


Preliminary Phytochemical Analysis: In-Vitro Comparative Evaluation of Anti-arthritic and Anti-inflammatory Potential of Some Traditionally Used Medicinal Plants

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Sundas Farooq¹, Ghazala Shaheen¹, Hafiz Muhammad Asif¹, Muhammad Rahil Aslam¹ , Rabia Zahid¹, Sehrish R. Rajpoot¹, Sana Jabbar², and Farah Zafar¹

Abstract

Background: *Colchicum autumnale*, *Strychnos nux-vomica* and *Aloe barbadensis* are the medicinal plants clinically utilized for the management of rheumatic disorders. **Purpose:** The present work was focused to evaluate the *in-vitro* anti-arthritic and anti-inflammatory activities of *Colchicum* (*Colchicum autumnale*), *Nux-vomica* (*Strychnos nux-vomica*), and *Aloe-vera* (*Aloe barbadensis*). **Research Design:** Primarily, the aqueous-ethanolic extracts of these medicinal plants were phytochemically screened followed by Fourier Transform Infrared (FTIR) analysis. Anti-arthritic activity by protein denaturation method and anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method at the concentration of 125, 250, and 500 µg/mL along with standard were performed. **Results:** Phytochemical screening revealed that alkaloids, saponins, terpenoids, phenols, and anthraquinones were found in all the extracts, and organic acids, amine group, aromatic or aliphatic compounds, esters and halogens, and phenolics were identified by FTIR. Protein denaturation method revealed that colchicum, nux-vomica, and aloe-vera showed maximum 98.5%, 99.6%, and 72.3% of inhibition at 500 µg/mL compared with that of standard drug, that is, Diclofenac sodium. Membrane stabilization method showed that colchicum, nux-vomica, and aloe-vera showed maximum 40.20%, 35.67%, and 40.1% protection at 500 µg/mL when compared with standard drug. **Conclusion:** It is concluded from the current study that extracts of colchicum, nux-vomica, and aloe-vera showed more potent effect and thus can be used as alternative options for the management of inflammatory and arthritic ailments.

Keywords

medicinal plants, phytochemical analysis, antiarthritic effect, anti-inflammatory effect

Introduction

Rheumatic ailments as well as chronic degenerative ailments that affect tissues and joints are associated with constant, recurring, or indeed persistent pain, inflammatory condition, and physical disability.¹ They harm the locomotor mechanism that contributes to impairment of the patient which impacts healthcare, social services, and reduces the individual's living standard^{2,3} that also influence about 30 to 40 % population in Europe.⁴ Such abnormalities include muscle pain, weakness, limited mobility, inflammation, or even diminished standard of living are associated with this condition. The incidence of rheumatic illnesses including muscle pain varies approximately

14 and 36 % in advanced countries.⁵ Treatment of inflammatory rheumatic conditions that consist of synthetic disease modifying

¹University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
²Department of Eastern Medicine, Qarshi University, Lahore, Pakistan

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Corresponding Author:

Muhammad Rahil Aslam or Hafiz Muhammad Asif, University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Khawaja Fareed Campus, Bahawalpur 63100, Pakistan.
Email: rahil.aslam17@gmail.com and Muhhammad.asif@iub.edu.pk



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antirheumatic drugs (sDMARDs) and glucocorticoids is generally effective as well as aimed at improving the illness. These commercially available treatment options are just causing economic exploitation and are also associated with severe adverse effects.^{6,7} For example, methotrexate also produces ulcers of the mouth.⁸ For that reason, the herbal medicine or natural medicine, having plant origin, may play effectual role in the treatment of arthritis and other inflammatory conditions.^{6,7}

Furthermore, because of the efficacy and cost-effectiveness of herbal medicines, people from developing countries such as India, Bangladesh, and Pakistan have relied on conventional medicinal systems to treat several ailments as alternate for healthcare. Local practice of medicinal herbs for treating numerous sicknesses is really popular in various areas of Pakistan.⁹ The developing world today is indeed prone towards alternative and complementary medicines originating primarily from natural sources. Over one quarter of certified synthetic drugs are initially expected to have been extracted from herbs.¹⁰ Herbs for medical purposes are being used throughout history as a treatment option for pain relief.¹¹

