International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.15 | Issue 1 | Jan - Mar -2025
www.ijpir.com
DOI : https://doi.org/10.61096/ijpir.v15.iss1.2025.64-76

ISSN: 2231-3656
Print: 2231-3648

Research

Evaluation Of Anti-Pyretic Potentials Of 70% Ethanolic Extract of *Cuscuta Reflexa* Using *In Vitro Studies*

K. Ponnudurai[1*], Dr. N. Venkateshan[2], R. Pandeeswari[1], P. Ponabinaya[1], K. Priyadharshini[1]

[1]Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626 126, India.*
[2]Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626 126, India.

*Author for Correspondence: Dr. K. Ponnudurai, M.Pharm., Ph.D.,
Email: drponnuduraik@gmail.com

Published on: 18 Mar 2025

Published by:
DrSriram Publications

Abstract

In present study, the antipyretic activity of 70% ethanol extracts from *Cuscuta reflexa* Roxb. (Cuscutaceae) was evaluated using Invitro methods. The extracts started reducing the elevated temperature in a dose related manner. The 70% ethanolic extract reduced 79 % and 83.8% respectively as compared to reference drug paracetamol (96.5%). It was therefore concluded that 70%of the ethanolic the extracts of C. reflexa has antipyretic activity, the ethanol extract was found to be slightly potent.

Keywords: Antipyretic, *Cuscuta reflexa*, phytochemical analysis, prostaglandin, *In vitro* methods.

INRODUCTION

The parasitic climber *Cuscuta reflexa* Roxb. (Cuscutaceae), also called Akashabela, Amarabela in Hindi, Swarnalata in Bengali, and Akakhi-lata in Assamese, is found up to 3000 meters in the Indian plains. The plant has long been utilized for a variety of therapeutic uses in India. The seeds have sedative, emmenagogue, and diuretic properties; they are also helpful for splenic and liver diseases, prolonged fevers, griping, and hiccups. In ophthalmia, the whole plant infusion is administered, and in biliousness, the decoction is used as a purgative. The stem has purgative properties. In addition to various purgative decoctions, the plant juice was administered. India's rural residents utilize the plant's juice to treat jaundice, and they apply a warm paste to rheumatism, gout, and other afflicted areas[1].

Cuscuta reflexa, commonly known as oriental dodder, is a parasitic plant species in the genus *Cuscuta* is a member of the Convolvulaceae family. It is a thread-like, leafless plant that parasitizes other plants to obtain its nutrients because it lacks chlorophyll.

Because of their therapeutic potential, medicinal plants have been used in traditional medicine for ages. The search for new drug candidates to treat a variety of illnesses has resulted in the discovery of *Cuscuta reflexa*, also known as the dodder plant, but also as devil's hair, witch's hair, love vine, amarbel, or akashabela. In addition to being a widespread climber, *Cuscuta reflexa* is a parasitic weed. It develops into a homoparasite. Commonly referred to as dodder or amarbel, *Cuscuta reflexa* is a perennial parasitic twining herb of the Convolvulaceae family that lacks roots and leaves. It lacks chlorophyll and is unable to produce food on its own by *Cuscuta* is a member of the Convolvulaceae family. It is a thread-like, leafless plant that parasitizes other plants to obtain its nutrients because it lacks chlorophyll.

Because of their therapeutic potential, medicinal plants have been used in traditional medicine for ages. The search for new drug candidates to treat a variety of illnesses has resulted in the discovery of *Cuscuta reflexa*, also known as the dodder plant, but also as devil's hair, witch's hair, love vine, amarbel, or akashabela. In addition to being a widespread climber, *Cuscuta reflexa* is a parasitic weed. It develops into a homoparasite. Commonly referred to as dodder or amarbel, *Cuscuta reflexa* is a perennial parasitic twining herb of the Convolvulaceae family that lacks roots and leaves. It lacks chlorophyll and is unable to produce food on its own by[2].

Pharmacological activities

Effect on Cardiovascular system

In a series of experiments, alcoholic extracts of his plant caused a fall in blood pressure on dog. This action was not blocked by atropine, merpyramine or propranolol, thus it could not be exerted through cholinergic, histaminergic or adrenergic mechanism. An ethanolic extract of the stem of *Cuscuta reflexa* caused a dose-dependent decrease in arterial blood pressure and heart rate in pentothal anaesthetized rats, and this effect was not blocked by atropine. Hypotensive and bradycardiac effects of *Cuscuta reflexa* were found to be independent of cholinergic receptor stimulation or adrenergic blockage.

Antidiabetic effect

The methanol and aqueous extracts (200 and 400 mg/kg body wt) showed significant reduction in blood glucose during OGTT in diabetes rats at 3h. The treatment also resulted an improvement in body weights, decreased Hb1c and restored lipid profile. Methanolic extracts of *Cuscuta reflexa* has significant antidiabetic effects and improves metabolic alterations.

Antioxidant activity

In Vitro antioxidant activity of *Cuscuta reflexa* stem extract by estimating degree of non-enzymatic haemoglobin glycosylation was measured calorimetrically at 440 nm. Ethyl acetate fraction of ethanolic extract showed higher activity than other fractions Synthesized phytochelatins and carried out modulation of antioxidants in response to cadmium stress in *Cuscuta reflexa*. The effects of cadmium on growth, the antioxidative enzymes namely catalase peroxidase glutathione reductase, glutathione and phytochelatins were found in callus and seedling of *Cuscuta reflexa*.

Antipyretic activity

At the dose of 400mg/kg body weight the aqueous and ethanol extract reduced 79% and 83.8% respectively of the elevated rectal temperature as compared to reference drug Paracetamol (96.5%) after 6 hours of treatment. It appears that the antipyretic activity of *Cuscuta reflexa* may be due to inhibition of prostaglandin synthesis. Again the extracts contain flavonoids and saponins, the antipyretic potential of which has been reported.

