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Anti-inflammatory, Antibacterial and Acaricidal Activities of Various Leaf Extracts of Bitter Apple, *Citrullus colocynthis* Schrad (Cucurbitaceae)

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Abstract

The present study involved the investigation of various leaf extracts of *Citrullus colocynthis* (bitter apple) *viz.* alcoholic, acetone and chloroform for their *in vivo* anti-inflammatory and *in vitro* antibacterial and acaricidal activities. For *in vivo* evaluation, doses of the extracts were taken as 50 mg/kg and 100mg/kg and for *in vitro* experiments, concentrations @5% and 10 % were used in the study. The antibacterial activity was studied against gram +ve (*Staphylococcus aureus*) and gram –ve (*Escherichia coli*) bacteria and was compared with standard drug (ampicillin).For anti-inflammatory activity; fifty rats were divided into 10 groups of five animals each. Initially the normal paw volumes of all the animals were noted down. All animals were injected in the sub-planter region of the right hind paw with 1% carrageenan (in normal saline) @ 0.1 ml to induce inflammation. The acaricidal activity of the alcoholic leaf extract was investigated by Adult immersion test against engorged adult females of *Rhipicephalus microplus* (Canestrini, 1888) (Acari: Ixodidae). No dose-dependent acaricidal activity of the extracts was demonstrated as there was no mortality of the ticks and absence of significant inhibition of oviposition by any of the concentrations of the extracts. Also the results revealed no significant antibacterial activity but all the extracts demonstated significant anti-inflammatory activity with maximum activity in alcoholic extract at higher concentration.

Keywords: Antibacterial, Anti-inflammatory, *Citrullus colocynthis, Rhipicephalus microplus*, Wistar Rats

1. Introduction

Plants have been most important sources of traditional medicines ever since the dawn of human civilization. Chemically, medicinal plants may have secondary metabolites like alkaloids, glycosides, steroids or other groups of compounds which have marked pharmaceutical action as anticancer, antimalarial, antidiabetic, antidy senteric, etc. In spite of tremendous developments in the allopathic medicines during the 20th century, plants still remain as one of the major sources of drugs in modern as well as traditional system of medicine throughout the world. Medicinal plants have been curing various disorders in humans and are considered an integral part of the Indian traditional medicinal system, better known as the Ayurvedic system of medicine[1]. It is estimated that about 80,000 species of plants are utilized by the different systems of Indian medicine[2]. Plant based bioactive compounds have been known to exert pronounced effect on human health, apart from having little or no side effects[3]. The World Health Organization has estimated that 80% of people in some Asian and African countries

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use traditional medicines for some part of their primary health care. Currently, 25 to 50% of all pharmaceuticals dispensed around the world are of plant origin and only few among them have been used as antimicrobials. Important drugs like Quinine, theophylline, penicillin G, morphine, paclitaxel, digoxin, vincristine, doxorubicin, cyclosporin, and vitamin A are cornerstones of modern pharmaceutical care which are derived from natural products[4]. Therefore, plants become a great source of interest in modern system of therapeutics as drugs, nutraceuticals, pharmaceutical intermediates, folk medicines[5].

Since ancient times, several diseases have been treated by administration of plant extracts. Interest in ethnopharmacy as a source of bioactive compounds has increased worldwide, particularly in the search for anti-inflammatory and antibacterial drugs. Plants with antibacterial and anti-inflammatory activities have become more interesting because some of them are part of the arsenal of modern medicine and many people are aware of problems associated with the over-prescription and misuse of usual drugs[6]. Increasingly adverse drug reactions to the synthetic antibiotics and the increasing resistance of some pathogens to synthetic antibiotics, has been another argument against the use of these chemicals as therapeutics.

