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Assessment of the antioxidant and anti-inflammatory activities of *Ipomoea pes-tigridis* L. leavesS. Sameemabegum[♦], T. Prabha*, S. Sribhuvanewari, T. Ravisankar**, B. Pavithra** and S. Somasundram**

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Abstract

According to ethnomedical information, the herb *Ipomoea pes-tigridis* L. which belongs to the Convolvulaceae family, is known as "Tiger foot" or "Morning glory". The goal of this study was to look at a hydroalcoholic extract of *I. pes-tigridis* leaves for its total phenol, total flavonoid, antioxidant, and anti-inflammatory properties. The contents of total phenol and total flavanoids were determined by the aluminium chloride assay method and the folincia-calteau assay method, respectively. Antioxidant activity was assessed using the DPPH method, the nitric oxide scavenging method, hydrogen peroxide, and the ferrous reducing power assay method. Anti-inflammatory activity was evaluated by the HRBC membrane stabilisation method and the protein denaturation assay method. In DPPH, nitric acid, hydrogen peroxide, and reducing power assays, *I. pes-tigridis* demonstrated a dose-dependent radical scavenging effect. The extract's IC₅₀ for free radicals was 88.82 mcg/ml, 165.96 mcg/ml, 138.47 mcg/ml, and 247.89 mcg/ml in the DPPH, nitric oxide, hydrogen peroxide, and reducing power assay methods, respectively. Also, the IC₅₀ for inhibition achieved by the extract was 88.43 mcg/ml and 189.43 mcg/ml in the membrane stabilisation and protein denaturation assays. According to the findings, the hydroalcoholic extract of *I. pes-tigridis* has significant antioxidant and anti-inflammatory activities that are dose-dependent and are correlated with phenol and flavonoidal compounds. Hence, the plant has promising antioxidant and anti-inflammatory properties due to phenolic and flavonoidal compounds.

1. Introduction

Today's medications are either made synthetically or from substances found in nature. Significant occurrences over the past three decades have already led to profound shifts in the public and scientific perceptions of herbal medicine. Millions of people around the world have developed an appreciation for all things natural as a result of their unhappiness with the efficiency and price of contemporary medicine (Ebadi, 2002; Agarwal *et al.*, 2006). Fruits and vegetables are high in the phytonutrients flavonoids and polyphenols. Colored vegetables and fruits are very rich sources of flavonoids and polyphenolic compounds, which are called phytonutrients. Flavonoids, like other phytonutrients, are typical antioxidants with anti-inflammatory properties (Sivakumar *et al.*, 2022). Many medicinal plants are reported to have antioxidant, antidiabetic, hepatoprotective and neuroprotective properties, antibacterial, and anti-inflammatory effects (Bhatt *et al.*, 2019; Duraisamy *et al.*, 2021).

In ethnomedicine, the herb *Ipomoea pes-tigridis* L. (Convolvulaceae) was referred to as a spreading or twining herb. It is an annual herb that can be found practically everywhere in India, growing up to 4000 feet above sea level from the plains to the coast and frequently

in fertile lands. The climber blooms all year long. The plant can be found pretty much everywhere in India, mainly in fields, bushes, hedges, grasslands, waste regions, and near the seashore. It flourishes during the monsoon in North India and stays lush and succulent for 3-4 months. Additionally, tropical East Africa and tropical Asia are home to it (Chopra *et al.*, 1956). It serves as a remedy for boils, carbuncles, and dog bites. As a poultice, the leaves were used to treat boils, sore and carbuncles. A purgative effect of the plant root was discovered (Mathew *et al.*, 1981) and stem used for the treatment of cancer and tumours (Chowdhury *et al.*, 2014). The leaf powder used to get relief when smoked for asthma. Various authors reported *I. pes-tigridis* possess different therapeutic effect. Benzenepetroleum ether extract of *I. pes-tigridis* leaves possess antioxidant, cytotoxicity, and neuropharmacological activity (Hossain *et al.*, 2017). The present study was focused on evaluating the antioxidant activity, anti-inflammatory effects, and their correlation between phenolic and flavonoidal compounds of hydroalcoholic extract of *I. pes-tigridis* leaves (Figure 1).

