Macro propagation and Assessment of the Anti-inflammatory potential of *Plectranthus caninus* – an *in vitro* approach

Mohankumar Harshini, Soundararajan Deepa and Raju Prabakaran*
PG and Research Department of Botany, PSG College of Arts & Science, Coimbatore, Tamil Nadu, INDIA
***raju.prabakaran@gmail.com**

Abstract
Plectranthus caninus, a member of the Lamiaceae family, possesses a range of therapeutic properties. Despite its inability to produce seeds, this species efficiently propagates through vegetative plant components. A recent study investigated the efficacy of vegetative propagation of P. caninus and evaluated the anti-inflammatory properties of an ethanolic leaf extract obtained from vegetatively propagated plants. Without any treatment, stem cuttings of P. caninus demonstrated a successful initiation of shoots and roots, achieving a propagation rate of 75%. Over a span of 60 days, the plants exhibited significant growth with a maximum plant height of 37cm, shoot length of 26.2cm and root length of 12.6cm.

The anti-inflammatory potential was assessed through the albumin denaturation assay, HRBC membrane stabilization assay and heat-induced hemolysis assay. The ethanolic leaf extract of P. caninus displayed maximum percentage inhibition at 200 mg/mL, with values of 78.70 ± 0.83, 77.7 ± 0.66 and 79.8 ± 0.63 respectively. Comparative analysis with the standard (diclofenac sodium) revealed similar results. In conclusion, the anti-inflammatory potential found in the leaves of P. caninus holds promise for the development of a novel plant-based anti-inflammatory drug.

Keywords: *Plectranthus caninus*, anti-inflammatory, macro propagation, HRBC.

Introduction

Plants serve as a remarkable source of various products including food, fodder, fuel and medicine, owing to the presence of specific chemical compounds that play a crucial role in treating specific disorders. The group of plants used for such purposes is referred to as medicinal and aromatic plants. Plants are unique components of nature showing a remarkable diversity and variation among the different species that are influenced by variable environmental conditions. Vegetative propagation can be most effectively done in a controlled environment. Therefore, photoautotrophic micropropagation will replace the conventional vegetative propagation method, provided that the production cost per propagation is comparable to that of the traditional greenhouse approach. The efficiency of propagation and costs associated with maintaining stock plants are variable, being affected by the weather conditions in greenhouse vegetative propagation. Thus, vegetative propagation under a controlled and pathogen-free environment (i.e. photoautotrophic micropropagation) can be a feasible alternative.

Humans and mammals respond to a variety of hostile foreign agents like pathogens, toxic chemicals or physical damage to the tissue through inflammation[2]. The processes associated with the inflammatory response are complex but important aspects which have been exploited for the screening of anti-inflammatory compounds[10]. Today, the search for natural compounds rich in anti-inflammatory properties is escalating due to their medicinal importance in controlling many related chronic disorders (cancer, diabetes, arthritis, hypertension etc) [14]. Typical inflammatory diseases such as rheumatoid arthritis, asthma, colitis and hepatitis are among the causes of death and disability in the world[1]. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents[6].

Lamiaceae formerly called Labiatae, is the mint family of flowering plants consisting of 236 genera and more than 7,000 species, the largest family of the order Lamiales. The Lamiaceae predominantly consists of herbs or shrubs, often characterized by aromatic, herbage, quadrangular stems and verticillate inflorescences. Lamiaceae is distributed nearly worldwide and many species are cultivated for their fragrant leaves and attractive flowers. The family is particularly important to humans for herb plants useful for flavour, fragrance, or medicinal properties. It is also distributed in Peninsular India, especially in Idukki district in Kerala. *Plectranthus caninus* has numerous medicinal properties. The plant is recognized for its diuretic properties. It is used in the treatment of teeth and gum disorders. Extracts from its roots serve as a remedy for cough. It has cytotoxic and antitumor-promoting activity and can be used in the treatment of cancer[12]. *P. caninus* has a historical application in managing coughs in Kenya[4] and has been reported to possess antimicrobial activity[19].