There are many medicinal plants which are being used traditionally in clinical practice to treat arthritic conditions, for example, *Azadirachta indica* (leaves), *Piper nigrum* (seeds), *Zingiber officinale* (rhizomes), *Withania somnifera* (erial parts) etc.¹² *Colchicum autumnale* (Colchicum) is a perennial herb commonly known as Colchicum or Suranjan tulkh which belongs to the family Colchicaceae and have alkaloids mainly colchicine, colchicoresin, demo-colchicine those which have anti-inflammatory, analgesic, phlegmagogue effects and therefore commonly used to treat arthralgia, gout, headache etc.¹³ *Strychnos nux-vomica* (Nux-vomica) belongs to Loganiaceae family having various alkaloids (brucine and brucine N-oxide) traditionally used for inflammatory diseases including arthritis.¹⁴ *Aloe barbadensis* (Aloe-vera) commonly called Aloe-vera belongs to the family Asphodelaceae have many valuable active constituents like vitamins, enzymes, anthraquinone glycosides, phenols, sugars, amino acids, hormones, and salicylic acid. Pharmacological properties of aloe-vera are widely acknowledged worldwide which includes wound healing, anti-aging, anti-inflammatory, antioxidant, and immuno-modulatory effects.¹⁵

Therefore, the present work was focused to evaluate the *in-vitro* anti-arthritic and anti-inflammatory activities of Colchicum (*Colchicum autumnale*), Nux-vomica (*Strychnous nux-vomica*), and Aloe-vera (*Aloe barbadensis*). Primarily, the aqueous-ethanolic extracts of these plants were phytochemically screened followed by Fourier Transform Infrared (FTIR) analysis.

Material and Methods

Procurement of Plant

Corms of Colchicum and seeds of Nux-vomica were collected from the local market of Bahawalpur and leaves of Aloe-vera were collected from the lawns of Khwaja Fareed Campus, the Islamia University of Bahawalpur, identified from the

Department of Pharmacognosy, the University of Karachi. Voucher numbers for identified plant specimens were A-132, A-134, and A-131. The study was approved by the ethical committee of University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, the Islamia University of Bahawalpur (1460/UCCM Dated 23-04-2019).

Extraction Process

The collected plants materials were washed with double distilled water and dried under shade. The seeds of Nux-vomica were immersed in excess of water for 5 days, to make them detox, in milk for 2 days followed by their boiling in milk, for its detoxification. Colchicum and Nux-vomica were grinded and weighted in specific proportions to sock in ethanol for making 70:30 aqueous ethanolic extract. The filtrate was placed for 72 hours with occasional stirring. Freshly cut leaves of Aloe-vera were taken and the epidermis was removed with knife. Pulp was separated and small pieces were added into ethanol and distilled water in specific proportions and agitated on sonicator for one hour followed by filtration, first with mucilin cloth, second with Whattman's filter paper grade no. 01. For preparation of extract, evaporation of ethanol was done with the help of rotary evaporator with revolution 70 rpm and temperature 45°C. Aloe-vera filtrate was lyophilized and the extract was placed at room temperature for three to 4 days for desiccation. Dried extracts were placed in jars and preserved for later analysis.

Preliminary Phytochemical Analysis of Plant Extracts

Preliminary qualitative phytochemical screening was carried out to identify the active constituents of plant extracts such as alkaloids, flavonoids, cardiac glycosides, terpenoids, carbohydrates, protein, fats, steroids, phenols, saponins, tannins, and anthraquinones with the following methods Dragondroff's test, lead acetate test, Legal's test, copper acetate test, Molish and Benedicts test, ninhydrin test, Salkowski test, ferric chloride test, froth test, and gelation test, respectively.^{16,17}

Fourier Transform Infrared

Dried extracts of Colchicum, Nux-vomica, and Aloe-vera were inserted into the spectroscope (Shimadzu, Japan) having a scanning range 400-4000 cm^{-1} along with a resolution of 4 cm^{-1} .

