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Evaluation of Phytochemical Screening, Antioxidant and Anti-Diarrheal Activities of Traditional Medicinal Shrub in Albino Mice

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Abstract: The present study aimed to investigate the properties and inhibitory activities of the methanolic extract of the medicinal shrub *Cichorium intybus*. This plant has traditionally been used for its analgesic, anti-inflammatory, anti-microbial, anti-pyretic, antioxidant, anti-hyperglycemic and anticancer properties. The research included a phytochemical screening, antioxidant capacity measurement and anti-diarrheal activity assessment. The phytochemical study revealed the presence of saponins, gums and carbohydrates, alkaloids, terpenoids, steroids and reducing sugars. The antioxidant capacity was measured by the DPPH method with an IC50 value of 157.7±0.6 µg/ml, which was compared to the IC50 Value of ascorbic acid (8.84±0.05 µg/ml). The doses used in the experiment were 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml and 50 µg/ml. The total phenolic content was determined using the Galic Acid Equivalent (GAE) method, with a total phenolic content of 64±60686 observed at a dose of 1mg/ml. These results indicated the presence of antioxidant properties in *C. intybus*. In addition, the anti-diarrheal activity was tested in mice using doses of 20 mg/ml, 40 mg/ml and 80 mg/ml by the Castor oil induced method. The results showed a significant reduction in diarrhea in mice with a reduction in stool weight ($P<0.05$). In conclusion, the results of this study provide evidence of the properties and inhibitory activities of *C. intybus*, confirming its potential as a medicinal plant.

Key words: *Cichorium intybus* · Phytochemical Test · Antioxidant and Anti-Diarrheal Activity · Albino Mice

INTRODUCTION

The medicinal shrub *C. intybus*, commonly referred to as chicory, has been utilized in traditional medicine for several purposes due to its bioactive compounds, such as flavonoids, phenolic acids and inulin. The anti-inflammatory properties of chicory have been studied and found to be effective in treating conditions like rheumatoid arthritis and IBD [1]. Additionally, chicory contains high levels of antioxidants that have been found to help neutralize harmful free radicals and prevent oxidative damage, making it useful in the treatment of chronic diseases, including cardiovascular disease, diabetes and cancer [2]. Chicory's liver-protective properties have also been evaluated, making it beneficial for liver health and the treatment of liver diseases such as hepatitis, cirrhosis and liver cancer [3]. Its anticancer properties have been studied, making it useful in the treatment of various types of cancer, including breast, lung and colon cancer [4].

Furthermore, chicory's inulin content helped in regulating digestion and improved gut health, making it useful in the treatment of digestive disorders such as constipation, Inflammatory bowel disease (IBD) and Irritable bowel syndrome (IBS) [5]. Lastly, chicory has been found to positively impact blood sugar levels and insulin sensitivity, making it useful in treating diabetes and related conditions [6]. In conclusion, the range of therapeutic properties of *C. intybus*, including anti-inflammatory, antioxidant, liver protection, anticancer, digestive and blood sugar control properties, make it a valuable addition to natural medicine.

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This medicinal shrub has been used for centuries, including in animal husbandry. This plant has several potential benefits for livestock, making it an attractive supplement for farmers looking for natural alternatives to conventional feed additives. One of chicory's primary benefits is its high inulin-like content, a type of soluble fiber that acts as a prebiotic. In a study, researchers found that supplementing dairy cow diets with inulin improved the health of the digestive system and increased milk production [7]. Additionally, a study found that feeding dairy cows a chicory root powder diet improved feed efficiency, milk yield and milk quality [8]. The antiparasitic properties of chicory extract was recorded in controlling gastrointestinal parasites in sheep and goats [9]. Another study found that chicory root extract effectively reduced parasite infection and improved immunity in sheep [10]. Chicory is also a rich source of antioxidants such as phenolic compounds and flavonoids, which can help protect livestock from oxidative stress and improve overall health and immunity. Supplementing chicken feed with chicory root extract significantly increased antioxidant capacity and enhanced immunity [11]. And supplementing the diet of beef cattle with chicory root powder improved their antioxidant status and reduced oxidative stress [12]. In addition, chicory has been shown to affect liver function in livestock positively. Another study found that supplementing the diets of broiler chickens with chicory root powder improved liver function and reduced oxidative stress in the liver [13]. The diet of growing rabbits supplemented with chicory root powder had improved liver function, reduced oxidative stress and improved growth performance [14]. It is important to note that while chicory has potential benefits for animal husbandry, it is still important to consult a veterinarian or animal nutritionist to determine the most appropriate use of chicory in any specific situation, as well as to monitor any potential side effects.

