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ANTIPYRETIC ACTIVITY OF METHANOLIC EXTRACT OF *CONYZA CANADENSIS*

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ABSTRACT:

The most abundant and selective source of bioactive secondary metabolites is found in plants. Throughout human history, people have used several medicinal plants as traditional remedies to treat their health issues. Medicinal plants are significant source of producing compounds which is great importance for the healthof individuals and communities. A wide range of pharmacological actions are known to be possessed by C. canadensis due to its inclusion of antioxidants, antiplatelet, and anticoagulant chemicals. The plant has medicinal properties (haemostatic, antirheumatic, anti-inflammatory, diuretic). It is astringent, stimulant, haemostatic and diuretic, also used in diarrhoea, dysentry, uterine haemorrhages, dropsy, gravel, cystitis, calculus, bronchial catarrh and haemoptysis. Methanolic extract of Conyza Canadensis linn. showed antipyretic activity but the maximum anti-pyretic activity was given by the standard drug paracetamol and the methanolic extract of Conyza canadensis showed antipyretic activity nearly equal to that of the standard drug.

Keywords: C. Canadensis, Antipyretic activity, methanolic extract

INTRODUCTION

Plants are richest source of bioactive secondary metabolites in a most effective way and with specific selectivity. Medicinal plants are significant source of producing compounds which is great importance for the healthof individuals and communities.[1] Plants are able to synthesise a variety of useful chemical constituent groups with intriguing biological activities.[2] Free radicals are associated for several diseases including cancer, diabetes mellitus, arthritis, ageing and liver disorder. Plants constitute of various naturals products that are important form medicinal point of view.[3] Conyza canadensis belongs belong to family Asteraceaewhich contain more than fifty species. Conyza are distributed throughout the world. Genus conyza some species have a lot of pharmacological properties. Traditional point ofview, pharmacological applications including treatment of sore smallpox and other skin related diseases.[4] Isolated secondary metabolites include those that have been shown to have biological activity, such as anti-inflammatory properties.[5] Conyza sumatrensis is another species is used in the treatment of facial pimples and stomach disorder.[6] The current research work directed toexplore chemical constituents and antioxidant action of Conyza bonarensis. Horseweed is regarded as an annual weed with an upright stem that can reach a height of seven feet. From a clump of basal leaves that eventually wither, this stem arises. This herb has lance-shaped, deeply green leaves that emerge alternately. The leaves can have jagged edges and widely dispersed, scratchy bristles with a white colour. Between July and November, horseweed develops an abundance of tiny flower heads in loose bunches. The flower heads have tiny, compact yellowish core blooms and tiny, lavender to greenish white ray flowers. These blossoms mature into white, tufted fruits or achenes that each contain a single seed.[7]

Pyrexia/Fever - Pyrexia or Fever is an elevation of core body temperature that exceeds the normal daily variation and occurs in conjunction with an increase in the hypothalamicset point. In normal adults, the average oral temperature is 37°C (98.6°F). Body temperature above the usual range of normal, can be caused by abnormalities in the brain itself or by toxic substances that affect the temperature - regulating centers.[8]

PLANT PROFILE[9,10]

Synonyms - Canadian Fleabane, Colt's tail, Fleabane, Hogweed, Horseweed.
Family: Asteraceae
Scientific Name - Conyza canadensis (L.)
Preferred Common Name - Canadian fleabane

Figure 1 : Conyza canadensis (L.)[11]

Other Scientific Names - Erigeron Canadensis, Erigeron Pusillus, Trimorpha Canadensis (L.)

Uses - A wide range of pharmacological actions are known to be possessed by C. canadensis due to its inclusion of antioxidants, antiplatelet, and anticoagulant chemicals. The plant has medicinal properties (haemostatic, antirheumatic, anti-inflammatory, diuretic).

