IIUC Journal of Science and Engineering
Vol.-1, Issue-1, December 2023, pp. 09-18
ISSN: 3005-5873

In vitro antimicrobial and antiarthritic effects of methanolic extract of *Zanthoxylum rhetsa* leaves

Mohammed Abu Sayeed, Md. Sekendar Ali,
U Swe Sing Chowdhuy, Ameerul Islam, Naiya Ruzzama,
Muzammil Ahmad and Afrina Azad
Department of Pharmacy
International Islamic University Chittagong (IIUC), Bangladesh

Article info

Keywords
Antiarthritic
Antimicrobial
Pathogenic microorganism
Zanthoxylum rhetsa

Abstract

The antimicrobial study of *Zanthoxylum rhetsa* leaves methanol extract was evaluated by the disc diffusion method against some pathogenic organisms. It exhibits the activity against almost all the test bacterial and fungal species with the zone of inhibition from mild to moderate in different concentrations- 100, 300 and 500 µg/disc where microbial species exhibits from minimum 10 to maximum 27 mm zone of inhibition. The Antiarthritic test have also done by using six concentrations for UV absorbance of test solution, product control solution, test control solution and percentage of inhibition of methanolic extract of *Z. rhetsa* with comparison of standard Diclofenac sodium in different concentrations like 31.75 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL where different concentrations showed an increase in all the parameters that was significant from test, control and standard groups. The present study indicates that, the extract has both antibacterial and antifungal activity from mild to moderately in different concentrations and has significantly antiarthritic activities.

Corresponding author
Mohammed Abu Sayeed
Department of Pharmacy, International Islamic University Chittagong, Kumira, Chattogram, Bangladesh - 4318. Email: sayeed@iiuc.ac.bd

1. Introduction

According to a World Health Organization (WHO) report, medicinal plants are a readily available, affordable, and traditionally appropriate source of primary health care for more than 80% of Asia's population (World Health Organization, 2019; Patwardhan & Partwardhan, 2005;. Patwardhan, 2005). An antimicrobial substance is an agent that inhibits bacterial as well as fungal growth or kills them by maximizing the spectrum (Pelaez, 2006). However, as knowledge of the causative agents of various infectious diseases has grown, the term "antibiotic" has come to refer to a broader range of antimicrobial

compounds, including antimicrobial, antifungal, and other compounds.

Generally, the elderly people of our society face a common problem of Arthritis (Jakobsson & Hallberg, 2002; Laidmäe, Leppik, Tulva, & Hääl, 2009). Approximately one fifth of the world's population suffers from this debilitating disease (Murray, Lopez, & World Health Organization, 1996; Organization, 2001; Wittchen et al., 2011). The management of arthritis and other related conditions involves the use of different classes of drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease modifying anti-rheumatic drugs (DMARDs) etc. (Radu & Bungau, 2021; Thakur, Riyaz, Patil, Kaur, Kapoor, & Mishra, 2018). The most common problem with NSAIDs is gastrointestinal side effects, which include gastric mucosa irritation, belching, gastric ulceration, and bleeding. (Sostres, Gargallo, Lanas, & therapy, 2013). Long term use of NSAIDs may damage renal and hepatic functions, predisposing the patient to cardiovascular disorders (Bindu, Mazumder, & Bandyopadhyay, 2020; Risser, Donovan, Heintzman, & Page, 2009). As a result, a continuous search for alternative drugs from plants and other natural sources is required. For the treatment of arthritis, a variety of medicinal substances are used. Treatment typically begins with medications that have the least side effects, with additional medications added if they are ineffective (Donahue et al., 2008; van Laar et al., 2012). Based on the information presented above, the *Z.rhetsa* leaves was selected for evaluating its antibacterial and antiarthritic activity in the management of infectious as well as some non-infectious diseases like arthritis, etc.

2. Materials and methods

2.1 Chemicals and reagents

Kanamycin, Fluconazole, BSA Solution (Bovine serum albumin) 5%, Diclofenac sodium powder. All required reagents were of analytical grade.

