narra j

Original Article

Identification and evaluation of antimicrobial and anti-arthritis activities of hydroethanolic extract of *Rubus ellipticus* leaves

Anjana Kumari[1], Vinit Prakash[2], Dinesh Gupta[3], Lokender Kashyap[4], Rajat Goyal[5], Hitesh Chopra[6], Rupesh K. Gautam[7*], Sandip Chakraborty[8], Deepak Chandran[9] and Kuldeep Dhama[10]

[1]Department of Applied Sciences and Humanities, Ambala College of Enginnering and Applied Research, Devsthali, Ambala, India; [2]Department of Applied Sciences, Global Group of Institutes, Amritsar, India; [3]Department of Silviculture and Forestry, University of Agriculture and Technology, Banda, India; [4]Department of Agriculture, Maharishi Markandeshwar (Deemed to be University), Ambala, India; [5]MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Ambala, India; [6]Chitkara College of Pharmacy, Chitkara University, Rajpura, India; [7]Indore Institute of Pharmacy, IIST Campus, Indore, India; [8]Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Agartala, India; [9]Department of Veterinary Sciences and Animal Husbandry, Amrita School of Agricultural Sciences, Amrita Vishwa Vidyapeetham University, Coimbatore, India; [10]Division of Pathology, ICAR-Indian Veterinary Research Institute, Bareilly, India

*Corresponding author: rupeshgautammmu@gmail.com

Abstract

Rubus ellipticus is a native plant to India's tropical and subtropical regions and has been used as a traditional medicinal. The aim of study was to identify and evaluate the antimicrobial and anti-arthritis activities of hydroethanolic extract of *R. ellipticus* leaves (HEERE). The leaves were collected from the Narkanda Valley, India and were shade-dried and finely ground to produce the powder. The hydroethanolic extract was utilized for phytochemical analysis to determine the existence of carbohydrate, phenolic, terpenoid, flavonoid, saponin, glycoside, tannin, protein, and alkaloid. The HEERE was futher analyzed by gas chromatography-mass spectrometry (GC-MS) for the characterization of the phytoconstituents. The antimicrobial activity was tested against *Escherichia coli*, *Staphylococcus aureus* as well as *Aspergillus niger*. To assess its anti-arthritic activities, different doses of HEERE were given orally to complete Freund's adjuvant (CFA)-induced albino Wistar rats for twenty-one days. The GC-MS analysis of hydroethanolic extracts from leaves detected and identified the presence of 33 phytochemical compounds. HEERE showed significant effects against *E. coli*, *S. aureus*, and *A. niger* strains at 600 ppm. Our data indicated that HEERE 200 mg/kg was more effective than 50 mg/kg as anti-arthritis. Paw volume, ankle-joint diameter, the number leucocytes, and erythrocyte sedimentation rate (ESR) were all significantly reduced in experimental rats. Furthermore, when compared to respective standard drugs, the body weight, erythrocyte, hemoglobin, and synobium healing effect have all improved. These data demonstrated the potential of *R. ellipticus* for the long-term investigation of antimicrobial and anti-arthritic properties.

Keywords: *Rubus ellipticus*, phytochemical, GC-MS, antimicrobial, anti-arthritis

https://narraj.org/

Introduction

While chemical-based synthetic medications are approached cautiously due to their adverse effects, traditional herbs are gaining attention for being naturally derived, environmentally friendly, and minimal adverse effects. Consequently, despite the advantages and disadvantages

Received: April 19, 2023 - Accepted: September 19, 2023 - Published online: December 22, 2023

of modern synthetic medications, people increasingly opt for plant-based herbal remedies [1]. Medicinal plants, such as traditional herbs, contain a high concentration of bioactive compounds, which have been linked to several therapeutic potentials such as antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [2-6].

The increasing utilization of bioactive derivatives for managing various diseases necessitates a critical examination of their perspective. Medicinal plants are a complex blend of various phytochemicals that are typically derived from crude plant extracts [7]. Many species of plants contain a large number of bioactive compounds, however, only a small fraction of them have been studied and proven to significantly contribute to bioactive mediators. The usage of the appropriate methodology of screening is crucial in the discovery of novel compounds [8]. Extraction and characterization of numerous bioactive compounds from various medicinal plants have led to the development of stable medicinal agents with higher therapeutic profiles [9]. Gas chromatography-mass spectrometry (GC-MS) techniques have been employed in the initial screening of herbal plants, assisting in the identification of bioactive compounds found in the plants [10,11].

