

ANTIMICROBIAL, LARVICIDAL AND ACARICIDAL ACTIVITIES OF THE ETHANOLIC EXTRACT OF ANDROGRAPHIS PANICULATA AND CARICA PAPAYA LEAVES

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ABSTRACT

The present study has been designed to evaluate the antimicrobial, larvicidal and acaricidal activities of the ethanolic extract of *Andrographis paniculata* and *Carica papaya* leaves. Antimicrobial activity of the extract was evaluated against five pathogenic bacterial and two fungal strains. The activity was compared with a standard antibiotic Erythromycin. Larvicidal activity of the extract was evaluated as the percentage mortality of mosquito larvae using different concentrations of the extract. Acaricidal activity was evaluated against the cattle tick *Rhipicephalonus annulatus*. The results of the present study are presented and discussed. The findings of the present study, clearly demonstrates the antimicrobial, larvicidal and acaricidal activities of the ethanolic extract of *Andrographis paniculata* and *Carica papaya*.

Keywords: Acaricide, *Andrographis paniculata*, Antimicrobial agent, *Carica papaya*, Larvicide.

INTRODUCTION

Plant drug *Rasayana* has always played a vital role to treat several diseases of human beings. The potential of plants as a source for new drugs is still largely unexplored. *Andrographis paniculata* (Burm.f) Nees belongs to the family Acanthaceae is widely used in traditional and folkloric medicines for a wide spectrum of ailments. The plant is bitter, acrid, cooling and laxative. It shows antipyretic, antiperiodic, anti-inflammatory [1-3], hepato protective, gastro

protective [4-6], anti-allergic [7], anticancer [8-10], anti-microbial[11-12], antimalarial[13], and Ovicidal [14-15] activities.

Carica papaya Linn. is the most widely cultivated fruit bearing plant with many pharmacological activities. Various pharmacological actions and medicinal uses of different parts of papaya are well reported in the ancient literature. The plant shows antimicrobial[16-22], anthelmintic [23], anti-amoebic [24], antimalarial [25], antifungal [26], diuretic,hepatoprotective and immunomodulatory [27-31] activities. Keeping in view of the available literature, the present study has been designed to evaluate the antimicrobial, larvicidal and acaricidal activities of the ethanolic extract made up of *A. paniculata* and *C. papaya* leaves.

MATERIALS AND METHODS

Collection Of Plant Materials

The leaves of *Andrographis paniculata* and *Carica papaya* were collected from Nemmeli village, Thanjavur district. The collected plant materials were authenticated by the Botanist Dr.K.Kandavel, S.T.E.T Women's College, Mannargudi.

Preparation Of The Extract

The collected leaves were washed in tap water and distilled water to remove impurities. The leaves were shade dried ($28\pm 2^\circ\text{C}$), ground and sieved to get fine powder from which the extract was prepared. Ethanolic extract of the plant material was obtained by taking 50g of dried leaf powder in a container, to which 250 ml of ethanol was added and kept for 24 hours with periodic shaking, then filtered and the filtrate was collected. This procedure was repeated thrice with fresh volumes of ethanol. The filtrates were pooled. The pooled extract was concentrated in a rotary vacuum evaporator at 40°C and evaporated to dryness. It was stored at 4°C in an air tight bottle (Jang *et al.*, 2002).

Anti Microbial Activity

Using an aseptic technique placed a sterile swab into the broth culture of a specific organism and then gently removed excess liquid by gently pressing the swab against the inside of the tube. Using the swab streaked the Mueller-Hinton agar plate. To obtain uniform growth, streaked the plate with the swab in one direction and then rotated the plate 90° and streaked the plate again in that direction. Repeated the rotation 3 times. Allowed the plate to dry for approximately 5 minutes. Discs impregnated with standard antibiotic Erythromycin and plant

extracts of different concentrations are gently pressed to the agar plates to ensure that the disc is attached to the agar. Plates should be incubated overnight at an incubation temperature of 37°C. After the plates have been incubated, there should be a noticeable "clearing" zone around each of the antibiotic discs. The diameter of each zone should be measured and recorded in millimeters (mm).