2.2 Plant materials

The plant parts were collected from hilly areas of Cox's Bazar district, Bangladesh. Confirmed and authenticated by Professor Dr.Shaikh Bokhtear Uddin, ethnobotanist of Chittagong University, Bangladesh.

2.3 Preparation of extraction

The freshly collected matured leaves were kept in the shade at room temperature for 21 days before being dried in an oven at 40-45°C for 5 days. The materials were ground with a grinder. At room temperature, 430 g of powder (Leaves) was extracted with 1200 mL of methanol. It was left to stand for 10 days, shaking and stirring occasionally. Cotton or double rings

filter paper was used to filter the liquid contents. The filtrate was then evaporated at temperature less than 50°C (Alam et al., 2021; Khan et al., 2020).

2.4 Microbial cultures

Gram positive and Gram negative species such as *Bacillus azotoformans, Bacillus cereus, Staphylococcus aureus, Lactobacillus casei, Corynebacterium species, Vibrio cholera, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhi* were chosen to investigate antibacterial activity. *Candida albicans, Blastomyces dermatitidis,* and *Trichoderma spp.* were used for antifungal activity as test organisms.

2.5 Experimental methods

Evaluation of in vitro antimicrobial activity: Due to versatile acceptability, the disc diffusion technique was used to performthestudy and evaluation of antiarthritic activity: Method based on protein denaturation inhibition.

2.5.1 Antibacterial activity test

The disc diffusion method was used for both antibacterial and antifungal assays (Guha et al., 2021). The test samples were prepared by dissolving the extract (10 mg/mL) of the samples in methanol. The discs (samples & standard antibiotic) were dissolved with different concentrations of extract and subsequent drying of solvent followed by placingthem into the agar medium contained in Petridishes. Kanamycin 30 µg/disc was used as standard.The plates were then inverted and kept in a refrigerator for about 24 h at 4°C. Finally, the dishes were incubated at 37°C for overnight which is about 8-18h duration. The extract's antimicrobial sensitivity was demonstrated by measuring the zone of inhibition in millimeters (mm).

2.5.2 Antifungal activity test

The weighted amount of potato slice was boiledwith a required amount of distilled water for 30 minutes and applied for course filtration by the help of cotton. Dextrose and nutrient agar powder were properly mixed with potato filtrate in a conical flask. Sufficient quantity of distilled water was adjusted for desired formulation of medium. The pH of the medium was adjusted to 5.6 and then sterilized in the steam heat sterilizer, autoclave. After the preparation of the test plates, application of sample and standard discs on the medium of petridishes were done. The petridishes were then subjected to diffusion into the solid medium at 4°C before being incubated at 28°C for 1-2 weeks (Parekh & Chanda, 2008).

2.5.3 Antiarthritic activity test

The UV absorbance of test solution, product control solution, test control

solution and the percentage of inhibition of methanol extract of the *Z. rhetsa* (MEZR) and standard Diclofenac sodium were observed in different concentrations like 31.75µg/mL, 62.5µg/mL, 125 µg/mL, 250µg/mL, 250µg/mL, 500 µg/mL and 1000µg /mL (Table 3 and Table 4). By using following equation, the antiarthritic activity of theextractcanbe calculated (Amraoui, Mayouf, Charef, Baghiani, & Arrar, 2019).

Percent of inhibition of protein denaturation =

$$100 - \frac{\text{Absorbace of test solution} - \text{Absorbace of Product control}}{Absorbence\ of\ test\ control} \times 100\%$$

3. Results and discussion

2.1 Antibacterial activity test

In antibacterial activity test, different concentrations of the extract were given effect against all pathogenic bacteria ranges from 11 mm to 25 mm zone of inhibitions. But the maximum zones of inhibitions were observed 25 mm by 500 µg/disc of extract against *Corynebacterium species*.

