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Antioxidant, Elemental and Pharmacological Study of Medicinally important *Plantago amplexicaulis.* L

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Natural products have played a critical role in medicine due to their ability to bind and modulate cellular targets involved in disease. Medicinal plants hold a variety of bioactive scaffolds for the treatment of multiple disorders. *Plantago amplexicaulis* is annual and perennial herb plant of family Plantaginaceae. *P. amplexicaulis* is grown in the Punjab plains, Malwa and Sindh and extending Southern Europe. *P. amplexicaulis* has been used widely since ancient time, to manage a wide range of diseases including constipation, coughs and wounds. The present research work was carried out to investigate the elemental analysis, antioxidant, anti-inflammatory and analgesic activities of the methanolic and ethanolic extracts of *Plantago amplexicaulis* L. The results reveled that the elemental analysis showed that Calcium was highest (50.16 %), and Cu is low (0.00025 %). The results of analgesic activity ethanol and methanol extract produced increased inhibition of the number of writhing with maximum inhibition observed at maximum dose (400mg/kg) ($p<0.001$) as compared to the standard drug diclofenac sodium at 20mg/kg. Administration of ethanol and methanol extract of *P. amplexicaulis* decreased paw edema significantly ($p<0.001$) at hour 3 (0.56±0.07 and 0.51±0.03) at dose of 200mg/kg and 400mg/kg body weight respectively but less than standard drug Ibuprofen at 20mg/kg. The result revealed that methanol extract of *Plantago amplexicaulis* showed highest (80.47±3.23%) antioxidant activity as compared to the ethanol extract (76.63±2.13%) at 400mg/ml but less than the standard drug ascorbic acid followed by the methanolic extract (32.62 µg/ml). The results clearly show that *P. amplexicaulis* has the ability to treat many diseases due to its efficiency against a verity illness.

Keywords: Elemental analysis, antioxidant, anti-inflammatory, analgesic activities, *P. amplexicaulis.*

INTRODUCTION

Medicinal plants are enhanced with one or more distinct constituents, or even more distinct elements, which may have a connection to pharmacological therapy. Since both organic and inorganic components is crucial for maintaining human physiological functions, it is crucial to measure the concentration of these elements in medicinal plants. Different illnesses can be caused by both an oversupply of trace elements and a shortage of them (Melkegna & Jonah, 2021). According to writing reviews, fundamental elements play a significant part in a variety of diseases. The macro- and micronutrient compositions of seasonal aromatic and healthy plants from diverse nations has uncovered by numerous studies. Elemental components are necessary for the physiological functioning of DNA and cells. Alkaloids, amines, glycosides, and other arranged elemental constituents are normalized by them (Jyothsna *et al.,* 2021).

Elemental analysis of medicinal plants is a critical field in the discipline of pharmacognosy, which concerns the study of drugs derived from natural sources. This process involves identifying and quantifying the elemental composition of medicinal plants, which includes both macro-elements (such as calcium, magnesium, potassium, and sodium) and trace elements (such as iron, zinc, copper, manganese, and molybdenum) (Cicero, 2017). These elements are essential to the biological activities of medicinal plants, influencing both their therapeutic properties and toxicity. For instance, minerals like calcium and magnesium are necessary for several biological processes, including bone health and muscle function, while trace elements like iron and zinc play critical roles in enzyme function and immune response (Mortimer and Presley, 2016).

Many phytochemicals, including tannins, coumarins, quinones, anthraquinones, amine, triterpenoids, lignans, flavonoids, and many more secondary metabolites, are abundant in plants and are excellent sources of ROS

scavengers. They contain antioxidant chemicals, which has linked to the healing of wounds, the prevention of cancer, the reduction of inflammation, and the lowering of blood pressure in numerous studies (Asraoui et al., 2021). It has demonstrated that eating a diet high in natural antioxidants lowers the risk of cancer, age-related diseases, and cardiovascular disease. Over the past ten years, researchers have researching on using natural phytochemicals, used to treat ailments and is found in herbs, green vegetables, beans, berries, cherries, and crops. To ascertain their usefulness as a pharmacological agent, many secondary plant metabolites with scant research investigations is thoroughly investigated (Owoade *et al.,* 2021). One of the main classes of secondary metabolites found in plants that produce staple foods is polyphenols and flavonoids, which serve a variety of biological purposes. Flavonoid is powerful natural antioxidants that neutralise free radicals, prevent them from damaging cells and tissues, and as a result, stop the development of pathological processes. This is true despite of the differences in their chemical structure and composition (Ydyrys *et al.,* 2021).

