

Pharmaco-Chemical characterization and Acaricidal Activity of Ethanolic Extract of *Chassalia Curviflora* (Wall ex Kurz.) Thwaites

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ABSTRACT

Introduction: *C. curviflora*, an important ethno-medicinal plant used by the Kurichia local people in Western Ghats region of Wayanad is yet to be explored pharmacologically. It is used as paste on the body of cattle and birds for curing skin diseases. **Objectives:** To characterize the pharmaco-chemical features and to study the acaricidal effect of ethanolic extract of *C. curviflora* on engorged female ticks of *R. (B.) annulatus*. **Methods:** The pharmaco-chemical features such as physico-chemical, proximate, phytochemical, fluorescence, and HPTLC profiling were carried out using standard techniques. The pulverized leaves were subjected to soxhlet extraction using ethanol. The ethanolic extract at different concentrations (10% to 1.25%) was tested against ticks using adult immersion test (AIT). **Result:** The preliminary phytochemical investigation showed high contents of saponins, alkaloids and flavonoids. The HPTLC profiling of ethanolic extract showed the presence of 14 polyvalent components. Based on AIT, the extract at 10% revealed 43.76% of inhibition of fecundity (IF) and 29.16%

of adult tick mortality. **Conclusion:** The results revealed that the extract has some active compounds that may influence in the reproductive system of female ticks.

Key words: *Chassalia curviflora*, Acaricidal activity, Pharmaco-chemical, HPTLC, *R. (B.) annulatus*, Fecundity.

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INTRODUCTION

Plants produce an amazing array of organic chemicals with an enormous diversity of structural types, essential for their growth. Medicines of herbal origin are preferred because of their fewer side effects compared to synthetic medicine.¹ The medicinal plants continues to play a significant role in providing valuable pharmaceutical products in health care system² in addition to its application in cosmetic, agricultural and food industry.³

Ticks are blood sucking hematophagous ectoparasites, transmitting many diseases affecting livestock, humans and companion animals.⁴ They cause significant loss in livestock industries through reduction of milk production, weight gain and reproductive efficacy⁵ and also increased the incidence of life threatening diseases in human. Chemical acaricides are used for control of tick population throughout the world. However, development of acaricide resistant ticks,⁶ toxicity to non-target living organisms and environmental pollution⁷ are some of their drawbacks. Survey of published literature reveals many reports on the potential of plant extracts against ticks⁸.

The species, *Chassalia curviflora* (Wall ex Kurz.) Thwaites is a flowering, tropical woody plant member of coffee family, Rubiaceae. The different parts of the plant is used to cure ear and eye disease, headache, skin diseases, ulcers, phlegm, rheumatism, jaundice, pneumonia, wounds and sour throat.⁹ The phytochemical studies dealing with secondary metabolites are meager in *C. curviflora*, but some of the *Chassalia* species have

proved to be a source of macrocyclic peptides, also known as cyclotids.¹⁰ In another study, Thenmozhi and co-workers (2013) detected presence of phenols, tannins and flavonoids in the fruits of *C. curviflora*. Recently, the GC/MS analysis of methanolic extract of *C. curviflora* revealed the presence of 69 phyto-constituents.¹¹ Experimentally, the genus *Chassalia* was reported for its anti-hypertensive,¹¹ antibacterial¹² antimicrobial, insecticidal and cyto-toxic activities.¹³ Currently, very limited reports exist on the chemical nature and pharmacological properties of this plant species. Therefore, the present investigation focuses on characterization of the pharmaco-chemical features and acaricidal properties of the ethanolic extract of leaves of *C. curviflora* against *R. (B.) annulatus* ticks.

MATERIAL AND METHODS

Collection and identification of plant material

The taxonomically identified *Chassalia curviflora* (Wall. ex Kurz.) Thwaites plant leaves were collected from Western Ghats region of Kozhikode, Kerala, India. A herbarium for morphological studies was prepared, authenticated and a voucher specimen (No: CALI-6806) deposited at the Department of Botany, Calicut University Herbarium, Kozhikode, Kerala.