1.1 Description

Conyza canadensis (L.) is a member of the tribe Astereae. It prefers undisturbed sites and is a particular problem in low tillage systems such as orchards, plantations but also insome agricultural crops. It may be controlled by tillage at a suitable growth stage, but otherwise, it has developed resistance to many herbicides in a large number of countries. It has been introduced internationally as a contaminant of cereals, forage seeds and cotton, and there is a risk of further similar introduction to countries where it is not yet established. It could become a problem invasive in protected areas, though may be controlled naturally as it is an early-successional species, often being replaced by perennial grasses It is an annual herb growing to 1.5 m and having a short taproot with laterals and several narrow, simple, alternate leaves. It has many capitula, less than 1 cm wide, in a long, paniculate inflorescence. There are numerous, small female florets, sitting in several rows, with a tubular-filiform corolla. Hermaphrodite florets are few, fertile and yellow. C. canadensis is an erect annual with a long taproot and one or more stems arising from a basal rosette, it is usually about 1 m high but may be much taller. Leaves are up to 10 cm long and about 1 cm wide with some shallow teeth, clear green (not grayish as in other common Conyza species), almost glabrous on the surfaces, but with some scattered hairs. Leaf margins ciliate and with longer conspicuous hairs towardsthe leaf base. Flower heads are very numerous on short pedicels, only 2-3 mm in diameter when fresh (broader in pressed specimens), involucral bracts about 5 mm long, glabrous (figure 1) . Disc florets yellow, contrasting with distinct white ray florets which are 0.5 to 1mm long, the latter distinguishing C. canadensis from other common weedy Conyza species.

1.2

1.3 Distribution

C. canadensis is indigenous to North America, but is now found globally as an omnipresent weed on cultivated ground and waste places, and is also widely distributedin Hungary and spread to Europe and later to Asia and Australia, including tropical regions such as in the Americas. However, in Africa it is so far restricted to north and south subtropical. In Bhutan, it is restricted to higher elevations, over 2000 m, but is apparently not so restricted in Central America. C. canadensis is a weed of agriculture and forestry in temperate and subtropical climates and at higher elevations in some parts of the tropics. It is associated with perennial crops, fallows and field borders and, in annual crops, is favoured by reduced tillage.

1.4 Biology and Ecology

1.5

- **Genetics -** C. canadensis is a diploid species, with a chromosome number of 2n=18. This is in contrast with several other species of Conyza, which were ascertained as allopolyploids. As there is a tendency in plants to increasing ploidy levels, it may be assumed that C. canadensis is an ancestral member of the genus, and other species may have arisen via hybridization events.
- **Physiology and Phenology -** C. canadensis is predominantly an annual plant, germinating in autumn and persisting as a rosette of leaves over the winter before bolting and flowering the following spring. It may, however, behave as a biennial in temperate climates, but rarely, if ever, persists for a second season after flowering. Seeds need a temperature of 10-25°C and require light for germination. Establishment occurs mainly in occasionally disturbed situations. Intensive cultivations for annual crops apparently bury most of the seed and greatly reduce emergence, while in completely uncultivated situations other vegetation tends to interfere with its establishment. After establishment asa rosette, elongation of the stem is inhibited by short days but occurs rapidly under longerday conditions.
- **Reproductive Biology -** Seed production can be immense, up to 250,000 seeds per plant,and seed dispersal by wind is made highly efficient. Seed size is small, but also variable.
- **Environmental Requirements -** C. canadensis is native to an area with broad climatic amplitude, though is most common in temperate and Mediterranean zones., it can even befound in tropical regions, though generally at higher altitudes. There is little evidence for preference regarding soil type, with C. canadensis apparently able to grow in a wide range of soil types.

1.6 Prevention and Control

1.7

- **Cultural Control -** In traditional farming, C. canadensis is controlled satisfactorily by tillage, hand-weeding and also suitable crop rotation. In more developed systems,

non- chemical methods include the use of living mulches of, for example, Trifolium subterraneum. Other mulches, living or inert, are observed to increase control of C. canadensis, which may be expected as seeds require light for germination. The use of cover crops may also have a similar effect, due to direct competition for light and possible water and plant nutrients, and were shown to be effective in controlling C. canadensis in apple orchards. It is, however, surprisingly resistant to destruction by soil solarization.

- **Chemical Control -** C. Canadensis is normally susceptible to most of the herbicides used to control annual broad-leaved weeds, including 2,4-D and dicamba. However, it has developed widespread resistance to paraquat and the triazines. The mechanisms of resistance have been studied in detail, including the dual resistance to both herbicide groups. Paraquat resistance has been shown to be controlled by a single dominant gene. One previously isolated report of resistance to glyphosate is now supported by others, confirming the increased resistance of this species to such herbicides normally effective along with a wide range of other alternatives depending on the particular croppingregime. These include sulfonylurea herbicides, cyanazine, sulfallate, glufosinate, oxyfluorfen, hexazinone, tebuthiuron, amitrole, asulam, oryzalin, clopyralid and imazapyr. Inconsistent results are reported with diuron, metribuzin, bentazon and acifluorfen, while poor results have been reported with oxadiazon and imazethapy.

