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CONTENTS

Antioxidant and anti-inflammatory properties of sun-dried leaves and fruits of wild *Pyracantha crenulata* (D. Don) M. Roem.

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ABSTRACT: *Pyracantha crenulata* (D. Don) M. Roem. (locally known as Ghingharu in Kumaun region) is a medicinally important wild fruit crop endemic to Himalayan hills. A laboratory experiment was conducted to evaluate the antioxidant properties of methanolic and aqueous extracts of leaves and fruits of the plant employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant power (FRAP) and Anti-inflammatory assays. The total phenolic and flavonoid contents were also calculated. Highest antioxidant activity was observed in the leaves (methanolic extract) for which the DPPH scavenging activity had the lowest Inhibitory concentration$_{50}$ (IC_{50}) value (26.12 µg/mL) and FRAP activity was the highest (27.63 µg Gallic acid equivalents/ mg extract). The methanolic extract of leaves also showed the highest protein denaturation inhibition percent with the lowest IB_{50} value (44.77µg/mL). Moreover, the maximum phenolic (16.217µg Gallic acid equivalents/mg) and flavonoid content (24.487 µg/mg Quercetin equivalents/mg) were also possessed by the methanolic extract of leaves. The study concluded that the dried leaves of *P. crenulata* possess significantly higher antioxidant properties than the dried fruits. Hence, not just the fruits but leaves of *P. crenulata* can also be used for medicinal purpose.

Key words: Medicinal plant, pharmacology, phytochemistry, *Pyracantha crenulata*, wild fruit

Many common and age-related degenerative diseases are the result of oxidative damage created by reactive oxygen species (ROS) that are generated as a byproduct of normal metabolic activities (Touyz, 2004). Controlled production of ROS in organisms is essential for the activity of signal transduction pathways but high levels of ROS can damage and modify cellular proteins, lipids, and nucleic acids that may promote many degenerative diseases and can weaken the immune system. Human body has powerful enzymatic and non-enzymatic antioxidant systems that control oxidative damage induced by ROS, but stress factors and aging can make these mechanisms inefficient (Seifried *et al.*, 2007). Therefore, supplementation of natural antioxidants through diet becomes a requirement in today's life. Fruits, vegetables and medicinal plants contain various types of antioxidants which exhibit potent antioxidant activity. Their consumption has been found to offer protection against degenerative diseases (Wong *et al.*, 2006). Dietary antioxidants can augment cellular defences and help to prevent oxidative damage to cellular components. The medicinal importance of many plants, throughout the world, is due to their potent antioxidant activities (Amarowicz and Pegg, 2019).

Pyracantha crenulata (D. Don) M. Roem., a wild fruit shrub belonging to the family Rosaceae, is of great importance among the medicinal plant wealth endemic to Himalayan hills. Its fruits are consumed by wild animals as well as humans. These are valued for their pleasant taste and cardiotonic properties. The consumption of fruits is believed to lower the risk of heart attack and hypertension, arteriosclerosis. The dried fruits are consumed with curd to cure dysentery (Sultana *et al.*, 2017; Singh *et al.*, 2018). Several biological activities have been reported in *P. crenulata* (such as antioxidant, anti-inflammatory, antimicrobial, larvicidal, and cytotoxic properties), most of which are attributed to their fruits (Sharifi-Rad *et al.*, 2020). The use of leaves to make herbal tea has been reported and a few studies are there that showed anti-inflammatory and antibacterial properties of leaves of *P. crenulata* (Saklani and Chandra, 2014, Sultana *et al.*, 2017,

Semwal *et al.*, 2020). Owing to the traditional medicine uses of *P. crenulata* the present study was aimed to assess the antioxidant properties of both sun-dried leaves and fruits of *P. crenulata* using methanol and water as solvents for extraction via DPPH scavenging activity and ferrous reducing antioxidant power assay (FRAP assay). Anti-inflammatory activity, the total flavonoid and phenolic contents were also assessed.

