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Evaluation of in vitro antioxidant activity of extracts & fractions of flowering thistle

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Abstract

The flowering thistle (*Cirsium arvense*) has been traditionally used for its medicinal properties, particularly in the management of diabetes and other health conditions. This study aims to evaluate the in vitro antioxidant activity of ethanol, aqueous, and hydro-alcoholic extracts, as well as the fractions of the flowering thistle plant. Using various radical scavenging assays, including DPPH, superoxide, ABTS, hydrogen peroxide, and nitric oxide, the extracts demonstrated significant antioxidant activity. The study found that the ethanolic extract exhibited the highest antioxidant potential, with a comparable IC50 value to the standard ascorbic acid in several assays. These findings suggest that *Cirsium arvense* possesses potent antioxidant properties, supporting its traditional use as a therapeutic agent.

Keywords: *Cirsium arvense*, flowering thistle, antioxidant activity, DPPH, radical scavenging, in vitro, extracts.

Introduction

Antioxidants play a critical role in protecting cells from oxidative stress caused by free radicals, which contribute to various diseases, including diabetes, cancer, and cardiovascular disorders [5][6]. The ability of plant-based compounds to scavenge free radicals has attracted considerable attention in pharmacological research [7]. *Cirsium arvense*, commonly known as flowering thistle, is a medicinal plant used in traditional medicine for its purported benefits in treating diabetes and other ailments [8][9]. Despite its widespread use, scientific studies evaluating its antioxidant potential remain limited [10].

The plant parts, particularly the aerial sections, are rich in phytochemicals such as flavonoids, phenolic compounds, and alkaloids, which are known for their antioxidant properties [11][12]. Various studies have indicated that plant extracts can effectively neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS), contributing to their therapeutic effects in oxidative stress-related diseases [13]. The goal of this study was to assess the antioxidant activity of different extracts and fractions of *Cirsium arvense* using in vitro assays, including DPPH radical scavenging, superoxide anion scavenging, ABTS radical cation scavenging, hydrogen peroxide scavenging, and nitric oxide scavenging activities.

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Material and Method

Plant material

We chose this plant for our study and our thesis because of its long history of usage by traditional healers in treating diabetes, as well as other information that has been documented. Parts of the flowering thistle plant were utilised as plant materials.

Collection, identification and authentication of plant material

Flowering thistle plants, both young and old, were gathered from the roadside area from Bulk amounts of freshly grown aerial portions of the Flowering thistle were gathered after proper identification. They were then cleaned with distilled water to remove any dust that had stuck to them, and then shade dried for 15 days. For later usage, the shade-dried materials were ground into a coarse powder using a mechanical grinder and stored in a deep freezer in a nylon bag.

METHODS

Preparation of extracts

The yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios (Jackson et al, 1996). The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with alcohol and water using a Soxhlet apparatus. The yield of the plant extracts ethanol (95%) and aqueous measured about 20 g each, after evaporating the solvent using water bath. The standard extracts obtained from *Flowering thistle* were packed in a air tight container and then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening (Akueshi et al, 2002).

Determination of In vitro Anti-oxidant activity

The antioxidant potential of the extracts was evaluated using various in vitro assays, including:

DPPH Radical Scavenging: The ability of the extracts to scavenge DPPH radicals was measured by monitoring the reduction in absorbance at 518 nm. The IC50 values were calculated based on the concentration required to scavenge 50% of the DPPH radicals [7].

Superoxide Radical Scavenging: The scavenging activity against superoxide radicals was assessed by measuring the reduction in absorbance at 560 nm [8].

ABTS Radical Cation Scavenging: The scavenging ability of the extracts against ABTS radicals was determined by measuring the reduction in absorbance at 833 nm [9].

Hydrogen Peroxide Scavenging: The extracts' ability to neutralize hydrogen peroxide was evaluated by measuring absorbance at 230 nm [10].

Nitric Oxide Scavenging: The nitric oxide scavenging activity was assessed by measuring absorbance at 540 nm [11].

Experimental Setup and Data Analysis

Each assay was conducted in triplicate, and results were expressed as mean ± standard error of the mean (SEM). The IC50 values for each extract were calculated and compared to the standard antioxidant, ascorbic acid [12].

Results and Discussion

Experimental findings on the radical scavenging activity of flowering thistle extracts against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) According to Shirwaikar et al. (2006), DPPH is a stable free radical that may be reduced to diamagnetic molecules by reacting with the right reducing agent, such as hydrogen. The number of electrons taken up determines the colourless stoichiometric conversion from the solution.

