Pharmacological evaluation of *Alternanthera pungens* Kunth for its antipyretic, analgesic, and antispasmodic activities

Hashma[1], Hussan Ara Begum[1*], Naseem Rafiq[2], Humera Shamshad[1], Behram Ali[1]

[1]Department of Botany, Abdul Wali Khan University Mardan, 23200 KP, Pakistan

[2]Department of Zoology, Abdul Wali Khan University Mardan, 23200 KP Pakistan

Correspondence: Dr Hussan Ara Begum

Abstract

The aim of this study was to investigate the pharmacological, phytochemical, and antioxidant potential of *Alternanthera pungens* Kunth. The ethanolic extract of the plant was used for evaluating its pharmacological, phytochemical, and antioxidant activities. In the antipyretic activity test, the ethanolic extract at a concentration of 300mg/kg reduced the temperature to 97.33b±0.48 °C from 100.0b±0.83 after four hours, compared to the standard drug (paracetamol) that reduced the temperature to 99.37c±0.15 from 103.3c±0.33. In the analgesic activity test, the extract at a concentration of 300mg/kg reduced the amount of writhing to 6a±1.45297 every five minutes, with an 86.04% inhibition, compared to the standard drug aspirin that reduced writhing to 8a±.57735, with an inhibition of 81.39%. In the antispasmodic activity test, the ethanolic extract showed significant results with 76.90% inhibition at a dose of 300mg/kg, while the standard drug (atropine sulphate) showed 94.15% inhibition. The qualitative investigation revealed that the ethanolic extract of *Alternanthera pungens* contained proteins, carbohydrates, flavonoids, alkaloids, phenols, tannins, saponins, glycosides, and steroids, while terpenoids were absent. In the quantitative analysis, the ethanolic extract at a concentration of 150µg/ml showed the highest amount of phenolic content (4.18bc±0.66), and the maximum flavonoid content was 4.66c±0.66.

Key words: pharmacological, phytochemical, antipyretic, analgesic, *Alternenthera Pungens*, medicinal plants, qualitative, antioxidant.

Introduction

Phytochemicals are physiologically active natural substances found in plants that have more health advantages than macronutrients and micronutrients Ha (Hasler *et al.,* 1999). They aid in the resistance of disease and harm to plants, as well as contributing to their colour, fragrance, and taste. Plant cells are protected by phytochemicals, which are chemical molecules that protect them from pollutants, stress, dehydration, UV radiation, and diseases (Gibson *et al.,* 1998). Recently, it has become clear that when their dietary intake is large, they play a role in protecting human health. It has catalogued more than 4,000 phytochemicals and classified them according to their protective functions, physical properties and chemical properties (Morrison *et al*., 1996).

There are an estimated 265,000 plant species on the planet, yet only half of them have been studied for their medical potential and chemical composition. Around 80% of the population in underdeveloped nations relies on medicinal plants to treat various diseases, however this is an estimate from ten years ago. In affluent countries, 60 percent of the population uses these plants to solve various health concerns, including 40-50 percent of Germans, 42 percent of Americans, 48 percent of Australians, and 49 percent of French. The fact that at least 25% of the medicines in the present pharmacopoeia are of plant origin demonstrates the importance of these therapeutic plants (Sharif *et al*., 2018).

Nearly 80% of the world's population relies on indigenous herbal remedies (THD) According to the World Health Organization (WHO), to cure numerous diseases in underdeveloped countries. There are 422,000 floral species on the earth. Only 5,000 species have been separated by phytochemistry to evaluate their active components, out of around 50,000 that are utilized as medicinal plants. Plant species and their compounds are used in 25% of medicines in developed countries. As a result, pharmaceutical corporations produce a great number of clinical medications, yet herbal medicine and phytomedicines are still used in various parts of the world (Shinwari 2010).

Native communities in many parts of Pakistan have known the traditional uses of local plants for hundreds of years. These plants are used to treat a wide range of ailments, including headaches, gastrointestinal difficulties, and cuts and wounds (Bhardwaj and Gakhar, 2005). Commercial harvesting of several significant plants is utilized to extract various sorts of active compounds.

Despite the fact that the systems of Unani and Ayurvedic [Oriental Medicines] differ and are primarily based on the medicinal characteristics of plants, the unique wealth of indigenous knowledge is in threat. The use of traditional knowledge reflects the values embodied in the traditions. In Pakistan, there are around 6000 medical plant species, of which 600 are recognized therapeutic. Local therapists and hakims, who provide health advice in rural areas, suggest these herbs (Shinwari 2010). Half of the 6000 species are found in the northern hemisphere (3000 species), with 124 species having therapeutic significance. Pakistan is home to 4940 flowering plant species (if cultivated flowering plants are included, the number becomes 5738). Unfortunately, just 10% of Pakistan's plant species have therapeutic use (Sharif *et al*., 2018). Approximately 80% of Pakistan's rural population relies on the Unani medicinal system, which is based on medicinal species or their products. Pakistan's diverse biodiversity is divided into nine distinct biological areas, the northernmost of which contains unique biodiversity. There is a diversity of medicinal plants in northern Pakistan that are economically vital to indigenous tribes. Herbal remedies have been based on the medicinal species found in these places since human history. The region has a vast range of aromatic and medicinal plants due to its diverse climatic conditions and unique botanical topography. People in Pakistan's hilly regions employ medicinal plants to treat a variety of ailments. Herbal items are also used to offer shelter, fuel, food, health, and other necessities. In comparison to other countries, ethnobotany is a relatively new field in Pakistan. Several scientists from various parts of the country have contributed significantly to this study (Malik *et al*., 2019).

