Journal of Pharmaceutical Research International

33(47B): 715-720, 2021; Article no.JPRI.74406
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

In vitro Evaluation of Anti-Inflammatory Activity of *Symplocos racemosa* Using Protein Denaturation Assay

K. Janani[1], R. V. Geetha[2*] and S. Rajeshkumar[3]

[1]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 77, Tamilnadu, India.
[2]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 77, Tamilnadu, India.
[3]Nanotechnology Research Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 77, Tamilnadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author RVG designed the study, performed the statistical analysis, and wrote the first draft of the manuscript. Author KJ managed the analyses of the study. Author KJ managed the literature searches. Author SR provided guidance for doing research, data verification, manuscript correction. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47B33174
Editor(s):
(1) Dr. Dharmesh Chandra Sharma, G. R. Medical College & J. A. Hospital, India.
Reviewers:
(1) Shalyda Md Shaarani, Universiti Malaysia Pahang, Malaysia.
(2) Ashish Kumar Dixit, All India Institute of Medical Sciences, India.
(3) Shashwati Sen, Mohak Hospital, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74406

Original Research Article

Received 02 August 2021
Accepted 08 October 2021
Published 04 November 2021

ABSTRACT

Introduction: Recently there is considerable awareness and interest in the field of herbal medicine due to its natural origin and lesser side effects compared to Allopathy. Selected herbal plants like *Symplocos racemosa*, commonly known as lodhra, are found mainly in plains and lower hills of Bengal. The word 'Lodhra' means 'Propitious'. *Symplocos racemosa* is an important Indian traditional drug used in many Ayurvedic and herbal formulations for treatment of liver as well as uterine disorders and leucorrhea. Ethnobotanical Literature indicates use of *Symplocos racemosa*

**Corresponding author: E-mail: geetha@saveetha.com;*

in treatment of eye disease, skin disease, ear disorders, liver and bowel complaints, tumours,uterine disorders, spongy and bleeding gums, asthma, fever, snakebite, gonorrhoea and arthritis.

Aim: To analyse the anti-inflammatory activity of *Symplocos racemosa* using protein denaturation assay.

Materials and Methods: 2 g of Lodhra bark powder is mixed with 100 ml distilled water & boiled for 20 min at 50°C. The extract is filtered using whatman filter paper & concentrated to 10 ml.1 ml each of Bovine serum albumin is added to various fixations of plant extract (10µL - 50 µL) and the anti - inflammatory activity was evaluated by analysing the percentage inhibition.

Results: From this study, it is evident that Lodhra has significant anti-inflammatory activity. At 50µℓ concentration, the plant extract shower higher anti- inflammatory activity of 76%.

Conclusion: *Symplocos racemosa* extract has proved to exhibit effective anti- inflammatory activity. Further studies have to be carried to analyse the other properties of this herb, which can be incorporated successfully in the pharmaceutical industry.

Keywords: Anti-inflammatory activity; Symplocos racemosa; in vitro study; protein denaturation assay; simple extraction technique.

1. INTRODUCTION

Ayurveda is the most ancient medical system practised in India [1]. Recently there is considerable awareness and interest in the field of herbal medicine due to its natural origin and lesser side effects compared to Allopathy [2]. Selected herbal plants like *Symplocos racemosa*, commonly known as lodhra, are found mainly in plains and lower hills of Bengal [3] . *Symplocos* racemosa grow up to 6-8.5m tall, found in the plains and lower hills throughout north and East India, ascending in the Himalayas up to an elevation of 1400 m, Bengal, Assam and Chota Nagpur [4]. *Symplocos* is a genus of ericales, containing about 250 species native to Asia, Australia and the Americas. About 68 species are found in India, of which only a few are of economical importance. The word 'Lodhra' means 'Propitious' [5]. The Lodhra leaves appear simple, alternate, spiral; petiole up to 1.5cm long, plano convex in cross-section, glabrous;lamina 6.5-12.5 3-4.3cm, oblateolate to narrow elliptic, narrow apex, canaliculate midrib, 6-12 pairs of secondary nerves [6]. The Lodhra bark is greyish in colour, lenticellata and blaze creamy.

