Hunan Daxue Xuebao/Journal of Hunan University Natural Sciences
ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/KZT86
Vol: 60 | Issue: 05 | 2023
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ANTIOXIDATIVE EFFECTS OF PSYCHROPHYTES AGAINST COLD INDUCED PAW OEDEMA; AN IN VIVO AND IN VITRO APPROACH

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ABSTRACT

Oxidative stress ensues when disturbance occurs between body's antioxidants and reactive oxygen species ROS and this imbalance ushers in a series of ailments and other deleterious side effects. Antioxidants are cell's defense against the ROS as they can either quench their thirst of electrons or can impede the whole chain reaction. Plants possess plethora of secondary metabolites and are a renowned source of antioxidants because of the aforementioned compounds. The point of present research was to investigate the in vivo and in vitroantioxidant effects of 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 obtained from Minimerg, Pakistan on Dry Ice-Induced Paw inflammation in Albino Wistar Rats. DPPH radical scavenging activity was done along with antioxidant enzymes levels which include catalase, cyclooxygenase-2 (COX-2), superoxide dismutase (SOD), interleukin-6 (IL-6) and nitric oxide (NO_2). Statistically, regarding DPPH radical scavenging activity % age inhibition was maximum of 91.88% for leaves of P.cernua and minimum 29.34 % for leaves of P.roxburghii at the same concentration (100µL). % age DPPH inhibition was also observed to increase with increasing concentration. Catalase activity was maximum for leaf extracts of S.forsterianum (16.26 U/ml) and minimum for leaf extract of B.ciliata (8.27 U/ml). SOD was maximum for roots of P.roxburghii(50.26 U/ml) and minimum for roots of C.pellucida (10.47U/ml). NO_2 activity was maximally inhibited by roots of B.ciliata (0.546 µmol/L) while it was minimally inhibited by roots of P.cernua and R. chalepensis (1.788 µmol/L both). Maximum COX-2 inhibition was seen in the in the leaf extracts of S. forsterianum (23.25 U/ml); while minimum COX-2 inhibition was observed in the leaf extract of C.pellucida (70.98 U/ml). As far as IL-6 is concerned, maximum inhibition of Interleukin-6 (IL-6) was demonstrated by root extracts of S.forsterianum(32.50

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pg/L), while minimum inhibition of (IL-6) was demonstrated by root extracts of C.pellucida (180.00pg/L)These crude extracts could be purified and can be a beneficial source of antioxidants.

Keywords: *Antioxidants, Psychrophytes, Ice-induced inflammation, ROS, DPPH, catalase, COX-2*

1. INTRODUCTION

Nearly all stresses, whether biotic or abiotic, can generate a generic stress reaction and this reaction is termed as oxidative stress. Oxidative stress can be detrimental to cellular constituents and may lead to their debilitation (Chevallier *et al.*, 2020). Terminology of oxidative stress was first coined by Sies (Sies *et al.*, 1985) to chiefly explain derangement in the harmony of antioxidants and reactive oxygen ROS. The definition of oxidative stress has seen many modifications and presently oxidative stress is defined as "an event where a transient or permanent perturbation in the ROS balance-state generates physiological consequences within the cell, for which the precise outcome depends on ROS targets and concentrations" (Cervantes Gracia *et al.*, 2017). Oxidative stress can culminate in unredeemable chemical alterations(Caliri *et al.*, 2021).

Free radicals are extremely reactive molecules or atoms and contain one or more than one unpaired electron/ electrons in their valence shell. These radicals are generated when oxygen reacts with certain distinct molecules(Wang *et al.*, 2021). Intracellular generation of free radicals occur when they gain or lose an electron. Thus, they can either behave as an oxidant or can act as reductants (Tavsan and Kayali, 2019). Terminology of reactiveoxygen species (ROS) and reactive nitrogen species (RNS) is attributed to non-radical and radical by-products of nitrogen and oxygen which are reactive in nature (Manbir *et al.*, 2022). Reactive oxygen and nitrogen species (RONS) are synthesized in all cells that are aerobic in nature and play a meaningful part in the process of aging and age related ailments (Salminen, 2021). An ever increasing scientific documentation reveals that enzymes called as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases generate substantial quantities of ROS in the human body (Waghela *et al.*, 2021). Other enzymes involved in the production of copious amounts of ROS include oxidases containing flavoproteins and superoxide dismutase (Jakubczyk *et al.*, 2020). Still other in vivo generators of ROS includes endothelial cells, cytochrome P_{450} monooxygenase system of enzymes, xanthine oxidases, monocytes, neutrophils, nitric oxide (NO) synthases and lastly lipoxygenases (Sahoo *et al.*, 2022).Malfunctioning of mitochondria also plays a pivotal role in increased aggregation of RONS (Iuchi *et al.*, 2021).