There is a wide variation in pain's intensity, regularity, and duration, as well as in its definitions and pathophysiological processes. It is difficult to express the concept of pain in a clear and concise manner. Pain is a mental, sensory, and unpleasant experience which is typically brought on by tissue damage. Although NSAIDs and opiates are often used as analgesics to reduce pain without altering consciousness, they are also responsible for a number of adverse effects. NSAIDs cause gastrointestinal irritation, ulceration, bleeding, and other side effects, whereas opiates frequently lead to dependence and tolerance. For this reason, scientists have demonstrated interest in finding analgesic medications with fewer adverse effects that come from natural sources (Hossen *et al.,* 2021).

Usually, analgesic and anti-inflammatory medications is used to treat pain. These medications target and alter both peripheral and central nociceptive pathways to reduce pain. The existing analgesics and anti-inflammatories have a notable ability to reduce pain, but they are not widely available, affordable, and have serious side effects which are constipation, drowsiness, dizziness, stomach trouble, skin itching or rash, and dry mouth. For instance, acetylsalicylic acid, which is present in aspirin and naproxen, can result in indigestion, stomach ulcers, renal damage, hepatotoxicity, stroke, cerebral hemorrhage, and Reye's syndrome, among other adverse effects. In addition, acetaminophen and ibuprofen can harm your kidneys and stomach. Furthermore, opioid analgesics lead to addiction, constipation, a compromised immune system, nausea, sleepiness, sweating, melancholy, itching, and euphoria (Reza et al., 2021). Therefore, there is a critical need for a secure, available, manageable, and effective alternative for pain management given the high prevalence rates of pain around the world and the limitations of current medications. Medicinal herbs are widely accessible, reasonably safe, cost-effective, and has a long history of use in folklore. In actuality, more than 40% of people from affluent countries and more than 80% of the world's population use traditional medicines derived from plants for their basic healthcare requirements, including pain management (Muchonjo *et al.,* 2021).

Non-steroidal anti-inflammatory Drugs (NSAIDs) are a widespread class of analgesic and anti-inflammatory medications that work by inhibiting COX to reduce inflammation-related symptoms. Unfortunately, they have negative effects on the lining of the gastrointestinal tract, blood coagulation, and the kidneys due in part to the inhibition of the necessary enzyme COX-1 (Ayertey *et al.,* 2021).

Plantago amplexicaulis L belongs to family Plantaginaceae and commonly known as Spighwol *P. amplexicaulis* annual or perennial, or mostly acaulescent herbs, grows up to meter two feet in four inches per year. This specie is anemophilous and hermaphrodites (Ahmed *et al*., 2022). They are distributed in Darra Adam Khel, KPK Pakistan, and also in, Algeria, Tunisia, Egypt. Arabia, Iran, and Western India. *P. amplexicaulis* is traditionally used for the treatment of intestinal and as a demulcent in cases of dysentery. They are said to be useful in the treatment of intermittent fevers and pulmonary affections. The present study is aimed to investigates various bioactive compounds, elemental analysis, antioxidant, anti-inflammatory and analgesic activities of medicinally important *P. amplexicaulis* L.

MATERIALS AND METHODS

Plant Collection

Fresh plants i-e *Plantago amplexicaulis* were collected, from Punjab plains. The plant was thoroughly cleaned with water without being squeezed to eliminate trash and dust, and it was then air-shaded dried at room temperature for a week. The air-dried plant was ground using electric grinder and the plant was given voucher no 02 and is preserved in herbarium, Qurtuba university while powder is used for the various Pharmacognotical and pharmacological activities. We separate leaves, stem, and root in small amount from the whole plant to investigate elements in these three parts. The air-dried powder of *P. amplexicaulis* was soked in methanol and leave over night for 24 hours and covered the beaker with aluminum sheet. After 24 hours we mix the plant and collect the extract with the help of filter paper this process is continues for 5 days. We use rotary evaporator to collect pure drug extract from methanol. After this process we used water bath to evaporate the remaining particles of methanol. We collect pure drug extract which are black in color.