The Rosaceae family contains around 750 species and has primarily been employed as traditional medicinal, and ornamental plants in various Asian regions. *Rubus ellipticus* (*R. ellipticus*), belonging to the Rosaceae berry plant family, is a plant species native to India's tropical and subtropical regions, exhibiting potential antimicrobial and anti-arthritic properties [12,13,14]. The aim of this study was to identify and evaluate the antimicrobial and anti-arthritis activities of the hydroethanolic extract of *R. ellipticus* leaves (HEERE).

Methods

Plant material

R. ellipticus leaves were collected in the Narkanda Valley, India, in August 2020 and were identified by the Himachal Pradesh State Biodiversity Board, Shimla, India, under the specimen number HPSBB/3055, and the specimen was finally acquiesced at the Biodiversity Board in Herbarium Department, Shimla, India.

Extraction of *R. ellipticus* leaves

R. ellipticus leaves were shade-dried and finely ground to produce a powder of 800 grams by utilizing hydroethanolic (4:6) via a Soxhlet extractor for around 72 hours, following the standard protocol [15]. Afterward, the extract was concentrated with a rotary evaporator using Rotavapor (BUCHI, Flawil, Switzerland) and kept in a glass vial sealed with a Teflon-coated cap at 4°C in the refrigerator for further investigation.

Photochemical analysis

The phytochemical analysis of HEERE was performed following the standard procedures outlined in previous studies [3,15]. The existence of carbohydrates, phenolic, terpenoids, flavonoids, saponins, glycosides, tannins, proteins, and alkaloids was determined in the crude extract.

Gas chromatography-mass spectrometry (GC-MS)

The Agilent 7890B gas chromatograph and Agilent 5977B mass detector (Agilent Tech, California, USA) were employed for the GC-MS analysis. The equipment was equipped with an auto-injector and a fused silica capillary column. Samples were injected at a split ratio of 50:1, with helium serving as the carrier gas at a rate value of 1 ml/minute. The injector's temperature was fixed to 280°C. The temperature of the oven was primarily set at 80°C for 2 minutes, then changed to 220°C at 10°C/minute with no hold and augmented to 310°C at 20°C/min held for 10 minutes and with a solvent delay of five minutes. The flow rate of the column was set at 1 ml/minute. The chromatography of a single sample took 30 minutes. The following parameters were set for the mass spectrometer's operation, namely ion source temperature of 230°C, electron energy (70eV), MS Quad temperature of 150°C, and scanning range of m/z, 25–1000 amu. Matching fragmentation patterns and library searches were used to identify the metabolites.

Antimicrobial activity evaluation

The well-diffusion method was used to assess antimicrobial activity [16]. The Microbial Type Culture Collection (MTCC) from the Institute of Microbial Technology, Chandigarh, India, provided two bacterial strains of *Escherichia coli* (MTCC 452) and *Staphylococcus aureus* (MTCC 737), and one fungal strain of *Aspergillus niger* (MTCC 1344). These strains were kept in the freezer at 4°C until they were utilized. Potato dextrose agar (PDA) and nutrient agar (NA) were used in the study. The bacterial inoculum was made by growing the freeze-dried cells in NA for 24 hours at 37°C, and the fungal strain was made by growing freeze-dried cells in PDA for 72 hours at 30°C. The test organisms were inoculated on a solidified agar plate with a micropipette and dried for about 10 minutes. A sterilized steel borer was utilized to make the 4-wells in each Petri dish containing agar and then filled with the test compound and standard solutions. The solvent used for the sample preparation was dimethyl sulfoxide (DMSO). The test sample was prepared in DMSO at various concentrations (i.e., 200, 400, and 600 ppm). Ciprofloxacin and fluconazole (both from the Central Drug House Pvt. Ltd., India) were used as positive controls, respectively for bacteria and fungus. The negative control was DMSO, and positive reference standards were ciprofloxacin/fluconazole at 100 ppm for all selected strains.

For the microbial assay, all work was done under aseptic conditions. The bacteria plates underwent incubation at 37±1°C for 24 hours, whereas the fungal plates underwent incubation at 30±1°C for 72 hours. The anti-microbial potential of the test compounds was calculated using the average diameter of the zone of inhibition surrounding the wells in millimeters (mm). Each antimicrobial activity was replicated three times, and the standard deviation of the bacterial and fungal growths corresponding to a specific sample was reported.

Animal preparation and oral acute toxicity test

Albino rats (Wistar strain) of *Rattus norvegicus* from both genders, with a weight range of 200–250 grams, were employed in the study as test samples and housed in standard temperature conditions (24–28°C), and in a relatively humid environment (60–70%) with a 12:12 light and dark cycle. The animals were fed a standard pellet diet and an unlimited supply of water. The protocol was approved by the Institute of Animal Ethics Committee of MM College of Pharmacy (deemed to be University), Maharishi Markandeshwar, India, following the guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPSCEA).

