PHYTOCHEMICAL ANALYSIS, SAFETY PROFILE, ANALGESIC, AND ANTI-INFLAMMATORY EFFECT OF ETHANOL EXTRACT OF ALLIUM FISTULOSUM L.
Shireen Nazir¹, Syeda Afroz¹, Hafiza Tauseef², Hafiza Afsheen², Riffat Farooqui³, Aisha Rizvi¹

¹Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
²Department of Pharmacology, Bahria University Medical and Health Science, Karachi, Pakistan
³Department of Pharmacology, Dow University of Health Science, Karachi, Pakistan
*Corresponding Author: Dr. Syeda Afroz E-mail. safroz@uok.edu.pk

Abstract
The study was conducted to evaluate the phytochemical, safety profile, analgesic, and anti-inflammatory effects of A. fistulosum. Fifty mice were divided into 5 groups randomly to determine the analgesic activity by using the tail-flick method and hot plate method respectively. Normal saline 5 ml/kg was given to group 1 orally whereas A. fistulosum was administered orally to groups 2, 3, and 4 at a dose of 200, 400, and 600 mg/kg respectively. Aspirin 300 mg/kg was given orally as a standard drug to a group of 5 animals. Acetic acid-induced writhing test were carried out in mice to evaluate peripheral analgesic activity. Animals were divided into 5 groups. Group 1 act as a control group. Group 2 received Diclofenac Sodium whereas group 3, 4 and 5 received 200mg/kg, 400mg/kg, and 600mg/kg of ethanol extract of A. fistulosum respectively. An anti-inflammatory effect was carried out on 50 rats. 1% carrageenan was used to induce edema in the paw. Group 1 acted as a control group whereas the other three groups received A. fistulosum at a dose of 200, 400, and 600 mg/kg. The fifth group was given the standard drug Ibuprofen. The phytochemical evaluation was performed to reveal the constituents present in the ethanol extract of A. fistulosum. The screening demonstrates that pharmacologically active components such as flavonoids, carbohydrates, amino acids, glycosides, phenols, and tannins were present. The increase in latency period has been seen in all three groups of animals receiving A. fistulosum which shows an analgesic effect. The paw thickness of animals treated with ethanol extract at all three doses was also found to be significantly reduced. The safety profile of A. fistulosum was accessed by Lorke’s method which was found safe at the treated dose. In conclusion, the study shows the analgesic and anti-inflammatory effects of Ethanol extract of A. fistulosum, which was predominantly attributable to the presence of phytochemical compounds like flavonoids.

Keywords: Allium fistulosum L., Analgesic, Anti-inflammatory, Phytochemical

INTRODUCTION

ALLIUM FISTULOSUM

For thousands of years, herbs have been major contributions to the quality of life. Herbal medicine is the world’s oldest and most extensively utilized form of medicine (1). Plants have long been used as important ingredients in spices, teas, cosmetics, colors, and medications. Herbs can refer to the bark, roots, leaves, seed, flowers, and fruit of trees, shrubs, and woody vines as well as herbaceous plants (2). In practically every culture, herbal medicine is an important aspect of traditional medicine (3).

Plants were originally the world’s major source of all medications, and they continue to provide people with new treatments. Herbal compounds and their derivatives account for more than half of all pharmaceuticals used in clinical trials around the world (4). Plants have an imperative role in the
development of novel therapeutics and higher plant-derived medications continue to hold a significant place in contemporary medicine. Several chemicals used in modern medicine have intricate structures, making chemically synthesizing these bioactive substances at a low-cost challenging (5).

*Allium fistulosum* which is sometimes known as Spring onion is often believed to be a type of scallion. Welsh onion leaves are spherical, hollow, and inflated for 6 to 20 inches in length. The scape (flower stem) is hollow as well. The plant has only slightly expanded bulbs that are exceedingly long and covered in a dry membrane, onion-like scales that extend aboveground for some distance. The bulbs range in color from white to pink. The black, pointy seeds look a lot like onion seeds.

The Welsh onion is frost resilient, although it will not survive a severe freeze. Some kinds, which are adapted to northern latitudes, lose their leaves in the winter. In the fall, winter, or spring, Welsh onion seeds or divisions can be sown (transplants). The perennial type must be propagated because it does not generate the seed.

