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Characterization of the bioactive components of *Chloris virgata* Sw. and assessment of its antimicrobial and anti-arthritic potential

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ABSTRACT

The goals of this study are to characterize the bioactive ingredients present in the formulation of three parts of *Chloris virgata* Sw. and to assess their antimicrobial and anti-arthritic properties. *C. virgata* Sw. extracts (methanol, hydroethanol, and aqueous) were characterized using ultraviolet-visible (UV-Vis) spectroscopy and Fourier transform infrared spectroscopy (FTIR) techniques. Antimicrobial activity was measured with the disc diffusion method, and anti-arthritic activity was determined by an *in vitro* protein denaturation assay. The *in-vivo* study was performed on methanol extract, and the biochemical and hematological parameters were investigated by Freund's complete adjuvant model. UV-visible spectroscopy revealed the presence of flavonoids, terpenoids, phenols, alkaloids, and saponins, whereas FTIR results show functional groups like carboxylic acids, alkenes, amides, carbohydrates and proteins, ether, and esters. Methanol and hydroethanol extracts showed higher antimicrobial activity at 400 µg/mL against *Escherichia coli, Bacillus subtilis,* and *Aspergillus niger* in comparison to the standards streptomycin and fluconazole at 10 µg/mL. *In-vitro* results of the methanol extract clearly show 72.58% inhibition as compared to other extracts. *In-vivo* anti-arthritic evaluation was performed with methanol extract, and it showed remarkable paw reduction in the treated group on 21st day at a high dose (2.2 ± 0.1) as compared to indomethacin (1.74 ± 0.114). Hematological parameters such as hemoglobin, red blood cells (RBC), white blood cells (WBC), neutrophils, lymphocytes, and platelets, and biochemical parameters like alkaline phosphatase (ALP) (51.36 ± 9.113 IU/L), aspartate aminotransferase (AST) (64.65 ± 10.011 IU/L), and alanine aminotransferase (ALT) (129.35 ± 13.53 IU/L) were restored to normal in the treated. The findings indicated that *C. virgata* extracts under UV spectroscopy and FTIR revealed significant bioactive components, which are determined to be significant contributors to their antioxidant and antibacterial properties.

1. INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory condition that mostly affects the joints. It causes mild swelling, and excessive and atypical synovial development, which finally alters the shape of the joint [1]. RA affects women more frequently than men, and although it can strike at any age, it usually appears between the ages of 50 and 60. Traditional remedies have been used as a form of healthcare since the prehistoric era and are frequently used to treat a wide range of disorders. Traditional remedies are safe for use at any age and have few negative effects [2]. To lead a healthy lifestyle, Ayurveda is extremely important. Animals, plants, and microorganisms all contribute to the synthesis of physiologically active phytochemical substances that are used in the creation of pharmaceuticals. These are classified as primary and secondary constituents depending on their role in plant metabolism. Many sophisticated techniques can be used to validate the presence of phytoconstituents in medicinal plants. Ultraviolet-Visible (UV-Vis) spectrometry and Fourier transform infrared spectroscopy (FTIR) are two effective techniques for identifying and determining the structure of phytochemicals [3].

Biologic agents, disease-modifying anti-rheumatic medicines (DMARDs), and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce inflammation and regulate symptoms [4], although they have been reported to have some side effects. According to estimates made by Arya et al. [5], about 43% of arthritis patients in India use herbal remedies to manage their condition. *Chloris virgata* Sw. of the Poaceae family is one of the weeds of warm weather in the eastern part of Australia, and its occurrence can be easily seen throughout the mainland of Australia. Resistance or tolerance to glyphosate, dispersal through flood and wind, and greater production of seeds are some factors that resulted in the prevalence of *C. virgata* Sw. in both non-cropping and cropping seasons [6,7]. Different transcriptomic analyses were done on *C. virgata* Sw. which reported the major genes responsible for their fastest growth and germination, especially in the major grasslands of Mongolia [8].

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An intradermal or subdermal injection of Freund's complete adjuvant (FCA) has been broadly used for the induction of arthritis in rats. However, its application to direct arthritis in mice has been reported occasionally, with less success [9]. Adjuvant directed arthritis has several limitations for an animal model; especially the disorder that resulted after the injection due to the resulting stress and severe discomfort. The polyarthritis caused by FCA is an attempt to result in less drastic arthritis by eliminating some of the complicating factors of this model. Major modifications include its injection locally, either around or into the tibiotarsal joint. These modifications allow the occurrence of localized arthritis without targeting the mobility of poor animals [10]. Panda et al. [11] reported that the decoction forms of *C. virgata* Sw. are employed by tribal people in Odisha, India, to treat a variety of rheumatic conditions. In rural Tamil Nadu, India, residents reportedly utilize *C. virgata* Sw. to treat rheumatism, inflammation, discomfort, and stomach aches. According to numerous studies, protein denaturation is a crucial factor in the development of rheumatoid arthritis. Therefore, the purpose of this study was to investigate all possible ideas and methods for future sources of herbal medicines to treat arthritis, as well as to characterize the bioactive components in *C. virgata* Sw. plant extracts using UV and FTIR.