The test samples of various doses (50, 100, and 200 mg/kg) of HEERE were dissolved in 0.2% w/v carboxy methyl cellulose and 2% Tween 80. Methotrexate (0.5 mg/kg) was also given orally as a standard drug in the form of a suspension [17]. The rats were divided into six groups with six rats from each group. Group I: normally operated + normal saline (0.9% normal saline orally); Group II: complete Freund's adjuvant (CFA) 0.1 ml, injected subcutaneously; Group III: CFA + methotrexate (0.5 mg/kg, orally); Group IV: CFA + HEERE (50 mg/kg body weight, once a day, orally); Group V: CFA + HEERE (100 mg/kg body weight, once a day, orally) and Group VI: CFA + HEERE (200 mg/kg body weight, once a day, orally). Group I acted as the negative control, while Group II served as the arthritic control.

The Organization for Economic Cooperation and Development (OECD) guideline 42518 was employed to test acute oral toxicity in the rats. The rats fasted for 16 hours before the experiment and had unlimited access to water. Six groups of rats were given the test samples orally at doses of 50 mg/kg, 500 mg/kg, and 2,000 mg/kg, respectively. The rats were continuously observed for 3 hours to detect any behavioral changes and for 7 days to look for signs of critical and short-term toxicological indications such as coma, respiratory depression, diarrhea, salivation, convulsion, and perspiration [18].

Anti-arthritis activity evaluation

The condition of arthritis was induced in all groups of rats via subcutaneous injection of 0.1 ml (0.1% w/v) suspension of killed *M. tuberculosis* bacteria, homogenized in the liquid paraffin into the left hind paw (except the normal control group). Intraperitoneal injection of thiopentone sodium (40 mg/kg) [18] was used to anesthetize the rats. The drug treatment began on the 1st day and lasted until the twenty-first. The paw volume was measured by a digital plethysmometer Model 7140 (UGO Basile, Gemonio, Italy) on the first, seventh, fourteenth, and twenty-first days.

An anti-inflammatory response was defined as the percentage inhibition of edema as that of normal control. Methotrexate (0.5 mg/kg, once daily) [18] was used as a controlled drug. The ankle-joint diameter and body weight were measured using a verniercaliper and a digital weighing balance AUX220 (Shimadzu, Kyoto, Japan) on the first, sixth, eleventh, sixteenth, and twenty-first days [19].

For the biochemical evaluation, the rats with were anesthetized with thiopentone sodium (60 mg/kg) and sacrificed at the end of the protocol. The blood samples were collected from each rat by retro-orbital plexus puncture and placed in vials containing ethylenediaminetetraacetic acid (EDTA) for biochemical analysis such as red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and erythrocyte sedimentation rate (ESR) count [19].

After the sacrifice, the rat's knee joints were separated and well-preserved in 10% formalin for histopathology examination of the synovium. After the fixation and decalcification, the sample was cut into 45 m pieces and provided staining with eosin and hematoxylin for microscopic estimation [20,21].

Statistical analysis

The data were accessible as the mean standard deviation of the mean (SEM). ANOVA test was utilized for comparison of the statistical significance of the means, followed by Tukey's multiple comparison tests. The $p<0.05$ was considered to be statistically significant. All statistical analysis was performed on SPSS version 20 (SPPS, IBM Inc, New York, US).

Results

Phytochemical analysis

The existence of flavonoids, tannins, phenols, carbohydrates, saponins, terpenoids, phenolic compounds, proteins, oil, and fats was observed in the phytochemical analysis of the HEERE (**Table 1**). Phytochemicals of alkaloids and glycosides were not found.

Table 1. Phytochemical constituents of HEERE

Phytochemical compounds	Chemical tests	Hydroethanolic extract
Alkaloids	Dragendorff's test	---
	Wagner's test	---
Glycosides	Legal's test	---
	Keller-Killiani test	---
Flavonoids	Shinoda's test	+++
	Alkaloid's test	+++
Tannins and Phenols	Ferric chloride test	+++
Carbohydrates	Fehling's test	--+
	Molisch's test	--+
Protein	Ninhydrin test	-++
	Biuret's test	-++
Terpenoids	Hager's test	-++
Saponins	Froth test	+++
Oil and fats	Saponification test	---

---: Absent; --+: slightly present; -++: moderately present; +++: intensely present

Gas chromatography-mass spectrometry (GC-MS) analysis

A total of 33 phhytochemical constituents were found in HEERE. The most prominent compound found in the HEERE were gallic acid (13.69%), protocatechuicacid (10.12%), quercetin (6.09%), glyceric acid (5.10%), malic acid (4.19%), 4-coumaric acid (3.91%), 2-butene-dioic acid (3.88), erythritol (3.82%), galactaric acid (3.55%), and palmitic acid (3.39%) (**Table 2**).