Symplocos racemosa is an important Indian traditional drug used in many Ayurvedic and herbal formulations for treatment of liver as well as uterine disorders and leucorrhea [7]. *Symplocos racemosa* belongs to the unite Eric family of symplocaceae, known as Lodhra in Sanskrit; it is a small evergreen tree, found throughout the tropics and subtropical countries [8]. Ethnobotanical Literature indicates use of *Symplocos racemosa* in treatment of eye disease, skin disease, ear disorders, liver and bowel complaints, tumours,uterine disorders, spongy and bleeding gums, asthma, fever, snakebite, gonorrhoea and arthritis [9]. Majority of the phytopharmacological reports on stem bark of the plant include anti-cancer,hepatoprotective, anti-oxidant, anti-androgenic, anti-diabetic, wound healing and anti-inflammatory effects [10].

Phytochemicals studies indicated the presence of many phenolic glycosides like symploca-side, triterpenoids like betulinic acid, acetyl oleanolic acid and oleanolic acid and flavonoids like Quertin,anthrasinins like 3-mono glucofuranoside of 7-O methyl leucopelargonidin glycosides, symposides, tannins like allergic acid, alkaloids like loturine, loturidine and colloturine [11-13]. Inflammation is the body's first response to infection or injury and is critical for both innate and adaptive immunity [14,15]. It can be considered as part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants [16]. Natural components and phytoconstituents are able to interfere with the inflammatory mechanisms by preventing prolonged inflammation which promotes health [17]. *Symplocos racemosa* has a wide spectrum of uses like anti-androgenic, anti -inflammatory, antiulcer, hepatoprotective, wound healing activities. Our team has extensive knowledge and research experience that has translate into high quality publications [18–22].

This study particularly aims at analysing the anti-inflammatory properties of *Symplocos racemosa.*

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

Powdered *Symplocos racemosa* is purchased commercially from herbal health center, in Chennai. 2 grams of the herbal powder is dissolved in 100 ml of distilled water. Then, the mixture was boiled for 20 minutes at 50 °C in a heating mantle. Afterwards, The extract is filtered finely using a whatman filter paper and allowed to stand undisturbed for about 20 minutes. 10 ml of the filtered plant extract is obtained by placing it in a hot water bath for up to 10 minutes and used for performing an anti-inflammatory assay.

2.2 Evaluation of Anti Inflammatory Activity Using Albumin Denaturation Assay

1 ml of 1% Bovine serum albumin (BSA) was mixed with 400 µL of aqueous crude extract in different concentrations (10 µl, 20 µl, 30 µl, 40 µl and 50 µl). The pH of the reaction mixture was adjusted to 6.8 using 1N HCl. The reaction mixture was incubated at room temperature for 20 min and then heated to 55°C for 20 min in a water bath. The mixture was cooled to room temperature and the absorbance value was recorded at 660 nm in an UV Spectrophotometer. BSA mixture with 30% methanol solution was used as a control. Diclofenac sodium in different concentrations was used as a standard. The experiment was performed in triplicate.

Percentage age of inhibition of protein denaturation was calculated using the following formula:

$$\%\text{Inhibition} = \text{Control O.D} - \text{Sample O.D} * 100 / \text{ControlO.D.}$$

Where,

Control O.D = Optical density of control.
Sample O.D = Optical density of test sample.

2.3 Statistical Analysis

By means of SPSS software version 22, the association between the concentration and percentage of inhibition of protein denaturation for the Symplocos racemosa plane extract as well as diclofenac was assessed using paired t tests. The p value was found.

3. RESULTS

In order to determine the anti-inflammatory activity of the *Symplocos racemosa* plant extract, we have taken different concentrations (10 µl, 20 µl, 30 µl, 40 µl and 50 µl) and compared it with the standard anti-inflammatory drug (Diclofenac). The percentage of inhibition increases gradually, as we raise the concentration of the plant extract. The results depicted the biosynthesised *Symplocos racemosa* has a percentage of inhibition of protein denaturation upto 76%, which is close to the standard drug used (86%) (Fig. 1). At the wavelength of 660nm, absorbance values were calculated using a UV spectrophotometer (Table 1). The results were statistically analysed by SPSS software through chi square tests. P value is 0.220($p>0.05$), which is statistically insignificant. Thus, there exists not a noteworthy statistical difference between the standard drug and the plant extract, this proved the good anti-inflammatory activity of *Symplocos racemosa*.

