Section A-Research paper

IN-VITRO EVALUATION OF ANTI-ARTHRITIC POTENTIAL OF SOME TRADITIONAL MEDICINAL PLANTS

Ruchi Singh [1], Syed Misbahul Hasan [2*], Amit Verma [3]

1. Research Scholar, Integral University,Lucknow.
2. Faculty of Pharmacy, Integral University,Lucknow
3. Department of Pharmacy, Himalayan Institute of Technology and Management, Lucknow

4.Corresponding author Name
Prof. Syed Misbahul Hasan
Dean, Faculty of Pharmacy
Integral University, Lucknow

Abstract

Introduction: Rheumatoid arthritis is a globally distributed systemic autoimmune disorder which mainly affects joints. It has a higher prevalence in the industrial area due to environmental risk factors or genetic factors. An encouraging source of novel chemicals with strong anti-inflammatory properties may be found in the enormous diversity of the plant kingdom. Natural remedies have made a tremendous contribution to the advancement of modern medicine. Recent worldwide reviews of conventional medicine have been influenced by extensive study on numerous plant species and their active medicinal components. The aim of the current study was to compare and assess *in-vitro* anti-arthritic effects of four solvent (petroleum ether, ethylacetate, methanol, water) extracts of three plants *Commiphoramukul, Nyctanthusarbortristis, Eucalyptus globulus* against the denaturation of protein inhibition assay *in-vitro*. Methods:In order to evaluate the anti-arthritic property, the test extracts at various concentrations were incubated with egg albumin under highly regulated laboratory conditions and then evaluated for absorbance to determine the anti-arthritic activity. Results: The percentage inhibition of protein denaturation was calculated. The current findings showed that all test extracts inhibited protein (albumin) denaturation in a concentration-dependent manner. Conclusion: It was determined that the methanolic extract of *Commiphoramukul* was the most effective of all extracts. Further, all of the extracts have a significant anti-arthritic impact against *in-vitro* protein denaturation inhibitionassay.

Keywords: Rheumatoid arthritis, *In-vitro*activity, TNF-α, Protein denaturation, Interleukins.

1. INTRODUCTION

Herbal treatment shows the tremendous impact on chronic ailments. Rheumatoid arthritis is a systemic, chronic autoimmune reaction that affects several joints and is associated with socioeconomic consequences, progressive disability, systemic complications and early

death. Rheumatoid arthritis and autoimmune thyroiditis are two instances of uncommon autoimmune illnesses. [1] The condition is made more difficult by its unidentified etiology and an uncertain prognosis. However, better knowledge and comprehension of the disease's biology, bring up the creation of new medications with promising results. In order to achieve clinical remission, the current treatment plan calls for the start of intensive medication as soon as the disease is diagnosed and ongoing monitoring of the disease's activity. [2]

Inflammation of the joints carried out an infectious, metabolic or constitutional condition referred to as arthritis. This condition is frequently accompanied by pain, swelling, and stiffness from an infection, trauma, degenerative changes, metabolic abnormalities or other reasons. A number of diseases including arthritis are caused by free radicals damaging biomolecules like lipids, proteins and nucleic acids. [3]

An encouraging source of novel chemicals with strong anti-inflammatory properties may be found in the enormous diversity of the plant kingdom. The main advantages of herbal therapies appear to be their low cost, low incidence of serious side effects and apparent efficacy. There are many plants which are used in the treatment of arthritis. Here most important four plants are comparatively discussed.

Nyctanthes arbor-tristis (NAT) Linn, a member of the Oleaceae family, is frequently referred to as "Night Jasmine" in English and "Harsingar" in Hindi because of its overnight aroma. [4, 5]. The juice from the decoction of the leaves is frequently used in traditional medicine to treat rheumatism and inflammatory diseases. NAT has already been found to have hepatoprotective, anti-inflammatory, antimalarial, antioxidant, antibacterial, antifungal, antidiabetic, anticancer, antiviral, immunostimulant, and CNS depressing activities. [6, 7]