ROS have been insinuated as major factors in the pathogenesis of numerous ailments due to their role in inactivation of enzymes, oxidation of proteins and lipids and mutilation of DNA. Diseases caused by ROS stretches from endocrine diseases like diabetes mellitus, atherogenic ailments like coronary diseases to neurodegenerative ailments like Alzheimer's and cancers(Henkel *et al.*, 2018).Subtle cerebral and neural afflictions that can enhance the risk of epilepsy in animals and humas are associated with expeditious generation of RONS (Terrone *et al.*, 2020). Oxidative stress perseveres during epileptogenesis in the forebrain of animal models and this has been confirmed by the increase of its markers (Pauletti *et al.*, 2019). Conspicuously, increased markers of oxidative stress can be detected in the duo of blood and brain of patients suffering from epilepsy (Shehta *et al.*, 2022). RONS initiates production of "oxidation-specific epitopes" by activating numerous alterations in various macromolecules such as proteins, lipids and DNA. These epitopes once generated, bring about the cellular inflammation which ultimately leads to cellular dysfunction (Lugrin *et al.*, 2014). Upsurge in the generation of ROS also causes disruption in biosignalling molecules which can lead to the commencement of apoptosis or unanticipated initiation. Consequently, enhancement of mitochondrial membrane permeability with subsequent increased pouring of cytochrome c from mitochondria into cytosol is seen. Uncoupling of oxidative phosphorylation can also ensue as a result

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of escalation of malondialdehyde (MDA)which is generated during the peroxidation of lipids (Puzanowska-Tarasiewicz *et al.*, 2008). ROS generated after degradation of ferritin by the process of autophagy also assist in the cellular death by a process named as ferroptosis (Hou *et al.*, 2016).

Simply put, antioxidants are molecules that inhibit the decay of oxygen. They have the ability to squelch or retard oxidation of other chemical compounds. In oxidation reactions, electrons are removed from a particular substance and shifted to the oxidizing agent. Antioxidants play a pivotal role in neutralizing free radicals before they can pose a threat to the cells (Bisht, 2018). Although synthetic antioxidants are available in the market but they carry with them strong side effects including hepatotoxicity. For this reason, there is greater predilection towards the discovery of natural antioxidants and one of the primary sources of antioxidants are plants(Imelda *et al.*, 2022). Plants are exceptional source of bioactive compounds which are named as phytochemicals and these chemicals play the role of antioxidants by hunting down RONS. Numerous plants can be employed therapeutically for ailments associated with RONS (Mfotie Njoya, 2021). Despite the fact that numerous plants lack proper documentation regarding their toxicity profiles, across the board cerebration is that medicinal compounds extracted from plants are comparatively safer than their synthetic equivalents (Nxumalo *et al.*, 2021). There has been an ever increasing demand for plant-derived antioxidant compounds that can curtail the adverse effects of ailments(Mani *et al.*, 2021).

Psychrophytes are defined as “Any plant that tolerates, or thrives in a cold climate, especially in arctic or alpine conditions”. These plants thrive under climates where the average annual temperature remains constantly below the freezing point of water i.e. 0 °C (Kotlyakov and Komarova, 2006). Numerous psychrophytes including those belonging to genus *Bergenia, Rumex, Cuscuta, Lyonia, Pinus, etc.* have been extensively used in folklore due to their far-reaching medicinal abilities(Bisht *et al.*, 2019; Gautam *et al.*, 2015; Man and Samant, 2011; Secim-Karakaya *et al.*, 2021; Shankar *et al.*, 2015). Present study is done to evaluate the effect of antioxidant effects of *Bergenia ciliata, Crassula pellucida, Ruta chalapensis, Rumex nepalensis, Sedum forsterianum, Palhinhaea cernua and Pinus roxburghii* on Dry Ice-Induced Paw Edema in Albino Wistar Rats.

2. MATERIAL AND METHODS

2.1 Collection and identification of Plants

Plants were collected in triplicates from Minimerg, Pakistan (34.7908°N 75.0799°E), stored at -80 °C to prevent stress, identified by informed taxonomists for voucher number at Department of Botany, Government College University, Lahore, and preserved at the University of Lahore, Lahore, with their botanical numbers.

2.2 Preparation of extracts

For the preparation of extracts, the leaves and roots of each plant were cut into small pieces separately, ground by hand in a pistil and mortar to increase the surface area accessible for contact, and centrifuged for 15 minutes at 3000 rpm utilizing centrifuge machine (M-800-LT) to obtain the supernatant. The supernatant (1 mg/mL) and residue were then preserved at -80 °C for subsequent use with traditional names and short labels (Table 1).

2.3 Ethical commendation

The Institute of Molecular Biology and Biotechnology's (IMBB) Ethical Committee in the University of Lahore granted the ethical nod of approval.