- **Biological Control -** There has been consideration of biological control possibilities, the insects of interest including the tephritid Procecidochares australis and the coleopteran Agrilus pulchellus [Engyaulus pulchellus] which is known to attack other Conyza/Erigeron species; but there are no reports of any practical progress.

1.8 LITERATURE REVIEW

1.9

Conyza canadensis (L.) Cronq. (formerly Erigeron Canadensis L.; Although horseweed, also known as canadian fleabane (Asteraceae), is native to North America, it is now widely diffused around the world, including Hungary. Worldwide, this plant's aerial portions and roots have been used as traditional or official herbal medicines to treat gastrointestinal ailments, most notably dysentery and diarrhoea, as well as acting as a diuretic.[1] Horseweed has also been used in Chinese traditional medicine to relieve arthritis pain, swellings, and sores.[2] Additionally, horseweed's volatile oil has been used to treat cystitis and bronchitis.[3] It was discovered that C. canadensis included hydrocarbons from 4,5 sesquiterpenes, sterols, sphingolipids, triterpenes, flavonoids, and C10 acetylenes.[8–9] The essential oil of juvenile and mature plants collected in Washington State, USA, was previously studied by Hrutfiord et al.[10] They discovered and validated the presence of twenty-five compounds, including monoterpenes, sesquiterpenes, and acetylenes, as well as the prevalence of limonene (67.25%).[12] In a sample of aerial parts collected in France, 18 compounds were detected

with limonene as the main constituent (76.0%).[13] The analysis of the essential oil of aerial part of horseweedgrowing in Japan led to the detection of 47 volatile components of which 91.0% were terpenoid.[14] Worldwide infectious diseases are one of the major causes of deaths and responsible for approximately one-half of all the deaths in tropical countries.[15] New, effective and safe therapeutic agents and strategies are demanding issues to cope with the infectious diseases. These medicinal plants are ingested as decoctions, teas and juice preparations to treat respiratory infections or as a poultice and applied directly on the infected wounds or burns. So far extensive work has been done and still going on for the search of bioactive compounds to treat microbial infections as more effective and safer therapeutic agents. Genus Conyza, belonging to the family Asteraceae, comprises about 50 species, which are found in the tropical and warm regions.[16] The plant Conyza canadensis is commonly known as Canada fleabane, bitterweed and horseweed etc. It is found in all warm countries, but is presumed to be of American origin and spread on the rest of the globe because of its import from that continent. Conyza canadensis (syn. Erigeron canadensis), (Asteraceae) is an annual herb that is distributed throughout world including Pakistan. Conyza canadensis is used locally as traditional vegetable and sweetening agent in northern areas of Pakistan and rest of the countries. This plant is reported to be astringent, stimulant, hemostatic, and diuretic, also used in diarrhea, dysentery uterine hemorrhages, dropsy, gravel, cystitis, calculus, bronchial catarrh, and hemoptysis in folk medicine. In Africa, it is used for the treatment of ringworm and eczema. This plant is traditionally used in folk medicines in the northern areas of Pakistan for the treatment of various pathological conditions including its use in acute pain, inflammation, fever and especially the microbial infections including urinary infections, respiratory tract infections, diarrhea and dysentery. Current study was designed to identify the potential bioactive compounds exhibiting significant antibacterial and antifungal activities possibly responsible for its folk use in infectious diseases.[17]

1.10 MATERIAL AND METHODS

1. Plant material

Plant material was collected wild from a village of Neemuch District, Madhya Pradesh in month of October – November. The aerial parts of the plant were air-dried under shade for 2 weeks at room temperature. The dried plant material was later on chopped, pulverized and stored in a polyethylene bag under refrigeration for further experimentation**.**

2. Extraction of plant material[19]

For a duration of 12 days, the 1.5 kg of dried and powdered leaves were macerated in methanol. After filtration, the process was repeated three times using 2.5 L methanol each time. The combined filtrates were concentrated by evaporation at 40 ◦C to afford crude methanol extract (89.4 g, 5.96 % (w/w)).