*Commiphoramukul*is a member of the Burseraceae family. It possesses anti-inflammatory, analgesic, and anti-hyperlipidemic effects. The primary component of several Ayurvedic weight-loss regimens is guggulu. [8] The extract of this gum, also known as gugulipid or guglipid, has long been produced in Ayurvedic medicine. Guggulu contains bitter compounds, resin, gum, and essential oils. The main chemical components of guggulu include Z-guggulsterone, E-guggulsterone, guggullignans I & II, gugglutetrols, mukulol, allylcembrol, C-27 guggulusterols I, II, III, Z-guggulusterol, E-guggulusterol, etc. These chemicals antibacterial activity is the cause of various pharmacological activities, including analgesic, anti-inflammatory, wound cleansing, and healing. [9]

Guggul (*Commiphoramukul*), a valuable botanical medicine, has been prescribed in Ayurveda for centuries to cure a variety of diseases. Guggulipid is produced by several extracts of this substance with guggulsterone as the active ingredient responsible for its therapeutic action. The herb's pharmacological, phytochemical, and morphological profile has been studied *in-vivo*, *in-vitro* and in clinical trials. Guggul contains flavonoids, terpenes, phytosterols and other compounds that have biological actions such as anti-inflammatory,

anti-obesity, antineoplastic andantidiabetic. [10]

Eucalyptus globulus is an Australian evergreen tree that belongs to the Myrtaceae family. [11]
Apart from anti-inflammatory action eucalyptus oil (EO) has several benefits.[12] The aromatic components of EO are used to make analgesic, anti-inflammatory, and antipyretic medications.[13] The antioxidants pinene and 1, 8-cineole, both of which have potent antioxidant and radical-scavenging abilities are found in EO.[14] Juergens et al. looked into the function of eucalyptol as a cytokine inhibitor in human blood monocytes and discovered that it blocks the synthesis and production of tumour necrosis factor TNF-α, interleukin-1, leukotriene B_4, and thromboxane B_2, indicating that eucalyptol is a potent cytokine inhibitor that can be helpful for long-term treatment of bronchial airway inflammation.[15]

2. MATERIALS AND METHODS

The purpose of the current study was to support the traditional use of extracts of Nyctanthesarbortristis, Commiphoramukul and Eucalyptus globulus in rheumatism. The primary goals of the study were to compare the antiarthritic effects of various plant extracts. The anti-arthritic activity was assessed by using an *in-vitro* protein denaturation inhibition test. The current investigation also tended to identify the extract's most potent anti-arthritis components

2.1. Collection of material

The *Nyctanthesarbortristis*and *Eucalytus globulus* leaves were collected from my home garden situated in Barabanki, Uttarpradesh and *Commiphoramukul*gum was purchased from the market and all the plants are authenticated by taking the help of ataxonomist (National Botanical Research Institute, Lucknow) and with the help of chemicaltest.

2.2. Preparation of theextracts

Individual plant leaves were dried in the shade, processed into a fine powder by machine, and stored in a tight container for later use.
The dried, powdered leaves of Nyctanthesarbortristis, Eucalyptus globulus, and sticky powdered Commiphoramukul gum were successively extracted for 10-15 hours in a soxhlet apparatus using petroleum ether, methanol, ethylacetate, and water. The respective extracts were encoded as follows:

NP, NE, NM, NW: Petroleum Ether, Ethylacetate, Methanol and Water extracts of *Nyctanthesarbortristis*.

CP, CE, CM, CW: Pet. Ether, Ethylacetate, Methanol, and Water extracts of *Commiphoramukul.*

EP, EE, EM, EW: Pet. Ether, Ethylacetate, Methanol, Water extracts of *Eucalyptus globulus.*

2.3. Phytochemical Analysis

Preliminary phytochemical research revealed that the primary phytochemical components of *Nyctanthesarbortristis*, *Eucalyptus globulus* leaves and *Commiphoramukul*gum extracts include alkaloid, glycoside, steroid, triterpenoid, and flavonoid, which are often responsible for theanti-arthritic effect.

2.4. *In- vitro* anti-arthritic activity by protein denaturation inhibitionassay

This protein denaturation inhibition assay used in this study was taken from Arya D. *et al.* [16]. A brief overview of the procedure is provided.

2.4.1. Preparation of the testsolution

The test solution (0.5 ml) was prepared by using 0.45 ml of Bovine serum albumin (2mg/ml aqueous solution) and 0.05 ml of test solution in various concentrations (10,50, 100, 200, 400, 800, 1000 μg/ml).