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2.4 Sorting and grouping of Rats

Total 102 albino Wistar Rats (250–300 g) of both genders were maintained in stainless steel restrainer boxes at the IMBB's animal house at The University of Lahore in Lahore under monitored humidity and temperature parameters (18–26°C) with unrestricted access to nutritious food (poultry feed no. 1) as well as water.

The following groups of animals were formed, each with half males and half females:

a) Vehicle= No infection and no intervention
b) Negative control group= Induced with inflammation by dry ice
c) Positive control group= Received Diclofenac sodium (Sami Pharmaceuticals, 25mg/ml), after dry ice-induced inflammation
d) Experimental group-I (EG-I)= Dry ice induced inflammation + P1L
e) Experimental group-II (EG-II)= Dry ice induced inflammation + P1R
f) Experimental group-III (EG-III)= Dry ice induced inflammation + P2L
g) Experimental group-IV (EG-IV)= Dry ice induced inflammation + P2R
h) Experimental group-V (EG-V)= Dry ice induced inflammation+ P3L
i) Experimental group-VI (EG-VI)= Dry ice induced inflammation+ P3R
j) Experimental group-VII (EG-VII)= Dry ice induced inflammation+ P4L
k) Experimental group-VIII (EG-VIII)= Dry ice induced inflammation+ P4R
l) Experimental group-IX (EG-IX)= Dry ice induced inflammation+ P5L
m) Experimental group-X (EG-X)= Dry ice induced inflammation+ P5R
n) Experimental group-XI (EG-XI)= Dry ice induced inflammation+ P6L
o) Experimental group-XII (EG-XII)= Dry ice induced inflammation+ P6R
p) Experimental group-XIII (EG-XIII)= Dry ice induced inflammation+ P7L
q) Experimental group-XIV (EG-XIV)= Dry ice induced inflammation+ P7R

2.5 Induction of Dry ice-induced inflammation in Limbs

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

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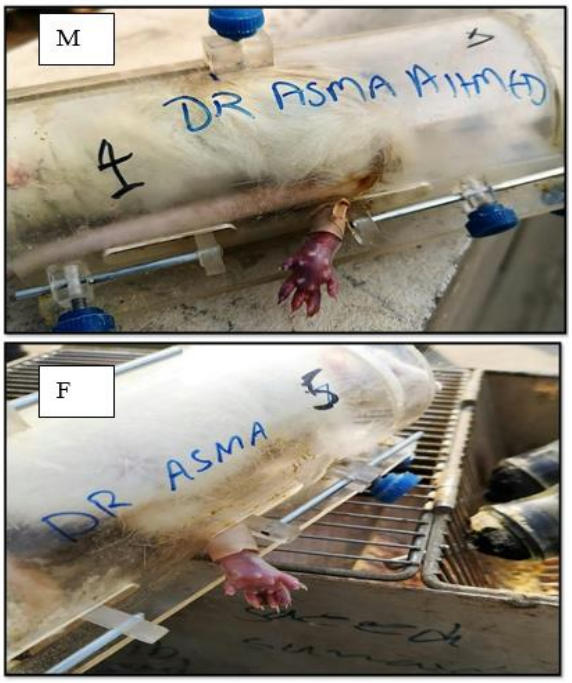

Figure 1: Cold-induced injury on plantar surfaces of rats after application of dry ice

M= Male F= Female

2.6 Induction of Anti-inflammatory drug / Plant extracts

With the aid of 1 cc syringes, 0.06 cc plant extract and Diclofenac sodium were injected on the plantar surface of the hind limbs of rats of all experimental groups and the positive control group, respectively, after one day of dry ice application. On the negative control group, no plant extract or anti-inflammatory pharmacotherapy was applied (Figure 1). Animals were monitored daily for changes and indications of toxicity across the whole study.

2.7 Determination of *In vitro* Antioxidant Activity

The technique was used to assess the antioxidant activity of the samples using the DPPH radical scavenging test (Brand-Williams *et al.*, 1995). 200 ml of ethanol (99.99 %) was added, followed by the addition of 0.0088g of DPPH solution in the flask and kept on stirring on an orbital shaker for around 15 to 20 minutes and to prevent oxidation, aluminium foil was placed on top of flask, till the appearance of purple color solution. Three test tubes were used for each sample, and each test tube contained 3mL of the DPPH solution. Then 1.0 mL of 20, 60, 100 µL of sample extract was added and measured the absorbance at 517nm by keeping a blank DPPH, whose had maximum absorption of 1.1488. The antioxidant activity of extracts was calculated using the formula:

$$\text{DPPH \%Inhibition activity} = \frac{Ao - Ac}{Ao} \times 100$$ Where:

A_o = Absorbance without extract A_c = Absorbance with extract

2.8 Determination of *In vivo* Antioxidant Activity

After recuperation, all the rat groups were anatomized and their blood samples were consigned to the laboratory for assessment of antioxidant enzymes levels.