MATERIALS AND METHODS

The fresh leaves and ripe fruits of *P. crenulata* (Fig. 1) were collected in July, 2022 from Kafara naugaon, Almora, Uttarakhand (1,646 m ASL, 29.6007 ° N, 79.5800 ° E) during the time of fruit ripening and seed maturation (Shah *et al.*, 2006). Plant material (Leaves and fruits, 500g each) was collected from approximately six individual healthy shrubs present in the area. The plant material was washed, sun-dried and stored in the airtight containers. Pulverized material was used for the experiments.

Preparation of plant extracts

The dried material of fruits and leaves was ground with an electric grinder (Bajaj, Majesty GX4 500 W). The coarse fruit material was further pulverized with pestle and mortar to obtain a fine textured powder. Two solvents, methanol and water were used for preparing crude extract. Four grams of fine powder of the sample in 400 mL of solvent (methanol and water, 1:100, w/v) was subjected to extraction by using the Soxhlet apparatus (Khera Instrument Pvt. Ltd.). The liquid extract obtained from the Soxhlet apparatus was first condensed using a rotary evaporator (U- tech) and then poured into Petri plates in thin films for further evaporation. The crude extract was obtained by scraping off the dried material from the Petri plates and it was stored at 4°C for further analyses. The yield of extracts was determined by using the following formula:

$$\text{Yield (\%)} = \frac{\text{Total crude extract(g)}}{\text{Dried powder (g)}} \times 100$$

The working solution was prepared by mixing 10 mg of the crude extract in 10 mL of the solvents. Samples were designed as follows- LM= Methanolic extract of leaves, LW= Aqueous extract of leaves, FM= Methanolic extract of fruits, FW= Aqueous extract of fruits.

Qualitative phytochemical analysis

For screening of the presence or absence of phytochemicals in the leaf and fruit extracts of *P. crenulata*, standard tests described by Harborne (1973) were applied.

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) scavenging assay

DPPH free radical scavenging activity is used to assess antioxidant activity by assessing the change in violet colour of the DPPH solution. DPPH is a free radical that forms a diamagnetic molecule by receiving free radicals. On addition of an antioxidant, the colour of DPPH solution fades from violet to bleached (yellow) as antioxidant molecules due to the hydrogen-ion-donating capacity quench the DPPH free radicals, decreasing the absorbance of the solution (Amarowicz *et al.*, 2004). DPPH radical scavenging activity (%) of the plant extract was analyzed according to the method used by Shen *et al.* (2010). One mL of standard (butylated hydroxytoluene, BHT) or plant extract (20 to 100 µg/mL concentrations of leaf extracts and standard; 200 to 1000 µg/mL conc. of fruit extracts) or solvent (for control) was mixed with 3 mL of 0.1 mM DPPH solution. The reaction mixture was incubated in dark for 30 min. The absorbance of the reaction was measured using UV-Vis Spectrophotometer (Genesys, Thermo-Fisher) at 517 nm. The scavenging activity was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(ac - as)}{ac} \times 100$$

Where, ac= the absorbance of the control sample and as= the absorbance of the plant sample reactions. Calibration curves were plotted using the percentage values of DPPH free radical scavenging activity (response) against the concentrations (dose) of plant samples and standard. The inhibitory concentration$_{50}$ (IC_{50}) values were determined by linear regression analysis of the 'dose-response' curve (Patel and Patel, 2011). The IC_{50} value of a sample with respect to DPPH assay is the concentration at which the

sample shows 50% of the free radical scavenging. The low IC_{50} value of a sample represents the high antioxidant property.

Fe^{3+} Reducing Antioxidant Power (FRAP) assay

Reducing power is an evaluation of a substances' ability to donate electrons in the antioxidant activity. When Fe^{3+} is reduced, it produces a blue colour. The higher reducing capacity is shown by an increase in absorbance (Gohari *et al.*, 2011). The Fe^{3+} reducing power of the plant extract was assayed by using the method suggested by Gohari *et al.* (2011). Fresh FRAP reagent was prepared by mixing acetate buffer (300 mM, 3.6 pH), 2, 4, 6-tripyridyl-s- triazine (10 mM in 40 mM HCl solution), and $FeCl_3.6H_2O$ (20 mM) in the ratio of 10:1:1. 1 mL of extracts (concentrations same as used for DPPH assay) was reacted with 2 mL of reagent. The absorbance was determined at 593 nm using UV-Vis Spectrophotometer (Genesys Thermo-Fisher) after 30 min of incubation at 37ºC. The FRAP values were expressed in µg gallic acid equivalents (GAE) determined by using a standard curve of the Gallic acid that was made by the same method.