Bitter apple originally bore the scientific name *Coloc ynthiscitrullus* but is now classified as *Citrullus colocynthis* [Family: Cucurbitaceae], is a perennial herb usually trailing and commonly known as bitter cucumber, desert gourd, vine of Sodom or wild gourd, Indrayan in Hindi, Pikkumutti in Malayalam, RakhaleShasa in Bengali, Paparabudam in Telugu and Kaudatumma in Punjabi.The angular leaves are alternately located on long petioles. Each leaf is almost 5 to 10 centimetres in length and has around 3 to 7 lobes. The upper surface of the leaves are fine green in colour and the lower surface is comparatively pale. Each bitter apple plant produces around 15 to 30 globular fruits having a diameter of almost 7 to 10 centimetres. The outer portion of the fruit is covered with a green skin having yellow stripes. The fruits may also be yellow in colour. The ripe fruits are characterized by a thin but hard rind. The fruits have a soft, white pulp which is filled with numerous ovate compressed seeds. The yellow-coloured flowers appear singly at leaf axils. They are monoecious; the pistils andstamens are present in different flowers of the same plant. They have long peduncles. Eachflower is also comprised of a yellow campanulate. The corolla has five lobes andthe calyx is parted five ways. The female flowers are easily identified from the males bytheir villous, hairy ovary. The seeds are around 6 mm in size, smooth, compressed and ovoid-shaped. They are located on the parietal placenta. The seedsare light yellowish-orange to dark brown in colour. The Bitter Apple plant has a large perennial root that sends out long and slender, angular, tough, rough vine-like stems. The stems are normally spread on the ground and have a tendency to climb over herbs and shrubs by their axillary branching tendrils. *Citrullus colocynthis* is found throughout India and Ceylon, both wild and cultivated. It is alsoindigenous in the Arabia, west Asia, tropical Africa, Mediterranean region. An important property of *C. colocynthis* is that it has a major role in hair growth stimulation[7]. Fruit or root with or without nux-vomica is rubbed into a paste with water and applied to boils and pimples[3]. Cucurbitacin glucosides found in this plant seem to be potentially important for therapy against breast cancer cells because of their ability to modify cell morphology and signaling, and to induce apoptosis and changes in mitochondrial membrane potential[8]. Fruits are used against tumors of gastrointestinal tract. It is more pronouncedly used as anticancerous drug. It is effective in leukemia and joint pains. In *Citrullus colocynthis* there is a cyanogenic glycoside compound (amygdalin) found generally in kernels and other parts of the plant. The dried pulp of *Citrullus colocynthis* L. has been used for constipation, edema, bacterial infections, cancer & diabetes. Fruits of Citrullus colocynthis L. contain seventeen compounds, were broadly identified and divided in to five classes: alcohols, ketones, epoxy compounds, hydrocarbons and an acid[9] .In the light of above, the present study investigated the potential of different leaf extracts of *Citrullus colocynthis* as an anti-inflammatory, antibacterial and acaricidal agent.

2. Material and Methods

2.1 Plant Material and Extraction

The collection of plant was done from the sources as per their availability i.e. *Citrullus colocynthis* were procured from the cotton belt of Punjab (mainly Fazilka and Abohar). After collection the leaves were authenticated by a botanist of Collaborative Research Centre in Veterinary Ayurveda, GADVASU. The leaves were shade dried at room temperature. After drying, leaves were powdered using a grinding machine in the department. The powdered leaves were weighed and stored in airtight plastic jars for further analysis. Different types of extracts of the plant were prepared using various menstruumviz ethanol, acetone and chloroform by maceration technique. 100 grams of powdered leaves was soaked in 1 litre menstruum at room temperature for 48-72 hours and stirred at frequent time intervals. After maceration, the extract was initially filtered using muslin cloth and then re-filtered again using Whatmann filter paper No 1. The filtrate obtained was evaporated in oven at a temperature of 40°C. The residue obtained was lyophilized and kept at 4°C in air tight bottles

until used. Percent yield for different solvents viz. ethanol, acetone and chloroform was found to be 3.8%, 3% and 2.8% respectively.