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2.8.1 Estimation of catalase activity.

10 ml serum was added to 2.80 ml of 50 mM potassium phosphate buffer (pH 7.0), and the reaction was initiated by adding 0.1 ml of fresh 30 mM hydrogen peroxide. Decomposition rate of hydrogen peroxide was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to calculate catalase activity (Atawodi, 2011).

2.8.2 Estimation of superoxide dismutase (SOD).

Xanthine-oxidase system was used to generate a superoxide flux, and nitro blue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of the enzyme providing 50% inhibition of NBT reduction. Results are expressed as U/ml (Sun *et al.*, 1988).

2.8.3 Determination of nitric oxide concentration

100 µl of each of seven concentrations of $NaNO_2$ (100µM to 5µM) were mixed with the cell supernatants in a 96-well plate by keeping phosphoric acid solution (5%) as blank. Then 100 µl of Griess reagent was added and reacted in the dark for 5 minutes, followed by the measurement of absorbance at 540 nm with an ELISA reader. The $NaNO_2$ standard curve was prepared by plotting concentration against absorbance, and the regression equation, as well as correlation coefficient (γ), was obtained for quantization of NO_2^- in sample extracts (Yamamoto *et al.*, 1998).

2.8.4 Cyclooxygenase assays (COX-2)

The COX-2 assay described by Noreen *et al.*, (1998) with slight modifications was followed, in which four controls were run. Two were background in which the enzyme was inactivated with HCl before the addition of arachidonic acid, and two were solvent blanks. 200 µM indomethacin was included in each test assay as a standard. Assay was performed in duplicate with double determinations for each sample per assay. Percentage inhibition by the tested compound was calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank.

2.8.5 Determination of IL-6

Cytokine levels were determined by ELISA kit method according to the manufacturer's instructions. The concentration of IL-6 was measured using a DuoSet ELISA development kit following the standard procedure.

2.9 STATISTICAL ANALYSIS

All the data obtained was analyzed by using GraphPad v 8.0 (for Windows, GraphPad Software, San Diego, California USA).

3. RESULTS

3.1 Identification of Plants

The plants identified in the current investigation were assigned Botanical numbers and taxonomic classification (Table 1).

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Table 1: Classification of psychrophytes of Pakistan used in current study

P= Plant, L= Leaf, R= Roots

Plant name	Division	Class	Order	Family	Genus	Species	Common Names	Figures
P.cernua	Tracheophyta	Lycopodiopsida	Isoetales	Chaloneriaceae	*Palhinhaea*	*Cernua*	Nodding Club Moss	2 (a)
B.ciliata	Tracheophyta	Magnoliopsida	Saxifrages'	Saxifragaceae	*Bergenia*	*Ciliata*	Fringed elephant's ears	2 (b)
R.chalepensis L.	Tracheophyta	Magnoliopsida	Sapindales	Rutaceae	*Ruta*	*Chalepensis*	Fringed rue	2 (c)
R.nepalensis	Tracheophyta	Magnoliopsida	Caryophyllales	Polygonaceae	*Rumex*	*Nepalensis*	Nepal dock	2 (d)
C.pellucida	Tracheophyta	Magnoliopsida	Saxifragales	Crassulaceae	*Crassula*	*Pellucida*	Stonecrop	2 (e)
P.roxburghii	Tracheophyta	Pinopsida	Pinales	Pinaceae	*Pinus*	*Roxburghii*	Chir, Chil	2 (f)
S.forsterianum	Tracheophyta	Magnoliopsida	Saxifragales	Crassulaceae	*Sedum*	*forsterianum*	Rock stonecrop	2 (g)