3. Phytochemical tests[19]

A variety of kinds of natural compounds were screened out of the plant material extract. One gramme of plant extract was diluted with two millilitres of distilled water, filtered, and then ferric chloride reagents were added to the filtrate to test for tannins. To test for alkaloids, 1 g of methanolic extract was boiled, filtered, and treated with 10 ml of 1% HCl along with Mayer's reagent. The presence of flavonoids was determined using magnesium turnins, potassium hydroxide solution, and 1% aluminium chloride solution in methanol concentrated HCl. By mixing 1 ml of acetic anhydride with 0.25 g of the sample's methanolic extract and 1 ml of H_2S0_4, steroids were screened. The presence of steroids was indicated by the colour changing from violet to blue or green. One gramme of extract was added to two millilitres of benzene, filtered, and then an ammonia solution was added to conduct the anthraquinones test. A sheet of filter paper was dampened with NaOH and placed over a test tube containing a boiling plant extract solution in order to identify coumarins. The filter paper was considered to have passed the coumarin test if it subsequently displayed any yellow fluorescence when exposed to UV light. The extract was treated with petroleum ether and then extracted with $CHCl_3$ in order to look for any sterols or terpenes. Following acquisition, concentrated HCl and acetic anhydride were applied to the CHCl3 layer. The presence of terpenes or sterols was shown by the colour changes from pink to purple and green to pink, respectively.

4. Subjects and maintenance[20]

Male rats (30-40 days of age, 100 -140 g) were used. The animals were housed in groups of two animals in a cage at controlled temperature (Table 1) . The animals were transferred to the experimental room 2 h before the experiments for acclimation to the environment. The Committee on the Utilization and Care of Laboratory Animals at our Institute authorized the experiments.

1.10. **Table no. 1 – Animals used for experiment**

Group	**Treatment**
Group I	Animals treated as normal.
Group II	Animals were treated as Controlled group and received vehicle only.
Group III	Animals were given extract of C. canadensis. (Dose 100 mg/ kg).
Group-IV	Animals were treated with standard drug Paracetamol (Dose 100 mg/kg).

5. Drugs[20]

Commercially available dried baker yeast (Saccharomyces cerevisiae,) was suspended in pyrogen-free 0.9% NaCl in a water bath at 37 ◦C for 5 min. Acetaminophen was suspended in 5% Tween 80 and 0.9% NaCl. Test drug (crude extract) was also suspendedin 5% Tween 80 and 0.9% NaCl.

6. Rectal temperature measurement[21]

Rectal temperature (TR) was measured by inserting a lubricated thermistor (Digital thermometer) into the rectum of the animal, which displayed the temperature with a 0.1
◦F precision.

7. Dose [21]

1.10.

1.10. Table no. 2 illustrated the dose given to subjects according to different groups.

Table no. 2 – Dose given to subjects

S. No.	Animal	Weight	Drug	Dose (per Kg)
1.	Group I (Normal)	110 g	Vehicle only	-
2.	Group II (Controlled)	100 g	Baker's yeast + Vehicle (5% Tween 80 in 0.9% NaCl)	100 ml
3.	Group III (Drug Treated)	120 g	Baker's yaest + C. canadensis extract	100 mg
4.	Group IV (Standard Drug)	140 g	Baker's yaest + Acetaminophen	100 mg

8. Baker's yeast impact on rectal temperature[22]

Baker's yeast (100 mg/kg, intraperitoneal) was delivered into the animals right after the first basal rectal temperature was recorded (Table 2). Up to six hours, TR variations were monitored every hour and reported as a variance from the basal value. Since it has been previously reported that handling and temperature measuring-related stress alter rectal temperature, these animals were habituated to the injection and measuring procedure for 2 days before experiments were carried out. In these sessions, the animals were subjected to the same temperature measuring procedure described above, and were injected intraperitoneally (i.p.) with 0.9% NaCl (10 ml/kg).

8.1 Effect of habituation on basal rectal temperature and on baker yeast-induced fever After determining the effective pyrogenic dose of yeast in previously habituated animals, we evaluated whether habituation was necessary for the appearance and for the reliabilityof the pyrogenic response. The animals were subjected to two, one or no

habituation sessions and, in the day of the experiment, injected with the pyrogenic dose of yeast(100 mg/kg). TR changess were represented as a variance from the basal value and recorded every hour for a maximum of 12 hours.

9. Effect of antipyretics on basal rectal temperature[22]

Antipyretic test (yeast induced pyrexia) - The antipyretic activity was determined in male rats (100-140 g). There were four groups of animals (n=4). Every group had an overnight fast and had unrestricted access to drinking water. First group is given a vehicle alone; second group is given saline as a control; third group is given 100 mg/kg of extract/fraction; and fourth group is given paracetamol as a routine medication. After taking the average temperature with a digital thermometer, each mince was given an injection of baker's yeast suspension (100 mg/kg) to induce pyrexia. The rectal temperature was measured after eighteen hours, and the appropriate groups received the above doses by injection. Rectal temperature was recorded periodically in every hour of drugs administration for 6 h, and after the fourth TR measurement they were injected with vehicle (5% Tween 80 in 0.9% NaCl, 5 ml/kg), acetaminophen (100 mg/kg). TR was monitored hourly for six hours following the medication injections.