The in-vitro DPPH scavenging capabilities of ethanolic and aqueous extracts of Flowering thistle's aerial parts are detailed in table-3.20, along with the corresponding IC50 values and percentage scavenging findings.

The amount of DPPH radical that the test extracts depend on the concentration. A colourless α-α-diphenyl-β-picryl hydrazine is produced when antioxidants react with DPPH, a persistent free radical that is purple in colour. By monitoring a drop in absorbance at 518 nm, one could determine the amount of DPPH that was lowered. The ethanolic, aqueous, and hydro-alcoholic extracts had IC50 values of 35.21, 30.11, and 32.9511 εg/ml, respectively. In contrast, ascorbic acid's IC50 value of 18.53 µg/ml considerably decreased the DPPH radical by bleaching. One

possible conclusion drawn from the data is that the free radical DPPH can be scavenged by hydrophilic and hydrophobic extracts of the aerial portions of Flowering thistle.

Table - 1: **Effect of extracts of *Flowering thistle*on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay**

Sample	Concentration µg/ml	% inhibition	IC 50 value µg/ml
	20	29.21 ± 2.13	
	30	38.53 ± 1.85	
Ethanolic Extract	60	59.21 ± 2.36	35.21
	80	83.82 ± 2.68	
	100	88.18 ± 1.35	
	20	25.21 ± 2.03	
	30	50.53 ± 1.65	
Aqueous extract	60	61.21 ± 2.36	30.11
	80	88.82 ± 2.39	
	100	90.18 ± 1.35	
	20	32.56 ± 2.32	
	30	61.06 ± 1.69	
Hydro-alcoholic extract	60	89.36 ± 1.98	32.95
	80	98.91± 2.13	
	100	113.58 ± 1.35	
Ascorbic acid	--	--	18.53

Values are expressed in MEAN ± S.E.M (n =3).

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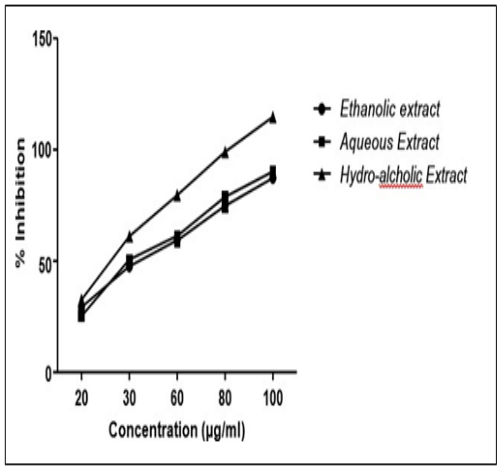

Fig. 1: Effect of extracts of *flowering thistle* on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

Even though it is not a radical species, hydrogen peroxide contributes to oxidative stress. It is possible that biological systems rely on the production of even trace amounts of H2O2. The cellular iron complexes that exist naturally are thought to combine with H2O2 in living organisms to produce very reactive hydroxyl radicals, which might be the source of many of its harmful effects.

Hydrogen peroxide scavenging of the Flowering thistle is test extracts shown in table

3.21 While ascorbic acid strongly and concentration-dependently scavenges H2O2 radical, the IC50 values for flowering thistle, aqueous, and hydro-alcoholic extracts were 56.02, 56.23, and 51.50 µg/ml, respectively. Despite this, the aforementioned investigations show that ethanolic and water-based extracts of flowering thistle aerial parts have great promise as free radical scavengers.

Radiation scavenging test for hydrogen peroxide (H2O2) and flowering thistle extracts

Table 2: Effect of various extracts of *Flowering thistle* on hydrogen peroxide (H2O2) radical scavenging assay

Sample	Concentration µg/ml	% inhibition	IC $_{50}$ value µg/ml
Ethanolic Extract	20	11.16 ± 2.12	56.02
	30	23.12 ± 1.62	
	60	56.25 ± 1.12	
	80	81.15 ± 1.23	
	100	83.25 ± 1.62	
Aqueous extract	20	16.12 ± 2.13	56.23
	30	29.15 ± 1.31	
	60	55.21 ± 2.32	
	80	69.15 ± 2.21	
	100	85.28 ± 1.91	
Hydro-alcoholic extract	20	18.23 ± 0.93	51.50
	30	35.68 ± 1.56	
	60	63.51 ± 0.85	
	80	88.92 ± 2.18	
	100	93.38 ± 1.63	
Ascorbic acid	--	--	21.31

Values are expressed in MEAN ± S.E.M (n =3).