Spasmolytic action

Aqueous and alcoholic extracts of *Cuscuta reflexa* stem have got a relaxant and spasmolytic action on small intestine of guinea pig and rabbit. Also, the extracts exhibited acetyl choline-like action

Anti-HIV activity

The crude water extracts of *Cuscuta reflexa* exhibited anti-HIV activity that could be due to combinatory effects with compounds of different modes of acion.

Antitumor activity

Administration of Aqueous and ethanol extracts of *Cuscuta reflexa* whole plant at at doses of 200 and 400 mg/kg body weight resulted in a significant ($p<0.05$) decrease in tumour volume and viable cell count but increased non-viable cell count and mean survival time, thereby increasing the life span of the tumour-bearing

mice. Restoration of haematological parameters -RBC, Hb, WBC, and lymphocyte count to normal levels in extract treated mice was also observed.

Anti-arthritic and nephroprotective effect

Antiarthritic activity of Aqueous and Methanol extracts of *Cuscuta reflexa* was evaluated in vivo using formaldehyde and turpentine oil-induced arthritis models and *In Vitro* using formaldehyde and turpentine oil-induced arthritis models and *In Vitro* using protein denaturation methods. AMECR at 600mg/kg significantly reduced paw edema and joint swelling with maximum inhibition of 71.22% at the 6 hour for turpentine oil and 76.74% on the 10 day for formaldehyde. Likewise *In Vitro* results corroborate significant concentration dependent increase in % protection at 800 against both bovine serum albumin (89.30%) and egg albumin (93.51%) denaturation. This result shows that AMECR provides protection against arthritis and nephrotoxicity that might be due the existence of phytoconstituents.

Anti-inflammatory activity

Alcoholic and aqueous extract of stem of *Cuscuta reflexa* were evaluated for their anti-inflammatory activity in carrageenan induced paw edema model in rats, and compared to the activity of the standard drug, Ibuprofen. These extracts were given orally at a concentration of 100, 200 and 400 mg/kg bd. Wt. before carrageenan injection. Both the extracts with medium and higher doses i.e 200mg/kg and 400 mg/kg have reduced edema volume by 47.27%, 72.72% and 57.72%, 80.00% respectively at 5th has compared to standard drug Ibuprofen 96.36%. Thus this study revealed that the selected extracts of *Cuscuta reflexa* exhibited a significant anti-inflammatory activity in carrageenan induced paw edema model in rats.

Antimicrobial activity

Ethanolic whole plant extracts obtained from *Cuscuta reflexa* were screened against Gram positive (Bacillus subtilis and Staphylococcus aureus) and Gram negative (Escherichia coli and Salmonella typhi) bacteria to evaluate their antimicrobial activity. Of the four concentrations of plant extract tested (200 µg/mL 300 µg/mL, 400 µg/ml or 500 µg/ml), 500 µg/ml elicited the greatest zones of bacterial inhibition across three of the bacteria. In contrast, the growth of Salmonella typhi was not halted regardless of extract concentration. Overall, although the greatest antimicrobial activity was demonstrated to be against E.coli at concentration of 500 µg/ml. (24.6), upon comparison to the other bacteria, both B. cereus and S. aureus reduced similar zones of inhibition upon comparison to their positive antibiotic control the ethanolic extract of *Cuscuta reflexa* contains myriad of compounds such alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenolic compounds and steroids. The authors determined that it is the flavonoid, glycosides contained within the plant which are responsible for the inherent antimicrobial activity. This preliminary investigation suggests that the ethanolic extracts from *Cuscuta reflexa* do possess significant antimicrobial properties.

Hair growth activity

The petroleum ether and ethanolic extract of *Cuscuta reflexa* were given at the dose 250 mg/kg in male swiss albino rats. Cyclophosphamide (125 mg/kg) was used to induce alopecia. This study was shown to be capable of promoting follicular proliferation or preventing hair loss in cyclophosphamide-induced hair fall[3].

Key features of *cuscuta reflexa*

Appearance

Cuscuta reflexa is a yellowish to reddish, twining, and thread-like plant. It has no leaves or stems of its own, instead it relies on the host plant for its sustenance. The plant forms dense tangled masses that are typically seen wrapped around the host plant. The stems are thin and often appear as delicate vines or threads that can grow up to several meters in length.

Parasitic Nature

Cuscuta reflexa is an holoparasite, meaning it lacks chlorophyll and cannot photosynthesize. Instead, it attaches itself to a host plant via specialized structures called haustoria. These structures penetrate the host plant's tissues to extract water, nutrients, and sometimes even sugars. The plant is highly parasitic and can severely weaken or even kill the host plant if left unchecked.

Host Plants

This species parasitizes a wide range of plants, including crops, shrubs, and trees. Common host plants include legumes, grasses, and some ornamental species. It has been reported to infest a variety of plants, causing significant agricultural damage.

Flowers and Reproduction
Cuscuta reflexa produces small, bell-shaped flowers that are usually white or pale yellow. The flowers grow in clusters along the stems and are pollinated by insects. The plant reproduces by seeds, which are dispersed to new locations, where they germinate and form new parasitic growths.

Ecological Role
As a parasitic plant, *Cuscuta reflexa* can have both positive and negative ecological impacts. It can regulate the population of certain plants by weakening or killing them, but it can also contribute to biodiversity by providing a niche for other organisms. However, its impact on agriculture can be damaging, as it affects crop yields and can result in significant economic losses.