1.10. Effect of antipyretics on baker yeast-induced Hyperthermia[23]

1.10.

The animals had their basal TR measured and were injected with a pyrogenic dose of baker yeast (100 mg/kg). TR changes were recorded every hour up to 6 h, when antipyretic acetaminophen (100 mg/kg), was administered. The TR was monitored over the following 6 h.

RESULTS AND DISCUSSION

Table No. 3 and Plot 1 illustrated the methanolic extract of Conyza Canadensis a dose of 100 mg/kg caused a significant lowering in rectal temperature of hyperthermic rats. This decrease persisted when an assessment was made 6 h after test drug administration and the efficacy was comparable to that of Paracetamol at a dose of 100 mg/kg (Table No. 3 and Plot 1).

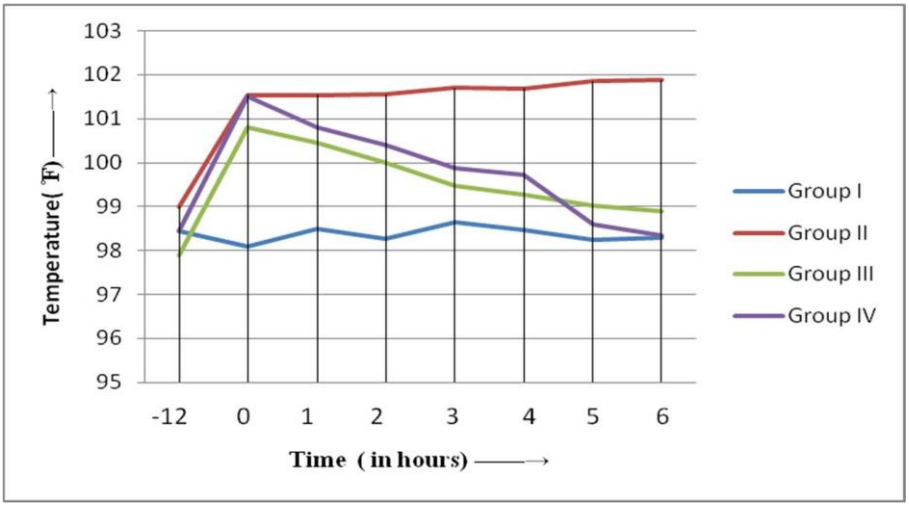
Table no. 3 - observations of rectal temperature

Treatment	Mean rectal temperature (in ^{o}F)							
	Before yeast	After yeast						
	-12 h	0h	1h	2h	3h	4h	5h	6h
Group I	98.438	98.101	98.492	98.276	98.636	98.474	98.258	98.302

Group II	98.996	101.534	101.535	101.552	101.696	101.687	101.84	101.876
Group III	97.898	100.796	100.438	100.004	99.48	99.278	99.01	98.896
Group IV	98.438	101.512	100.796	100.4	99.878	99.716	98.6	98.34

1.11

1.12 Plot No. 1



1.13 Discussion

Fever can be brought on by an infection or one of the aftereffects of inflammation, graft infection, tissue damage, or other medical conditions. Antipyretics are medications that lower high body temperatures. The generation and loss of heat must be carefully balanced in order to regulate body temperature, and the hypothalamus controls the set point at which this temperature is maintained. In fever this set point is elevated and drugs like paracetamol don‟t influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature. The search for effective antipyretic herbal therapies has gained impetus recently due to the availability of antipyretics like aspirin, paracetamol, nimosulide, etc. have toxic effect to the various organs of the body. The antipyretic effect of non-steroidal anti-inflammatory medicines is produced by inhibiting prostaglandin synthesis in the hypothalamus. The anti-pyretic action of Conyza canadensis linn may be due to the inhibition of prostaglandin synthesis.

CONCLUSION

Methanolic extract of Conyza Canadensis linn. showed antipyretic activity but the maximum anti-pyretic activity was given by the standard drug paracetamol and the methanolic extract of Conyza canadensis showed antipyretic activity nearly equal to that

of the standard drug. To pinpoint the precise mechanisms of action and identify the active ingredients causing this activity, more research is necessary.

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