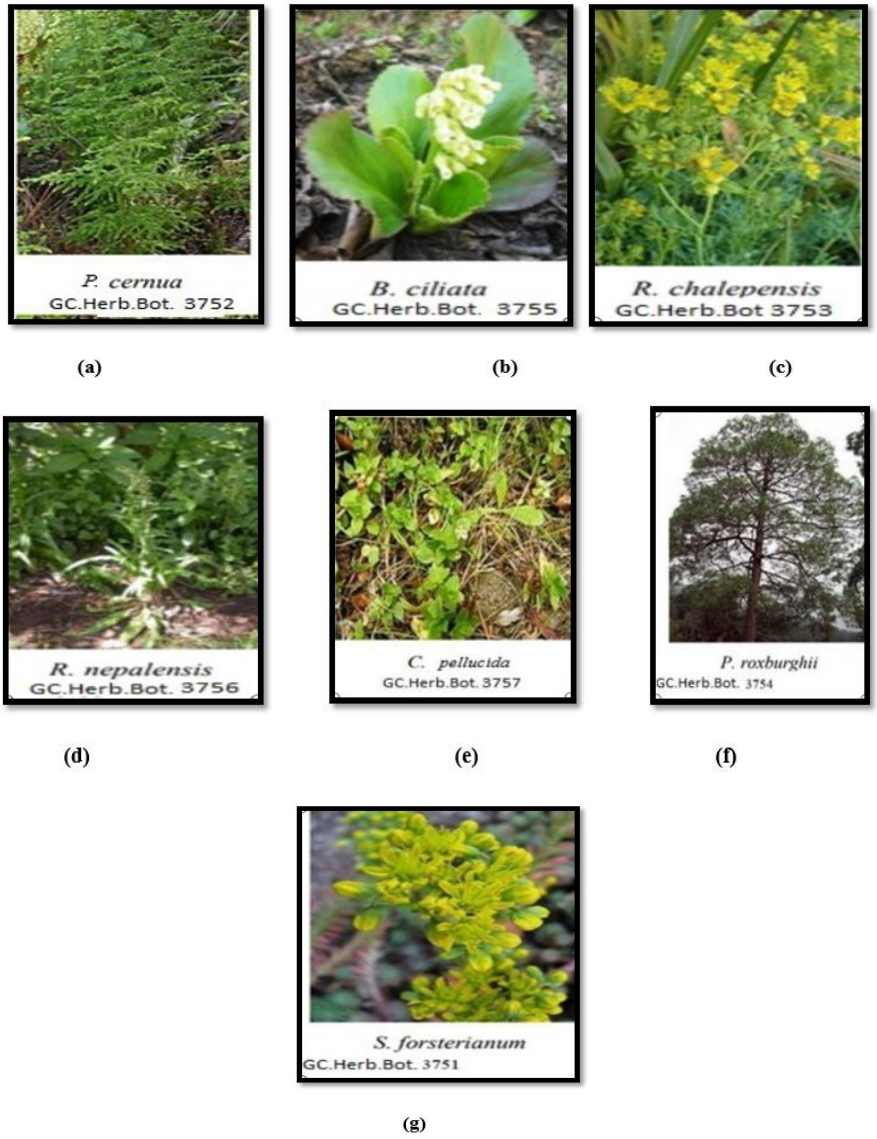

Figure 2: Identified plants used in the study (Plants are mentioned with scientific names and their botanical numbers . GC= Government College

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3.2 *In vitro* Antioxidant activity of leaf and root extracts

Antioxidants are compounds that can delay inhibiting the oxidation process. The reaction took place in the presence of atmospheric oxygen or reactive oxygen species (ROS). Highest content of antioxidants were present in the leaf extract of *P.cernua* and this effect increased in a dose dependant manner (18.37, 77.38, and 91.88 %) of extract concentrations and in the same manner second-highest antioxidant potential was observed by the root extracts of *P.cernua* (13.97, 63.57, and 91.25 %). Least antioxidant potential was observed by the leaf extract of the *P.roxburghii* (3.87, 14.53, and 29.34%) and the activity was seen increasing with the rise of extract concentration (Figure 2).

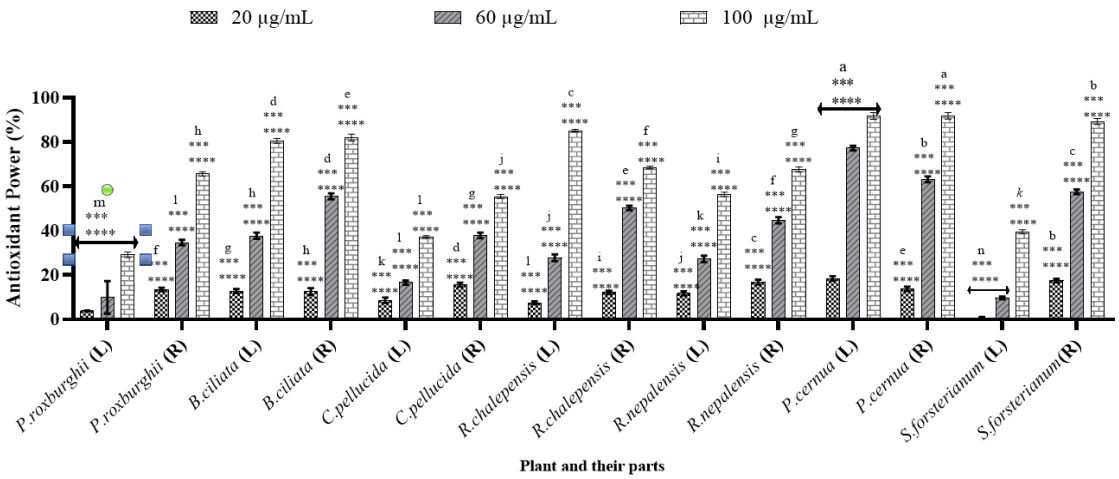

Figure 2: Comparison of the in vitro antioxidant (DPPH scavenging) activity of leaf and root extracts of plants