Table 2. Gas chromatography-mass spectrometry (GC-MS) analysis of HEERE

Peak no	Retention time (minute)	Compound name	Area (%)	Molecular weight (ug)
1	5.778	1,3-Propanediol	0.30	76
2	6.084	Glycolic acid	0.27	76
3	8.381	4-Hydroxybutanoic acid	0.15	104
4	8.592	Benzoic acid	0.12	122

Peak no	Retention time (minute)	Compound name	Area (%)	Molecular weight (ug)
5	8.865	Glycerol	4.27	92
6	9.22	L-Proline	3.19	115
7	9.476	Butanedioic acid	0.80	118
8	9.643	Glyceric acid	5.10	106
9	9.966	2-Butenedioic acid	3.88	144
10	10.127	Pipecolic acid	0.67	129
11	10.956	β-Alanine	0.22	89
12	11.389	Decanoic acid	0.17	172
13	11.495	Citramalic acid	0.11	148
14	11.723	Malic acid	4.89	134
15	11.834	Erythritol	4.82	122
16	12.118	Aspartic acid	0.42	133
17	12.157	5-Oxoproline	0.69	129
18	12.363	2,3,4-Trihydroxybutyric acid	0.71	136
19	13.481	Levoglucosan	3.51	161
20	13.731	Dodecanoic acid	0.09	200
21	14.415	Arabitol	2.86	152
22	15.505	Citric acid	4.62	192
23	15.544	Protocatechoic acid	10.12	154
24	16.823	4-Coumaric acid	3.91	164
25	16.923	Gallic acid	13.69	170
26	17.663	Galactaric acid	3.55	210
27	17.78	Palmitic Acid	3.39	256
28	18.047	Myo-Inositol	0.66	180
29	18.441	Caffeic acid	0.42	180
30	19.142	Stearic acid	0.69	284
31	20.838	1-Monopalmitin	0.77	330
32	21.628	Glycerol monostearate	0.54	358
33	23.569	Quercetin	6.09	302

Antimicrobial activity

All tested microorganisms were inhibited by the HEERE, with the zone of inhibition ranging from 1.9±0.2 to 8.0±0.15 mm for bacterial strains and 1.4±0.4 to 4.5±0.1 mm for fungal strains. The HEERE was more effective againts *S. aureus* (8.0±0.15 mm) than *E. coli* (6.4±0.4mm) at 600 ppm concentration. While at the same concentration, a maximum zone of inhibition 4.5±0.1mm was observed for the fungal strain (*A. niger*) (**Table 3**).

Table 3. Antimicrobial activity of HEERE using well-diffusion method

Samples	HEERE doses (ppm)	Zone of inhibition Mean±SD (mm)
E. coli	200	1.9±0.2
	400	3.1±0.11
	600	6.4±0.4
S. aureus	200	2.8±0.19
	400	4.2±0.4
	600	8.0±0.15
A. niger	200	1.4±0.2
	400	2.9±0.31
	600	4.5±0.1
Ciprofloxacin	100	20.8±0.1
Fluconazole	100	18.3±0.1

Anti-arthritis activity

Acute toxicity studies have demonstrated that the HEERE was risk-free. The treated rats appeared normal, with no significant changes in behavioral or neurological responses up to 2,000 mg/kg body weight of HEERE. Similarly, no change in body weight, adaptation, mortality, or toxicity reaction was observed at any of the doses until the study's conclusion. As a result, therapeutic doses of *R. ellipticus* of 50, 100, and 200 mg/kg were chosen. In the chronic CFA model, swelling and redness were observed in the injected paw after 24 hours. From the seventh day until the end of the study, the methotrexate-treated group had a decrease in paw volume. All HEERE doses of 50, 100, and 200 mg/kg decreased paw volume and thickness throughout the 21st day of the study. The 200 mg/kg dose of HEERE outperformed the 100 and 50 mg/kg doses

and was almost identical to methotrexate. From the first day to the end of the research investigation, the CFA-induced paw volume gradually increases (**Table 4** and **Table 5**).