Material and Methods

Collection of plant materials: The plant material was collected from Palamalai hills (11.7355° N, 77.7494° E) at an altitude of 1623 m, Coimbatore district, Tamil Nadu, India. The plant was then used for various experiments to develop the explant preparation and regeneration protocol.

Vegetative Propagation: *Plectranthus caninus* was selected for vegetative propagation. The plant is efficiently reproducing through its mature stem cuttings. A disease-free, healthy and mature *P. caninus* plant was identified and utilized as a source of stem cuttings for the development of new individuals resembling their parent plants. A well-prepared mixture of soil, vermicompost and sand in a 3:1:1 ratio, along with coir pith, supported the initiation of new shoots and roots, facilitating the development of new plants. Due to its succulent nature, excess water adversely affects its growth. Fifty poly bags, each measuring 13 cm in length and 8 cm in width, were selected and filled with a mixture of fertile soil, manure and sand in equal proportions. This served as a medium for regeneration using 10 cm deep stem cuttings individually planted in an herbal garden.

After a few days of stem-cutting propagation, new bud development is initiated, gradually transforming the plant into a new one similar to its parent. The germination percentage is calculated using the formula:

$$\text{Percentage Inhibition} = \left(\frac{\text{No of plants germinated}}{\text{Total no of plants planted}} \text{ X } 100 \right)$$

Preparation of Solvent Extracts: The powdered plant material was soaked in approximately 400 mL of ethanol and water. It is kept separately on an electrical shaker for forty-three hours at room temperature. The mixtures were filtered into conical flasks using Whatmann filter paper no.1. The filtrate was then concentrated on a rotary evaporator at 50°C to yield semi-solid masses whose weights were determined. The extracts were stored in a refrigerator at 4°C.

Inhibition of Albumin Denaturation Assay: The reaction mixture (5 mL) consisted of 0.5 mL of egg albumin (from fresh hen's egg), 2.5mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of the test extract (HEPA) to achieve final concentrations of 50 and 100 mg/mL[8]. A similar volume of double-distilled water served as a control. The mixtures were incubated at 37±2ºC in a BOD incubator for 15 minutes and then heated at 70ºC for 5 minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as blank. Diclofenac at the final concentration of (50, 100mg/mL) was used as a standard drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the formula:

$$\text{Percentage Inhibition} = 100 \times \left(\frac{Vt}{Vc} - 1 \right)$$

where *Vt* is the absorbance of the test sample and *Vc* is the absorbance of the control.

Human Red Blood Cell (HRBC) Membrane Stabilization Method: The blood was collected from a healthy human volunteer who had not taken any NSAIDS for 2 weeks before the experiment. Blood was mixed with an equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm[5]. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared (70,140 and 210 mg/mL) using distilled water and to each concentration, 1 mL of phosphate buffer, 2 mL hypo saline and 0.5 mL of HRBC suspension were added[18]. It was incubated at 37^{0}C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm.

Diclofenac (50, 100 and 150 mg/mL) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicate and the mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection was calculated using the formula:

$$\text{Percentage Inhibition} = \left(100 - \frac{\text{OD of drug} - \text{treated sample}}{\text{OD of control}} \right) \text{X } 100$$

The experiment was performed in triplicate for all test samples.

Heat-induced haemolysis: The reaction mixture (2 mL) consisted of 1 mL of test sample solution and 1 mL of 10 % RBCs suspension[7]. Diclofenac sodium was taken as a standard drug. All the centrifuge tubes containing the reaction mixture were incubated in a water bath at 56°C for 30 minutes. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm. The percentage of haemolysis was calculated by using the following formula:

$$\text{Percentage Inhibition} = \left(100 - \frac{\text{OD of drug} - \text{treated sample}}{\text{OD of control}} \right) \text{X } 100$$

The experiment was performed in triplicate for all test samples.

