RESEARCH ARTICLE

Assessment of Carrageenan-induced Anti-Inflammatory Activity of *Gaultheria fragrantissima* Wall. and *Byttneria herbaceae* Roxb. collected from Idukki District, Kerala, India on Albino Wistar Rats

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Received: 12th May, 2020; Revised: 08th June, 2020; Accepted: 14th August, 2020; Available Online: 25th September, 2020

ABSTRACT

The tribal people in Idukki use the plants *Byttneria herbaceae* Robb. and *Gaultheria fragrantissima* (*G. fragrantissima*) Wall. for the treatment of inflammations related to the skin and rheumatoid arthritis, respectively. The ethanol extract of *B. herbaceae* and methanol extract of *G. fragrantissima* were investigated for anti-inflammatory effects at the dose (p.o.) of 200 and 400 mg/kg in animal models, albino Wistar rats. The extracts of *G. fragrantissima* and *B. herbacea* reduced the carrageenan-induced edema by 55.15 and 57.57% on oral administration of 200 and 400 mg/kg, respectively. Animals treated with the *G. fragrantissima* and *B. herbaceae* (200 and 400 mg/kg, p.o.) decreased the volume of pleural exudates to 0.18 ± 0.07 mL and 0.16 ± 0.05, and inhibited the migration of leukocytes to 0.55 ± 0.07 × 10^3, 0.51 ± 0.04 × 10^3, respectively, on carrageenan-induced pleurisy in rats. The results suggested that the extracts can be an active source of substances with effective anti-inflammatory activities.

Keywords: Anti-inflammatory activity, *Byttneria herbaceae*, *Gaultheria fragrantissima*.

International Journal of Pharmacognosy and Phytochemical Research (2020); DOI: 10.25258/phyto.12.3.3

How to cite this article: Narayanan DP, Rexliene MJ, Suresh S. Assessment of Carrageenan-induced Anti-Inflammatory Activity of *Gaultheria fragrantissima* Wall. and *Byttneria herbaceae* Roxb. collected from Idukki District, Kerala, India on Albino Wistar Rats. International Journal of Pharmacognosy and Phytochemical Research. 2020;12(3):138-142.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

B. herbaceae Roxb. and *G. fragrantissima* Wall. are the two traditional medicinal plants belongs to the family Malvaceae and Ericaceae, respectively, used by indigenous people in Idukki district, Kerala, India, for the treatment of inflammatory disorders. *B. herbaceae* is an endemic medicinal plant of peninsular India and used to treat various ailments, such as, dysentery, impaction, leprosy, fracture of limbs, asthma, leucorrhoea, wound, swellings, and body pain.[1] The genus *Gaultheria* comprises approximately 134 species, used mainly as a traditional medicine to cure rheumatism and relieve pain. *G. fragrantissima* is an aromatic plant widely used in Indian folk medicine and the essential oil extracted from *G. fragarntissima* is one of the most exported oils from Nepal. Reports have demonstrated that the plants have shown analgesic, antibacterial, antioxidant, and anti-inflammatory activities.[2,3] Intensive studies are less regarding the anti-inflammatory activity of the plants *B. herbaceae* and *G. fragrantissima*.

Inflammation is a non-specific biological expression of the body against aggressive agents, such as, microorganisms, damaged cells, or irritants. The primary response of the body is acute inflammation and is characterized by the increased movement of plasma and innate immune systems of the cells, such as, neutrophils and macrophages, from the blood into the injured tissues. The significant symptoms of inflammation are noticed by rising in blood flow, vasodilatation, and elevated cellular metabolism, the liberation of soluble mediators, cellular influx, and leakage of fluid.[4] If an inflammatory agent is there, cell membranes promote the activation of phospholipase A2, followed by deliverance of arachidonic acid and inflammatory mediators, such as, cytokines, histamine, serotonin, leukotrienes, and prostaglandin that make a rise in vascular permeability, thus, allowing the migration of leukocytes to the site of inflammation.[5] The carrageenan-induced inflammation is acute, well studied, non-immune, and highly reproducible. The cardinal symptoms of inflammations that develop just after subcutaneous injection, resulting from the action of pro-inflammatory agents bradykinin, histamine, tachykinins, complement, and reactive oxygen and nitrogen species are edema, hyperalgesia, and erythema. Different phytocompounds tested have revealed notable anti-inflammatory activity reports on skin inflammation, cardiovascular inflammation, joint inflammation, and other inflammatory diseases.[6] Several