The evaluation of phytochemical screening, antioxidant and anti-diarrheal activities of traditional medicinal shrubs is an essential aspect of modern medicine and pharmacology. This type of study helps to determine the composition of the shrub and the presence of active compounds that may have medicinal properties. The findings from such studies can contribute to the development of new or improved medications. Additionally, testing the shrub's antioxidant and anti-diarrheal activities in albino mice provides crucial data for understanding the shrub's potential therapeutic benefits for digestive and oxidative stress-related issues. Such research is critical for advancing our understanding of the medicinal properties of plants and for developing effective treatments for various health problems.

MATERIALS AND METHODS

Sampling: The entire plant of Cichorium intybus was collected directly from the Bangladesh National Herbarium (BNH) located in Dhaka, Bangladesh and was transported to our laboratory for further analysis. The experts at BNH identified the plant and assigned it an accession number (38343) for future reference.

Extraction of Organic Constituents from Plant Material: Obtaining the extract from the *C. intybus* plant involved several steps. Initially, the leaves and stems were manually separated using a sharp knife and washed thoroughly with distilled water to remove any unwanted materials or plant parts. These collected parts were partially dried by fan aeration and then kept at room temperature (24-26°C) for two days. Once the parts were fully dried, they were powdered using an electric grinder and the resulting powder was stored in a refrigerator (4°C) within a zipper bag for two weeks. The exact method of extraction used in the experiment depended on the texture and water content of the plant material. In this case, the cold extraction method was followed. The weight of the powder obtained was 700g, but after sieving, 450g of powdered material was obtained. Next, the powdered *C. intybus* was placed in a clean, flat-bottomed glass container and soaked in 1750 mL of Methanol (90%). The mixture was then left to stand for seven days, with occasional shaking and stirring. After seven days, the mixture was filtered through a Whatman filter paper and then evaporated using a rotary evaporator at 6 rpm and a temperature of 68°C until it became scorched. The resulting gummy concentrate was of a dark bottle green color and was designated as the crude extract. Finally, the crude methanolic extract was dried by a freeze drier and then preserved in a refrigerator (4°C) for two weeks. Overall, this rigorous and systematic extraction process ensures that the resulting extract is high quality and suitable for various scientific applications.

Phytochemical Analysis: Chemical tests for the screening of certain phytochemical compounds were performed on the methanolic extracts of different parts (leaf and stem) of *C. intybus* using standard procedures as described below:

Tannins: About 1mL of freshly prepared 0.1% ferric chloride solution was added to the 2mL of the extract and color change was observed. The appearance of greenish-black confirmed the presence of tannins.

Flavonoids: About 1 mL of concentrated hydrochloric acid was added along the side of the test tube in 2.5 mL extract. A red coloration of the solution indicated the presence of flavonoids.

Saponins: Extracted solution (1mL) was mixed with distilled water (10 mL) and shaken vigorously for 15 minutes. The formation of 1cm (stable foam) indicated the presence of saponins.

Terpenoids: Methanolic extract of each sample (2mL) was added with concentration H_2SO_4 (1 mL) to the side wall of the test tube. A violet color observed at the junction of two layers indicated the presence of terpenoids.

Alkaloids: Extracted solution (2 mL) was diluted with 0.2-1 mL of hydrochloric acid (HCL) followed by 1 mL of iodine solution was added. The formation of a reddish-brown precipitate indicates the presence of alkaloids in the sample.