Results

Vegetative propagation: The stem cuttings of *P. caninus* without any treatment, effectively initiated shoots and roots. All cuttings were checked intermittently for the development of shoots (Figure 1). The highest germination percentage was 75%. After 60 days, the plants displayed remarkable growth, demonstrating significant differences in parameters such as plant height, root length and shoot length with a maximum height of 37cm, a length of the shoot was 26.2cm and a maximum root length of 12.6cm. The results of the growth analysis are represented in table 1.

Inhibition of Albumin Denaturation Assay: Ethanoic leaf extracts of *P. caninus* at the concentration of 50mg/mL showed 65.90 ± 0.51 % inhibition. They are compared with

standard (diclofenac sodium) which showed 77.49 ± 0.74 % inhibition in 50mg/mL. The percentage of inhibition was found to be increased at a higher concentration which showed 78.70 ± 0.83 % inhibition in 200 mg/mL. The inhibition was a little higher when compared to the standard drug (Figure 2a).

Human Red Blood Cell (HRBC) Membrane Stabilization Method: The ethanolic extracts of *P. caninus* show a minimum inhibition of 41.46 ± 0.56 in 50 mg/mL and the maximum inhibition was demonstrated in 200mg/mL. The yield value is compared to the standard diclofenac sodium. The percentage of inhibition is 81.65 ± 0.74 % in standard. The results are graphically represented in figure 2b.

Table 1
Growth analysis of *Plectranthus caninus* through vegetative propagation

S.N.	Root length (cm)	Shoot length (cm)	Plant height (cm)	No. of leaves per plant	No. of branches per plant
1.	9.6	11.6	21.2	10	1
2.	3.6	12.9	16.5	7	1
3.	12.3	19.8	32.1	19	2
4.	8.7	18.3	27	21	3
5.	3.8	7.1	10.9	13	1
6.	5.8	18.4	24.2	26	2
7.	9.2	17.4	26.6	33	3
8.	6.4	19.9	26.3	19	2
9.	12.6	20.7	33.3	37	4
10.	9.2	5.4	14.6	13	2
11.	4.6	7.6	12.2	17	2
12.	5.4	8.3	13.7	23	1
13.	10.8	26.2	37	32	3

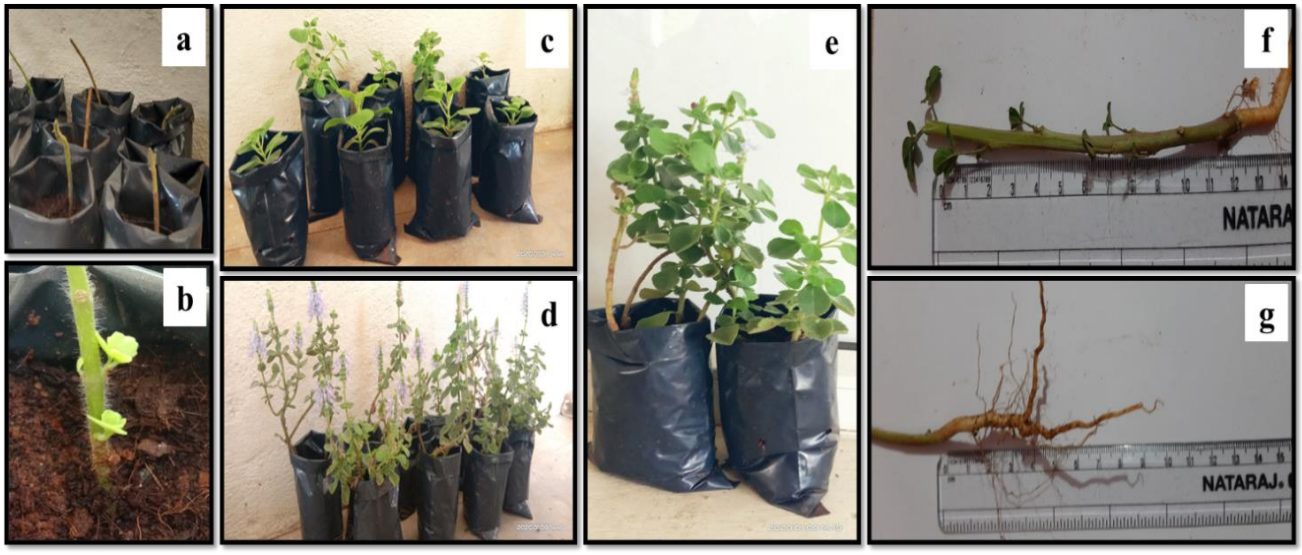

Figure 1: (a) Stem cuttings of *Plectranthus caninus*, (b) Initiation of nodal development (c) Young plants (d) Inflorescence of plants (e) Mature plants (f) Shoot measurement (g) Root measurement