This species' flavor and odor are strikingly similar to that of related common onion, *Allium cepa*, as well as hybrids (tree onions) that exist between the two. *A. Fistulosum*, on the other hand, does not yield bulbs and has leaves that are hollow and scapes (fistulosum means "hollow"). A huge variety of Fistulosum types, like the Japanese Negi, look like leeks, while smaller varieties look like chives. *A. fistulosum* can grow in clumps to make perpetual evergreen clumps. It can also be planted as an attractive plant in a bunch. Although it is a common meal in Asia, where there are many kinds, it is usually produced on a smaller scale in Europe and America, mostly for its edible leaves, which can be gathered all year if the weather is mil.

*A. fistulosum L.* is a major vegetable product, grown from Siberia to tropical Asia as well as in Japan, Korea, and China. *A. fistulosum* belongs to the Alliaceae family. It has been used as an herbal remedy for many ailments such as headaches, diarrhea, and colds. Phytochemical studies reported that organ sulfur compounds (6) and polyphenol compounds (7) were present in Welsh onion. Previous studies have demonstrated the antiungal, ant oxidative, anti-hypertensive, anti-platelet, and anti-obesity effects of Welsh onion (8,9,10,11). *A. Fistulosum* contains the amino acid, phenols, coumaric, fericulic acids, glycosides, β-sitosterol, campesterol, stigma sterol, and flavonoid (7), D-limonene (12). Minor bioactive constituents such as TMS derivative, thymol dichloroacetic acid, α-pinene, and 1-Buten-3-yne, 1- chloro-, (Z) are also present (12).

Current research is being conducted to determine the anti-inflammatory and analgesic effects of medicinal plants. Pain and inflammation are almost always linked, even though they are two different conditions. Pain is said when there is discomfort sensory and emotional experience due to potential tissue damage. An increase in blood vessel permeability and the movement of proteins, fluid, and leukocytes from the circulation to the site of tissue damage characterize inflammation, which is the body's immunological response to injury. Pain and inflammation are defense mechanisms that occur in response to tissue damage but if it persists for longer periods, they can lead to various ailments such as neurodegenerative disease, atherosclerosis, and inflammatory bowel disease. Various non-steroid anti-inflammatory drugs are currently available to treat pain and inflammation which are known to have serious consequences like cardiovascular effects. Anti-inflammatory effects have been found in natural products, as proven by their usage in the treatment of ailments like cancer, malaria, and diabetes. Several studies conducted over the last several decades have revealed that several plants show anti-inflammatory effects. Curcumin, resveratrol, and capsaicin are plant-derived compounds that reduce inflammation by decreasing COX-2, prostaglandins, and inflammatory pathways, as well as lowering the levels of cytokines like TNF, IL-1, and IL-6. Active organo-sulphur components such as ajoene, alliiin, and allicin are present in garlic, lowering pro-inflammatory cytokines while increasing anti-inflammatory IL-10 levels (13).

Natural chemicals derived from marine flora, such as those found in coral and algae, inhibit IL-6, TNF-α, and NO production while suppressing COX-2, iNOS, and NF-B activity (14,15). Flavonoids, polyphenol bioactive components found in many plants and vegetables, are another class of natural compounds originating from plants. The term Flavonoid comes from the Latin word which means "yellow," and characterizes the color of these compounds in their natural condition (16,17,18). Flavonoids are secondary metabolites found in a variety of herbs, fruits, stems, roots, flowers, barks, tea, grains, and wine.
that give the plants color and protection while also making them safe to eat. They are also known as 'phytonutrients. These compounds constitute a critical component that can be produced as a therapeutic entity because of their broad spectrum of biological activity and appealing features such as antiviral and anti-inflammatory actions. The purpose of our research is to determine the phytochemical, safety profile, anti-inflammatory, and analgesic effects of A. fistulosum.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

A. fistulosum was purchased from the local market. The plant was submitted to the herbarium department, at the University of Karachi to investigate the species. Voucher number C.H.No.95605 was issued. The study was approved by the University of Karachi, Board of Advanced Studies, and Research.