Table 4. Effect of HEERE on paw thickness

Groups	0 day	1st day	6th day	11th day	16th day	21st day
Negative control	0.40± 0.05	0.40± 0.03	0.45± 0.08	0.41± 0.06***	0.42± 0.05***	0.40± 0.09***
Arthritic control (CFA)	0.42± 0.018	0.78± 0.031	1.64± 0.84	1.89± 0.36	2.07± 0.15	2.26± 0.09
CFA+methotrexate 0.5mg/kg)	0.36± 0.05	0.80± 0.04	1.89± 0.53	1.57± 0.80**	0.86± 0.11***	0.47± 0.19***
CFA+HEERE 50 mg/kg	0.34± 0.06	0.82± 0.14	2.36± 0.53	2.08± 0.22	1.89± 0.15	1.66± 0.91*
CFA+HEERE 100 mg/kg	0.36± 0.10	0.81± 0.03	2.22± 0.80	1.83± 0.16	1.54± 0.13**	1.29± 0.11***
CFA+HEERE 200 mg/kg	0.37± 0.03	0.80± 0.37	2.13± 0.50	1.67± 0.20*	1.27± 0.11***	0.85± 0.05***

Data was presented in mean±SD and analyzed by one-way ANOVA which is followed by Tukey's multiple comparison test. All treated groups and the normal group were compared with the Arthritic control group.
* Statistically significant at $p<0.05$
** Statistically significant at $p<0.01$
*** Statistically significant at $p<0.001$

Table 5. Effect of HEERE on paw volume

Groups	0 day	1st day	7th day	14th day	21st day
Negative control	1.83±0.05	1.81±0.08	1.79±0.79***	1.82±0.55***	1.14±0.74***
Arthritic control (CFA)	1.84±0.07	1.99±0.05	2.29±0.11	2.46±0.87	2.62±0.89
CFA + methotrexate 0.5mg/kg	1.85±0.06	2.14±0.12	1.90±0.32*	1.70±0.43**	1.21±0.07***
CFA + HEERE 50 mg/kg	1.86±0.01	2.34±0.13	2.22±0.45ns	2.16±0.39ns	2.06±0.48**
CFA+HEERE 100 mg/kg	1.87±0.05	2.23±0.15	2.17±0.90ns	2.13±0.80ns	2.01±0.16**
CFA+HEERE 200 mg/kg	1.86±0.04	2.21±0.12	2.14±0.38 ns	1.90±0.63*	1.32±0.24***

Data was presented in mean±SD and analyzed by one-way ANOVA which is followed by Tukey's multiple comparison test. All treated groups and the normal group were compared with the arthritic control group.
* Statistically significant at $p<0.05$
** Statistically significant at $p<0.01$
*** Statistically significant at $p<0.001$

The WBC and ESR levels increased in the arthritic control group, while Hb and RBC levels decreased. In hematological analysis, all HEERE doses (50, 100, and 200 mg/kg) exhibited significant results ($p<0.05$, $p<0.01$, and $p<0.001$). The HEERE at 200 mg/kg produced the best results, with lower WBC and ESR levels and higher Hb and RBC levels (**Table 6**).

Table 6. Effect of HEERE on hematology parameters

Groups	Hb (g/dl)	WBC (10^9/L)	RBC (million/µl)	ESR (mm)
Negative Control	14.18±0.51***	8.46±0.59***	6.88±0.11***	3.29±0.47***
Arthritic Control (CFA)	7.79±0.42	14.96±0.49	3.06±0.58	10.23±0.15
CFA+Standard (Methotrexate 0.5mg/kg)	13.30±0.54***	8.09±0.72***	6.97±0.49***	3.65±0.10***
CFA+HEERE 50 mg/kg	9.55±0.10 ns	13.03±0.52 ns	4.92±0.43 ns	8.26±0.70**
CFA+HEERE 100 mg/kg	11.03±0.75*	12.84±0.43**	5.46±0.84*	7.74±0.17***
CFA+HEERE 200 mg/kg	12.55±0.82**	9.93±0.70***	6.31±0.17**	5.48±0.38***

Data was articulated in mean±SD and analyzed by one-way ANOVA which is followed by Tukey's multiple comparison test. All treated groups and the normal group were compared with the arthritic control group.
* Statistically significant at $p<0.05$
** Statistically significant at $p<0.01$
*** Statistically significant at $p<0.001$

Histopathology

Histopathology of the ankle joint of negative control and arthritic control rats exposed intact synovium and synovial lining morphology, without inflammation, and the influx of inflammatory

cells. HEERE at 200 mg/kg inhibited all histological findings of arthritis significantly more than at 100 and 50 mg/kg (**Figure 1**).

