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HELOTROPHIUM VELUTINUM LEAF EXTRACT: PHYSICO-CHEMICAL AND ANTIPYRETIC PROPERTIES.

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ABSTRACT

Researchers investigated the phytochemical screening and antipyretic activity of *Heliotropium velutinum* L's (H. velutinum L) ethanolic leaf extract in rats under Brewer's yeast-induced hypothermia. Several areas of Tamilnadu were selected for collection of H. velutinum leaves and the leaves were cut into small pieces and shade dried for a period of two weeks. A Soxhlet apparatus was used to extract 96% ethanol from dried powdered leaves (200 gm). Based on a standard phytochemical screening procedure, alkaloids, glycosides, carbohydrates, phytosterols, flavonoids, and saponins were identified, but fixed oil and gums-mucilage were not detected. For the experiment, albino rats were taken weighing (300-350g) and were divided into four different groups with each group containing seven animals. A 4% aqueous suspension of gum Acacia was administered orally as vehicle to group 1, ethanolic leaf extracts of *H. velutinum* were administered in groups 2 and 3, along with 4% aqueous suspensions of gum Acacia, and paracetamol 30 mg/kg was given orally as a standard treatment. Rectal temperature was markedly elevated after 20 hours following subcutaneous injection of yeast suspension. The rectal temperature of rats was decreased in a dose-dependent manner by *H. velutinum* extract treatment. A significant reduction in body temperature was observed at a dose of 600 mg/kg and lasted up to 240 minutes after it was administered, as a result of this effect. Within 60 minutes of administration, the antipyretic effect began and lasted for 240 minutes. At 120, 180, and 240 minutes, yeast elevated rectal temperatures were significantly reduced by alcohol extract and paracetamol 30 mg/kg compared to the control group.

Key words: *Heliotropium velutinum*, Physiochemical screening, anti-pyretic

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INTRODUCTION

An herbal medicine is one of the types of dietary supplements that are available. A wide variety of herbal products are available for sale, including tablets, capsules, powders, teas, extracts, fresh or dried plants.

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There are approximately 80% of people all over the world that take herbal medicines as a form of treatment. Phytomedicine is the practice of treating illness and disease with plants' seeds, berries, roots, leaves, bark, or flowers.

It is widely accepted that herbal medicine has been used outside the conventional medical system for many centuries. Health problems can be treated with herbal medicines. The most productive source of drug leads has been natural products including plants, animals, and minerals. In clinical and preclinical study, a number of compounds derived from natural sources are being investigated, particularly for their effects on inflammation, cardiology, diabetes, obesity, antimalaria, antiviral, and neoplastic diseases.

A large number of tribes live in the North East of India, which has such a rich floristic diversity that they are totally dependent on forest plants in order to lead a complicated life. They depend heavily on the local vegetation for food, fuel, fodder, medicine, cordage and a number of other household necessities like food, fuel, fodder, medicine and cordage, among other things. About 60% of the total population lives in tribal areas in this region. However, tribal populations comprise over 75% of the population in Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, and Assam. It is often a secondary effect of infection, malignancy or other diseases to cause pyrexia or fever as a secondary consequence. Infectious agents and damaged tissue cannot survive in the environment created by the body's natural defenses. When tissue becomes infected or damaged, it produces proinflammatory mediators, which stimulate the hypothalamus to elevate body temperature by synthesizing prostagland in E2 (PG E2).

Methodology

Authentication of *Heliotropium velutinum*

It was during the month of May and June that we collected the entire plant of *Heliotropium velutinum* Linn from Tamilnadu. In a note book, data such as the plant's height, flower color, and soil condition were recorded after they were thoroughly washed in running water. A botanist from the Botanical Survey of India (BSI) verified the authenticity of the specimen.

Soxhlet Extraction

Heliotropium velutinum L, dried and pulverized, and taken in Soxhlet apparatus as 650 g. The material was defatted in ethanol and heated at 80°C to extract. Extracts obtained were filtered and treated by rotary vacuum evaporators at reduced temperatures, then concentrated and stored at 5°C. In terms of dried whole plant of *Heliotropium velutinum*, yield was 6.00% (w/w). It was decided to dry the extract in a desiccator and then study it in greater detail.

Experimental procedure

In order to collect young Albino rats weighing in between 150 g and 300 g, healthy albinos were purchased. Polypropylene cages were used for individual animal housing and the temperature and humidity were maintained at 30±6C and 50% respectively with half a day and night cycle. Pellet chew feed and water included in the standard diet were fed to the animals as well. Animals were used in all experiments according to the guidelines of the Institutional Animal Ethics Committee for the care and use of animals.

PHYTOCHEMICAL SCREENING

Qualitative tests were performed on the ethanolic extract to assess the presence of different phytoconstituents, such as alkaloids, glycosides, saponins, flavonoids, carbohydrates, amino acids, sterols, gums, and mucilage. In order to identify the various constituents of the concentrated extract, chemical tests were performed according to the methods listed below.