Figure 1: Habitat and leaves of *Ipomoea pes-tigridis* L.

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2. Materials and Methods

2.1 Collection and preparation of plant extract

The plant *Ipomoea pes-tigridis* L. leaves were collected from the roadside area of Madurai. The plant was identified and authenticated by Dr. L. Stephen, Botanist, American College, Madurai and also by Dr. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirapalli. The voucher specimen (PARC/ 2012/1222) was kept for future reference. The fresh plant material collected was thoroughly cleaned and air-dried. The fresh plant material underwent a thorough cleaning and air drying. After that, it was homogenised and converted into a coarse powder. Then, it was extracted with 70% ethanol by a triple maceration process. The extract was collected and evaporated to dryness in the open air. The dried extract was used for further studies.

2.2 Estimation of total phenolic content

The 70% ethanolic extract of *I. pes-tigridis* was used to determine the amount of phenolic acid using the Folin-Ciocalteu reagent assay (Singleton *et al.*, 1979). 1 ml of a stock solution and 0.5 ml of Folin-Ciocalteu reagent (1N) were combined in a test tube and let to stand for 15 min. To this, 1ml solution of 10% sodium carbonate was then added. Finally, 10 ml of distilled water was added to the mixture. After 30 min of standing at room temperature, the total phenol content was measured spectrophotometrically at 760 nm. Gallic acid served as a benchmark substance. Gallic acid, a common medication, was used to create a calibration curve of concentration versus absorbance at various concentrations (2, 4, 6, 8, and 10). In order to calculate the total phenol content, regression analysis was used. The result was represented as mg of gallic acid equivalent/g of extract (mg GAE/g).

2.3 Analysis of total flavonoid content

The total flavonoid concentration was estimated using the aluminium chloride colorimetric method (Chang *et al.*, 2002; Siddique *et al.*, 2010). For a stock solution of 1mg/ml, an aliquot of quercetin was dissolved in ethanol. Additional dilutions were performed to obtain concentrations between 20 and 100 mg/ml. In each volumetric flask, 1 ml of the above standard solution was placed. After adding 0.1 ml of potassium acetate solution, 0.1 ml of aluminium chloride solution and 2.8 ml of ethanol, the final volume was brought to 5 ml by adding distilled water. At 415 nm absorbance was measured after 20 min. As a control, a sample devoid of aluminum chloride was used. Using linear regression analysis, it is possible to determine how much flavonoids are present. The mg of quercetin equivalents/g of extract was used to express the overall flavonoid content.

2.4 Evaluation of antioxidant activity

2.4.1 DPPH assay method

According to the method of Pavithra *et al.* (2020), with little modification, DPPH scavenging activity was performed. 4 ml of DPPH were added to the 1 ml test solutions of different concentration. It was recommended to carry out the reaction for around 30 min in the dark. Finally, by using ethanol as blank, whereas ascorbic acid as a reference standard, the absorbance of sample combinations was measured at 517 nm. The following formula used to get percentage scavenging:

$$\text{Percentage inhibition} = [(\text{Control} - \text{Test})/\text{Control}] \times 100$$

2.4.2 Nitric oxide scavenging assay method

The procedure described by Karpagavalli *et al.* (2021), was followed with just minor alterations for the activity that scavenges nitric acid. Using this procedure, 1 ml of sodium nitroprusside in phosphate buffer (pH 7.4) was combined with 1 ml of plant extracts at various concentrations, and the mixture was then incubated at 25°C for 30 min. 0.5 ml of naphthyl ethylene diamine dihydrochloride and 1 ml of sulphanilamide in phosphoric acid were added to 1.5 ml of the incubated mixture. Reagent served as a blank for the measurement of absorbance at 546 nm, while ascorbic acid served as the reference standard. In order to determine the proportion of nitric oxide radical scavenging that was stopped, the following formula was used:

$$\% \text{ inhibition} = [(\text{Control} - \text{Test})/\text{Control}] \times 100$$