Pharmaco-chemical characterization

Evaluation of physicochemical parameters

The physicochemical parameters such as the percentage of loss on drying (LOD), total ash, foreign content, moisture content, acid soluble ash, water soluble ash and alcohol soluble ash were determined as per the Indian Pharmacopoeia, (1996).¹⁴

Determination of extractive values

Coarsely chopped 5 g of leaf powder was subjected to maceration for 24 h in a closed flask using 100 mL of solvent (such as alcohol, chloroform, ethyl acetate, hexane, petroleum ether and distilled water) frequently shaken during the first 6 h, allowed to stand for 18 h and filtered rapidly using Whatman's No: 42 filter paper. In a tared flat bottom shallow dish, 25 mL of filtrate was evaporated to dryness, dried at room temperature and weighed. Percentage of soluble extractive fraction was calculated with reference to the air dried powder.

$$\text{Percentage of extractive value} = \frac{\text{Weight of flask with extract} - \text{Weight of empty flask}}{\text{Weight of sample}} \times 100$$

Proximate analysis

The proximate analysis of ash, crude fibre, crude protein, carbohydrate, crude fat, dry matter and moisture content of the powdered leaves of *C. curviflora* were done using standard proximate analysis techniques.¹⁵

Preparation of ethanolic extract

The collected plant leaves were cleaned by washing in running water. The leaves were dried at room temperature for two weeks. The dried leaves (100 g) were powdered in a plant sample grinder at controlled temperature and used for extraction using ethanol in a soxhlet extraction apparatus attached with a rotary vacuum evaporator (Buchi, Switzerland). Solvents were removed using rotary vacuum evaporator at 175 mbar at a temperature ranging from 40°C to 60°C.

Preliminary phytochemical screening

Preliminary phytochemical screening of crude drug powder was done as per standard procedure described by Harbone, (1991)¹⁶ for various phyto-constituents such as steroids, terpenoids, alkaloids, tannins, phenolic compounds, flavanoids, carbohydrates and amino acids.

High performance thin layer chromatography (HPTLC)

High performance thin layer chromatography (HPTLC) analysis was carried out on a HPTLC (Camag, Switzerland) system with ethanolic extract of *C. curviflora*. Chromatographic separation was performed on Merck TLC plates precoated with silica gel 60 F₂₅₄ (20×10 cm with 200 µm layer thicknesses) from E. Merck, Germany. The 10 µL of extract (2 mg/mL) was applied onto the plates as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland) using Camag Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber hexane: ethyl acetate (8:2). Scanning was performed using Camag TLC scanner 3 at 254 nm and 366 nm through fluorescence mode and operated by win CATS software (version 1.4.1, Camag). Plates were visualized under UV 254, UV 366 nm and in visible light.

Bioassay test

Adult immersion test (AIT)

Adult immersion test was performed based on Drummond *et al.* (1973).¹⁷ Fully engorged adult female *R. (B.) annulatus* were collected

Table 1: The phytochemical constituents in ethanolic leaves extracts of *C. curviflora*

Phyto-constituents	Reagents/Chemicals	Observations
Alkaloids	Mayer's test	++ ve
	Dragendroff's test	++ ve
	Hager's test	+ve
	Wagner's test	+++ve
Carbohydrates	Molisch's	+ve
	Foam test	+++ve
Saponins	Forth test	+++ve
	Salkowski's test	+ve
Phytosteroids	LibermannBurchard's test	+ve
	Ferric chloride test	+ve
Phenols	Lead acetate test	+ve
	Ferric chloride	-ve
Tannins	Lead acetate test	+++ve
	Alkaline reagent test	+++ve
Flavanoids	Millon's test	-ve
	Biuret's test	-ve
	Ninhydrin's test	-ve
Protein & amino acids	Salkowski's test	+ve
	Spot test	+ve
Fixed oils and fats	Saponification test	+ve