2. MATERIALS AND METHODS

2.1. Plant Identification and Authentication

The three parts (leaves, stem, and roots) of *C. virgata* Sw. were collected from the Banasthali Vidyapith, Rajasthan, India, in 2019. The plant herbarium was submitted (voucher number: BURI 1392/2021; plantarum number: Fl. Ind. Occid. 1: 203 1797) and it was further identified and authenticated by the botanist of the Department of Bioscience and Biotechnology.

2.2. Preparation of Plant Extracts with Different Solvents

The plant sample was washed thoroughly and shade dried for one week, then ground into fine powder form and stored in an airtight container. About 30 g of powder (10 g from each leaf, stem, and root) was successively taken to carry out the successive extraction with different solvents [methanol, hydroethanol (50:50), and aqueous] using a soxhlet apparatus for 1-2 working days. The obtained extracts were filtered and dried using a rotary evaporator. The dried extracts were kept at 4°C for further experimental use [12]. For characterization and antimicrobial analysis, all three extracts (methanol, hydroethanol, and aqueous) of the whole plant *C. virgata* Sw. were taken. For *in-vitro* protein denaturation and *in-vivo* anti-arthritic studies, only the selected methanol extract of the whole plant of *C. virgata* Sw. was used.

2.3. Characterization of *C. virgata* Sw. Extracts

2.3.1. UV-Vis spectroscopic analysis

The plant extracts were dissolved in the respective solvent and centrifuged for 10 min at 3000 rpm. The supernatant was then filtered and diluted at a 1:10 ratio with the same solvent. The extracts were scanned with a Labman-UV1900 at a wavelength range of 200-1100 nm [13].

2.3.2. FTIR analysis

Subashini et al. [14] described a method for performing FTIR analysis. It was carried out in order to determine the functional groups present in *C. virgata* Sw. extracts and identify them based on peak values in the infrared radiation region.

2.4. Antimicrobial Activity of *C. virgata* Sw. Extracts

2.4.1. Test microorganisms and their inoculum preparation

The pure cultures of the test organisms were provided by the Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, India. *Bacillus subtilis* (*B. subtilis;* MTCC441), *Escherichia coli*, (*E. coli*; MTCC739), and *Aspergillus niger* (*A. niger*; MTCC282) were the bacterial and fungal strains employed in the investigation.

Table 1: Measure of zone of inhibition among different extracts of *C. virgata* against bacterial strains.

Plant extracts	Concentration (1 mg/ml)	Tested bacterial strains	
		E. coli	*B. subtilis*
Methanol	50	7.05 ± 3.04	6.07 ± 3.5
	100	8.1 ± 3.39	9.07 ± 5.23
	200	9.25 ± 4.01	12.8 ± 7.40
	400	9.78 ± 0.01	13.1 ± 6.40
Hydroethanol	50	4.6 ± 2.65	5.32 ± 3.07
	100	5.32 ± 3.07	6.82 ± 3.94
	200	6.02 ± 3.47	8.35 ± 4.82
	400	8.37 ± 4.83	12.12 ± 7.00
Aqueous	50	NZ	NZ
	100	4.6 ± 2.65	5.35 ± 3.09
	200	7.4 ± 0.08	6.82 ± 3.94
	400	6.77 ± 3.91	9.07 ± 5.23
Streptomycin (standard)	10 µg/ml	22 ± 0.871	25 ± 0.654

Notes: All the results are expressed as mean ± SD (n = 5). NZ: no zone of inhibition.

Table 2: Measure of zone of inhibition among different extracts of *C. virgata* against fungal strains.

Plant extracts/ Standard	Concentration (1 mg/ml)	*A. niger*
Methanol	50	5.32 ± 0.08
	100	6.82 ± 0.08
	200	9.07 ± 0.81
	400	12.1 ± 0.12
Hydroethanol	50	4.57 ± 2.64
	100	6.1 ± 3.52
	200	8.35 ± 4.82
	400	9.82 ± 5.67
Aqueous	50	NZ
	100	3.97 ± 2.99
	200	4.57 ± 2.64
	400	5.35 ± 3.09
Fluconazole (standard)	10 µg/ml	21 ± 2.23

Notes: All the results are expressed as mean ± SD (n = 5). NZ: no zone of inhibition.