PREPARATIONS OF EXTRACT

5 kg of A. fistulosum were initially rinsed with distilled water and then kept in a dried shade for 5-7 days. The material was blended and soaked in Ethanol at room temperature for 10-12 days. It was then strained through muslin cloth followed by filtration. The residual solvent was evaporated with a rotary evaporator at 40°C under decreased pressure. A portion of ethanol extract and its fraction were also used for determining its analgesic and anti-inflammatory effects.

SELECTION OF ANIMALS

Experimental animals Adult Albino mice (20-25 g) and rats (200-250 g) of either sex was bought from the local market. Animals were kept at a temperature of 25±3 °C in ventilated laboratory cages with dark and light cycles of 12 hr. in the Pharmacology Department, University of Karachi.

ANALGESIC EFFECTS

TAIL FICK TEST

A total of 50 mice were distributed into five groups. Group 1 animals were given 5 ml/kg of normal saline orally, whereas groups 2, 3, and 4 were given Ethanol extract of A. fistulosum at a dose of 200, 400, and 600 mg/kg, respectively. Animals of group 5 were administered the standard drug Aspirin at a dose of 300 mg/kg orally. The tail-flick technique, as described by Sewell and Spencer, was used to test the analgesic efficacy of A. fistulosum (19). The baseline and latency times at 0hr, 1hr, and 2hr were determined by immersing the tail tip of mice at 50°C in a water bath. The mean increase in latency time was then calculated to find out the analgesic activity of ethanol extract of A. fistulosum.

HOT PLATE METHOD

The analgesic activity of A. fistulosum was also determined using a hot plate test. The mice were placed on a hot plate at a temperature of 50°C. Reaction time was recorded at 0 min, 30 min, 60 min, 90 min, and 120 min when the mice flicked and licked their paws (20).

WRITHING RESPONSE INDUCED BY ACETIC ACID

Five groups of mice, each containing n=10 animals, were chosen for acetic acid-induced writhing. The animals in group 1 were given 5 ml of distilled water (p.o). Diclofenac Na 5 mg/kg (p.o.) was given to group 2, whereas A. fistulosum 200, 400, and 600 mg/kg were given to groups 3, 4, and 5 respectively. Intraperitoneal injection of 1 %v/v acetic acid solution (0.1 ml/10g) was used to produce abdominal tightness after 30 minutes of testing and standard medications. The number of writhing movements noticed between 5 and 20 minutes following acetic acid injection was noted and recorded for each animal.

The effectiveness of the treatment was determined by the percentage of acetic acid writhes that were inhibited.
ANTI-INFLAMMATORY EFFECT

The anti-inflammatory effect was tested on n=50 rats who were divided randomly into five groups. Group 1 was given normal saline 5ml while the other three groups received ethanol extract of A. fistulosum (21). The fifth group was given the standard drug Ibuprofen. After oral administration of ethanol extract of A. fistulosum, edema was induced in the rat hind paw by sub plantar injection of 0.1 ml carrageenan. Edema was induced in the rat’s hind paw by a sub planter injecting 0.1ml carrageenan after oral administration of ethanol extract of A. fistulosum. The sign of inflammation was observed by immersing the hind paw in a Plethysmometer tube resulting in the displacement of water which was detected by the platinum electrode. The paw edema was then measured at intervals of 1 hr., 2hr, 3hr, 4hr, 5hr, and 24 hr. The following calculation was used to compute the % inhibition of edema:

\[
\text{Paw edema of control} - \text{Paw edema of treated} \times 100
\]

\[
\text{Paw edema of control}
\]

ACUTE TOXICITY TEST

Thirty (n=30) mice were separated into three groups and given 10 mg/kg, 100 mg/kg, and 1000 mg/kg of A. fistulosum. Mice were kept deprived of food overnight for 12 hr. followed by the administration of ethanol extract of A. fistulosum on the next day. Acute toxicity symptoms such as sedation, muscle spasm, convulsion, muscle spasm, diarrhea, toxic extension, loss of righting reflex, writhing, and salivation were observed. Mortality rates were monitored in animals for 48 hrs (22).

PHYTOCHEMICAL TESTS

Phytochemical screening was determined by ethanol extract of A. fistulosum using different chemical reagents (21, 23).

For carbohydrate test, the test sample was mixed in an equal ratio with Fehling solutions A and B. After that, the mixture was heated for a few minutes at 55 °C. Carbohydrate presence was confirmed by the formation of a reddish-orange precipitate.