Anti-inflammatory assay

To assess the percent inhibition of protein denaturation the method described by Bahuguna *et al.* (2023) was used. 2 mL of diclofenac sodium (standard) or extracts (extract concentrations were same as used for DPPH assay) was mixed with 2.8 mL of phosphate buffer (pH = 6.4). For protein, egg albumin (isolated from chicken eggs) was chosen and 0.2 mL of isolated albumin was added to the reaction mixture. Thus, 5 mL of the total volume of reaction mixture was prepared. The reaction mixture was incubated at 37 °C for 15 min, and then at 70 °C for 5 min. The absorbance was determined at 660 nm using UV-Vis Spectrophotometer (Genesys, Thermo-Fisher). The inhibition in protein denaturation activity (%) was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{(bc - bs)}{bc} \times 100$$

Where, bc= the absorbance of the control sample and bs= the absorbance of the plant sample reactions. Denaturation of protein is one of the causes of inflammation. In the inhibition of protein denaturation assay, the saline buffer causes the denaturation of the protein (egg albumin). Powerful antiinflammatory substances added to the test samples are likely to inhibit the denaturation of albumin protein. The high inhibition percent of denaturation of egg albumin protein denotes the high antiinflammatory activity (Kar *et al.*, 2012). The inhibitory concentration$_{50}$ (here denoted by IB_{50}) is the concentration required to inhibit 50% of the protein denaturation reaction. It was determined according to the above mentioned method that was used to determine IC_{50} values.

Quantitative Phytochemical analysis

Total phenolic content (TPC)

Folin-Ciocalteu reagent was used for determining the total phenolic content according to the method of Velioglu *et al.* (1998). The phenolic estimation method by Folin-Ciocalteu reagent-based assay

Table 1: Qualitative phytochemical screening of *P. crenulata* extracts

	Test used	Plant metabolite	*P. crenulata* extracts			
			LM	LW	FM	FW
1.	Mayer's test	Alkaloids	-	-	-	-
2.	H_2SO_4 test	Flavonoids	+	+	+	+
3.	Ferric chloride test	Phenols	+	+	+	+
4.	Molisch's test	Carbohydrate	+	+	+	+
5.	Ninhydrin test	Protein	+	+	+	+
6.	Lead acetate	Tannins	+	+	+	+
7.	HCl test	Quinones	-	-	-	-
8.	Keller-Kiliani test	Cardiac Glycosides	+	+	+	+

Where, LM= Methanolic extract of leaves, LW= Aqueous extract of leaves, FM= Methanolic extract of fruits, FW= Aqueous extract of fruits

Table 2. IC_{50} values of DPPH free radical scavenging activity in standard vs. *P. crenulata* extracts

Samples	IC_{50} (µg/mL)
Standard: butylated hydroxytoluene (BHT)	20.22
Methanolic extract of leaves	26.12
Aqueous extract of leaves	49.76
Methanolic extract of fruits	676.73
Aqueous extract of fruits	894.81

Table 3: IB_{50} values of anti-inflammatory activity in standard vs. *P. crenulata* extracts

Samples	IB_{50} (µg/mL)
Standard: diclofenac sodium	18.91
Methanolic extract of leaves	44.77
Aqueous extract of leaves	63.28
Methanolic extract of fruits	790.15
Aqueous extract of fruits	1017.26

Table 4: Total Flavonoid content (TFC, µg QE /mg extract). Among groups (Mean ± SE) ANOVA was applied. Significant difference (p< 0.05) is indicated by different lowercase letters according to DMRT