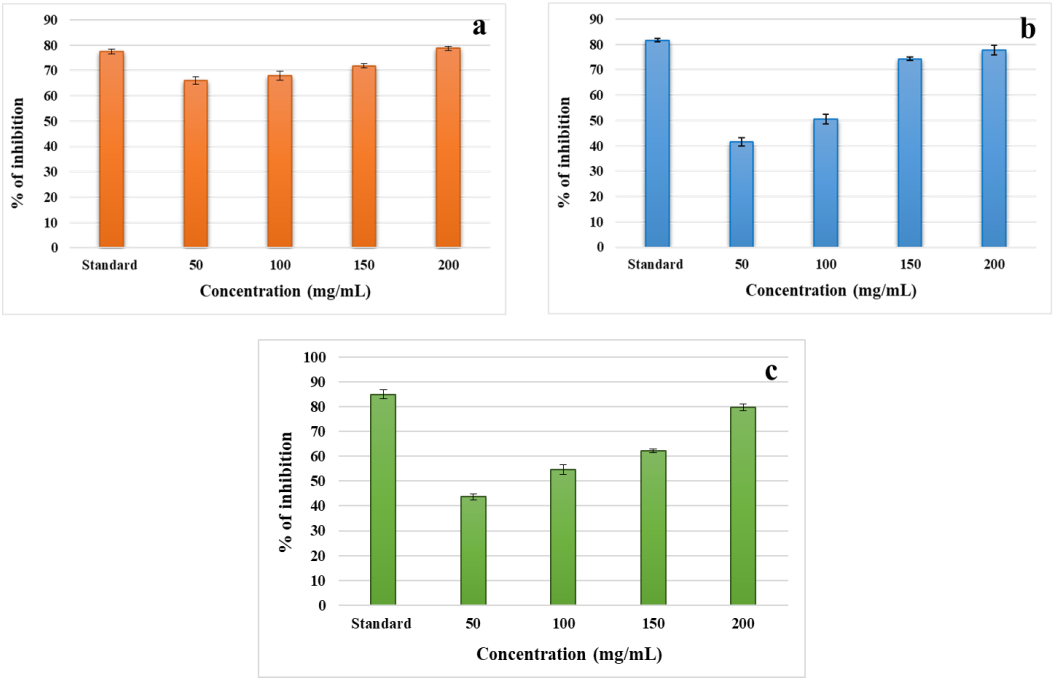

Figure 2: Graphical representation of inhibition percentage in the ethanolic leaf extract of *Plectranthus caninus* (a) Albumin denaturation assay (b) HRBC Membrane Sterilization assay (c) Heat-Induced Haemolysis assay

Heat-induced haemolysis method: The plant ethanolic extracts inhibited the heat-induced haemolysis of RBCs to various degrees. The highest percentage of inhibition was 79.8 ± 0.63 % at 200mg/mL. They were compared with standard diclofenac sodium which showed a maximum inhibition of 85 ± 0.64 %. The inhibition of plant samples is eventually equal to the standard drug (Figure 2c).

Discussion

The plants produce seeds efficiently during their favourable season. Simultaneously, it is noted that some plants are not capable of producing seeds and propagating using their vegetative plant parts[3]. The current study depends on the investigation of vegetative propagation using stem cutting of *P.caninus*. The stem cuttings are planted in poly bags filled with red soil, sand, vermicompost and coir pith for developing into new individuals. Stem cuttings started to develop new buds on their nodular region. These buds further develop the shoot system and roots from the nodular part of the stem at the base, deep in the soil within poly bags. Low temperatures and high moisture levels support the process of root and shoot development[15].

