, M.A., Patil, B.M., 2007. Evaluation of anti-inflammatory activity of Methanol extract of *phyllanthus amarus* in experimental animal models. Indian J.Pharmaceutical sciences. 1,33-36.
43. Anastasia Kotanidov, Angeliki xagorari, Eleni Bagli, Panagiota kitsanta, Theodore Fotsis, 2002. Luteolin redules lipopolysaccharide – induced lethal toxicity and Expression of pre-inflammatory molecules in mice. Am. J. Respir. Crit. Care med. 165,818 - 823.
44. Yasukawa, K., Takido, M., Ikekawa, T., Shimada, F., Takeuchi, M., Nakagawa, S., 1991. Relative inhibitory activity of berberine – type alkaloids against 12-0-tetradecanyl phorbol 13-acetate induced inflammation in mice, Chem. Pharm. Bull 39,1462-1465.
45. Chi – Likuo, Chin – Wenchi, Tsung – Yun Liu, 2003. The anti-inflammatory potential of berberine *in vitro* and *in vivo*, Cancer Letters. 203,127 – 137.
46. Fukuda, K., Hibiya, Y., Mutoch, M., Koshiji, M., Akao, S., Fujiwara, H., 1999. Inhibition by berberine of cyclooxygenase – 2 – transcriptional activity in human colon cancer cells. J.Ethanopharmacol. 66,227- 233.
47. Misik, V., Bazakova, L., Malekova, L., Kostalova, D., 1995. Lipoxygenase inhibition and antioxidant properties of proteberberine and a morphine alkaloids isolated from *Mahonia aquifolium*. Planta med. 61, 372-373.
48. Narayanan, N., Thirugnanasambantham, P., Viswanathan, S., Kannappa Reddy, M., Vijayasekaran, V., Sukumar, E., 2000. Antipyretic, antinociceptive and anti-infammatory activity of *Premna herbacea* roots. Fitoterapia. 71, 147- 153.
49. Dimo, T., Agathe, L. F., Nguelefack, T.B., Asongalem, E.A., Kamtchouting, P., 2010. Anti-inflammatory activity of leaf extract of *Kalanchoe Crenata*. Indian Journal. Pharmacol. 38, 115-119.
50. Pullaiah T., Murthy K.S.R. & Rani S.S., 2004. Floristic analysis of Andhra Pradesh, India, In: Rama Swami N. *et al*. (eds.) Gleanings in Plant Sciences p. 43-52.

Figures and Tables:

Fig. 1 . Effect of growth regulators on plant regeneration from leaf derived callus explants.

A. Callus formation in leaf explant on MS medium containing IAA (5.0 mg/l)
B. Microshoot initiation in callus cultured in MS medium containing BAP (0.5 mg/l), KN (0.1 mg/l)
C. Microshoot proliferation in callus cultured in MS medium containing BAP (3.0 mg/l), KN (0.5 mg/l), D. Shoot proliferation in MS medium containing BAP (3.0 mg/l), KN (0.5 mg/l), E. Root formation in regenerated plantlets in MS medium containing NAA (1.0 mg/l), F. Plants transferred to plastic pots containing soil mixture,
G. Plants survived in earthen pots under green house condition

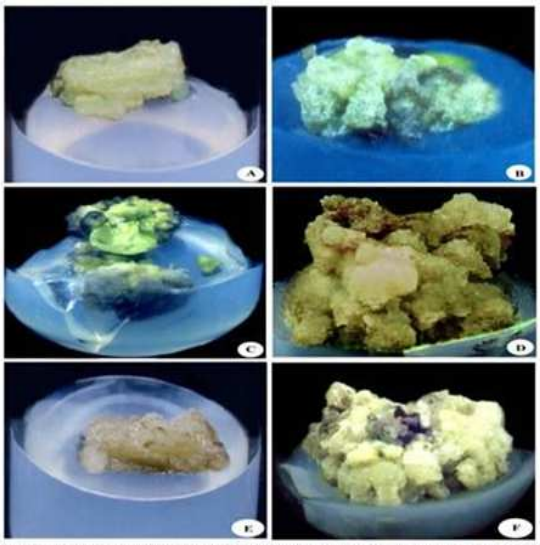

Fig: 2. Effect of different auxins on callus induction in different explants of *Premna serratifolia* L.