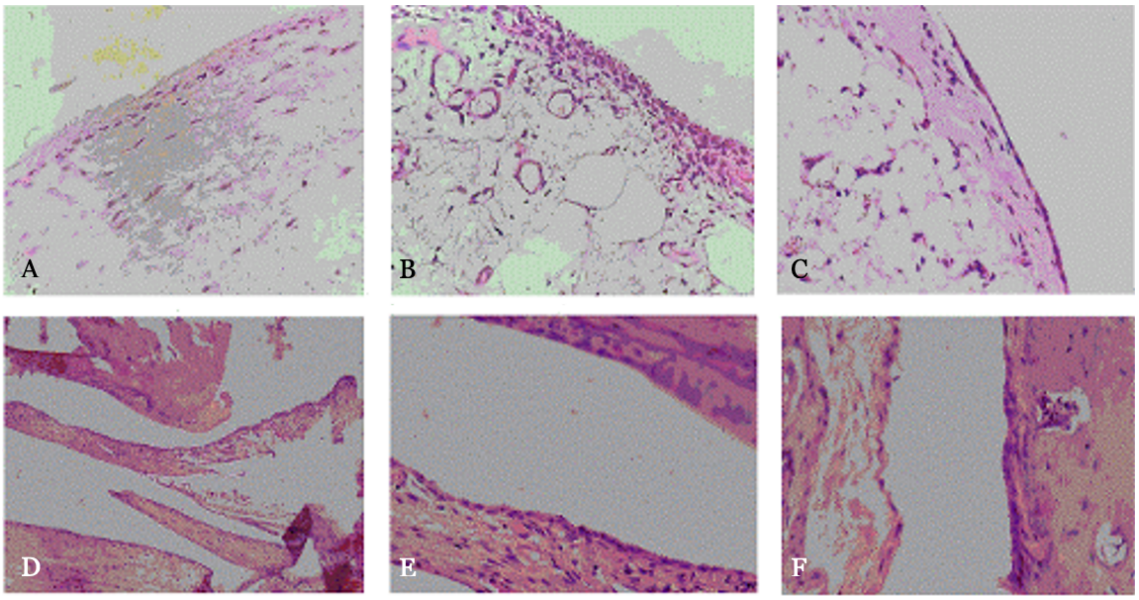

Figure 1. Histopathology of ankle-joint of Wistar rats (A) Group I: normally operated + normal saline; (B) Group II: Complete Freund's adjuvant (CFA) 0.1 ml, injected subcutaneously); (C) Group III: CFA + methotrexate (0.5 mg/kg, orally) standard; (D) Group IV: CFA + HEERE (50 mg/kg body weight, once a day, orally); (E) Group V: CFA + HEERE (100 mg/kg body weight, once a day, orally); and (F) CFA+ HEERE (200 mg/kg body weight, once a day, orally. Original magnification 40×, DXIT 1200, Nikon, Japan.

Discussion

The present study investigated phytochemical constituents and in-vivo anti arthritis activity of HEERE. Phytochemical analysis was done by the GC-MS. Several studies confirmed similar phytochemical constituents found in *R. ellipticus* [17,18]. In line the study's findings, thirty-three phytochemical constutuents were identified in *R. ellipticus* leaves by GC-MS analysis in previous studies [22,23,24]. The HEERE was found to have the highest levels of gallic acid, protocatechoic acid, and quercetin in our study. However, these constituents have already been reported from the genus *Rubus* by numerous studies [12,22-26].

The HEERE have shown antimicrobial activities against *E. coli, S. aureus*, and *A. niger*. The maximum zone of inhabitation was observed in *S. aureus*, rather than *E. coli*, and *A. niger* due to phenols and flavonoids as a major constituent Phenols and flavonoids are responsible for the destruction of the membrane of microorganisms first and making the cells more delicate. Resulted in impairment of proton pumps and loss of H^+- ATPase in damaged membranes can disrupt the normal cellular function of the microorganism ultimately lead to cell death. The antimicrobial activity findings of this study also aligns with several other prior studies [17,18,27]. Several herbal plants deliver relief from the various maladies and symptoms that are similar to those provided by traditional medicinal agents [28]. The CFA or Adjuvant induced arthritis in a tested rat model was chosen for the study because it is a common and widely employed animal model for inflammation and arthritis [29]. Reduced paw swelling is the most important factor in determining the degree of inflammation, and activity of anti-inflammatory and anti-arthritic drugs [30, 31]. It is also one of the simplest, most sensitive, and fastest methods.The decrease in paw thickness is thought to be due to a decline in the release of inflammatory mediators, and it is evidence of any drug's anti-inflammatory and anti-arthritic action in CFA-induced arthritis [32].

R. ellipticus is commonly employed in traditional medicines to treat inflammation [33]. As a result, the current study has been confirming the efficacy of *R. ellipticus* in reducing paw swelling as well as regulating the synobium cell line. Previous research has confirmed that the phenolic, flavonoids [34-36] have a broad range of anti-arthritic activity due to the presence of

gallic acid, procatechoic acid, p-coumaric acid, Quercetin, and Kaempferol,which is similar to our findings [37-39].