2.4.3 Hydrogen peroxide assay method

The following solutions were combined: 3.8 ml of 0.1M phosphate buffer solution (pH 7.4), 0.2 ml of hydrogen peroxide solution, and 1ml of various concentrations of plant extract (40, 80, 120, 180, and 200 mg/ml). After 10 min, the reaction mixture's absorbance was measured at 230 nm using ascorbic acid as a standard and a reaction mixture without any sample as a blank. The formula was applied to calculate the hydrogen peroxide inhibition percentage. The percentage inhibition is equal to $100/(\text{A control} - \text{A sample})$. In order to derive the linear regression equation, a concentration versus % inhibition graph was generated (Karpagavalli *et al.*, 2021).

2.4.4 Reducing power assay method

Phosphate buffer (2.5 ml), potassium ferricyanide (2.5 ml), and 1ml of various quantities of hydroalcoholic extract solution were combined and incubated for 20 min at 50°C. Trichloroacetic acid (2.5 ml) was added to the mixture, after it had been incubated, and it was centrifuged at 3000 rpm for 10 min. With ascorbic acid as a reference and phosphate buffer as a blank solution, 1.5 ml of supernatant solution, 1.5 ml of distilled water, and 0.5 ml of ferric chloride were used to measure the absorbance at 700 nm in the UV-Visible spectrophotometer (Koleva *et al.*, 2002; Ruch *et al.*, 1989). The formula used to determine the decreasing power was $(\text{A control} - \text{Asample})/\text{Acontrol} \times 100 = \% \text{ inhibition}$.

2.5 Evaluation of anti-inflammatory property by membrane stabilisation assay

Anti-inflammatory activity was carried out utilising the Sadique *et al.* (2010) method as modified by Oyedapo and Famurewa (1995); Oyedapo *et al.* (2002).

2.5.1 Preparation of HRBC suspension in isosaline

Healthy volunteers who had abstained from NSAID use for two weeks previous to the experiment's start had their blood drawn. Alsever's solution, which contains 2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride was combined with an equal volume of blood before being centrifuged at 3000 rpm. The packed cells were then rinsed in isosaline before being prepared with 10% v/v erythrocyte suspension in isosaline.

2.5.2 Experimental procedure

In order to prepare a stock solution containing 2 mg/ml of *I. pes-tigridis* hydroalcoholic extract, ethanol was used as the solvent. This was followed by adding different amounts of the extract (0.2

to 1 ml) to 2 ml of hyposaline, 1 ml of phosphate buffer, and 0.5 ml of HRBC suspension in isosaline. The remaining capacity was then filled with isosaline to a total of 4.5 ml. The control was made in the same way as described above, with the exception that the drug was left out, and the drug control was made in a similar way but without HRBC suspension. The reaction mixture was heated in a water bath to 56°C for 30 min, after which the tubes were chilled. At 560 nm, the absorbance of the released haemoglobin was measured. Additionally, diclofenac sodium was employed as a benchmark. The formula for percentage membrane stabilisation was used to calculate the substance percentage activity in membrane stabilisation:

(A test – A product control) 100/Acontrol = % membrane stabilization

2.6 Evaluation of anti-inflammatory activity by protein denaturation method

2.6.1 Preparation of test solutions

The 0.45 ml of bovine serum albumin (5% w/v aqueous solution) was added with 0.05 ml of various concentrations of hydroalcoholic extract. The test control solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of distilled water. Additionally, the product control consists of 0.45 ml of distilled water and 0.05 ml of extract (various concentrations of hydroalcoholic extract in µg/ml) whereas, standard solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of diclofenac sodium solution (Gunathilake *et al.*, 2018).