Larvicidal Assay

Laboratory reared IV instar larvae of *Aedes Egypt* were tested with different concentrations (0.25, 0.50, 0.75, 1.00, 2.00, 3.00mg/ml) of the ethanolic extract of *Andrographis paniculata* and *Carica papaya* according to the standard WHO procedure (1981). A series of 250ml glass beakers are taken and 100ml of tap water was taken in each beaker. Different concentrations of the ethanolic extract were added to the beakers. A control was also maintained without the addition of plant extract. About 10 larvae were introduced into each beaker. The treatments were replicated three times. Mortalities were reported after 8-24 hours of the exposure period. The moribund and dead larvae in three replicates were combined and expressed as percentage mortality for each concentration. Dead larvae were acknowledged when they failed to move after probing with a needle. Moribund larvae were those unable of rising to the surface within reasonable period of time. The percentage mortality was calculated by using Abbott formula (Abbott, 1925)

$$\text{Mortality \%} = \frac{\text{No of mortality}}{\text{Total number of ticks}} \times 100$$

Acaricidal Activity

The acaricidal activity of plant extract was determined by direct contact application using filter paper. 1ml of different concentrations of the plant extracts were applied to filter papers (90mm diameter) respectively. After drying each filter paper was placed in the petridish and 10 parasites were dipped in the respective concentration placed in each petridish and incubated for 24 hours at 26°C and 80% relative humidity in the dark. Ticks were studied with a dissecting microscope and mortality rates of groups were recorded. The mortality rate was recorded 8-24 hours post inoculation (hpi) by counting dead ticks. Dead ticks were diagnosed based on three Criteria: signs of cuticle darkness and hemorrhagic skin lesions, leg movement, halted Malpighian tube movement. The main criterion to diagnose the death of ticks was the lack of Malpighian tube movement, which is very clean in *R. (B) anulatus*. The second criterion was the lack of movement of legs tested with a paint brush with ticks placed

in an inverted position under a stereo microscope lamp. The third criterion was a change of cuticle color and hemorrhagic skin lesions demonstrated by dead ticks. The collected data were recorded. All treatments were replicated three times and reported as the average for calculation of % mortality. The following formula was used:-

$$\text{Mortality\%} = \frac{\text{No of mortality}}{\text{Total number of ticks}} \times 100$$

RESULTS AND DISCUSSION

Plants are important source of potentially useful structure for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay. Some of these observations have helped in identifying the active principles responsible for such activities and in developing drugs for the therapeutic use in human beings. In the present study qualitative phytochemical analysis of the ethanolic extract of *Andrographis paniculata* and *Carica papaya* leaves revealed the presence of alkaloids, tannins, flavonoids, saponins, steroids, phenols, anthocyanins, anthroquinone, cardiac glycoside and reducing sugar (Table1).

The antimicrobial activity of the ethanolic extract of *Andrographis paniculata* and *Carica papaya* leaves was evaluated by the disc diffusion method against 5 bacterial and 2 fungal strains namely *Clostridium perfringens*, *Serratia marscenens*, *Alkaligenes faecalis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Microsporium ferruginem* and *Trichophyton interdigitate*. The highest zone of inhibition was obtained against the fungal strain *Trichophyton interdigitate*. The antimicrobial activity of the extract was more than standard antibiotic Erythromycin for *Clostridium perfringens* and *Alkaligenes faecalis*. In the present study, the synergistic effect of *Andrographis paniculata* and *Carica papaya* leaves on antimicrobial activity was clearly demonstrated through zone of inhibition. Hence, the ethanolic extract made from the leaves of both the plants has potential antimicrobial activity. The indiscriminate use of synthetic insecticides has caused environmental contaminations and toxicity to living organisms [32], indicating the need for the development of products that are not hazardous to the environment, target-specific and biodegradable. Thus, the development of new insecticides from plant extracts sources can be an alternative for the control of insect pests.