Elemental Analysis

An atomic absorption spectrophotometric analysis of the root, stem, and leaves of *Plantago amplexicaulis* will be performed. 0.5g of the powdered plant sample and 10ml HNO3 will be mixed and left overnight. The next day, 4ml of per chloric acid was added and was placed on hot plate. When the test material's initial yellow color changed to white fumes, digestion was accomplished. 100ml of distilled water was added, and the solution was then put into plastic bottles and filtered using filter paper. Atomic absorption spectrometers were used to determine the components in the solution contained in these bottles (Zafar *et al.,*2010).

RESULTS

Antioxidant Activity:

DPPH Free-radical Scavenging Assay:

Free radical scavenging assay utilizing 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) Sigma-Aldrich of *Euphorbia parviflora* extracts was carried out following the procedures (Souri *et al*., 2022). A 25 mg of dry crude extracts was added in distilled methanol and 50 ml diluted solution was prepared. This solution serves as base for development of various solutions for test to be performed i.e. 50 µg/ml, 100 µg/ml, 150µg/ml. Ascorbic acid was taken as standard drug. In a test tube, about 5 ml of each solution was taken to which 1 ml of 0.001 M of DPPH solution was added up to it. All of the solutions were kept in absence of light for half an hour. In controlled solution, methanol extract of about 5 ml was added to about 1 ml of DPPH. After the completion of incubation period the mixtures were observed for the antioxidant activity potential using Optima UV Visible spectrophotometer at wavelength of 517 nm. The experiments were performed and observed in triplicate. Percentage of DPPH inhibition was determined by using the formula:

$$(\%)\ \text{Scavenged DPPH} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Anti-inflammatory Activity

Carrageenan-induced inflammation in mice will be used to test the metholic extract of *Plantgo amplexicaulis* for its anti-inflammatory properties (winter et al.. 1962) with few modifications. Male albino mice weighing around 22 g will be separated into five groups (5 mice/kg), with group II receiving plant extracts at doses of 100, 200, and 300 mg/kg body weight in the right hind paw if each animal, after 30 minutes of oral administration of the teat components, one percent carrageenan will be injected to the right. The volume of the paw edema will be measured using a plethysmometer after 1, 2, 3, 4, and 5 hours.

Analgesic Activity

Koster et al., (1959) will be investigated to test the analgesic effect of the methanol extract with a few minor adjustments. The mail albino mice (approximately 22 g in weight) will fast for two hours before to the experiment's start. Animals will be split into five groups, each with five mice. A standard medicine (diclofenac sodium, 10 mg/kg i.p.) will be administered to group II, which served as the positive countable group, while normal saline (10 ml/kg i.p.) will be put into group one, which functioned as the negative control. Groups III, IV, and V will receive trace amounts of plant extract (100, 200, and 300 mg/kg i.p.). Each mouse will get one injection of acetic acid into the peritoneal cavity after 30. The result will be recorded by counting the number of writings within the next 10 minutes. The following formula will be used to get the % activity:

% analgesic effect = 100 - (NO of writhing in control animals X 100) and tested animal NO of writhing.

Elemental Activity

In this research study, the elemental analysis of the plant *plantago amplexicaulis* was carried out by atomic absorption spectrometry (AAS). For this purpose, the sample of leaves, stem and root was taken. These elements found within the leaves, stem and root along with their quantity. Additionally, substantial positive relationships between the contents of soils and plants were found, indicating this species' capacity for intake and transfer to aboveground components. Although the model of this pathway is not thought to be completely validated, tungsten can enter the plant cell through altering the mechanism for transfer into the cytoplasm. Because there was a positive association between in the plant parts, our results for *Plantago* species are consistent with the projections. This correlation was an indication of the synergistic effect of their biological functions which is shown below in table 4 Arslan *et al.* (2018). The present results showed that calcium was highest (0.613 mg/L) in roots and lowest in (0.518 mg/L) in stem. Cd was highest in (0.037 mg/L) roots and lowest (0.002 mg/L) in stem. Pb was highest in stem (0.50 mg/L) and lowest (0.00 mg/L) in root. Fe was highest in leaves (16.56mg/L) and lowest (0.866 mg/L) in root. Ca was highest in leaves (51.16 mg/L) and lowest (47.10 mg/L) in stem. K was highest in leaves (39.28 mg/L) and lowest (11.62 mg/L) in stem. Mg was highest in stem (5.355 mg/L) and lowest (4.878 mg/L) in root. Mn was highest in leaves (0.594 mg/L) and lowest (0.257mg/L) in stem. Cu was highest in leaves (0.090 mg/L) and lowest (0.00 mg/L) in roots.