Table 3: *In-vitro* protein denaturation of *C. virgata* extracts and standard diclofenac sodium.

Conc. (µg/ml)	% inhibition			
	Diclofen ac sodium	Methanol extract	Hydroethanol extract	Aqueous extract
0	39.88 ± 0.011	26.09 ± 0.021	17.30 ± 0.007	14.36 ± 0.021
100	51.02 ± 0.023	34.01 ± 0.012	25.51 ± 0.016	23.02 ± 0.012
250	63.04 ± 0.032	39.88 ± 0.019	32.40 ± 0.013	27.12 ± 0.010
500	69.94 ± 0.024	50.73 ± 0.020	47.65 ± 0.018	39.88 ± 0.020
1000	78.59 ± 0.031	61.58 ± 0.011	58.65 ± 0.013	48.38 ± 0.015
2000	86.95 ± 0.022	72.58 ± 0.022	68.91 ± 0.016	60.11 ± 0.012

Notes: All the results are expressed as mean ± SD (n = 5).

2.4.2. Antimicrobial activity (Disc diffusion method)

On the nutrition and potato dextrose agar plates, 250 µL of the standardized inoculum of microbial species was applied using a sterile cotton swab. On the infected agar plates, the extract-impregnated discs were deposited in varied quantities (ranging from 50 to 400 µg/mL) for antibacterial and antifungal purposes. Using the standards of fluconazole and streptomycin (10 µg/disc), the sensitivity of various bacterial and fungal species was assessed. For 24 h at 37°C (bacteria) and 72 h at 27°C (fungi), all petri dishes were incubated. The inhibitory zone diameter was measured in millimeters [15].

2.5. *In-vitro* Anti-arthritic Study

2.5.1. Protein denaturation inhibition assay

The method described by Djuichou et al. [16] was used to measure the proportion of denaturized protein that was inhibited by employing bovine serum albumin (BSA). At 660 nm, the absorbance was measured, and the inhibition percentage was computed using the following formula:

% Inhibition = (Control Abs -Sample Abs) ×100/ Control Abs.

2.6. *In-vivo* Anti-arthritic Study

2.6.1. Experimental animals

Male albino Wistar rats weighing between 150 and 200 g were purchased and in-vivo study was also done at Bilwal Medchem and Research Laboratory Pvt. Ltd. Rats were handled in accordance with the recommendations made by CPCSEA (BMRL/AD/ CPCSEA/ IAEC/2022/3/2).

2.6.2. Acute toxicity study

Organization for Economic Co-operation and Development (OECD) standards 423 were followed while evaluating the acute toxicity of the methanol extract of *C. virgata* Sw.

2.6.3. FCA-induced arthritis

For the purpose of studying FCA-induced arthritis, rats were divided into six groups, each containing five rats (n = 5). FCA was injected subcutaneously into the left hind paw in an amount of around 0.1 mL. The standard drugs indomethacin and methanol extract of *C. virgata* Sw. were subsequently administered daily until the 21st day following the second day of FCA injection. The body weight change was also noticed on the first, seventh, fourteenth, and twenty-first days [17]. Group 1- Normal control rats; Group 2- Arthritic control rats; Group 3- Arthritic rats + standard drug indomethacin at 10 mg/kg body weight (b.w.), orally (p.o.); Group 4- Arthritic rats + methanol extract of *C. virgata* Sw. at 200 mg/kg b.w., p.o.; Group 5- Arthritic rats + methanol extract of *C. virgata* Sw. at 300 mg/kg b.w., p.o.; and Group 6- Arthritic rats + methanol extract of *C. virgata* Sw. at 400 mg/kg b.w., p.o.

2.6.4. Evaluation of hematological and serological parameters

Blood samples were taken on the study's last day (day 22) from each animal group using a retro-orbital puncture into two tubes. One tube is coated with EDTA. Shaking the samples must combine the blood and EDTA right away. Following that, an EDTA tube was sent through an automated Rayto, China hematoanalyzer to measure hemoglobin, red blood cells (RBCs), neutrophils, lymphocytes, platelets, and white blood cells (WBCs) [18]. To separate the serum, the anticoagulant-free blood tube was centrifuged at 2500 g for 10 min. Using the Abcam diagnostic kits, the levels of alkaline phosphatase (ALP),

Table 4: Effect of *C. virgata* methanol extract on change in body weight in FCA-induced arthritic rats.