In the present study, mosquito larvicidal activity was investigated using the ethanolic extracts of *Andrographis paniculata* and *Carica papaya* leaves. Larvicidal activity of the extract was

evaluated through the percentage mortality of the mosquito larvae at various concentrations. Larval mortality was exhibited in a dose dependent manner. Mortality rate was evaluated at 6 hours, 12 hours, 24 hours post inoculation. At minimum concentrations, maximum larval mortality was produced only after 24 hours. When the concentrations of the extract are increased, 100% mortality was exhibited after 12 hours/6hours in a dose dependent manner. The findings of the present study justify the use of *Andrographis paniculata* and *Carica papaya* leaves as a herbal larvicide.

Many essential oils are known to possess various bio-efficacies such as acaricidal, ovicidal, repellent, anti-feeding and biocidal activities against various arthropod pests. Cardiac glycoside was found to have an acaricidal effect .In the present study, acaricidal activity of the ethanolic extract of *Andrographis paniculata* and *Carica papaya* was evaluated against the cattle tick *Rhipicephalus annulatus*. It is one of the most important bovine ticks species. The various concentrations of the extract exhibited acaricidal activity in a dose dependent manner. Parasite mortality was examined at 8hours, 16 hours and 24 hours post inoculation. When the concentration of the extract is increased, the parasite mortality was more even at 8 hours. The findings of the present study, correlates with the previous findings [33].From the present findings, it was evident that the aqueous and ethanolic extracts of *Andrographis paniculata* and *Carica papaya* was a potent acaricide.

Table1: Qualitative phytochemical analysis of the ethanolic extract of *Andrographis paniculata* and *Carica papaya* leaves.

S.NO	COMPOUNDS	RESULT
1	Alkaloids	+
2	Tannins	+
3	Flavonoids	+
4	Saponins	+
5	Steroids	+
6	Phenols	+
7	Anthocyanins	+
8	Anthroquinones	+
9	Cardiac glycosides	+
10	Reducing sugar	+

+ Presence

Table 2: Antimicrobial activity of the ethanolic extract of *Andrographis paniculata* and *Carica papaya* leaves.

S.No	Microorganisms	Zone of Inhibition(mm)	
		Erythromycin 50µg/ml	Sample 100µg/ml
1	<i>Clostridium perfringens</i>	15	18
2	<i>Serratia marscenens</i>	16	10
3	<i>Alkaligenes faecalis</i>	10	13
4	<i>Enterococcus faecalis</i>	13	12
5	<i>Klebsiella pneumonia</i>	15	13
6	<i>Microsporum ferruginem</i>	13	11
7	<i>Trichophyton interdiginem</i>	29	24

Table 3: Larvicidal effect of ethanolic extract of *Andrographis paniculata* and *Carica papaya* leaves at different concentrations.

S.No	Concentration Mg/ml	Initial No of larvae	Larval Mortality			Mortality%
			8h	16h	24h	
1	Control	10	-	-	-	0%
2	0.25	10	-	2	3	30%
3	0.50	10	1	3	5	50%
4	0.75	10	4	7	8	80%
5	1.00	10	6	10	-	100%
6	1.50	10	8	10	-	100%
7	2.00	10	10	-	-	100%
8	3.00	10	10	-	-	100%

Table: 4 Acaricidal effect of ethanolic extract of *Andrographis paniculata* and *Carica papaya* leaves at different concentrations.

S.No	Concentration mg/ml	Initial No of parasites	Parasite Mortality			Mortality%
			8h	16h	24h	
1	Control	10	-	-	-	0%
2	0.25	10	1	2	3	30%
3	0.50	10	1	3	5	50%
4	0.75	10	3	5	7	70%
5	1.00	10	6	10	-	100%
6	1.50	10	8	10	-	100%
7	2.00	10	10	-	-	100%
8	3.00	10	10	-	-	100%

CONCLUSION

It can be concluded that the present study showed that some indigenous plant based products are very promising against microbes and ectoparasites mosquitoes, and can be used as, antimicrobials, insecticides and acaricides. They offer a safer alternative to synthetic chemicals and can be obtained easily at a very low cost. *Andrographis paniculata* and *Carica papaya* can be used alone or in combination with other products for effective protection against microbial, mosquito and ticks borne diseases. Even though the plant extract have shown, larvicidal and acaricidal activities, their potential under field conditions needs to be evaluated. In future, studies should be designed to isolate the active principles and their mechanism of action has to be elucidated.

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