2.6.2 Experimental method

All the above test samples were adjusted to pH 6.3 using 1N

hydrochloric acid. They performed 20 min of incubation at 37°C, 3 min of heating at 57°C, and then a cooling period. 2.5 ml of phosphate buffer (pH 6.3) was added with the all prepared above solutions. With the help of UV spectrophotometer (416 nm) the absorbance was measured using phosphate buffer (pH 6.3) as a blank (Karpagavalli *et al.*, 2019). By using the formula, the percentage inhibition of protein denaturation was determined:

$$\text{Percentage inhibition} = 100 - \left\{ \frac{\text{OD of test solution} - \text{OD of control}}{\text{control}} - 1 \right\}$$

Protein denaturation at 100% is represented by the control. Both the extract and diclofenac sodium were displayed on a graph using concentration versus % inhibition of protein denaturation.

3. Results

3.1 Estimation of total phenolic content

The results of the total phenolic content in a 70% ethanolic extract of *I. pes-tigridis* were displayed in Table 1 and Figure 2. For gallic acid, the linear regression equation was $y = 0.092x - 0.028$, and the correlation coefficient was 0.994. Using the mentioned regression equation, it was determined that the phenolic content of the ethanolic extract was 51.41 mg GAE/g of extract.

3.2 Estimation of total flavonoid content

The presence of total flavonoid content in the hydroalcoholic extract of *I. pes-tigridis* is shown in Table 2 and Figure 3. The linear regression equation was found to be $0.0307x - 0.0432$ and using the linear equation, it was found that total flavonoid content (mg quercetin equivalent/g) of hydroalcoholic extract of *I. pes-tigridis* was calculated to 23.59.

Table 1: Total phenol content in hydroalcoholic extract of *I. pes-tigridis*

S.No.	Conc. of gallic acid in µg/ml	Absorbance at 760 nm	Conc. of 70% ethanol extract in µg/ml	Absorbance at 760 nm*	Amount of total phenolic content intermsmg GAE/g of extract*
1	2	0.229 ± 0.010	50	0.257 ± 0.001	44.833 ± 0.44
2	4	0.452 ± 0.006	100	0.672 ± 0.004	58 ± 0.471
3	6	0.695 ± 0.005		Average	51.41 ± 5.37
4	8	0.918 ± 0.028			
5	10	1.162 ± 0.031			

Values are presented as*mean ± SEM (n = 3).

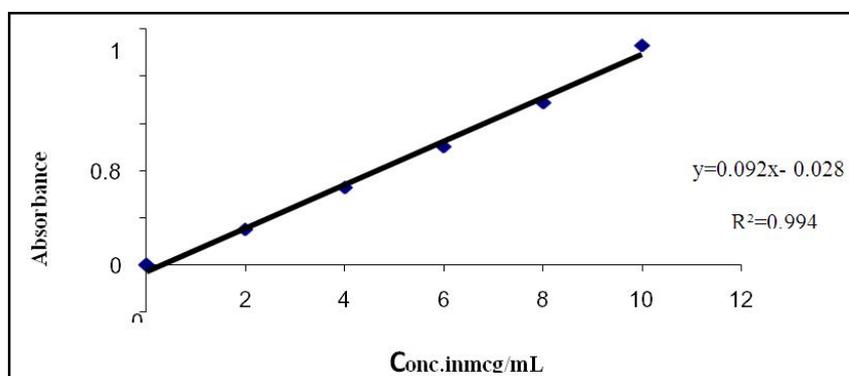


Figure 2: Calibration curve of gallic acid.