Medicinal Uses
In some traditional medicinal practices, parts of *Cuscuta reflexa* are used for their purported health benefits. It is believed to have various properties such as anti-inflammatory, anti-viral, and anti-cancer effects, though these claims require more scientific validation. However, it should be noted that due to its parasitic nature, it should be used with caution, and its safety for human consumption is not well-established.

Distribution
Cuscuta reflexa is native to tropical and subtropical regions of Asia but has spread to other parts of the world, including Africa and the Americas, often due to human activities.

Control Measures
Controlling *Cuscuta reflexa* can be challenging due to its parasitic nature. Strategies typically involve removing infested plants, using herbicides, and employing crop rotation or resistant plant varieties. Preventing the spread of seeds and ensuring that host plants are not overly stressed are key methods to reduce infestation[4].

Traditional importance of *cuscuta reflexa*
In traditional systems of medicine such as Ayurveda, Unani, and folk remedies, *Cuscuta reflexa* has been used extensively for its therapeutic potential. The plant has been employed to treat various conditions, including:
Fever: Used as a natural antipyretic agent to reduce elevated body temperature.
Liver disorders: Applied for jaundice and hepatoprotective purposes.
Skin conditions: Used to treat itchiness, eczema, and other dermatological issues.
Digestive health: Promotes relief from constipation and indigestion.
Eye disorders: Infusions and pastes of the plant are applied to improve vision or treat eye infections.
The use of *Cuscuta reflexa* in traditional medicine highlights its role in holistic healing practices[5].

Phytochemistry of *cuscuta reflexa*
Cuscuta reflexa is a rich source of bioactive phytochemicals, which include:
Flavonoids: Potent antioxidants known for anti-inflammatory and antipyretic effects.
Alkaloids: Compounds that may contribute to the plant's therapeutic activity, including pain and fever relief.
Glycosides: Known for their role in modulating inflammatory responses.
Phenolic compounds: Act as antioxidants and anti-inflammatory agents.
Steroids: Possess immunomodulatory properties.
The diverse chemical composition of *Cuscuta reflexa* is key to its wide range of pharmacological effects, making it a subject of interest for scientific research[6].

Medicinal properties of *cuscuta reflexa*
The medicinal properties of *Cuscuta reflexa* are supported by its various pharmacological actions, including:

1. Antipyretic Activity: The plant has shown potential in reducing fever by modulating the body's thermoregulatory centre and inhibiting prostaglandin synthesis.
2. Anti-inflammatory Action: It helps reduce inflammation, a common cause of fever.
3. Antioxidant Properties: Neutralizes free radicals, thereby preventing cellular damage associated with inflammation and fever.
4. Hepatoprotective Effect: Protects the liver from toxins, often implicated in systemic fever conditions.
5. Antimicrobial Activity: Effective against various pathogens, indirectly aiding in fever reduction by addressing the root cause[1].

Role in fever management
Fever, or pyrexia, is a common symptom of various illnesses caused by infections, inflammation, or immune responses. Conventional antipyretic drugs, such as paracetamol and ibuprofen, often have side effects

like gastrointestinal irritation or hepatotoxicity with prolonged use. *Cuscuta reflexa* presents a promising alternative with its natural antipyretic properties. Studies have demonstrated its ability to reduce fever in experimental models, such as yeast-induced pyrexia in rats. These findings suggest that the plant's bioactive compounds inhibit the production of prostaglandins, which are central to the fever response [7].

Mechanism of antipyretic action

Hypothalamic Regulation

The hypothalamus, a part of the brain, controls the body's temperature regulation. When the body detects infection or inflammation, the hypothalamus raises the body's set point temperature to fight off pathogens (a process known as fever). Antipyretic agents act on the hypothalamus to bring the body's temperature set point back to normal, thereby reducing fever.

Prostaglandin Inhibition

Many antipyretic agents work by inhibiting the production of prostaglandins, which are chemicals in the body that promote inflammation and fever. Specifically, they block the enzyme cyclooxygenase (COX), which is responsible for the synthesis of prostaglandins.

By inhibiting COX enzymes, the production of fever-inducing prostaglandins is reduced, helping to lower the fever [8].

Antipyretic properties in *cuscuta reflexa*

There is some anecdotal evidence and traditional medicinal usage suggesting that *Cuscuta reflexa* might have antipyretic properties. In traditional medicine, it has been used for various ailments, including fever, due to its believed anti-inflammatory and cooling effects. However, the scientific evidence supporting the specific antipyretic activity of *Cuscuta reflexa* is limited. Potential Active Compounds: Some studies suggest that compounds found in *Cuscuta reflexa*, such as flavonoids, alkaloids, and saponins, might contribute to its anti-inflammatory and fever-reducing effects. These compounds could potentially interact with the body's immune response, influencing the inflammatory pathways involved in fever[9].

Mechanism of protein denaturation

The mechanism of protein denaturation in relation to antipyretic properties involves the stabilization of proteins and the suppression of inflammation-induced fever pathways. Here's a detailed explanation:

Protein Denaturation

Proteins lose their natural structure (denaturation) under stress conditions such as high temperature, inflammation, or oxidative stress. Denatured proteins trigger the release of pro-inflammatory mediators like cytokines and prostaglandins, which contribute to the onset of fever by acting on the hypothalamus to elevate body temperature.

Role of Antipyretic Agents

Antipyretic agents act by inhibiting the pathways leading to inflammation and protein denaturation.

Specifically, they: Prevent the denaturation of proteins by stabilizing their structure under stress conditions. Reduce the release of pro-inflammatory mediators, thereby controlling the cascade of events leading to fever.