Test for alkaloidal content

It is necessary to dissolve 60mg of solvent free extract with a few ml of dilute hydrochloric acid and filter this extract. Following are some of the acid-based reagents that have been used to test the filtrate:

- **Mayer's test**
  A few drops of Mayer's reagent should be added to a few ml of the filtrate by the side of the test tube, along with a few drops of filtrate. An orange PP indicates a positive test. The Wagner Test consists of the following steps: By the side of the test tube, a few drops of Wagner's reagent are added to a few ml of filtrate that has been diluted. In the case of a positive test, the result will appear on a reddish-brown ppt.
- **Hager test**
  By the side of the test tube, Hager's reagent is added to 2 or 3 ml of filtrate. Precipitates of yellow color indicate a positive test.
- **Dragendorff's test**
  The Dragendorff's reagent is added by the side of the test tube to a few ml of filtrate and is then mixed with two or three ml of filtrate. Test results are indicated by a prominent yellow ppt.

Test for carbohydrates

We dissolve 200 mg of the extract in 6 ml of water and filter it through cheesecloth before using it. There are several tests that are conducted on the filtrate after it has been filtered.

- **Molish's test**
  3 ml of the filtrate is added to a test tube with 2ml of cone.H2SO4 slowly added following along the side of the test tube, the mixture is shaken well and allowed to stand for two minutes. The next step of the procedure is to add three drops of alcoholic sol of alpha-naphthol to the test tube. The presence of carbohydrate is indicated by a violet ring.
- **Feling's test**
  In this experiment, 2 ml filtrate is added to 2 ml of Fehling's solutions A & B, the mixture is heated on a water bath for 3 minutes, and the presence of red ppt indicates the presence of sugar in the sample.
- **Benedict's test**
  When 2 ml Benedict reagent and 1 ml filtrate are heated on a boiling water bath for 3 minutes, a characteristic colour ppt indicates the presence of sugar.
- **Barfoed's Test**
  After heating the mixture on a water bath for 3 minutes, 2ml of filtrate is added to 2ml of barfoed reagent, which indicates the presence of sugar.

Test for Saponins

There is one dosage of 60 mg of extract diluted in 30ml of water, made up with 30ml of water. An accumulation of 3 cm of foam indicates saponin is present after shaking for 20 minutes.

Test for phenols

Ferric Chloride test

A few drops of 6% ferric chloride solution are added to the dissolved extract 60 mg in 5 ml of distilled water. The presence of phenolic compounds is indicated by a dark green color.

Test for gelatin:

A solution of 60% sodium chloride is added to 5 ml of distilled water and 60 mg of extract are dissolved. Phenolic compounds are indicated by white precipitate.

Test for Glycosides and Flavonoids

An extract of 60mg is hydrolyzed with concentrate hydrochloric acid for 120 minutes in a water bath, then filtered and tested as follows:

Borntrager's Test

Hydrolysate is added to 4ml of chloroform and shaken, separating the chloroform layer and adding 15% ammonia solution. Glycoside is detected by pink color.

Lead acetate's test

This solution is prepared by dissolving 60mg of the extract in water and then adding 4ml of a 15% solution of lead acetate to this solution. The presence of flavonoids is indicated by a bulky white precipitate.

Reduction of magnesium & hydrochloric acid

Six ml of alcohol is added to the 60mg extract along with fragments of magnesium ribbon and HCL acid drop wise. A pink to crimson color indicates the presence of flavanols glycosides.

Test for Alkaline Reagent

It is treated with a solution of 20 % Ammonium hydroxide in order to get a solution of aqueous extract. The presence of flavonoids is indicated by yellow fluorescence.

Aqueous's Sodium Hydroxide Test

By treating an aqueous solution of the extracts with sodium hydroxide solution, the cyanine was turned into blue to violet (as an anthrocyanine), the flavones turned yellow, and the flavanones turned orange to yellow.

Concealed Sulfuric Acid Test

In aqueous solution, if cyanine (Anthrocyanines) is added to the aqueous solution, then flavones (yellow), flavanones (orange), and cyan (crimson) will result.

Protein and Amino Acid test

Million's test

The presence of proteins and amino acids has been determined by adding three ml of filtrate plus a few drops of Millon's reagent.

Test for Biurets

3% copper sulphate solution is added to an aliquot of filtrate. This mixture is then fed with 2ml of 99.9% ethanol and potassium hydroxide pellets on top. An ethanolic layer that is pink indicates that protein is present.

Ninhydrin test

Ninhydrin solution (15 mg in 300 ml of acetone) is diluted with aqueous filtrate to form two

Legal's test

An alkaline solution is made with 15% NAOH using 60mg of extract dissolved in pyridine and a sodium nitroprusside solution added.

Killer Killani's test

We added ferric chloride and concentrated sulphuric acid to a GAA extract of the drug. The junction between the two layers is reddish brown, while the upper layer turns blueish green.