Anti-Oxidant Activity

The ethanolic and methanolic extract of *Plantago amplexicaulis* were studied for antioxidant activity by DPPH method. The result revealed that methanol extract of *Plantago amplexicaulis* showed highest (80.47±3.23%) antioxidant activity as compared to the ethanol extract

(76.63±2.13%) at 400mg/ml but less than the standard drug ascorbic acid followed by the methanolic extract (32.62 µg/ml) the scavenging activity of extract was done. Antioxidant capabilities, which reduce redox imbalances or neutralise the effects of free radicals, have been discovered in many herbs. Antioxidants are well known for their ability to scavenge reactive species (ROS and NOS) by acting as substrates for oxidative processes, preventing the chain reactions that could otherwise cause cell injuries. *Plantago* species are frequently utilised in both conventional and modern medicine. These species have motivated numerous researchers to examine the pharmacological effects of their extracts, including their anti-inflammatory, antioxidant, and wound-healing activities which is shown in table 3. Farcaș *et al.* (2019).

Anti-Inflammatory Activity

The result of the anti-inflammatory activity is shown in table 2. The inflammation caused by carrageenan increased with time and reached a maximum after 3 hours (1.35±0.10). Administration of Ibuprofen (20mg/kg body weight) significantly (p<0.001) reduced planter edema at 3h following the carrageenan administration (0.1ml 1% sol). Administration of ethanol and methanol extract of *Plantago amplexicaulis* decreased planter edema significantly (p<0.001) at hour 3 (0.56±0.07 and 0.51±0.03) at dose of 200mg/kg and 400mg/kg body weight respectively but less than standard drug Ibuprofen at 20mg/kg. Chronic wounds can be healed with crushed types of leaves. Recent ethno-pharmacological research has shown that *Plantago* is utilised around the globe to cure a variety of ailments. *Plantago* seeds and leaves include lipids, vitamins, caffeic acid derivatives, flavonoids, iridoid glycosides, alkaloids, and other organic compounds, which contribute to the variety of medical uses of this plant. Each ingredient has particular therapeutic characteristics. The propagation and migration of oral epithelial cells were examined in vitro to test *Plantago's* potential for healing wounds. The results showed that *Plantago* extracts had a positive impact on epithelial cell proliferation, indicating that it may have wound-healing qualities. Shareef *et al..* (2022).

Analgesic activity

In this test the *plantago amplexicaulis* ethanol and methanol extracts demonstrated statistically significant peripheral analgesic action in a manner depending on dose in this test. All doses used (200 and 400 mg/kg, respectively) in comparison to the adverse control (p0.05, p0.001, respectively). As compared to the usual medication diclofenac sodium at 20mg/kg, the ethanol and methanol extract enhanced the number of writhing that was inhibited, with the largest inhibition being observed at the highest dose (400mg/kg) (p 0.001). However, the methanol extract induced more inhibition than the ethanol extract (400 mg/kg) (p 0.001), but less than the common medication diclofenac sodium. Pretreatment of the animals included distilled water (10ml/kg) as control and the conventional medication diclofenac sodium (20mg/kg). The animals were placed on a hot plate 30 minutes after administration. When the animals licked their paws and attempted to flee, the reaction times of 0, 30, 60, 90, and 120 minutes were noted. A cut off time of 20 seconds was considered positive which is shown in table 1 Barua *et al.* (2011)

Table 1:Analgesic activity of ethanolic and methanol extract of plant by writhing method

Treatment	Dose (mg/kg)	Average no. of Writhings
Normal Saline	10mL/kg	40.52±2.05
Diclofenac Sodium	20 mg/kg	8.46 ±2.51***
Ethanol	200 mg/kg	25.42±4.22**
	400 mg/kg	16.50±3.04***
Methanol	200 mg/kg	23.32±3.02**