Alternenthera Pungens Kunth belongs to the Amaranthaceae family and is native to Central America. It has also been reported from tropical nations such as India and Pakistan. It has a mat-like shape and is a perineal herb. It has a hairy stem that is 10-50 cm long and prostrate, with green leaves that are 0.5–4.5 cm long and 0.3–2 cm broad. Flowers have no stalks, and spikes with spiky bracts and bracteoles are sparsely velvety (Naidu *et al*., 2012). *Alternenthera Pungens* is used as painkiller, to cure stomachache and swellings. Plant also has antimicrobial, antioxidant and phytochemical properties (Jakhar *et al*., 2017). To investigate qualitative and quantitative phytochemical analysis of *Alternenthera Pungens* and monitor its different Pharmacological activities.

Material and methods

3.1 Plant Collection and Identification

Plant that are in good condition *Alternenthera Pungens* Kunth leaves were obtained in 2019 from the district Buner. With the help of the Pakistan Flora and available literature, the plant was identified and confirmed. After cutting into pieces, plant leaves were cleaned with tap water and dried in the shade for about 20 days at 20-30 °C room temperature.

Figure 1. *Alternenthera Pungens* Kunth

3.2 Extract preparation

A clean glass container was filled with about 250 g of finely ground plant material, which was soaked in 800 mL of ethanol. After that, the container was sealed and maintained for 15 days. It was shaken and stirred every now and then during this time. Cotton was used to filter the final mixture. Whatman filter paper was used to filter it (Hasan *et al*., 2017).

3.3 Pharmacological effects

All pharmacological actions, such as analgesic, antipyretic, and anti-spasmodic were performed in ethanol and doses were 150mg/kg and 300mg/kg of *Alternenthera Pungens* Kunth. Mice were placed into four groups, each with three mice, for each activity, with the first group receiving a normal saline solution as a negative control, the second group serving as a standard (positive control) for the administration of standard drugs, the third group administering ethanolic

concentrations at doses of 150 mg/kg, and the ethanolic fractions of 300mg/kg formed the fourth group.

3.3.1. Animals used in research

Albino mice weighing 25 to 30gm in both sexes were used. Mice were placed in polypropylene cages in a sanitary environment following ethical roles. The room was kept at a constant 25-30°C temperature. A 12-hour light/dark cycle was implemented for the animals. All of the animals were equally provided rodent pellet diet and free access to water (Thakuria *et al*.,

3.3.2. Drugs

Standard medications in the experiment were aspirin (Bayer, Germany), paracetamol (Glaxo Smith Kline, United Kingdom), diclofenac sodium (Pfizer, United States), and atropine sulphate.

3.3.3. Chemicals

Normal saline (Immunasol NS, A.Z. Pharmaceuticals Co. Pak), carrageenan (Lewis Labs, U.S.), Acetic acid, 95 percent methanol (Merck, Germany) (Petrochemical International Limited), charcoal, brewer's yeast (Lewis Labs, U.S.),

3.4 Analgesic Effect

Induced by acetic acid Analgesic activity was determined using writing tests.

3.4.1. Writhing Test caused by Acetic Acid

Using an acetic acid-induced writhing effect, the ethanolic extract of *Alternenthera Pungens* was examined for analgesic activity in mice, as described by Srivastava *et al*., (2013); Franzotti *et al*., (2000) with minor alterations. A total of four groups of mice were created. Normal saline (10ml) was given to the control group (group I). A 1.cc acetic acid solution was administered to the mice in the 2nd, 3rd and 4th groups and ten minutes later the number of writhing's was observed. The mice in Group II were given positive controls of 1 cc aspirin (10 mg/kg), the number of writhing was counted every 10 minutes and 1.cc ethanol plant extracts of 150 mg/kg and 300 mg/kg was given to group III and IV, respectively. The number of writhes after twenty minutes was noted for the ethanolic extracts from plants of both doses and aspirin (standard). It was then calculated the inhibition in writhes. Finally, the analgesic inhibition % was computed using the following formula.