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saponins tested have exhibited remarkable anti-inflammatory, antipyretic, and antinociceptive activities probably of their nonglycosidic moiety, the sapogenin, but also many diverse activities have also been reported, such as, anti-allergic, antifungal, analgesic, and others.[7-10] Moreover, many plant extracts from different countries have been found to be useful in animal models of inflammation.[11]

The relevant method used for analyzing inflammatory changes to irritants and antigenic challenges is footpad edema or paw swelling. Generally, test compounds were evaluated for acute anti-inflammatory activity by observing their ability to reduce or prevent the formation of carrageenan-induced paw swelling. In this study, experiments were conducted to validate the knowledge of tribal people regarding the anti-inflammatory activity of *B. herbaceae* and *G. fragrantissima*. Many plant extracts have been confirmed to be useful in animal models of inflammation.[12-15]

MATERIALS AND METHODS

Collection of Plant Material and Extraction

The whole plant of *B. herbaceae* and leaves of *G. fragrantissima* were collected from Marayur and Munnar, Kerala, India, during May and June. The specimens were identified and authenticated by the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, with the number BSI/SRC/5/23/2019/Tech-145 and BSI/SRC/5/23/2018/Tech-1671. The whole plant of *B. herbaceae* and leaves of *G. fragrantissima* were washed, shade dried, and powdered at room temperature. The powder was subjected to reflux extraction with ethanol and methanol. The concentrated extracts were stored at 4˚C for further use.

Animals used

Male albino rats (180 ± 5 grams) were acquired from animal houses, K. M. College of Pharmacy, Madurai, and kept in standard laboratory conditions. They were maintained in standard laboratory conditions and given a standard diet and water, *ad libitum*. All animal experimental protocols are approved by the Institutional Animal Ethics Committee and were followed by the guidelines of the Committee for Control and Supervision of Experiments on Animal (CPCSEA), Government of India.

Phytochemical Screening of Plant Extract

The qualitative phytochemical analysis reveals the information on the presence or absence of different classes of secondary metabolites. The ethanol extract of *B. herbaceae* and methanol extract of *G. fragrantissima* were subjected to various phytochemical tests (Table 1) for the presence of steroids, alkaloids, flavonoids, saponins, coumarins, tannins, terpenoids, glycosides, quinones, and phenolic compounds described by Harborne[16]; Trease and Evans[17]; Sofowora[18]; Zohra *et al*.[19]; Joseph *et al*.[20]

Acute Inflammation

The carrageenan-induced rat paw edema is used generally as an experimental model of inflammation in search of a new anti-inflammatory drug. The anti-inflammatory ability of the plant extracts was investigated by the rat paw edema method induced by carrageenan.[21] Albino Wistar rats (180 ± 5 grams) were used. The rats were divided into five groups of five animals each. Normal saline was given to group I and treated as a negative control. Group II rats were treated with carrageenan (1% w/v) in saline in the sub-planter region of the right hind paw. Indomethacin (10 mg/kg, bw) was administered to rats in group III and was considered as standard. Rats from group IV and V were given two doses of plant extracts (*G. fragrantissima* 200 and *B. herbaceae* 400 mg/kg bw). By injecting 0.1 mL of 1% (w/v) carrageenan solution, prepared in normal saline, acute paw edema was induced. After an hour, 0.1 mL, 1% carrageenan suspension in 0.9% NaCl solution was injected into the subplantar tissue of the right hind paw. The circumference of the linear paw will be measured at the hourly interval for 4 hours. The perimeter of the paw was measured by using vernier calipers. Measurements were taken at 0 to 4 hours after the administration of the carrageenan.

