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Evaluation of In-vitro vector control activity of *Physalis angulata.*

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Abstract

The present study was undertaken to evaluate anthelmintic and larvicidal activity of crude ethanolic leaf extract of *Physalis angulata* belonging to family Solanaceae. *Pheretima posthuma* was used as the test worms. Various concentrations of ethanolic extracts were tested in the anthelmintic screening, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate was included as standard reference and distilled water as control. In the case of larvicidal activity the study was conducted on *Culex quniquefasicatus* species of mosquito larvae and the rate of larval mortality was calculated. The results indicated that the crude ethanolic extract significantly demonstrated paralysis and also caused death of the helminthes especially at higher concentration of 50 mg/ml, as compared to standard reference piperazine citrate. Similarly very optimistic results were observed for *Culex quniquefasicatus* species of mosquito larvae and LC_{50} value was calculated as 51.8802 mg/l.

Keywords: Anthelmintic, larvicidal, *Physalis angulata, Pheretima posthuma, Culex quniquefasicatus*

1. Introduction

Ethanopharmacology got its prominence as a science of relationship between primitive society and there environment. *Physalis angulata* L., Family- Solanaceae commonly known as Cutleaf Ground-Cherry is one such commonly used ethno botanical plant. P.angulata is an annual herb indigenous to many parts of the tropics, including the Amazon. It can be found on most continents in the tropics, including Africa, Asia, and the Americas. It grows up to 1 m high, bears small, cream-colored flowers, and produces small, light yellowish-orange, edible fruit sometimes referred to as cutleaf groundcherry. Fruit is about the size of a cherry tomato, and like tomatoes, it contains many tiny edible seeds inside P.angulata propagate easily from the many seeds the fruit contains; spontaneous clumps of plants can be found along river banks and just about anywhere the soil is disturbed and the canopy is broken.

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Organic extracts of the whole plant exhibits Immunomodulatory, Anti-inflammatory, anticancer, antinociceptive, trypanocidal, antimycobial, molluscidal, Antigonorrheal and Antioxidant effects[1-9]. Substantial scientific evidences are now recognizing multitude of these medicinal uses. Phytochemical investigation of *P.angulata* has led to elucidation of many novel chemical compounds primarily it constitutes of a seco-steroidal compound Physalin. Purified secosteroids have shown to inhabit lymphocyte function and allogeneic transplant rejection[10].

Studies have led to the indication that *P.angulata* exerts powerful anti-inflammatory by interfering with cyclooxygenase pathway, lymphocyte proliferation, NO, and TGF- beta production[11]. Anti hepatoma activity of Physalis extracts on apoptosis in human Hep G2 cells was conducted and results conclude that this potent activity is associated with mitochondrial dysfunction[12]. The extensive survey of literature revealed that *P.angulata* has diverse pharmacological spectrum which needs further clinical and animal evaluation. *P.angulata* has all the attributes to be termed as the hidden "Holy Grail of Medicine".

During the past decade there have been major efforts to plan, implement, and sustain measures for reducing the burden of human disease that accompanies helminth infections. Further impetus was provided at the Fifty-fourth World Health Assembly, when WHO Member States were urged to ensure access to essential anthelminthic drugs in health services located where the parasites - schistosomes, roundworms, hookworms, and whipworms - are endemic.

The Assembly stressed that provision should be made for the regular anthelminthic treatment of school-age children living wherever schistosomes and soil-transmitted nematodes are entrenched. Helminth infections are among the most common infections in man, affecting a large proportion of the world's population.

In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosnophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas and some of them can develop in temperate climates. Parasitic diseases cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis. These infections can affect most populations in endemic areas with major economic and social consequences.

Since the discovery of DDT, control of disease-causing mosquito species has been almost completely based on synthetic organic insecticides. Following DDT, conventional pesticides such as malathion and pyrethroids are generally used for mosquito control. But the extensive use of synthetic organic insecticides during the last five decades has resulted in environmental hazards. Besides, this also caused the development of physiological resistance in the major vector species. This has necessitated the need for search and development of environmentally safe, biodegradable, low cost and indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situation (ICMR bulletin, 2003)[13].

