RESEARCH ARTICLE

Journal of Ecophysiology and Occupational Health, *Vol 23(4), DOI: 10.18311/jeoh/2023/34716, 305-316, December 2023*

ISSN (Online) : 0972-4397
ISSN (Online) : 0974-0805

Phytochemical Studies, Anti-Oxidant and Anti-Inflammatory Properties of Four Medicinal Plants

Ayushi Sharma* and Anjana Goel

Department of Biotechnology, GLA University, Mathura - 281406, Uttar Pradesh, India; ayushi.sharmabsc15@gmail.com

Abstract

Rheumatoid arthritis, a chronic autoimmune disorder, is characterized by polyarthralgia and joint dysfunction resulting from autoimmune responses that target self-neoepitopes. These attacks lead to the activation of macrophages and other defense cells. By identifying these self-epitopes as biomarkers in RA, researchers have gained valuable insights into the disease's pathogenesis. Over the years, research has focused on investigating the potential of medicinal plants as anti-inflammatory agents. In this study, a hydroethanolic extract of *Saraca asoca*, *Ficus benghalensis*, *Chenopodium album,* and *Nyctanthes arbor-tristis* leaves was prepared by Soxhlet apparatus. Various concentrations of extracts were utilized to assess phytochemical analysis, GC-MS, *in-vitro* anti-oxidant, and anti-inflammatory activities. The highest scavenging potential was exhibited by Chenopodium album, as assessed by the DPPH assay (90.32 ± 3.2 %) and H_2O_2 (86.00 ± 1.94 %). Similarly, the Nyctanthes arbor-tristis showed (92.23 ± 0.83 %) inhibition in membrane stabilization assay, and (91.49 ± 1.03 %) in protein denaturation inhibition capacity. GCMS analysis showed various phytoconstituents in extracts. In order to confirm its therapeutic potential for treating RA, Nyctanthes arbor-tristis may be a better drug candidate that needs to be further researched for mechanistic studies.

Keywords: Anti-Inflammatory Agents, Anti-Oxidant Activities, *Nyctanthes arbor-tristis,* Phytochemical Analysis, Rheumatoid Arthritis

1. Introduction

The progressive destruction of bones and joints, as well as other organs, characterizes the course of rheumatoid arthritis, a prevalent chronic inflammatory autoimmune disease of the joints. About 1% of people worldwide are affected by rheumatoid arthritis. Joints are attacked by the body's immune system in rheumatoid arthritis, an autoimmune disorder[1]. Joint destruction, deformity, and significant disability are hallmarks of the disease's advanced stages. Bone loss, skeletal abnormalities, and synovitis are hallmarks of this condition[2].

Drugs such as Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), corticosteroids, Disease-Modifying Antirheumatic Drugs (DMARDs), and biologicals have all been used to alleviate the discomfort associated with rheumatoid arthritis. Ulcers, cancers, and infections are just some of the serious adverse effects of these medications[3]. Therefore, it is important to discover new treatments for rheumatoid arthritis that are efficient, cost-effective, helpful, and safe. There is still a significant amount of healing power in the traditional plants used all over the world. The majority of the world's population uses traditional medicine, which often involves the use of plant extracts or their components, to meet their primary healthcare needs. There is still the option of turning to traditional medicine instead of Western medicine[4]. Herbal supplements taken in conjunction with disease-modifying anti-rheumatic drugs are becoming increasingly accepted as a treatment option for rheumatoid arthritis. Pain and inflammation, such as that caused by arthritis, can be treated with a number of different plants. Antioxidants,

**Author for correspondence*

Article Received on: 08.08.2023 Accepted on: 07.11.2023 Revised on: 10.11.2023

compounds that treat arthritis, and anti-inflammatory compounds can all be found in abundance among plants[5].

When different enzymes are activated, proteins lose their structure or become denatured, tissues migrate, and tissues break down[6]. One common technique for measuring anti-inflammatory efficacy involves the hypotonicity and heat-induced hemolysis of erythrocytes. Because of their similarities, the effects of drugs on erythrocyte stabilization could be extrapolated to the stabilization of the lysosomal membrane[7]. Activated neutrophils, proteases, and histamines are just some of the pro-inflammatory markers that have been shown to be released at the site of tissue damage due to the lysis of lysosomal membranes during chronic inflammation[8].

About 80% of people around the world rely on medicines made from plants. Herbal medicines play a significant role in India's conventional medical practice[9]. The primary objective of the present study was to assess the antioxidant and anti-inflammatory capabilities of a hydroethanolic extract derived from medicinal plants. This investigation is rooted in the historical utilization of plant extracts for the treatment of various ailments. The outcomes of this research hold the promise of further enhancing the already substantial medicinal value of these plants.

2. Materials and Methods

2.1 Sampling and Leaf Extraction

Plant leaves were collected from GLA University, Mathura (U.P.) for this study. Collected leaves were shadow-dried and extracted for the process. We used a reflux apparatus to remove the phytoconstituents in a hydroethanolic solvent and then concentrated the extract in a rotary vacuum evaporator (Yamato Scientific Co., Japan) at 45°C. For in-vitro testing, the final concentrated extract was stored at -20°C.

2.2 Phytochemical Analysis

Using Kokate's method, qualitative chemical analyses were performed on each of the extracts in order to determine the phytoconstituents present in each of the samples[10].

2.3 *In-vitro* Antioxidant Activities

The antioxidant power of various plant extracts was measured in terms of their ability to scavenge free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide, with the results being compared to those of standard anti-oxidant drugs (ascorbic acid).

2.4 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay

The antioxidant capacity of the extracts was evaluated using a tweaked version of the method developed by Moradi *et al*[11]. A hydroethanolic solution of DPPH (3.4mg/100 mL) was added to a 50 L aliquot of the sample solutions to determine their concentrations (25-400 g/mL). For a period of one hour at 37 degrees Celsius, the reaction mixture was kept in the dark. How quickly the extracts bleached out the purple showed how well they neutralized free radicals. The reaction mixture's absorbance was measured using a UV-visible spectrophotometer (Agilent 8453, Germany). Ascorbic acid, derived from vitamin C, served as the standard. The following methodology was employed to determine the extent to which DPPH could be reduced.

Scavenging activity percentage =
(Abs control – Abs sample)/Abs control × 100

2.5 Hydrogen Peroxide Scavenging Assay

The extract's ability to scavenge hydrogen peroxide (H_2O_2) was measured using Ruch *et al*.'s method[12]. Extracts (25-400 g/mL) were diluted to a concentration of 0.1 mL in Eppendorf tubes with 50 mM phosphate buffer (pH 7.4), and then 2 mM H_2O_2 solution was added. After letting the mixture react for 10 minutes, the absorbance at 230 nm was measured while the mixture was vortexed. Ascorbic acid was used as a positive control. Extracts' H_2O_2 scavenging efficacy was calculated using the following formula: where A0 and A1 are the absorbances of the control and sample, respectively.