In Vitro Anti-Arthritic Activity by Protein Denaturation Method

In vitro evaluation of anti-arthritic activity by protein denaturation method was assessed as previously described methods.^{18,19} The reaction mixture was prepared by adding

egg albumin 0.2 mL, phosphate buffer saline (PBS, pH 6.4) 2.8 mL, and different concentrations of plant extracts or Diclofenac sodium 2 mL (200 µg, 400 µg, and 600 µg/mL) to make total volume up to 5 mL. Distilled water 2 mL, buffer 2.8 mL, and 0.2 mL albumin were taken as control. The mixture was incubated at 37°C for 15 minutes and further heated at 70°C for 5 minutes. Absorption was taken at 660 nm while using spectrophotometer followed by blank. The inhibitory percentage was analyzed by the under given formula. Percentage inhibition = $[\text{Abs control} - \text{Abs sample} \div \text{Abs control}] \times 100$ where Abs stands for absorption.

In-Vitro Anti-Inflammatory Activity by Human Red Blood Cell Membrane Stabilization Method

In-vitro anti-inflammatory activity by HRBC membrane stabilization method as reported previously with little modifications were carried out.²⁰ Blood was taken from healthy human volunteers and Alsever solution was added in equal volume. Blood was centrifuged at 3000 rpm for 30 minutes. 10% V/V RBC suspension was prepared with iso-saline solution. Plasma was decanted and packed cells were washed with iso-saline. Reaction mixture is prepared by adding phosphate buffer, RBC suspension, hypotonic saline solution, and different concentrations of plant extracts (125, 250, and 500 µg/mL). Total volume = 3 mL. Same is prepared for different concentrations of Diclofenac sodium. Control is 2 mL distilled water with phosphate buffer and RBC suspension. The reaction mixtures are incubated at 37°C for 30 min. Centrifugation was done at 3000 rpm for 20 min. Absorption was taken at 570 nm from UV visible spectrophotometer and spectra were taken. Percentage protection was calculated by following formula¹⁹

Percentage protection = $100 - [(\text{absorption sample} \div \text{absorption control}) \times 100]$

Statistical Analysis

The data was expressed as Mean with standard error of Mean (SEM), analyzed by SPSS version 20. One-way analysis of variance (ANOVA) was performed by using post-hoc Tukey test.

Results

Phytochemical Screening

The phytochemical evaluation carried for three medicinal plants revealed that alkaloids, saponins, terpenoids, phenols, and anthraquinones were commonly found in all the extracts (Table 1).

Fourier Transform Infrared Analysis

Colchicum. Figure 1 shows the FTIR spectra of aqueous-ethanolic extract of Colchicum. The peaks at 3959.89 to

3730.20 show the presence of organic acids (–COOH group). The peak at 3340.92 and 3155.48 refers to the presence of amine group (NH₂). The peak from 2923.46 to 2169.72 denotes the presence of aromatic compounds with C–H bonds or aliphatic compounds with –CH bonds. The peaks from 1943.13 to 1587.99 show the presence of ester bonds which can be C = N, C = C or O. The peaks at 990.3 to 628.94 refer to the presence of halogens with carbon group C–X.

Nux-vomica. Figure 2 shows the spectra of aqueous-ethanolic extract of Nux-vomica. The peak at 3896.13 and 3615.04 shows the presence of alcoholic groups (–OH group) with phenolic compounds. The peak at 3401.04 and 3356.82 shows the presence of NH₂ (amine group). The peak from 3123.38 to 2772.89 denotes the presence of aromatic compounds with –CH bonds or aliphatic compounds with –CH bonds. The peak from 1933.20 to 1516.42 is referred as ester peaks (C=N, C=C, O). The peak from 1098.06 to 628.17 shows the presence of halogen with carbon group (C–X).