For flavonoid test, sodium hydroxide was added to ethanol extract. After adding a few drops of dilute acid, the color changed from intense yellow to colorless. This demonstrates the presence of flavonoids in the solution.

For proteins test, 4% sodium hydroxide was added with 1% copper sulfate in a test tube containing ethanol extract. The solution was changed to purple or pink which shows protein is present in the solution.

For alkaloids test (Dragendroff’s test), dilute hydrochloric acid was added to a sample extract, which was then filtered and tested with Dragendroff’s reagent. The presence of alkaloids is indicated by the appearance of an orange-red precipitate.

For terpenoids test, chloroform and concentrated sulphuric acid were mixed in a sample extract. The presence of a reddish-brown tint confirms that terpenoid is present in the liver.

For glycosides test (Keller Kiliani test), sulphuric acid, ferric chloride, and glacial acetie acid are added to the test sample. The presence of cardiac glycosides can be seen by the presence of a reddish-brown layer.

For phenols test, ferric chloride solution is added to a test sample resulting in the formation of violet color thus confirming the presence of phenols.

For steroids test, chloroform was first added to the test sample, then acetic acid and concentrated Sulphuric acid is added to the solution. The presence of steroids is indicated by a change in color from violet to green.

For amino acid test, ninhydrin reagent was added to ethanol extract which was then boiled for 5 minutes. The development of blue color indicates that Amino acid is present.

For tannis test, after concentrating in a water bath, a 5 percent ferric chloride solution was then added to a test sample. The presence of violet color, which indicates the presence of tannins, was then tested.
STATISTICAL ANALYSIS

The data were analyzed using SPSS software 20.0 and a one-way ANOVA test with a significance level of p<0.05.

RESULTS

ACUTE TOXICITY

*A. fistulosum* is safe at the selected dose as no mortality was observed. No acute toxicity symptoms such as sedation, muscle spasm, convulsion, muscle spasm, diarrhea, toxic extension, loss of righting reflex, writhing, and salivation were monitored.

PHYTOCHEMICAL SCREENING

Table I shows the phytochemical screening of ethanol extract of *A. fistulosum*. The occurrence of pharmacologically active components such as flavonoids, carbohydrates, amino acids, tannins, glycosides, and phenols is shown by phytochemical analysis.

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Result</th>
<th>Table I. Phytochemical screening of A. fistulosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate Fehling’s test</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Flavonoid Alkaline reagent</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Protein Biuret’s test</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alkaloids Dragendorf’s reagent</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Terpenoids Sulphuric acid and chloroform</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glycosides Keller Killiani test</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Phenols Ferric Chloride test</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Steroids Libemann-burchard reaction</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Amino acid Ninhydrin test</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Tannins Ferric Chloride test</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**Key:** + present, - absent, ++ present in greater amount

ANALGESIC EFFECT

TAIL FLICK METHOD

Fig. 1 shows the analgesic effect of *A. fistulosum* using the Tail Flick method at 200 mg/kg, 400 mg/kg, and 600 mg/kg. *Allium fistulosum* has been compared to the control group. It has been seen that there is an increase in the latency period in all three groups. In group 1 the mean reaction period in 0 hr. is 0.87±0.02 whereas there is a highly significant increase in the latency period at 1 hr, 2 hr, and 4 hr i.e., 2.67 ± 0.77, 4.75±1.03, and 4.22±1.03 respectively. There is a significant increase in latency period at 3hr i.e., 3.67±0.77. Group 2 shows a highly significant increase in latency period at 1hr, 2hr, 3hr, and 4 hr i.e., 3.40±0.45, 4.43±0.87, 4.40±0.45, and 4.83±0.87 respectively. Group 3 shows a highly significant increase in latency period at 1hr, 3hr, and 4 hr i.e., 4.87±0.33, 5.87±0.33 and 6.83±0.76 respectively while there is a significant increase in latency period at 2 hr. This indicates that all three groups show the analgesic effect when treated with *A. fistulosum* (Table II).