Groups	0 day	7th day	14th day	21st day	Mean change inbody weight
Normal control	158.6 ± 2.516	160.06 ± 2.081	160.4 ± 1.504	165.1 ± 0.854	3.93%
Arthritic control	133.8 ± 3.164[a]	136.11 ± 3.200[a]	140.07 ± 8.620[a]	137.77 ± 8.161[a]	2.9%
Standard indomethacin	172.03 ± 5.078[c]	180.28 ± 6.475[d]	185.2 ± 10.362[a]	192.88 ± 6.233[c]	12.11%
Methanol extract of *C. virgata* (200 mg/kg)	157.24 ± 14.89[b]	149.42 ±7.895[b]	154.07 ± 10.625[b]	165.9 ± 10.043[b]	5.5%
Methanol extract of *C. virgata* (300 mg/kg)	162.14 ± 12.485[c]	164.95 ± 4.741[c]	16.752 ± 10.043[c]	171.77 ± 5.366[b]	5.9%
Methanol extract of *C. virgata* (400 mg/kg)	173.27 ± 13.047[c]	174.08 ± 7.523[d]	179.91 ± 8.963[d]	187.08 ± 9.116[c]	7.97%

Notes: All the results are expressed as mean ± SD (n = 5). [a]$p < 0.05$ vs. normal control, [b]$p < 0.01$ vs. normal control, [c]$p < 0.05$ vs. arthritic control, and [d]$p < 0.01$ vs. arthritic control.

aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the serum sample were determined. The ALP, AST, and ALT concentrations were expressed as IU/L of serum.

2.7. Statistical Analysis

The mean and standard deviation (SD) of all data were calculated in triplicate. Using the IBM 8 SPSS (20) statistical program, one-way analysis of variance (ANOVA), post-hock analysis, and Dunnet's test were performed for each of the factors under investigation. At $p < 0.05$ and $p < 0.01$, the values were deemed statistically significant.

3. RESULTS AND DISCUSSION

3.1. Characterization of *C. virgata* Sw. Extract by UV-Vis Spectroscopic

The primary bands were detected at absorption peaks, and the presence of flavonoids, phenols, saponins, terpenoids, and alkaloids in *C. virgata* Sw. extracts was indicated by the absorption bands [208 (0.628), 256 (2.268), 362 (1.083), 463 (0.436), and 622 (1.189)] obtained in the UV-Vis region [Figure 1]. The present study results were consistent with earlier research for alkaloids, flavonoids, terpenoids, and related glycosides by Rawat and Garg [19]. The current findings were also found to be compatible with the report of Yadav et al. [20]. The presence of various phytocompounds in *C. virgata* Sw. was also supported by the studies of Abbasi et al. [21] and Uddin et al. [22] using the leaf extracts of *Rhamnus virgata* and *Berberis balochistanica*.

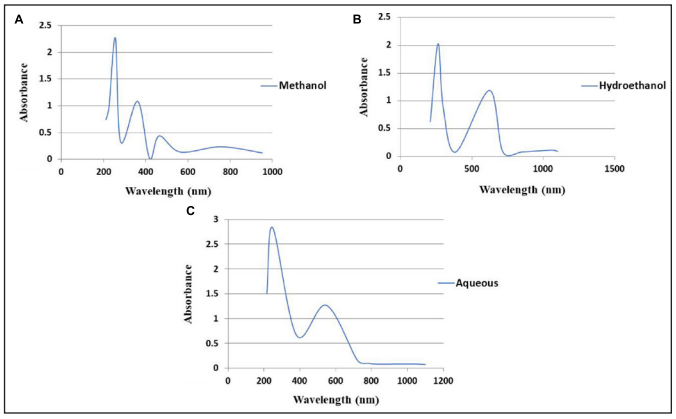

Figure 1: UV-Vis spectrum of *C. virgata* extracts. **(A)**. methanol; **(B)**. hydroethanol; and **(C)**. aqueous extract.