Aloe-vera. Figure 3 shows the spectra of aqueous-ethanolic extract of Aloe-vera. The peak at 3927.50 shows the presence of organic acids (–COOH) group. The peaks at 3739.73, 3643.39 and 3626.53 show the presence of –OH group (alcoholic compounds with phenolic group). The peak at 2921.65 and 2853.56 refers to the presence of aromatic compounds –CH bonds or aliphatic compounds with –CH bonds. The peaks at 1706.67, 1636.32, and 1587.88 are referred as ester peaks (C = N, C = C, O). The peaks at 989.86 to 620.31 denotes the presence of halogen compounds with carbon group (C–X).

In Vitro Antiarthritic Activity

In vitro antiarthritic activity was carried out using protein denaturation method and percentage of inhibition was calculated as summarized in Table 2. Dose dependent increase in the percentage inhibition was observed and all the extracts showed maximum inhibition at the concentration of 500 µg/mL except Aloe-vera that showed maximum inhibition at 250 µg/mL. Strongest effect was observed by the Colchicum as compared to the standard Diclofenac sodium followed by Nux-vomica that indicated the anti-arthritis effect by all the extracts (Table 2 and Figure 4).

In Vitro Anti-Inflammatory Activity

The anti-inflammatory activity was determined by HRBC membrane stabilization method and percentage of protection was calculated as summarized in Table 2. Dose dependent increase in the percentage of protection was observed and all the extracts showed maximum inhibition at the concentration of 500 µg/mL. Strongest effect was observed by Colchicum

Table 1. Summary of Phytochemical Analysis of Medicinal Plants.

Plants name	Alkaloids	Flavonoids	Cardiac glycosides	Terpenoids	Carbohydrates	Proteins	Fats	Steroids	Phenols	Saponins	Tannins	Anthraquinones
Colchicum	+	+	+	+	-	+	+	+	+	+	+	+
Nux-vomica	+	-	-	+	-	-	-	-	+	+	-	+
Aloe-vera	+	-	-	+	-	Traces	Traces	-	+	-	+	+

(+) indicates the presence and (-) indicates the absence of constituents

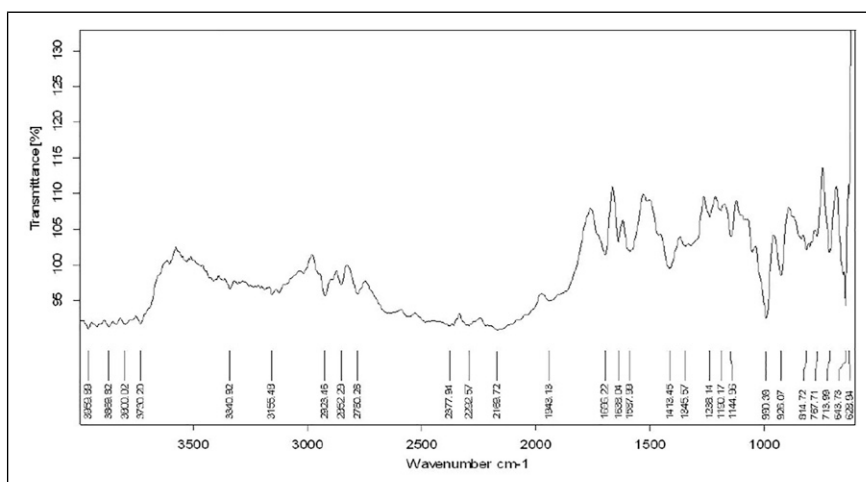


Figure 1. Fourier Transform Infrared spectra of aqueous-ethanolic extract of *Colchicum autumnale*.