<table>
<thead>
<tr>
<th>Groups &amp; Doses (mg/kg)</th>
<th>Reaction time (sec)</th>
<th>Table II. Analgesic Activity of A. fistulosum by tail flick method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td>0.93±0.02</td>
<td>1.56 ± 0.82</td>
</tr>
<tr>
<td><em>A. fistulosum</em> 200</td>
<td>0.87±0.02</td>
<td>2.67±0.77**</td>
</tr>
<tr>
<td><em>A. fistulosum</em> 400</td>
<td>0.92±0.76</td>
<td>3.40±0.45**</td>
</tr>
<tr>
<td><em>A. fistulosum</em> 600</td>
<td>0.94±0.22</td>
<td>4.87±0.33**</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1.42±0.06</td>
<td>2.98±0.66**</td>
</tr>
</tbody>
</table>

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Fig. 1. Analgesic activity of A. fistulosum by tail flick method

HOT PLATE METHOD

Fig. 2 shows the analgesic effect of A. fistulosum using hot plate method at 200 mg/kg, 400 mg/kg, and 600 mg/kg. It has been seen that there is an increase in the latency period in all three groups. In group 1 there is a significant increase in latency period at 30 and 90 min i.e., 9.35±0.35 and 9.34±0.37 respectively. There is a highly significant increase in latency period at 1 hr and 2 hr i.e., 11.31±0.67 and 8.45±0.42 respectively in animals receiving 200mg/kg of A. fistulosum. Group 2 shows a highly significant increase in latency period at 30 min and 1 hr i.e., 10.40 ± 0.55 and 8.33 ± 0.56 respectively while there was a significant increase in latency period at 60 min and 90 min. Group 3 shows a significant increase in latency period at 60 min and 90 min. There was also a highly significant increase in reaction time at 30 min and 2 hrs i.e., 10.89±0.45 and 9.10±0.46 respectively in animals that received 600mg/kg of A. fistulosum. However, it was less than aspirin (Table III).

Table III. Analgesic activity of A. fistulosum by hot plate method

<table>
<thead>
<tr>
<th>Groups &amp; Doses (mg/kg)</th>
<th>Reaction time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Control</td>
<td>6.25±1.35</td>
</tr>
<tr>
<td>A. fistulosum 200</td>
<td>5.85±0.42</td>
</tr>
<tr>
<td>A. fistulosum 400</td>
<td>7.35±0.42</td>
</tr>
<tr>
<td>A. fistulosum 600</td>
<td>7.85±0.82</td>
</tr>
<tr>
<td>Aspirin</td>
<td>7.40±1.10</td>
</tr>
</tbody>
</table>

Fig. 2. Analgesic activity of A. fistulosum by hot plate method
ACETIC ACID-INDUCED WRITHING

The test was demonstrated to find out the peripheral analgesic effect of *A. fistulosum*. Table III shows that the number of writhing was highest in the control group and lowest in animals receiving the highest dose of *A. fistulosum* by inducing 50% inhibition. The test drug at 200mg/kg and 400mg/kg shows 23.8% and 34% inhibition respectively. The standard drug Diclofenac sodium shows 46% inhibition (Table IV).

### Table IV. Analgesic Activity of *A. fistulosum* by Acetic acid-induced writhing response

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>No. of writhing</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>92.3 ± 24.75</td>
<td></td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>5</td>
<td>51.3 ± 23.5</td>
<td>46%</td>
</tr>
<tr>
<td><em>A. fistulosum</em></td>
<td>200</td>
<td>77.6 ± 28.3</td>
<td>23.8%</td>
</tr>
<tr>
<td><em>A. fistulosum</em></td>
<td>400</td>
<td>61.3 ± 12.4</td>
<td>34%</td>
</tr>
<tr>
<td><em>A. fistulosum</em></td>
<td>600</td>
<td>43.4 ± 22.5</td>
<td>50%*</td>
</tr>
</tbody>
</table>

n= 10, Mean ± SEM, Significant relative to control reading; *p < 0.05 was considered significant

Fig. 3. Acetic acid induced writhing

ANTI-INFLAMMATORY EFFECT

Fig. 4 shows the anti-inflammatory effect of *A. fistulosum* when an irritant was used to induce paw edema due to induction of inflammation. Group 1 shows a significant decrease in paw edema at 2hr, 5hr, and 24 hr when the animal received 200mg/kg. Group 2 shows a significant decrease in paw edema at 1hr, 2hr, and 24hr whereas there is a highly significant decrease in paw edema at 5 hr with 52.70 % inhibition when the animal was treated with 400mg/kg extract. Group 3 shows a significant decrease in paw edema at 2 hr, 3 hr, and 4 hr whereas there is a highly significant decrease was observed in paw edema in group 3 at 5hr and 24 hr with 70.27% and 73.52% inhibition when treated with 600mg/kg dose (Table V).