Samples	Concentration	
	200 µg/mL	500 µg/mL
Methanolic extract of leaves	15.53 ± 0.22^{f}	33.43 ± 0.31^{g}
Aqueous extract of leaves	6.01 ± 0.11^{c}	14.5 ± 0.19^{e}
Methanolic extract of fruits	5.53 ± 0.12^{c}	10.71 ± 0.20^{d}
Aqueous extract of fruits	0.40 ± 0.15^{a}	1.49 ± 0.2^{b}

relies on the transfer of electrons from phenolic compounds to phosphomolybdic or phosphotungstic acid complexes to form blue coloured complexes (Sánchez-Rangel *et al.*, 2013). The reaction mixture was prepared with 0.5 mL of extracts (concentrations same as used for DPPH assay) and 0.2 mL Folin-Ciocalteu reagent. After 5 min., 7% sodium carbonate (Na_2CO_3) was added to the mixture. The absorbance was measured at 765 nm after 1 hr. of incubation using UV-Vis Spectrophotometer (Genesys, Thermo-Fisher) and the final values were expressed in ¼g gallic acid equivalents (GAE) using a gallic acid standard curve that was made by the same method.

Total flavonoid content (TFC)

The total flavonoid content in the samples was analyzed by the method used by Chang *et al.* (2002). The extracts of leaves and fruits (200 and 500 µg/mL concentrations) were mixed with 2% aluminium chloride. The aluminnium chloride assay utilises $AlCl_3$ as a complexing agent. Flavonoids bind metal to ions Al (III) and make yellow coloured complexes (Shraim *et al.*, 2021). Absorbance of the samples was measured at 420 nm after 60 min of incubation using UV-Vis Spectrophotometer (Genesys, Thermo-Fisher). The final values were expressed in µg quercetin equivalents (QE) by using a quercetin standard curve.

Each experiment consisted of three replications and was repeated twice. The results were interpreted as the Mean ± SE (standard error). One way ANOVA followed by Duncan's Multiple Range Test (significance level of 5%) was applied in the results using SPSS 16.0 version, Graphs were made using Microsoft Excel.

RESULTS AND DISCUSSION

In this study, the maximum yield was obtained for the aqueous and methanolic extracts of the leaves (26.08% and 24.73%) and the minimum yield was for the aqueous extract of fruits (15.72%) The yield for methanolic extracts of fruits was 24.08%. Overall, methanol gave better extraction yield for both leaves and fruits of *P. crenulata* using the Soxhlet apparatus. Different solvents extract different quantities of metabolites from different plants based on their polarity (Naudiyal and Kumar, 2023). Leaves of *Epipremnum aureum* yielded 3.33% extract *in* methanol and 7.11% extract in water solvents (Sherikar and Mahanthesh, 2015). Whereas, the extraction yields of 23.7% and 11.6% for *Ixeris polycephala* and *Youngia japonica* were found using the same aqua-methanol solvent for both plants (Rawat and Rao, 2020). The extraction yield of the fruits of *Prunus laurocerasus* was 42.8 g/100 g, which exceeded that of the leaves at 38.6 g/100 g (Karabegovi *et al.*, 2014). The aggregate sample of fruits and leaves of *P. crenulata* in the study done by Sati (2017) had the extractive values of 12.50 and 15% in water and ethanol respectively.

Qualitative phytochemical analysis

Standard tests were used for phytochemical screening of important plant metabolites. Table 1 briefs result from the preliminary phytochemical screening. The findings of this study revealed

a b

Fig. 1: a) Sun-dried fruits and b) Leaves of *Pyracantha crenulata*

presence of alkaloids, flavonoids, tannins, phenolics and cardiac glycosides in the priliminary investigation.These classes of secondary metabolites are very potent against a number of diseases. The presence of above-mentioned components and sterols and terpenoids in the aggregate sample extract of leaves and fruits was also reported by Sati (2017) in their study.