H_2O_2 scavenging activity percentage =
(Abs control – Abs sample)/Abs control × 100

2.6 *In-Vitro* Anti-Inflammatory Activities

By using the BSA denaturation assay, and HRBC membrane stabilization to test the effects of various plant extracts on arthritis, the effects were compared to those of common anti-inflammatory medications (diclofenac).

2.7 Inhibition of Protein Denaturation

The methodology was slightly modified from a previously reported procedure by Pavithra *et al*[13]. The reaction mixture included 100 ml of 5% Bovine Serum Albumin (BSA) aqueous solution and 100µl of aqueous extracts of *Ficus benghalensis* (final concentrations of 100, 200, 400, 800, and 1000 g/ml). The sample was incubated for 20 minutes at 37°C before being heated, was raised in a hot water bath for 10 minutes to 70°C. After 10 minutes of cooling under running water, the mixture's turbidity and absorbance were measured at 660nm. Distilled water served as the blank. The outcomes were contrasted against methotrexate. The following formula was used to calculate the protein denaturation inhibition:

Protein denaturation inhibition (%) =
(Abs control – Abs sample)/Abs control X 100

2.8 Membrane-stabilizing Effects

Vallabh Deshpande *et al.*[14] provided the basis for the technique, which was adapted here. Human volunteers were blood-collected after they had gone NSAID-free for two weeks. The reaction mixture contains 2ml of hypotonic saline (0.25% NaCl), 1ml of 0.15 M phosphate buffer (pH 7.4), 1 ml of test solution (100-500 g/ml), and 0.5 ml of 10% HRBC in normal saline, and has a total volume of 4.5ml. The combination was kept in an incubator at 56°C for 30 minutes. The test tubes were placed under a running faucet for 20 minutes to cool them down. The absorbance of the supernatant at 560nm was then measured after centrifuging the reaction mixture for 10 minutes at 3000rpm. The drug methotrexate was widely used. The percentage membrane stabilization activity was calculated as per the following formula

Membrane-stabilizing (%) =
(Abs control – Abs sample)/Abs control X 100

2.9 Gas Chromatography-Mass Spectroscopic Analysis

Thermo GC-Trace Ultra Version: 5.0 and Thermo MS DSQ II were used to do a GC-MS analysis of the leaf extract of a medicinal plant. The equipment has a 30mm 0.25mm ID 0.25m film DB 35 – MS Capillary Standard non-polar column. Helium is used as the carrier gas at a rate of at least 1.0 ml/min. The injector was set to 250°C, and this is how the oven temperature was set: 60°C for 15 minutes, and then slowly went up to 280°C over the next 3 minutes. The Willey and NIST libraries, as well as a comparison of their retention indices, were used to figure out what the parts were. The parts were figured out by comparing them to the ones in the computer library (NIST and Willey) that were connected to the GC-MS instrument. The results were then put into a table.

3. Result

3.1 Phytochemical Analysis

The qualitative phytochemical analysis of plant extracts has shown the presence of alkaloids, flavonoids, phenols, steroid proteins, Saponins, tannins, carbohydrates, terpenoids, glycosides and as shown in Table 1.

3.2 *In vitro* Antioxidant Activities

The production of ROS leads to more oxidative stress, which can cause autoimmune diseases like RA. As lipid peroxidation gets worse, the activities of antioxidant enzymes and the Na+/K+-ATPase get worse. Increasing the levels of ROS can also harm immunoglobulins and alter the DNA repair mechanisms. Di Dalmazi *et al.*[15] have shown that ROS oxidative stress plays an important role in the progression of RA. DPPH and H_2O_2 assays were used to test the potential antioxidant activity of crude extracts from *Saraca asoca*, *Ficus benghalensis*, *Chenopodium album*, and *Nyctanthes arbor-tristis*. The inhibitory effect was compared to that of the standard ascorbic acid. *Chenopodium album* showed the most activity, and this activity increased with the amount of extract used. At the highest concentration tested (100 mg/mL), *Chenopodium album* showed the most ability to fight free radicals. In the DPPH assay, *Chenopodium album* had a percentage of inhibition of 90.32 ± 3.2 %, which was a little bit higher than the standard ascorbic acid (95.53 ± 1.4 %) and showed more activity than the other plant extracts (Figure 1(a)). *Chenopodium album* also had the highest ability to get rid of H_2O_2 (86.00 ± 1.94 %), which was the same as ascorbic acid (92.24 ± 2.02 %) (Figure 1(b)).

3.3 *In Vitro* Anti-Arthritic Activities

The protein denaturation method and the HRBC membrane stabilization method were used to study the anti-arthritic effects of different concentrations

Table 1. Results of phytochemical screening of plant extracts

Chemical Constituents	*Saraca asoca*	*Ficus benghalensis*	*Chenopodium album*	*Nyctanthes arbor-tristis*
Alkaloids	-	-	+	+
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	-
Tannin	+	+	+	-
Terpenoids	-	+	+	+
Carbohydrate	+	+	+	+
Glycoside	-	+	+	+
Protein	+	+	+	+

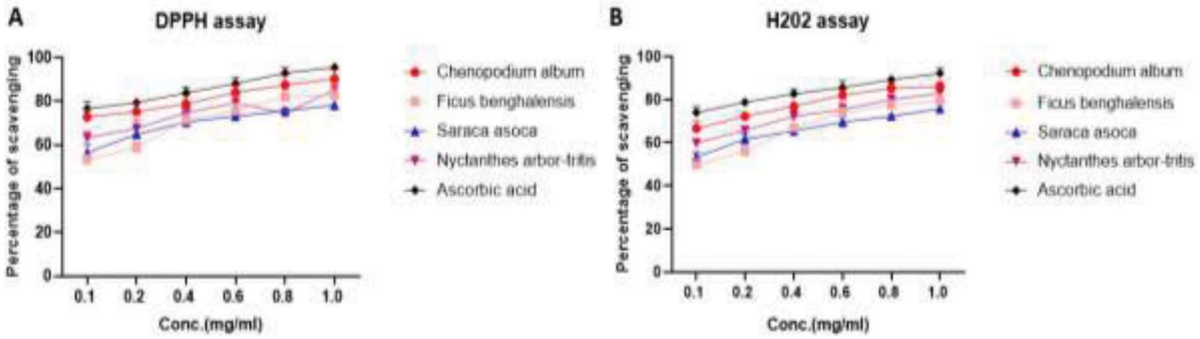

Figure 1. In-vitro antioxidant activity of Nyctanthes arbor-tristis (DPPH free radical scavenging assay **(a)** and Hydrogen peroxide scavenging assay assay **(b)**).