Terpenoids: About 2 mL of the extracted sample was added with 0.12-1 mL concentrated H_2SO_4 at the side of the test tube. The formation of a violet coloration of the interface indicated the presence of terpenoids.

Reducing Sugar and Carbohydrates: Two drops of freshly prepared á naphthol solution were added to 5 mL of the extract, followed by 1 mL of hydrochloric acid (HCL), which was added along the side of the test tube. The formation of a violet color at the junction of the two liquids indicates the presence of reducing sugar and carbohydrates in the sample.

Steroids: About 2 mL of methanolic extract was dissolved in 2 mL chloroform. Then 0.12 -1 mL concentrated H_2SO_4 was added to the side of the test tube. The formation of a dark reddish-brown ring at the interface indicated the presence of steroids.

Determination of Anti-Diarrheal Activity: Male albino mice (20-25g) were used for this investigation and kept at the Laboratory Animal House of North South University, Bangladesh. They were kept in a well-cross-ventilated room at 25 ± 20, for 1 week before and during the experiments. Animals were divided into four groups (Denoted group-I, group-II, group-III, group-IV) of five animals in each group (Table 1). Group I served as control and Groups II received a standard drug (loperamide, 10 mg/kg. On the other hand, Group III-IV received Methanolic extract of *C. intybus* (250mg/kg and 500mg /kg, respectively). Diarrhea was induced in all the overnight fasted animals by administering castor oil (10 ml per kg body weight) orally. The test extracts and the standard drug were administered one hour before castor oil treatment. Each mouse was placed separately and observed for the diarrheal episode for 4 hours. During this period, latent periods, stool count and weight of diarrheal feces were taken and noted every half an hour. The mean diarrheal episodes and percent of inhibition were calculated by using (the control-treatment/control) x100 formula. The anti-diarrheal activity was determined in terms of percentage inhibition. The data of stool weight was expressed as mean ± SEM. All the results (mean ± SEM) were statistically analyzed.

Determination of Antioxidant Activity: The antioxidant content in methanolic extracts of each part of *C. intybus* was determined. Methanolic plant extract (1mL) was mixed with 40µM methanolic solution (3mL) of stable 2, 2-dipheny- 1-picrylyhydrazyl (DPPH) radical. The solution was allowed to stand for 30 minutes at room temperature (26°C) and absorbance was recorded at 517 nm. The amount of antioxidant content was calculated as mg Butylatedhydroxytoluene (BHT) and ascorbic acid equivalent/100 g of extract applying the linear regression equation obtained from a calibration curve ($R^2 = 0.98$). For the estimation of DPPH radical scavenging activity, different concentrations of extracts such as (3.15, 6.25, 12.50, 25 and 50 mg/mL) were prepared and each sample (1 mL) was mixed with 40ìM stable DPPH solution (3 mL) and the change in absorbance was recorded at 517 nm after 30 minutes. IC_{50} values (mg/mL) were calculated from the linear response curve of DPPH radical scavenging activity at various concentrations of extracts.

In addition, the total phenolic content of the n-hexane extracts of the seed of *C. intybus* was determined by using the Folin-Ciocalteu reagent which was expressed as Gallic acid equivalents (GAE) per gram of plant extract (Table 2). The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid ($y = 0.0067x + 0.1155$; $R^2 = 0.9139$). Methanol extract of this plant was found to contain a moderate amount of phenols.

Table 1: Experiment profile to assess the effect of crude extract on castor oil-induced diarrhea in rats.