Table 2: Total flavonoid content of plant extract by aluminium chloride method

S. No.	Conc. of quercetin in µg/ml	Absorbance at 415 nm	Conc. of ethanol extract in µg/ml	Absorbance at 415 nm	Amount of total flavonoid content in terms mg quercetin in equivalent/g of extract
1	20	0.589 ± 0.01	100	0.039 ± 0.0003	26.80 ± 0.066
2	40	1.151 ± 0.04	200	0.082 ± 0.0034	20.39 ± 0.060
3	60	1.710 ± 0.09			
4	80	2.390 ± 0.03			
5	100	3.112 ± 0.03			
Average					23.59

Values presented as *mean of three readings ± SEM.

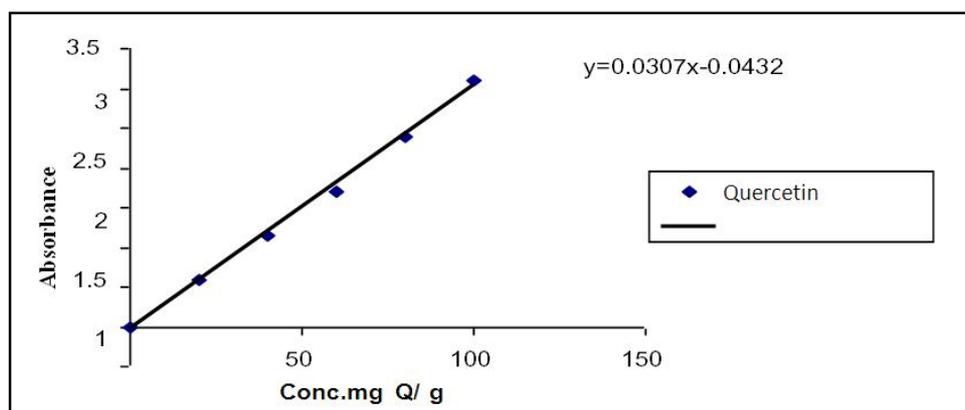


Figure 3: Calibration curve of quercetin.

3.3 Antioxidant activity

3.3.1 DPPH assay method

Ascorbic acid and 70% ethanolic extract of *I. pes-tigridis* were evaluated for their capacity to neutralise free radicals in DPPH

(Figure 4). It can be seen that an inhibition percentage for ascorbic acid at a concentration of 200 µg/ml was 96.38 and for the hydroalcoholic extract it was 85.18. Regression analysis was used to determine the ascorbic acid IC_{50} , which was discovered to be 19.69 µg/ml for ascorbic acid and 88.82 µg/ml for plant extract.

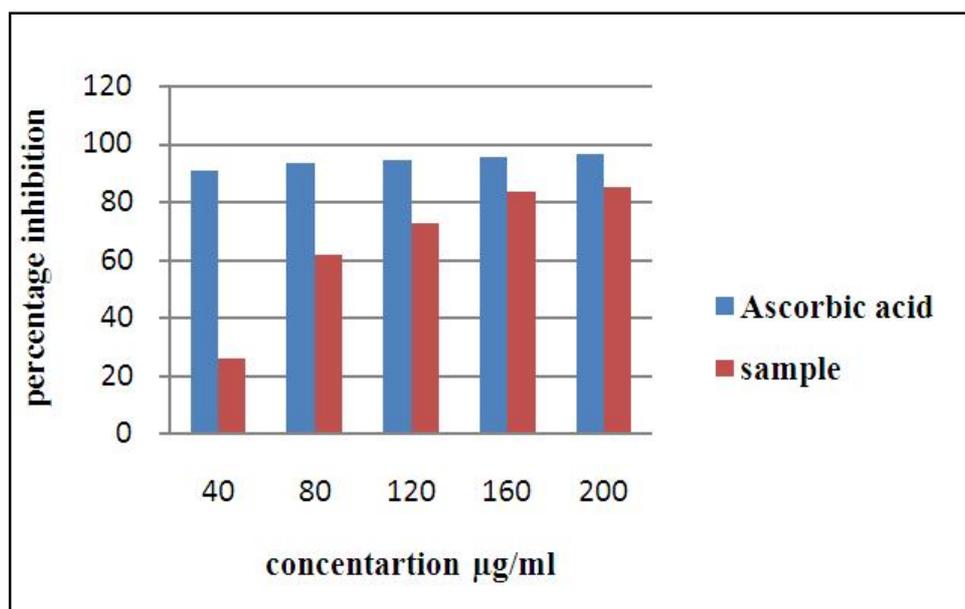


Figure 4: Antioxidant activity of *I. pes-tigridis* by DPPH assay.