Where L: extracts of leaves of plants; R: extracts of roots of plants. *** Present comparison among different concentrations (highly significant difference at p= 0.0003) while **** represent comparison among psychrophytes and their parts (leaves and roots) (extremely significant difference at p< 0.0001)

3.3 *In vivo* antioxidant activity of Psychrophytes

Serum catalase activity was found to be maximum in leaf extracts of *S.forsterianum* (16.26 U/ml) closely followed by root extract of *R.nepalensis* (16.17 U/ml) and was minimum by leaf extracts of *B.ciliata* (8.27 U/ml).The extract also produced a dose-dependent increase in the serum catalase activity as the group treated with 400 mg/kg of plant extracts had significantly (< 0.05) higher catalase activity as compared to others. Minimum catalase level was shown by the leaf extract of *P.cernua* (8.27 U/ml). A dose-dependent increase in the serum level of superoxide dismutase activity was also shown as serum superoxide dismutase activity of the groups treated with plant extracts was significantly (< 0.05) higher as compared to negative control group. Maximum SOD activity was observed by the leaf extract of *P.roxburghii* (50.264150 U/ml) while least was observed by root extract of *C.pellucida* (10.471698 U/ml). Maximum NO_2 inhibition was seen by the root extracts of *B.ciliata* (0.54659 µmol/L) while minimum inhibition was observed by the root extracts of *P.cernua* and *R.chalepensis* (1.78884 µmol/L each). Maximum COX-2 inhibition was shown by the leaf extracts of *S.forsterianum* (23.25 U/L) whereas least COX-2 inhibition was observed by the leaf extract of *C.pellucida* (70.98 U/L). Maximum inhibition of Interleukin-6 (IL-6) was demonstrated by root extracts of *S.forsterianum* (32.50 pg/L) whereas minimum inhibition of IL-6 was demonstrated by root extracts of *C.pellucida* (146.67 pg/L) (Table 2 and Figure 3).

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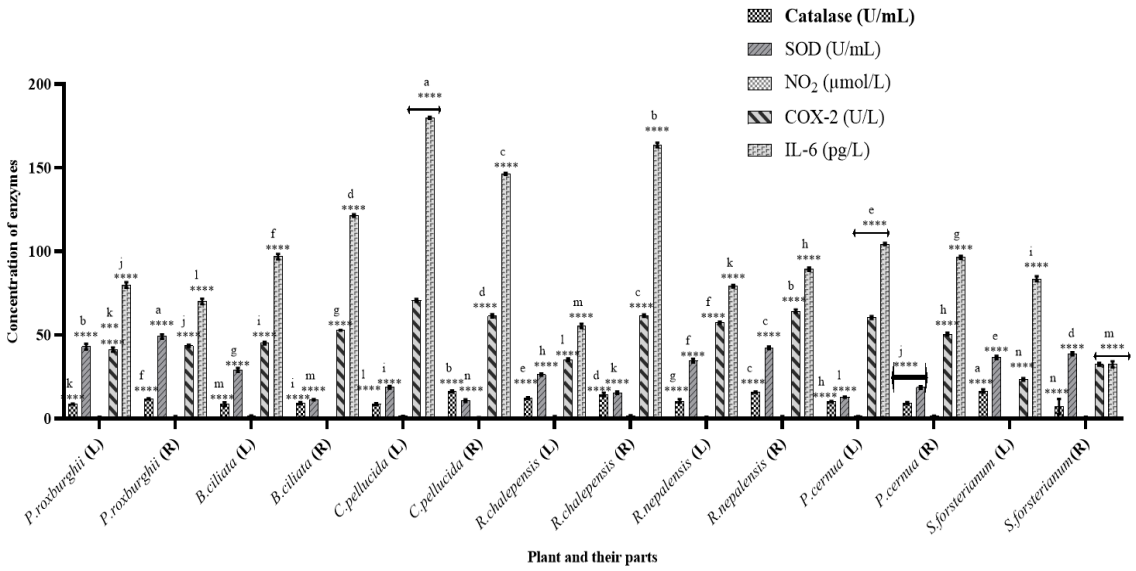

Figure 3: In vitro antioxidant potential of Psychrophytes of Pakistani origin

Where L: Leaves; R: Roots. **** represent comparison among psychrophytes and their parts (leaves and roots) (extremely significant difference at p< 0.0001). SOD: Superoxide dismutase; COX-2: Cyclooxygenase 2; IL-6: Interleukin 6; NO_2= Nitrogen dioxide

DISCUSSION

Free radicals, which comprises of reactive nitrogen species (RNS) and radical oxygen species (ROS), are outgrowths of cellular metabolism and are implicated in biological mechanisms including immunity, differentiation, mitogenic response, and cytokine generation as well as cellular redox modulation(Sun *et al.*, 2020). ROS can cut both ways, ROS mediate redox signalling pathways that induce plant growth, development, and stress tolerance when present below the cut-off point. Under extreme duress, on the contrary, the rate of ROS generation rapidly rises, outpacing the capacity of antioxidant scavengers, causing an oxidative burst that damages biomolecules and throws off cellular redox equilibrium (Sachdev *et al.*, 2021).