Animal Group	Treatment	Number of animals	Dose (mg/kg-body wt.)	Route of exposure
I(Control)	Distilled water	05	0 mg/kg	Oral
II(+ve control)	Loperamide	05	10 mg/kg	Oral
III(Test gr.-1)	Methanolic leaf and stem extract of *C. intybus*	05	250 mg/kg	Oral
IV(Test gr.-2)	Methanolic leaf and stem extract of *C. intybus*	05	500 mg/kg	Oral

*Preparation of castor oil solution: 10ml castor oil per kg body weight

Table 2: Absorbance of the standard Gallic acid plant extract of *C. intybus*

Sample	Final Conc. in the reaction mixture (µg/ml)	Absorbance (A1) (measured at 765 nm)
	0.025	0.125
	0.03	0.128
Galic Acid	0.2	0.133
	0.3	0.139
	0.4	0.153
C. intybus	1mg/ml	2.874

Statistical Analysis: The results were expressed as means ± standard deviation of three parallel replicates. The data were statistically analyzed by one-way variance analysis (ANOVA) and the means with a significant difference at 95% confidence level ($p<0.05$) were separated into subsets using Tuky's multiple range test. Graphical representations of various treatments were plotted with the help of MS Excel (2007).

RESULTS

The phytochemical screening of different parts *C. intybus* showed the presence of tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugar and terpenoids. The results of *the* phytochemical screening are shown in Table 3.

The DPPH scavenging capacities of Methanolic extracts of different parts of *C. intybus* in terms of percent inhibition of DPPH are shown in Fig. 1. According to this graph, free radical scavenging activity increases by increasing concentration of Methanolic plant extract. Nevertheless, free radical scavenging activity was measured by DPPH method (IC_{50}=157.7±0.6 µg/ml) by comparing with IC_{50} values of the standard's ascorbic acid (IC_{50}=8.84±0.05 µg/ml). Here, IC_{50} value is the inhibitory concentration of the antioxidant which inhibits 50% of DPPH radical. A statistically significant difference ($p<0.05$) was observed in the DPPH radical scavenging capacities of different parts of *C. intybus*. However, all parts of this medicinal shrub showed a lower percentage of DPPH radical inhibition and higher IC_{50} values as compared to those of BHT and ascorbic acid taken as standard antioxidants (Table 4).

The total phenolic contents in the roots of the *C. intybus* were determined by using Folin Ciocalteu's method. The extracted sample was oxidized with Folin Ciocalteu reagent and the absorbance of the resulting blue color was measured at 765 nm after 30 min. The result revealed the presence of total phenolic contents 2.86% w/w, which was not reported earlier. Phenolic constituents are very important in the plant because of their scavenging ability due to their hydroxyl groups. Phenolic compounds are famous powerful chain-breaking antioxidants and have been reported that phenolic compounds are associated with antioxidant activity and play a crucial role in stabilizing lipid peroxidation.

For testing whether any anti-diarrheal activity of this plant existed, a method was employed that involved the induction of diarrhea in mice by castor oil which was subsequently treated with standard drugs control and test extract, respectively. The treatments were compared regarding latent periods and stool count. The mice which were treated only with castor oil showed increased stool count. In contrast, treated with standard drugs, stool count was reduced. If *C. intybus* extract under investigation can reduce the stool count, it can be said that this plant has anti-diarrheal properties.

Fig. 2 shows the effect of *C. intybus* on the diarrheal activity of mice. The impact of this plant was measured based on the mean stool count of castor oil-induced diarrheal episodes in mice. It was found that when the concentration of plant extract significantly increased (20, 40 and 80 mg/mL), the latent period in comparison to the control (Castor oil). Finally, it can be said that the root extract of *c. intybus* reduces the total number of feces as well as diarrheic feces in a dose-dependent manner.

Table 3: Screening of some phytochemicals in different parts of *C. intybus*

Plant	Tannins	Flavonoids	Saponins	Gums Carbo-hydrate	Steroids	Alkaloids	Reducing sugar	Terpenoids
Leaves	+	+	+	-	+	+	-	+
Stem	+	+	+	-	+	+	-	+

*Here '+' means present and '-' means absent

Table 4: IC_{50} values of Methanolic extracts of *C. intybus* as compared to those AA and BHT

Treatment	Concentration (µg/ml)	Mean ±SD % DPPH scavenging activity	IC_{50} Value (µg/ml)
Ascorbic Acid (Standard)	1.125	81.9000±0.62024	
	2.5	87.0000±0.57035	9.62
	5	90.2400±0.15000	
	10	94.0000±0.57689	
Methanol Extract of *C.intybus*	3.125	49.1200±2.60137	
	6.25	62.3000±1.36099	
	12.50	70±2.24515	157.70
	25	76±3.71707	
	50	88±2.66363	