Conclusion

A total of 33 phytochemical constituents were found in the hydroethanolic extract of *R.ellipticus* leaves. The HEERE demonstrated potential antimicrobial properties with the zone of inhibition ranging from 1.9±0.2 to 8.0±0.15 mm for bacterial strains and 1.4±0.4 to 4.5±0.1 mm for fungal strains. The acute toxicity studies have demonstrated that the HEERE was risk-free with no significant changes in behavioral or neurological responses up to 2,000 mg/kg body weight of HEERE. All HEERE doses of 50, 100, and 200 mg/kg decreased paw volume and thickness throughout the study. The 200 mg/kg dose of HEERE was almost identical to methotrexate for anti-anthritis activity.

Ethical approval

The protocol was approved by the Institute of Animal Ethics Committee (MMCP-IAEC-85) and the guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPSCEA) were followed.

Competing interests

The authors declare that there is no conflict of interest.

Acknowledgments

All authors are thankful to their organizations for their full support during the research work.

Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

How to cite

Kumari A, Prakash V, Gupta D, *et al.* Identification and evaluation of antimicrobial and anti-arthritis activities of hydroethanolic extract of *Rubus ellipticus* leaves. Narra J 2023; 3 (3): e152 - http://doi.org/10.52225/narra.v3i3.152.

References

1. Konappa N, Udayashankar AC, Krishnamurthy S, *et al.* GC-MS analysis of phytoconstituents from Amomumnilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. Sci Rep 2020;10(1):1-23.
2. Prakash V, Kumari A, Kaur H, *et al.* Chemical constituents and biological activities of genus picrorhiza: An update. Indian J Pharm Sci2020;82(4):562-577.
3. Prakash V, Kumari A, Kaur H, *et al.* Green synthesis, characterization and antimicrobial activities of copper nanoparticles from the rhizomes extract of Picrorhizakurroa. Pharm Nanotechnol 2021;9(4):298-306.
4. Prakash V, Kumari A, Kaur H, *et al.* Chemical composition and anti-microbial activity of essential oil from aerial parts of *Origanum vulgare* L. from North-Western Himalayas. J Essent Oil-Bear Plants 2021;24 (2):177-185.
5. Prakash V, Kaur H, Kumari A, *et al.* Phytochemicals and biological studies on cycas revolute thunb.: A review. Adv Trad Med 2021;21(3):389-404.
6. Malongane F, McGaw LJ, Mudau FN. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: A review. J Sci Food Agri 2017;97:4679-4689.
7. Pandey MM, Rastogi S, Rawat AK. Indian herbal drug for general healthcare: An overview. Internet J Altern Med 2008;6:1-4.

