IJMPR 2024, 8(7), 42-46

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Review Article
SJIF Impact Factor: 5.273

TRADITIONAL USES, DETERMINATION OF IN- VITRO ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY, AND HYDROGEL FORMULATION FROM THE LEAVES OF ZANTHOXYLUM ARMATUM DC

Salam Malemnganba Singh, Jabir Hussain Choudhury* and Mustaque Ahmed

Rahman Institute of Pharmaceutical Sciences and Research, Tepesia, Assam 782402.
B.pharm 8th sem

Article Received on: 30/04/2024
Article Revised on: 20/05/2024
Article Accepted on: 10/06/2024

***Corresponding Author**
Jabir Hussain Choudhury
Rahman Institute of Pharmaceutical Sciences and Research, Tepesia, Assam 782402.

ABSTRACT
Zanthoxylum armatum DC of the Rutaceace family also known as Mukthrubi in Manipuri or Indian prickly ash has an important role in the history of Manipuri and Indian history of medicine. Here the plants is collected from the hills of Manipur to analyse the traditional use, in-vitro anti-inflammatory and anti-oxidant activity and hydrogel formulation from the leaves of *Zanthoxylum armatum DC*. The dried leaves were crushed into powder and macerated to obtained the extracts. further the extract are use to determine the in-vitro anti-inflammatory and anti-oxidant activity and for hydrogel formulation too. After further experiment the leaves of zanthoxylum is said to have all the above activity.

KEYWORDS: Zanthoxylum armatum *DC, Mukthrubi, Tejpal, tejowati, hydrogel, anti-inflammatory, anti-oxidant.*

INTRODUCTION

Zanthoxylum armatum DC. (syn. Z. alatum Roxb.) of the Rutaceace family is an important medicinal plant which is commonly known as Indian Prickly Ash, Nepal Pepper or Toothache tree. Local names of this plant are: Tejpal (Hindi), Tejo Wati (Sanskrit), Mukthrubi (Manipuri) and Timur (Nepal).[1-12] Synonyms of Z. armatum are Z. planispinum, Timur, Timber, Toothache Tree, Winged prickly ash. Z. armatum is found in India in the state of Meghalaya, Mizoram and Manipur. There are 11 species and genus of the Z. armatum that's mainly found as medicinal plants, Z. budrunga, Z. oxyphyllum, Z. oval folium, Z. acanthopodium, Z. planispinum, Z. armatum, Z. nitidium, Z. rhesta, Z. simulans, Z. avicennae and Z. limonella. Out of these, 4 species are Z. armatum DC., Z. acanthopodium DC., Z. oxyphyllum Edge, and Z. budrunga are present in India.[13]

Fig. 1: *Zanthoxylum armatum DC.*

Distribution

Zanthoxylum armatum is distributed in the hills of Manipur including Imphal East, West, Jiribam, Maram, Mao, Moreh, Ukhrul at an altitude ranging from 100m to 7500. Also founds in Jammu and Kashmir from altitude of 1000 to 1200 m and in Orissa and Andra Pradesh at 1200m. Genus found in different place of the world like Eastern and Southeast Asia. In southeast Asia India, Bangladesh, Bhutan, Vietnam, Cambodia, Thailand, Malaysia, etc. This plant is mostly found in warmer and tropical region. The location for growth of these plants are valleys, mountains, forests etc.

Description

It is a small tree or large spiny shrub. Leaves are distinctively trifoliolate with the leaf-stalk winged. Leaflets are stalk less, Natural regeneration usually occurs through seeds but the seeds undergo strong dormancy and may take few months to years for germination. Freshly harvested seeds are best for the large-scale cultivation. The seeds are sown in August-September in polybags in nursery or main field. The seeds germinate in 20–30 days after sowing. Stem cuttings may also be planted in the nursery during monsoon.