The anti-inflammatory activity was calculated by using the relation% inhibition of edema = $(T - T0)/T \times 100$

Where T = thickness of paw in the control group and T0 = thickness of paw edema in the test compound treated group.

Carrageenan-induced Pleurisy in Rats

The animals were divided into five groups of five rats in each group, as explained in the carrageenan-induced paw edema model,[13,14] and each pretreated with plant extracts (*G. fragrantissima* 200 and *B. herbaceae* 400 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.), or normal saline (0.1 mL). All the animals have received 0.25 mL of an intrapleural injection of 1% carrageenan on the right side of the thorax after one hour. By ether inhalation, the animals were sacrificed 3 hours after carrageenan injection. Into the pleural cavity, 1 mL of heparinized Hank's solution was injected and slowly massaged to mix its contents. The fluid was aspirated out of the cavity and the exudates were collected. With the help of the Neubauer chamber, the number of migrating leukocytes in the exudates was investigated. The values of each experimental group were expressed as mean ± SEM and compared with the control group.

Statistical Analysis

The results of anti-inflammatory activity were demonstrated as a mean increase in paw diameter ± SD. One-way analysis

Table 1: Preliminary phytochemical analysis of *B. herbaceae* and *G. fragrantissima*

Phytochemicals	*B. herbaceae (EE)*	*G. fragrantissima (ME)*
Steroids	+	+
Alkaloids	-	+
Flavanoids	+	+
Saponins	-	+
Coumarins	+	+
Tannins	-	+
Terpenoids	+	+
Glycosides	-	+
Quinones	+	+
Phenolic compounds	+	+

EE: ethanol extract; ME: methanol extract; + = present; - = absent

of variance (ANOVA) using GraphPad Prism InStat was used to analyze the results. Differences were considered statistically significant at $p < 0.05$ are compared to control.

RESULTS

The qualitative analysis of the ethanol extract of *B. herbaceae* and methanol extract of *G. fragrantissima* was analyzed using standard procedures. The results showed the presence of phytochemicals differently. The extracts of *G. fragrantissima* exhibited the presence of all the tested secondary metabolites, such as, steroids, alkaloids, flavonoids, saponins, coumarins, tannins, terpenoids, glycosides, quinones, and phenolic compounds. But the extracts of *B. herbaceae* were devoid of alkaloids, saponins, tannins, and quinones, and showed the presence of other phytochemicals, like steroids, flavonoids, coumarins, tannins, terpenoids, and phenolic compounds. The results are shown in Table 1.

The effect of extracts of the whole plant of *B. herbaceae* and leaves of *G. fragrantissima* on edema in rats induced carrageenan is shown in Table 2. The results observed indicated that these plant extracts have significant anti-inflammatory activity in rats. The methanol extracts of *G. fragrantissima* reduced the edema induced by carrageenan by 55.15% on oral administration of 200 mg/kg, whereas; the ethanol extract of *B. herbaceae* reduced the edema by 57.57% on oral administration of 400 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 59.39%.

The effect of ethanol extract of *B. herbaceae* and methanol leaf extract of *G. fragrantissima* on carrageenan-induced pleurisy in rats is shown in Table 3. The volume of pleural exudates in the toxic control group was 0.38 ± 0.08 mL. Animals treated with the *G. fragrantissima* and *B. herbaceae* (200 and 400 mg/kg, p.o.) decreased the pleural exudates to 0.18 ± 0.07 mL and 0.16 ± 0.05. Treatment with indomethacin (10 mg/kg, p.o.) produced the exudates of 0.15 ± 0.04 mL. The leukocyte count for the control group was found to be 4.16 ± 0.38 × 103 cells/mL. Animals treated with *G. fragrantissima*, *B. herbaceae*, and standard, produced a leukocyte migration of 0.55 ± 0.07 × 10^3, 0.51 ± 0.04 × 10^3, and 0.45 ± 0.06 × 10^3 cells/mL, respectively.