2.4.2. Preparation of test control solution

This solution (0.5 ml) was prepared by using 0.45 ml of Bovine serum albumin (2 mg/ml aqueous solution) and 0.05 ml of distilled water.

2.4.3. Experimental

All the prepared solutions were adjusted to pH 6.3 with saline phosphate buffer. The sampleswere incubated at 37°C for 15 mins and then heated at 70°C for 3 mins. After cooling the samples 1.5ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for thecontrol test 0.05 ml distilled water was used instead of extracts. [16]

The following formula was used to determine the % inhibition of protein denaturation. Percentage inhibition = (100× (Abs Treated/Abs Control)-1)

Results from several test extracts were compared with those from the Control, which shows 100% protein denaturation. The % suppression of protein denaturation by different extracts is shown in Table 2.

3. RESULTS AND DISCUSSION

Different plant fraction is collected and calculated their extractive values shown in Table: 1

Table 1: Extractive value of the different Plant fractions

% Yield(w/w) % Yield or Extractive value (w/w) of the plant fraction obtained			
Extract fraction	**NYC**	**COM**	**EUC**
Petroleum Ether	6.51	5.13	1.47
Ethyl Acetate	6.46	5.18	2.82
Methanol	7.70	6.66	5.21
Water	5.80	3.90	3.34

- **NYC:***Nyctanthesarbortristis*, **COM:** *Commiphoramukul*, **EUC:***Eucalyptusglobulus*

In the phytochemical screening of various extract of different plants *Nyctanthesarbortristis*, *Eucalyptus globulus* leaves and *Commiphoramukul*gum has shown thepresence of phytochemical constituents such as alkaloid, glycoside, Steroid, triterpenoid and flavonoid etc

Table 2: Phytochemical screening of selected plant extract

Solvent Phytoconstituents	Pet. Ether			Ethyl acetate			Methanol			Water		
	NYC	COM	EUC	NYC	COM	EUC	NYC	COM	EUC	NYC	COM	EUC
Alkaloids	-	-	-	+	+	-	+	+	+	+	-	-
Steroids	+	+	+	-	-	-	+	+	-	-	-	-
Carbohydrates	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	-	-	+	+	+	+	+	+	+	-	-	-
Proteins	-	-	-	+	+	-	+	+	-	-	-	-
Glycosides	-	-	-	-	-	-	+	+	+	+	-	-
Flavonoids	-	-	+	+	+	+	+	+	+	-	-	+
Terpenoids	+	+	+	-	-	-	-	+	-	-	+	-

- **Presence of phytoconsituent indicated by "+" and absence by "-"**

Whenever these extracts were tested for *in-vitro* anti-arthritic efficacy through protein denaturation inhibition assay, the methanol extract of Commiphoramukul showed the greatest inhibition when compared to the other extracts. The presence of alkaloids, steroids, tannins, proteins, glycosides, flavonoids, and terpenoids in the ethanol fraction explains its high activity. At 1000 µg/ml, the percentage inhibition of various extracts was found to be526.984% (CP), 188 % (NP), 65.656% (EP), 174.603 % (CE), 71.717% (NE), 95.294 % (EE),653.968% (CM),147.474% (NM),128.235% (EM),95.238% (CW),149.412% (NW), 66.666 % (EW). All the extracts show dose-dependentresponses as tabulated in Table 2. All the extracts show dose-dependent responses are also shown in the Figure.

conc. (µg/ml) ↓ / % Inhibition →	Petroleum ether			Ethyl acetate			Methanol			Water		
	CP	**NP**	**EP**	**CE**	**NE**	**EE**	**CM**	**NM**	**EM**	**CW**	**NW**	**EW**
0	0	0	0	0	0	0	0	0	0	0	0	0
250	126.984	29.411	2.020	50.793	-1.010	14.117	147.619	11.111	11.764	38.095	42.352	3.030
500	258.730	83.529	29.292	101.587	28.282	49.411	306.349	57.575	49.411	69.841	49.412	57.575
1000	526.984	188	65.656	174.603	71.717	95.294	653.968	147.474	128.235	95.238	149.412	66.666

Table 3: *In-vitro* anti-arthritic activity of different plant extracts in different solvents