### Table V. Anti-inflammatory activity of ethanol extract of *A. fistulosum*

<table>
<thead>
<tr>
<th>Groups &amp; Doses (mg/kg)</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.81±0.08</td>
<td>0.83±0.01</td>
<td>0.78±0.01</td>
<td>0.77±0.01</td>
<td>0.74±0.01</td>
<td>0.68±0.01</td>
</tr>
<tr>
<td><em>A. fistulosum</em> 200</td>
<td>0.80±0.02</td>
<td>0.60±0.02*</td>
<td>0.49±0.02</td>
<td>0.42±0.03</td>
<td>0.40±0.03*</td>
<td>0.36±0.03*</td>
</tr>
<tr>
<td><em>A. fistulosum</em> 400</td>
<td>0.78±0.01*</td>
<td>0.66±0.01*</td>
<td>0.61±0.02</td>
<td>0.45±0.02</td>
<td>0.35±0.02**</td>
<td>0.24±0.02*</td>
</tr>
<tr>
<td><em>A. fistulosum</em> 600</td>
<td>0.73±0.02</td>
<td>0.58±0.02*</td>
<td>0.48±0.02*</td>
<td>0.39±0.03*</td>
<td>0.22±0.03**</td>
<td>0.18±0.03**</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.79±0.03</td>
<td>0.59±0.02*</td>
<td>0.48±0.02**</td>
<td>0.38±0.03*</td>
<td>0.31±0.03**</td>
<td>0.11±0.03**</td>
</tr>
</tbody>
</table>

0.20% 40.67% 38.07% 50.64% 58.10% 64%
DISCUSSION

Due to the adverse effects caused by traditional treatments, pain management is a worldwide concern. Pain can be effectively managed with acetaminophen and NSAIDs. While preclinical studies show that COX-2 selective inhibitors are beneficial, studies reveal that they cause a variety of side effects, including cardiac and renal problems (24), and non-selective COX inhibitors cause gastrointestinal ulcers and kidney injury (25). In mice and humans, acetaminophen has been found to cause liver damage (26, 27). This necessitates the development of medications with fewer adverse effects, allowing the patient’s comorbidities to be taken into account while selecting the optimal alternative. As a result, researchers have been looking for natural substances that have fewer side effects on patients, and flavonoids have received more attention in recent years.

Thus, the analgesic and anti-inflammatory effects of A. fistulosum, as well as acute toxicity, were investigated. At 10, 100, and 1000 mg/kg of ethanol extract of A. fistulosum, no indications of acute toxicity were noticed, and no mortality was observed up to 1000 mg/kg, indicating that these extracts are safe.

The analgesic and anti-inflammatory activity of ethanol extract of A. fistulosum were carried out in rats and mice. Thermal and neuronal-induced algesia was evaluated using tail-flick and hot plate tests. Both the tests show that A. fistulosum inhibits the supraspinal centers and spinal reflexes which indicates that these extracts affect CNS (28). The hot plate test is a standard way to assess centrally acting analgesics that affect spinal reflexes. It involves the transmission of pain via nociceptors from the periphery to the brain via the spinal cord. Inhibition of phospholipase A2 inhibits the breakdown of arachidonic acid, which suppresses the pain-producing prostaglandins. The extracts, particularly the A. fistulosum have analgesic and promising anti-inflammatory properties.

The acetic acid-induced writhing test is carried out to evaluate the peripheral analgesic activity. The test has long been used to evaluate novel drugs for antinociceptive and anti-inflammatory effects. Acetic acid is thought to cause pain sensation by promoting the release of prostaglandins and lipoxygenase products into the peritoneum, which stimulates the nociceptive neurons on the sensory nerve fibers (29). The results also revealed that their analgesic efficacy was comparable to Diclofenac Sodium, indicating the presence of phytoconstituents in the fractions that had analgesic activity as the dose is increased. As a result of the findings, we believe that the pharmacological mechanism for the plant’s analgesic impact is linked to a reduction in prostaglandin synthesis due to their inhibitory role in the lipoxygenase and/or cyclooxygenase pathways (30).