2.2 Antibacterial Potential

Different extracts of the plant were evaluated for their antibacterial activity against gram +ve (*Staphylococcus aureus*) and gram –ve (*Escherichia coli*) bacteria. These activities were compared with standard drug (ampicillin) against these microorganisms. The agar well diffusion method was followed to test the antibacterial activity of the different plant extracts. The sterilized media were poured into pre-sterilized petri plates (in triplicate) at a depth of approximately 4 mm (15 ml) and allowed to solidify and the bacterial colonies grown on the selective media were swabbed over the solidified media. Then the wells were cut using a sterile well punch. Different plant extracts dissolved in corresponding solvents (water for aqueous and DMSO for other extracts) at concentrations 5% and 10% were delivered into the wells. DMSO (50 µl), and ampicillin (10µg/ml) were maintained as negative and positive controls, respectively. Incubation was done at 37° C for 24 h. Following incubation the zones of inhibition were measured and noted down.

2.3 Anti-inflammatory Activity

The present investigation was conducted on 50 rats aged 3-4 months, weighing 180-200 grams, at Guru AngadDev Veterinary and Animal Sciences University, Ludhiana. The animals were purchased from small animal colony, GADVASU. The animals were acclimatized to the environment for ten days before starting experiment and kept in cages under standard laboratory conditions of temperature (27–30°C), with a 12-h light cycle. All animals were fed commercial rat pellets procured from Ashirwad industries, Mohali (Punjab). Feed and water were given *ad libitum* to the animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide reference no. VMC/13/1786-1806 dated 4/4/13 and was conducted in accordance with ethical committee guidelines. After a washout period of 20 days the experiments were repeated on the same rats for different pharmacological activities.

Fifty rats were divided into 10 groups of five animals each. Initially the normal paw volume of all the animals was noted down. All animals were injected in the sub-planter region of the right hind paw with 1% carrageenan (in normal saline) @ 0.1 ml to induce inflammation as per method of 11. Group 1 served as control without any treatment. Group II was administered meloxicam orally @1mg/kg as a standard drug to serve as positive control. Group III and IV were administrated aqueous extract @ 50 mg/kg and 100 mg/kg orally, respectively. Group V and VI were administered with alcoholic extract @50 mg/kg and 100 mg/kg, respectively. Similarly, group VII and VIII with acetone extract and group IX and X with chloroform extracts @50 mg/kg and 100 mg/kg orally. The administration of different types of plant extracts and the standard drug was done half an hour before induction of inflammation by carrageenan. The change in paw volume was determined by the water displacement method. In this method a glass beaker filled with water was placed on electronic balance. Before start of experiment, right hind paw of individual rat was dipped up to tibio-tarsal joint and reading was noted. It was initial paw volume. This paw volume was equal to water displaced by dipped paw. Thereafter all groups were administered with suitable drugs and extracts as described above. Readings were noted at duration of 1 h, 2 h, 3 h, 4 h, 5 h and 6 h respectively and the anti-inflammatory activity was determined as the change in the paw volume at the respective time intervals.

2.4 Acaricidal Activity

Fully engorged adult *Rhipicephalus microplus* (Acari: Ixodidae) female ticks were collected from Bhatinda region. For evaluation of anti-tick activity of the ethanolic leaf extracts, adult immersion test (AIT) was conducted as per Drummond *et al.* (1973) and Sharma *et al.* (2012) was adopted. Briefly, the adult engorged female ticks were washed thoroughly thrice with tap water and kept on filter paper for drying. These ticks were distributed randomly into various groups of ten each. A dose dependent response study was conducted by exposing adult ticks to various concentrations (0.625, 1.25, 2.5, 5.0 and 10.0%) of alcoholic extract of *Citrullus colocynthis* and in each concentration ten ticks were maintained. All the ticks were immersed for 2 min while control ticks were immersed in carboxymethylcellulose solution and then transferred to the petri dishes padded with Whatmann filter paper no. 1. After 24 h, the ticks were weighed individually and transferred to glass vials covered with muslin cloth and kept in desiccators placed in incubator maintained at 28 ± 1 °C and 85 ± 5% relative humidity. The effect of the extracts on tick mortality was recorded and ticks which did not oviposit even after 14 days post-treatment were considered as dead[12]. The ticks which survived after exposure in various concentrations were reared subsequently for generating the data on the efficacy of extracts on inhibition of oviposition.