One way ANOVA followed by dunnet's t-test. Values are mean ± SEM. n=5, in each group *p<0.05, **p<0.01, ***p<0.001

Table 2:Anti-inflammatory activity of ethanol and methanol extract of plant

Treatment	Dose (mg/kg)	1 hour	2 hours	3 hours
Ibuprofen	20 mg/kg	0.60±0.04	0.52 ±0.04*	0.44±0.02*
Ethanol extract	200 mg/kg	0.84±0.06	0.75 ±0.08	0.67±0.05
	400 mg/kg	0.72±0.05	0.66±0.26	0.56±0.07
Methanol extract	200 mg/kg	0.80±0.02	0.71±0.04	0.60 ±0.08
	400 mg/kg	0.68±0.06	0.62±0.04	0.51 ±0.03

One way ANOVA followed by dunnet's t-test. Values are mean ± SEM. n=5, in each group *p<0.05, **p<0.01, ***p<0.001

Table 3:The antioxidant activity of plant extracts

Sample	Conc. (µg/ml)	% DPPH radical scavenging activity	
			IC_{50} (µg/ml)
Ascorbic acid	200	73.30±1.52	12.56
	400	89.32±2.03	
Ethanol extract	200	58.37±1.06	80.45
	400	76.63±2.13	
Methanol extract	200	63.67±1.38	32.62
	400	80.47±3.23	

Table 4: Elemental analysis of *Plantago amplexicaulis.*

Plant parts	Zn	Cd	Pb	Fe	Co	Ca	K	Mg	Mn	Cu
Leaves	0.56±0.0541	0.015±0.0188	0.00±0.159	16.56±0.218	0.00±0.0215	51.16±0.272	39.28±6.855	5.056±0.0603	0.594±0.0569	0.090±0.0027
Stem	0.518±0.0136	0.00±0.0168	0.50±0.144	0.931±0.0160	0.00±0.0380	47.10±0.244	11.62±1.215	5.355±0.3266	0.257±0.0315	0.00±0.0050
Root	0.613±0.0541	0.037±0.0093	0.00±1.067	0.866±0.0766	0.00±0.0074	50.16±0.327	22.22±8.802	4.878±0.0554	0.374±0.0526	0.00±0.0012
Mean value	**0.550**	**0.025**	**0.50**	**14.5**	**0.0215**	**50.16**	**25.50**	**4.878**	**0.400**	**0.00025**

CONCLUSIONS

The purpose of this study is to evaluate the *plantago amplexicaulis* conventional uses, botanical characteristics, pharmacological activity, and toxicological effects. The canon of medica, a reliable pharmacopoeia, and other texts from the 10th to the 18th centuries are used to gather the therapeutic properties of *P. amplexicaulis* in this reviewing study. Electronic data bases including Scopus, Med Lane, and Web of Science were also explored research. *P. amplexicaulis* has been commonly used to treat a wide range of illnesses, such as constipation, coughing, and wounds. *P. amplexicaulis* is also used cure numerous diseases it is used for analgesic, anti-inflammatory, antiviral, antioxidant etc. Various preparations of *P. amplexicaulis* have been recommended, including roosted seeds, decoction, syrup, liniment, gargle, and rectal enema. Each ailment has a vaginal suppository, ocular, and nasal drop provided by a TPM scholar. Recent studies have proven some of its traditional qualities, including as wound healing, antipyretic, antitussive, anti-effective, anti-hemorrhagic, anti-inflammatory, diuretic, laxative, astringent, and hemostatic. Phytochemical chemical investigation shows that *P. amplexicaulis* contains volatile substances, tetra phenoids, phenolic acids, and flavonoids. Advanced pharmacological research has validated that *P. amplexicaulis* is used traditionally, but more research is still needed because this plant has the potential to be used to make a variety of natural medicines.

Recommendation

As a result of the current research and information from the literature review, the following recommendations are made.

As our result shows that *Plantago amplexicaulis* plant has important for medicinal purposes. Also further studied must be done and people must be aware of the useful utilization of this plant on medicinal and business side. In future advanced tools study must be done to explore much more knowledge in medicinal community.

Supplementary materials

The supplementary material / supporting for this article Not applicable.

Author contributions

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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