Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

"IN VIVO ANTI-INFLAMMATORY POTENTIAL OF PSYCHROPHYTES AGAINST COLD-INDUCED PAW INFLAMMATION IN ALBINO WISTAR RATS"

HASAN AKBAR KHAN
Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore, Lahore, Pakistan.

ASMA AHMED
Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore, Lahore, Pakistan. Correspondence: asma.ahmed@imbb.uol.edu.pk, asma.ahmad.aridian@gmail.com

REHANA BADAR
Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore, Lahore, Pakistan.
Department of Biological Sciences, Superior University, Lahore, Punjab, Pakistan

NOMAN KHALIQUE
Department of Zoology, the University of Punjab, Lahore, Punjab, Pakistan.

SYEDA ZAINAB ALI
Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore.

Abstract

Injuries occurring at high altitudes or extremely cold environments vary from those involving the central nervous system to those that affect the peripheral regions of the body with frostbite being one of the examples. Herbal medicines although have been in usage for the vast amount of time but have become in vogue nowadays and have now been used in conjunction with western medicines for more effective control with the added advantage of having lesser side effects. The In current study, aqueous extracts of roots and leaves of psychrophytes (plants that grow below 0°C), Bergenia ciliata, Crassula pellucida, Ruta chalapensis, Rumex nepalensis, Palhinhaea cernua, Sedum forsterianum and Pinus roxburghii from Minimerg, Pakistan was used to cure dry ice induced inflammation on plantar surf the ace of hind paw of albino Wistar rats (250-300g of both genders). Paw thickness (cm) was measured for 10 connective days, followed by the histopathology of skin. Statistically analyzed results showed that the leaf extract of P.roxburghiia showed significant reduction (0.7±0.01 cm) in paw thickness, followed by the root extract of C. pellucida(0.55±0.02 cm), root extract of P.cernua (0.7±0.03 cm) and leave extract of S.forsterianum(0.45±0.03 cm). Histological examination performed on male and female rats with application of leaves extracts of P.roxburghii showed no signs of acute inflammation but signs of mild chronic inflammation were observed. Dense reepithelization with no granulation tissue formation was seen in male rat while limited reepithelization with partial granulation tissue formation was observed in the female rat. Moderate collagen deposition and fibroblast maturation was seen in male while mild collagen deposition and fibroblast maturation was observed in female rat. Neovascularization of up to 5/HPF (High power field count) was also observed in male while it was absent in female. These crude extracts could be refined for the isolation and characterization of active compound behind this study.

Keywords: Psychrophytes, Cold-induced inflammation, Anti-inflammatory plant extracts

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

1. INTRODUCTION

Cold weather injuries are designated to incorporate injuries that impact central nervous system such as hypothermia and those that principally impact the peripheral regions of the body and these include injuries such as frostbite. The injuries that principally impact the peripheral regions of the body are further sub classified into non-freezing cold injuries and freezing cold injuries. Freezing cold injuries are characterized by damage which occurs due to exposure to temperatures below freezing point i.e. -0.55 °C (Imray and Oakley, 2005). Non-freezing cold injuries are identified by a gradual decline in the tissue temperature without direct freezing(Kuht et al., 2019).These are traditionally related with occupational environments such as military, employees of cold storage facilities, fishermen along with the harbor workers (Zafren, 2021) . Nevertheless, there has been a steady rise in the number of civilians who have been affected by these injuries in USA (Friedman et al., 2020). This rise can be linked to progressive increase in the people interested in high altitude sports such as skiing and mountaineering(Rao and Mohan, 2021).

Living things that thrive in cold conditions are classified into psychrophiles and psych tolerant organisms. Psychrophiles are those living things that thrive best at temperatures less than 15°C (upper limit of 20°C), whereas psych tolerant organisms thrive at temperatures of 20-25 °C, but they can also withstand temperatures below 0°C (Morita, 1975).

Entire process of healing of the wound is broken down into three phases which consist of the process of inflammation, formation of the tissue on the wound site and finally the remodeling of the newly formed tissue. The first stage of inflammation involves phases of aggregation of platelets at the wound site, agglomeration, and the migration of leukocytes (Burnet et al., 2022). The second phase of wound healing comprises of reformation of epithelial tissue, reformation of blood vessels on the affected site, formation of fibrous tissue and finally the contraction of wound. Final stage of remodeling comprises of the reaction of dermis to the injury and it retaliates with production of collagen and proteins of extracellular matrix in order to facilitate the ontogenesis of normal epithelial tissue and maturation of scar tissue. All This stage can last for 12-24 months and by the end of it, the affected tissue reverts back to its pre-injury state(Singh et al., 2017).

Various chemicals found in plants are used in primary healthcare due to their medicinal properties. Flavonoids from plant extracts are employed for wound healing. These chemicals facilitate the process of healing by either preventing the progression of wound complications such as necrosis or preventing the onset right from the start. They also tend to minimize lipid peroxidation by increasing the blood circulation. Furthermore, Flavonoids also expedite the abridgment and reepithelization of wounds owing to their

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

antimicrobial properties (Carvalho et al., 2021). Triterpenes are also employed to stimulate the process of wound healing by the virtue of their astringent and antimicrobial characteristics (Ticona et al., 2022). Tannins are another compounds being employed for stimulating wound healing owing due to their astringent and antimicrobial characteristics(Wu et al., 2023). Saponins are also productive in stimulating wound recovery owing to their antimicrobial and antioxidant characteristics (Baky et al., 2022). Sterols and phenols are beneficial in inhibiting lipid peroxidation due their ability to track down free radicals and it is because of this characteristic that they facilitate the reduction in the progression of necrosis. Furthermore, they also increase the vascularity of the cells(Yadav et al., 2018). This time-honored plant centric medical practice continues to be a pivotal part of our health system. These plants pave the way for discovery and development of they play dual role in the development of newer drugs(Umar et al., 2021).

Medicinal importance of psychrophytes used in current study has been enlisted in Table 1 along with the phytocompounds of respective plants. Aim of current work was to determine the anti-inflammatory activity of Bergenia ciliata, Crassula pellucida, Ruta chalapensis, Rumex nepalensis, Palhinhae acernua, Sedum forsterianum and Pinus roxburghii against dry ice-induced paw inflammation in albino Wistar rats by keeping diclofenac sodium as positive control.

Table 1: Various compounds and medicinal uses of Psychrophytes used in the current study

Plant name	Local name	English name	Family	Compounds	Uses	Citations
P.cernua a	Billi booti	Nodding Club-Moss	Lycopod iopsida	Alkaloids, bitter cucurbitacin glycosides, arvenin, I and II and cucurnitacin, B, D, E, I and L, sterols, ß-amyrin, rutin, n-hexacosane, lacceric acid, triterpene sanagalligenone, anagalligenin, carbohydrates and enzymes	Antimicrobial, anti-inflammatory, analgesic, behavioral, antioxidant, cytotoxic, molluscicidal**,** taenicidal, expectorant, oestrogenic and other pharmacological effects	(Giang et al., 2021; Ortega et al., 2004)
B.ciliata	Bud Mawa	Fringed elephant's ears	Rutacea e	Polyphenols, antioxidant, β-carotene, lavonoids and phenolic acids	Rheumatism, fever, mental disorders, dropsy, neuralgia, menstrual problems, convulsions, and	(El-Hawary et al., 2016)

ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

					other bleeding and nervous disorders	
R.chalapensisL.	Fringed rue	Fringed rue	Rutaceae	Polyphenols, Phenolics, Proteins, Flavonoids	Wound healing activity, anti-inflammatory activity, treat malaria, gonorrhea, poisoning, hepatitis, constipation, sciatic neuralgia, hypertension, migraine, rheumatism, breast cancer, stomach distention, earache, liver diseases, hemorrhoids, typhus, rabies	(Iauk et al., 2004; Ouerghemmi et al., 2017)
R.nepalensis	Nepal dock	Nepal dock	Polygonaceae	Flavonoids, Vitamins, Alkaloids, Carotenoids	Anti-oxidant, diarrhea, dysentery	(Antoniades et al., 2013; Bisht et al., 2019)
C.pellucida	Calico kitten plant	Stonecrop	Crassulaceae	Ursolic acid, daucosterol, b-sitosterol-3-O-b-Dgalactopyranoside, apigenin, apigetrin, and apiin were isolated from some species of the same genus.	Antibacterial, anti-inflammatory, antioxidant, antihypertensive, antidiabeitc activity was shown by other members of the same genus	(Hassan et al., 2021; Odukoya et al., 2022; Sadhana et al., 2018)
P.roxburghii	Chir, Chil	Chir, Chil	Pinaceae	B-secretase 1 and cholinesterase, pinene, flavoanoids	Anti-inflammatory, anticancer, antioxidant activities.	(Bhardwaj et al., 2022; Hou et al., 2019; Labib et al., 2017)
S.forsterianum	Stonecrop	Rock stonecrop	Crassulaceae	Alkaloids, flavonoids, glycosides, tannins, saponins, anthra-quinones, steroids and triterpenoids	Hypocholestrolemic, anti-hypertensive anti-oxidant, anticancer	(Gong et al., 2020; Qi et al., 2022; Taheri et al., 2020)

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

2 MATERIAL AND METHODS

2.1 Collection and identification of plant

Plants were collected in triplicates from Minimerg, Pakistan (34.7908°N 75.0799°E), stored at -80 °C to protect them from stress, identified by expert taxonomist at Department of Botany, Government College University, Lahore, and kept at the University of Lahore, Lahore with their botanical numbers.

2.2 Preparation of extract

To prepare extracts, leaves (L) and roots (R) of each plant were cut down into small pieces separately, hand ground by pistil and mortar to increase the surface area available for contact and were centrifuged at 3000 rpm by Centrifuge machine (M-800-LT) for 15 minutes after the addition of distilled water to obtain supernatant which was further subjected to evaporation while supernatant (1 mg/mL) and residue were saved -80 °C for further use with common names and short labels.

2.6 Ethical Approval

Ethical approval for the experiment was taken by the Ethical Committee, Instituted of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore.