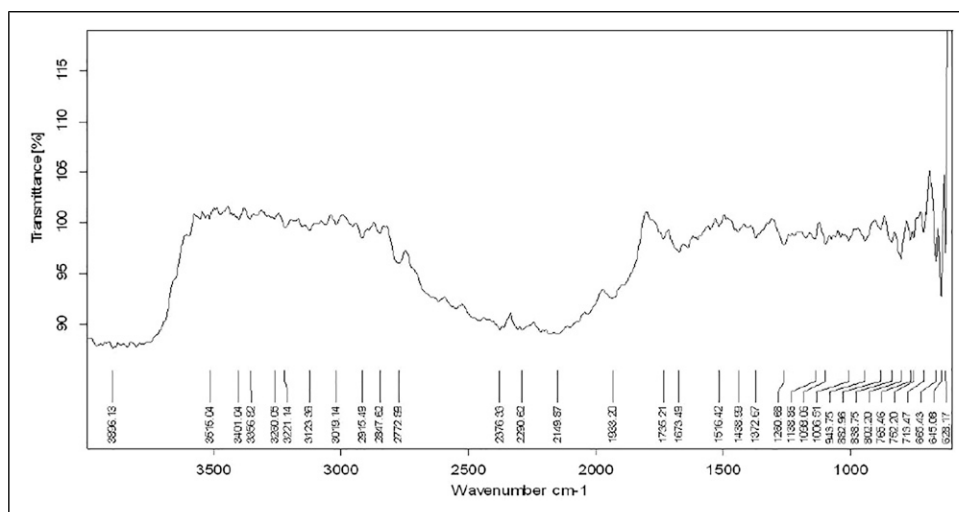


Figure 2. Spectra of aqueous-ethanolic extract of *Strychnos Nux-vomica*.

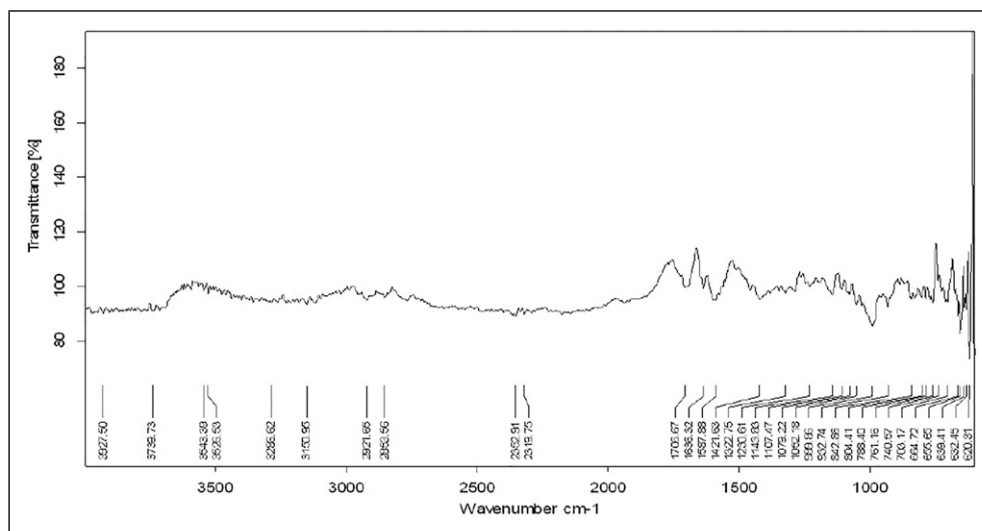
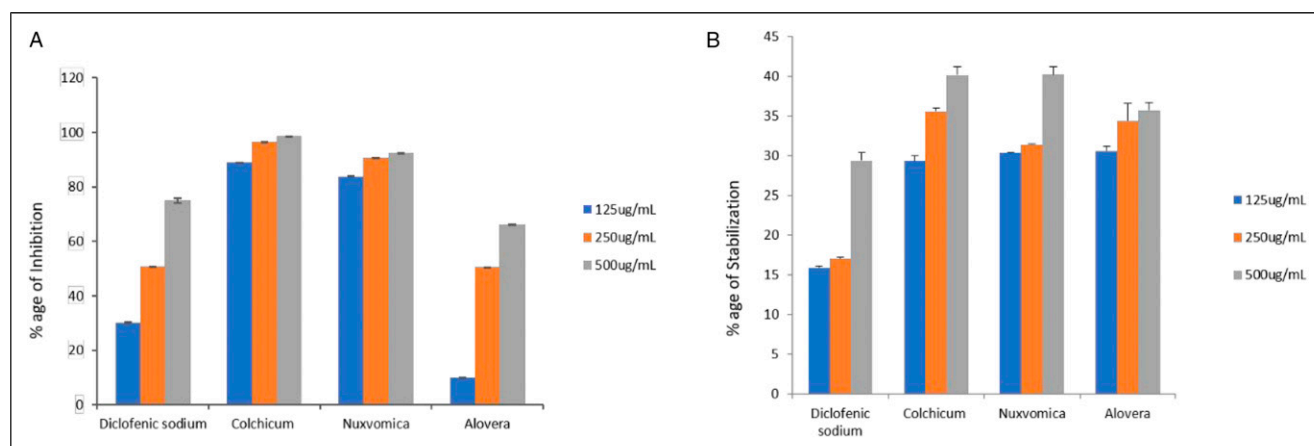