Traditional uses

These species are used as a medicinal plant and they are more effective against diseases and more curable without having any side effect. *Zanthoxylum armatum* is widely

used as a medicine from ancient time to cure various diseases such as toothache and problems related to tooth, asthma, gum bleeding, fever, dyspepsia, and tonics and also use for Phytochemistry, pharmacological activities, diseases, traditional uses.[14]

The bark, fruits and seeds of *Zanthoxylum armatum DC* are extensively used in indigenous system of medicine as a carminative, stomachic and anthelmintic. The fruit and seeds are employed as an aromatic tonic in fever and dyspepsia. An extract of the fruits is reported to be effective in expelling round worms. Because of their deodorant, disinfectant and antiseptic properties, the fruits are used in dental troubles, and their lotion for scabies. They are also used to ward-off houseflies. The fruit part of the plant may use to purify the water, also used as insect repellent. The wood of this pant may be very heavier and strong then it is used for walking sticks. *Zanthoxylum armatum DC* also gives and showed work against antioxidants, antinociceptive, antifungal, anti-inflammatory, hepatoprotective, pesticides, anthelmintic, antiproliferative etc.[15] The leaves *of Zanthoxylum armatum* is widely used as a food ingredient in Manipur for different dishes and it gives various health benefits too. The dried seeds are also widely used as a spice in various dishes in Manipur. The extract of these plant such as leaves, barks, seeds is used for reducing pains and inflammation in the body so it is considered to be a very important plant in Manipur household.

Determination of In- vitro* anti-inflammatory and anti-oxidant activities of *zanthoxylum armatum

1. *In- vitro* anti-inflammatory activity

The stock solution was prepared by using the extract in ethanol at concentration 1mg /ml and suitable dilutions (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml,100µg/ml) were made to get the test solution.

Inhibition of albumin denaturation method

The in-vitro anti-inflammatory activity of extracts was evaluated by using inhibition of albumin denaturation technique. Inhibition of protein denaturation method was followed by **SINGH et al. 2020.** with some changes. The reaction mixture (5ml) consisting of 1ml of (0.1%) of bovine serum albumin fraction, 1ml tris-HCL buffer pH 7.8 solution and 1 ml of test solutions incubated at 37%C for 20 minutes, followed by heating at 72°C for 2-4 minutes in the water bath for denaturation. After cooling the sample at room temperature, the turbidity was recorded by spectrophotometrically at 660nm. Aspirin was taken as a standard solution. 1ml of distilled water with 1ml (0.1%) bovine serum albumin fraction and 1ml buffer solution was taken as control.[16-19]

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{absorbance of test}}{\text{Absorbance of control}} \times 1oo$$

2. In- vitro anti-oxidant activity of zanthoxylum armatum

A. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH)radical scavenging activity

The antioxidant activity of zanthoxylum armatum is estimated on the basis of the radical scavenging effect of the stable DPPH free radical according to the method described by Blois (1958) with a minor change. Butylated hydroxy anisole (BHA) was used as a reference standard .0.5ml of DPPH solution in ethanol (0.1Mm) was mixed with 3ml of extract and 3ml of extract prepared in various concentration (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100 µg/ml) respectively the standards were incubated for 30 minutes at 37.c. Absorbance was measured at 517nm using uv- visible spectrophotometer. Control reading was also taken and IC50 value was determined.[19-22]

The scavenging effect of DPPH free radical was calculated using the following equation.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{absorbance of test}}{\text{Absorbance of control}} \times 1oo$$

B. Ferric reducing anti-oxidant power (FRAP) ASSAY

The total antioxidant potential of a sample was determined using ferric reducing ability of the FRAP assay (El JEMLI et al.2016). The principal of this method was based on the reduction of potassium ferricyanide to its ferrous coloured form in the presence of anti-oxidant. Briefly, 100micro litre of each of the ethanolic extracts at various concentrations(10 µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100 µg/ml), and 250microliter of phosphate buffer (0.2M, pH6.6) and 250microliter of potassium ferricyanide 1% were mixed and incubated for 30min at 50.c. and the reaction were stopped by adding 250µl of 10%(w/v)trichloro acetate acid and mixed in a microcentrifuge tube for 10min at 3000RPM to reduce ferricyanide into ferrocyanide. Finally, 250µl of the upper layer of the above reaction mixture was mixed with 250µl of distilled water and added freshly prepared 50µl of 0.1% of −ferric chloride solution in the above solution. The absorbance was measured at 700nm after keeping in a room temperature for 5 minutes. Gallic acid was taken as a standard. The antioxidant capacity was calculated by following formula.[23-27]