% inhibition = writhing value of tested drug / Writhing value of negative control

3.5 Antipyretic activity

To test for antipyretic activity, plant extract of various doses was utilized, by using the Brewer's yeast induce pyrexia method (Bhowmick *et al*., 2014). The mice were separated into four groups during antipyretic activity. Mice in all groups were given access to water but were denied food for six hours before to start the experiment. Normal temperature for all mice was measured prior to yeast administration. A Saline Injection was administered to mice of Group I (control group), whereas mice of Groups II, III and IV were treated with 1.cc brewer's yeast solution. After 18 hours of yeast treatment, all mice temperatures was checked (with digital thermometer). The mice were given regular 1.cc medicine with 500mg (paracetamol) medicine with 150mg/kg in the second group. To make a paracetamol solution In 50 mL of water, one 500 mg paracetamol pill is dissolved. Dose of 1.cc ethanol extract plants were administered in groups III and IV at doses of 150 mg/kg and 300 mg/kg each. Rectal temperatures were assessed at 1 hour, 2 hours, 3 hours and 4 hours compared with paracetamol following injecting the concentration of ethanol extracts. In order to compute the antipyretic percentage reduction, the following formula was employed.

$$\% \text{ reduction} = B - Cn / B - A \times 100$$

B is the temperature after pyrexia induction

Cn is the temperature after 1, 2, 3, 4, and

A is the usual body temperature.

3.6 Antispasmodic Activity

The antispasmodic activity of the Charcoal meal test model was determined using the approach of (Taiwo *et al*., 2000). The mice were divided into 4 groups, with the first receiving 10 mL of normal saline with the use of a feeding tube, the 2nd, 3rd, and 4th groups were administered 4 ml charcoal through the mouth. The inactive charcoal solution was made by dissolving 5-gram chemicals in 20ml distilled water. The second group of mice were administered standard (atropine sulphate) 10mg/kg after twenty minutes. The mice received plant extract amounts in the third and fourth groups, respectively, at doses of 150mg/kg and 300mg/kg. The mice were dissected after 20

minutes to measure the overall intestinal length, as well as the length of time the charcoal meal moved through the intestine to compare the extract concentration to a standard of low or high antispasmodic action. The given standard formula was used to calculate the initial transit percentage (percent) of antispasmodic action.

Intestinal transit (%) = D/L ×100

D is the length of the charcoal meal and

L is the whole length of the intestine (cm).

3.7 Analysis of plant chemicals

A study (qualitative and quantitative) on phytochemical analyses was conducted by following standard methods.

3.7.1. Test for proteins.

Millon's analysis

When 2ml Millon's reagent was taken and combined with crude extract, a white precipitate formed become red, which showed the presence of protein following gentle heating (Yadav and Agarwala 2011).

3.7.2. Test for carbohydrates

Test of Benedict

A reddish-brown precipitate showed the existence of carbohydrates when raw extract was heated by the reagent of Benedict 2ml (Yadav and Agarwala 2011).

3.7.3. Test for flavonoids.

Test using alkaline reagent

2 mL of crude extract was combined with 2 mL of a 2% NaOH solution. The intense yellow color became colorless when several drops of diluted acid were introduced suggesting flavonoid content. (Agarwala and Yadav 2011).

3.7.4. Test for Alkaloids

Wagner's test

Wagner's test Along the walls of the test tube, to a few ml of plant extract a few drops of Wagner's reagent will be added. The occurrence of a reddish-brown precipitation shows a positive test (Banu *et al*., 2015)

3.7.5. Ferric chloride test for Phenolic chemicals

The extract is dissolved in 5 ml of clean water (50 mg). Adding a few drops of neutral ferrous chloride solution (5% ferric chloride). The presence of phenolic compounds is shown in a dark green color (Banu *et al*., 2015).

3.7.6. Test for tannins

To see if any precipitations or color changes had occurred, a few drops of 10% ferric chloride were added, and 1 mL of cool filtrate was distilled to 5 mL with distilled water. With tannins, a bluish-black or brownish-green precipitate has been seen (Ajayi *et al*., 2011).

3.7.7. Test for saponins

Frothing test was used to assess the presence of saponins. Each plant's crude dry powder was violently agitated with distilled water for 10 minutes before being categorized for saponin concentration as follows: The absence of froth indicates the absence of saponins, while the presence of saponins is indicated by stable foam more than 1.5 cm (Vaghasiya *et al*., 2011).

3.7.8. Glycoside's test

Test of Salkowski

2 mL chloroform was added to the crude extract. After that, 2 mL of H_2SO_4 was added slowly and gently shaken. A steroidal glycoside component, i.e., a reddish-brown coloring, was discovered (Yadav and Agarwala 2011).

3.7.9. Test for steroid

Test was carried out using 2 ml of chloroform, combining crude extract. The mixture was then filled with 2ml each of concentrated H2SO4 and acetic acid. The appearance of a greenish tint showed that steroids occur (Yadav and Agarwala 2011).

3.7.10. Terpenoids test

The extract of raw material was diluted in chloroform of 2 mL and evaporated till dry. After adding 2ml of concentrated H2SO4 this was heated for 2 minutes. A greyish colour showed that terpenoids are present (Yadav and Agarwala).

3.9 Quantitative analysis

3.9.1. Estimation of Flavonoids

The combined samples were of 0.5 mL (1 mg/mL), of aluminum chloride of 10 percent and potassium acetate of 0.1 ml. (1M). In order to get a volume of 5mL, the mixture was added 4.3mL of 80% methanol. Spectrophotometrically, 415nm after it was vortexed, the absorption

of this combination was measured. The total flavonoid content in the sample was determined by the amount of optical density (Daffodil *et al*., 2013).