3. Results

3.1 Antibacterial potential

The results for evaluation of antibacterial activity of leaf extracts of *Citrullus colocynthis* revealed absence of activity against *E.coli* and *S.aureus* of any of the leaf extracts as the diameter of zone of inhibition was non-significant when compared to the control (Tables 1 and 2).

Table 1. Effect of different leaf extracts of *Citrullus colocynthis* on antibacterial activity against *E. coli* (Measurements on Zone of inhibition (mm)

Groups	Dose (concentration)	Zone of inhibition (mm)
DMSO	--	2 ± 0.33^{b}
Standard Drug	10 ug/ml	18.6 ± 0.33^{a}
alcoholic	50 mg/ml	2.6 ± 0.66^{b}
	100 mg/ml	3 ± 0.50^{b}
Acetone	50 mg/ml	3 ± 0.57^{b}
	100 mg/ml	3 ± 0.66^{b}
Chloroform	50 mg/ml	2 ± 0.50^{b}
	100 mg/ml	2.3 ± 0.33^{b}

Values lacking a common superscript in given column differ significantly from each other ($p<0.05$).

Table 2. Effect of different leaf extracts of *Citrullus colocynthis* on antibacterial activity against *S. aureus* (Measurements on Zone of inhibition (mm))

Groups	Dose (concentration)	Zone of inhibition (mm)
DMSO	--	1.66 ± 0.33^{b}
Standard Drug	10 ug/ml	29.6 ± 0.33^{a}
alcoholic	50 mg/ml	3 ± 0.57^{b}
	100 mg/ml	2.6 ± 0.67^{b}
Acetone	50 mg/ml	2.3 ± 0.66^{b}
	100 mg/ml	2.6 ± 0.33^{b}
Chloroform	50 mg/ml	1.6 ± 0.33^{b}
	100 mg/ml	2 ± 0.00^{b}

Values lacking a common superscript in given column differ significantly from each other ($p<0.05$).

3.2 Anti-inflammatory Activity

For observing the anti-inflammatory potential of the extracts under study, Carrageenan 0.1 ml was injected sub-planter to induce inflammation in all the animals. Group I served as control and group II served as standard. In the standard group meloxicam @ 1 mg/kg was administered orally. In group III, group V and group VII the alcoholic, acetone and chloroform extracts were given respectively, @ 50 mg/kg whereas in group IV, group VI and group VIII respectively these extracts were given @ 100 mg/kg orally. In all the groups, treatments were given half an hour before the sub-planter injection of carrageenan. The results for the change in paw volume on administration of various leaf extracts of *Citrullus colocynthis* on carrageenan-induced inflammation in right paw of Wistar rats are given in the Figure 1.

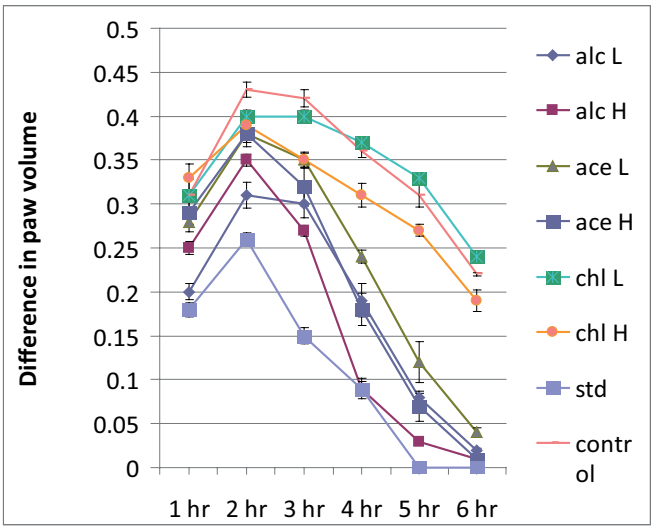


Figure 1. Effect of different leaf extracts of *Citrullus colocynthis* on carrageenan-induced paw odema in rats.

