ANTHELMINTIC ACTIVITY OF LEAVES OF TWO SPECIES OF *CADABA* FORSK.

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Abstract
The aqueous and ethanol extracts of leaves of *Cadaba fruticosa* and *Cadaba trifoliata* were evaluated for *in vitro* anthelmintic activity using anthelmintic tests on larvae of *A. galli and H. contortus*. Alcohol extracts of *C. trifoliata* at 50 mg/ml possess significant larvicidal activity against *A. galli* and *H. contortus*.

Keywords: Anthelmintic, *Cadaba fruticosa*, *Cadaba trifoliata*, earthworm, wormicidal, vermifuge.

Introduction

Cadaba fruticosa (L.) Druce and *Cadaba trifoliata* (Roxb.) Wt. & Arn. are wasteland plants belonging to the family Capparaceae. They have been used widely in India in traditional medicine to treat many ailments and recommended for the treatment of pyrexia, eczema, syphilis as an aperient, antiphlogistic, emmenagogue and vermifuge [1]. Anthelmintic activity of these two species on adult earth worm has been reported by the authors elsewhere [2]. Antimicrobial activity of *C. fruticosa* has been demonstrated by Selvamani *et al* [3]. In the past literatures of *C. fruticosa* alcohol extract have been used to evaluate various activity due to the presence of alkaloids and other phenolic compounds in alcohol extract. Hence, in this study also alcohol extract is chosen. Aqueous extract was also used, to compare the activity of alcohol and aqueous extracts. An attempt is taken up to substantiate the vermifuge property of these plants on larvae of *Ascaridia galli* and *Haemonchus contortus* besides its reported wormicidal activity.

Prevalence of human helminthias in India is as high as 70 to 90 % of which ascariasis is most common, and it exacts a heavy toll on human health and productivity. Although effective anthelmintics are currently available for most infections, the search for novel compounds is essential in view of the development of resistance as noted in animals [4]. In this study, we evaluated the larvicidal activity of ethanol and aqueous extracts of *C. fruticosa* and *C. trifoliata* in larvae or eggs of *A. galli* and *H. contortus.*

Material and methods

Plant material

Leaves of *C. fruticosa* and *C. trifoliata*. were collected from Tirunelvely, Tamil Nadu, South India, in September 2005. Specimens were identified with the help of available literature and confirmed by Dr.S.N.Yoganarasimhan, Research Coordinator, Department of Pharmacognosy, M.S.Ramaiah College of Pharmacy, Bangalore, India. The voucher specimens (015 and 016) are deposited in the Department of Pharmacognosy, M.S.Ramaiah College of Pharmacy, Bangalore. The leaves of both plants were shade dried separately at 25- 30° C for 5 days and powdered. Both powders (100 g) each were extracted with 200 ml of absolute alcohol and water in a Soxhlet apparatus. It was then evaporated to dryness under reduced pressure. (Yield of *C. fruticosa* 14.2 % and *C. trifoliata* 8 %w/w for ethanol extract and *C. fruticosa* 14.60 % and *C. trifoliata* 7.04 % w/w for aqueous extract). Different concentrations of alcohol and aqueous extracts of both plants were prepared in Tween 80 as suspending agent.

A. galli was obtained from the freshly slaughtered birds. Eggs were isolated from the worm and used. Larvae of *H. contortus* was isolated from sheep and used. Both were authenticated by the Head, Parasitology Department, Veterinary College, Bangalore, India. Piperazine citrate and albendazole were used as standard drugs.

Larvicidal activity

Larvicidal activity was performed using the method described by Diehl[5]. The crude extracts were suspended in 1ml of 1% Tween 80 and diluted with demineralised water to final concentration from 1.5 to 0.0015 mg/ml in wells of flat-bottomed microtitre plates. Agar 140 µl (45 – 50°c) containing 2 % amphotericin B was added to the wells, and 80 eggs were transferred on to the agar. The microtitre plates were kept in humid atmosphere (90 %) for six days at 27°c. Normal development of the larvae was monitored from wells containing eggs in water. The number of unhatched eggs and the

number of larvae were counted. The developmental stage of larvae and their mobility were also noted. The larvicidal (LC_{100}) concentration was defined as the lowest concentration of the extract or the standard drug that was still able to block completely the normal larval development. The tests were repeated three times with all four extracts that showed some anthelmintic effect. Statistical tests were performed using one way ANOVA and the standard deviation presented.

Results and Discussion

Anthelmintic tests for alcohol and aqueous extracts of both plants showed some activity against larvae of *A. galli* and *H. contortus* and presented in Table-1. The larvicidal potency ranges from 48.92– 583.68 mg/ml for *H. contortus* and 52.96– 467.23 mg/ml for *A. galli*. Ethanol extract of *C. fruticosa* produced 52.10 and 61.54 mg/ml against *H.contortus* and *A.galli* which is almost four folds less than the activity of the tested standards (piperazine and albendazole) and aqueous extract produced very feeble larvicidal activity whereas for *C trifoliata* both alcohol and aqueous extracts, produced similar larvicidal activity in comparison with the standards. The observed anthelmintic activity of extracts on live earth worms also support the observed larvicidal activity against the tested the larvae[2].

Helminthic infections of the gastrointestinal tract of humans and animals have been recognized to adversely affect the health standards of large population with a consequent lowering of resistance to other diseases. In the search for compounds with anthelmintic activity, a number of substances have been screened using different species of worms, larvae of different species. As per Siddha literature *C. fruticosa* (Vizhuthi) and *C. trifoliata* (Mara vizhuthi) are known to possess various medicinal properties [6, 7]. In this study, we have evaluated the larvicidal effect of both alcohol and aqueous extracts of *C. fruticosa* and *C. trifoliata* on *A. galli* and *H. contortus*. Alcohol extracts of both plants and aqueous extract of *C. trifoliata possess* significant larvicidal activity.

C. fruticosa contains alkaloids like cadabacine, cadabalone, cadabine, flavonoids like quercetin, stachydrine, in addition to tannins and sterols. Whereas no reports are available on the constituents of *C. trifoliata* [8]. Tannins in the extracts may be responsible for the observed activity, because tannins can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite may cause death [9, 10]. Polyphenolic compounds are reported to interefere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[11]. It is possible that tannins contained in the extracts produced the observed effects. The larvicidal activity against *A. galli* and *H. contortus* suggests that it might be effective against parasitic infections of human being. However, further isolation of active constituents and finding the possible mechanism of action would confirm this statement.

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TABLE-1 VALUES OF LARVICIDAL CONCENTRATIONS (mg/ml) OF PLANT EXTRACTS

Treatment	LC100 of H. contortus (mg/ml)	LC100 of A. galli (mg/ml)
Control 1%Tween 80 solution	--	--
Alc. C. fruticosa (ecf)	52.10 ± 1.2*	61.54 ± 2.2*
Aq. C. fruticosa (acf)	583.68 ± 11.2*	467.23 ± 8.2*
Piperazine citrate	15.24 ± 0.8*	15.48 ± 1.02*
Albendazole	12.34 ± 0.2*	13.27 ± 0.4*
Alc. C. trifoliata (ect)	49.03 ± 1.62*	52.96 ± 1.25*
Aq. C. trifoliata (act)	48.92 ± 1.12*	62.02 ± 1.36*

* indicate all the groups including piperazine citrate and albendazole were significant at $p < 0.01$ when compared to control (Tween 80).

Larvicidal activity of plant extracts