The reducing power activity was determined by using following equation –

$$\% \text{ reduction activity} = \frac{\text{absorbance in test} - \text{absorbance in control}}{\text{absorbance of test}} \text{x } 100$$

3. Hydrogel formulation

Leaves of *zanthoxylum armatum* extracts were tried with polymer Carbopol 940. The following few combinations with Carbopol 940 resulted in the best gel formulation, which was smooth and Stable.

Method for preparation of gel containing extract

1g of Carbopol 940 was dispersed in 50 ml of distilled water with continuous stirring. 5ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by Heating on water bath. Cool the solution, then to that added Propylene glycol 400. Further required quantity of zanthoxylum armatum leaves extract was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water.

Finally full mixed ingredients were mixed properly to the Carbopol 940 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of control sample without adding any *zanthoxylum armatum* leaves extract.

Formulation

The method above describe above the formula were tabulated in Table l. Along with control sample gel were prepared with addition of 2.5g, 5g of *Zanthoxylum armatum* leaves extract to prepared 2.5% and 5% *Zanthoxylum armatum* leaves gel respectively. Table: Different formulation prepared with this ingredient along with quantity:

Evaluation for hydrogel formulation

1. Physical Evaluation: Physical parameters such as colour and appearance were checked.
2. Measurement of pH: pH of the gel was measured by using ph. meter.
3. Spreadibility: Spreadibility was determined by the apparatus which consist of wooden block, which was provided by a pulley at one end. By this method spreadibility was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on this ground slide. The gel was then sandwiched between this slid and another glass slide having the dimension of fixed ground slide and provide with the hook. A 1kg weighted was placed on the top of the two slides for mins to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80gms. With the help of string attached to the hook and the time (in second) required by the top slide to cover a distance of 7.5cm be noted. A shorter interval indicates better spreadibility. Spreadibility was calculated using the following formula:

CONCLUSION

In conclusion, the exploration of Zanthoxylum armatum DC leaves has revealed a treasure trove of traditional uses, coupled with promising scientific findings regarding its in-vitro anti-inflammatory and anti-oxidant properties. Through meticulous research, we've unveiled a deeper understanding of its potential therapeutic benefits, shedding light on its role in combating inflammation and oxidative stress. Moreover, the development of hydrogel formulations offers exciting prospects for harnessing these properties in practical applications, ranging from pharmaceuticals to cosmetics. As we continue to delve into the intricate mechanisms of natural remedies like Zanthoxylum armatum DC, we pave the way for novel treatments that merge traditional wisdom with modern scientific innovation, ultimately enriching our arsenal against various health challenges.

Declaration

We declare that we do not have any conflict of interest.our aim is to deliver objectives and unbaised information and we maintained the integrety throughout the articles.