3.2 Antifungal activity test

The zones of inhibition were compared with the results obtained with standard kanamycin (Table I). The antifungal was determined with same concentrations against four fungi species. Fluconazole100µg/disc was used as standard. Maximum reading given by the 500µg/disc of extract was 27 mm zone of inhibition against *Trichoderma spp.* (Table II).

3.3 Antiarthritic activity test

Also the antiarthritic activity of the extract at different concentrations was observed and compared with standard drug (Table III and IV).

Table I.

Antibacterial activity of extract of Z. rhetsa & standard Kanamycin

Groups	Test organisms	Diameter of zone of inhibition(mm)			
		MEZR		Standard *(Kanamycin)*	
		100µg/disc	300µg/disc	500µg/disc	30µg/disc
Gram positive	*Lactobacillus casei*	14	19	24	25
	Corynebacterium species	12	15	25	30
	Bacillus cereus	13	17	23	35
	Staphylococcus aureus	11	14	24	25
Gram negative	*Escherichia coli*	13	18	22	34
	Bacillusazoto formans	12	16	21	35
	Salmonella typhi	14	20	24	30
	Vibrio cholera	12	17	23	36

Table II
Antifungal activity of methanol extractof Z. rhetsa & standard Fluconazole

Test organisms	Diameter of zone of inhibition(mm)			
	MEZR		Standard (Fluconazole)	
	100µg/disc	300µg/disc	500µg/disc	30µg/disc
Candida albicans	10	15	23	35
Cryptococcusneoformans	14	19	21	30
Blastomycesdermatitidis	11	16	26	35
Trichodermaspp	13	18	27	40

Table III
Antiarthritic result of methanolic extract of Z. rhetsa

Conc. (µg/ml)	Absorbance			% of inhibition of extract
	Test solution	*Product control solution*	*Test control solution*	
31.75	0.117	0.062	0.063	12.698
62.5	0.144	0.092	0.063	17.460
125	0.181	0.135	0.063	26.984
250	0.225	0.181	0.063	30.158
500	0.337	0.299	0.063	39.682
1000	0.436	0.401	0.063	44.444

Table IV
Antiarthritic result of standard Diclofenac sodium

Conc. (µg/ml)	Absorbence			% of inhibition Diclofenac Na
	Test solution	*Product control solution*	*Test control solution*	
31.75	0.149	0.119	0.063	52.380
62.5	0.163	0.137	0.063	58.730
125	0.189	0.164	0.063	60.317
250	0.299	0.279	0.063	68.253
500	0.494	0.482	0.063	80.952
1000	0.655	0.647	0.063	87.301

The antibacterial activity of the extract of *Z. rhetsa* extract exhibits activity against almost all the test microbial species with the zone of inhibition from mild tomoderate indifferent concentrations- 100, 300 and 500 µg/disc where microbial species exhibits from minimum 11 to maximum 25 mm which is approximately nearer to standard kanamycin. The antifungal activity of extract of *Z. rhetsa* exhibits activity against all the fungal species from mild to moderate like *Candida albicans, Cryptococcus neoformans, Blastomyces dermatitidis,* and *Trichoderma spp*. with the zone of inhibition from 10 to 27 mm. *Cryptococcus neoformans* and *Trichoderma spp* were inhibited in the sample concentration of 500 µg/dis compared to standard from 21 to 27 mm. The

antimicrobial property of an extract is determined by the extracting solvent, effective solubility and miscibility of the active component in the test medium, vulnerability of the test organisms, and assessment technique (Gidudu et al., 2011). In gram-positive bacteria, antibacterial drugs can damage the bacterial cell wall, causing cytoplasm leakage and coagulation. Phytocompounds in the methanol extract, like others, may inhibit a variety of bacterial metabolic processes, including cell wall, DNA, lipid, and/or protein synthesis. (Frassinetti, Gabriele, Moccia, Longo, & Di Gioia, 2020).