3.3.2 Nitric acid scavenging assay

Figure 5 illustrates the findings for the ascorbic acid and *I. pes-tigridis* hydroalcoholic extract's percentage improvement in reducing power on potassium ferric cyanide.

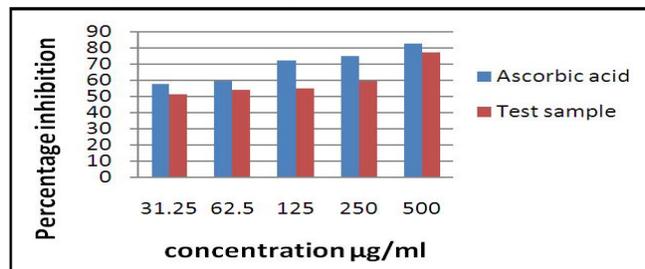


Figure 5: Nitric oxide neutralization by *I. pes-tigridis* and ascorbic acid

3.3.3 Reducing power assay method

In a reducing power assay method, ascorbic acid showed a 99.03 per cent inhibition at 500 µg/ml, whereas the extract showed an 88.62 per cent inhibition at the same dose (Table 3). Correlation analysis was used to determine an inhibitory activity, which was determined to be 16.97 µg/ml, 247.89 µg/ml for ascorbic acid and hydroalcoholic extract, correspondingly (Table 3).

Table 3: Comparative reducing power of *I. pes-tigridis* and ascorbic acid

S.No.	Conc (µg/ml)	Reducing power of ascorbic acid (%)	Reducing power of plant extract (%)
1	31.25	20.87 ± 0.001	16.66 ± 0.01
2	62.5	34.46 ± 0.002	18.79 ± 0.02
3	125	53.40 ± 0.002	35.10 ± 0.05
4	250	85.92 ± 0.001	54.25 ± 0.07
5	500	99.03 ± 0.001	88.62 ± 0.01
	IC ₅₀	16.97 µg/ml	247.89 µg/ml

Values expressed as 'mean of three readings' ± SEM.

3.3.4 Free radicals scavenging activity by hydrogen peroxide

In the method for measuring the ability of free radicals to scavenge hydrogen peroxide, a concentration of 200 µg/ml resulted in a percentage inhibition of 86.56 for ascorbic acid and 76.80 for the extract. Minimum inhibitory concentration (IC₅₀) was determined with the use of regression analysis. Ascorbic acid's IC₅₀ was discovered to be 93.32 µg/ml and hydroalcoholic extract's IC₅₀ to be 138.47 µg/ml, respectively (Table 4).

Table 4: *I. pes-tigridis* and ascorbic acid against hydrogen peroxide

S.No.	Conc. in µg/ml	Percentage inhibition	
		Test sample	Ascorbic acid
1	40	14.84 ± 0.06	18.81 ± 0.002
2	80	19.84 ± 0.09	23.52 ± 0.001
3	120	29.72 ± 0.12	47.85 ± 0.002
4	160	67.69 ± 0.32	72.18 ± 0.001
5	200	76.80 ± 0.18	86.56 ± 0.003
	IC ₅₀	138.47 µg/ml	93.32 µg/ml

Values expressed as * mean of three readings ± SEM.

3.4 Anti-inflammatory activity

3.4.1 HRBC membrane stabilization assay method

Table 5 and Figure 6 display the effectiveness for the % reduction of diclofenac sodium and extract on membrane stability. It can be seen that the percentage membrane stabilisation for the extract was 76.14 and the percentage for diclofenac sodium was 88.23 at a concentration of 444.44 mg/ml, respectively.