2.7 Selection and grouping of Rats

Total 102 Albino Wistar Rats of weight 250-300 g (half male and half female) were kept at the animal house of IMBB, The University of Lahore, Lahore in stainless steel restrainer boxes, at controlled temperature (18-26°C) and humidity (40-60 %) with free access to food (poultry feed no. 1) and water. Animals were divided into following groups (half male and half female) and all animals, excluding vehicle, were induced with dry ice prior to the induction of control drug and/ plant extract;

1. Vehicle with no inflammation and no treatment.
2. Negative control group (Only dry ice induced inflammation).
3. Positive control group (25mg/mL Diclofenac sodium, Sami).
4. Experimental group-I (EG-I) with P1L extract.
5. EG-II with P1R extract.
6. EG-III with P2L extract.
7. EG-IV with P2R extract.
8. EG-V with P3L extract.
9. EG-VI with P3R extract.
10. EG-VII with P4L extract.
11. EG-VIII with P4R extract.
12. EG-IX with P5L extract.
13. EG-X with P5R extract.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

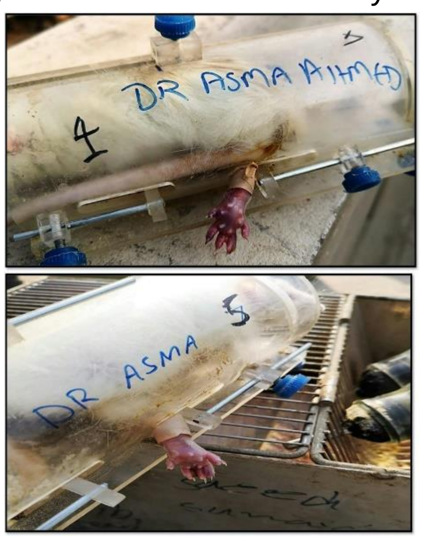
14. EG-XI with P6L extract.
15. EG-XII withP6R extract.
16. EG-XIII withP7L extract.
17. EG-XIV with P7R extract.

2.8 Induction of Dry ice-induced inflammation in Limbs

Dry ice was applied for 30-60 seconds on the plantar surface of rat's hind limb of all animals excluding vehicle to induce dry ice-induced inflammation(Ben Sghaier et al., 2018), and after the induction, shifted the rats to custom designed restrainers where only their hind feet were fixed outside the restrainer boxes, mouth in upward direction near to food and water bottles and tails in downward position to keep their feces and urine out of boxes and to maintain cleanliness (**Figure 1**).

2.9 Induction of Anti-inflammatory drug / Plant extracts

After one day of application of dry ice, 60 µL (0.06 cc) of plant extracts and Diclofenac sodium were injected on plantar surface of rat limb of all experimental groups and positive control group respectively with the help of 1 cc syringes, while no plant extract or anti-inflammatory drug was applied on the negative control group. During the trial, the animals were checked daily for changes and indicators of toxicity.



A

B

Figure 1: Cold-induced injury on plantar surfaces of rats after application of dry ice
(A) Male (B) Female

2.10 Measurement of the changes in dry ice-induced paw thickness of rats

The rats' paw thickness was measured in centimeters by using a pre-calibrated vernier caliper(Ingco® vernier caliper HVC 01200) for ten consecutive days (0-10 days)(Jijith and Jayakumari, 2020). During the trial, the animals were checked daily for changes and indicators of toxicity. Paw thickness was calculated by using following formula;

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

Paw thickness before application of dry-ice= A (cm)
Every subsequent measurement was noted after subtracting value of A
Results are expressed as mean± SEM at p≥ 0.05 by applying Two-way ANOVA on Graph Pad Prism 8.0.

2.11 Histopathological Studies

After 10 days, rats (both treated and controlled) were sacrificed after being anesthetized by 75 mg/kg intraperitoneal Ketamine injection(Tebbutt et al., 2006). Skin from plantar surfaces of all the rats was removed and kept in formalin solution for the preparation of slides. Three to four µm thick histological cross sections were made after fixation, stained with hematoxylin and eosin (H & E) and examined at 40X under compound microscope. In histological examination, scoring system mentioned by Abramov (Abramov et al., 2007) was used for checking the extent of wound healing.

3. RESULTS

3.1 Identification of Plants

The plants identified in the current investigation were assigned Botanical numbers (Table 2, Figure 2).

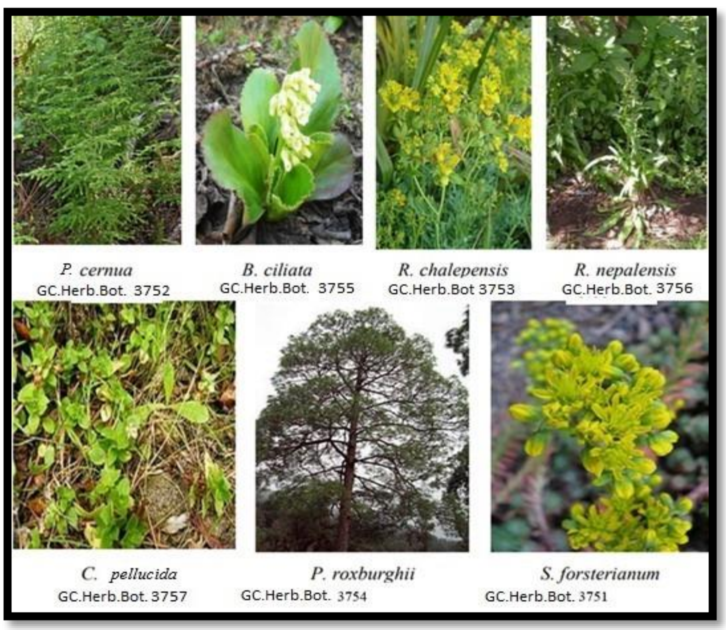


Figure 2: Psychrophytes of Pakistani basis with botanical or identification number GC. Herb. Bot= Herbarium of Botanical Garden at Government College University, Lahore, Pakistan

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

Table 2: Classification of identified Psychrophytes of Pakistan used in our study

Plant name	Division	Class	Order	Family	Genus	Species	Short labels and animal groups
P.cernua	Tracheophyta	Lycopodiopsida	Lycopodiales	Lycopodiaceae	Palhinhaea	Cernua	P1L (EG-I), PIR (EG-II)
B.ciliata	Tracheophyta	Magnoliopsida	Saxifragales	Saxifragaceae	Bergenia	Ciliata	P2L (EG-III),P2R(EG-IV)
R.chalepensis L.	Tracheophyta	Magnoliopsida	Sapindales	Rutaceae	Ruta	Chalepensis	P3L (EG-V), P3R (EG-VI)
R.nepalensis	Tracheophyta	Magnoliopsida	Caryophyllales	Polygonaceae	Rumex	Nepalensis	P4L (EG-VII),P4R (EG-VIII)
C.pellucida	Tracheophyta	Magnoliopsida	Saxifragales	Crassulaceae	Crassula	Pellucida	P5L (EG-IX), P5R (EG-X)
P.roxburghii	Tracheophyta	Pinopsida	Pinales	Pinaceae	Pinus	Roxburghii	P6L (EG-XI), P6R (EG-XII)
S.forsterianum	Tracheophyta	Magnoliopsida	Saxifragales	Crassulaceae	Sedum	Forsterianum	P7L (EG-XIII), P7R (EG-XIV)

L= Leaf R= Roots

3.2 Anti-inflammatory activity of Psychrophytes

Crude extracts of almost all plants have shown anti-inflammatory potential in both male and female rats (Figure 4).

3.2.1 Control Groups

Vehicle showed normal paw size from day 0 to day 10, which showed zero-centimeter changes in paw thickness of both male and female rats respectively. Paw thickness increased in negative control group, which showed that inflammation increased with the passage of time (Paw thickness before dry ice application or on day 0 was 1.3±0.01cm and 1.28±0.02 cm, which had been increased on day one or after application of dry ice as 3.3±0.04 cm and 3.1±0.06 and it reached up-to 3.28±0.05 and 3.2±0.06 on day 10 in female and male rats respectively), with loss of nails as well. Positive control group showed significant reduction in paw inflammation/ thickness from day 3 to day 10 (paw thickness after application of dry ice was 2.8±0.02 cm and 2.7±0.01 cm, but reduction of inflammation after application of diclofenac sodium, was 0.8±0.02 cm and 0.7±0.01 cm, while on day 10, paw thickness reduced to 0.3±0.09 and 0.2±0.08 in female and male rat respectively). Negative control group showed very small reduction in paw inflammation/ thickness from day one to day 10 (paw thickness after application of dry ice was 3.3±0.04 cm and 3.1±0.06 and on day 10 it was 3.28±0.05cm and 3.2±0.06 cm in female and male rats respectively(Table 3 and Figure 3).

Histological examination performed under 40 X on male vehicle showed no signs of acute or chronic inflammation. Mild reepithelization and granulation tissue formation was seen in male dense reepithelization and partial granulation tissue formation was observed in females. Abundant collagen deposition and thorough granulation tissue maturation was seen in male while abundant collagen deposition and moderate granulation tissue maturation was observed in female vehicle. Neovascularization was also observed in male while it was absent in female {Figure 5 (i, ii)}. Histological examination performed on positive control male and female showed dense signs of acute inflammation but chronic inflammation was not seen. Partial reepithelization and granulation tissue formation was seen along with limited collagen deposition and granulation tissue maturation in both male and female rats. Neovascularization was not observed in both {Figure 5 (iii, iv)}.Histological examination performed negative control male rat showed

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

moderate signs of acute inflammation but chronic inflammation was not seen while signs of heavy acute and limited chronic inflammation were observed in female rat. Reepithelization and limited granulation tissue formation was seen in male and female rats. Collagen deposition and limited granulation tissue maturation were seen in both sexes. Neovascularization was also observed in both {Figure 5 (v, vi)}.