Mechanism in Protein Denaturation Assay

Substances that inhibit protein denaturation mimic the action of standard antipyretic drugs like aspirin. These substances bind to proteins, protecting them from thermal or chemical stress and maintaining their functional structure. By stabilizing proteins, they reduce the availability of denatured proteins that act as triggers for inflammation and fever.

Connection to Antipyretic Action

Inhibition of protein denaturation is indicative of a compound's ability to interfere with inflammatory processes. This stabilizing effect reduces the synthesis of pyrogenic (fever-inducing) substances, thereby lowering body temperature. In summary, the prevention of protein denaturation reflects a compound's ability to stabilize cellular processes, suppress inflammation, and exhibit antipyretic properties[7].

Mechanism of heat-induced haemolysis

The mechanism of heat-induced haemolysis in relation to antipyretic properties is based on the ability of a compound to stabilize cell membranes and reduce inflammatory responses. Here's how it works:

Heat-Induced Haemolysis
Haemolysis Mechanism
When red blood cells (RBCs) are exposed to heat, their membranes become destabilized, leading to rupture and release of intracellular components.

Role of Antipyretic Agents
Antipyretic agents stabilize RBC membranes, preventing heat-induced damage. By maintaining the integrity of the cell membrane, these agents reduce the release of inflammatory mediators that are responsible for triggering pyrexia.

Mechanism in Heat-Induced Haemolysis Assay
The assay measures the ability of a compound to protect RBCs from haemolysis under heat stress. Substances that inhibit haemolysis act by:
Membrane Stabilization: Strengthening the lipid bilayer and proteins in the RBC membrane.
Anti-inflammatory Action: Reducing the activation of pathways that lead to the synthesis of fever-inducing substances.

Connection to Antipyretic Action
By stabilizing RBC membranes, compounds prevent the release of inflammatory signals that exacerbate fever. This mechanism mimics the action of standard antipyretics, such as aspirin, which also reduce inflammation and stabilize cellular components. In summary, the ability to inhibit heat-induced haemolysis indicates a compound's membrane-stabilizing and anti-inflammatory properties, which contribute to its antipyretic effects[10].

Materials and methods

Plant material

The entire plants of *Cuscuta reflexa* Roxb. were collected during the month of November 2024 from the Ayyanar Falls is located 6km west of Rajapalayam, a city and municipality in Virudhunagar District in the Indian State of Tamil Nadu. The species was identified by Dr.R. Murugan,Ph.D, Assistant Professor, Centre for Research and Postgraduate Studies in Botany in Ayyan Nadar Janaki Ammal College. The plant material was shade dried at room temperature (24-26 °C) and ground mechanically into a coarse powder.

Drugs and chemicals

All the chemicals used were of analytical grades, obtained from commercial suppliers. Paracetamol was brought from Kumaran Medical, Rajapalayam. Double-distilled water from all-glass still was employed throughout the study.

Preparation of extracts

The powdered plant materials were extracted with 70 % v/v ethanol separately by using Soxhlet extraction method. Then the extracts were filtered and concentrated using rotary vacuum evaporator at 45 °C. The semisolid mass thus obtained were stored in desiccator until further use.[10]

Extraction yield
The yield of 4 grams of extract from 70 grams of *Cuscuta reflexa* plant material represents approximately 5.71% (Calculated as 4 gms/70gms×100). This percentage reflects the amount of the plant's bioactive constituents that were successfully extracted through the Soxhlet method.

Preliminary phytochemical analysis

The extracts were qualitatively analysed for presence of different phytoconstituents as per usual methods**.**
Preliminary phytochemical screening of *Cuscuta reflexa* involves identifying the presence of various bioactive compounds, which may contribute to its anti-pyretic activity. The following tests are commonly conducted:

Alkaloids test
Dragendorff's Test
1. Preparation of Dragendorff's reagent: Mix 0.5 g of bismuth nitrate with 10 mL of distilled water. Add 10 mL of potassium iodide solution (10% w/v) to the mixture. Stir well to dissolve the reagents.
2. Preparation of the test sample: Prepare a solution of the test sample (plant extract) in distilled water.
3. Adding the reagent: Add 1-2 drops of Dragendorff's reagent to the test sample solution.
4. Observation: Observe the resulting mixture for the formation of a precipitate or colour change[14].

Flavonoids test
Flavonoids are known for their anti-inflammatory and antioxidant activities, which can help in reducing fever.
Alkaline Reagent Test
Add sodium hydroxide (NaOH) to the extract. A yellow colour indicates the presence of flavonoids. Upon adding an acid (HCl), the colour should revert to colourless, confirming the presence of flavonoids[15].

Tannins test
Tannins can also have an anti-inflammatory effect, which may contribute to reducing fever.
Ferric Chloride Test
Add ferric chloride (FeCl3) to the extract. A blue or greenish-black colour indicates the presence of tannins[16].

Saponins test
Saponins have anti-inflammatory and immune-modulating properties, both of which can contribute to antipyretic effects.
Foam Test:
Shake the extract with water. If stable foam forms, it indicates the presence of saponins, which are known to reduce fever in some cases.
Foam Test: Persistent froth upon shaking in water indicates saponins.
Lead acetate test:
Add 10% lead acetate solution to the extract.
Observation: A white or yellowish precipitate indicates the presence of saponin[17].

Terpenoids test
Terpenoids have anti-inflammatory properties and are sometimes associated with fever reduction.
Liebermann-Burchard Test
Add acetic anhydride and concentrated sulfuric acid to the extract. A blue or green colour indicates the presence of terpenoids.
Liebermann-Burchard Test: Green or blue colour formation indicates steroids[18].