Medicinal plants have become a necessary part of human society since civilization started. Medicinal plants are the boon of nature to treat several ailments of human beings. Plants contain bioactive molecules and thus can provide lead structures for the development of alternative medicines to counter currently available toxic commercial drugs with better effectiveness and increased safety. Inflammation is an intricate response of an organism's vascular tissue when subjected to a wide array of external potentially harmful stimuli such as irritants, damaged cells and foreign pathogens(Levick *et al.*, 2007). Mechanisms associated with inflammatory responses usually involve arachidonic acid and its metabolites can be produced wither by a cyclooxygenase pathway or lipoxygenase pathway (Wang, Fu, *et al.*, 2019). The mechanisms involved in these processes and customarily engage reactive oxygen species (Torres *et al.*, 2018). Accordingly, safeguarding against ROS with the help of secondary metabolites of plants would inadvertently safeguard against inflammation (Naz *et al.*, 2020). Numerous studies have highlighted the importance of employment of antioxidant potential of secondary metabolites of plants for the management of ailments induced by oxidative stress (Pawlowska *et al.*, 2019). Because of their potential to guard against oxidative stress, flavonoids and other phenolic compounds rank among the most valuable chemical components of phytochemical constituents(Yu *et al.*, 2015).

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The present study showed that *in vitro* antioxidant potential of plant extracts as depicted by %age DPPH inhibition was found maximum in the extracts of *P.cernua* which was around 91.88% while the content of antioxidant was found many a times lower in *C.pellucida*but the provided contraction of extracts was the same in all. According to the present experimentation, plant parts of *P.cernua* were having higher amount of antioxidants and these results were further compared with the outcomes of the studies conducted by others which suggested that leaf extract of this plant is blessed with higher content of antioxidants (Porquis *et al.*, 2018).Moreover, previous experimentation also confirmed the presence of antioxidants in the said plant (Baang *et al.*, 2015). The antioxidant properties of *P.cernua*have also been exhibited by Giang et. al who also discovered novel antioxidants in the aforementioned plant (Giang *et al.*, 2022). With reference to *P.roxburghii,* although its leaf extracts showed lesser % inhibition (29.34%) but the root extracts gave much better results (65.85%) at the same concentration level (100 μg/ml). This can be attributed to the fact that root extracts exhibit greater antioxidant activity as compared to leaves extracts as exhibited by Tshabalala et. al (Tshabalala *et al.*, 2020) while working on *Moringa oleifera*plant.

In relation to *in vivo* antioxidant capabilities of plant extracts; catalase activity was found to be maximum in leaf extracts of *S.forsterianum.* It has also been exhibited by other researchers who found that catalase activity of the aforementioned plant genus increased when exposed to stressful conditions (Habibi and Hajiboland, 2012). Recently published studies have also highlighted the beneficial role of *Sedum* species in the management of oxidative stress-induced ailments such as gastric ulcer owing to its enhanced catalase activity(da Luz *et al.*, 2021). Root extracts of *R.nepalensis*were a close second to *S.forsterianum's* with regards to catalase activity. Numerous species belonging to genus *Rumex* have exhibited this characteristic of enhanced catalase activity and have been used in the treatment of ailments because of the said characteristic(Qazi *et al.*, 2022).

Superoxide dismutase (SOD) are a family of metalloenzymes and act as frontline warriors in human body's fight against free radicals (Younus, 2018). Among our plants, highest SOD was depicted by the root extracts of *P.roxburghii.* Analogous findings of *P.roxburghii*have been made by Labib et. al, (Labib *et al.*, 2017) who also highlighted increased malondialdehyde (MDA), myeloperoxidase (MPO) and tumor necrosis factor-α (TNF-α) levels with accompanying enhancement in activities of superoxide dismutase (SOD) and catalase (CAT) enzymes.Similar findings were also seen in other species of *Pinus* genus by Bhardwaj et. al, (Bhardwaj *et al.*, 2022).Another contemporary study conducted by Singh et. al, (Singh *et al.*, 2021) used*P. gerardiana's* nutextracts in diabetic rats and they displayed appreciable increase in SOD activity along with enhanced total thiol groups, total serum as well as hepatic antioxidant capacity(p <0.001).