3.3 Acaricidal Activity

The results showing the effects of different concentrations of the alcoholic leaf extracts of *Citrullus colocynthis* on the engorged adult females of *R. microplus* are shown in Table 3.

Table 3. Acaricidal activity of different concentrations of leaf extracts of *Citrullus colocynthis* against *Rhipicephalus microplus*

Concentration	Total number of ticks	Number of ticks died	Percent mortality
0.625	10	0	0
1.25	10	0	0
2.5	10	0	0
5	10	0	0
10	10	0	0
control	10	0	0

4. Discussion

For antibacterial activity, three different extracts of *Citrullus colocynthis* i.e. alcoholic, acetone and chloroform at the concentrations of 50 mg/ml and 100mg/ml were used to study their antibacterial activity against clinical isolates of gram +ve (*Staphylococcus aureus*) and gram –ve (*Escherichia coli*) bacteria. Ampicillin (10ug/ml) was used as standard drug and DMSO was used as control. There was no significant antibacterial activity demonstrated by any of the leaf extracts of the plant against the clinical isolates. Similar results were obtained in the evaluation of antibacterial activity of ethanolic leaf extracts of *Citrullus colocynthis* against *E.coli* and *P.aeruginosa*[13,14].

In the evaluation of anti-inflammatory activity of leaf extracts of *Citrullus colocynthis*, almost all the extracts showed the dose dependent anti-inflammatory activity. In almost all the extract-treated groups, the inflammation started to decrease after 2 hours of treatment with maximum decrease shown by group IV (alcoholic extract) and also the same group achieved normal values at 5 hours which showed significant activity of the extract when compared to control group in which normal values were obtained beyond 7 hours of treatment. In the standard group, the paw volume came to normal value after five hours whereas in almost all the extract treated groups the values came to normal after six hours of carrageenan injection. The oedema and inflammation induced by carageenan is proved to be mediated by histamine and 5HT during first 1h, after which increased vascular permeability is maintained by the release of kinins upto 2:30h and from 2:30 to 6 h the mediators appear to be prostaglandins, the release of which is closely associated with the migration of leukocytes into the inflamed site[15]. The anti-inflammatory activity depicted in the present study may be attributed to the presence of therapeutically active flavonoids like apigenin, quercetin, naringein and luteolin which might have inhibited the release of inflammatory mediators in Carageenan model.

In the experiment conducted for evaluation of acaricidal potential of the plant extract, it was observed that there was no dose-dependent effect on the mortality of the ticks by any of the concentrations of the extract after 14 days of treatment. Also it was observed that there was no inhibition of oviposition by any of the concentrations of the extract revealing absence of acaricidal property in the different concentrations of the extracts used in the study.

5. Conclusion

The present study demonstrated absence of antibacterial activity in the different leaf extracts of the plant against *E. coli* and *S. aureus* at the extract concentrations of 50 and 100 mg/ml as there was negligible zone of inhibition when compared to the standard antibiotic ampicillin. In the study for acaricidal activity of the extracts against *Rhipicephalus (B.) microplus*, there was no mortality of the ticks exposed to different concentration of the plant extract. Although the investigation revealed significant anti-inflammatory effect of all plant extracts with maximum activity in the alcoholic extract of *Citrullus colocynthis*. It can be concluded that *Citrullus colocynthis* can act as promising medicinal plant with substantial anti-inflammatory activity which could be utilized in several medical applications because of its effectiveness and safety.

6. References

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