8. Keskes H, Belhadj S, Jlail L, *et al*. LC-MS-MS and GC-MS analyses of biologically active extracts and fractions from *Tunisian Juniperusphoeniceleaves*. PharmBiol 2017;55:88-95.
9. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri District (MP). Int J Life SciSci Res 2017;3:844-847.
10. Satapute P,Murali KP, Kurjogi M, *et al*. Physiological adaptation and spectral annotation of arsenic and cadmium heavy metal-resistant and susceptible strain Pseudomonas taiwanensis. EnvironPollut 2019;251:555-563.
11. Juszczak AM, Zovko-Koncic M, Tomczyk M. Recent trends in the application of chromatographic techniques in the analysis of Luteolin and its derivatives. Biomol 2019;9:731.
12. Badhani A, Rawat S, Bhatt ID, *et al*. Variation in chemical constituents and antioxidant activity in yellow himalayan (*Rubus ellipticus* Smith) and hill raspberry (RubusniveusThunb.). J Food Biochem 2015;39(6):663-672.
13. Vadivelan R, Bhadra S, Ravi AVS, *et al*. Evaluation of anti-inflammatory and membrane stabilizing property of ethanol root extract of *Rubus ellipticus* Smith in Albino rats. J NatRemedies 2009;9(1):74-78.
14. Graham J, Woodhead M. Rubus, in wild crop relatives: Genetic, genomic and breeding resources, C. Kole, Ed. 2011;6:179-187.
15. Kaur H, Kumari A, Kumar M, *et al*. Phytochemicals analysis of sarcotesta layer of *Cycas revolutea Thunb*. Fruit through GC-MS. Int J Adv Sci2020;29:5111-5118.
16. Sethi P, Khare R, Choudhary R. Complexes of pyrimidine thiones: Mechanochemical synthesis and biological evaluation. Asian J Chem2020;32(10):2594-600.
17. Ringmichon CL, Gopalkrishnan B, Dixit AP. Ethno-pharmacognostical studies on root bark of *Rubus ellipticus* Smith. from Manipur. Int J Pharmacogn Phytochem 2013;2(2):223-228.
18. Prasanth SC, Chandran P. Phytochemical and antimicrobial analysis of leaf samples of different Rubus species. Intl J Chem Tech Res 2017;10(4):359-368.
19. Kumar S, Bhatia M, Garg LN, *et al*. Acute and chronic inflammation studies of *Strobilanthes callosus* leaves extract on rat model. Inflammo pharmacology 2013;21(3):233-239.
20. Mbiantcha M, Almas J, Shabana SU, *et al*. Anti-arthritic property of crude extracts of *Piptadeniastrum africanum* (Mimosaceae) in complete Freund's adjuvant-induced arthritis in rats. BMC Complement Altern Med 2017;17(1):111.
21. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2001;1(2):87-93.
22. Krauze-Baranowska M, Głod D, Kula M, *et al*. Chemical composition and biological activity of Rubusidaeus shoots–a traditional herbal remedy of Eastern Europe. BMC Complement Altern Med 2014;14:1-12.
23. Tallini LR, Pedrazza GP, de Bordignon SA, *et al*. Analysis of flavonoids in Rubuserythrocladus and *Morus nigra* leaves extracts by liquid chromatography and capillary electrophoresis. Rev BrasFarmacogn 2015;25:219-227.
24. Muniyandi K, George E, Sathyanarayanan S, *et al*. Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activities of Rubus fruits from Western Ghats, India. Food Sci Hum Wellness 2019;8:73-81.
25. Belwal T, Pandey A, Bhatt ID, *et al*. Trends of polyphenolics and anthocyanins accumulation along ripening stages of wild edible fruits of Indian Himalayan region. Sci Rep 2019;9:1-11.
26. Sparzak B, Merino-Arevalo M, Vander Heyden Y, *et al*. HPLC analysis of polyphenols in the fruits of Rubusidaeus L. (Rosaceae) Nat Prod Res2010;24:1811-1822.
27. Latha R, Sarkar T, Jansi RS, *et al*. Evaluation of antimicrobial efficiency and alpha-glucosidase inhibition of *Rubus ellipticus smith*. Leaf extracts and its phytochemical analysis. Asian J Pharm Clin Res 2015;8(2):422-426.
28. Verpoorte R. Exploration of nature's chemodiversity: The role of secondary metabolites as leads in drug development. Drug Discov 2019;3:232-238.
29. Arulmozhi S, Mazumder PM, Sathiyanarayanan L, *et al*. Anti-arthritic and antioxidant activity of leaves of Alstoniascholaris Linn. R.Br. Eur J Integr Med 2011;3:83-90.
30. Patil KS, Suryavanshi J. Effect of *Celastrus paniculatus* Willd. seed on adjuvant induced arthritis in rats. Pharmacogn Mag 2007;3(11):177.
31. Ekambaram S, Perumal SS, Subramanian V. Evaluation of antiarthritic activity of *Strychnos potatorum* Linn seeds in Freund 's adjuvant induced arthritic rat model. BMC Complement. Altern Med 2010;10:56.
32. Paval J, Kaitheri S, Potu B, *et al*. Comparing the anti-arthritic activities of the plants Justiciagendarussaburm F. and Withania somniferalinn. Int J Green Pharm2009;3:281.
33. Pandey Y, Bhatt SS. Overview of Himalayan yellow raspberry (*Rubus ellipticus* Smith.): A nutraceutical plant. J Nat ApplSci 2016;8(1):494-499.

34. Elisha IL, Dzoyem JP, McGaw LJ, *et al*. The anti-arthritic, anti-inflammatory, antioxidant activity and relationships with total phenolics and total flavonoids of nine South African plants used traditionally to treat arthritis. BMC ComplemAlter Med2016;16(1):1-10.
35. Shokry AA, El-Shiekh RA, Kamel G, *et al*. Anti-arthritic activity of the flavonoids fraction of ivy leaves (*Hedera helix* L.) standardized extract in adjuvant induced arthritis model in rats in relation to its metabolite profile using LC/MS. BiomedPharmacother 2022;145:112456.
36. Ticona LA, Serban AM, Ticona DA, *et al*. Anti-inflammatory and anti-arthritic activities of aqueous extract and flavonoids from *Tripodanthus acutifolius* leaves in mice paw oedema. Planta Medica International Open 2021;8(2):e43-55.
37. Jean-Gilles D, Li L, Ma H, *et al*. Anti-inflammatory effects of polyphenolic-enriched red raspberry extract in an antigen-induced arthritis rat model. J Agric Food Chem2012;60(23):5755-5762.
38. Kroes BV, Van den Berg AJ, Van Ufford HQ, *et al*. Anti-inflammatory activity of gallic acid. Planta Med 1992;58(06):499-504.
39. Saleem A, Saleem M, Akhtar MF. Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: An ethnomedicinal plant of Moringaceae family. S Afr J Bot2020;128:246-256.