In this case, the favourable ecological conditions help the plant stem to bear the new buds in the nodular part and roots developed in the stem base in the poly bags. The influence of organic and inorganic on quality and yield components is boosted to the productivity potential with combined application in microbial and chemical fertilizers which had a great influence at all the growth stages of the crop. Significant differences in all parameters like plant height, number of leaves, leaf area and number of branches are due to the combined application of microbial fertilizer and chemical fertilizer[9]. In the investigation, the utilization of vermicompost has a considerable impact on plant development, causing significant contrast in parameters like plant height, root length, number of leaves and number of branches.

Regular cultivation process of varied medicinal and aromatic plants not only supports economic benefits to the farmers/cultivars but also plays a significant role in the conservation of such flora in nature. Propagation using vegetative parts/stem cutting of the plant[3] supports further protection of the rich fragrant having effective potential for therapeutic characteristics to treat a few issues and is conceivably used among the general public in multifield bearings.

Inflammation covers protective and reparative responses in damaged tissue, either caused by infection, disease, autoimmune disorders, industrial chemicals, or radiation[13]. In the present study, the anti-inflammatory activity of the ethanolic extracts of *P. canines* was evaluated using *in vitro* measures prompted by chemical substances. During inflammation, lysosomal hydrolytic enzymes are released into the sites which cause damage to the surrounding organelles and tissues, creating a variety of disorders. The protective effect on heat and hypotonic saline-induced erythrocyte lysis is known to be a very good index of anti-inflammatory activity for any sample[17].

The present investigation in the *in vitro* anti-inflammatory tests by human red blood cell membrane stabilization is because of reproducibility. The results were looked at by standard diclofenac sodium which has a standard worth. Such action hinders the hypotonic arrangement that incites cell lysis. This additionally demonstrated that with

increment focus, the cell haemolysis rate likewise diminished. The denaturation of proteins is the main cause of inflammation[16].

In this investigation, ethanolic extracts *of P. caninus* were effective in inhibiting heat-induced albumin denaturation seen as 78.70 ± 0.83% at 20mg/mL focus and contrasted with standard diclofenac sodium which has the capacity to inhibit the protein denaturation. The erythrocyte membrane against lysis induced by heat aspirin offered significant protection against the damaging effects of heat solution[11]. In this investigation, ethanolic extracts of *P. caninus* are effective in inhibiting the heat-induced haemolysis of HRBC at different concentrations and standard diclofenac sodium showed maximum inhibition of 81.65 ± 0.76 %. The essential oils are rich in plants which are aromatic and are medicinally used as therapeutics to treat various diseases and disorders. The extracts tested in this work possessed better anti-inflammatory activity.

Conclusion

This study demonstrates the successful macro propagation of *P. caninus* through stem cuttings. The optimized medium in polybags, comprising of sand, red soil, vermicompost and coir pith facilitated root commencement and shoot elongation. The propagation through mature stem cuttings serves conservation and supports future generations. The anti-inflammatory potential of ethanolic leaf extracts from *P. caninus*, as indicated by various assays, supports its potential use in traditional medicine for managing inflammatory conditions. This plant can be used for new drug discoveries and also can serve as an alternative to steroidal drugs used for inflammation.

Acknowledgement

We thank the PG and Research Department of Botany for providing the lab facility to conduct the experiment.