DPPH radical scavenging assay

The DPPH scavenging activity in all the tested samples increased in a concentration-dependent manner. The IC_{50} values were determined for each sample (Table 2). The methanolic extracts of both the leaves and fruits showed higher scavenging activity than their aqueous counterparts (Fig.2). The leaves, however, showed significantly higher antioxidant activity than the fruits in both solvents. In the present study, the methanolic extract of the leaves showed minimum IC_{50} value *i.e.* 26.12 µg/mL that is very close to the IC_{50} value of standard (BHT, 20.22 µg/mL). The aqueous extract of fruits showed the maximum IC_{50} value *i.e.* 894.81 µg/mL and hence exhibited the minimum antioxidant activity. Our results are in accordance with the previous study done on *P. crenulata*. by Guglani *et al.* (2021) where alcoholic extract of leaves of *P. crenulata* exhibited maximum antioxidant activity with the lowest IC_{50} values for ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (0.029 mg/mL) and DPPH (0.047 mg/mL) antioxidant assays. In their study, the alcoholic extracts of the plant samples showed better antioxidant potential than the aqueous extracts. They concluded that the leaves of *P. crenulata* had the highest antioxidant activity followed by the roots, stem and the fruits. The results can also be compared to the IC_{50} values for DPPH assays in the common medicinal plants used in herbal decoction. For the extracts of *Ocimum sanctum* (ethanol-water), the IC_{50} value was 34.2 µg/mL (Gupta *et al.*, 2012), and for the aqueous-acetone extract of Mentha haplocalyx, it was 45.7 µg/mL (She *et al.*, 2010).

Fe^{3+} reducing antioxidant power (FRAP) assay

The ferrous-reducing power shown by all four extracts increased with the incresing concentration of the extracts (Fig.3). Leaf methanolic extract at 100µg/mL conc. had a higher FRAP value i.e.,

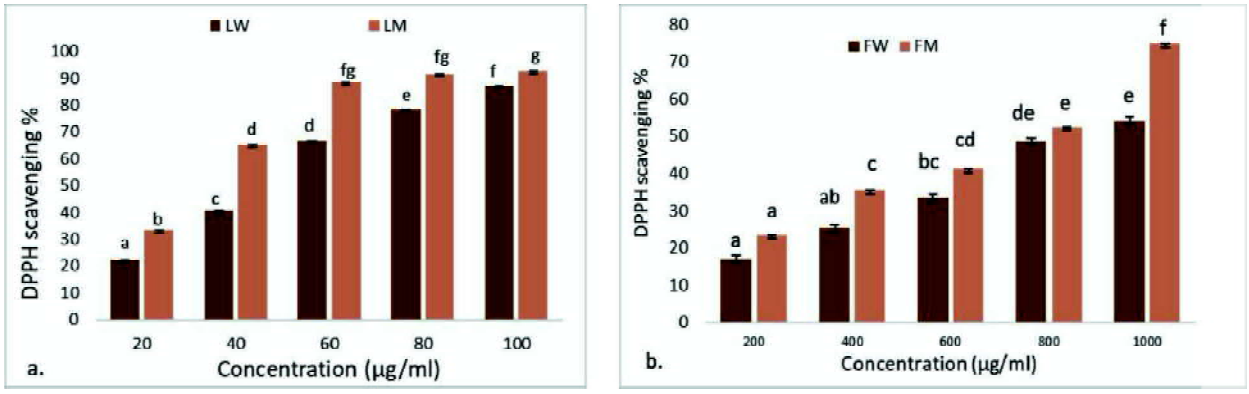

Fig. 2: DPPH free radical scavenging activity (%) shown by methanolic and aqueous extracts of a.) leaves and b.) fruits of *P. crenulata* at varying concentration. Among groups (Mean±SE) ANOVA was applied. Significant difference (p< 0.05) is indicated by different lowercase letters according to DMRT

Where, LM= Methanolic extract of leaves, LW= Aqueous extract of leaves, FM= Methanolic extract of fruits, FW= Aqueous extract of fruits.