Effect of fractions of *flowering thistle* on 1, 1-diphenyl-2-picrylhydrazyl (DPPH), super oxide, ABTS and Nitric oxide radicals scavenging assay

Table 2 displays the percentage scavenging findings and corresponding IC50 values for the chloroform fraction of Flowering thistle as it relates to the radical scavenging capacities of the plant against DPPH, superoxide, ABTS, and nitric oxide radicals, respectively.

In phytomedicine, the evaluation of bioactive fractions' scavenging properties has frequently made use of DPPH, a stable free radical. Many see the DPPH radical as a prototypical stable lipophilic radical. Many compounds' free-radical scavenging or hydrogen-donating capabilities, as well as the antioxidative activity of plant extracts or fractions, have been investigated using this process. Using 1, 1-diphenyl 1-2- picrylhydrazyl (DPPH) free radicals, the scavenging activity of chloroform and aqueous fractions of Flowering thistle was assessed. The results demonstrated that the chloroform and water fractions had strong antioxidant properties, with IC50 values of 61.89 and 63.88 µg/ml, respectively. Although the IC50 value of the reference medication ascorbic acid is 10.05 µg/ml

While oxidation is an essential part of life, the regular metabolism of oxygen constantly produces free radicals like superoxide. It is well-known that excess levels of this superoxide radical are detrimental to cellular components, leading to tissue damage and a host of illnesses. The capacity to decrease NBT is a measure of the superoxide radicals produced from dissolved oxygen through PMS-NADH coupling. The ability of the plant fraction and the reference chemical to quench superoxide radicals in the reaction mixture is indicated by the reduction in absorbance at 560 nm. Based on the IC50 values (88.86 and 95.53 µg/ml), the chloroform and aqueous fractions were determined to be more effective superoxide anion scavengers than ascorbic acid. Based on the quantification of the absorbance of the radical cation ABTS•+ at 833 nm, the 2, 2 azobis-(3-ethylbenzothiozoline-6-sulphonic acid (ABTS) assay measures the concentration of the compound. Instead of repeatedly generating the radical in the presence of antioxidants, as is the case in conventional assays, this one prepares the radical cation before adding the antioxidant test system (Youdim and Joseph, 2001). The results, obtained that chloroform and aqueous fractions with IC50 of 115.29 and 118.82 µg/ml, where ascorbic acid had IC50 of 8.81µg/ml, significantly showed activity. which is either by inhibiting or scavenging the ABTS radicals in a concentration dependant manner.

Endothelial cells, macrophages, neurons, and other cells produce nitric oxide (NO), a key chemical modulator that controls numerous physiological processes. Hydrogen Reactivity Species like oxygen reacts with excess NO to generate RNS like NO2, N2O3 and peroxynitrite, together ROS and RNS attack and damage cellular molecules including lipids, protein, nucleic acids and carbohydrates (Pacifici and Davies, 1991).The chloroform and aqueous fractions with IC50 value of 82.12 and 83.51µg/ml where ascorbic acid had IC50 value of 8.92µg/ml significantly scavenges RNS. Potentially protecting biomolecules from oxidative stress are the test fractions with radical scavenging capabilities. Additionally, it may scavenge free radicals because it contains antioxidant principles that inhibit the production of reactive nitrogen species (RNS) by competing with oxygen for reactions involving nitric oxide.

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Table -3: Effect of fractions of *Flowering thistle* on 1, 1-diphenyl-2-picrylhydrazyl (DPPH), super oxide, ABTS and Nitric oxide radicals scavenging assay

Sl. No	Concentration (µg/ml)	Chloroform fraction				Aqueous fraction			
		DPPH (% inhibition)	Superoxide radical (% inhibition)	ABTS (% inhibition)	Nitric oxide radical (% inhibition)	DPPH (% inhibition)	Superoxide radical (% inhibition)	ABTS (% inhibition)	Nitric oxide radical (% inhibition)
1	5	15.58 ± 0.33	11.85 ± 0.19	8.01 ± 0.30	12.11 ± 0.63	16.35 ± 0.82	13.03 ± 0.86	10.21 ± 0.85	13.85 ± 1.53
2	10	23.58 ± 0.12	19.52 ± 0.15	13.81 ± 0.28	22.08 ± 0.38	23.32 ± 0.53	21.85 ± 0.82	16.68 ± 1.03	26.11 ± 2.06
3	20	38.98 ± 0.19	28.11 ± 0.02	21.22 ± 0.11	29.16 ± 0.29	38.13 ± 1.05	29.83 ± 1.32	23.93 ± 0.53	32.5 ± 1.03
3	30	38.55 ± 0.25	33.56 ± 0.03	32.10 ± 0.22	38.13 ± 0.26	36.28 ± 0.93	33.56 ± 1.80	33.83 ± 0.39	39.38 ± 0.85
5	80	68.63 ± 0.28	58.33 ± 0.16	38.38 ± 0.12	58.38 ± 0.63	65.62 ± 0.18	39.29 ± 0.26	36.52 ± 0.12	36.65 ± 0.35
6	160	83.52 ± 0.16	81.03 ± 0.16	58.58 ± 0.13	83.56 ± 1.85	81.12 ± 1.13	69.03 ± 0.53	56.82 ± 0.88	85.3 ± 0.19
IC_{50} (µg/ml)		61.89	88.86	115.29	82.12	63.88	93.53	118.82	83.31
Ascorbic Acid IC_{50} (µg/ml)		10.05	15.53	8.81	8.92	10.05	15.53	8.81	8.92