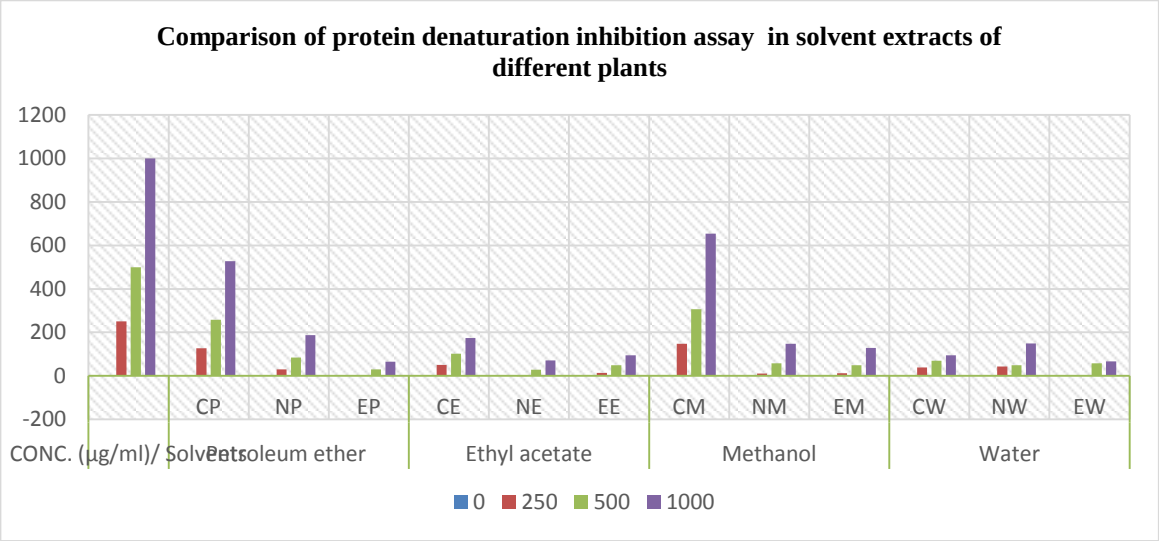

Figure: Graph plotted between concentration (y-axis) vs % inhibition (x-axis)

The above figure depicts the dose-dependent response of several plant extracts. A contributing factor to rheumatoid arthritis is protein denaturation. Protein denaturation may contribute to autoantigen generation in certain arthritic conditions. [17] Changes in the electrostatic binding of hydrogen, hydrophobic molecules, and disulfide molecules are most likely to cause denaturation. The results of this study indicate that *Commiphoramukul* has the ability to control the production of autoantigens that are involved in the prevention of protein denaturation. The secondary metabolite of *Commiphoramukul*appears to have a wide spectrum of effects, according to several studies. Further research is needed to identify the

active principles responsible for the anti-arthritic potential as well as *in-vivo* testing to determine the specific mechanism of action that contributes to the *Commiphoramukul's*arthritic potential.

4. **Conclusion:** This is the first in-vitro assessment of the anti-arthritic efficacy of many plant extracts in diverse solvents. When compared to other plant extracts, the methanolic extract of Commiphoramukul resin demonstrated the most anti-arthritic effect in-vitro. Secondary metabolites found in plants include flavonoids, glycosides, tannins, alkaloids, triterpenoids, and phenolics. As a result, successful active component isolation may aid in the identification of new lead compounds in the investigation of anti-inflammatory and anti-arthritic drugs. This paved the way for the development of Commiphoramukul's multifarious application in herbal medicine and set the framework for the creation of novel, potent therapies for arthritis and inflammation.

Acknowledgement: The authors are grateful to the Faculty of Pharmacy, Integral University, Lucknow for extending their support and providing facilities to complete this work.Integral University provided the MCN No.: IU/R&D/2022-MCN0001589 for publication of this article.

Reference

[1] D.L. Jacobson, S.J. Gange, N.R. Rose, N.M. Graham, Epidemiology and estimated population burden of selected autoimmune diseases in the United States, Clin Immunol Immunopathol 84 (1997) 223-243. https://doi.org/10.1006/clin.1997.4412

[2] G.S. Firestein, Evolving concepts of rheumatoid arthritis, Nature 423 (2003) 356–361. https://doi.org/10.1038/nature0166

[3] Z. Rasheed, Hydroxyl radical damaged immunoglobulin G in patients with rheumatoid arthritis: biochemical and immunological studies. Clin Biochem 41 (9) (2008) 663-9. https://doi.org/10.1016/j.clinbiochem.2008.02.013.