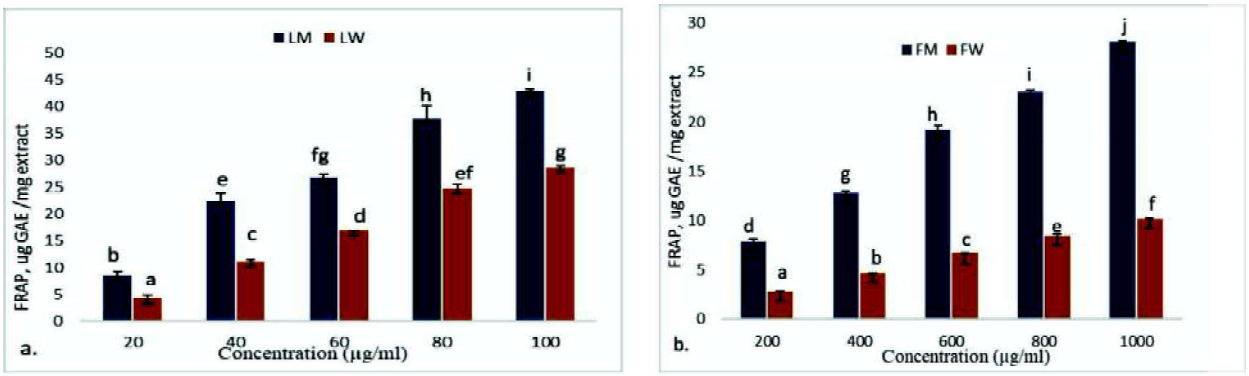

Fig. 3: Fe^{3+} reducing power (FRAP, µg GAE /mg extract) of methanolic and aqueous extracts of a.) leaves and b.) fruits of *P. crenulata* at varying concentrations. Among groups (Mean±SE) ANOVA was applied. Significant difference (p< 0.05) is indicated by different lowercase letters according to DMRT

Where, LM= Methanolic extract of leaves, LW= Aqueous extract of leaves, FM= Methanolic extract of fruits, FW= Aqueous extract of fruits.

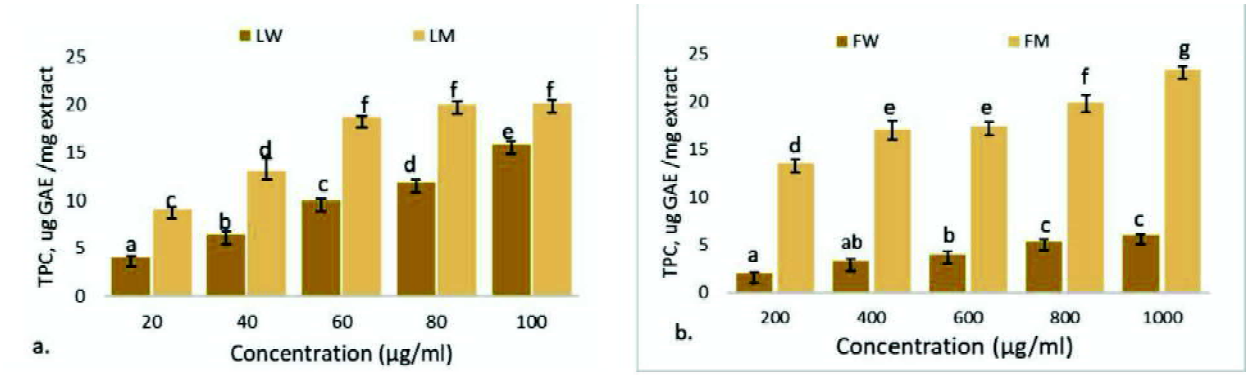

Fig. 4: Total phenol content (TPC, µg GAE /mg extract) in methanolic and aqueous extracts of a.) leaves and b.) fruits of *P. crenulata* at varying concentrations. Among groups (Mean±SE) ANOVA was applied. Significant difference (p< 0.05) is indicated by different lowercase letters according to DMRT

Where, LM= Methanolic extract of leaves, LW= Aqueous extract of leaves, FM= Methanolic extract of fruits, FW= Aqueous extract of fruits.