DISCUSSION

There is a necessity to focus on the scientific exploration of plant extracts based drugs having fewer side effects because of the rise in the use of Non Steroidal Anti-Inflammatory Drugs (NSAID's) and their reported common side-effects. So, there is a continuous search for natural drugs, which can provide relief to inflammation. Medicinal plants and their isolated compounds are used worldwide to treat various inflammatory

Table 2: Effect of plant extracts on carrageenan-induced rat paw edema

Treatment	*Dosage (mg/kg, p.o.)*	*Mean increase in paw volume (mL)*	*% decrease in paw volume*
Normal control	10 mL/kg saline	0.94 ± 0.08	-
Toxic control	0.1 mL, 1% carrageenan	3.3 ± 0.2[*a]	-
Standard control	10 mg/kg indomethacin	1.34 ± 0.12[*b]	59.39%
Treatment control	200 mg/kg *G. fragrantissima*	1.48 ± 0.16[*b]	55.15%
Treatment control	400 mg/kg *B. herbaceae*	1.4 ± 0.14[*b]	57.57%

Values are expressed as mean ± SEM
Values were compared by using ANOVA, using GraphPad Prism InStat, followed by Newman-Keul's multiple range tests
[*a] = Values are significantly different from normal control G1 at $p < 0.01$; [*b] = Values are significantly different from toxic control G2 at $p < 0.01$
- = No decrease in paw volume

Table 3: Effect of plant extracts on carrageenan-induced pleurisy in rats

Treatment	*Dosage (mg/kg, p.o.)*	*Pleural exudates (mL)*	*Leukocytes (× 10^3 cells/mL)*
Normal control	10 mL/kg saline	0.11 ± 0.02	0.36 ± 0.03
Toxic control	0.1 mL, 1% carrageenan	0.38 ± 0.08[*a]	4.16 ± 0.38[*a]
Standard control	10 mg/kg indomethacin	0.15 ± 0.04[*b]	0.45 ± 0.06[*b]
Treatment control	200 mg/kg *G. fragrantissima*	0.18 ± 0.07[*b]	0.55 ± 0.07[*b]
Treatment control	400 mg/kg *B. herbaceae*	0.16 ± 0.05[*b]	0.51 ± 0.04[*b]

Values are expressed as mean ± SEM
Values were compared by using ANOVA, using GraphPad Prism InStat, followed by Newman-Keul's multiple range tests
[*a] = Values are significantly different from normal control G1 at $p < 0.01$; [*b] = Values are significantly different from Toxic control G2 at $p < 0.01$

conditions, such as, skin and lung inflammations in traditional medicine.[22] The carrageenan-induced inflammation is a biphasic phenomenon.[14] Release of histamine and 5-hydroxytryptamine is the primary phase of edema. Kinin-like substances maintained the plateau phase, and the second accelerating phase of swelling is due to prostaglandin-like substances. The study of these mediators included in different phases is essential for evaluating the mode of drug action. The tests carried out with the ethanol extract of *B. herbaceae* and methanol extract of *G. fragrantissima* in the pleurisy model showed that both the plant extracts act as an inhibitor of leukocyte migration and the formation of pleural exudates when given orally, which strengthens the reports of Mickami *et al*.[15] that explained the inhibition of leukocyte migration and pleural exudates formation by plant extracts. Recent works of *G. fragrantissima* demonstrated that the plant has a high content of methyl salicylate and its glycosides, which are responsible for the anti-inflammatory efficiency of the plant.[3] Compared with the chemical compounds of *G. fragrantissima* collected from Nepal, the present work found the similarities in phytocompounds qualitatively. But the quantitative variation of compounds may occur in the extracts and it has to be studied. The reports, like Jerkovic,[23] found the difference in the yield of essential oils from *Artemisia vulgaris*, which was higher in the case of *A. vulgaris* collected from Croatia than the French plants. Among the *Gaultheria* species, *G. nummularioides* and *G. yunnanensis* have been the most studied species and are the rich source of steroids and flavanoids compounds.[3] Zhang *et al*.[24] demonstrated that the inflammatory effect of *G. yunnanensis* is due to the inhibition of production of pro-inflammatory cytokines, nitric oxide (NO), and reactive oxygen species (ROS) by two methyl glycosides in the plant.