Based of the above findings and traditional claims of the plant an in-vitro anthelmintic and larvicidal assay was conducted to prove them.

2. Materials and methods

Plant collection and authentication: The plant was collected in the month of November and December from the surrounding areas of Nalgonda and Ranga Reddy district ,A.P, India. The plant was identified and authenticated by Department of Botany, Osmania University, Hyderabad. Preparation of Herbarium was submitted and the plant was certified as *Physalis angulata* L. ; Family – Solanaceae; Voucher no : 00490 (OUAH)

2.1. Collection of worms and larvae: Indian earthworm Pheretima posthuma (Annelida) were collected from the culture environment water logged areas of soil at the Nizam College of science, Osmania University, Hyderabad.The larvae of *Cx. quniquefasicatus* 3^{rd} & 4^{th} stage instar larvae which were procured from the Dept. of Zoology, Osmania University, Hyderabad.

2.2. **Plant extraction[14]:**
The leaves of the plant was dried for several days and powdered with the help of an electric grinder and extracted exhaustively with ethanol. The liquid extract was evaporated in vacuum to yield 14.59%w/w.

2.3. Preliminary phytochemical screening
The preliminary chemical tests for the ethanolic leaf extract showed presence of steroids, flavonoids , tannins and phenols.

3. Anthelmintic assay[15]

3.1. *Preparation of test sample*

Samples for in-vitro anthelmintic study were prepared by dissolving and suspending 2.5 g of crude ethanolic extract fractions in 25 ml of distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 25 and 50 mg/ml. The anthelmintic assay was carried as per the method of Ajayieoba E. O. et al with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[16-18].
Three different concentrations of 10, 25 and 50 mg/ml in distilled water were taken in petriplates and six earth worms of same size were placed in each plate. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^0 C. Piperazine citrate (10 mg/ml) was used as reference standard and distilled water as the control.

3.2. *Larvicidal assay* [19]

Drug samples for larvicidal activity were prepared by making a stock solution which was serially diluted in water. Test concentrations are then obtained 25.50, 100.150 and 200 mg/l of the appropriate dilution. The larvicidal assay was carried as per the W.H.O guidelines for larvicidal activity with minor modifications. Batches of approximately 25 third or fourth instar larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups or vessels, each containing 100–200 ml of water along with drug concentrations. The test containers were held at 25–28^{o}C and preferably a photoperiod of 12 h light followed by 12 h dark (12L: 12D). After 24 hr exposure, larval mortality was recorded. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed. The results were recorded where the LC50, LC90 and LC99 values, and slope were also plotted.

4. *Data analysis*

Data from all replicates were pooled for analysis. LC50 and LC90 values were calculated from a log dosage–probit mortality regression line using biological statistical program BIOSATAT 2008, Professional package by Analyst soft .Inc, U.S.A.

5. Results and discussion

Tribals of Andhra Pradesh use *P.angulata* for its anthelmntic properties[20]. These traditional claims have been proven in this experiment where the plant has shown to exhibit potent anthelmintic activity. It showed a response time of 9 min and 17 min for paralysis and death respectively. The reference drug Piperazine citrate showed the same activity of 19.26 and 63.25 minutes at 10mg/ml respectively.*Physalis angulata* has exhibited anthelmintic activity in dose dependent manner taking shortest time for paralysis (P) and death (D) with 50mg/ml concentration (Table no.1& fig no.1). As R^2 is closer to one the extract shows good co-relation among death time taken at different concentration there by it can be said that the activity is dose dependent in nature. LC_{50} calculation was done using Probit Analysis. (Biostat 2008 professional software). The end anthelmentic activity of the extract is shown in fig 3.

Regarding the larvicidal activity the percentile mortality values of instar larvae treated with different concentration of the leaf extract of *P.angulata* at the end of 24 hr are represented Table no (2,3,4) for *C.quinquefasciatus* . The regression equations (based on probit analysis) between the concentration of leaf extract and 24 h per cent mortality of 3rd and 4th instar larvae of *C. quinquefasciatus* are represented in Fig no 2. The LC50 value was calculated as 51.8802. The end larvicidal activity of the extract is shown in fig 4.