Table 5: HRBC membrane stabilization of *I. pes-tigridis*

S.No.	Conc. (µg/ml)	Membrane stabilization of diclofenac (%)	Membrane stabilization of extract (%)
1	88.88	34.25 ± 0.07	38.65 ± 0.03
2	177.77	41.42 ± 0.16	56.02 ± 0.12
3	266.66	52.98 ± 0.04	64.84 ± 0.21
4	355.55	61.09 ± 0.13	76.34 ± 0.26
5	444.44	72.14 ± 0.27	88.23 ± 0.16

Values expressed as *mean of three readings ± SEM.

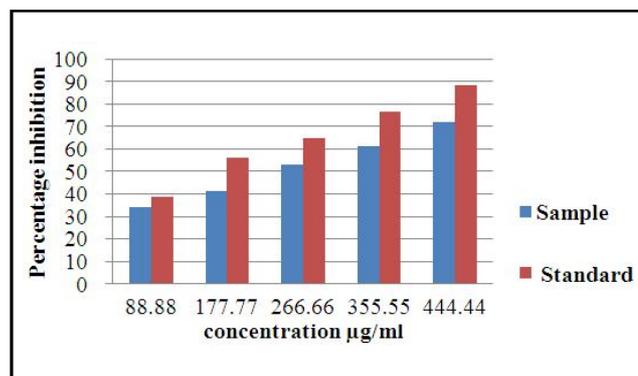


Figure 6: Percentage of HRBC membrane stabilization by *I. pes-tigridis*.

3.4.2 Protein denaturation assay

Table 6 represents the findings of the anti-inflammatory technique that involves inhibiting protein denaturation, and Figure 7 shows a graphical representation. At a concentration of 500 mg/ml, the percentage inhibition of protein denaturation of plant extract was 82.98, while diclofenac was 97.01.

Table 6: Effect of plant extract and diclofenac on inhibition of protein denaturation

S.No	Conc. in µg/ml	Percentage inhibition	
		70% ethanol extract	Diclofenac
1	31.25	28.56 ± 2.35	32.48 ± 2.44
2	62.5	42.78 ± 2.16	53.85 ± 1.11
3	125	53.45 ± 1.45	75.64 ± 1.21
4	250	68.96 ± 1.89	90.60 ± 2.12
5	500	82.98 ± 3.32	97.01 ± 1.39
	IC ₅₀	189.63 µg/ml	110.32 µg/ml

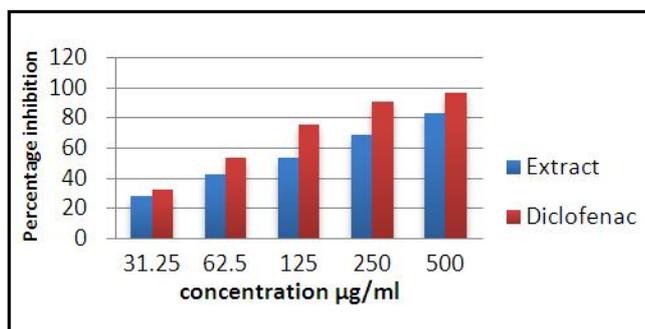


Figure 7: Percentage inhibition of protein denaturation of *I. pes-tigridis*.