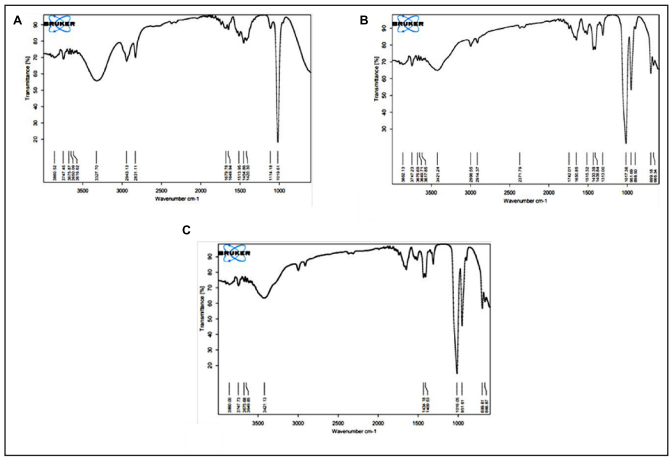

Figure 2: FTIR analysis of *C. virgata* extracts. **(A)**. methanol extract; **(B)**. hydroethanol; and **(C)**. aqueous extract.

3.2. Characterization of *C. virgata* Sw. Extracts by FTIR Spectroscopy

Functional groups found in *C. virgata* Sw. at 3,676 and 3,421 (alcohol); 3,747 (hydroxyl); 1,017, 2,831, 3,327, and 1,114 (carboxylic acid); 1,454 and 2,997 (alkene); 1,649 (amide); 3,420 (carbohydrates and proteins); and 1,019 (ether and esters) are confirmed by FTIR analysis as depicted in Figure 2. The identified phytochemicals are advantageous to medical fields, such as alkenes, which are used as a starting point for the production of alcohols, while aldehydes are crucial for the fruit ripening process. Carboxylic acids have potent antibacterial properties and are also effective in treating headaches, fever, pain, rheumatic joint aches, ulcers, and jaundice, while amides are helpful in protein synthesis. The present study and the one conducted by Pharmawati and Wrasiati [23] are extremely comparable.

3.3. Antimicrobial Assay

3.3.1. Antibacterial activity of C. virgata Sw. extracts

Antibacterial results revealed that all three extracts of the experimental plant had the potential to inhibit the growth of bacteria. The methanol extract had the greatest zone of inhibition at 400 µg/mL for *E. coli* and *B. subtilis*, and no zone of inhibition was observed at 50 µg/mL for either of the two hydroethanol and aqueous extracts. However, the extracts zones of inhibition against *E. coli* and *B. subtilis* at 10 µg/mL were somewhat lower than those of standard streptomycin [Table 1 and Figures 3 and 4]. In support of the present study, the effectiveness of different extracts of *Argemone mexicana* against various pathogenic

Table 5: Effect of *C. virgata* methanol extract on paw edema thickness of rats.

Groups	0 day	7th day	14th day	21st day
Normal control	5.33 ± 0.929	5.54 ± 0.413	5.64 ± 0.365	5.75 ± 0.406
Arthritic control	5.34 ± 0.181[b]	7.84 ± 0.089[a]	8.44 ± 0.181[b]	8.92 ± 0.130[c]
Standard Indomethacin	5.72 ± 0.192[d] (31%)	5.95 ± 0.238[c] (54%)	5.89 ± 0.083[b] (66%)	5.78 ± 0.114[a] (80%)
Methanol extract of *C. virgata* (200 mg/kg)	5.72 ± 0.192[d] (19%)	6.9 ± 0.122[c] (24%)	5.93 ± 0.238[b] (39%)	5.86 ± 0.223[a] (51%)
Methanol extract of *C. virgata* (300 mg/kg)	5.26 ± 0.270[d] (24%)	6.16 ± 0.089[c] (34%)	5.91 ± 0.151[b] (43%)	5.84 ± 0.192[a] (58%)
Methanol extract of *C. virgata* (400 mg/kg)	5.1 ± 0.141[d] (26%)	5.98 ± 0.238[c] (39%)	5.89 ± 0.187[b] (54%)	5.81 ± 0.1[a] (75%)

Notes: All the results are expressed as mean ± SD (n = 5). [a]$p < 0.05$ vs. normal control, [b]$p < 0.01$ vs. normal control, [c]$p < 0.05$ vs. arthritic control, and [d]$p < 0.01$ vs. arthritic control.