4. DISCUSSION

From the advent of mankind, several medicinal plants have been exploited for various research purposes to investigate their properties which can be applied in industrial and medical fields. A lot of artificial drugs like NSAIDs which are used against inflammation are effective, but have many side-effects like gastrointestinal and renal damage. *Symplocos racemosa* has been used in standardisation of Ayurvedic formulations and can pave the way for global acceptance of traditional medicinal systems [23].

Table 1. Tabulation showing the percentage of inhibition of protein denaturation for the *Symplocos racemosa* plant extract at various concentrations in 10, 20, 30, 40 and 50 µl

Concentration (µl)	Wavelength (nm)	Absorbance	% of inhibition
10	660	0.63	37
20	660	0.61	39
30	660	0.56	44
40	660	0.49	52

50	660	0.24	76

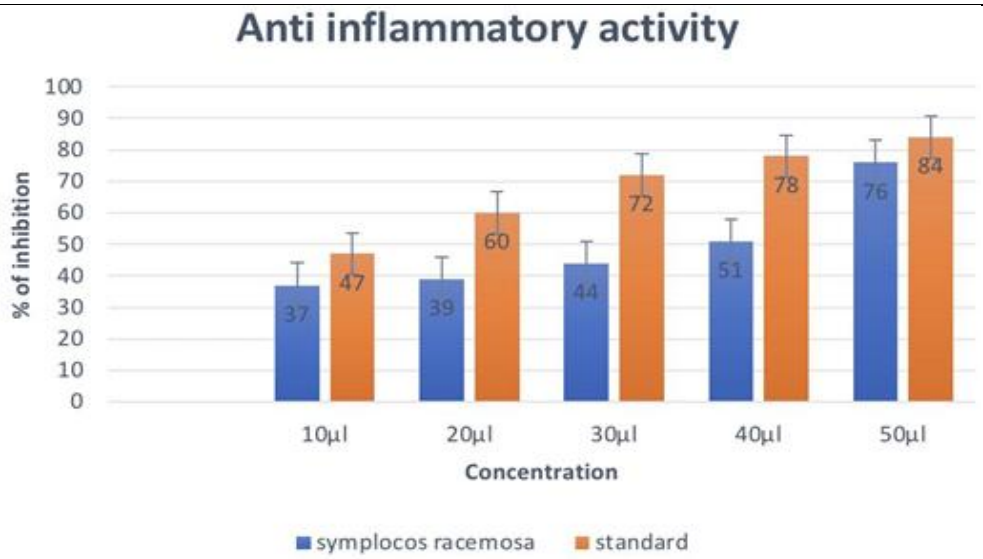

Fig. 1. The bar graph represents the comparison between the *Symplocos racemosa* and the standard anti-inflammatory agent. The X axis represents the percentage of inhibition of protein denaturation and the Y axis represents the concentration of the *Symplocos racemosa* plant extract in microlitres and the standard anti-inflammatory agent (Diclofenac). P value=0.220 (>0.05), which is statistically insignificant

A study has been reported that the methanolic extract of leaves of *Symplocos racemosa* in the concentration of 1000 milligram/ml exhibited 67 % protection against inflammation [24]. Another study revealed that the bark of *Symplocos racemosa* showed significant anti-inflammatory activity at 300 and 500 mg/kg doses. L. Kaviya et al., has proved that at 50 µl concentration, 81% maximum anti-inflammatory activity was shown by Lodhra bark and the efficiency of standard drugs to nearly 90% . Suppression of inflammation is by spatially and temporarily controlled production of mediators. Chronic inflammation is associated in case of cardiovascular diseases, atherosclerosis, Type two diabetes, Rheumatoid arthritis and several types of cancers. Inflammatory biomarkers involve cytokines, acute phase reactants… The elevators in inflammation involve interleukins IL-6, C-reactive proteins, lipoprotein associated phospholipases. Infectious, Non-infectious agents, damaged cells, toxic compounds, pathogens trigger inflammatory signalling pathways. Commonly, Nuclear factor kappa-light chain enhancer of activated B cells (NF-KB), Mitogen-activated protein kinase (MAPK), Janus kinase(JAK) signal transducer and activator of transcription (STAT) pathways are associated. Huong et al., in his study had revealed that the genus symplocaceae inhibits the NO production and the expression of iNOS and COX-2 proteins, thus acting against inflammation [25].