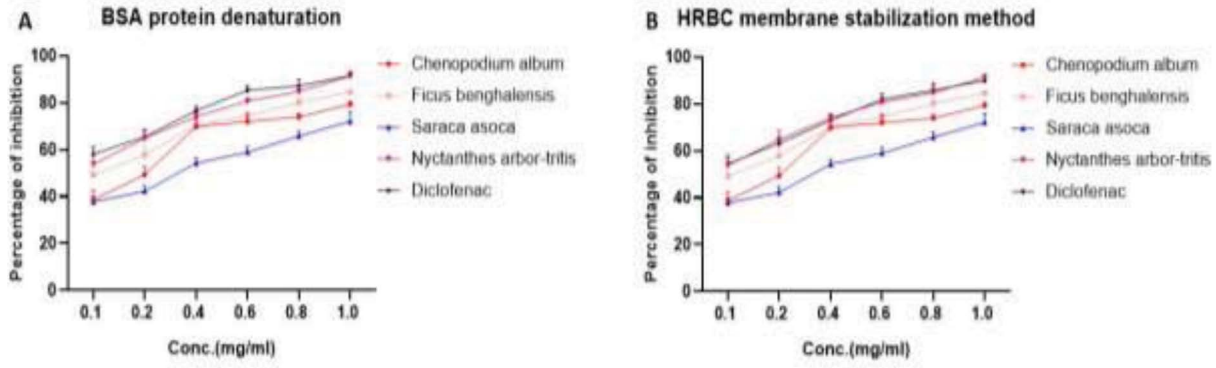

Figure 2. *In-vitro* anti-arthritic activity of *Nyctanthes arbor-tristis* (BSA protein denaturation **(a)** and HRBC membrane stabilization **(b)**).

of Hydroethanolic Extract (HE) of leaves of *Ficus benghalensis* L. in a test tube. The anti-arthritic activity was measured by how well it stopped proteins from breaking down and stopped membranes from becoming more stable when the solution was less salty. The hydroethanolic extracts of *Saraca asoca*, *Ficus benghalensis*, *Chenopodium album*, *Nyctanthes arbor-tristis*, and diclofenac were tested at concentrations of 100, 200, 400, 600, 800, and 1000 g/ml to see how well they stopped proteins from getting messed up and how well they kept membranes

from getting messed up. At different concentrations, HE of *Saraca asoca*, *Ficus benghalensis*, *Chenopodium album*, and *Nyctanthes arbor-tristis* can stop proteins from breaking down. It showed that 1000 mg/ml of HE of *Nyctanthes arbor-tristis* and 1000 mg/ml of diclofenac had the most effect at (91.49 ± 1.03 %) and (91.59 ± 1.97 %), respectively. Stopping hypotonicity caused the HRBC membrane to break, so the anti-arthritic activity was measured by how well the HRBC membrane held together. At different concentrations, HEs of plant can stop the breakdown of HRBC caused by heat. It showed that the maximum inhibition was (92.23 ± 0.83) at 1000 mg/ml of HE of *Nyctanthes arbor-tristis* and (89.92 ± 2.09 %) at 1000 mg/ml of diclofenac. As the concentration goes up, the ability to stop proteins from breaking down and protect and stabilize membranes also goes up. So, the anti-arthritic effect of extracts depended on how much was used.

Table 2. Name, retention time, peak area, mol. formula, mol. weight of phytocompounds in the hydroethanolic extract of *Saraca asoca*

S.No	Chemical Compound	R.T	Area	Medicinal properties	Reference
1	1,2-Ethanediol	0.590	12.69	Anti-tumor activity	[16]
2	Hydroxylamine	0.735	6.90	Carcinostatic activity, Antibacterial	[17]
3	dl-Glyceraldehyde	0.779	2.94	Neuroprotective effects	[18]
4	Acetic acid	1.816	1.14	Anti-inflammatory, anti-bacterial	[19]
5	Acetamide	4.166	0.09	Anti-cancer, Anti-diabetic, and anti-inflammatory	[20]
6	2-ethoxyethyl ethyl ester	11.426	0.18	Anti-microbial and anti-inflammatory properties	[21]
7	2-Dodecanol	14.592	0.03	Anti-oxidant and anti-inflammatory activity	[22]

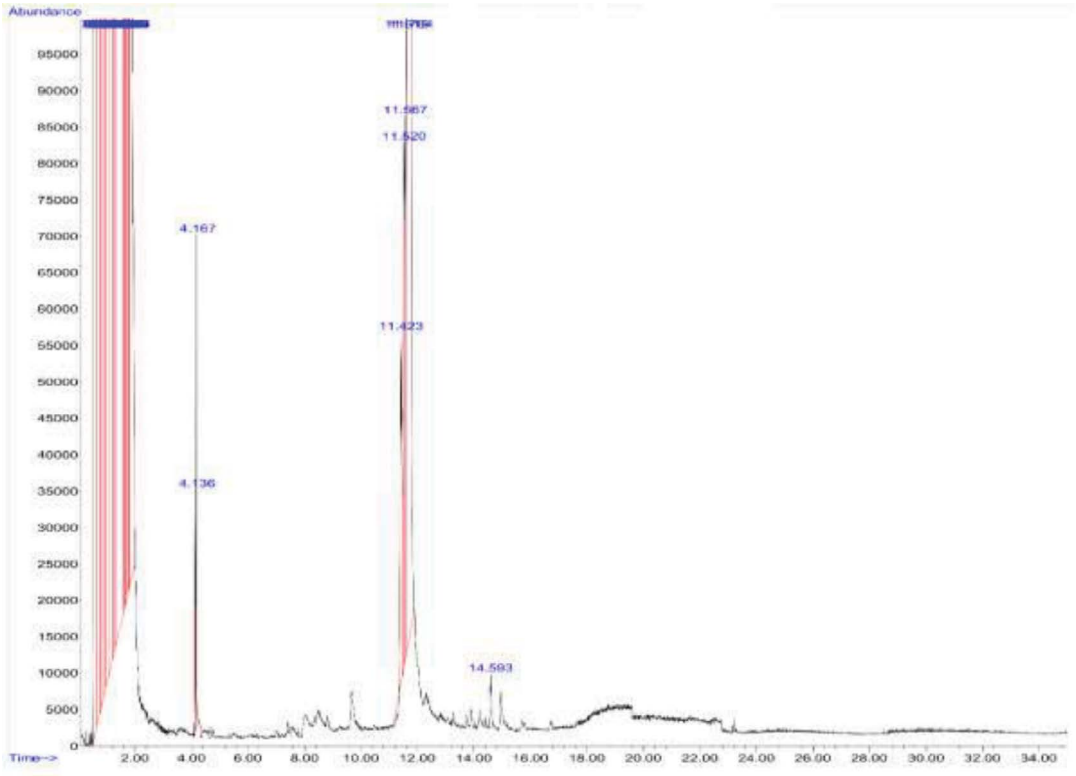

Figure 3. GCMS chromatogram of *Saraca asoca.*

Table 3. Name, retention time, peak area, mol. formula, mol. weight of phytocompounds in hydroethanolic extract of *Chenopodium album*