But in the case of *B. herbaceae*, so far, there are no previous reports regarding the phytocompounds responsible as anti-inflammatory agents. The preliminary phytochemical screening of different extracts of different parts of *B. herbaceae* by Somkuwar *et al*., strengthens the present findings.[25] Sarkar *et al.* demonstrated the anti-edemogenic activity of hydroalcoholic extract of *B. herbaceaee* via inhibition capillary permeability, probably due to the presence of histamine receptor type 1.[26] Sarkar[27] compared the various fractions of *B. herbaceae* root extract against the capillary permeability, which is the significant feature of inflammation mediated by histamine and histamine receptor type 1, and found that the n-butanol fraction showed the highest inhibition probably due to the maximum amount of alkaloids in that fraction.

CONCLUSION

The extracts of the whole plant of *B. herbaceae* and leaves of *G. fragrantissima* possess significant anti-inflammatory activity in rats. Future studies including the purification, isolation of compounds, and the analysis of the biochemical pathways may result in the formulation of a potent anti-inflammatory agent with less side effects and a better therapeutic index.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Microbiology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India, for providing the facilities.

REFERENCES

1. Sharma T; Acharya R. Review on Ethnomedicinal Claims, Pharmacological activity and Phytochemical constituents of Samarakhadyam (*Byttneria herbaceae* Roxb.). Journal of Drug Research in Ayurvedic Sciences. 2018; 3(3):173-180. Available from: DOI: 10.5005/jp-journals-10059-0051
2. Liu WR, Qiao WL, Liu ZZ, Wang XH, Jiang R, Li SY, Shi RB, She GM. *Gaultheria*: Phytochemical and Pharmacological characteristics. Molecules. 2013; 18: 12071-12108. Available from: doi: 10.3390/molecules181012071.
3. Pandey BP, Rupak T, Anil U. Chemical composition, antioxidant and antibacterial activities of essential oil and methanol extract of *Artemisia vulgaris* and *Gaultheria fragrantissima* collected from Nepal. Asian Pacific journal of Tropical Medici ne 2017; 10 (10): 952-959. Available from : https://doi.org/10.1016/j.apjtm.2017.09.005
4. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. Clinical and Experimental Immunology. 2007; 147 (2): 227–235. Available from: DOI: 10.1111/j.1365-2249.2006.03261.x
5. Dassoler M, Schwanz M, Beset F, Moreira EA, Gutierrez L. Phytochemical profile and pharmacological test of *Averrhoa carambola* L.(Oxalidaceae). Brazilian Journal of Phytomeedicine. 2004;2:4-8.
6. Francesco M, Rossa R, Haroon K, Nicola M. Medicinal plants with anti-inflammatory activities. Natural Products Research. 2016; 30 (12): 1343-1352. Available from: http://doi.org/10.1080/14786419.2015.1062761
7. Hostettmann K, Marston A. Saponins. Cambridge University Press, Cambridge, New York, 1995. Available from: https://doi.org/10.1007/978-1-4899-1367-8_12
8. Milgate J, Roberts DCK. The nutritional and biological significance of saponins. Nutrition Research. 1995; 15(8): 1223–1249. Available from: https://doi.org/10.1016/0271-5317(95)00081-S
9. Lacaille-Dubois MA, Wagner H. A review of the biological and pharmacological activities of saponins. Phytomedicine. 1996; 2(4): 363–386. Available from: https://doi.org/10.1016/S0944-7113(96)80081-X
10. Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. British Journal of Nutrition. 2002; 88: 587–605. Available from: DOI: 10.1079/BJN2002725
11. Oguntibeju OO. Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. J. Inflamm. Res. 2018; 11:307-317. Available from: doi: 10.2147/JIR.S167789
12. Sarkhel S. Evaluation of the anti-inflammatory activities of *Quillaja saponaria* Mol. saponin extract in mice. Toxicology Reports. 2015; 2: 1-3. Available from: https://doi.org/10.1016/j.toxrep.2015.11.006
13. Vinegar R, Triad JF, Selph JL, Voelker FA. Pathway of onset, development and decay of carrageenan pleurisy in the rat. Federation Proceedings 1982; 41:2588-95. Available from: PMID :6806127