To lessen the side-effects and toxicity, Biologically prepared herbs act as an excellent alternative for commercially available synthetic drugs. Medicinal plants have a wide range of phytochemicals like secondary metabolites which are potent and safe to use. Understanding the role and mechanisms of low-grade inflammation, both as a contributor to those chronic diseases as well as to the overall health status is essential to provide clues for the development of innovative, more efficient, therapeutic and preventive strategies. The interplay between inflammation and metabolic imbalances will have its vast impact on important homoeostatic mechanisms like the Intrinsic circadian rhythm, Autophagy and cell senescence . The limitations of this study is that it made use of only one herb and analysed its anti-inflammatory activity. Moreover, No trials were performed against microbes to test its efficacy. In future, these drawbacks could be satisfied by using more herbs and analysing the other therapeutic properties of *Symplocos racemosa.*

5. CONCLUSION

In this study, *Symplocos racemosa* (Lodhra bark) extract has been prepared using a simple extraction method. This extract has been proved

to exhibit significant anti-inflammatory activity with increasing concentration. It can act as a better alternative when compared to commercial steroidal and non-steroidal anti-inflammatory drugs prevailing in the society. Further studies have to be carried out to analyse other properties of *Symplocos racemosa* that can be incorporated successfully in the medical and pharmaceutical industry.

CONSENT

It is not applicable.

ETHICAL APPROVAL

For conducting the study, Ethical clearance was obtained from the institutional review board.

ACKNOWLEDGEMENT

- Saveetha Dental College and Hospitals
- K K traders, Mayiladuthurai, Tamilnadu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Telles S, Singh N. Awareness about bibliographic databases among students of Ayurveda and qualified Ayurveda practitioners. Journal of Ayurveda and Integrative Medicine 2012;3:59. Available:https://doi.org/10.4103/0975-9476.96516
2. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, et al. Indian medicinal herbs as sources of antioxidants. Food Res Int. 2008;41:1–15.
3. Sood H, Kumar Y, Gupta VK, Arora DS. Bioprospecting the antimicrobial, antibiofilm and antiproliferative activity of Symplocos racemosa Roxb. Bark phytoconstituents along with their biosafety evaluation and detection of antimicrobial components by GC-MS. BMC Pharmacol Toxicol. 2020;21:78.
4. Farooq U, Khan S, Naz S, Khan A, Khan A, Ahmed A, et al. Three new anthraquinone derivatives isolated from Symplocos racemosa and their antibiofilm activity. Chin J Nat Med. 2017;15:944–9.
5. Acharya N, Acharya S, Shah U, Shah R, Hingorani L. A comprehensive analysis on Symplocos racemosa Roxb: Traditional uses, botany, phytochemistry and pharmacological activities. J Ethnopharmacol. 2016;181:236–51.
6. Jung M, Choi J, Chae H-S, Cho JY, Kim Y-D, Htwe KM, et al. Flavonoids from Symplocos racemosa. Molecules. 2014;20:358–65.
7. Durkar AM, Patil RR, Naik SR. Hypolipidemic and antioxidant activity of ethanolic extract of Symplocos racemosa Roxb. in hyperlipidemic rats: An evidence of participation of oxidative stress in hyperlipidemia. Indian J Exp Biol. 2014;52:36–45.
8. Wakchaure D, Jain D, Singhai AK, Somani R. Hepatoprotective activity of Symplocos racemosa bark on carbon tetrachloride-induced hepatic damage in rats. J Ayurveda Integr Med. 2011;2:137–43.
9. Ahmad VU, Rashid MA, Abbasi MA, Rasool N, Zubair M. New salirepin derivatives from Symplocos racemosa. J Asian Nat Prod Res. 2007;9:209–15.
10. Abbasi MA, Ahmad VU, Zubair M, Nawaz SA, Lodhi MA, Farooq U, et al. Lipoxygenase inhibiting ethyl substituted glycoside from Symplocos racemosa. Nat Prod Res. 2005;19:509–15.
11. Khare CP. Symplocos racemosa Roxb. Indian Medicinal Plants. 2007;1–1. Available:https://doi.org/10.1007/978-0-387-70638-2_1581
12. Ali M, Bhutani KK, Srivastava TN. Triterpenoids from Symplocos racemosa bark. Phytochemistry. 1990;29:3601–4. Available:https://doi.org/10.1016/0031-9422(90)85284-m.
13. Prabha NSDML, Sreejit N, Lakshmi Prabha M. Wound healing properties of symplocos racemosa. International Journal of Innovative Research in Medical Science 2016;1. Available:https://doi.org/10.23958/ijirms/vol01-i01/04
14. KJ, Janani K, Preetha S, Jeevitha, Rajeshkumar S. Green synthesis of Selenium nanoparticles using Capparis decidua and its anti-inflammatory activity. International Journal of Research in Pharmaceutical Sciences. 2020;11:6211–5. Available:https://doi.org/10.26452/ijrps.v11i4.3298
15. Francis T, Rajeshkumar S, Roy A, Lakshmi T. Anti-inflammatory and Cytotoxic Effect