Phytosterol Detection

Test for Libbermann-Burchards

Using acetic anhydride and 60mg of the extract, make 3ml of the extract. The solution is then diluted by adding 3-4 drops of conc. sulphuric acid over the side of the test tube. Phytosterols can be detected by an array of color changes.

Table 1. Pyrexia caused by yeast

An Approach To Treatment	Dosage (mg/kg)	Ideal Temperature	Induced Pyrexia in yeast 20 hrs later, Rectal Temperature (°c)	Temperature Of The Rectal After Extract Treatment (°C)			
				60 Min	120 Min	180 Mins	240 Mins
Control	0	36.30 ±0.005	39.09 ±0.008	39.0 ±0.009	39.09 ±0.008	39.08 ±0.008	39.09 ±0.005
***H. velutinum* ethanolic extract**	255	36.32 ±0.007	39.08 ±0.009	38.8 ±0.006	38.04 ±0.008	37.95 ±0.005	37.66 ±0.009

***H. velutinum* ethanolic extract**	550	36.32 ±0.007	39.06 ±0.008	38.9 ±0.007	38.56 ±0.004	37.64 ±0.006	37.36 ±0.004
Paracetamol	30	36.33 ±0.008	39.07 ±0.007	38.0 ±0.008	38.37 ±0.003	37.76 ±0.005	36.90 ±0.004

Fat and Oil Detection

Spot's Test

The extract was pressed between two filter papers separately and a small quantity was removed from each filter paper. There is a possibility that fixed oil has stained the paper, which indicates that fixed oil has been present.

Saponification's test

Combine 0.6N alco. KOH with phenolphthalein and add a few drops to a small quantity of extracts. The mixture needs to be heated on a water bath for 120 minutes. The presence of fats and oils is indicated by the formation of soap or partial neutralization of alkali.

Mucilage and Gum

A solution of 200 mg of the extract mixed with 10ml of distilled water is dissolved in a solution of 30ml of absolute alcohol that is steadily stirred to dissolve the extract. The presence of gums or mucilage is indicated by white or cloudy precipitation.

Coumarin Detection

It is necessary to dissolve 60mg of the extract in 15ml of absolute alcohol, and then add a few drops of ferric chloride to this solution. Coumarin exhibits a greenish fluorescence.

Antipyretic Activity of Yeast

There has been a study that investigated the antipyretic activity of Brewer's yeast in rats induced with fever (30% Brewer's yeast). A uniform diet of 300-350g rats was given until 1 day before drugs were given. To induce pyrexia in rats, a 30% suspension of dried yeast in 3% gum Acacia in normal saline was subcutaneously injected at a dose of 30 ml/kg of body weight after the rectal temperature was measured by a digital thermometer placed 2.0 cm in the rectum. Study rats whose temperature rose by at least 2°C after yeast injection were taken for analysis. Each of the groups of animals was treated in accordance with the following procedures:

Group A:

Gum Acacia suspension in 4% aqueous suspension (2ml/300g) is taken orally as a vehicle.

Group B:

Leaves of *H. velutinum* were extracted with ethanol 4% aqueous suspension of gum Acacia and taken orally in doses of 350 mg/kg (2ml/300g).

Group C: The oral administration of an ethanolic leaf extract of the leaves of *Heliotropium velutinum* is 600 mg/kg (2ml/300g), and a 4% aqueous suspension of the gum Acacia is used.

Group D: Taking Paracetamol 30 mg/kg (2ml/300g) orally with 4% aqueous suspension of gum Acacia fluid to reduce the symptoms of the cold. During the first four hours following drug administration, the rectal temperature was recorded every hour.

Result

Several chemical tests showed that *Heliotropium velutinum* Linn ethanolic extract contained alkaloids, glycosides, carbohydrates, phytosterols, flavonoids, saponins, but no fixed oil or gum. Table 1 shows the effects of ethanolic leaf extract of the plant *H. velutinum* on rectal temperature in rats over a period of time. After 20 hours of subcutaneous yeast injection, the rectal temperature was markedly elevated.

In rats treated with *H. velutinum* extract at doses of 350, 600 mg/kg, rectal temperature decreased dose dependently. In the 240 minutes following administration of 350 mg/kg of the extract, the body temperature was significantly lowered (37.66 ± 0.009). After the administration of 600 mg/kg, the effect was maximal, and significant lower body temperature was observed up to 240 minutes after the administration of the compound ($P<0.002$) (37.36 ± 0.004). A significant antipyretic effect was observed after 1 hour of administration of the drug, and lasted for 240 minutes after the drug was administered. A yeast elevated rectal temperature was reduced significantly by standard drug paracetamol 30 mg/kg as well as tested drug *H. velutinum* extract at 120,180 and240 minutes.

CONCLUSION

Based on the observation that the average percentage of antipyretic activity increased with the concentration of ethanolic leaf extract of *H. velutinum* (600mg/kg) compared with the control, the results of the present study suggest that the ethanolic leaf extract significantly reduces pyretic rats' temperature. In addition to the analgesic effect, flavonoids in ethanolic leaf extract of *H. velutinum* may contribute to the antipyretic activity.

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