S.No	Chemical compound	R.T	Area	Medicinal properties	Reference
1	Hydroxylamine	0.568	1.73	Anti-inflammatory properties, anti-cancer properties, and antibacterial	[23]
2	Methylamine	0.595	3.77	Neurogenic, antioxidant, cholinergic, and neuroprotective properties	[24]
3	1-Butanol	0.806	12.33	Anti-microbial activity	[25]
4	1,2-Ethanediol	1.762	19.07	Anti-cancer activity	[26]
5	Acetic acid	4.101	0.17	Antimicrobial properties, antibacterial, antifungal, antioxidant and anticancer activities	[27]
6	2-Acetylbenzoic acid	11.302	0.02	Anti-inflammatory property	[28]
7	Benzoic acid	11.410	0.02	Antialgal, antimicrobial, antimutagenic, hypoglycaemic, antiestrogenic, anti-inflammatory, anti-platelet aggregating, nematocidal, antioxidant, antiviral properties	[29]

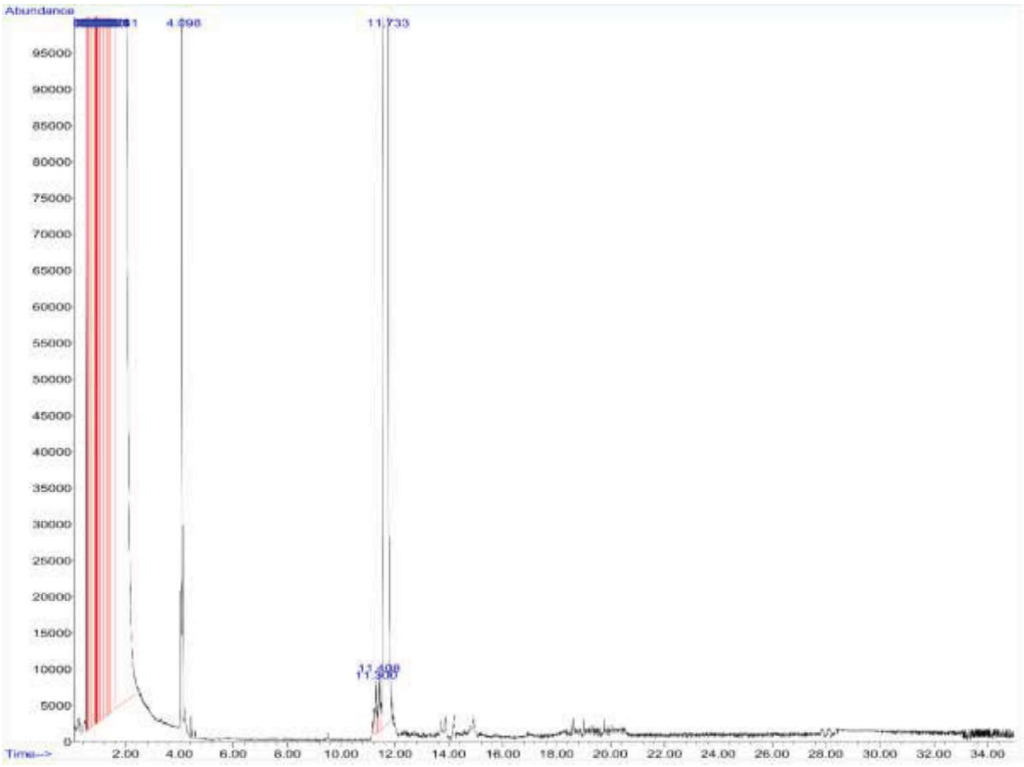

Figure 4. GCMS chromatogram of *Chenopodium album.*

3.4 GCMS Analysis

Mass spectrum interpretation GC-MS was performed using the National Institute of Standards and Technology's (NIST) database, which contains more than 62,000 patterns. The spectrum of the unknown component was compared to the library of known component spectra at NIST. This study was conducted to identify the bioactive compounds present in the HE of plant material by means of Gas chromatography and Mass spectrometry. The formula, concentration (peak area %), Molecular Weight (MW), and Retention Time (RT) of the active ingredients are shown in Table 2 and Figure 3. Table 2 and Figure 4

Table 4. Name, retention time, peak area, mol. formula, mol. weight of phytocompounds in the hydroethanolic extract of *Ficus benghalensis*

S.No	Chemical compound	R.T	Area	Medicinal properties	Reference
1	Methylamine	0.352	3.05	Neurogenic, antioxidant, cholinergic, and neuroprotective	[30]
2	Hydroxylamine	0.741	11.04	Antioxidant property	[31]
3	1,2-Ethanediol	2.156	7.01	Anti-tumor	[32]
4	Dimethylamine	4.177	0.02	Anti-inflammatory and antiviral	[32]
5	Methylglyoxal	4.231	0.10	Anti-bacterial and Anti-inflammatory properties	[33]
6	N, N-Dimethyl-2-aminoethanol	4.285	0.39	Anti-oxidant and Anti-microbial	[34]
7	Cyclopropyl carbinol	4.355	0.03	Anti-bacterial and Anti-fungal	[35]
8	N-Hydroxycarbamic acid	6.235	0.12	Antimicrobial activity	[36]
9	4-Amino- dl-3-Aminobutyric acid	6.386	0.06	It plays a crucial role in regulating neuronal excitability throughout the brain and is involved in various physiological processes and activity	[37]
10	2-Formylhistamine	10.443	0.05	Anti-inflammatory activity	[38]
11	3-Chloro-N-methyl propylamine	11.037	0.03	Insecticidal activities	[39]
12	Azetidin-2-one 3	13.711	0.23	Antitubercular, antifungal activity, and antibacterial, as well as anticonvulsant, antioxidant, anti-inflammatory, and antidepressant activity	[40]
13	Cathinone	13.862	0.06	Anti-depressant activity	[41]
14	2-Formylhistamine	14.116	0.03	Anti-cancer activity	[42]
15	2-Amino-1-(o-methoxyphenyl) propane	14.187	0.06	Anti-inflammatory activity	[43]