of Arrow Root Mediated Selenium Nanoparticles. Pharmacognosy Journal. 2020;12:1363–7. Available:https://doi.org/10.5530/pj.2020.12.188

16. Anti-Inflammatory Activity of Silver Nanoparticles Synthesised Using Hing Oil: An In Vitro Study. International Journal of Pharmaceutical Research. 2020;12. Available:https://doi.org/10.31838/ijpr/2020.sp1.422
17. Swathy S, Roy A, Rajeshkumar S. Anti-inflammatory activity of Ginger oleoresin mediated Silver nanoparticles. Research Journal of Pharmacy and Technology. 2020;13:4591. Available:https://doi.org/10.5958/0974-360x.2020.00808.2
18. PradeepKumar AR, Shemesh H, Jothilatha S, Vijayabharathi R, Jayalakshmi S, Kishen A. Diagnosis of Vertical Root Fractures in Restored Endodontically Treated Teeth: A Time-dependent Retrospective Cohort Study. J Endod. 2016;42:1175–80.
19. Dhinesh B, Isaac JoshuaRamesh Lalvani J, Parthasarathy M, Annamalai K. An assessment on performance, emission and combustion characteristics of single cylinder diesel engine powered by Cymbopogon flexuosus biofuel. Energy Convers Manage. 2016;117:466–74.
20. Lekha L, Kanmani Raja K, Rajagopal G, Easwaramoorthy D. Schiff base complexes of rare earth metal ions: Synthesis, characterization and catalytic activity for the oxidation of aniline and substituted anilines. J Organomet Chem. 2014;753: 72–80.
21. Soh CL, Narayanan V. Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery—A systematic review. Int J Oral Maxillofac Surg. 2013;42:974–80.
22. Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. J Adv Pharm Technol Res. 2013;4:78–83.
23. Govindarajan N, Cheekala UMR, Arcot S, Sundaramoorthy S, Shanmugam M, Duraisamy R, et al. Comparative Powder Microscopic and HPTLC Studies on Stem barks of Symplocos racemosa Roxb. and Symplocos crataegoides Ham. Pharmacognosy Journal. 2016;8:346–51. Available:https://doi.org/10.5530/pj.2016.4.7
24. Vadivu R, Lakshmi KS. *In vitro* and *In vivo* anti-inflammatory activity of leaves of Symplocos cochinchnensis (Lour) Moore ssp laurina. Bangladesh Journal of Pharmacology. 2008;3. Available:https://doi.org/10.3329/bjp.v3i2.956
25. Huong TT, Tram LH, Minh TT, Van Thong N, Giang DH, Dang NH, et al. Investigation of anti-inflammatory lignans from the leaves of *Symplocos sumuntia* Buch-Ham ex D Don (Symplocaceae). Tropical Journal of Pharmaceutical Research. 2017;16:2191. Available:https://doi.org/10.4314/tjpr.v16i9.21

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74406