Values are expressed in MEAN ± S.E.M (n =3)

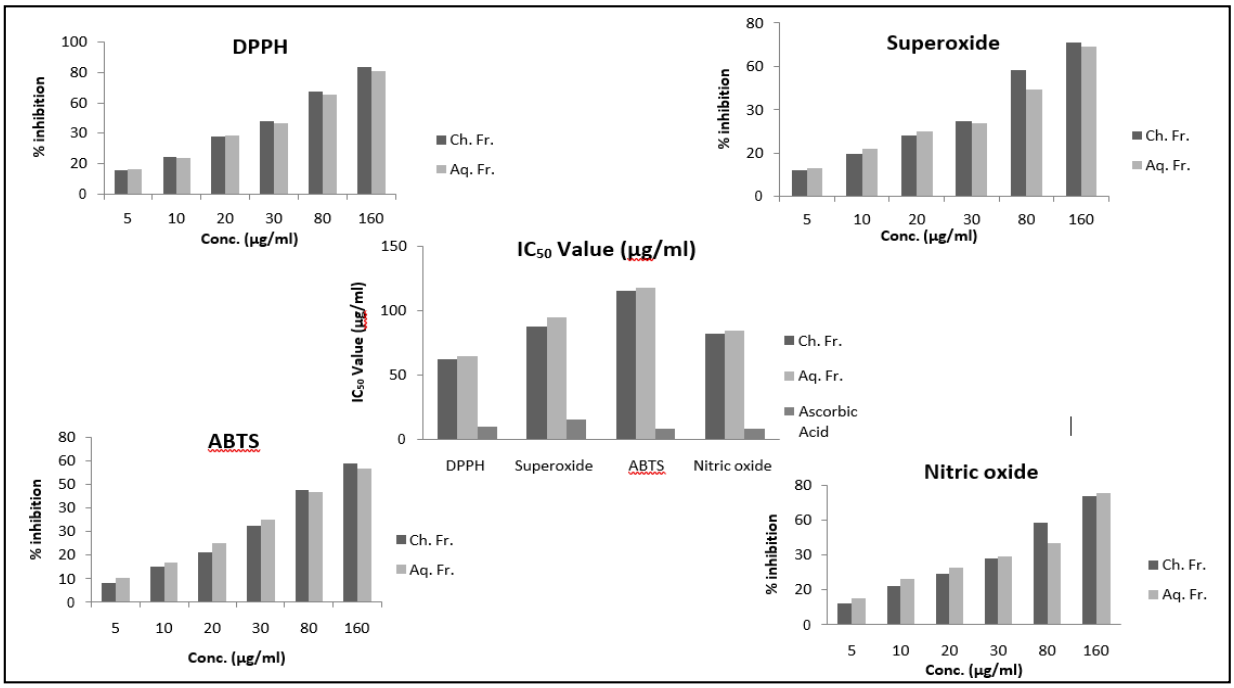

Fig. 2: Effect of fractions of *Flowering thistle* on 1, 1-diphenyl-2-picrylhydrazyl (DPPH), super oxide, ABTS and Nitric oxide radicals scavenging assay

Conclusion

The findings of this study demonstrate that *Cirsium arvense*, particularly its ethanolic and aqueous extracts, exhibit significant in vitro antioxidant activity. The plant's potential as a natural antioxidant agent may provide support for its traditional medicinal uses, particularly in managing oxidative stress-related conditions such as diabetes. The bioactive compounds in the plant's extracts, including phenolic acids and flavonoids, contribute to its radical scavenging abilities, making it a valuable resource for future pharmacological research

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