Figure 3. Spectra of aqueous-ethanolic extract of *Aloe barbadensis*.

Table 2. Percentage Absorption and Inhibition by Anti-Arthritic Activity.

	Conc. $\mu\text{g}/\text{mL}$	Anti-arthritic activity by protein denaturation method	Anti-inflammatory activity by human red blood cell membrane stabilization method
		Percentage inhibition %	Percentage protection%
Colchicum	125	88.95 \pm .0296	30.31 \pm .6339
	250	96.44 \pm .0512	31.37 \pm .3911
	500	98.51 \pm .0296	40.20 \pm .6369
Nux-vomica	125	83.83 \pm .1025	30.58 \pm .0783
	250	90.55 \pm .0783	34.36 \pm .1356
	500	92.30 \pm .1067	35.67 \pm .0783
Aloe-vera	125	10.02 \pm .0296	29.33 \pm .6492
	250	75.72 \pm 11.3825	35.58 \pm 2.1912
	500	66.16 \pm .0512	40.17 \pm .4144
Diclofenac sodium	125	30.07 \pm .3256	15.89 \pm .1848
	250	50.74 \pm .0296	17.05 \pm .1356
	500	74.98 \pm .8112	29.36 \pm .0296

**Figure 4.** In vitro anti-arthritic and anti-inflammatory activities of Colchicum autumnale, Strychnous Nux-vomica, and Aloe barbadensis (“A” Percentage of inhibition by egg albumin protein denaturation method, “B” Percentage of stabilization by human red blood cell membrane stabilization method).

and Aloe-vera followed by Nux-vomica as compared to the standard diclofenac sodium as shown in Table 2 and Figure 4.

Discussion

The medicinal plants for therapeutic purposes are used since ancient times and are the principal source of many modern medicines for the treatment of different diseases.²¹ Aqueous-ethanolic extracts of three plants, that is, Colchicum, Nux-vomica, and Aloe-vera were screened phytochemically and the presence of different chemical constituents were confirmed. It was found that Colchicum possesses alkaloids, flavonoids, glycosides, fats, proteins, saponins, tanins, terpenoids, phenols, steroids, and anthraquinones. Nux-vomica contains alkaloids, phenols, saponins, terpenoids, and anthraquinones, whereas Aloe-vera comprises of alkaloids, terpenoids, tanins, anthraquinones, proteins, and fats (traces). Alkaloids are reported to

have anti-inflammatory effect²¹ and colchicine in Colchicum; strychnine and brucine in Nux-vomica are alkaloids in nature.^{22,23} Similarly, barbaloin is glycoside and Aloe emodin in Aloe-vera is anthraquinone in nature²⁴ that are reported to have anti-arthritic and anti-inflammatory potential.²⁵

We also performed FTIR analysis to find out functional groups present in these plant extracts and found the presence of organic acids, amine groups, aromatic compounds with C-H bonds or aliphatic compounds, esters and halogen compounds with carbon group (C-X), and phenolic compounds. As the chemical compounds found in the extracts are reported to have antiarthritic and anti-inflammatory activity, we sought to investigate these crude extracts to determine the aforementioned pharmacological effects *in vitro*.