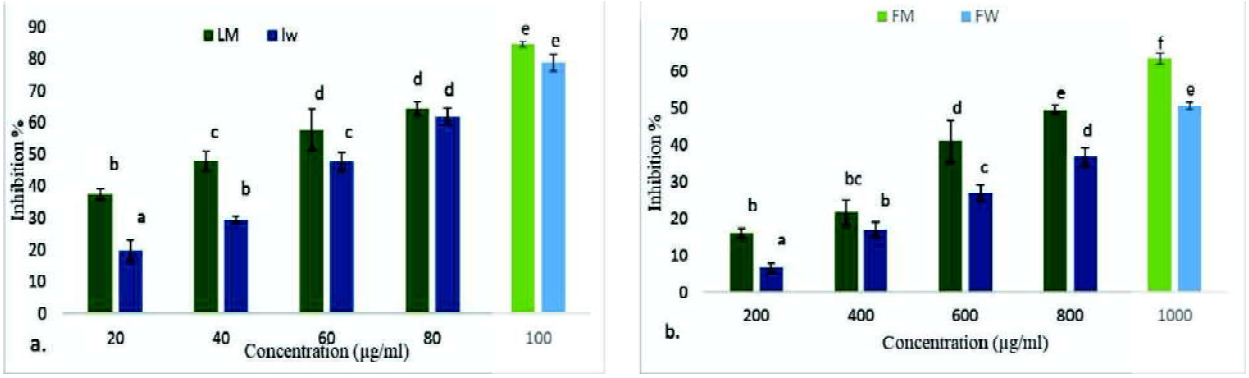

Fig. 5: Inhibition of protein denaturation (%) shown by methanolic and aqueous extracts of a.) leaves and b.) fruits of *P. crenulata* at varying concentrations. Among groups (Mean±SE) ANOVA was applied. Significant difference (p< 0.05) is indicated by different lowercase letters according to DMRT

Where, LM= Methanolic extract of leaves, LW= Aqueous extract of leaves, FM= Methanolic extract of fruits, FW= Aqueous extract of fruits.

42.74±0.27 µg GAE (Gallic acid equivalent) /mg extract, than the leaf aqueous extract (28.53 ±0.3 µg GAE /mg extract) at the same extract conc. Similarly, the fruit methanolic extract at 1000 µg/mL conc. had a higher FRAP value of 27.93±0.09 µg GAE /mg extract than the fruit aqueous extract (10.19±0.14 µg GAE /mg extract). The highest average FRAP value in our study was 27.63±1.04 µg GAE /mg extract, that was shown by the methanolic extract of leaves followed by the FRAP average 17.09± 0.22 µg GAE /mg extract, shown by the aqueous extract of leaves. This value was close to the FRAP average shown by the methanolic extract of fruits (17.81± 0.22 µg GAE /mg extract) and the lowest FRAP average was of the aqueous extract of fruits (6.54±0.1 µg GAE /mg extract). In another study the average FRAP value in the aqueous extract of leaves of *Epipremnum aureum* was 1.93 which was higher than that found in the methanolic extracts, i.e. 1.716 (Sherikar, and Mahanthesh, 2015). This disparity in the FRAP values may stem from the uneven distribution of phytochemicals across different parts such as leaves and fruits in different plants, which might extract out in varying amounts in different solvents.

Antiinflammatory assay

The concentration dependant increase in the inhibition (%) of albumin denaturation was observed among all the samples (Fig.5). The IB_{50} of the leaf methanolic extract was the lowest among all the plant samples (44.77 µg/mL) but it was more than double to the value of standard's 18.91 µg/mL (Table 3). Similar to DPPH assay, here also the higher inhibion percentage was shown by methanolic extracts of leaves and fruits. The lowest inhibition (6.6± 1.32%) at 200 µg/mL extract concentration was shown by the aquous extracts of fruits and the highest inhibition (84.50±0.81%) was shown by the methanolic extracts of leaves at 100 µg/mL extract concentration. A similar trend was reported by Akoto *et al.* (2020) where the inhibition (%) of egg albumin denaturation by using the ethanol and hexane extracts of fruits of *Ocimum basilicum* was close to 6 and 26% respectively at 1000 µg/mL extract concentration. In another study by Bahuguna and Rawat, (2014) to assess the antiinflamatory property of ripe or semiripe fruits of *P. crenulata,* the egg albumin denatutration inhibition assay was performed. They used the fruit juice extract and fruit semisolid mass extract of *P. crenulata*. The fruit juice extract at 50 µg/mL showed inhibition of 96.65% whereas fruit semisolid mass extract at the same concentration exhibited 91.07% inhibition (Bahuguna and Rawat, 2014). This suggests that various extraction and storage methods can lead to significantly different results.