from the naturally infested calves, washed with distilled water, dried on an absorbent paper and was used for adult immersion test (AIT). The different concentrations of ethanolic extract (10% to 1.25%) were prepared in methanol. Four replicates, each with six ticks, were used for each concentration. The groups of six ticks selected randomly based on the size were weighed before the experiments and were immersed for two minutes in the respective dilution in a 50 mL beaker containing 10 mL extract. Ticks were recovered from the solution, dried using absorbent paper and placed in a separate plastic specimen tube (25×50 mm). The tubes were incubated at 28 ± 2°C and 80 per cent relative humidity in a BOD incubator. Methanol was used as control. Adult tick mortality was observed up to 15th day post-treatment. After oviposition, the eggs laid by the female ticks were collected and weighed. Eggs were kept under the same incubation conditions in a BOD incubator for the next 30 days.

The index of egg laying (IE) and percentage inhibition of fecundity (IF) were calculated as follows.

Index of egg laying/fecundity (IE)=weight of eggs laid (g)/weight of females (g)

Per cent inhibition of fecundity (% IF)=[(IE control group-IE treated)×100]/IE control group. Hatching of eggs was observed visually.

Statistical analysis

Analysis of data was performed.¹⁸ Data were expressed as the mean ± SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. Duncan's test was used for post-hoc analysis. A value of P<0.05 was considered significant.

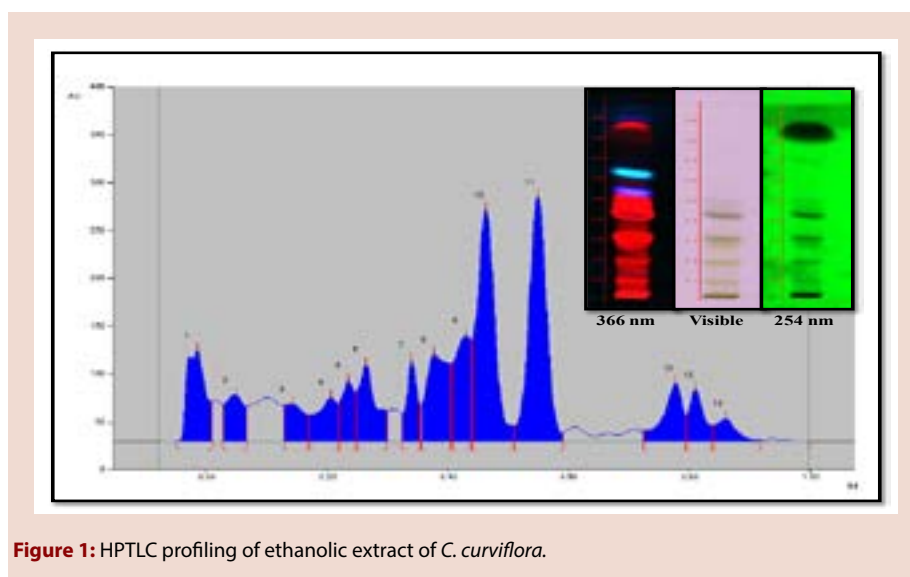


Figure 1: HPTLC profiling of ethanolic extract of *C. curviflora*.

Table 2: Effects of ethanolic leaves extract of *C. curviflora* against *R. (B.) annulatus*