3.9.2. Estimation of phenolic compounds

100 mg test separate has been weighed cautiously and weakened into 100 ml of multiple times refined water (TDW). 1 ml of this arrangement has been put to the cylinder, trailed by the expansion of 0.5 ml of 2N Folin Ciocalteu and 1.5 ml of 20% of Na2CO3, with a TDW, lively shaking volume at long last expanding to 8 ml and afterward estimating assimilation at 765 nm inside 2 hours. Using a standard calibration curve constructed from varied diluted quantities of gallic acid, the total phenolic content was determined (Gresolin *et al*., 2013).

3.10 Antioxidant Activity

The method of (Rangkadilok *et al*., 2007; Daffodil *et al*., 2013) with slight changes will be used to estimate free radical scavenging for leaf extracts of chosen plants using 1,1-diphenyl-2-picrylhydrazyl (DPPH).

3.10.1 DPPH Radical Slavenging Activity.

DPPH is a steady free extremist used to test the revolutionary rummaging limit of the cell reinforcement part. This method depends in the foundation of the non-revolutionary DPPH structure when DPPH within the sight of hydrogen-giving cancer prevention agent is diminished into a methanol arrangement. All concentrates were assessed utilizing 1, 1-diphenyl-2-picryl-hydrazyl. In a short, a 0.1mM DPPH arrangement in methanol was created, and 1mL of this arrangement was added to 3 mL of the concentrate arrangement at different focuses (50,100 and 150g/mL). The combinations were joined and held at room temperature for 30 minutes. The retention was estimated with a 517 nm UV-VIS spectrophotometer. The norm for examination was ascorbic corrosive. Higher free extremist searching movement is shown by lower absorbance upsides of the response blend. The capacity to rummaging the DPPH extremist was determined by utilizing the accompanying recipe. DPPH searching impact

(% Inhibition) = {(A0 – A1)/A0) *100}.

Results

4.Pharmacological Activities

4.1 Antipyretic Activity

The antipyretic activity of *Alternenthera Pungens* Kunth was evaluated in ethanolic extracts by the brewer's yeast induced pyrexia in albino mice. Second group (G2) Injected with Standard Paracetamol (10mg/kg) and 1.cc of our selected doses of extract 150mg/kg and 300mg/kg were injected to group three and four (G3 and G4). The control group had given values of 98.65b±0.03 prior to injection of brewery yeast. Pyrexia in the mice recorded 103.7c±0.20 after the injection of brewer's yeast. The measurement was then obtained as 98.90c±0.16 using the standard medication paracetamol (10mg/kg) and after eighteen hours the temperature was 103.3^{c}±0.33 and temperature of control G1 after 1,2,3, and 4 hours was 103.6^{c}±0.19, 103.6^{d}±0.18, 103.6^{d}±0.18 and 103.5^{d}±0.18.Temprature of standard after 1^{st} ,2^{nd} ,3^{rd} ,and 4^{th} hour was 102.3^{c}±0.37, 101.5^{c}±0.34, 100.6^{c}±0.36 and 99.37^{c}±0.15.Reading of group three (G3) ethanolic extract concentration (150mg/kg)of *Alternenthera Pungens* Kunth before the injection of brewery yeast was 93.53^{a}±0.74 . After eighteen hours of injection the reading was 96.66^{a}±1.27 and temperature after 1,2,3 and 4 hours was 95.96^{a}±0.90, 95.20^{a}±0.75, 94.76^{a}±0.78 and 93.90^{a}±0.70. Reading of group four (G4) ethanolic extract concentration (300mg/kg) before the injection of brewery yeast was 96.73^{b}±0.37 and 100.0^{b}±0.83 after eighteen hours of injection. The reading was 99.13^{b}±0.81, 98.16^{b}±0.50, 97.46^{b}±0.43 and 97.33^{b}±0.48 after 1^{st},2^{nd} 3^{rd} and 4^{th} hour.