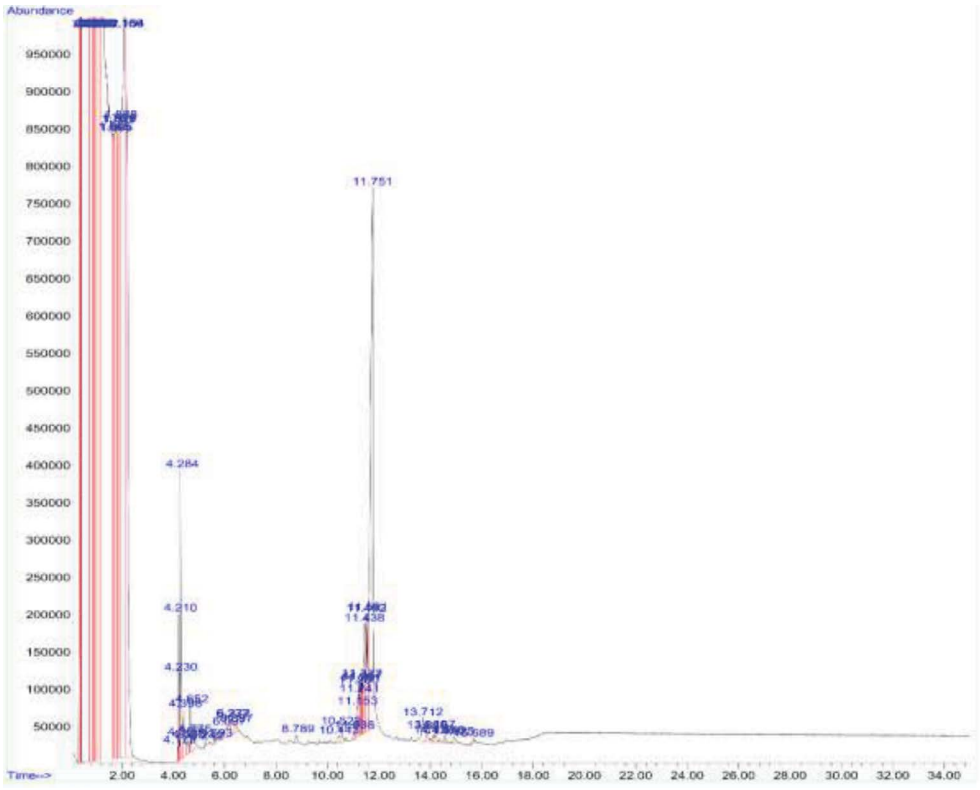

Figure 5. GCMS chromatogram of *Ficus benghalensis.*

Table 5. Name, retention time, peak area, mol. formula, mol. weight of phytocompounds in the hydroethanolic extract of *Nyctanthes arbor-tristis*

S.No	Chemical compound	R.T	Area	Medicinal properties	Ref
1	Methylamine	0.568	3.78	Insecticide, Fungicide, antioxidant	[44]
2	Hydroxylamine	0.633	5.50	Carcinostatic activity, Antibacterial	[45]
3	Methylglyoxal	4.409	0.26	Antibacterial activity	[46]
4	Cyclopropyl carbinol	13.749	0.07	Anti-diabetic, anti-oxidant activity	[47]
5	1-Methyl-2-phenoxyethylamine	13.906	0.03	Antinociceptive properties	[48]
6	3-Azabicyclo3.2.2nonane	18.298	0.03	Anti-cancer and anti-viral property	[49]
7	1,3-Propanediamine	18.379	0.01	Anti-oxidant property	[50]
8	Phenylpropanolamine	18.514	0.01	Anti-bacterial, anti-oxidant	[51]

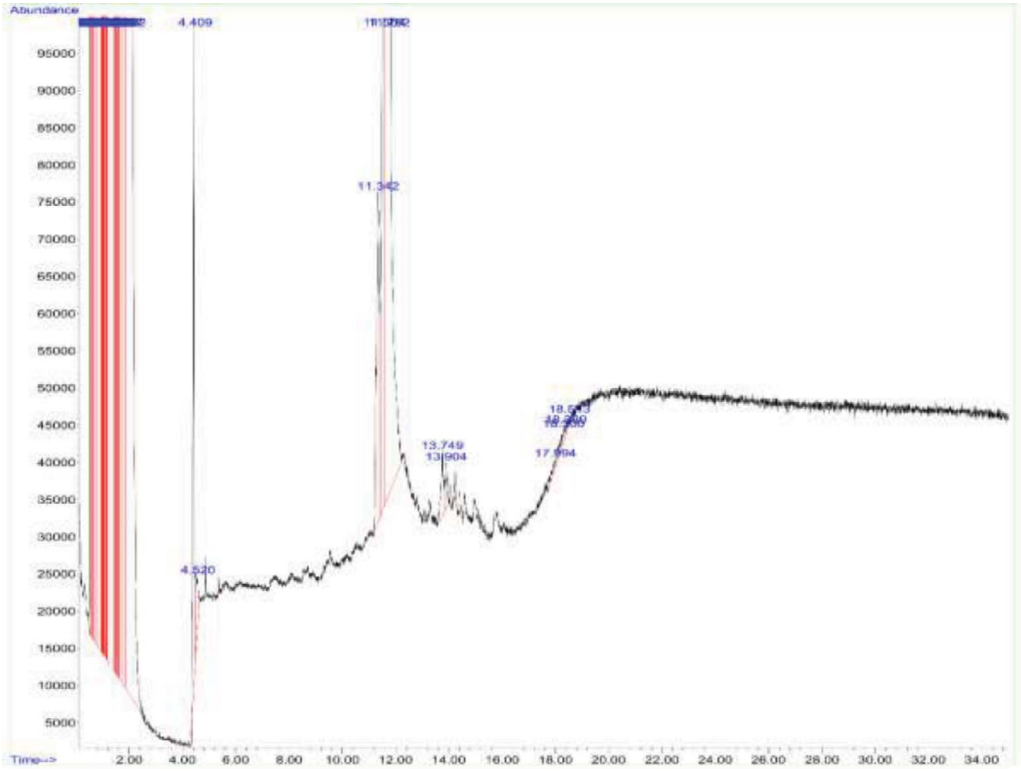

Figure 6. GCMS chromatogram of *Nyctanthes arbor-tristis*.

show that the HE of *Saraca asoca* contains 7 bioactive phytochemical compounds, while Table 3 and Figure 2 show that the HE of *Chenopodium album* contains 8 bioactive phytochemical compounds, Table 4 and Figure 5 show that the HE of *Ficus benghalensis* contains 15 bioactive phytochemical compounds, and Table 5 and Figure 6 show that the HE of *Nyctanthes arbor-tristis* contains 9 bioactive phytochemical compounds.