Acaricide	Mean ticks weight per replicate \pm SEM (g)	Mean % adult mortality within 15 days \pm SEM	Mean eggs mass per replicate \pm SEM (g)	Index of fecundity \pm SEM	Percentage Inhibition of Fecundity (%)	Hatching % (Visual)
Methanol	0.972 \pm 0.016 ^{bc}	0 \pm 0 ^a	0.374 \pm 0.024 ^b	0.386 \pm 0.029 ^b	0	100
10%	0.941 \pm 0.012 ^{ab}	29.162 \pm 14.230 ^b	0.204 \pm 0.046 ^a	0.217 \pm 0.049 ^a	43.76	100
5%	0.985 \pm 0.004 ^c	0 \pm 0 ^a	0.310 \pm 0.023 ^b	0.314 \pm 0.022 ^b	18.42	100
2.5%	0.996 \pm 0.014 ^{abc}	0 \pm 0 ^a	0.322 \pm 0.031 ^b	0.334 \pm 0.031 ^b	13.26	100
1.25%	0.931 \pm 0.010 ^a	0 \pm 0 ^a	0.341 \pm 0.013 ^b	0.366 \pm 0.016 ^b	5.05	100

n= 4, values are Mean \pm SEM, means bearing different superscripts a, b or c (P<0.05), indicates significant difference when compared with control.

RESULTS

Pharmaco-chemical characterization

The results showed that the per cent of ash content was 7.57%. The per cent of foreign content in the crude drug powder was 0.29%. Besides the percentage of acid, water and alcohol insoluble ash were 56.84%, 15.03% and 10.59% respectively.

The per cent extractive yields of the powdered leaves of *C. curviflora* determined using different solvents such as ethanol, chloroform, petroleum ether, ethyl acetate, hexane and water were 7.66 \pm 0.08%, 4.28 \pm 0.33%, 3.61 \pm 0.10%, 6.60 \pm 0.05%, 1.65 \pm 0.31% and 16.41 \pm 0.75% respectively.

Proximate analysis indicated that dry matter, carbohydrate, moisture, crude fibre, crude protein, total ash and crude fat were 87.16 \pm 0.16 %, 54.10 \pm 0.05 %, 11.75 \pm 0.16%, 21.87 \pm 0.05 %, 11.41 \pm 0.28 %, 12.83 \pm 0.16% and 03.61 \pm 0.10 % respectively.

The phytochemical screening carried out on the ethanolic leaf extract of *C. curviflora* revealed the presence of pharmacologically active constituents such as alkaloid, carbohydrate, saponins, phyto-steroids, triterpenoids, phenolic compounds, flavonoids, fixed oils and fats (Table 1). However, it did not reveal the presence of tannins, proteins and amino acids.

The ethanolic extracts of leaves of *C. curviflora*, when subjected to HPTLC profiling revealed 14 polyvalent compounds (Figure 1) with the solvent system hexane: ethyl acetate (8:2). Out of these polyvalent

compounds with *Rf* values 0.5, 0.44, 0.36, 0.41, and 0.25 were more prominent peaks with area per cent of 20.05% (5573.4 AU), 19.30% (5365.5 AU), 9.08% (2523.4 AU), 7.55% (2099.8 AU), and 6.19% (1720.1 AU) respectively.

The result of adult immersion test (AIT) for *C. curviflora* are represented in Table 2. The parameters such as adult tick mortality, inhibition of fecundity and hatching rates of ova were assessed. The adult tick mortality and hatching rate at the maximum concentration tested were 29.16% and 100%. The maximum inhibition of fecundity (43.76%) was noted at highest tested concentration of 10%.

DISCUSSION

During last few decades there has been an increasing surge in the study of medicinal plants and their traditional uses in different parts of the world. Herbal remedies are considered as one of the oldest form of health care known to mankind on this earth. The species *C. curviflora* is an important ethno-medicinal plant. The Kurichia local people in Western Ghats region of Wayanad, used the plant paste on the body of cattle and birds for curing skin diseases,¹⁹ and the plant is also used to treat wound healing and snake bite. This plant has been confused with other species of similar plants due to their relative similarities. Therefore, it is necessary to establish the pharmaco-chemical parameters and standards in order to properly identify the plant.

In the present study, we evaluated the pharmaco-chemical characteristic and acaricidal activity of the ethanolic extracts of leaves of *C. curviflora*.