Previous literature reported that one of the factors which is involved in the causation of rheumatoid arthritis is denaturation of proteins.²⁶ These findings prompt us to

evaluate the anti-arthritic potential by protein denaturation method for 125, 250, and 500 µg/mL of concentration of aforementioned medicinal plants derived crude extracts. For Colchicum, we observed the 88.4, 96.0, and 98.5% of inhibition and Nux-vomica expressed 83.83, 90.55, and 92.30 % inhibition of protein denaturation while Aloe-vera revealed 10.2, 75.72, and 66.16 % inhibition, respectively. All the extracts showed comparable effects as of standard drug, that is, Diclofenac sodium having 30.7, 50.74, and 74.98 % of inhibition. Previously, H. Rehman et al.¹⁸ worked on *Oryza sativa* (variety: Joha Rice) and found comparable 75.0, 80.31, and 84.15 %, respectively, at same concentration that are in line with our findings. Mechanism of denaturation can be due to changes in hydrogen, electrostatic, disulfide, and hydrophobic bonding in structure of peptides. It is evident from various studies that many anti-inflammatory drugs exhibit dose-dependent ability of inhibition for thermally induced protein denaturation.²⁷ Protein denaturation method in our study is expected to involve such mechanism.

The cell vitality depends upon the integrity of their membranes. Exposure of RBCs to injurious substances, such as hypotonic medium, causes the lysis of membrane which leads to hemolysis and oxidation of hemoglobin. The hemolytic effect of hypotonic solution is related to excessive fluid accumulation within the cell, resulting in the rupture of membrane. Such kind of injury to membrane causes secondary damage through lipid peroxidation due to free radicals.²⁸ It is thus anticipated that compounds possessing membrane-stabilizing properties should offer significant cell membrane protection against injurious agents.²⁹ Thus, taking in view the critical role of cell membrane protection, we aimed to investigate the membrane stabilizing effect of Colchicum, Nux-vomica, and Aloe-vera crude extracts by HRBC membrane stabilization method at the concentration of 125, 250, and 500 µg/mL. Results showed that percentage protection of Colchicum is 30.31, 31.37, and 40.20% of protection. Similarly, Nux-vomica expressed remarkable 30.58, 34.36, and 35.67% of protection while Aloe-vera revealed 29.33, 35.58, and 40.17% of protection and was comparable with the standard drug, that is, Diclofenac sodium that had 15.89, 17.05, and 29.36% of protection. Compounds with membrane-stabilizing properties are considered reliable for their ability to restrict the release of phospholipases that are responsible for triggering the formation of inflammatory mediators.³⁰ In this study, absorbance of hemoglobin is taken by spectrophotometer in HRBC membrane stabilization method. When RBCs are exposed to hypotonic medium, hemoglobin inside the cell membrane is lysed.³¹ Due to the addition of membrane stabilizing agents, the cell membrane stability is increased, and thus, less absorbance is noted by spectrometer. The medicinal plant extracts used in our study, namely, Colchicum, Nux-vomica, and Aloe-vera has shown comparable

membrane stabilizing property with the standard that may be due to the inhibition of the release of phospholipases.³²

Conclusion

We concluded that aqueous-ethanolic extract of Colchicum, Nux-vomica, and Aloe-vera were found to be more potent in inhibition of egg albumin denaturation and protection of HRBC membrane stabilization and is comparable with Diclofenac sodium suggesting that these plants can be used as alternative options in the management of inflammatory and arthritic conditions. Further studies are warranted to find out the phytochemicals responsible for the potential and their molecular targets underlying disease recovery process.

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Declaration of Conflicting Interests

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ORCID iD

Muhammad Rahil Aslam  <https://orcid.org/0000-0003-0684-9147>

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