Glycosides test
Glycosides, especially cardiac glycosides, can influence inflammation and fever response.
Keller-Killiani Test
Add a few drops of ferric chloride (FeCl3) and then concentrated sulfuric acid (H_2SO_4) to the extract. A reddish-brown layer at the interface indicates the presence of glycosides.
Keller-Killiani Test: Brown ring formation at the interface suggests cardiac glycosides[19].

Carbohydrates test
Mix the plant extract with Benedict's reagent (copper (II) sulphate, sodium carbonate, and sodium citrate). Heat the mixture and observe for a green, yellow, or brick-red precipitate, indicating the presence of reducing sugars[20].
Benedict’s Test: Red, green, or yellow precipitate suggests reducing sugars

Proteins and amino acids test
Biuret Test
Mix the plant extract with biuret reagent (copper (II) sulphate, sodium hydroxide, and tartrate). A purple colour indicates the presence of proteins**.**
Ninhydrin Test
Mix the plant extract with ninhydrin reagent. A blue or purple colour indicates the presence of proteins[21].
These tests help in identifying the phytochemicals responsible for *Cuscuta reflexa*'s potential anti-pyretic activity.

Anti-pyretic activity
Cuscuta reflexa has been traditionally used to treat fever and other inflammatory conditions. The plant's anti-pyretic activity has been attributed to its bioactive compounds, including flavonoids, alkaloids, and glycosides.
The exact mechanism of action of *Cuscuta reflexa* anti-pyretic activity
1. Inhibition of prostaglandin synthesis: *Cuscuta reflexa* bioactive compounds may inhibit the synthesis of prostaglandins, which are mediators of inflammation and fever.
2. Modulation of the hypothalamic-pituitary-adrenal (HPA) axis: *Cuscuta reflexa* may modulate the HPA axis, which plays a crucial role in regulating body temperature.

3. Antioxidant activity: *Cuscuta reflexa*'s bioactive compounds may exhibit antioxidant activity, which can help reduce oxidative stress and inflammation[22].

***Invitro* studies**

INVITRO studies have demonstrated the anti-pyretic activity of *Cuscuta reflexa*:

The Protein Denaturation Test is used to evaluate the pyrogenic activity of a substance by measuring its effect on protein stability. Pyrogens, particularly endotoxins, can cause protein denaturation, which is an indirect indicator of their presence.

Procedure for protein denaturation test to detect pyrogenic activity

Materials required

1.Test sample (suspected pyrogenic substance)
2.Egg albumin or bovine serum albumin (BSA) solution
3.Phosphate buffer (pH 6.4)
4.Distilled water
5.Aspirin (standard anti-inflammatory drug, positive control)
6.Incubator or water bath (set at 37°C)
7.UV-Visible spectrophotometer

Preparation of the reagents

Egg Albumin Solution

Prepare a 5% v/v solution of fresh egg albumin in distilled water.

Phosphate Buffer

Prepare a 0.2 M phosphate buffer (pH 6.4).

Standard Drug (PARACETAMOL) Solution

Prepare a standard paracetamol solution (50 µg/mL)

Test procedure

Test substance was dissolved in phosphate buffer at various concentrations (e.g., 10, 20, 30, 40, 50 µg/mL). Control was prepared without the test sample (only egg albumin and buffer). Positive control was prepared using paracetamol. An egg albumin solution of 0.2ml was taken in each test tube. Phosphate buffer of 2.8ml was added to maintain pH. The test tubes are incubated in a water bath at 37°C for 15-30 minutes. After incubation, the absorbance was measured at 660 nm was using a UV-Vis spectrophotometer. A higher absorbance indicates greater protein denaturation, suggesting pyrogenic activity [23].

Calculation of inhibition of protein denaturation

The percentage inhibition of protein denaturation is calculated using the following formula:

Percentage Inhibition = Absorbance Of Contol – Absorbance Of Test/Absorbance Of Contol × 100

Where,

Absorbance of Control: The absorbance of the control group (without the test sample).

Absorbance of Test: The absorbance of the test substance[24].

Heat-induced haemolysis assay for antipyretic property

This assay evaluates the ability of plant extracts or compounds to stabilize red blood cells (RBCs) against heat-induced haemolysis, which is associated with anti-inflammatory and antipyretic properties.

Materials required

1. Blood Sample: Fresh human or animal blood (preferably from a healthy donor or obtained from a lab source).
2. Phosphate Buffered Saline (PBS): pH 7.4
3. Plant Extract/Test Sample: Prepared in a suitable solvent (e.g., water, methanol, or DMSO).
4. Centrifuge
5. Water Bath (Set at 54°C)
6. Spectrophotometer/UV-Vis Spectrometer
7. Eppendorf Tubes or Test Tubes

Procedure

A fresh blood was collected and transferred to a centrifuge tube containing an anticoagulant (K3 EDTA). It was Centrifuged at 3000 rpm for 10 minutes at room temperature. The supernatant (plasma) was discarded and

the pellet (RBCs) was washed for three times with phosphate-buffered saline (PBS, pH 7.4). The washed RBCs was resuspended in PBS to make a 10% v/v RBC suspension.1 mL of the 10% RBC suspension was taken in different test tubes.1 mL of plant extract/test sample at different concentrations (100, 200, 400µg/mL) was added. A control tube containing only PBS and RBC suspension (no plant extract). A standard drug control (paracetamol) at a known concentration was taken. All tubes were incubated in water bath at 54°C for 30 minutes. After incubation, immediately cool the tubes to room temperature. It is Centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and the absorbance was measured at 540 nm using a spectrophotometer.
The absorbance correlates with the degree of haemolysis[25].

Calculation of haemolysis inhibition (%)

To determine the percentage inhibition of haemolysis caused by the test substance, use the following formula:

Percentage Of Haemolysis = (OD sample/OD control) ×100

Where,

OD of sample: Absorbance of the test sample.