3.2.2 Experimental groups

3.2.2.1 Treated with extracts of P.roxburghii

Paw thickness after application of dry ice was 2.76±0.05 cm and 2.8±0.02cm in female and male rats respectively. Reduction of injury after application of leaf extract of P.roxburghii was statistically significant ($p<0.0001$) (2.76±0.05 cm and 2.8±0.02 cm before extract application to 0.7±0.01 cm and 1.05±0.02 cm on day 10^{th} of extract application) in female and male rats respectively. Moreover, in case of root extract, paw thickness after application of dry ice was 2.6±0.01 cm and 2.9±0.01 cm in female and male rats respectively. It showed significant reduction ($p<0.0001$) (2.6 ±0.01 cm and 2.9±0.01 cm before extract application to 1.18±0.01 cm and 1.1±0.01 cm on day 10 of extract application) (Table 3 and Figure 3).

Histological examination performed on male and female rat with application of leaves extracts of P.roxburghii showed no signs of acute inflammation but signs of mild chronic inflammation was observed. Dense reepithelization with no granulation tissue formation was seen in male rat while limited reepithelization with partial granulation tissue formation was observed in female rat. Moderate collagen deposition and fibroblast maturation was seen in male while mild collagen deposition and fibroblast maturation was observed in female rat. Neovascularization of up to 5/HPF (High power field count) was also observed in male while it was absent in female {Figure 5 (vii, viii)}.

Histological examination performed on male rat with application of roots extracts of P.roxburghii showed no signs of acute inflammation but signs of mild chronic inflammation were present while female rat showed modest acute inflammation and signs of mild chronic inflammation. Intermediate reepithelization with partial granulation tissue formation was seen in male while moderate reepithelization and granulation tissue formation was observed in female. Moderate collagen deposition and fibroblast maturation was observed in both male and female rats. Neovascularization of up to 5/HPF and 6-10/HPF was observed in male and female rats respectively {Figure 5 (ix, x)}.

3.2.2.2 Treated with extracts of B.ciliata

Paw thickness after application of dry ice was 2.51±0.08 cm and 2.5±0.03 cm in female and male rats respectively. The reduction of injury after applying extract of B. ciliata leaves was significant (from 2.51±0.08 cm and 2.5±0.03 cm before extract application to 1.7±0.03 cm and 1.8±0.01 in female and male respectively on the 10^{th} day of extract application). Moreover, in case of root extract, paw thickness after application of dry ice was 2.38±0.03 and 2.65±0.01 cm. It showed reduction from 2.38±0.03 and 2.65±0.01 cm before extract application to 1.7±0.01 cm and 1.5±0.04 in female and male respectively on the 10^{th} day of extract application (Table 3 and Figure 3).

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

Histological examination performed under on male rat with application of leaves extract of B. ciliata showed dense signs of acute inflammation but signs of chronic inflammation was absent while mild signs of acute and chronic inflammation were observed in female rats. Scanty reepithelization with dense granulation tissue formation was seen in male while limited reepithelization with scanty granulation tissue formation was observed in female. Although collagen deposition was not observed but scanty fibroblast maturation was seen in male while moderate collagen deposition and fibroblast maturation was observed in female. Neovascularization of up to 5/HPF was observed in male while it was absent in female {Figure 5 (xi, xii)}.

Histological examination performed on male rat with application of roots extract of B. ciliata showed mild signs of acute and chronic inflammation while female showed no signs of acute or chronic inflammation. Scanty reepithelization with limited granulation tissue formation was seen in both sexes. Limited collagen deposition and fibroblast maturation was also seen in both sexes. Neovascularization was not observed in male while neovascularization of up to 10/HPF was observed in female rat {Figure 5 (xiii, xiv)}.

3.2.2.3 Treated with extracts of C.pellucida

Paw thickness after application of dry ice was 2.51±0.01 cm and 2.5±0.05cm in female and male rats respectively. The reduction of injury after applying C.pellucida leaves extract was significant (from 2.51±0.08 cm and 2.5±0.03 cm before extract application to0.72±0.03 cm and 0.85±0.02 in female and male rats respectively on the 10^{th} day). Moreover, in case of root extract, paw thickness after application of dry ice was 2.64±0.04 cm and 2.5±0.04 cm. It showed reduction from 2.64±0.04 cm and 2.5±0.04 cm before extract application to 0.55±0.02 cm and 0.5±0.02 cm in female and male respectively on the 10^{th} day of extract application (Table 3 and Figure 3).

Histological examination performed on male rat with application of leaves extracts of C.pellucida showed no signs of acute or chronic inflammation while signs of dense acute and limited chronic inflammation were observed in female. Dense reepithelization with absent granulation tissue formation was observed in male and female rats. Heavy collagen deposition and limited fibroblast maturation were seen in male while collagen deposition and fibroblast maturation were absent in female. Neovascularization was absent in male and female rats {Figure 5 (xv, xvi)}.

Histological examination performed on male rat with application of roots extracts of C.pellucida showed signs of dense acute inflammation with limited chronic inflammation while signs of dense acute inflammation were also observed in female but signs of chronic inflammation were absent. Limited reepithelization with dense granulation tissue formation was observed in male while scanty reepithelization with limited granulation tissue formation was seen in female. Moderate collagen deposition and limited fibroblast maturation were seen in male and female rats. Neovascularization of up to 6-10/HPF and 5/HPF was seen in male and female rats respectively {Figure 5 (xvii, xviii)}.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

3.2.2.4 Treated with extracts of R.chalepensis

In the case of leaf extract of R.chalepensis, paw thickness after application of dry ice was 2.51±0.03 cm and 2.5±0.02 cm in female and male rats respectively. The reduction of injury after applying extract was significant (from 2.51±0.03 cm and 2.5±0.02 cm before extract application to 1.7±0.01 cm and 1.6±0.04in females and male respectivelyon the 10^{th} day of extract application). Moreover, in case of root extract, paw thickness after application of dry ice was 2.5 ±0.01 and 2.9±0.06 cm in female and male rats respectively. It showed reduction from 2.5 ±0.01 and 2.9±0.06 cm before extract application to 1.73±0.03 cm and 1.8±0.03 in female and male rats respectively on the 10^{th}day of extract application (Table 3 and Figure 3).

Histological examination performed on male rat with application of leaves extracts of R.chalepensis showed signs of mild acute and chronic inflammation while inflammation was absent in female. Dense reepithelization with granulation tissue formation was seen in male but was absent in female. Limited collagen deposition and moderate granulation tissue maturation was seen in male while it was absent in female. Neovascularization of around 6-10/HPF was also observed in male which was absent in female {Figure 5 (xix, xx)}.

Histological examination performed on male rat with application of roots extracts of R.chalepensis showed signs of mild acute inflammation and moderate chronic inflammation while modest acute inflammation and mild chronic inflammation was observed in female. Dense reepithelization with partial granulation tissue formation was seen in male while scanty reepithelization with no granulation tissue formation was observed in female. Limited collagen deposition and fibroblast maturation was seen in male while collagen deposition and fibroblast maturation were absent in female. Neovascularization was not observed in both sexes {Figure 5 (xxi, xxii)}.

3.2.2.5 Treated with extracts of R.nepalensis

In the case of leaf extract of R.nepalensis, paw thickness after application of dry ice was 2.51±0.03 cm and 2.5±0.02 cm in female and male rats respectively. The reduction of injury after applying extract was significant (from 2.5±0.02cm and 2.5±0.01 before extract application to 1.98±0.03 cm and 1.45±0.03 cm in females and male respectively on the 10^{th} day of extract application). Moreover, in case of root extract, paw thickness after application of dry ice was 2.5±0.03 cm and 2.9±0.06 cm in female and male rats respectively. It showed reduction from 2.5±0.03 cm and 2.9±0.06 cm before extract application to 2.2±0.06 cm and 1.45±0.05 cm infemale and male rats respectively on the 10^{th} day of extract application (Table 3 and Figure 3).

Histological examination performed under on male rat with application of leaves extracts of R.nepalensis showed no signs of acute inflammation but signs of mild chronic inflammation were observed while signs of both acute and chronic inflammation were absent in female. Limited reepithelization with no granulation tissue formation was seen in male while scanty reepithelization with granulation tissue formation was observed in female. Abundant collagen deposition and moderate granulation tissue maturation was

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

seen in male while limited collagen deposition and fibroblast maturation was observed in female. Neovascularization was not observed in both sexes {Figure 5 (xxiii, xxiv)}.

Histological examination performed on male rat with roots extracts of R.nepalensis showed mild signs of acute inflammation and moderate signs of chronic inflammation while no signs of acute inflammation but mild signs of chronic inflammation were observed in female. Moderate reepithelization and mild granulation tissue formation was seen in male and female rats. Limited collagen deposition and granulation tissue maturation was seen in male while dense collagen deposition and moderate granulation tissue maturation was observed in female. Neovascularization was observed in male while it was absent in female rat {Figure 5 (xxv, xxvi)}.

3.2.2.6 Treated with extracts of P.cernua

In the case of leaf extract of P.cernua, paw thickness after application of dry ice was 2.5±0.03 cm and 2.51±0.02 cm in female and male rats respectively. The reduction of injury after applying extract was significant (from 2.5±0.03 cm and 2.51±0.02 cm before extract application to 1.6±0.01 cm and 1.45±0.04 cm in females and male respectively on the 10^{th} day of extract application). Moreover, in case of root extract, paw thickness after application of dry ice was 2.56±0.01cm and 2.64±0.01 cm in female and male rats respectively. It showed reduction from 2.56±0.01cm and 2.64±0.01 cm before extract application to 1.1±0.02 cm and 0.7±0.03 cm in female and male rats respectively on the 10^{th} day of extract application (Table 3 and Figure 3).

Histological examination performed under 40 X on male rat with application of leaves extract of P.cernua showed signs of severe acute and limited chronic inflammation while female rat exhibited no signs of acute inflammation but signs of mild chronic inflammation were observed. Dense reepithelization with limited granulation tissue formation was seen in male while limited reepithelization with granulation tissue formation was observed in female. Moderate collagen deposition and limited fibroblast maturation was seen in male while dense collagen deposition and moderate granulation tissue maturation was observed in female. Neovascularization was not observed in both sexes {Figure 5 (xxvii, xxviii)}.