A. Yellow friable callus initiation in stem explants in the MS medium containing IAA (1.0 mg/l), B. Light green friable callus proliferation in stem explants in the MS medium containing IAA (0.5 mg/l), C. Green friable callus formation in leaf explants in MS medium containing IAA (1.0 mg/l), D. Yellow friable callus proliferation in leaf explants in MS medium containing IAA (5.0 mg/l), E. Yellow green friable callus initiation from root explants in MS medium containing IAA (1.0 mg/l) f. Yellow friable callus proliferation in root explants in MS medium containing IAA (5.0 mg/l)

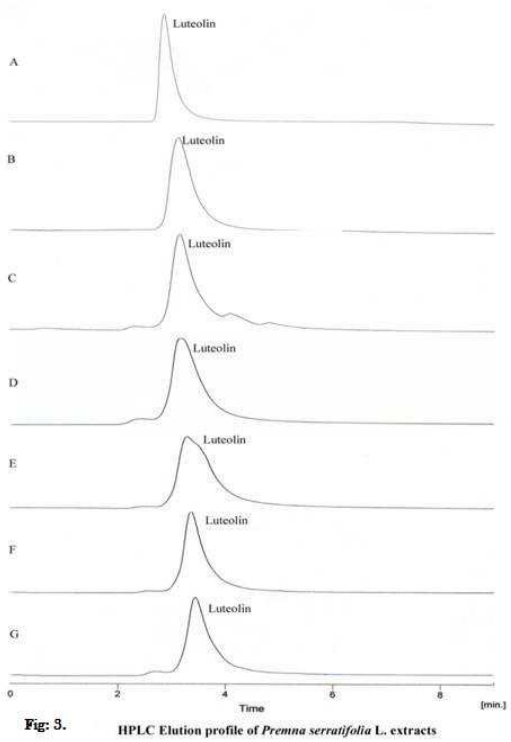

Fig: 3. HPLC Elution profile of *Premna serratifolia* L. extracts

A. Standard Luteolin, B. Leaf extract of *P.serratifolia*, C. Lead derived callus of *P.serratifolia*, D. Stem extract of *P.serratifolia*, E. Stem derived callus extract of *P.serratifolia*, F. Root extract of *P.serratifolia*, G. Root derived callus extract of *P.serratifolia*,

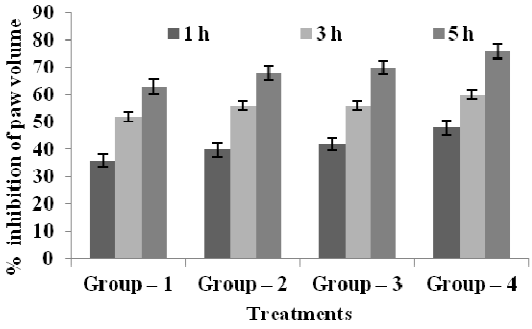

Fig: 4- Anti-inflammatory activity of root and root derived callus extract of *Premna serratifolia* L. against carageenan induced paw edema in Male Albino rats.

Group – 1 Control (Carageenan 0.5 ml/kg); **Group – 2** *P.serratifolia* root extract (100 mg/kg); **Group – 3** *P.serratifolia* root derived callus extract (100 mg/kg); **Group – 4** Indomethacin (10 mg/kg)

Table-1 Effect of various auxins on callus induction from different explants of *Premna serratifolia* L.

Growth regulators (mg/l)	Leaf	
	% of response	Callus growth (mg/fresh mass)
NAA (mg/l)		
1.0	40.0	98.5 ± 0.7^{e}
3.0	58.0	113 ± 0.0^{d}
5.0	73.0	145 ± 0.4^{bc}
7.0	70.0	122 ± 0.5^{cd}
IAA (mg/l)		
1.0	57.0	111 ± 0.0^{d}
3.0	77.0	139 ± 0.0^{c}
5.0	88.5	170 ± 0.6^{a}
7.0	82.0	155 ± 0.6^{b}
2,4-D (mg/l)		
1.0	37.0	90 ± 0.2^{e}
3.0	55.0	102 ± 0.7^{de}
5.0	50.0	99 ± 0.0^{e}
7.0	42.0	82 ± 0.9^{f}
IBA (mg/l)		
1.0	28	95 ± 0.5^{e}
3.0	44	109 ± 0.0^{de}
5.0	60	127 ± 0.0^{cd}
7.0	58	119 ± 0.4^{d}

Values are mean ± SE of three repeated experiments. Mean within a column followed by the same letters are not significantly different at 5.0 % probability level according to Duncan's multiple range test (DMRT).