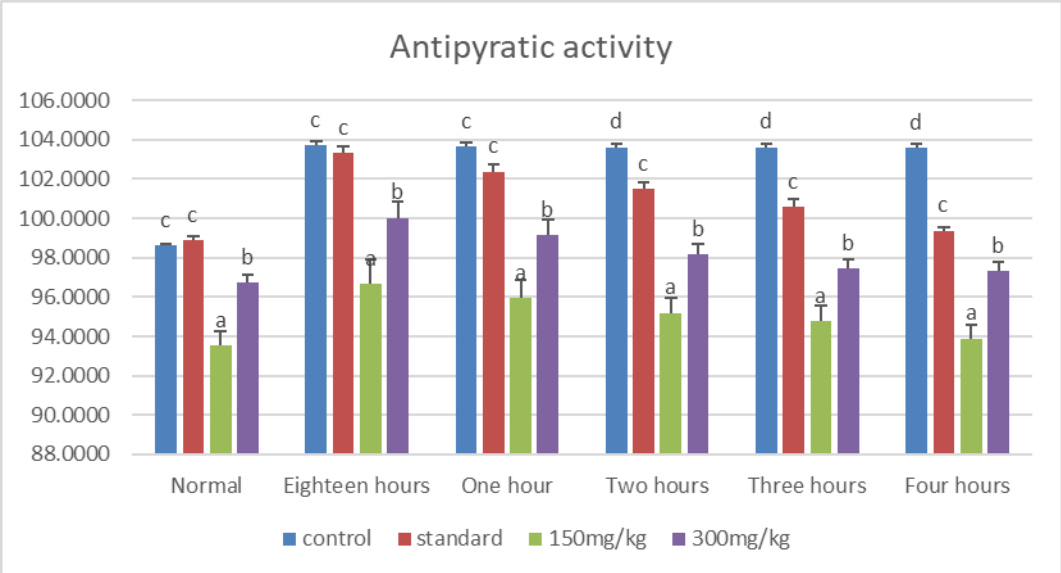

Fig. 1: Graphical presentation of *Alternenthera Pungens Kunth* antipyretic activity

4.2 Analgesic activity

In the present study analgesic activity of *Alternenthera Pungens* Kunth was carried out in ethanolic extract at different doses (150mg/kg and 300mg/kg). The results demonstrated substantial dosages dependent on writhing. The number of writhing's in control groups (G1) of the saline water in *Alternenthera Pungens* Kunth was (43b±2.000) It is shown that conventional medicine aspirin treated group (G2) shows 81.39% writhing (8a±.57735) inhibition number. Group 3(G3) showed the writhing number (6a±1.15470), at a dose of 150mg/kg in ethanol and had (86.04%) inhibition. A dose group (4th) inhibited percentage (300mg/kg) of ethanol (86.04%) and a five-minute number of writhings (6a±1.45297) was reported.

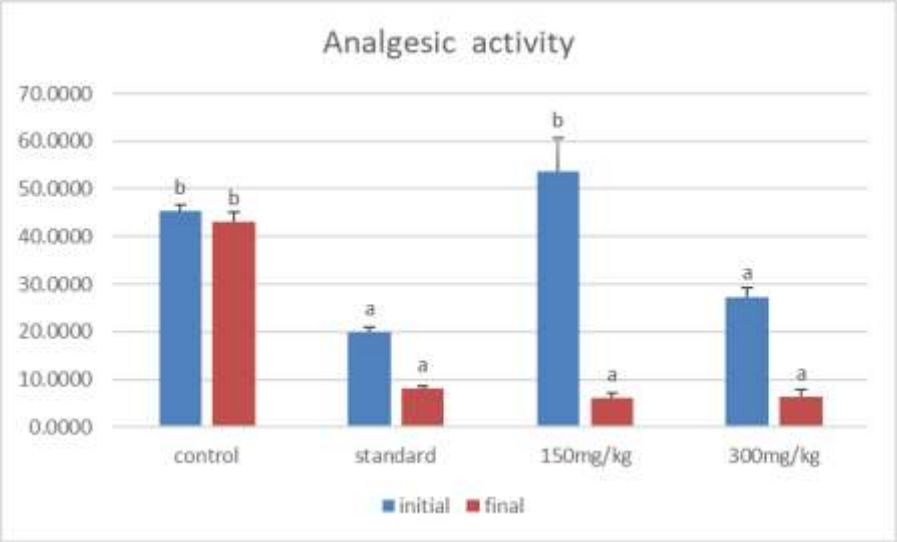

Fig. 3 Graphical representation of *Alternenthera Pungens* analgesic activity

4.3 Antispasmodic activity

During this action, the total intestinal length of Group I (control group) was measured at 45.3a±0.33; the total atropine sulphate (standard medication) of Group 2 was measured at 51.3b±2.07 and 48.3c±2.48 respectively; and the total intestinal length and charcoal meal length of Group 3 were measured at 20.8a±0. 16.. percent inhibition of control was 46% and standard were 94.15%. In group 3rd of *Alternenthera Pungens* Kunth extract concentration (150mg/kg) of the total intestinal length were 54.6^{b}±0.88 and charcoal meal length was 22^{a}±0.57 and showed

percent inhibition of 40.29%. total length of group 4th (300mg/kg) were $43.3^{a}\pm1.45$ and charcoal meal length was$33.3^{b}\pm1.20$ and showed percent inhibition of 76.90%.