Histological examination performed on male rat with application of roots extracts of P.cernua showed signs of severe acute inflammation and mild chronic inflammation while moderate signs of acute and chronic inflammation were observed in female. Limited reepithelization with dense granulation tissue formation was seen in male and female rats. Moderate collagen deposition and fibroblast maturation was seen in male while limited collagen deposition and moderate granulation tissue maturation was observed in female. Neovascularization was absent in male while neovascularization of around 6-10/HPF was observed in female {Figure 5 (xxix, xxx)}.

3.2.2.7 Treated with extracts of S.forsterianum

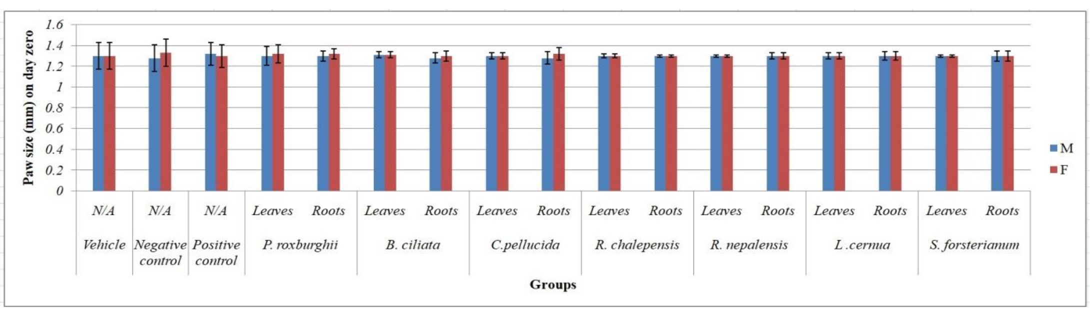
In the case of leaf extract of S.forsterianum, paw thickness after application of dry ice was 2.5±0.03 cm and 2.5±0.06 cm in female and male rats respectively. The reduction of injury after applying extract was significant (from 2.5±0.03 cm and 2.5±0.06 cm before extract

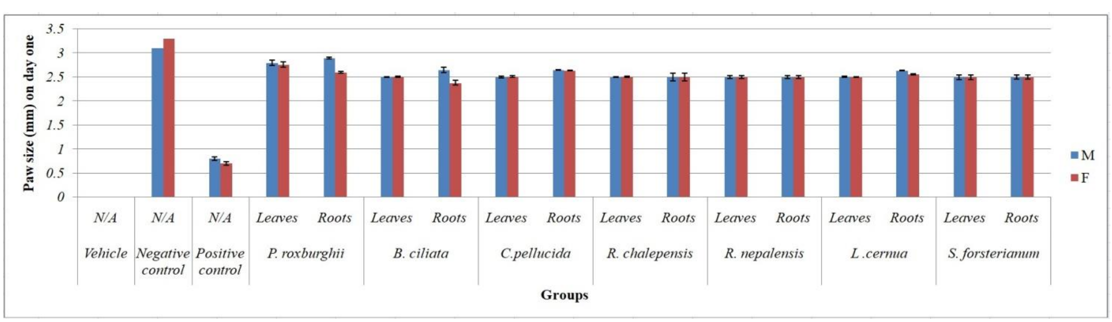
Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

application to 1.44±0.03 cm and 1.35±0.02 cm in females and male respectively on the 10th day of extract application). Moreover, in case of root extract, paw thickness after application of dry ice was 2.5±0.04 cm and 2.5±0.05 cm in female and male rats respectively. It showed reduction from 2.5±0.04 cm and 2.5±0.05 cm before extract application to 0.8±0.03cm and 1.1±0.03 cm in female and male rats respectively on the 10th day of extract application (Table 3 and Figure 3).

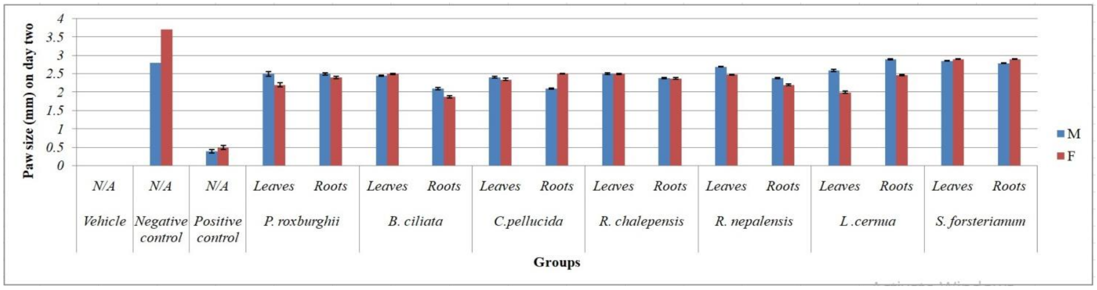
Histological examination performed under on male rat with application of leaves extracts of S.forsterianum showed signs of modest acute and chronic inflammation while signs of mild acute and modest chronic inflammation were observed in female.Limited reepithelization with dense granulation tissue formation was seen in male while limited reepithelization with moderate granulation tissue formation was observed in female. Limited collagen deposition and fibroblast maturation were observed in male while moderate collagen deposition and fibroblast maturation was seen in male. Neovascularization was absent in male while neovascularization of up to 6-10/HPF was seen in female {Figure 5 (xxxi, xxxii)}.

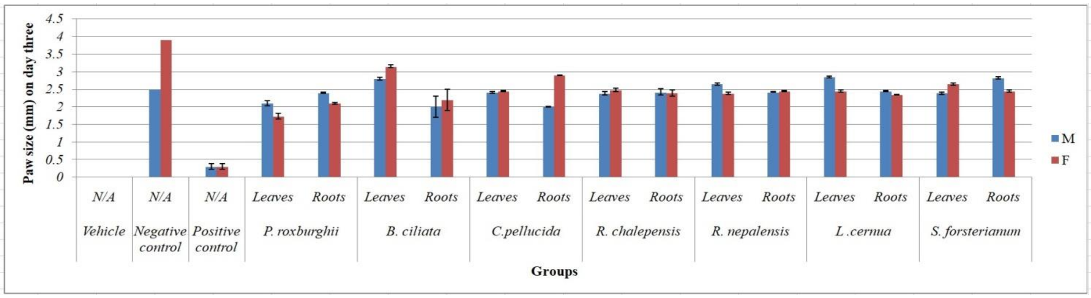
Histological examination performed on male rat with application of roots extracts of S.forsterianum showed dense signs of acute inflammation but signs of chronic inflammation was absent while signs of dense acute inflammation and mild chronic inflammation were observed in female. Scanty reepithelization with limited granulation tissue formation was seen in male while dense reepithelization with limited granulation tissue formation was observed in female. Collagen deposition and fibroblast maturation were absent in male. Although fibroblast maturation was alsoabsent in female but partial collagen deposition was present in it. Neovascularization was absent in male while neovascularization of up to 5/HPF was observed in female rat {Figure 5 (xxxiii, xxxiv)}.

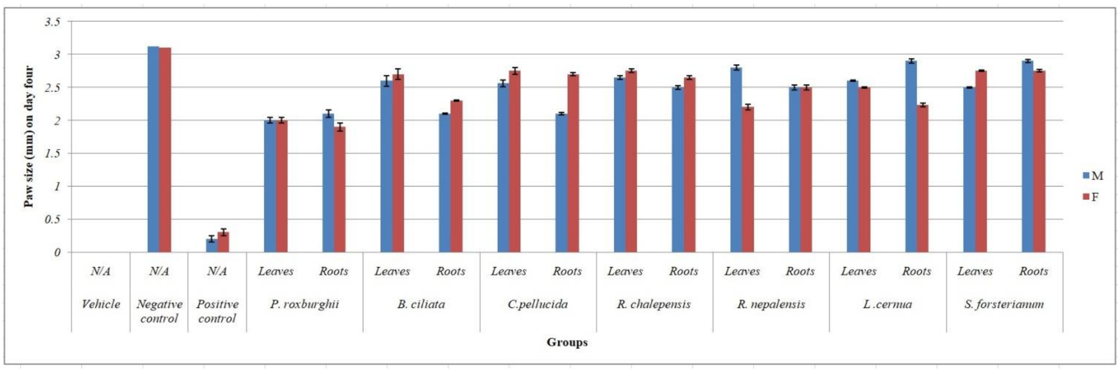


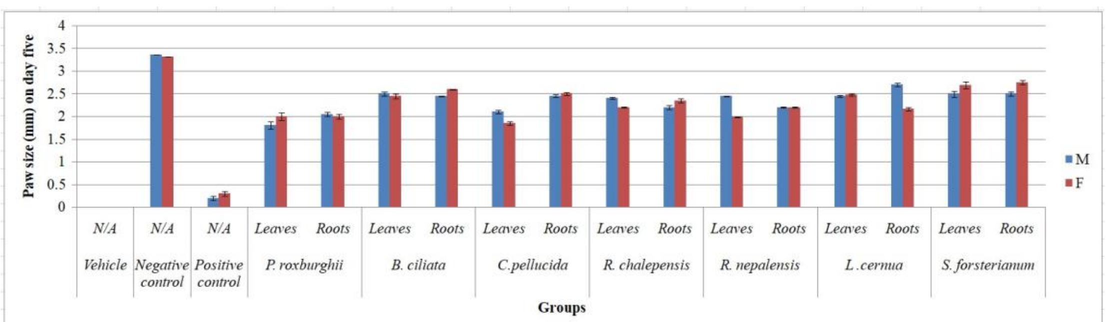


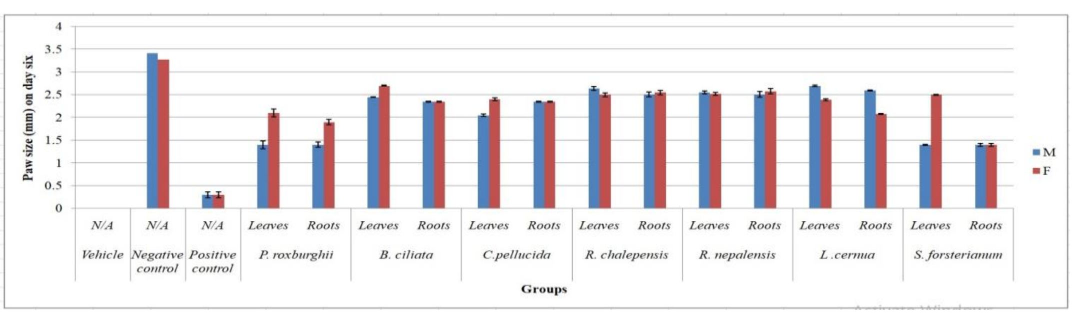
Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF











Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

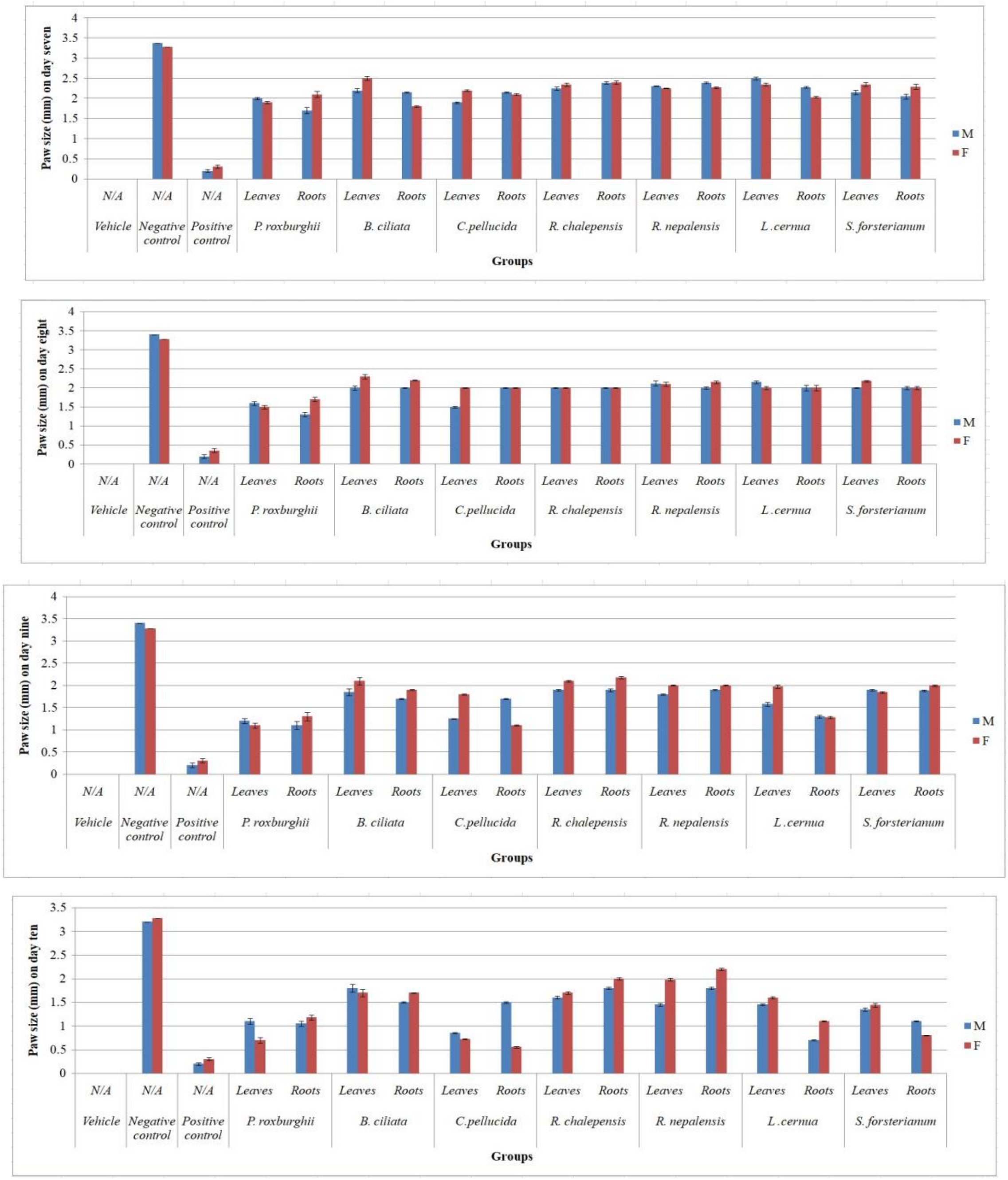


Figure 3: Experimental data of anti-inflammatory effect of psychrophytes against cold-induced injury in albino rats

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF


(A)

(B)

Figure 4: Cold-induced injury on plantar surfaces of rats after application of dry ice

(A) Male (B) Female

Table 3 Experimental data of anti-inflammatory effect of Psychrophytes' extracts against cold-induced injury in albino rats indicated by changes in paw thickness measured in centimeters for ten days