6. Conclusion

Phytochemical analysis of crude extract reveled presence of phenols. flavonoids, phenols and steroids.

It has been reported that some synthetic Phenols interfere with energy generation in helminth parasites by uncoupling oxidative phosphorilation[16]. Hence it is possible the extract of *P.angualata* could also produce similar effects.

The control of mosquito-borne diseases can be achieved either by killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centers of the vectors in the environment. The extract of Physalis angualta could be used for spraying in stagnant water bodies which are known to be the breeding grounds for mosquitoes acting as vector for a multitude of infectious diseases.

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Table: 1 Anthelmintic time profile for *P.angulata*

Concentration(mg/ml)	Time for paralysis(min)	Time of Death(min)
10	16.36±0.4	45.04±0.02
25	12.07±0.8	33.37±0.45
50	9.07±0.01	17.05±0.03
Piprazine citrate	19.26±0.62	63.25.±0.58

Results are expressed as Mean±SD from three set of observations.

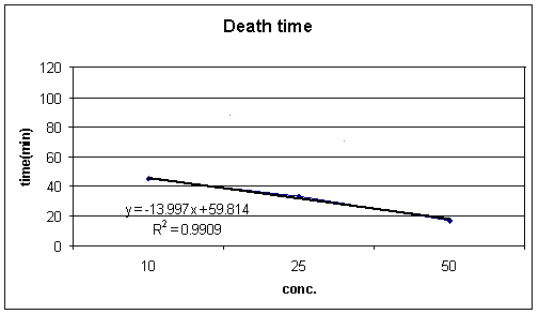

Fig no : 1 - The line represents death time for *P.angulata*

Table no.2 – Efficiency of ethanolic extract of leaf of *P.angulata* on *Cx.quiniquefasicatus*

Concentration (mg/l)	No. of exposed larvae	No. of dead larvae
Control	25	2
25	23	5
50	25	13
100	24	17
150	23	20
200	25	23

Table no: 3 – Results of Finneys analysis for the larvicidal activity of *P.angulata*

Log10[LC50]	1.715
LC50	51.8802
Standard Error LC50	8.0392
LC50 LCL	36.5455
LC50 UCL	66.9256
Log10[LC16]	1.288
LC16	19.4091
LC84	138.6752
LC100	182.0728
Significance Level	0.05
Log10[LC84]	2.142
Beta	2.3419
Alfa	0.9836
Standard Error Beta	0.4197
Standard Error Log10[LC50]	0.067

Fig: 3: Paralyzed worms in 50mg/ml conc. of in Ethanolic extract of P.angulata

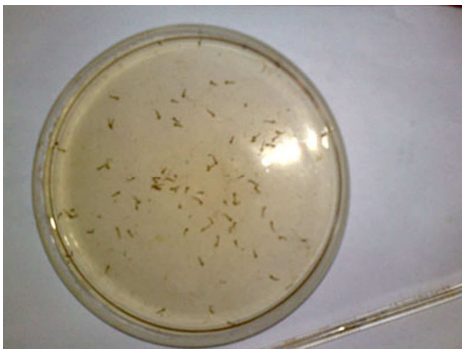

Fig: 4 Image of dead larvae at 24 hrs in the ethanolic extract of P.angulata.

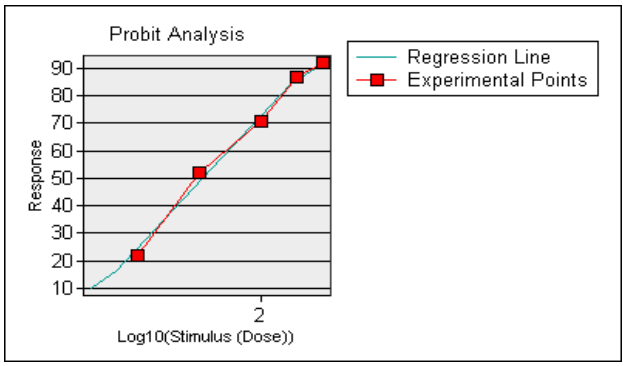

Fig no: 2 – Graph for Finneys analysis

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