OD of control: Absorbance of the control tube (RBCs with PBS, no extract)[26].

RESULTS AND DISCUSSION

The 70% ethanol extract of the whole plant showed the Presence of Alkaloids, Flavonoids, Saponins, Glycosides, Terpenoids, Tannins, Carbohydrates, Proteins and Amino acids (Table 1).

Table 1: Preliminary Phytochemical Screening of *CUSCUTA REFLEXA*

S. No	Test Parameters	Result
1	Alkaloids	PRESENT
2	Flavonoids	PRESENT
3	Tannins	PRESENT
4	Saponins	PRESENT
5	Terpenoids	PRESENT
6	Glycosides	PRESENT
7	Carbohydrates	PRESENT
8	Proteins and Amino acids	PRESENT

***IN VITRO* STUDIES**

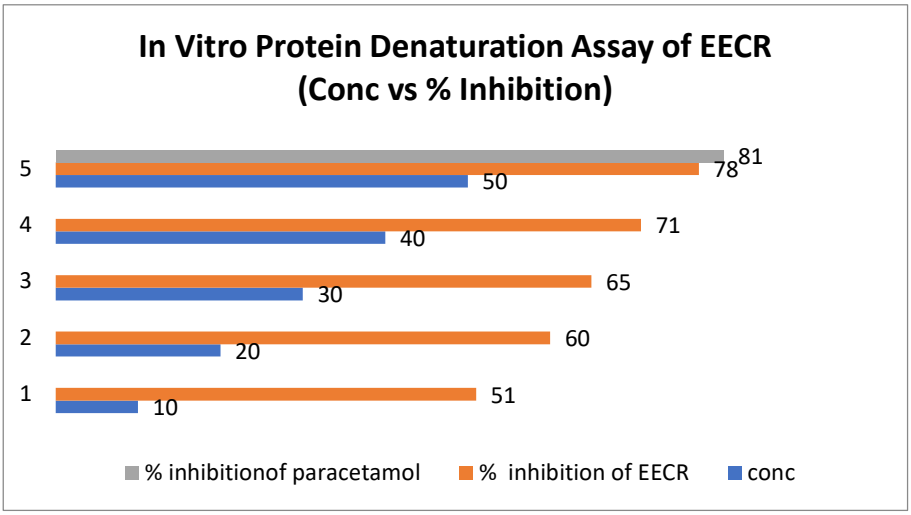
Anti-Pyretic Potentials Of 70% Ethanolic Extract Of *Cuscuta Reflexa Using In Vitro* Studies.

Protein denaturation assay are discussed below (Table 2).

Table 2: *In Vitro* Protein Denaturation Assay of EECR

S.NO	Drug Treatment	Absorbance OD	% Inhibition
1	**Control**	0.35±0.05	
2	EECR (10µg/ml)	0.17±0.04	51
3	EECR (20µg/ml)	0.14±0.02	60
4	EECR (30µg/ml)	0.12±0.05	65
5	EECR (40µg/ml)	0.10±0.04	71
6	EECR (50µg/ml)	0.07±0.01	78
7	Standard Paracetamol (50µg/ml)	0.06±0.01	81

Values are presented as mean ± Standard Deviation, n=3,



Heat induced haemolysis assay are discussed below (Table 3).

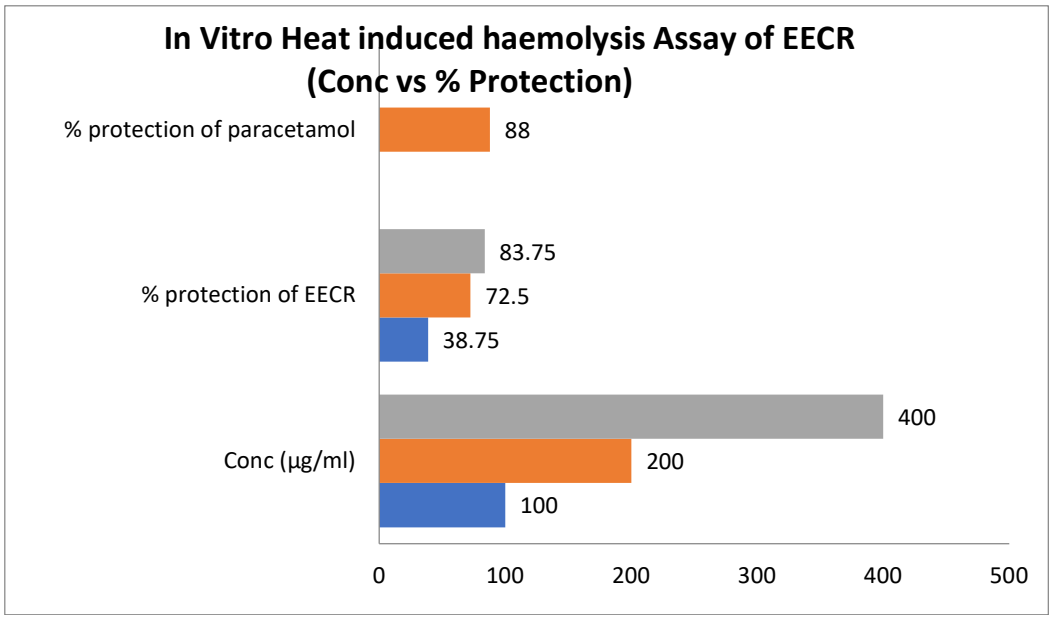
The results from the **in vitro protein denaturation assay** of the ethanol extract of *Cuscuta reflexa* (EECR) provide valuable insights into its potential anti-pyretic properties. This assay is commonly used to evaluate a substance's ability to inhibit protein denaturation, which is a key process in the inflammatory response. Denaturation of proteins, particularly in the case of albumin, can contribute to various inflammatory conditions, and substances that can prevent this denaturation may serve as potential anti-pyretic agents.