4. Discussion

Flavonoids have been shown to have beneficial effects against bacteria, inflammation, allergies, and viruses. Pharmacologically active alkaloids find widespread application in the pharmaceutical and recreational drug industries[52]. Saponins have been found to inhibit tumor growth and hypercholesterolemia in experimental

animals[53]. The polyphenolic family, of which tannins and phenols are part, has long been appreciated for its beneficial effects on health. According to Mehta *et al.*[54], terpenoids in hydroalcoholic extract and ethyl acetate fraction possess anti-arthritic properties. One of the first steps in identifying the active substances in medicinal plants is GC-MS analysis. As shown in 2, 3, 4, and 5 Tables, 7 bioactive phytochemical compounds from *Saraca asoca*, 8 bioactive phytochemical compounds from *Chenopodium album*, 15 bioactive phytochemical compounds from *Ficus benghalensis* and 9 bioactive phytochemical compounds from *Nyctanthes arbor-tristis* which shows various pharmacological activities.

ROS-induced oxidative stress contributes to the development of autoimmune disorders such as rheumatoid arthritis. As lipid peroxidation increases, the functions of both anti-oxidant enzymes and the Na+/K+-ATPase decrease. In addition to damaging immunoglobulins, increases in Reactive Oxygen Species (ROS) modify DNA mismatch repair pathways. According to these investigations[15], ROS oxidative stress plays an essential role in the pathogenesis of RA.

Denaturation of proteins in the synovial membrane and surrounding tissues is a well-known trigger of arthritic conditions. Some forms of arthritis might generate autoantigens because of protein denaturation[28,55]. By altering electrostatic, hydrophobic, hydrogen, and disulfide bonds in proteins, lysosomal lysis during inflammation may contribute to denaturation[55]. Anti-inflammatory drugs (NSAIDs) work to prevent the release of lysosomal enzymes by mitigating the injury to the lysosomal membrane. Environmental factors such as high temperatures, chemical medications, and hypotonic medium such as methotrexate, hydroxychloroquine, and diclofenac sodium can all compromise the RBC membrane integrity and lead to haemolysis[56]. Since RBC membranes are similar to lysosomal membranes, studying their protective function may shed light on their anti-inflammatory qualities. Substances that inhibit protein denaturation and aid in membrane stabilization are needed for the development of anti-arthritic drugs, as shown in Figure 5; NAT extract possesses these properties.

5. Conclusion

Medicinal plants with anti-rheumatic properties hold great promise as natural alternatives for managing rheumatic conditions. Through centuries of traditional use and modern scientific research, these plants have demonstrated their potential to alleviate inflammation, reduce pain, and improve the overall quality of life for individuals suffering from rheumatism. Furthermore, the integration of medicinal plants into conventional rheumatic treatments offers a holistic approach that can complement pharmaceutical interventions, potentially reducing the reliance on synthetic drugs and minimizing adverse effects. However, it's important to note that while medicinal plants show promise, their efficacy and safety should be thoroughly studied through rigorous clinical trials and research. Proper dosing, standardization, and potential interactions with other medications should also be considered.

6. Acknowledgments

GLA University, Mathura, India for providing the support to conduct this study.

7. References

1. Gharat S, Pandya A, Kulkarni D, Momin M. Unravelling the potential of nanocarriers to deliver Curcumin for the management of Rheumatoid Arthritis. Journal of Drug Delivery Science and Technology. 2023; 20:104742. https://doi.org/10.1016/j.jddst.2023.104742
2. Ding Q, Hu W, Wang R, Yang Q, Zhu M, Li M, Cai J, Rose P, Mao J, Zhu YZ. Signaling pathways in rheumatoid arthritis: Implications for targeted therapy. Signal Transduction and Targeted Therapy. 2023; 8(1):68. https://doi.org/10.1038/s41392-023-01331-9
3. Zhao JH, Ma S, Li CY, Zhang HC, Zhao LJ, Zhang ZY. Clinically approved small-molecule drugs for the treatment of rheumatoid arthritis. European Journal of Medicinal Chemistry. 2023; 256:115434. https://doi.org/10.1016/j.ejmech.2023.115434
4. Sharma A, Goel A. Pathogenesis of rheumatoid arthritis and its treatment with anti-inflammatory natural products. Molecular Biology Reports. 2023; 50(5):4687-706. https://doi.org/10.1007/s11033-023-08406-4
5. Han R, Ren HC, Zhou S, Gu S, Gu YY, Sze DM, Chen MH. Conventional disease-modifying anti-rheumatic drugs combined with Chinese Herbal Medicines for rheumatoid arthritis: A systematic review and meta-analysis. Journal of Traditional and Complementary Medicine. 2022; 12(5):437-46. https://doi.org/10.1016/j.jtcme.2022.01.005
6. Dharsana JN, Mathew M. Preliminary screening of anti-inflammatory and antioxidant activity of *Morinda umbellata*. International Journal of Pharmacy and Life Sciences. 2014; 5(8):12-6.

7. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). DARU Journal of Pharmaceutical Sciences. 2012; 20:1-7. https://doi.org/10.1186/2008-2231-20-76
8. Boniface PK, Verma S, Shukla A, Khan F, Srivastava SK, Pal A. Membrane stabilization: A possible anti-inflammatory mechanism for the extracts and compounds from *Spathodea campanulata*. Natural Product Research. 2014; 28(23): 2203-7. https://doi.org/10.1080/14786419.2014.930858
9. Khan MS, Ahmad I. Herbal medicine: current trends and future prospects. In New look to phytomedicine. Academic Press. 2019; 1:3-13. https://doi.org/10.1016/B978-0-12-814619-4.00001-X
10. Barupal T, Meena M, Sharma K. Inhibitory effects of leaf extract of *Lawsonia inermis* on *Curvularia lunata* and characterization of novel inhibitory compounds by GC–MS analysis. Biotechnology Reports. 2019; 23:e00335. https://doi.org/10.1016/j.btre.2019.e00335
11. Moradi MT, Karimi A, Alidadi S, Hashemi L. *In vitro* anti-adenovirus activity, antioxidant potential, and total phenolic compounds of *Melissa officinalis* L. (lemon balm) extract. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(9):1471-7.
12. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10(6):1003-8. https://doi.org/10.1093/carcin/10.6.1003
13. Joshi DG, Jat RK, Patil SB. Investigation of *in vitro* anti-arthritic activity of aqueous extracts of leaves of *Vitex negundo* L. using methotrexate as DMARDs. Journal of Pharmacognosy and Phytochemistry. 2021; 10(2):963-5. https://doi.org/10.22271/phyto.2021.v10.i2m.13926
14. Vallabh D, Jadhav VM, Kadam VJ. In-vitro anti-arthritic activity of *Abutilon indicum* (Linn.) sweet. Journal of Pharmacy Research. 2009; 2(4):644-5.
15. Di Dalmazi G, Hirshberg J, Lyle D, Freij JB, Caturegli P. Reactive oxygen species in organ-specific autoimmunity. Autoimmunity Highlights. 2016; 7(1):1-1. https://doi.org/10.1007/s13317-016-0083-0
16. Howis J, Bandzerewicz A, Gadomska-Gajadhur A. Rapid and efficient optimization of poly (1, 2-ethanediol citrate) synthesis based on magic squares' various methods. Gels. 2022; 9(1):30-8. https://doi.org/10.3390/gels9010030
17. Tódor IS, Marişca OT, Rugină D, Diaconeasa Z, Leopold LF, Coman C, Antonescu E, Szabó L, Iancu SD, Bálint Z, Leopold N. Photothermal property assessment of gold nanoparticle assemblies obtained by hydroxylamine reduction. Colloid and Polymer Science. 2020; 298:1369-77. https://doi.org/10.1007/s00396-020-04721-5
18. Enemor V, Oguazu C, Odiakosa A, Okafor S. Research article evaluation of the medicinal properties and possible nutrient composition of *Citrullus lanatus* (Watermelon) seeds. Res J Med Plant. 2019; 13(4):129-35. https://doi.org/10.3923/rjmp.2019.129.135
19. Mehrabani D, Ziaei M, Hosseini SV, Ghahramani L, Bananzadeh AM, Ashraf MJ, Amini A, Amini M, Tanideh NA. The effect of *Calendula officinalis* in therapy of acetic acid-induced ulcerative colitis in the dog as an animal model. Iranian Red Crescent Medical Journal. 2011; 13(12):884-94.
20. Al-Ostoot FH, Zabiulla, Salah S, Khanum SA. Recent investigations into the synthesis and pharmacological activities of phenoxy acetamide and its derivatives (chalcone, indole, and quinoline) as possible therapeutic candidates. Journal of the Iranian Chemical Society. 2021; 18:1839-75. https://doi.org/10.1007/s13738-021-02172-5
21. Issayeva UB, Akhmetova GS, Datkhayev UM, Omyrzakov MT, Praliyev KD, Ross SA. The search for biologically active compounds in the Series of N-ethoxyethylpiperidine Derivatives. Eurasian Chemico-Technological Journal. 2019; 21(2):125-33. https://doi.org/10.18321/ectj822
22. Lim CS, Wong WF, Rosli R, Ng KP, Seow HF, Chong PP. 2-Dodecanol (decyl methyl carbinol) inhibits hyphal formation and SIR2 expression in *C. albicans*. Journal of Basic Microbiology. 2009; 49(6):579-83. https://doi.org/10.1002/jobm.200900035
23. Lingfa L, Ankanagari S. GC-MS profiling of reproductive stage *Withania somnifera* for antimicrobial and anticancer phytochemicals. Biomedical and Pharmacology Journal. 2023; 16(1):197-211. https://doi.org/10.13005/bpj/2601
24. Lopez-Iglesias B, Perez C, Morales-García JA, Alonso-Gil S, Pérez-Castillo A, Romero A, López MG, Villarroya M, Conde S, Rodríguez-Franco MI. New melatonin–n, n-dibenzyl (n-methyl) amine hybrids: Potent neurogenic agents with antioxidant, cholinergic, and neuroprotective properties as innovative drugs for Alzheimer's disease. Journal of Medicinal Chemistry. 2014; 57(9):3773-85. https://doi.org/10.1021/jm5000613
25. Goniewicz ML, Havel CM, Peng MW, Jacob III P, Dempsey D, Yu L, Zielinska-Danch W, Koszowski B, Czogala J, Sobczak A, Benowitz NL. Elimination kinetics of the tobacco-specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. Cancer Epidemiology, Biomarkers and Prevention. 2009; 18(12):3421-5. https://doi.org/10.1158/1055-9965.EPI-09-0874
26. Azarang N, Movagharnejad K, Pirdashti M, Ketabi M. Densities, viscosities, and refractive indices of poly (ethylene glycol) 300+ 1, 2-Ethanediol, 1, 2-Propanediol, 1,