The Antiarthritic test have done by using extract of *Z. rhetsa* with comparison of standard Diclofenac sodium in different concentrations like 31.75, 62.5, 125, 250, 500 and 1000 $\mu g/ml$ where they showed an increase in all the parameters that was significant from test, control, and standard drugs. Inappropriate COX-2 expression has been linked to the development of inflammatory pathophysiology in gastrointestinal illnesses, central nervous system diseases, ischemia, and lung inflammation and fibrosis (Ansari et al., 2010; Keskek et al., 2006). Chronic inflammation, such as Rheumatoid arthritis (RA), can result from any aggravation of inflammatory triggers. RA is a chronic and disabling disease that affects between 0.3% and 1% of the world's population. This condition is projected to affect 9.6% of men and 18% of women over the age of 60. It restricts the movement of 80% of those affected, while 25% are unable to do daily living chores (Conditions, 2009; Gibofsky, 2012).

4. Conclusion

According to the current study, *Z. rhetsa* leaves (methanol extract) have significant antiarthritic activities at different concentrations and mild to moderate antimicrobial effects. These must be confirmed in human clinical trials before concluding that it is effective against arthritis. Before it can be considered effective, more research on the active ingredient responsible for antiarthritic action and different concentrations at which toxic manifestations of appear must be conducted.

Acknowledgement

The authors are sincerely grateful to the Department of Pharmacy, International Islamic University Chittagong for valuable supports during this research work.

References

Alam, S., Rashid, M. A., Sarker, M. M. R., Emon, N. U., Arman, M., Mohamed, I. N., & Haque, M. R. (2021). Antidiarrheal, antimicrobial and

antioxidant potentials of methanol extract of Colocasia gigantea Hook. f. leaves: evidenced from in vivo and in vitro studies along with computer-aided approaches. *BMC Complementary Medicine and Therapies*, *21*, 1-12. https://doi.org/10.1186/s12906-021-03290-6

Amraoui, N., Mayouf, N., Charef, N., Baghiani, A., & Arrar, L. (2019). Antioxidant, anti-inflammatory and anti-arthritic activities of methanol extract of Tamus communis L. roots. *Tropical Journal of Pharmaceutical Research*, *18*(7), 1499-1506. https://doi.org/10.4314/tjpr.v18i7.20

Ansari, N., Khodagholi, F., Ramin, M., Amini, M., Irannejad, H., Dargahi, L., & Amirabad, A. D. (2010). Inhibition of LPS-induced apoptosis in differentiated-PC12 cells by new triazine derivatives through NF-κB-mediated suppression of COX-2. *Neurochemistry International*, *57*(8), 958-968. https://doi.org/10.1016/j.neuint.2010.10.002

Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical pharmacology*, *180*, 114147. https://doi.org/10.1016/j.bcp.2020.114147

Conditions, N. C. C. f. C. (2009). *Rheumatoid arthritis: National clinical guideline for management and treatment in adults*. London: Royal College of Physicians (UK).

Donahue, K. E., Gartlehner, G., Jonas, D. E., Lux, L. J., Thieda, P., Jonas, B. L., ... & Lohr, K. N. (2008). Systematic review: Comparative effectiveness and harms of disease-modifying medications for rheumatoid arthritis. *Annals of Internal Medicine*, *148*(2), 124-134.

Frassinetti, S., Gabriele, M., Moccia, E., Longo, V., & Di Gioia, D. (2020). Antimicrobial and antibiofilm activity of Cannabis sativa L. seeds extract against Staphylococcus aureus and growth effects on probiotic Lactobacillus spp. *Lwt*, *124*, 109149. https://doi.org/10.1016/j.lwt.2020.109149

Gibofsky, A. (2012). Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. *The American journal of managed care*, *18*(13 Suppl), S295-302.