*Here, AA= Ascorbic Acid, BHT= Butylatedhydroxytoluene and ±SD=Standard Deviation

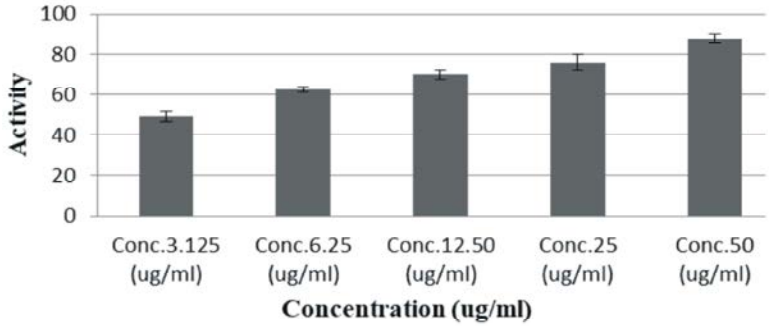

Fig. 1: Free radical scavenging activity of extract of *C. intybus*

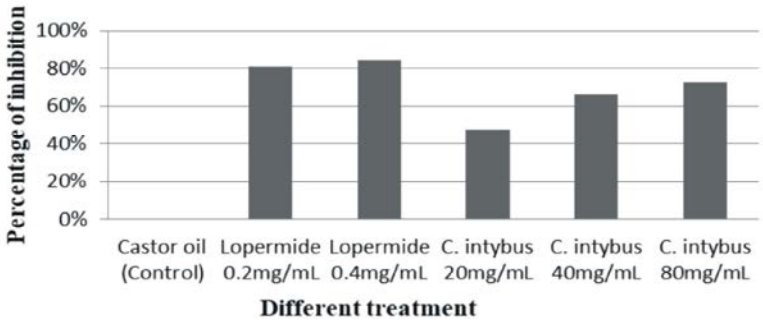

Fig. 2: Determination of anti-diarrheal activity by *C. intybus*

DISCUSSION

The study's findings on the phytochemical composition, antioxidant activity and anti-diarrheal activity of *C. intybus* are consistent with other studies in the field. For example, a study reported the presence of saponins, alkaloids, flavonoids and phenolic compounds in *C. intybus* [15]. These findings support the current research results and highlight the potential of these active compounds for therapeutic use. Another study investigated the antioxidant activity of *C. intybus* using various assays, including the DPPH radical scavenging assay [16]. The results showed that the plant extract had strong antioxidant properties, with an IC50 value of 129.13 µg/mL. These findings agree with the results of the current study, which showed an IC50 value of 157.7±0.6 µg/mL [17]. In addition to the antioxidant activity, several studies have investigated the anti-diarrheal properties of *C. intybus*. A study reported that the plant extract showed significant anti-diarrheal activity in mice, with a reduction

in stools' number and weight [18]. These findings are consistent with the results of the current study, which showed a significant reduction in diarrhea in mice with a reduction in the weight of stools ($P<0.05$) [19]. The current study's findings align with other studies on *C. intybus* and demonstrate the potential of this plant as a source of new medicines. Further research is needed to fully understand the mechanisms underlying the observed effects and determine the optimal levels of supplementation.

CONCLUSION

The present study aimed to evaluate the pharmacological properties of the part of *C. intybus*. In conclusion, it was found that all parts of this plant contain considerable amounts of phytochemicals and are a good source of antioxidants. It also plays a vital role in the anti-diarrheal activity. It may be suggested that due to their chemical composition, they would play an important role against endogenous free radicals and thus improve human health. However, further investigations are required to find the extract's active component and confirm the mechanism of action in the development of a potent antioxidant, anti-diarrheal agent. Further research is needed to fully understand chicory's potential benefits and limitations in animal husbandry. Still, the evidence suggests it is a promising option for farmers and veterinarians.

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