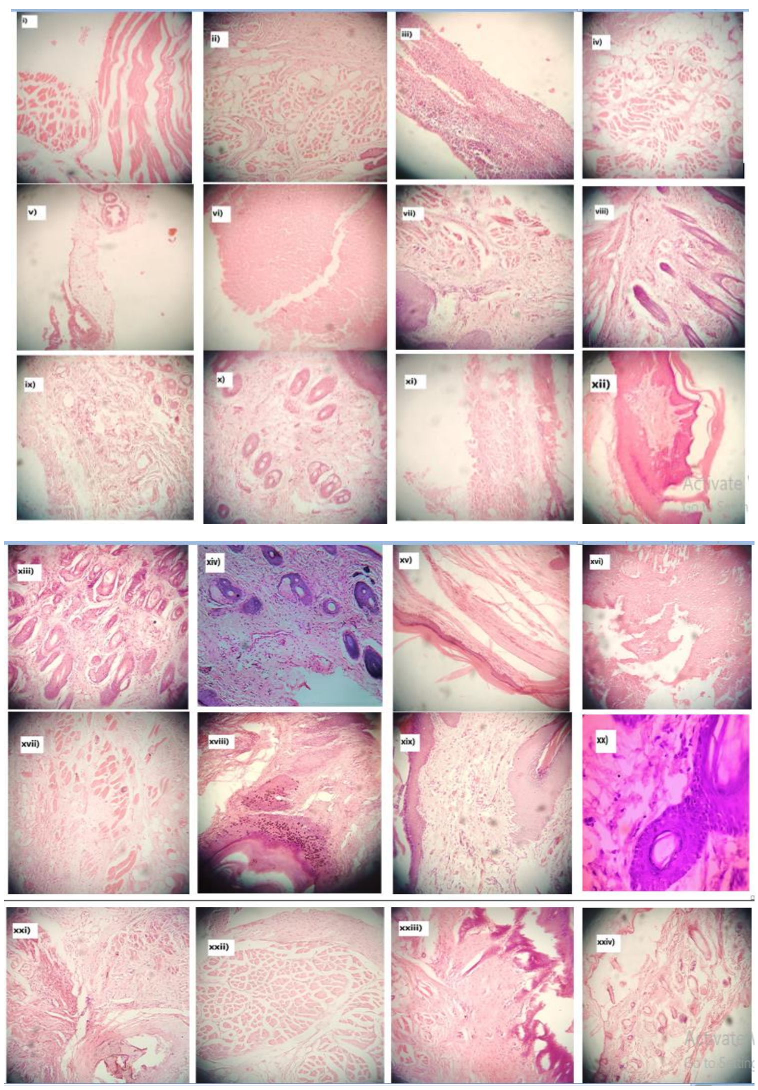
Groups	Extracts	Gender	Paw thickness in cm/ Days										
			A	B**	After application plant extract/ Diclofenic sodium**								
			Zero	One	Two	Three	Four	Five	Six	Seven	Eight	Nine	Ten
Vehicle control	Dist. W	F	1.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
		M	1.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Negative control	N/A	F	1.3	3.3±0.04	3.72±0.05	3.9±0.08	3.1±0.05	3.32±0.05	3.28±0.06	3.28±0.04	3.28±005	3.28±0.03	3.28±0.05
		M	1.28±0.02	3.1±0.06	2.82±0.06	2.5±0.08	3.12±0.04	3.36±0.08	3.42±0.09	3.38±0.03	3.4±0.05	3.4±0.06	3.2±0.06
Positive control	Diclofenic sodium	F	1.3±0.04	2.8±0.02	0.8±0.03	0.5±0.02	0.3±0.06	0.3±0.05	0.3±0.06	0.3±0.08	0.35±0.06	0.3±0.05	0.3±0.09
		M	1.32±0.05	2.7±0.01	0.7±0.01	0.4±0.04	0.2±0.08	0.2±0.05	0.3±0.01	0.2±0.05	0.2±0.06	0.2±0.08	0.2±0.08
P.roxburghii	L	F	1.32±0.02	2.76±0.05	2.2±0.03	1.7±0.3	2±0.01	2±0.01	2.1±0.01	1.9±0.02	1.5±0.01	1.1±0.01	0.7±0.01
	R	F	1.32±0.06	2.6±0.01	2.4±0.01	2.1±0.01	1.9±0.02	2±0.03	1.9±0.01	2.1±0.02	1.7±0.01	1.3±0.02	1.18±0.01
	L	M	1.3±0.04	2.8±0.02	2.5±0.03	2.1±0.02	2±0.05	1.8±0.04	1.4±0.03	2.0±0.02	1.6±0.02	1.2±0.01	1.1±0.01
	R	M	1.3±0.02	2.9±0.01	2.5±0.02	2.4±0.05	2.1±0.03	2.05±0.02	1.4±0.04	1.7±0.04	1.3±0.01	1.1±0.03	1.05±0.02
B.ciliata	L	F	1.31±0.02	2.51±0.08	2.5±0.02	3.15±0.09	2.7±0.03	2.45±0.04	2.7±0.05	2.5±0.04	2.3±0.01	2.1±0.02	1.7±0.03
	R	F	1.3±0.05	2.38±0.03	1.88±0.02	2.2±0.01	2.3±0.04	2.6±0.01	2.35±0.06	1.8±0.02	2.2±0.03	1.9±0.02	1.7±0.01
	L	M	1.31±0.04	2.5±0.03	2.45±0.01	2.8±0.03	2.6±0.04	2.5±0.01	2.45±0.03	2.2±0.01	2±0.06	1.85±0.03	1.8±0.01
	R	M	1.28±0.02	2.65±0.01	2.1±0.03	2±0.03	2.1±0.01	2.45±0.02	2.35±0.02	2.15±0.03	2±0.04	1.7±0.02	1.5±0.04
C.pellucida	L	F	1.3±0.05	2.51±0.01	2.35±0.02	2.45±0.01	2.75±0.03	1.85±0.04	2.4±0.01	2.2±0.02	2±0.07	1.8±0.01	0.72±0.03
	R	F	1.32±0.02	2.64±0.04	2.51±0.01	2.9±0.03	2.7±0.02	2.5±0.04	2.35±0.03	2.1±0.06	2±0.04	1.1±0.01	0.55±0.02
	L	M	1.3±0.06	2.5±0.05	2.41±0.01	2.41±0.03	2.56±0.01	2.1±0.07	2.05±0.01	1.9±0.05	1.5±0.02	1.25±0.04	0.85±0.02
	R	M	1.29±0.07	2.5±0.04	2.45±0.04	2.46±0.03	2.65±0.03	2.45±0.01	2.32±0.03	1.8±0.04	1.3±0.03	1±0.02	0.5±0.02
R.chalepensis	L	F	1.3±0.05	2.51±0.03	2.5±0.02	2.48±0.02	2.75±0.01	2.2±0.01	2.5±0.04	2.34±0.03	2±0.01	2.1±0.03	1.7±0.01
	R	F	1.29±0.04	2.5±0.01	2.38±0.02	2.39±0.03	2.65±0.04	2.35±0.03	2.55±0.05	2.4±0.03	2.25±0.04	2.18±0.02	1.73±0.03
	L	M	1.3±0.06	2.5±0.02	2.51±0.04	2.38±0.01	2.65±0.05	2.4±0.03	2.64±0.01	2.25±0.02	2±0.03	1.9±0.01	1.6±0.04
	R	M	1.31±0.02	2.5±0.05	2.39±0.03	2.42±0.04	2.5±0.02	2.2±0.02	2.51±0.02	2.39±0.01	2±0.05	1.9±0.03	1.8±0.03
R.nepalensis	L	F	1.29±0.06	2.5±0.02	2.48±0.05	2.38±0.03	2.2±0.03	1.98±0.01	2.52±0.01	2.25±0.02	2.1±0.01	2±0.04	1.98±0.03
	R	F	1.3±0.04	2.5±0.03	2.2±0.04	2.45±0.01	2.5±0.02	2.2±0.06	2.58±0.04	2.27±0.05	2.15±0.06	2±0.05	2.2±0.06
	L	M	1.31±0.05	2.5±0.01	2.7±0.02	2.65±0.04	2.8±0.04	2.45±0.01	2.55±0.05	2.31±0.03	2.12±0.01	1.8±0.02	1.45±0.03
	R	M	1.28±0.08	2.9±0.06	2.7±0.03	2.95±0.02	2.45±0.03	2.7±0.02	2.65±0.01	2.41±0.04	2.08±0.04	1.9±0.01	1.45±0.05
P.cernua	L	F	1.32±0.03	2.5±0.03	2±0.03	2.45±0.04	2.5±0.03	2.48±0.04	2.39±0.03	2.35±0.01	2±0.05	1.98±0.04	1.6±0.01
	R	F	1.29±0.06	2.56±0.01	2.47±0.03	2.35±0.06	2.23±0.02	2.16±0.05	2.08±0.02	2.03±0.03	2±0.04	1.28±0.05	1.1±0.02
	L	M	1.31±0.01	2.51±0.02	2.6±0.01	2.85±0.03	2.6±0.06	2.45±0.04	2.7±0.05	2.5±0.08	2.15±0.07	1.58±0.01	1.45±0.04
	R	M	1.28±0.04	2.64±0.01	2.9±0.03	2.45±0.02	2.9±0.04	2.7±0.06	2.6±0.07	2.28±0.05	2±0.01	1.3±0.03	0.7±0.03
S.forsterianum	L	F	1.31±0.03	2.5±0.03	2.9±0.01	2.65±0.02	2.75±0.05	2.69±0.04	2.5±0.06	2.35±0.08	2.18±0.07	1.85±0.04	1.44±0.03
	R	F	1.32±0.05	2.5±0.04	2.9±0.02	2.45±0.01	2.75±0.02	2.75±0.03	1.4±0.05	2.29±0.07	2±0.06	2±0.01	0.8±0.03
	L	M	1.28±0.06	2.5±0.06	2.86±0.04	2.39±0.02	2.5±0.03	2.49±0.01	1.4±0.05	2.15±0.01	2±0.08	1.9±0.07	1.35±0.02
	R	M	1.3±0.03	2.5±0.05	2.79±0.02	2.82±0.04	2.9±0.01	2.5±0.02	1.4±0.03	2.05±0.05	2±0.01	1.88±0.06	1.1±0.03

Where; **F**: Female Albino wistar rats; **M**: Male Albino wistar rats; **N/A**: nothing was administered; **L**: extracts of leaves of plants;**R**: extracts of roots of plants; ±SEM= value obtained after triplicate analysis, A= Paw thickness before the start of experiment (in

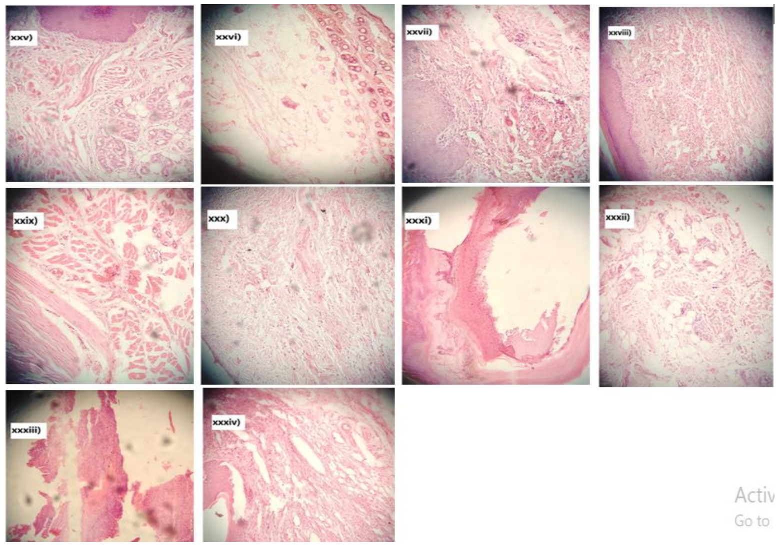
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

cm) or Paw thickness before application of dry ice, B= After application of dry ice, **= Values are mentioned after subtracting values of A

(A) **(B)**



Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF



(C)

Figure 5: Plantar surfaces after application of plant extract (A) male rat (B) female rat (C) Histopathology at 40X (400μm) of rat skin

Vehicle (i) Male (ii) Female, **Positive control** (iii) Male (iv) Female, **Negative control** (v) Male (vi) Female, **Treated with leaf extract of P.roxburghii**(vii) Male (viii) Female, **Treated with root extract of P.roxburghii**(ix) Male (x) Female, **Treated with leaf extract of B.ciliata**(xi) Male (xii) Female, **Treated with root extract of B.ciliata**(xiii) Male (xiv) Female, **Treated with leaf extract of C.pellucida**(xv) Male (xvi) Female, **Treated with root extract of C.pellucida**(xvii) Male (xviii) Female, **Treated with leaf extract of R.chalepensis**(xix) Male (xx) Female, **Treated with root extract of R.chalepensis**(xxi) Male (xxii) Female, **Treated with leaf extract of R.nepalensis**(xxiii) Male (xxiv) Female, **Treated with root extract of R.nepalensis** (xxv) Male (xxvi) Female, **Treated with leaf extract of P.cernua**(xxvii) Male (xxviii) Female, **Treated with root extract of P.cernua**(xxix) Male (xxx) Female, **Treated with leaf extract of S.forsterianum**(xxxi) Male (xxxii) Female, **Treated with root extract of S.forsterianum**(xxxiii) Male (xxxiv) Female

4. DISCUSSION

Inflammation and pain are two severe dissimilar illnesses that are intimately linked. The ubiquitous definition of pain is “Pain is a mutually recognizable somatic experience that reflects a person's apprehension of threat to their bodily or existential integrity” (Cohen et al., 2018). Meanwhile, inflammation is the body's natural response to foreign objects as well as damage, resulting in the migration of leucocytes and antibodies to the afflicted areas, as well as the formation of swelling and oedema(S Gad, 2018). Synthetic analgesics and anti-inflammatory medicines can help with pain, but due to their nonselective nature, they have a variety of negative side effects(Farhood et al., 2019).

Results of the current investigation revealed that C.pellucida extracts had one of the best anti-inflammatory potential. During the first four days no significant changes occurred.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

After 4th day the inflammation is decreasing significantly and on day 10th the inflammation was the lowest in root extract than that of leaves in male and female both. Similar results have been obtained from other species of C.pellucida as well. Another species of Crassula including Crassula arborescenshas been studied for their antiviral, antimicrobial and antioxidant activity by El-Hawary et. al (El-Hawary et al., 2016). Species of family Crassulaceae have been highlighted to possess anti-convulsant, anti-cancer, anti-microbial, antioxidant, anti-inflammatory, and antidiabetic characteristics (Hassan et al., 2021).Crassula ovata's leaves extracts were shown to have antihyperglycemic, antioxidant and antimicrobial activities antioxidant and antidiabetic activity(Chokhone et al., 2017). Crassula capitella leaves were shown to be effective against wounds(Mbhele et al., 2022).