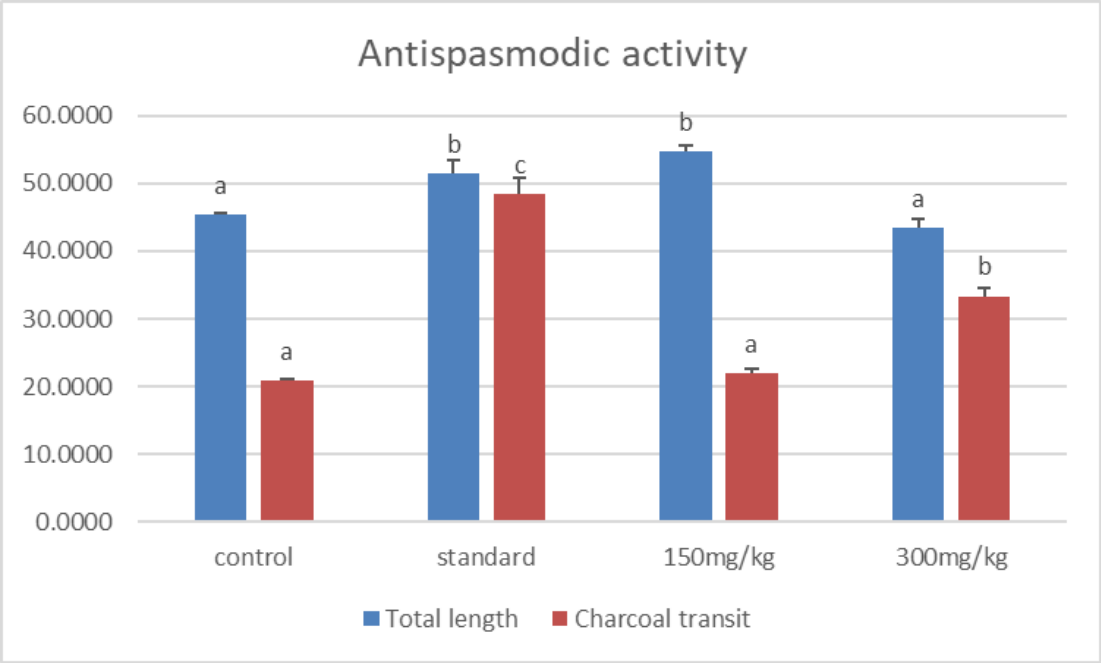

Fig .5. graphical presentation of *Alternenthera Pungens* Kunth

Phytochemical analysis

4.4.1. Qualitative analysis

All ethanolic concentrates of the plant contain flavonoids, alkaloids, phenols, tannins, starches, saponins, and glycosides. The ethanolic concentrate of the plant showed the absence of terpenoids.

Table. 4.4.1. Qualitative Analysis of Phytochemical of *Alternenthera Pungens* Kunth

S.No	Compounds	*Alternenthera Pungens* Kunth
1	Flavonoids	+
2	Alkaloids	+
3	Phenol	+
4	Tannins	+
5	Carbohydrates	+
6	Saponins	+
7	Glycosides	+
8	Terpenoids	-
9	Proteins	+
10	Steroids	+

The positive (+) sign indicates the presence of a component, whereas the negative (-) sign indicates the absence of a compound in a plant extract.

4.2 Qualitative analysis

4.2.1. Total flavonoid content (TFC) and Total phenolic contents (TPC)

Evaluation of total phenolic content was carried out in ethanolic extract of leaves of *Alternenthera Pungens* Kunth . Statistically analyzed data was compared with the gallic acid taken as standard for phenol determination having y = 0.0039, x = 0.3297 R^2 = 0. 8694. For analysis of statistical data quercetin was taken as standard. Maximum phenol concentration were detected in 150 µg/ml concentration in leaves of *Alternenthera Pungens* Kunth (4.18^{bc}±0.66) followed by concentration 100 µg/ml (3.09^{ab}±0.30) and 50 µg/ml (1.87^{a}±0.36) respectively. Maximum flavonoids were determined in *Alternenthera Pungens* Kunth 150 µg/ml concentration (4.66^{c}±0.66) followed by100 and 50 µg/ml (3.06^{ab}±0.16) and (1.74^{a}±0.08) .