REFERENCES

1. Singh, T. P., & Singh, O. M. Phytochemical and pharmacological profile of Zanthoxylum armatum DC.-an overview, 2011.
2. The Wealth of India A Dictionary of Indian Raw Materials and Industrial Products-Raw Materials Series, Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, 1976; 11: 18-21.
3. Jain SK and Dam N, Some ethnobotanical notes from Northeastern India, *Econ Bot*, 1979; 33: 52-56.
4. 3.Kala CP, Farooquee NA and Dhar U, Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India, *Biodivers Conserv*, 2004; 13: 453-469.
5. Grierson AJC and Long DJ, Flora of Bhutan including a record of plants from Sikkim, Edinburgh, Royal Botanic Garden, 1984.
6. Flora of China, by W Zheng-yi and PH Raven (Eds), EnglishEdn, Science Press, Beijing, 1994.
7. Flora of Taiwan, H Tseng-Chieng, Editorial Committee ofthe Flora of Taiwan, 1993; 2: 538.
8. Hara H, Stearn WT and Williams LHJ, An Enumeration of Flowering Plants of Nepal, Trustees of British Museum (Natural History), London, 1978.
9. Quisumbing E, Medicinal Plants of the Philippines, Manila, Bureau of Printing, 1951; 467-468.
10. Hartley TG, A revision of the Malesia species of *Zanthoxylum armatum* (Rutaceace), *J Arnold Arbor*, 1966; 47: 171-221.
11. Flora of Pakistan, Fascicles, by E Nasir and SI Ali (Eds), Islamabad, Karachi, 1970-2001, 1-200.
12. Flora of Japan, Angiospermae, Dicotyledoneae, Sympetalae, by K Iwatsuki, T Yamazaki, DE Boufford and H Ohba (Eds), Kodansha, Ltd., Tokyo, 1993; IIIa.
13. Verma, K. K., Kumar, B., Raj, H., & Sharma, A. A review on chemical constituents, traditional uses, pharmacological studies of Zanthoxylum armatum (rutaceae). Journal of Drug Delivery and Therapeutics, 2021; 11(2-S): 136-142.
14. Gupta S, Bhaskar S, Andola HC. Altitudinal Variation in Essential Oil in Leaves of Zanthoxylum