References

1. Abreu P., Matthew S., Gonzalez T., Costa D., Segundo M.A. and Fernandes E., Anti-inflammatory and antioxidant activity of a medical tincture from *Pedilanthus tithymaloides*, *Life Sci.*, **78**, 1578- 1585 **(2006)**

2. Aksoy L. et al, Free radical scavenging activity, total phenolic content, total antioxidant status and total oxidant status of endemic *Thermopsis turcica*, *Saudi J. Biol. Sci.*, **20(3)**, 235-239 **(2013)**

3. Dixena D. and Patel D.K., Mass vegetative propagation of an aromatic medicinal plant *Melissa officinalis* Linn for Ex-situ conservation in Herbal Garden, *J. Med. Plants*, **6(1)**, 06-10 **(2018)**

4. Githinji C.W. and Kokwaro J.O., Ethnomedicinal study of major species in the family Labiatae from Kenya, *J. Ethnopharmacol.*, **39**, 197-203 **(2003)**

5. Gopalkrishnan S., Kamalutheen M. and Ismail T.S., Anti-inflammatory and anti-arthritic activities of *Merremia tridentate*, *E. J. Chem.*, **6(4)**, 943-948 **(2009)**

6. Goudgaon N.M., Basavaraj N.R. and Vijayalaxmi A., Anti-inflammatory activity of different fractions of *Leucas aspera* Spreng, *Indian.J. Pharmacol.*, **35**, 397-398 **(2003)**

7. Gupta V., Chauhan S., Prakash A. and Mathur A., Evaluation of *in vitro* and *in vivo* anti-inflammatory activities of *Parthenium camphora*, *Recent Res. Sci. Technol.*, **5(1)**, 33-39 **(2013)**

8. Janakiraman D. and Somasundaram C., Evaluation of Anti-inflammatory effect of *Plectranthus amboinicus* leaf extract- An *in vitro* study, *Recent Res. Sci. Technol.*, **4(2)**, 229-232 **(2014)**

9. Latha P., Jeyaraman S. and Prabakaran R., Study effects of microbial and chemical fertilizer on yield components in brinjal (*Solanum melongena* Linn.) C. Var CO-2, *Int. J. Curr. Microbiol. Appl. Sci.*, **3(8)**, 817-822 **(2014)**

10. Laupattarakasem P. et al, An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis, *J. Ethnopharmacol.*, **85(2-3)**, 207-215 **(2003)**

11. Leelaprakash G. and Dass S.M., *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*, *Int. J. Drug Dev. Res.*, **3(3)**, 189-196 **(2011)**

12. Lukhoba C.W., Simmonds M.S.J. and Paton A.J., *Plectranthus*: A review of ethnobotanical uses, *J. Ethnopharmacol*, **103(1)**, 1-24 **(2006)**

13. Menon D.B., Sasikumar J.M. and Latha K., Anti-inflammatory and cytotoxic activity of methanolic extract of *Plectranthus hadiensis* stem, *Pharmacology Online*, **3**, 275-282 **(2011)**

14. Naik D.G., Majumdar A.M., Dandge C.N. and Puntambekar H.M., Anti-inflammatory activity of *Curcuma amada* Roxb. in Albino rats, *Indian J. Pharmacol.*, **32(6)**, 375-377 **(2000)**

15. Patel D.K., Vegetative Propagation Strategy of *Plectranthus amboinicus* (Lour) Spreng in Herbal Garden, *Adv. Plants Agric. Res.*, **5(4)**, 00190 **(2016)**

16. Reshma A.K. and Brindha P., *In vitro* anti-inflammatory, antioxidant and nephroprotective studies on leaves of *Aegle marmelos* and *Ocimum sanctum*, *Asian J. Pharm. Clin. Res.*, **7(4)**, 121-129 **(2014)**

17. Saha H. et al, Comparative evaluation of antimicrobial and anti-inflammatory activities of *Ocimum sanctum, Phyllanthus niruri* and *Cadaba fruticosa*: an *in vitro* approach with emphasis on detection of their bioactive compounds using GC-MS, *Int. J. Biol. Chem.*, **9(5)**, 235-248 **(2015)**

18. Sakat S., Juvekar A.R. and Gambhire M.N., *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn, *Int. J. Pharm. Sci.*, **2(1)**, 146-155 **(2010)**

19. Tadesse S. et al, Chemical composition and biological activities of the essential oil of *Plectranthus caninus* Roth, *Pharmacogn. J.*, **3(26)**, 1-7 **(2011)**.

(Received 15th February 2024, accepted 13th April 2024)