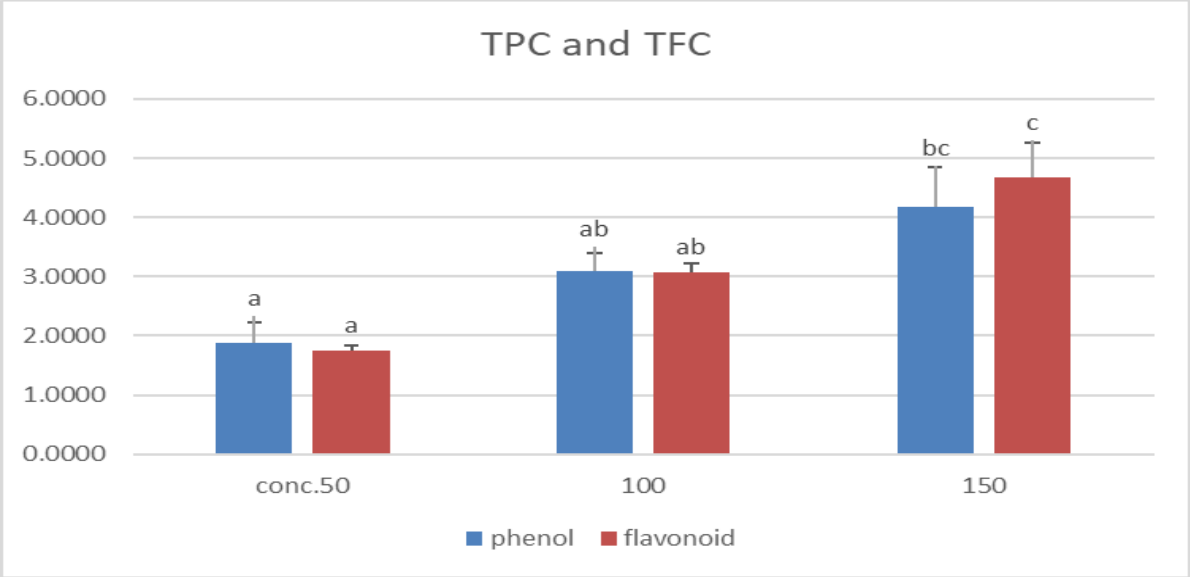

Fig. 6; Graphical presentation of TPC and TFC determination of *Alternenthera Pungens* Kunth

4.3 Antioxidant activity

Ethanolic extract of *Alternenthera Pungens* Kunth was checked for Antioxidant effect through 2,2-diphenyl-1-picrylhydrazine (DPPH) scavenging antioxidant assay. The extract showed significant antioxidant activity at all concentrations, ascorbic acid was used as a positive control for the activity. With concentrations of 50mg/ml, 100mg/ml, and 150mg/ml, the value for decrease rate was taken in Mean and Standard deviation (Mean ± SEM). *Alternenthera Pungens* Kunth significant values were 0.021^{a}±0.020, 0.008^{a}±0.004 and 0.010^{a}±0. 004.Maximum inhibition showed in standard 96.8% while 96.7% was the inhibition showed by 150mg/ml extract concentration followed by 100mg/ml and 50mg/ml 94.44% and93.44% respectively.

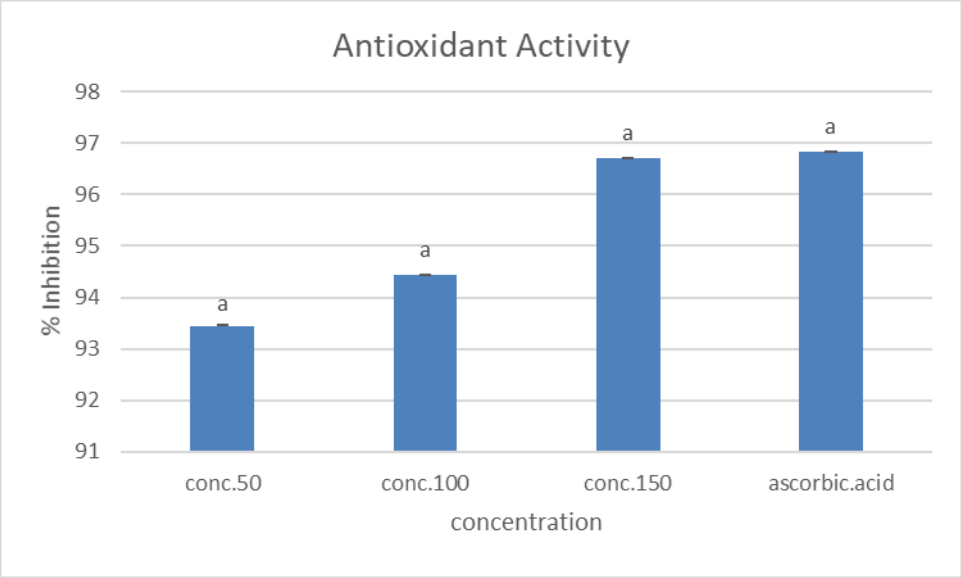

Fig10. Graphical presentation of antioxidant activity of *Alternenthera Pungens* Kunth ethanolic extract.

Discussion

In the present study ethanolic extract of *Alternenthera Pungens* Kunth was used to examine the phytochemistry, pharmacological potential and antioxidant activity. The ethanolic extract showed significant results in antipyretic, analgesic and antispasmodic activities. The phytochemical investigation both (qualitative and quantitative) showed that ethanolic concentrate of plant contains alkaloids, proteins, flavonoids, starches, phenols, tannins, saponins, glycosides, and steroids while terpenoids was missing in the plant. Therapeutic plants have bioactive mixtures

which are utilized for restoring different human sicknesses. It assumes a significant part in recovering. Healing plants have antifungal, antibacterial, and hostile to aggravation exercises. In therapeutic plants, Phytochemicals normally happen in leaves, vegetables, and roots with guard systems and insurance against various infections. Essential and auxiliary mixtures are phytochemicals. Essential mixtures contain chlorophyll, proteins, and normal sugars, while auxiliary mixtures contain terpenoid, alkaloid, and phenolic compounds. Terpenoids are available with an assortment of significant microorganisms, e.g., mitigating, anticancer, hostile to malarial, cholesterol restraint, against viral, and against bacterial combination (Wadood *et al*., 2013).