alatum Roxb. A High Value Aromatic Tree from Uttarakhand. J. Med. Plant Res, 2011; 5(3): 348-351.
15. Samant SS, Dhar U, Palni LMS. Medicinal plants of Himalaya: diversity, distribution and potential values. Himavikas, Gyan Prakash, Nainital, 1998; 13: 163-172.
16. Singh, A., Palariya, D., Dhami, A., Prakash, O., Kumar, R., Rawat, D. S., & Pant, A. K. Biological activities and Phytochemical analysis of *Zanthoxylum armatum DC*. leaves and bark extracts collected from Kumaun region, Uttarakhand, India. *J. Med. Herbs Ethnomed*, 2020; *6*: 1-10.
17. Leelaprakash G and Dass SM. In-vitro anti-inflammatory activity of methanol extract of Enicostemma axillare. International Journal of Drug Development and Research, 2011; 3(3): 189-196.
18. Williams LAD, Connar AO, Latore L, Dennis O, Ringer O, Whittaker JA, Conrad J, Vogler B, Rosner H, Kraus W. The in-vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Medical Journal, 2008; 57(4): 327-331.
19. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evalution of in-vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine, 2012; 178-180.
20. Shunmugaperumal T and Kaur V. In-vitro anti-inflammatory and antimicrobial activities of azithromycin after loaded in chitosan and tween 20-based oil-in-water macroemulsion for acne management. AAPS PharmSciTech, 2016; 17(3): 700-709.
21. Blois, M. S. Antioxidant determinations by the use of a stable free radical. Nature, 1958; 181(4617): 1199-1200.
22. Goldschmidt, S., and Renn, K., Chem. Ber, 1922; 55: 628.
23. Poirier, R. H., Kahler, E. J., and Benington, F., J. Org. Chem, 1952; 17: 1437.
24. Braude, E. A., Brook, A. G., and Linstead, R. P., J. Chem. Soc, 1954; 3574.
25. Blois, M. S., Biochim. Biophys. Acta, 1955; 18: 165.
26. El Jemli, M., Kamal, R., Marmouzi, I., Zerrouki, A., Cherrah, Y., & Alaoui, K. (2016). Radical-scavenging activity and ferric reducing ability of Juniperus thurifera (L.), J. oxycedrus (L.), J. phoenicea (L.) and Tetraclinis articulata (L.). Advances in Pharmacological and Pharmaceutical Sciences, 2016.
27. M. R. Loizzo, R. Tundis, M. Bonesi et al., "Radical scavenging, antioxidant and metal chelating activities of Annona cherimola Mill. (cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents," Journal of Food Composition and Analysis, 2012; 25, 2: 179–184.
28. L. Cherrat, L. Espina, M. Bakkali, R. Pagán, and A. Laglaoui, "Chemical composition, antioxidant and antimicrobial properties of *Mentha pulegium*, *Lavandula stoechas* and *Satureja calamintha* Scheele essential oils and an evaluation of their bactericidal effect in combined processes," *Innovative Food Science and Emerging Technologies*, 2014; 22: 221–229.
29. E. M. E. Ouariachi, I. Hamdani, A. Bouyanzer et al., "Chemical composition and antioxidant activity of essential oils of Thymus broussonetii Boiss. And Thymus algeriensis Boiss. From Morocco," *Asian Pacific Journal of Tropical Disease*, 2014; 4, 4: 281–286.
N. Chahmi, J. Anissi, S. Jennan, A. Farah, K. Sendide, and M. El Hassouni, "Antioxidant activities and total phenol content of *Inula viscosa* extracts selected from three regions of Morocco," *Asian Pacific Journal of Tropical Biomedicine*, 2015; 5, 3: 228–233.
30. Giri, M. A., & Bhalke, R. D. Formulation and evaluation of topical anti-inflammatory herbal gel. *Asian J Pharm Clin Res*, 2019; *12*(7): 252-255.
31. Dixit G, Misal G, Gulkari V, Upadhye K. Formulation and evaluation of polyherbal gel for anti-inflammatory activity. Int J Pharm Sci Res, 2013; 4: 1186-91.
32. ICH Guidelines. Stability Testing of New Drug Substances and Products, 1993; 27.
33. Singh M, Mittal V. Formulation and evaluation of herbal gel containing ethanolic extract of ipomoea fistulosa. Int J Sci Res, 2014; 3: 25-9.
34. Misal G, Dixit G, Gilkari V. Formulation and evaluation of herbal gel. Indian J Nat Prod Res, 2012; 3: 501-5.
35. Bhalke RD, Pal SC. Anti-inflammatory and antinociceptive activity of Pterospermum acer folium leaves. Asian J Pharm Clin Res, 2012; 5: 23 6.
36. Helal DA, El-Rhman DA, Abdel-Halim SA, El-Nabarawi MA. Formulation and evaluation of fluconazole topical gel. Int J Pharm Pharm Sci, 2012; 4: 176-83.
37. Jyothi D, Koland M. Formulation and evaluation of an herbal anti-inflammatory gel containing Trigonella foenum greacum seed extract. Int J Pharm Pharm Sci, 2016; 8: 41-4.
38. Sudipta D, Haldar PK, Pramanik G. Formulation and evaluation of herbal gel containing Clerodendrum infortunatum leaves extract. Int J Pharmtech Res, 2011; 3: 140-3.
39. Wood JH, Catacalos G, Liberman SV. Adaptation of commercial viscometers for special applications in pharmaceutical rheology – Severs extrusion rheometer. J Pharm Sci, 1963; 52: 375-8.
40. Goyal S, Sharma P, Ramchandani V, Shrivastava SK, Dubey PK. Novel anti-inflammatory topical herbal gels containing Withania somnifera and

Boswellia serrata. Int J Pharm Biol Sci Arch, 2011; 2: 1087-94.
41. Mishra US, Murthey PN, Mishra D, Sahu K. Formulation and standerdisation of herbal gel containing methanolic extract of Calophyllum inophyllum. Am J Pharmtech Res, 2011; 1: 276-89.
42. Jyothi D, Koland M, Priya S. Investigation of anti-inflammatory activity of ointments containing fenugreek extract. Asian J Pharm Clin Res, 2014; 7: 66-9.