4. Discussion

Dynamic oxygen species, such as free radicals, are recognised as agents involved in the pathogenesis of illness such as, diabetes, cancer, orthopathies, arteriosclerosis, Parkinson, asthma, inflammatory hepatotoxicity and Alzheimer's disease (Chiavaroli *et al.*, 2011; Chaves *et al.*, 2020). An antioxidant is a substance that has the ability to delay or inhibit oxidative damage to a particular molecule (Pour *et al.*, 2009; Das *et al.*, 2017). Natural antioxidants, either in the form of crude extracts or their chemical constituents, are very powerful in preventing the pernicious development caused by oxidative stress (Lakshmi *et al.*, 2015; Tiwari *et al.*, 2016). There are many different *in vitro* techniques for measuring antioxidant activity, therefore it is crucial to select the right one to figure out which species has the maximum antioxidant activity. The lysosomal enzymes released during inflammation cause a variety of disorders because the HRBC membrane resembles the lysosomal membrane, which affects the process of inflammation (Nagulsamy *et al.*, 2015). By inhibiting the release of lysosomal components including bactericidal enzymes and proteases, which cause additional inflammation and tissue damage when released extracellularly. The lysosomal membrane must be stabilised in order to reduce the inflammatory response. These enzymes' extracellular activity is thought to be connected to either acute or chronic inflammation (Cho, 1997). Antioxidants have been associated with hepatoprotective, anticancer, antidiabetic, and anti-inflammatory activities (Iwuelee *et al.*, 2007).

From the observation of research studies, the total phenolics and flavonoids of medicinal plants contribute to the antioxidant activities of the plant (Santharam *et al.*, 2007; Green *et al.*, 1982). The antioxidant power of the DPPH method is caused by the scavenging of hydrogen donation (Zhang *et al.*, 2011; Cai *et al.*, 2002). The sulphanilamide is diazotized by the nitrite ions created from nitroprusside in the nitric oxide scavenging process, which results in a pink colour with a maximum absorption at 546 nm (Mehta *et al.*, 2017). When using the reducing power assay method (FRAP), the sample reduces potassium ferricyanide to make potassium ferrocyanide, which is then combined with ferric chloride to produce the prussian blue colour (Rastogi *et al.*, 2018). In the hydrogen peroxide scavenging method, the extract's ability to convert hydrogen peroxide in to water increases with extract concentration (Ali *et al.*, 2020). With rising extract concentration, the extract's ability to scavenge free radicals will also rise (Nilima and Hande, 2011). The DPPH, nitric oxide, hydrogen peroxide, and reducing power assay methods revealed that the scavenging power

of the extract increased dose dependently. Superoxide, hydroxide and hydrogen peroxide radicals are produced abundance in many inflammatory conditions. These radicals significantly damage tissues either actively across oxidising action or obliquely through the establishment of H_2O_2 and OH radicals from oxygen, which starts lipid peroxidation and results in membrane breakdown. The formation of mediators and chemotactic factors then initiates the inflammatory process (Keser *et al.*, 2012). The extract demonstrated membrane stabilisation by preventing erythrocyte membranes, which are assumed to be analogous to the lysosomal membrane and are prone to hyposaline and heat-induced lysis (Alhakmani *et al.*, 2014). Antioxidant compounds from plants can minimise the generation of free radicals (Lewis, 1989; Diaz *et al.*, 2012). *I. pes-tigridis* ethanolic extract evaluated membrane stabilisation as well as protein denaturation, both of which are favourable for its anti-inflammatory properties. As a result, the findings of the current study suggested that the plant *I. pes-tigridis* leaves are promising source of anti-inflammatory drugs, as well as a source of natural antioxidant agents.

5. Conclusion

The effectiveness of excellent anti-inflammatory and antioxidant medications derived from naturally occurring product sources has recently been confirmed using a variety of experimental methodologies. The current endeavour is an effort to produce anti-inflammatory drugs for a number of chronic inflammatory and related disorders. The antioxidants found in the plants may be the cause of their anti-inflammatory activity. Plants containing phenolics and flavonoids may contribute to the antioxidant and anti-inflammatory properties. Their relationship is formed by the correlation observed between the two research processes. It can be concluded that the plant species studied possess promising antioxidant and anti-inflammatory activity, which may be due to the presence of phenolic and flavonoid compounds. Additional and thorough research is being done to isolate the individual flavonoid and phenolic compounds essential for this activity and to determine an underlying role for its anti-inflammatory effects.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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