[4] A. Jahan , M. Anis, I. Aref, Assessment of factors affecting micropropagation andex vitro acclimatization of *Nyctanthes arbor-tristis L*, Acta BiologicaHungarica 62 (1) (2011) 45–56. https://doi.org/10.1556/abiol.61.2011.1.4

[5] G.R. Rout, A. Mahato, S.K. Senapati, *In-vitro* clonal propagation of Nyctanthes arbor-tristis Linn. A medicinal tree, Horticulture Science (Prague) 34 (2007) 84–89. http://dx.doi.org/10.1007/s10535-008-0101-9.

[6] J. Agrawal, A. Pal, Nyctanthes arbor-tristis Linn—a critical ethnopharmacological review, J Ethnopharmacol 146 (2013) 645–658. http://dx.doi.org/10.1016/j.jep.2013.01.024.

[7] J. Singh, A.P. Singh, A.P. Singh, "Nyctanthes Arbor-Tristis: A Comprehensive Review", World J. Curr. Med. Pharm. Res. 3(4) (2021) 74-77. http://dx.doi.org/10.37022/wjcmpr.v3i4.181.

[8] L.V. Gopalasimha, Standardization of NavakaGuggulu – An Ayurvedic polyherbal formulation, Indian J. Tradit. Kn. 74 (2008) 542-547. http://hdl.handle.net/123456789/2398.

[9] N. Manjula, B. Gayathri, K.S. Vinaykumar, N.P. Shankernarayanan, R.A. Vishwakarm, A. Balakrishnan, Inhibition of MAP kinases by crude extract and pure compound isolated from Commiphoramukul leads to down regulation of TNF-a, IL-1h and IL-2, Int. Immunopharmacol 6 (2006) 122–132. http://dx.doi.org/10.1016/j.intimp.2005.07.001.

[10] R. Ragavi, A.S. Saritha, Commiphoramukul: An Overview, Research J. Pharm and Tech 11 (2018) 11.http://dx.doi.org/10.5958/0974-360X.2018.00589.9.

[11] T. Mei-Lin, L. Chih-Chien, Y. Wei-Chao-Hsun, Antimicrobial and anti- inflammatory activities of essential oil from five herbs-a review, BiosciBiotechnolBiochem 75(10) (2011) 1977-1983. https://doi.org/10.1271/bbb.110377.

[12] J. Silva, W. Abebe, S.M. Sousa, V.G. Duarte, M.I. Machado, F.J. Matos, Analgesic and anti-inflammatory effects of Essential oils of Eucalyptus, J Ethnopharmacol 89 (2003) 277-283.http://doi.org/10.1016/j.jep.2003.09.007.

[13] H.P. Singh, S. Mittal, S. Kaur, D.R. Batish, R.K. Kohli. Characterization and antioxidant activity of essential oils from fresh and decaying leaves of Eucalyptus tereticorn. J Agric Food Chem 57 (2009) 6962-6963. https://doi.org/10.1021/jf9012407.

[14] U.R. Juergens, M. Stober, H. Vetter, Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1.8-cineole) in human blood monocytes *in vitro*, Eur J Med Res. 3 (1998) 508-510.PMID: 9810029.

[15] L.J. Juergens, H. worth, U.R. Juergens,New Perspectives for Mucolytic, Anti-inflammatory and Adjunctive Therapy with 1,8-Cineole in COPD and Asthma: Review on the New Therapeutic Approach, Adv Ther. 37 (5) (2020) 1737–1753.https://doi.org/10.1007/s12325-020-01279-0.

[16] A. K. Dhakad, V. V. Pandey, S. Beg, J. M. Rawat, A. Singh, Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. *J. Sci. Food Agric.* 98 (3) (2018) 833–848. http://doi.org/10.1002/jsfa.8600.

[17] A. M. Uttra, U. H. Hasan. Anti-arthritic activity of aqueous-methanolic extract and various fractions of Berberis orthobotrys Bien ex Aitch. BMC Complement Altern Med. 17(1) (2017) 371. http://doi.org/10.1186/s12906-017-1879-9.

[18]