Table 6: Effect of *C. virgata* methanolic extract at three dosing levels and indomethacin on several parameters

Groups	Hb (g/deci L)	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Neutrophils (10^3/mm^3)	Lymphocytes (10^3/mm^3)	Platelets (lakhs/mm^3)
Normal control	14.44 ± 0.38	9.14 ± 0.66	7.97 ± 0.335	2.93 ± 0.24	5.24 ± 0.348	563.3 ± 9.07
Arthritic control	12.23 ± 0.016[a]	6.82 ± 0.013[d]	11.73 ± 0.0151[d]	6.83 ± 0.023[c]	10.41 ± 0.008[e]	821.6 ± 18.52[a]
Standard indomethacin	14.32 ± 0.008[e]	10.46 ± 0.0151[b]	6.54 ± 0.023[b]	3.74 ± 0.0240[a]	6.46 ± 0.0216[c]	625.4 ± 10.01[a]
Methanol extract (200 mg/kg)	12.82 ± 0.083[b]	7.58 ± 0.0678[c]	10.53 ± 0.0173[c]	6.17 ± 0.0070[b]	9.32 ± 0.032[d]	7.69 ± 17.10[a]
Methanol extract (300 mg/kg)	13.23 ± 0.015[c]	8.46 ± 0.1673[b]	6.26 ± 0.02[b]	9.75 ± 0.025[e]	5.55 ± 0.02[b]	714 ± 17.38[a]
Methanol extract (400 mg/kg)	14.03 ± 0.029[d]	9.72 ± 0.0192[a]	5.74 ± 0.015[a]	8.84 ± 0.016[d]	4.75 ± 0.013[a]	654 ± 20.69[a]

Notes: All the results are expressed as mean ± SD (n = 5). [a]$p < 0.05$ vs. normal control, [b]$p < 0.01$ vs. normal control, [c]$p < 0.05$ vs. arthritic control, and [d]$p < 0.01$ vs. arthritic control.

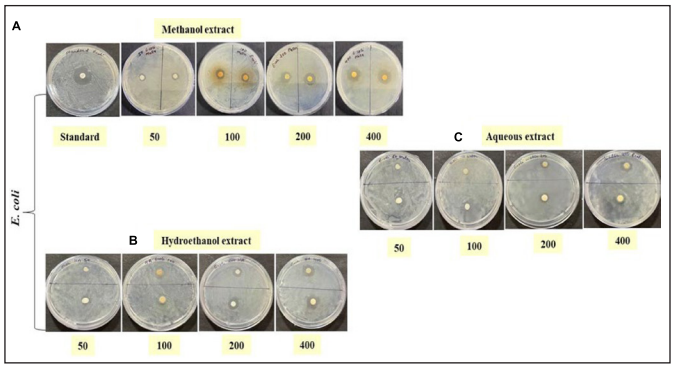

Figure 3: Antibacterial study of *C. virgata* against *E. coli*- **(A)**. standard (Streptomycin) and methanol, **(B)**. aqueous, and **(C)**. hydroethanol extract.

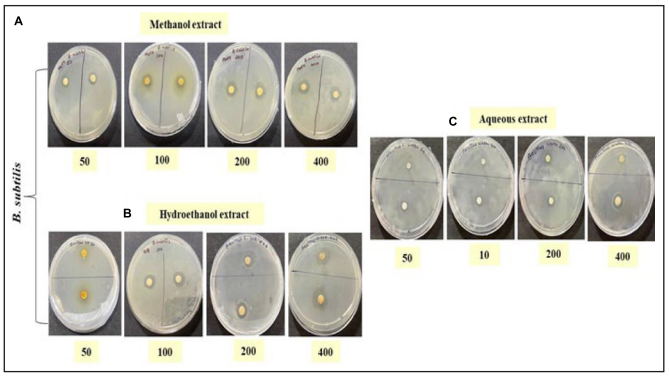

Figure 4: Antibacterial study of *C. virgata* against *B. subtilis*. **(A)**. methanol, **(B)**. aqueous, and **(C)**. hydroethanol extracts.

bacteria has also been investigated [24]. The conventional and green extracts of *Salvia sclarea* also represented the results in favor of the current study [25].

3.3.2. Antifungal activity of C. virgata Sw. extracts

In this screening investigation, it was found that *C. virgata* Sw. methanol and hydroethanol extracts inhibited *A. niger* at concentrations between 50 and 400 µg/mL, but the aqueous extract did not exhibit any zone of inhibition at 50 µg/mL concentration. All extracts had negligible inhibitory effects when compared to standard fluconazole at 10 µg/mL, although methanol and hydroethanol extracts exhibited a greater zone of inhibition against *A. niger* at 400 µg/mL [Table 2 and Figure 5]. Comparable studies were carried out by Farjana et al. [26] on the effectiveness of several leaf extracts against different pathogens that cause disease and found somewhere similar results to the present findings. Similarly, the results of Okla et al. [27] in determining the antifungal activity of various parts of *Avicennia marina* were in favor of the outcomes of the current findings.