The current investigation uncovered that the ethanolic concentrate of *Alternanthera Pungens* has solid antipyretic impacts against yeast-initiated pyrexia in mice (Table 4.1) and its percent restraint is similar with standard medication (paracetamol). The critical antipyretic worth of 300mg/kg remove was 97.33b±0.48 as tantamount with standard medication paracetamol with 99.37c±0.15. Antipyretics are the specialists that diminish the high temperature of the body. Yeast-instigated pyrexia is known as pathogenic fever and its etiologic attributes incorporate the creation of prostaglandins at a lower temperature at the warmth guideline focus. To appears to be the last pathway responsible for the production of prostaglandins, mainly the potent pyretic agent, for fever induced from a number of pyrogens. Antipyretic activity is usually one of the properties of non-steroidal anti-inflammatory drugs as a result of their inhibitory effect on central nervous system prostaglandin (Bhattacharya *et al*., 2010).

The acetic acid produces writhing model ethanolic extract exhibited a significant reduction in *Alternenthera Pungens* writhing at a dose of 300mg/kg (6^{a}±1.45297). When compared to the standard medicine aspirin, which prevented 81.39 % percent of writhing generated by acetic acid,. The analgesic property was assessed using an acetic acid-induced writhing technique. Pain is described as "an unpleasant sensory and emotional experience connected with real or potential tissue damage," according to the International Association for the Study of Pain (IASP). In recent decades, common pain relievers such as aspirin and morphine have become increasingly popular. In the majority of cases, analgesic medicines, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), can only relieve 50% of pain in roughly 30% of patients (Fan *et al*., 2014).

the ethanolic extract of *Alternenthera Pungens* has strong antispasmodic effects. After fifteen minutes of oral medication use, the major development of charcoal flow was studied. (Table.4.3.1) 300mg/kg antispasmodic value of extract was Significant 33.3^{b}±1.20 (76.90%) as comparable with standard drug atropine sulphate 48.3^{c}±2.48, (94.15%). Antispasmodics are medications that help to treat or prevent muscle spasms. These medications assist to recover the gastrointestinal muscle to its correct tone by lowering the intestinal hypercontractility of smooth muscles, therefore alleviating numerous stomach pains and symptoms. As a result, antispasmodics are commonly given for a variety of gastrointestinal disorders, including irritable bowel syndrome, which affects 10 to 25% of the general population. The most often available antispasmodic medicines (such as otilon and pinaverium) are antimuscarinic compounds (e.g., plant belladonna alkaloids and their synthetic analogues) and calcium channel blockers (N'Guessan *et al.*, 2015). In general discussion, the benefits and validity of the medications for antispasmodic effect and activities in both plants are evaluating. The current study was attempted to find out qualitative and quantitative phytochemical of *Alternenthera Pungens* Kunth in ethanolic extract. The qualitative investigation suggested that plant extract contained alkaloids, proteins, flavonoids, carbohydrates, phenols, tannins, saponins, glycosides and steroids while terpenoids were absent in plant ethanolic extract. The phytochemical part creation and optional metabolites of plants, for example, phenolic compounds, flavonoids, alkaloids, tannins, and other pressure quality reaction items, are related to their helpful potential (Mondal *et al*., 2019).

High amount of total phenolic content of Plant extract of *Alternanthera Pungens* Kunth was found 4.18bc±0.66 in 150µg/ml followed by 3.09ab±0.30 in 100µg/ml and1.87a±0.36 in 50 µg/ml. Total flavonoids were 4.66c±0.66 followed by 3.06ab±0.16 in 100µg/ml and 1.74a±0.08 in 50 µg/ml. Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables, and medicinal and aromatic plants, which are responsible for antioxidant activities (Phuyal et al., 2020).

The maximum antioxidant activity of *Alternenthera Pungens* Kunth was recorded in ethanolic extract at a dose of 150mg/ml was 0.010^{a}±0.004 (96.7%) followed by 0.008^{a}±0.004 (94.44%) in 100mg/ml and 0.021^{a}±0.020 (93.44%) of 50mg/ml. The data was statistically analyzed using ANOVA, the results showed that the antioxidant activity among ethanolic extracts with different concentration was significant. Recent research has confirmed that antioxidants are the most

effective tools to eliminate free radicals which cause oxidative stress and are possible protective agents that protect the cells from reactive oxygen spices and retard the progress of many diseases as well as lipid peroxidation. Additionally, they also possess anti-inflammatory, anti-viral and anti-cancer properties (Mondal *et al.*, 2019).

Conclusion and Recommendation

The present investigation revealed that *Alternenthera Pungens* Kunth ethanolic extract has a strong potential for pharmacological, phytochemical, and antioxidant activities. The presence of flavonoids, phenolic contents, and antioxidant abilities contributed to the greatest potential. Analgesic, antipyretic, and antispasmodic properties were found in the ethanolic extracts. This study encourages greater research into bioactive molecules that will aid in the development of various drugs with less diverse effects that are beneficial against various disorders. This study is expected to lead to greater research in the fields of bioactive chemical isolation and identification in the plants. This will aid in the development of innovative medicines with minimal side effects, cheap cost, and great efficacy in the treatment of many ailments.

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