1. **Dose-Dependent Inhibition**: The data clearly demonstrate that EECR exhibits dose-dependent inhibition of protein denaturation. As the concentration of the extract increases from 10 µg/ml to 50 µg/ml, the percentage of inhibition increases significantly, from 51% to 78%. This indicates that EECR has a potent capacity to inhibit protein denaturation at higher concentrations.
2. **Comparison with Standard (Paracetamol)**: At the highest concentration tested (50 µg/ml), EECR showed 78% inhibition of protein denaturation, while the standard drug (Paracetamol) at the same concentration (50 µg/ml) showed 81% inhibition. This suggests that EECR is nearly as effective as the standard drug in preventing protein denaturation, which highlights its potential as a valuable anti-pyretic agent.
3. **Statistical Significance**: The values presented as mean ± standard deviation (n=3) suggest that the results are consistent and reproducible, providing reliable evidence of the anti-pyretic potential of EECR. The use of multiple replicates (n=3) strengthens the validity of the findings.

Table 3: *In Vitro* heat induced haemolysis assay of EECR

S.NO	Drug Treatment	Absorbance	% Protection
1	CONTROL	0.81±0.05	
2	EECR (100µg/ml)	0.49±0.02	**38.75**
3	EECR (200 µg/ml)	0.22±0.04	**72.5**
4	EECR (400 µg/ml)	0.13±0.01	**83.75**
5	Standard Paracetamol (200 µg/ml)	0.09±0.03	**88**

Values are presented as mean ± Standard Deviation, n=3,



Interpretation of Results

EECR as a Potential Anti-pyretic Agent: The inhibition of protein denaturation is often associated with anti-pyretic and analgesic activities. The results from this assay suggest that *Cuscuta reflexa* has significant anti-pyretic properties, which could be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, and glycosides, known for their ability to modulate inflammatory pathways.

Dose-Response Relationship: The observed dose-response relationship implies that higher concentrations of EECR are more effective in preventing protein denaturation, which supports its potential use in treating inflammatory conditions at higher doses.

Potential for Drug Development: Given its promising results, EECR could serve as a candidate for the development of new anti-inflammatory drugs, especially considering its nearly comparable efficacy to a well-known anti-inflammatory drug like paracetamol.

The results from the **in vitro heat-induced hemolysis assay** of the ethanol extract of *Cuscuta reflexa* (EECR) provide important insights into its membrane-stabilizing properties, which are critical for its potential as an anti-inflammatory and protective agent. This assay evaluates the ability of a substance to protect red blood cells (RBCs) from lysis (breakdown) induced by heat, **Dose-Dependent Protection:** The data demonstrate a dose-dependent increase in protection against heat-induced hemolysis as the concentration of EECR increases. At 100 µg/ml, the protection is 38.75%, while at 400 µg/ml, protection reaches 83.75%. This indicates that higher concentrations of EECR provide better stabilization to RBC membranes, preventing hemolysis caused by heat.

1. **Comparison with Standard (Paracetamol):** At a concentration of 200 µg/ml, the standard drug paracetamol provides 88% protection, which is slightly higher than the 83.75% protection observed with EECR at 400 µg/ml. Although EECR's effectiveness at higher concentrations is very close to that of paracetamol, this suggests that *Cuscuta reflexa* could be a potent alternative or complementary agent for protecting cell membranes under stress conditions.
2. **Statistical Consistency:** The results, presented as mean ± standard deviation (n=3), indicate that the data are consistent and reproducible, further validating the reliability of the assay and the promising protective effect of EECR.

Interpretation of Results

Membrane-Stabilizing Effect of EECR: The ability of EECR to protect RBCs from heat-induced hemolysis suggests that it has membrane-stabilizing properties. This could be attributed to the presence of bioactive compounds in *Cuscuta reflexa*, such as flavonoids, saponins, and alkaloids, which are known to possess antioxidant and anti-inflammatory activities that help stabilize cell membranes during oxidative stress.

Potential Anti-inflammatory Properties: Since heat-induced hemolysis is a model of cellular damage that occurs in inflammatory conditions, the observed protection suggests that EECR may have therapeutic potential in managing inflammation or conditions involving oxidative stress. The compound could help protect cells from damage caused by excessive heat or inflammation.

Dose-Dependent Protection: The results also highlight a dose-response relationship, with higher concentrations of EECR providing greater protection. This suggests that higher doses of EECR may be necessary to achieve the maximum protective effect, which could be relevant in designing potential therapeutic applications.

CONCLUSION

Phytochemical screening helped to identify the key bioactive compounds in *Cuscuta reflexa* that might be responsible for its antipyretic effects. By detecting flavonoids, alkaloids, saponins, terpenoids, and glycosides, can correlate the presence of these compounds with potential fever-reducing properties. Based on the data from the protein denaturation assay, the ethanolic extract (EECR) shows a clear, dose-dependent inhibition of protein denaturation. As the concentration of EECR increases from 10 µg/mL to 50 µg/mL, the percentage inhibition rises from 51% to 78%. This is close to the standard drug (Paracetamol at 50 µg/mL), which exhibits 81% inhibition. Therefore, the extract demonstrates significant anti-denaturation activity, suggesting it may have protective or anti-pyretic potential comparable (though slightly lower) to that of Paracetamol under these experimental conditions. Based on the data, The Ethanolic Extract of *Cuscuta reflexa* (EECR) demonstrates a clear, dose-dependent membrane-stabilizing effect against heat-induced haemolysis of red blood cells. As the concentration increases (100 → 400 µg/mL), the percentage protection rises from about 39% to nearly 84%, approaching the protection level of the standard (Paracetamol 88%). This suggests that EECR has substantial membrane-stabilizing or anti-pyretic potential, comparable (though slightly lower) to Paracetamol at the concentrations tested.