3.4. *In-vitro* Protein Denaturation Assay

As with usual medication, all of the extracts of *C. virgata* Sw. shown measurable anti-arthritic efficacy. The methanol extract denaturized the protein most efficiently (72.58 ± 0.022%) at the maximum dose (2000 µg/mL), while hydro-ethanol and aqueous extracts showed 68.91 ± 0.016% and 60.11 ± 0.012% inhibition, respectively [Table 3]. The denaturation of methanol extract was somehow less effective in comparison to standard diclofenac sodium at the maximum dosage, but it is quite close to it. In light of this, Khandelwal [28] showed a substantial anti-arthritic impact of *Crossandra infundibuliformis* leaves, which was consistent with the findings of this study. Similarly,

Table 7: Effect of standard and methanol extract of *C. virgata* on various serological parameters.

Groups	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	44.13 ± 1.724[b]	57.76 ±1.569[a]	53.2 ± 1.708[b]
Arthritic control	144.71 ± 9.763[c]	113.0 ±25.78[c]	226.3 ± 25.71[c]
Standard indomethacin (10 mg/kg)	43.5 ± 7.844[a]	53.9 ± 10.34[a]	121.5 ± 38.79[a]
Methanol extract of *C. virgata* (200 mg/kg)	87.63 ± 16.08[c]	104.2 ± 12.49[c]	205.9 ± 26.53[b, c]
Methanol extract of *C. virgata* (300 mg/kg)	73.72 ± 10.92[b]	78.45 ± 9.783[b]	161.96 ± 11.86[b]
Methanol extract of *C. virgata* (400 mg/kg)	51.36 ± 9.113[a, b]	64.65 ± 10.011[a, b]	129.35 ± 13.53[a]

Notes: All the results are expressed as mean ± SD (n = 5). [a]$p < 0.05$ vs. normal control, [b]$p < 0.01$ vs. normal control, [c]$p < 0.05$ vs. arthritic control, and [d]$p < 0.01$ vs. arthritic control.

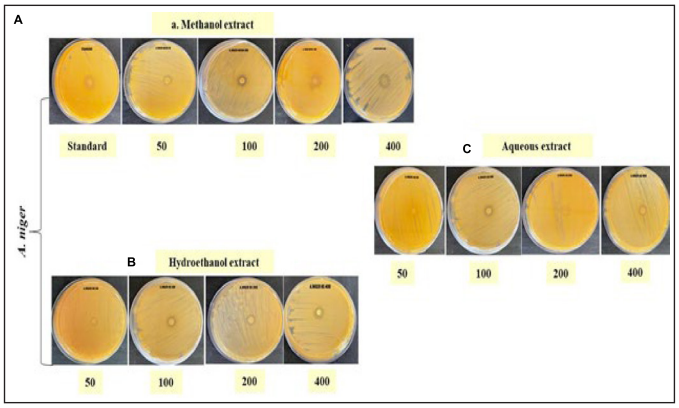

Figure 5: Antifungal activity of *C. virgata* against *A. niger*. **(A)**. standard (Fluconazole) and methanol, **(B)**. aqueous, and **(C)**. hydroethanol extracts.

Dharmadeva et al. [29] evaluated the *in-vitro* anti-inflammatory study of *Ficus racemosa* by using albumin denaturation methods, and the results obtained were quite comparable with the results of the current analysis.

3.5. *In-vivo* Anti-arthritic Analysis of *C. virgata* Sw. Methanol Extract

3.5.1. Acute toxicity study

Methanol extract dosages up to 2000 mg/kg b.w. do not exhibit any hazardous or fatal effects. Rat eyes, skin, salivation, diarrhea, and behavioral patterns were not altered in any way. The relative organ weight of the rat did not significantly change. As a result, treatment dosages of 200, 300, and 400 mg/kg b.w. of *C. virgata* Sw. methanol extract were used in the experiment.

3.5.2. Change in body weight

A substantial decrease in body weight was seen in the arthritic control rats after normal control and arthritic control rats were compared. Rats given all three dosages of *C. virgata* Sw. methanol extract and the standard treatment indomethacin experienced a significant ($p < 0.05$) increase in body weight compared to the rats in the arthritic control group [Table 4]. High, medium, and low dosages of the methanol extract of *C. virgata* Sw. as well as standard medication significantly ($p < 0.05$) increased body weight compared to the healthy control group [Table 4]. The methanol extract and conventional medication induced rise in body weight may be connected to the intestine's capacity to absorb nutrients. In a related study on *Tridax procumbens*, Petchi et al. [18] found that the arthritic control group displayed a substantial reduction in body weight compared to the control group. Similarly, the body weight was significantly lower in the arthritic rat, but during the last week of treatment with *Nigella sativa*, the weight of immunized rats tended to return to normal as observed in the present finding [30].