Quantitative phytochemical analysis

A strong positve coorelation of phytoconstituents with high antioxidant and antiinflamatory activities has been reported in many studies. Thus, quantifying phytoconstituents becomes crucial. This allows accurate assessment of the levels of these compounds in plant extracts. Stojiljkovi *et al.* (2016) conducted a study to evaluate the antioxidant activities of wild apple (*Malus sylvestris*) by employing DPPH and FRAP assays. They correlated the outcomes with the total phenolic content (TPC), total flavonoid content (TFC), total tannin (TT), and total anthocyanin (TA) contents of the plant. The study revealed a direct correlation, indicating that higher concentrations of secondary metabolites in the plant corresponded to increased antioxidant potential.

Phenolics are one of the largest classes of plant secondary metabolites. They are diverse in structure and are found containing hydroxylated aromatic rings. They are regarded as the molecules with the highest potential to neutralize free radicals and are responsible for attributing the antioxidant, anti-inflammatory, anticancerous activities in plants (Kabera *et al.*, 2014). The total phenolic content was calculated for five concentrations of all the samples as shown in Fig. 4. The methanolic leaf extract showed a total phenolic content of 20.13±0.37 µg GAE/mg extract at 100 µg/mL extract conc., whereas the aqueous leaf extract had a TPC value of 15.82±0.34 µg GAE/mg extract at the same extract conc. The methanolic fruit extract showed a total phenolic content of 23.37±0.35 µg GAE/mg extract at 1000 µg/mL conc., whereas the aqueous fruit extract had a TPC value of 6.04±0.08 µg GAE/mg extract at the same extract concentration. In the study

of Guglani *et al.*, (2021) on *P. crenulata* a similar trend was found for the plant parts and the and solvents used for extraction. The leaves exhibited maximum phenolic content (128 mg/g) in the ethanolic extracts, which was significantly higher than the TPC of aqueous counterpart of the leaf extract (77 mg/g). The TPC was the lowest for fruit, at 21 mg/g for aqueous extract and close to 40 mg/g for ethanolic extract.

Flavonoids are the most prevalent and ubiquitous class of polyphenolic compounds. Three groups-anthocyanin, flavones and flavonols represent the flavonoids of the plants. Flavonoids possess various biological activities such as antiinflammatory, antioxidant, antidiabetic, antirheumatic, anti-carcinogenic etc. The total flavonoid content was calculated for 200 and 500 µg/mL extract concentrations for all the four extracts (Table 4). The methanolic leaf extract showed the highest average value for TFC (24.48±0.22 µg QE/mg extract) whereas, the aqueous extract of fruits had the lowest average TFC value, i.e. 0.95±0.20 µg QE/mg extract. In the study of Guglani *et al.*, (2021) the TFC was higher for the ethanolic extract (23 mg/g) than the aquous extract (10 mg/g) of leaves. The flavonoid content 5.46 mg/g fw and phenol content 05.59 mg GAE/g fw, in the fresh fruits of *P. crenulata* was reported by Saklani *et al.* (2012) that can be juxtapositioned to the results we got from our study done in the dried fruits.

CONCLUSION

The findings of this study revealed presence of alkaloids, flavenoids, tannins, phenolics and cardiac glycosides in the priliminary investigation of the leaves and fruits of *P. crenulata*. Subsequently, detailed investigation on the antioxidant, antiinflammatory and quantitative phytochemical assays by using a range of concentraions of the extracts was performed. This provided a clear understanding of the potency of the medicinal plant at specific concentrations in the respective aspects. In the present study, methanolic extracts gave significantly higher extraction yield, total phytochemical contents and exhibited higher antioxidant and antiinflammatory activities than the aqueous extracts for both leaves and fruits. The leaves of *P. crenulata* exhibited superior antioxidant properties compared to the fruits. While the fruits are commonly utilized, there is limited usage of the leaves among the public. Our study validates the utilization of *P. crenulata* leaves for herbal tea preparation, highlighting the health-promoting benefits. Additionally, our findings suggest potential medicinal applications such as anti-aging, immunoprotective, and immunity-boosting properties associated with the consumption of these wild fruits and leaves. This study provides a foundational framework and suggests the need for further investigation to identify the active compounds responsible for the antioxidant and anti-inflammatory properties exhibited by the plant leaves.

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