14. Vinegar R, Schreiber W, Hugo RJ. Biphasic development of carrageenin edema in rats. Journal of Pharmacology and Experimental Therapeutics. 1969; 166:96-103. Available from: PMID : 5776026
15. MikamiT, Miyasaka E. Effects of several anti-inflammatory drugs on the various parameters involved in the inflammatory response in rat carrageenan induced pleurisy. European Journal of Pharmacology 1983; 95:1-12. Available from: doi: 10.1016/0014-2999(83)90261-3.
16. Harborne J. B. Phytochemical methods. Chapman and Hill, London. 1973. Available from: ISBN 978-94-009-5921-7
17. Tease and Evans. Pharmacognosy. 15th ed. Elsevier publication. 519-520. 2002. Available from : ISBN 13: 9788131200872
18. Sofowora, A. *Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa*, Spectrum Books Ltd, Ibadan, Nigeria, 1993. 134-156.
19. Zohra SF, Meriem B, Samira S, Muneer A.M.S. Phytochemical screening and identification of some compounds from mallow. J. Nat. Prod. Plant Resour. 2012; 2:512-516. Available from: Corpus ID: 46240712
20. Joseph BS, Kumbhare PH, Kale MC. Preliminary phytochemical screening of selected medicinal plants. International Research Journal of Science and Engineering. 2013; 1:55-62. CORPUS ID: 86243794
21. Winter CA, Risley EA, Nuns GW. Carrageenan-induced oedema in the hind paw of rat as an assay for anti-inflammatory activity, Proceedings of the Society for Experimental Biology and Medicine. 1962, 111; 544–547. Available from: https://doi.org/10.3181/00379727-111-27849
22. Rita de Cassia da Silveira e Sa, Andrade LN, Damiao Pergentino de Sousa. A review on Anti-Inflammatory Activity of Monoterpenes. Molecules. 2013; 18: 1227-125.Available from: doi: 10.3390/molecules18011227
23. Jerkovic I, Mastoid J, Milo M, Juteau F, Mastoid V, Ivan J. Chemical variability of *Artemisia vulgaris* L. essential oils originated from the Mediterranean area of France and Croatia. Flavour and Fragrance Journal. 2003; 18: 436-440. Available from: https://doi.org/10.1002/ffj.1246
24. Zhang D, Liu R, Sun L, Huang C, Wang C, Zhang DM, Tai-Zhang T, Du GH. Anti inflammatory Activity of Methyl Salicylate Glycosides Isolated from *Gaultheria yunnensis* (Franch.) Rehder. Molecules. 2011; 16(5): 3875-3884. Available from: https://doi.org/10.3390/molecules16053875
25. Somkuwar SR, Dongre UJ, Chaudhary R.R, Chaturvedi A. *In-vitro screening* of an Antioxidant Potential of *Byttneria herbacea R*oxb. International Journal of Current Microbiology and Applied Sciences. 2014; 3(8): 622-629. Available from: http://www.ijcmas.com
26. Sarkar L, Bhuvaneswari N, Samanta N K, Islam N, Sen T, Fukui H, Mizuguchi H, Karmarkar S. A report on anti-oedemogenic activity of *Byttneria herbaceae* roots-possible involvement of histamine receptor (Type1). Journal of Ethnopharmacology 2012; 140: 443-446. Available from: DOI:10.1016/Jj.jep.2012.01.013
27. Sarkar L, Bera R, SenT, Karmakar S. Comparative study of the fractions of a relatively unexplored plant- *Byttneria herbacea* on histaminergic inflammation. International Journal of Pharmacy and Pharmaceutical sciences. 2013; 5(3): 862-866. Available from: CORPUS ID: 4246389.