3-Propanediol, 1, 3-Butanediol, or 1, 4-Butanediol binary liquid mixtures. Journal of Chemical and Engineering Data. 2020; 65(7):3448-62. https://doi.org/10.1021/acs.jced.9b01189
27. Kumar H, Javed SA, Khan SA, Amir M. 1, 3, 4-Oxadiazole/thiadiazole and 1, 2, 4-triazole derivatives of biphenyl-4-yloxy acetic acid: Synthesis and preliminary evaluation of biological properties. European Journal of Medicinal Chemistry. 2008; 43(12):2688-98. https://doi.org/10.1016/j.ejmech.2008.01.039
28. Liu YF, Cao GM, Chen L, Li K, Lin XL, Xu XX, Le ZG, Yang GP. Synthesis of 3, 3′-Disubstituted Isobenzofuran-1 (3H)-Ones via Cs0. 5H2. 5PW12O40-catalyzed difunctionalization of carbonyls. Advanced Synthesis and Catalysis. 2022; 364(8):1460-4. https://doi.org/10.1002/adsc.202200081
29. Manuja R, Sachdeva S, Jain A, Chaudhary J. A comprehensive review of biological activities of p-hydroxy benzoic acid and its derivatives. International Journal of Pharmaceutical Science/Review Research. 2013; 22(2):109-15.
30. Lopez-Iglesias B, Perez C, Morales-García JA, Alonso-Gil, S, Pérez-Castillo A, Romero A, Rodríguez-Franco MI. New melatonin–n, n-dibenzyl (n-methyl) amine hybrids: Potent neurogenic agents with antioxidant, cholinergic, and neuroprotective properties as innovative drugs for Alzheimer's disease. Journal of Medicinal Chemistry. 2014; 57(9):3773-85. https://doi.org/10.1021/jm5000613
31. Heil J, Liu S, Vereecken H, Brueggemann N. Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. Soil Biology and Biochemistry. 2015; 84:107-15. https://doi.org/10.1016/j.soilbio.2015.02.022
32. Schlingemann J, Boucley C, Hickert S, Bourasseau L, Walker M, Celdran C, Chemarin T, Pegues C, Fritzsche M, Keitel J, Goettsche A. Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite. International Journal of Pharmaceutics. 2022; 620:121740. https://doi.org/10.1016/j.ijpharm.2022.121740
33. Terio V, Bozzo G, Ceci E, Savarino AE, Barrasso R, Di Pinto A, Mottola A, Marchetti P, Tantillo G, Bonerba E. Methylglyoxal (MGO) in Italian Honey. Applied Sciences. 2021; 11(2):831. https://doi.org/10.3390/app11020831
34. Wei LS, Wendy WE, Siong JY, Syamsumir DF. Characterization of antimicrobial, antioxidant, anticancer properties and chemical composition of *Sauropus androgynus* stem extract. Acta Medica Lituanica. 2011; 18(1):12-6. https://doi.org/10.6001/actamedica.v18i1.1808
35. Akbarizare M. GC-MS analysis and antimicrobial activity of an Iranian traditional medicinal smoke (Anbarnasara). Journal of Medical Microbiology and Infectious Diseases. 2021; 9(3):148-55. https://doi.org/10.52547/JoMMID.9.3.148
36. Bahnasawya AA, Ahmeda AS, Koraiemb AI. Synthesis of some antimicrobial pyrimidine nucleoside derivatives. Asw Science and Technology Bulletin. 2001; 1:46-60.
37. Chandra M, Sangwan NS, Kumar H, Singh AK, Kalra A. dl-2 Aminobutyric acid and calliterpinone are the potential stimulators of *Trichoderma* cellulase activities. Biomass and Bioenergy. 2014; 62:212-7. https://doi.org/10.1016/j.biombioe.2014.01.026
38. Bharate SB, Mahajan TR, Gole YR, Nambiar M, Matan TT, Kulkarni-Almeida A, Balachandran S, Junjappa H, Balakrishnan A, Vishwakarma RA. Synthesis and evaluation of pyrazolo 3, 4-b pyridines and its structural analogues as TNF-α and IL-6 inhibitors. Bioorganic and Medicinal Chemistry. 2008; 16(15):7167-76. https://doi.org/10.1016/j.bmc.2008.06.042
39. Deyno S, Mtewa AG, Hope D, Bazira J, Makonnen E, Alele PE. Antibacterial activities of *Echinops kebericho* mesfin tuber extracts and isolation of the most active compound, dehydrocostus lactone. Frontiers in Pharmacology. 2021; 11:608672. https://doi.org/10.3389/fphar.2020.608672
40. Popiołek L. The application of hydrazones and hydrazide-hydrazones in the synthesis of bioactive azetidin-2-one derivatives: A mini review. Biomedicine and Pharmacotherapy. 2023; 163:114853. https://doi.org/10.1016/j.biopha.2023.114853
41. Soares J, Costa VM, Bastos MD, Carvalho F, Capela JP. An updated review on synthetic cathinones. Archives of Toxicology. 2021; 95(9):2895-940. https://doi.org/10.1007/s00204-021-03083-3
42. Fayera S. Anti-Tuberculosis Activity and GC-MS Analysis of leaf extract of *Plantago lanceolata* L. Pure and Applied Chemistry. 2022; 10(3):1-4.
43. Singh G, Kumar A, Verma MK, Gupta P, Katoch M. Secondary metabolites produced by *Macrophomina phaseolina*, a fungal root endophyte of *Brugmansia aurea*, using classical and epigenetic manipulation approach. Folia Microbiologica. 2022; 67(5):793-9. https://doi.org/10.1007/s12223-022-00976-3
44. Lopez-Iglesias B, Perez C, Morales-García JA, Alonso-Gil S, Pérez-Castillo A, Romero A, López MG, Villarroya M, Conde S, Rodríguez-Franco MI. New melatonin–n, n-dibenzyl (n-methyl) amine hybrids: Potent neurogenic agents with antioxidant, cholinergic, and neuroprotective properties as innovative drugs for Alzheimer's disease. Journal of Medicinal Chemistry. 2014; 57(9):3773-85. https://doi.org/10.1021/jm5000613
45. Trnka J, Blaikie FH, Logan A, Smith RA, Murphy MP. Antioxidant properties of MitoTEMPOL and its

hydroxylamine. Free Radical Research. 2009; 43(1):4-12. https://doi.org/10.1080/10715760802582183

46. Hossain ML, Lim LY, Hammer K, Hettiarachchi D, Locher C. Monitoring the release of Methylglyoxal (MGO) from honey and honey-based formulations. Molecules. 2023; 28(6):2858. https://doi.org/10.3390/molecules28062858
47. Imsong L, Murali M. DNA barcoding, determination of bioactive compounds, antioxidant and anti-diabetic property in edible gastropod *Brotia Costula* (Rafinesque, 1833) Of Dimapur district, Nagaland.
48. Cruz-Salomón KD, Cruz-Rodríguez RI, Espinosa-Juárez JV, Cruz-Salomón A, Briones-Aranda A, Ruiz-Lau N, Ruíz-Valdiviezo VM. *In vivo* and *in silico* study of the antinociceptive and toxicological effect of the extracts of *Petiveria alliacea* L. Leaves. Pharmaceuticals. 2022; 15(8):943. https://doi.org/10.3390/ph15080943
49. Pulipaka AB. Intramolecular ring opening reactions of aziridines by π-nucleophiles. Ohio University; 2008. https://doi.org/10.1002/chin.200837138
50. Indraningrat AAG, Wijaya MD, Masyeni DAPS, Apsari PIB, Janurianti NMD, Daniswara D, Widhidewi NW. Evaluation of antioxidant and cytotoxic activities of ethyl acetate extracts of bacterial isolates associated with mangrove soil from the Ngurah Rai mangrove forest, Denpasar, Bali. In WICSTH 2022: Proceedings of the 2nd Warmadewa International Conference on Science, Technology and Humanity, WICSTH 2022, 28-29 October 2022, Denpasar-Bali, Indonesia (p. 18). European Alliance for Innovation. https://doi.org/10.4108/eai.28-10-2022.2328209
51. Wu C, Xiao Y, Jing Y, Yin Q, Bao Y. New insights into the solubilization of multicomponent crystals: A case study of pipemidic acid. Crystal Growth and Design. 2023; 23(5):3367-83. https://doi.org/10.1021/acs.cgd.2c01508
52. Sullivan RJ, Hagen EH, Hammerstein P. Revealing the paradox of drug reward in human evolution. Proc Biol Sci. 2008; 275(1640):1231-41. https://doi.org/10.1098/rspb.2007.1673
53. Johns T. Phytochemicals as evolutionary mediators of human nutritional physiology. International Journal of Pharmacognosy. 1996; 34(5):327-34. https://doi.org/10.1076/phbi.34.5.327.13254
54. Mehta A, Sethiya NK, Mehta C, Shah GB. Anti–arthritis activity of roots of *Hemidesmus indicus* R. Br. (Anantmul) in rats. Asian Pacific Journal of Tropical Medicine. 2012; 5(2):130-5. https://doi.org/10.1016/S1995-7645(12)60011-X
55. Volluri SS, Bammidi SR, Chippada SC, Vangalapati M. *In vitro* antioxidant activity and estimation of total phenolic content in methanolic extract of *Bacopa monniera*. Rasayan J chem. 2011; 4(2):381-6.
56. Held J, Mosheimer-Feistritzer B, Gruber J, Mur E, Weiss G. Methotrexate therapy impacts on red cell distribution width and its predictive value for cardiovascular events in patients with rheumatoid arthritis. BMC Rheumatology. 2018; 2:1-7. https://doi.org/10.1186/s41927-018-0012-0