The physicochemical studies were taken up with the crude powdered leaves of *C. curviflora*. The per cent of ash content gave an idea about the earthy or inorganic composite present along with the material. Whereas, the lowest value of foreign content indicated the presence of lesser amount of impurities. The extractive values provided preliminary information on the extent of polar and non-polar chemical constituents present in the crude drug. The yield was highest when extracted with water, followed by ethanol, ethyl acetate, chloroform, petroleum ether and hexane in descending order. The high water soluble extractive value suggested rich content of polar compounds. These extractive values gave an idea about the chemical constituents present in the crude drug as well as its usefulness in the determination of adulterated drug. The proximate analysis of leaves helped in the standardization, identification of crude drug and its nutritive value. On, phytochemical screening of ethanolic extract revealed the high content of alkaloids, saponins, and flavonoids. Further, HPTLC profiling indicated the presence of 14 polyvalent compounds. There are a few limited reports describing the chemical composition present in *C. curviflora* plant.^{20,11}

The acaricidal activity of the ethanolic extract of *C. curviflora* leaves were evaluated against adult engorged female ticks of *R. (B.) annulatus*. The extract at 10% concentration caused only 29.16% of adult mortality and 43.76% of inhibition of fecundity. To the best of our knowledge no published data exist to support the acaricidal activity of *C. curviflora*. However, there are large number of publications describing the effect of various other plant extracts on inhibition of fecundity/egg laying in ticks viz., *N. variegata*,²¹ *A. conyzoides*, *A. absinthium*,²² *T. patula*,²³ *C. serrata*.²⁴ Many plant extracts tested in our laboratory also revealed inhibition of fecundity/ blocking of egg laying effect.²⁵⁻²⁶

The pharmacological activity of the crude extracts can be due to the presence of active biomolecules present in them. The acaricidal/insecticidal activity of many plants was attributed to alkaloids,²⁷⁻²⁸ saponins²⁹ and flavanoids.^{30,25} Hence, further fractionation of bioactive molecules from the ethanolic extract of *C. curviflora* is essential for the identification of active principles that contributed to the acaricidal activity.

CONCLUSION

In this work the pharmaco-chemical and acaricidal properties of the ethanolic extract of leaves of *C. curviflora* were studied. High contents of saponins, alkaloids and flavonoids were observed in the plant extract. HPTLC profiling of ethanolic extract showed 14 polyvalent components. In, Adult immersion test, 43.76% of inhibition of fecundity (IF) and 29.16% of adult tick mortality were observed at 10% concentration of extract. The results showed that the extract have some active compounds that may influence in the reproductive system of female ticks. This new findings can be utilized to develop new drugs from the leaves of *C. curviflora*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper.

ABBREVIATION USED

GC/MS: Gas chromatography mass spectrometry; **LOD:** loss on drying; **HPTLC:** High performance thin layer chromatography; **ml:** milliliter; **h:**

hour; **g:** gram; **UV:** ultraviolet; **nm:** nanometer; **AIT:** Adult immersion test; **IE:** Index of egg laying; **IF:** Inhibition of fecundity; **SEM:** Standard error of mean; **ANOVA:** Analysis of Variance; **BOD:** Biological Oxygen Demand; **AU:** Absorbance units; **Rf:** Retardation/retention factor.

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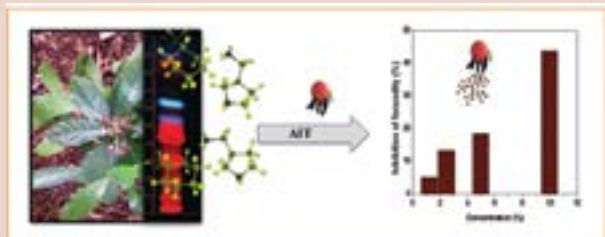
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PICTORIAL ABSTRACT



SUMMARY

- The plant leaves are rich in saponins, alkaloids and flavonoids.
- HPTLC profiling indicated the presence of 14 polyvalent compounds.
- The extract was highly effective in controlling laying of eggs by treated ticks.