In present study, the antipyretic activity of 70 % ethanol extracts from *Cuscuta reflexa* Roxb. (Cuscutaceae) was evaluated using *In Vitro* methods. The extracts started reducing the elevated temperature in a dose related manner. The 70% ethanolic extract reduced 51% to 78% respectively as compared to reference drug paracetamol (81%) for protein denaturation assay and 39% to 84% respectively as compared to reference drug paracetamol (88%) for heat induced haemolysis. It was therefore concluded that 70% of the ethanolic the extracts of *C. reflexa* has antipyretic activity, the ethanol extract was found to be slightly potent. From our study and the result obtained, We conclude that even the parasitic dooder can be an alternative medicine for the various aliments. Further more, the results obtained from our study would be good lead to the researchers who take *Cuscuta reflexa* as their research plant.

REFERENCES

1. Kirtikar KR, Basu BD. Indian medicinal plants. publisher not identified Basu, Bhuwaneśwari Âśrama; 1918.
2. Khory RN. The Bombay materia medica and their therapeutics. Periodical Expert Book Agency; 1986.
3. Baqar, S.R., Medicinal plants of Southern-West Pakistan. Periodical Expert Book Agency: New Delhi, 1992.
4. Khare, C.P. Indian medicinal plants: an illustrated dictionary. Springer: Berlin. Heidelberg, 2007.
5. Khandelwal. K.R. Practical pharmacognosy. Nirali Prakashan: Pune, 2005.
6. Harborne, J.B. Phytochemical methods - A guide to modern techniques of plant analysis. Chapman & Hall: London, 1998.
7. Ansari S.H. Essentials of Pharmacognosy. Birla Publications Pvt. Ltd.: New Delhi. 2008.
8. Sharma, P., & Agarwal, A. (2008). "Phytochemical and pharmacological properties of *Cuscuta reflexa* Roxb." *Phytochemistry Reviews, 7*(4), 495-499.
9. Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1956). "Glossary of Indian Medicinal Plants." *Council of Scientific and Industrial Research, New Delhi.*
10. Singh, S., & Singh, D. (2014). "Antimicrobial, anti-inflammatory and antipyretic effects of *Cuscuta reflexa*." *International Journal of Phytomedicine, 6*(2), 144-149.
11. Vijikumar S, Ramanathan K, Devi BP. *Cuscuta reflexa* Roxb—A wonderful miracle plant in ethnomedicine. Indian J Nat Sci. 2011;976:997.
12. Kumar, S., & Sharma, S. (2016). "Antidiabetic and antioxidant properties of *Cuscuta reflexa*." *Journal of Ethnopharmacology, 179*, 16-20.
13. Saraswati, S., & Sharma, M. (2019). "The role of *Cuscuta reflexa* in traditional medicine and its pharmacological actions." *Pharmacognosy Reviews, 13*(26), 18-22.
14. Raghavendra, S., & Nair, A. (2020). "Medicinal properties of *Cuscuta reflexa*: A review." *International Journal of Herbology, 9*(2), 77-83.
15. Kaur, S., & Walia, M. (2011). "Antipyretic activity of *Cuscuta reflexa* in experimental models." *Journal of Experimental Pharmacology, 3*, 35-40.
16. Joshi, S., & Khanna, S. (2012). "Phytochemical screening and antimicrobial activity of *Cuscuta reflexa* Roxb." *International Journal of Drug Development and Research, 4*(3), 92-98.

17. Prajapati, N. D., & Purohit, S. S. (2003). "A Handbook of Medicinal Plants: A Complete Source for Indian Medicinal Plants." *Agro Bios Publishers.*
18. Bose, S., & Bandyopadhyay, P. (2017). "Pharmacological properties of *Cuscuta reflexa*: A review of its use in traditional medicine and modern pharmacology." *Pharmacology and Therapeutics, 18*, 92-105.
19. Bhattacharya, S., & Roy, B. (2010). Preliminary investigation on antipyretic activity of *Cuscuta reflexa* in rats. Journal of Advanced Pharmaceutical Technology & Research, 1(1), 83–87.
20. Afrin, N. S., Hossain, M. A., & Saha, K. (2019). Phytochemical screening of plant extracts and GC-MS analysis of n-Hexane soluble part of crude chloroform extract of *Cuscuta reflexa* (Roxb.). Journal of Pharmacognosy and Phytochemistry, 8(2), 560–564.
21. Azad, A. K., & Mohamed, F. (2024). Determination of total phenolic and flavonoid content and evaluation of antioxidant activities of *Cuscuta reflexa*. Universal Journal of Pharmaceutical Research, 8(6).
22. Shahwar, D., Raza, M. A., Bukhari, I. H., & Abbas, G. (2015). *Cuscuta reflexa* and Carthamus oxyacantha: Potent sources of alternative and complementary drugs. BMC Complementary and Alternative Medicine, 15, 52.
23. "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis" by Jean B. Harborne (Chapman and Hall, 1998)
24. "Biochemistry Laboratory Manual" by Richard J. Cumport (Wiley, 2013)
25. "Pharmacognosy and Pharmacobiotechnology" by Ashutosh Rar (CRC Press, 2017)
26. Wang X, Li N, Ma M, Han Y, Rao K. Immunotoxicity in vitro assays for environmental pollutants under paradigm shift in toxicity tests. International Journal of Environmental Research and Public Health. 2022 Dec 24;20(1):273.