Cyclooxygenase 2 (COX-2) also known as Prostaglandin-endoperoxide synthase 2 or prostaglandin G/H synthaseis the enzyme required to breakdown arachidonic acid to PGH2 which eventually produces prostaglandins that are mediators of inflammation (Krisnamurti and Fatchiyah, 2019). The COX-2 enzyme plays no part in protecting the stomach lining and is only activated during inflammatory responses or malignancy. Therefore, specific COX-2 inhibition would substantially lessen the adverse limitations of conventional NSAIDs, such as GI tract impairment and hepatotoxic consequences (Sharma *et al.*, 2019). Among our plants maximum COX-2 inhibition was seen in the leaf extracts of *S.forsterianum* (23.25 U/ml). Bruna et. al, (da Luz *et al.*, 2019) investigated another species of *Sedum* genus namely *Sedum dendroideum*and exhibited its gastroprotective effects by nitric oxide production, GSH levels, and stomach wall mucus stabilization without underlying antisecretory effects. Second best result among our other plants was exhibited by leaf extract of *R.chalepensis* (35.00 U/ml). a study conducted by Kasem et. al, (Kacem *et al.*, 2015) showed that through the reduction of nuclear

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factor-B (NF-B) activation, therapy with *R.chalepensis* dramatically resulted in significant reduction of iNOS and COX-2 gene expression.

The rapid and temporary production of interleukin 6 (IL-6), in response to infections and tissue injury, aids in host immune response by stimulating acute phase actions, haematopoiesis, and immunological responses(Tanaka *et al.*, 2014).Among proinflammatory cytokines, several studies have indicated that perhaps the pleiotropic cytokine interleukin-6 (IL-6), which itself is generated by various cell types, contributes to the suppression of the apoptosis and proliferation of tumour cells(Waldner *et al.*, 2012). The expression of IL-6 is linked to a greater risk of colorectal cancer, according to numerous publications(Kraus and Arber, 2009). IL-6 expression has been shown to be upregulated in both tumour tissue and CRC patients (Tertis *et al.*, 2019). The physiological connection between IL-6-induced signalling and chronic inflammatory response through oxidative stress spurred on by the impact of reactive oxygen species on DNA repair mechanisms is considered to be the fundamental cause of CRC, according to increasing amounts of data(Yu *et al.*, 2020).The evidences demonstrates that IL-6 is essential for ovarian cancer metastasis, which encompasses the migration, multiplication, infiltration, survival, and chemoresistance of ovarian tumour cells, via inducing pathways like JAK/STAT3 and procedures like epithelial-to-mesenchymal transition(EMT)(Browning *et al.*, 2018). Among our plants maximum inhibition of Interleukin-6 (IL-6) was demonstrated by root extracts of *S.forsterianum* (32.50 pg/L). Study conducted by Wang et. al, (Wang, Zhang, *et al.*, 2019) demonstrated that *Sedum sarmentosum* extract exhibited their cytoprotective potential via inhibition of IL-6 levels.

Nitrogen dioxide (NO_2) is an oxidizing free radical that can often commence a variety of damaging processes in biological systems. It is hypothesized that both externally and intracellularly generated NO_2 may be associated with a variety of ailments. Although it is considered that peroxynitrite (ONOO—/ONOOH) is a significant endogenous reservoir of NO_2 radicals, other sources, including enzymatic ones, have also been reported(Kirsch *et al.*, 2002).By modulating Th1/Th2 maturation and triggering the JAK-STAT pathway, exposure to nitrogen dioxide (NO_2) induces airway inflammation(Ji *et al.*, 2015).NO2 levels have been used as a marker of oxidative stress (Marefati *et al.*, 2018). In our experiment, roots of *B.ciliata* showed maximum inhibition of the abovementioned oxidative marker. One of the most studied metabolite that has been isolated from *Bergenia* is berginin (Zafar *et al.*, 2019). Derivatives of berginin have been reported to be good inhibitors of nitric oxide (Qiu *et al.*, 2022).

4. CONCLUSION

These unprocessed extracts are used to isolate, analyze, and locate active compounds. In the future, they could well be processed and employed to alleviate cold-induced inflammation. In control groups, we employed the prescribed medication dicloran for both genders in the positive control group, demonstrating positive recovery, whilst the negative control group was not administered any drug therapy throughout the course of the study, and inflammation worsened with time in this particular group.

5. FUNDED BY

Present course of work was funded by HEC, Government of Pakistan, under NRPU grant No: 8175.

DOI 10.17605/OSF.IO/KZT86
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6. ACKNOWLEDGMENT

Authors are highly acknowledged to the Higher Education Commission (HEC) Pakistan and Government of Pakistan for providing funds under NRPU to complete this work. Moreover authors also acknowledge officers and soldiers of Pakistan Armed forces who provided their immense support for the collection of plants from LOC Minimerg Pakistan.

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