On the other hand, only leave extract of P.roxburghii in female showed very positive results. The presence of gallic acid and catechin in the leaves of P.roxburghii is thus confirmed. Gallic acid is a polyphenol that has antioxidant and neuroprotective properties. It may be present in practically every component of a plant in the environment. Catechin is a naturally occurring phenol with antioxidant properties. As a result, this plant has a lot of therapeutic benefits. Because they exhibit antibacterial, anti-inflammatory, anti-cancer, antitussive, antidiabetic, antilithiatic, antidiabetic, antibacterial, and other pharmacological qualities, there is a pressing need to register and assist these strains. There is an ever-growing need to find novel methods to capitalize on efficiencies that will benefit mankind in the long run. Some herbal medicines including B.ciliata, on the other hand, can be used therapeutically (Kour et al., 2019).

Additionally, in this experimental study it's also discovered that Bergenia is truly the important therapeutic plant. Bergenia has piqued the interest of scientists due to its numerous medicinal applications. In Bergen, a number of research have been carried out to assess the impact of genotype recognition and separation of phytochemical compounds, as well as medicinal activity. Bergenin is abundant in B.ciliata, and it has a range of medicinal properties when it is present (Ali et al., 2021; Bashir et al., 2022). These findings indicate that species/cell types that flourish at higher elevations collect a lot of secondary metabolites. Ploidy chromosomal flexibility tends to promote species adaptation, especially at higher altitudes. Rumex is also utilized to generate commercial variations for commerce and industry since it contains a large number of useful bioactive metabolites and has a diverse range of cell types and chemicals (Berillo et al., 2022).

The results of the botanical experiments indicated that plant components include flavonoids, phenolic content, and antioxidants. The presence of flavonoids, a class of chemicals well-known for their therapeutic potential, distinguishes these plants as medicinal plants. Furthermore, the same molecule is linked to the plant extract's antiviral, anti-estrogenic, antiviral, and anti-proliferative effects(Ullah et al., 2020). The highest quantity of TPC was found in B.ciliataroot extracts, while the least amount was found in L. cernua root extracts. The leaf extract of the R.chalepensisendowed with the greatest level of phenolic compounds as leaf extract of the R.chalepensis were showed greater content of phenolic compounds, according to the study. Leaf extracts of R.chalepensis have previously been found to contain flavonoids, which are responsible for biological

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

activity (Ouerghemmi et al., 2017). The phytochemicals in these plants, such as flavonoids, have been found to be employed in food (Loizzo et al., 2018). However, the findings of Porquis et al., (Porquis et al., 2018) differed from those of the current study. The current study was backed up by the findings of Recuenco et al., (Recuenco et al., 2016), who revealed that the TPC and TFC content of P.cernua is low when compared to other plants. Plant samples were subjected to qualitative phytochemical analysis, which is a crucial stage in the discovery and manufacture of new medications.

At various dosages of an alcoholic extract of P.roxburghiibark displayed anti-inflammatory and analgesic effect (analgesic activity was assessed using acetic acid-induced writhing and tail immersion tests in albino mice). Carrageenan-induced paw oedema and cotton pellet granuloma in Wistar albino rats were used to assess acute and chronic anti-inflammatory effects. The presence of polyphenolic chemicals in the extract was responsible for these actions. The results demonstrated varying degrees of binding of isolated ingredients with the different receptors studied, indicating the plant's analgesic and anti-inflammatory properties. According to the findings, the plant has potential as a natural analgesic and anti-inflammatory candidate (Gulilat et al., 2021).The isolation and structural elucidation of sixteen compounds, including two xanthones, six flavonoids, one diterpene, and two triterpenes, as well as five phenolic compounds, came from a comprehensive phytochemical examination. For the first time, some of them were separated from the reported plant. The findings revealed that the different separated elements bind to the different receptors in varying degrees, which probably explains the reported analgesic and anti-inflammatory effects. As a result of the synergistic effect of all of its components, some of them provide a relatively safe, natural analgesic and anti-inflammatory option (Labib et al., 2017).

These crude extracts may be processed and utilized to minimize cold-induced inflammation in the future, and they are also employed for active component identification, characterization, and isolation. In the control groups, we employed the documented medicine dicloran for both genders in the positive control group, which showed a high rate of recovery, but the negative control group was not treated for the duration of the trial, and inflammation worsened with time.

5. CONCLUSION

In the conclusion highest reduction showed by P.roxburghii (0.7±0.01) among the seven plants extracts from leaf and roots, extract of C.pellucida (0.55±0.02 cm), extracts of roots of P.cernua (0.7±0.03 cm) and extract of leaves of S.forsterianum (0.45±0.03 cm)showed the most reduction of paw thickness. These crude extracts could be refined and used to reduce the cold-induced inflammation in the future and these are further used for the isolation, characterization and for active compounds. In control groups we have used documented drugs dicloran for both genders in positive control group which show high recovery while the negative control group is not treated in the whole study and inflammation increased with time.

6. FUNDED BY

Present course of work was funded by HEC, Government of Pakistan, under NRPU Project No: 8715/Punjab/NRPU/R&D/HEC/2017.

7. ACKNOWLEDGMENT

Authors are highly acknowledged to the Higher Education Commission (HEC) Pakistan, Government of Pakistan for providing funds for the completion of this work as a part of Ph.D. project and for providing competent faculty and University as a forum to complete such tasks with peace of mind. Authors also highly acknowledged to all soldiers and officers posted on Minimerg, Pakistan from Pakistan Armed forces for their immense support during collection of plants utilized in current study.

REFERENCES

- Abramov, Y., Golden, B., Sullivan, M., Botros, S.M., Miller, J.-J.R., Alshahrour, A., Goldberg, R.P., et al. (2007), "Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model", Wound Repair and Regeneration, Vol. 15 No. 1, pp. 80–86.
- Ali, E., Hussain, K., Bukhari, N.I., Arshad, N., Hussain, A., Abbas, N., Arshad, S., et al. (2021), "Determination of Bergenin in Different Parts of Bergenia ciliata using a Validated RP-HPLC Method", Natural Product Sciences, The Korean Society of Pharmacognosy, Vol. 27 No. 1, pp. 54–59.
- Antoniades, V., Sioga, A., Dietrich, E.M., Meditskou, S., Ekonomou, L. and Antoniades, K. (2013), "Is copper chelation an effective anti-angiogenic strategy for cancer treatment?", Medical Hypotheses, Vol. 81 No. 6, pp. 1159–1163.
- Baky, M.H., Elsaid, M.B. and Farag, M.A. (2022), "Phytochemical and biological diversity of triterpenoid saponins from family Sapotaceae: A comprehensive review", Phytochemistry, Vol. 202, p. 113345.
- Bashir, K., Khan, M.F.A., Alhodaib, A., Ahmed, N., Naz, I., Mirza, B., Tipu, M.K., et al. (2022), "Design and Evaluation of pH-Sensitive Nanoformulation of Bergenin Isolated from Bergenia ciliata", Polymers, MDPI, Vol. 14 No. 9, p. 1639.
- Ben Sghaier, M., Louhichi, T., Hakem, A. and Ammari, Y. (2018), "Chemical investigation of polar extracts from Ruta chalpensis L. growing in Tunisia: Correlation with their antioxidant activities.", Journal of New Sciences, Vol. 49.
- Berillo, D., Kozhahmetova, M. and Lebedeva, L. (2022), "Overview of the Biological Activity of Anthraquinons and Flavanoids of the Plant Rumex Species", Molecules, Multidisciplinary Digital Publishing Institute, Vol. 27 No. 4, p. 1204.
- Bhardwaj, K., Sharma, R., Cruz-Martins, N., Valko, M., Upadhyay, N.K., Kuča, K. and Bhardwaj, P. (2022), "Studies of Phytochemicals, Antioxidant, and Antibacterial Activities of Pinus gerardiana and Pinus roxburghii Seed Extracts", BioMed Research International, Hindawi, Vol. 2022.
- Bisht, M., Sekar, K. and Arya, D. (2019), "Diversity, utilization pattern, threat status and conservation of medicinal plants in great Himalayan national park, Himachal Pradesh, western Himalaya", Asia Pacific Journal of Research, Vol. 1.
- Burnet, M., Metcalf, D.G., Milo, S., Gamerith, C., Heinzle, A., Sigl, E., Eitel, K., et al. (2022), "A Host-Directed Approach to the Detection of Infection in Hard-to-Heal Wounds", Diagnostics, MDPI, Vol. 12 No. 10, p. 2408.
- Carvalho, M.T., Araujo-Filho, H.G., Barreto, A.S., Quintans-Junior, L.J., Quintans, J.S. and Barreto, R.S. (2021), "Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action", Phytomedicine, Elsevier, Vol. 90, p. 153636.

❖ Chokhone, K., Talukdar, N., Sarma, M.P., Das, K. and Kalita, P.P. (2017), "Screening of phytochemicals and evaluation of anti-microbial, anti-oxidant and in-vitro antidiabetic activity ofCrassula ovata leaves", International Journal of Pharmaceutical Sciences and Research, International Journal of Pharmaceutical Sciences and Research, Vol. 8 No. 2, p. 859.

❖ Cohen, M., Quintner, J. and van Rysewyk, S. (2018), "Reconsidering the International Association for the Study of Pain definition of pain", Pain Reports, Vol. 3 No. 2, p. e634.

❖ El-Hawary, S.S., Mohammed, R., Abouzid, S., Ali, Z.Y., El-Gendy, A.O. and Elwekeel, A. (2016), "In-vitro cyclooxygenase inhibitory, antioxidant and antimicrobial activities of phytochemicals isolated from Crassula arborescens (Mill.) Willd.", International Journal of Applied Research in Natural Products, Healthy Synergies Publications, Vol. 9 No. 4, pp. 8–14.

❖ Farhood, B., Mortezaee, K., Goradel, N.H., Khanlarkhani, N., Salehi, E., Nashtaei, M.S., Najafi, M., et al. (2019), "Curcumin as an anti-inflammatory agent: Implications to radiotherapy and chemotherapy", Journal of Cellular Physiology, Vol. 234 No. 5, pp. 5728–5740.