3.5.3. Effect on paw diameter

Seven days later, there was a significant increase ($p < 0.05$) in the paw diameter of the arthritic control group of rats in contrast to the healthy control group. While the regular indomethacin group of rats showed a decline up until the last day. In comparison to the control group of arthritic rats [Table 5], the methanol extract of *C. virgata* Sw. action demonstrated ($p < 0.05$) a reduction of paw diameter at low, medium, and high doses, respectively. The inhibition by *C. virgata* Sw. methanol extract obtained on day 21 of treatment was effective and equivalent to the standard. This work is supported by Sarkar and Rai [17] investigation on the anti-arthritic properties of *Trichosanthes dioica* hydroalcoholic extract and Yadav et al. [31] of *Heteropogon contortus* methanol extract. Similarly, Singh et al. [32] also supported the significant effect of the methanol extract of *Calotropis procera* in reducing paw edema on the 17th, 21st, 24th, and 28th days.

3.5.4. Effect on hematological parameters

The total number of WBC, neutrophils, lymphocytes, and platelets in the arthritic control group II was significantly ($p < 0.05$) higher than in the healthy normal control group I. Nevertheless, the groups treated with indomethacin and methanol extract (GpIII to GpVI; Table 6) had significantly ($p < 0.05$) lower counts of neutrophils, lymphocytes, platelets, and total white blood cells. In the current investigation, rats with arthritis Gp II had lower RBC and Hb counts than control Gp I rats. The methanol extract of *C. virgata* Sw. for 21 days significantly ($p < 0.05$) improved the hemoglobin (Hb) content and RBC count towards normal in comparison to the arthritic control. This suggests that the extract has potential applications in arthritic disorders and indicates a significant improvement in the anemic state. Conditions that call for a WBC count include infectious and inflammatory disorders [33]. In their investigation, Rahman et al. [34] found that an ethanolic extract of *Aquilaria agallocha* leaves restored WBC and hemoglobin levels to normal, hence preventing rheumatoid arthritis. Similarly, treatment with crocetin raised hemoglobin, and red blood cells, along with a decrease in white blood cell count [35].

3.5.5. Effect on serological parameters

ALP, AST, and ALT levels in all arthritic rats were significantly ($p < 0.05$) higher than those in the healthy control group. The enzyme levels were considerably ($p < 0.05$) lower in rats in Groups 3 (standard) and 6 (high dosage) than in rats in the arthritic control group, and just slightly lower in Groups 4 and 5. The standard group had the lowest levels of ALP, AST, and ALT, measuring 43.5 ± 7.844, 53.9 ± 10.34, and 121.5 ± 38.79 IU/L, respectively, as compared to the extract-treated groups (Gp IV to GP VI) [Table 7]. This demonstrates the anti-arthritic effectiveness of the therapy. Kamal et al. [36] found a notable and significant rise in the liver enzymes (ALP, AST, and ALT) with *Aloe thraskii* extract, which has protective benefits against rheumatoid arthritis. The findings of the current study are consistent with the other investigations determining the action of FCA-induced arthritis in rats along with its treatment using phytocompounds such as silibinin [37]. Similarly, treatment with *Nyctanthes arbor-tristis* restored the elevated levels of AST and ALT in arthritis-induced rats [38].

4. CONCLUSION

Findings concluded that the extracts of *C. virgata* Sw. under FTIR and UV spectroscopy revealed the presence of significant bioactive components that were found to be important contributors to its antibacterial and anti-arthritic activities. Treatment with methanolic extract at a dose of 400 mg/kg of *C. virgata* possesses potentially useful anti-arthritic activity since it gives a positive result in controlling inflammation in rats induced with FCA. It has reduced body weight, paw edema swelling, and decreased inflammatory cytokine levels. The study validated the promising anti-arthritic activity of *C. virgata* Sw.; however, more research is needed to standardize the plant extract before it can be considered a viable treatment option alongside conventional medicines.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

7. ETHICAL APPROVALS

The study protocol was approved by the Institutional Animal Ethics Committee of Bilwal Medchem and Research Laboratory Pvt. Ltd., Rajasthan (Approval No.: BMRL/AD/CPCSEA/ IAEC/2022/3/2).

8. CONFLICTS OF INTEREST

The authors declare no conflicts of interest associated with this study.

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This study is not supported by any funding agency.

10. DATA AVAILABILITY

Data will be available from the corresponding author on special request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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