The use of carrageenan-induced inflammation to assess the anti-inflammatory effects of natural compounds is common. The most likely mechanism of action for carrageenan-induced inflammation is biphasic. The production of histamine, 5-hydroxytryptamine, and bra, bradykinin is responsible for the initial phase (0–1 h) of edema, which is not reduced by NSAIDs such as indomethacin or aspirin (31). In rat hind paw edema, higher levels of prostaglandins and inducible cyclooxygenase (COX-2) were seen during...
the second phase (1–6 h) of edema formation (32). In the exudate, a time-dependent increase in edema, nitrite/nitrate, and prostaglandins E2 (PGE2) has been seen. Local neutrophil infiltration in the inflamed area contributes to the development of inflammation by creating reactive oxygen species (ROS) and other inflammatory mediators.

The cytokine-inducible, calcium/calmodulin-independent variant of nitric oxide synthase produces nitric oxide (NO) which is another inflammatory mediator (iNOS). Vascular permeability and blood flow towards the inflamed region increased as a result of NO generation which also increases the release of pro-inflammatory cytokines. The presence of the NOS and COX pathways at the same time exacerbated the inflammatory response. *A. fistulosum* reduces the inflammation during the early and late stages of edema indicating that it may have mediated anti-inflammatory effects by decreasing the production of inflammatory mediators (32, 31).

Carbohydrates, flavonoids amino acids, glycosides, phenols, and tannins were found in the preliminary qualitative phytochemical examination of *A. fistulosum*. Because these secondary metabolites have a diverse spectrum of medicinal properties (33, 34), this species is likely to have a wide range of medical applications. *A. fistulosum* contains flavonoids that can be used as anti-inflammatory, antiplatelet, antimicrobial, and antihypertensive. Flavonoids such as quercetin and kaempferol inhibit both inflammatory and neuropathic pain through mechanisms involving the inhibition of cytokine production and prostaglandin. *A. fistulosum* shows a decrease in paw edema which is supported by Tsanova who reported that polyphenols help in lowering the pain in osteoarthritis and rheumatic arthritis (35). The study has shown that those polyphenol compounds inhibit inflammation and decrease the succession of lipid oxidation (36). Chist *et al.*, and Dorsch *et al.*, also described that *A. fistulosum* has anti-inflammatory activity on the immune system due to the presence of thiosulfinates and capaenes (36, 37). Quercetin, an important component of *A. fistulosum* has been shown to reduce inflammation, lung tightening, and blood sugar and cholesterol levels, among other biological actions (38).

The present study revealed that the therapeutic plant reduces the pain in all three doses when compared with the animal that received normal saline. The ethanol extracts of *A. fistulosum* showed a significant anti-inflammatory effect from 2 hrs to 24 hrs. However, the anti-inflammatory effect of *A. fistulosum* at 400 mg/kg was highly significant at 5 hours, while the anti-inflammatory effect of *A. fistulosum* at 600 mg/kg showed highly significant at 5 hrs and 24 hrs.

This result indicated that flavonoids reduced pain and inflammation by inhibiting leukocyte recruitment, oxidative stress, and pro-hyperalgesic mediator production (39). Furthermore, *A. fistulosum* contains allicin which inhibits the JNK and P38 pathways and modulates NF-κB expression thus showing an anti-inflammatory agent (40). The synergetic effect of the phytochemical ingredients found in *A. fistulosum* is important in lowering the production and synthesis of endogenous inflammatory mediators, which may help to alleviate pain and inflammation.

**CONCLUSION**

The findings of this investigation indicate that ethanol extract of *A. fistulosum* provides a beneficial therapeutic effect to reduce pain and inflammation. The plant is found to be safe but more clinical studies should be done. Furthermore, anti-inflammatory, and analgesic properties could be attributed to the presence of various phytochemical substances. Although more studies are required with an increased sample size since very limited information is available to date.

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