Gidudu, J., Sack, D. A., Pina, M., Hudson, M. J., Kohl, K. S., Bishop, P., ... & Zaman, K. (2011). Diarrhea: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*, *29*(5), 1053. https://doi.org/10.1016/j.vaccine.2010.11.065

Guha, B., Arman, M., Islam, M. N., Tareq, S. M., Rahman, M. M., Sakib, S. A., . . . Alqahtani, A. M. (2021). Unveiling pharmacological studies provide

new insights on Mangifera longipes and Quercus gomeziana. *Saudi Journal of Biological Sciences, 28*(1), 183-190. https://doi.org/10.1016/j.sjbs.2020.09.037

Jakobsson, U. L. F., & Hallberg, I. R. (2002). Pain and quality of life among older people with rheumatoid arthritis and/or osteoarthritis: a literature review. *Journal of clinical nursing*, *11*(4), 430-443. https://doi.org/10.1046/j.1365-2702.2002.00624.x

Keskek, M., Gocmen, E., Kilic, M., Gencturk, S., Can, B., Cengiz, M., ... & Koc, M. (2006). Increased expression of cyclooxygenase-2 (COX-2) in radiation-induced small bowel injury in rats. *Journal of Surgical Research*, *135*(1), 76-84. https://doi.org/10.1016/j.jss.2006.03.031

Khan, M. F., Kader, F. B., Arman, M., Ahmed, S., Lyzu, C., Sakib, S. A., . . . Capasso, R. (2020). Pharmacological insights and prediction of lead bioactive isolates of Dita bark through experimental and computer-aided mechanism. *Biomedicine and Pharmacotherapy, 131*, 110774. https://doi.org/10.1016/j.biopha.2020.110774

Laidmäe, V. I., Leppik, L., Tulva, T., & Hääl, M. L. (2009). Disease-related social and family life: people coping with rheumatoid arthritis in Estonia. *Critical Public Health*, *19*(1), 87-105. https://doi.org/10.1080/09581590802375871

Murray, C. J., & Lopez, A. D. (1996). *The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020: Summary*: World Health Organization and World Bank.

World Health Organization. (2001). The World Health Report 2001: Mental health: New understanding, new hope.

World Health Organization. (2019). *WHO global report on traditional and complementary medicine 2019*. World Health Organization.

Parekh, J., & Chanda, S. (2008). In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. *African Journal of Biotechnology*, *7*(23), 4349-4353.

Patwardhan, B., & Partwardhan, A. (2005). *Traditional Medicine: Modern Approach for affordable global health* (pp. 1-172). Switzerland: World Health Organization.

Patwardhan, B. (2005, June 22-24). *Traditional medicine: A novel approach for available, accessible and affordable health care. World Health Organization, 13*.

Pelaez, F. J. B. p. (2006). The historical delivery of antibiotics from microbial natural products—can history repeat? *, 71*(7), 981-990.

Radu, A. F., & Bungau, S. G. (2021). Management of rheumatoid arthritis: An overview. *Cells*, *10*(11), 2857. https://doi.org/10.3390/cells10112857

Risser, A., Donovan, D., Heintzman, J., & Page, T. (2009). NSAID prescribing precautions. *American Family Physician*, *80*(12), 1371-1378.

Sostres, C., Gargallo, C. J., Lanas, A. J. A. r., & therapy. (2013). Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. *15*(3), 1-8.

Thakur, S., Riyaz, B., Patil, A., Kaur, A., Kapoor, B., & Mishra, V. (2018). Novel drug delivery systems for NSAIDs in management of rheumatoid arthritis: An overview. *Biomedicine and Pharmacotherapy*, *106*, 1011-1023.

van Laar, M., Pergolizzi Jr, J. V., Mellinghoff, H. U., Merchante, I. M., Nalamachu, S., O'Brien, J., ... Raffa, R. B. (2012). Pain treatment in arthritis-related pain: Beyond NSAIDs. *The Open Rheumatology Journal*, *6*, 320-330. https://doi.org/10.2174%2F1874312901206010320

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., ... Steinhausen, H. C. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *European neuropsychopharmacology*, *21*(9), 655-679. https://doi.org/10.1016/j.euroneuro.2011.07.018