❖ Friedman, L.S., Abasilim, C., Fitts, R. and Wueste, M. (2020), "Clinical outcomes of temperature related injuries treated in the hospital setting, 2011–2018", Environmental Research, Vol. 189, p. 109882.

❖ Giang, V.H., Thuy, L.T., Cham, P.T., Vinh, L.B., Ban, N.K., Linh, T.M., Mai, N.C., et al. (2021), "Chemical constituents from Lycopodiella cernua and their anti-inflammatory and cytotoxic activities", Natural Product Research, pp. 1–7.

❖ Gong, G., Guan, Y.-Y., Zhang, Z.-L., Rahman, K., Wang, S.-J., Zhou, S., Luan, X., et al. (2020), "Isorhamnetin: A review of pharmacological effects", Biomedicine & Pharmacotherapy, Vol. 128, p. 110301.

❖ Gulilat, H., Saini, A.K. and Saini, R.V. (2021), "The comparative analysis of Pinus roxburghii phytoconstituents found at various Asian locations as determined by GC-MS and its medicinal properties", Medicinal Plants-International Journal of Phytomedicines and Related Industries, Society for Conservation and Resource Development of Medicinal Plants, Vol. 13 No. 3, pp. 396–408.

❖ Hassan, M.H.A., Elwekeel, A., Moawad, A., Afifi, N., Amin, E. and Amir, D.E. (2021), "Phytochemical constituents and biological activity of selected genera of family Crassulaceae: A review", South African Journal of Botany, Vol. 141, pp. 383–404.

❖ Hou, J., Zhang, Y., Zhu, Y., Zhou, B., Ren, C., Liang, S. and Guo, Y. (2019), "α-Pinene induces apoptotic cell death via caspase activation in human ovarian cancer cells", Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, International Scientific Information, Inc., Vol. 25, p. 6631.

❖ Iauk, L., Mangano, K., Rapisarda, A., Ragusa, S., Maiolino, L., Musumeci, R., Costanzo, R., et al. (2004), "Protection against murine endotoxemia by treatment with Ruta chalepensis L., a plant with anti-inflammatory properties", Journal of Ethnopharmacology, Elsevier, Vol. 90 No. 2–3, pp. 267–272.

❖ Imray, C.H.E. and Oakley, E.H.N. (2005), "Cold still kills: cold-related illnesses in military practice freezing and non-freezing cold injury", Journal of the Royal Army Medical Corps, Vol. 151 No. 4, pp. 218–222.

❖ Jijith, U.S. and Jayakumari, S. (2020), "An Apparatus for the determination of rat paw Edema during In vivo Evaluation of Anti-inflammatory agents", Research J. Pharm. and Tech, Vol. 13 No. 5, pp. 1–3.

❖ Kour, H., Raina, R., Verma, P.K., Pankaj, N.K. and Singh, S.P. (2019), "Phytochemical ingredients and pharmacological properties of Bergenia ciliata", Journal of Veterinary Pharmacology and Toxicology, Vol. 18 No. 1, pp. 1–10.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 02-2023
DOI 10.17605/OSF.IO/TNDVF

❖ Kuht, J.A., Woods, D. and Hollis, S. (2019), "Case series of non-freezing cold injury: epidemiology and risk factors", BMJ Military Health, British Medical Journal Publishing Group, Vol. 165 No. 6, pp. 400–404.

❖ Labib, R.M., Youssef, F.S., Ashour, M.L., Abdel-Daim, M.M. and Ross, S.A. (2017), "Chemical Composition of Pinus roxburghii Bark Volatile Oil and Validation of Its Anti-Inflammatory Activity Using Molecular Modelling and Bleomycin-Induced Inflammation in Albino Mice", Molecules, Multidisciplinary Digital Publishing Institute, Vol. 22 No. 9, p. 1384.

❖ Loizzo, M.R., Falco, T., Bonesi, M., Sicari, V., Tundis, R. and Bruno, M. (2018), "Ruta chalepensis L.(Rutaceae) leaf extract: chemical composition, antioxidant and hypoglycemic activities", Natural Product Research, Taylor & Francis, Vol. 32 No. 5, pp. 521–528.

❖ Mbhele, N., Ncube, B., Ndhlala, A.R. and Moteetee, A. (2022), "Pro-inflammatory enzyme inhibition and antioxidant activity of six scientifically unexplored indigenous plants traditionally used in South Africa to treat wounds", South African Journal of Botany, Vol. 147, pp. 119–129.

❖ Morita, R.Y. (1975), "Psychrophilic bacteria", Bacteriological Reviews, Vol. 39 No. 2, pp. 144–167.

❖ Odukoya, J.O., Odukoya, J.O., Mmutlane, E.M. and Ndinteh, D.T. (2022), "Ethnopharmacological Study of Medicinal Plants Used for the Treatment of Cardiovascular Diseases and Their Associated Risk Factors in sub-Saharan Africa", Plants, Multidisciplinary Digital Publishing Institute, Vol. 11 No. 10, p. 1387.

❖ Ortega, M.G., Agnese, A.M. and Cabrera, J.L. (2004), "Anticholinesterase activity in an alkaloid extract of Huperzia saururus", Phytomedicine, Elsevier, Vol. 11 No. 6, pp. 539–543.

❖ Ouerghemmi, I., Rebey, I.B., Rahali, F.Z., Bourgou, S., Pistelli, L., Ksouri, R., Marzouk, B., et al. (2017), "Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian Ruta chalepensis", Journal of Food and Drug Analysis, Elsevier, Vol. 25 No. 2, pp. 350–359.

❖ Porquis, H.C., Ang, A.M.G., Doblas, G.Z., Amoroso, V.B., Jacalan, D.R.Y., Batbatan, C.G. and Cruz, R.Y.D. (2018), "Anti-inflammatory, Antioxidant and Cytotoxicity Studies on Lycopodiella cernua (L.) J. Sm. in Bukidnon, Philippines", Asian Journal of Biological and Life Sciences, Vol. 7 No. 2.

❖ Qi, X., Lu, X., Sun, X., Lin, C. and Cui, C. (2022), "The regulatory effect of total flavonoids of Sedum aizoon L. on oxidative stress in type 1 diabetic mice", Current Research in Food Science, Vol. 5, pp. 1140–1147.

❖ Rao, V.L. and Mohan, N.V. (2021), "Impact of weather on sports and sport injuries", Int. J. Phys. Educ. Sports Health, Vol. 8, pp. 9–13.

❖ Recuenco, M.C., Lacsamana, M.S., Hurtada, W.A. and Sabularse, V.C. (2016), "Total phenolic and total flavonoid contents of selected fruits in the Philippines", Philippine Journal of Science, Vol. 145 No. 3, pp. 275–281.

❖ S Gad, S. (2018), "Effect of ginger as anti-inflammatory agent on serum nitric oxide, tumor necrotic factor α (TNF-α) and interleukin 4 (IL-4) in albino rats with carrageenan induced paw edema",Virol Immunol J, Vol. 2 No. 8, pp. 1-15.

❖ Sadhana, D., Parveen, S., Bukhari, N.I., Shehzadi, N., Qamar, S., Ijaz, A., Niazi, S.U., et al. (2018), "Bryophyllum pinnatum: Botanical Description, Vernacular Names, Parts Used, Traditional Uses, Phytochemical and Pharmacological Activities", Pakistan Journal of Pharmacy, Vol. 30 No. 1, pp. 3-7.

❖ Singh, S., Young, A. and McNaught, C.-E. (2017), "The physiology of wound healing", Surgery (Oxford), Vol. 35 No. 9, pp. 473–477.

❖ Taheri, Y., Suleria, H.A.R., Martins, N., Sytar, O., Beyatli, A., Yeskaliyeva, B., Seitimova, G., et al. (2020), "Myricetin bioactive effects: moving from preclinical evidence to potential clinical applications", BMC Complementary Medicine and Therapies, Vol. 20 No. 1, p. 241.

- ❖ Tebbutt, S., Harvey, M., Nicholson, T. and Cave, G. (2006), “Intralipid Prolongs Survival in a Rat Model of Verapamil Toxicity”, Academic Emergency Medicine, Vol. 13 No. 2, pp. 134–139.
- ❖ Ticona, L.A., Slowing, K., Serban, A.M., Bastante, M.H. and Hernáiz, M.J. (2022), “Wound healing, anti-inflammatory and anti-melanogenic activities of ursane-type triterpenes from Semialarium mexicanum (Miers) Mennega”, Journal of Ethnopharmacology, Elsevier, Vol. 289, p. 115009.
- ❖ Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.-H., et al. (2020), “Important Flavonoids and Their Role as a Therapeutic Agent”, Molecules, Vol. 25 No. 22, p. 5243.
- ❖ Umar, N.M., Parumasivam, T. and Toh, S.-M. (2021), “An Overview of Cutaneous Wounds and the Beneficial Roles of Medicinal Plants in Promoting Wound Healing”, Pharmaceutical Sciences, Tabriz University of Medical Sciences, Vol. 27 No. 4, pp. 489–502.
- ❖ Wu, K., Fu, M., Zhao, Y., Gerhard, E., Li, Y., Yang, J. and Guo, J. (2023), “Anti-oxidant anti-inflammatory and antibacterial tannin-crosslinked citrate-based mussel-inspired bioadhesives facilitate scarless wound healing”, Bioactive Materials, Vol. 20, pp. 93–110.
- ❖ Yadav, E., Singh, D., Yadav, P. and Verma, A. (2018), “Antioxidant and anti-inflammatory properties of Prosopis cineraria based phenolic rich ointment in wound healing”, Biomedicine & Pharmacotherapy, Vol. 108, pp. 1572–1583.
- ❖ Zafren, K. (2021), “Nonfreezing Cold Injury (Trench Foot)”, International Journal of Environmental Research and Public Health, Multidisciplinary Digital Publishing Institute, Vol. 18 No. 19, p. 10482.