Growth regulators (mg/l)	Leaf			
	No. of explants, callus responded	% of organogenesis	Mean no. of shoots	Shoot length (cm)
BAP				
0.5	33.2 ± 0.4^{bc}	38.6	1.8 ± 0.0^{de}	1.5 ± 0.5^{c}
1.5	43.7 ± 0.6^{b}	57.8	1.4 ± 0.4^{d}	1.9 ± 0.0^{b}
3.0	48 ± 0.5^{ab}	82.0	2.7 ± 0.1^{c}	1.5 ± 0.7^{a}
5.0	40.5 ± 0.8^{b}	67.5	2.0 ± 0.0^{cd}	1.0 ± 0.1^{ab}
KN				
0.5	10 ± 0.0^{e}	54.1	0.0 ± 0.0^{f}	0.0 ± 0.0^{d}
1.5	27.5 ± 0.7^{cd}	45.8	1.9 ± 0.0^{de}	0.7 ± 0.7^{c}
3.0	22.9 ± 0.5^{d}	38.1	2.7 ± 0.0^{de}	1.4 ± 0.0^{cd}
5.0	20 ± 0.0^{d}	33.3	1.5 ± 0.3^{e}	1.4 ± 0.7^{cd}
BAP + KN				
0.5 0.1	32.5 ± 0.0^{bc}	54.1	2.2 ± 0.7^{cd}	1.4 ± 0.1^{cd}
1.5 0.3	46.7 ± 0.5^{ab}	76.6	5.8 ± 0.5^{b}	2.5 ± 0.0^{c}
3.0 0.5	54 ± 0.9^{a}	90.0	9.4 ± 0.1^{a}	2.5 ± 0.4^{c}
5.0 0.7	50 ± 0.5^{a}	83.0	8.0 ± 0.8^{ab}	1.3 ± 0.9^{cd}
KN + IAA				
0.5 0.1	17.3 ± 0.7^{d}	28.8	0.0 ± 0.0^{f}	0.0 ± 0.0^{d}
1.5 0.3	30.1 ± 0.8^{c}	50.1	1.9 ± 0.0^{d}	0.5 ± 0.7^{c}
3.0 0.5	34 ± 0.0^{bc}	56.6	4.7 ± 0.7^{bc}	1.7 ± 0.9^{bc}
5.0 0.7	39.5 ± 0.2^{b}	65.8	5.3 ± 0.5^{b}	1.2 ± 0.0^{ab}

Table-2 Effect of different hormones on multiple shoot induction from Leaf callus of *Premna serratifolia* L.

60explants were taken for each experiment. Values are mean ± SE of three repeated experiments. Mean within a column followed by the same letters are not significantly different at 5.0 % probability level according to Duncan's multiple range test (DMRT)

Table-3 Effect of different auxins on efficient root induction on *Premna serratifolia* L.

Growth regulators (mg/l)	No. of shoots responded	% of rooting	Mean no. of roots/shoot	Root length (cm)
IBA				
0.5	17.2 ± 0.2^{bc}	57.3	$1.0 \pm 0.0_{c}$	0.7 ± 0.7^{cd}
1.0	20.7 ± 0.0^{ab}	69.0	1.2 ± 0.6^{c}	1.6 ± 0.3^{b}
2.0	19.0 ± 0.0^{b}	63.3	2.8 ± 0.6^{b}	2.1 ± 0.0^{bc}
NAA				
0.5	21.5 ± 0.0^{ab}	71.7	1.5 ± 0.5^{bc}	1.9 ± 0.7^{ab}
1.0	26.7 ± 0.8^{a}	89.0	3.4 ± 0.9^{a}	2.4 ± 0.0^{a}
2.0	25.0 ± 0.5^{a}	83.3	1.9 ± 0.0^{b}	2.0 ± 0.0^{ab}
IAA				
0.5	14.0 ± 0.5^{c}	46.7	0.5 ± 0.0^{d}	1.4 ± 0.4^{d}
1.0	19.5 ± 0.2^{b}	65.0	1.0 ± 0.4^{c}	0.6 ± 0.6^{cd}
2.0	20.0 ± 0.7^{ab}	66.7	0.9 ± 0.5^{b}	0.9 ± 0.8^{c}

Values are mean ± SE of three repeated experiments. Mean within a column followed by the same letters are not significantly different at 5.0 % probability level according to Duncan's multiple range test (DMRT).

Table-4 Anti-inflammatory activity of root and root derived callus extract of *Premna serratifolia* L. against carageenan induced paw edema in Male Albino rats

Treatments	**% Inhibition of paw volume**		
	1 h	**3 h**	**5 h**
Group – 1	36	52	63
Group – 2	40	56	68
Group – 3	42	56	70
Group – 4	48	60	76

Group – 1 Control (Carageenan 0.5 ml/kg); Group – 2 *P.serratifolia* root extract (100 mg/kg); Group – 3 *P.serratifolia* root derived callus extract (100 mg/kg); Group – 4 Indomethacin (10 mg/kg)