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PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTHELMINTIC EFFECTS OF *ACMELLA PANICULATA* PLANT EXTRACTS

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ABSTRACT

Development of resistant to most of commercially available anthelmintic became a sever problem worldwide. Pet ether, chloroform, ethyl acetate and methanol extracts of *Acmella paniculata* were evaluated for there anthelmintic activity using *Pheretima posthuma* model (Indian earthworm). The preliminary phytochemical analysis showed the presence of alkaloids, saponins, flavonoids and tannins in whole plant. Three concentrations (0.075, 0.15 and 0.22w/v) of each extracts were used for this study involve the determination of time of paralysis and time of death of the worm. Extracts obtained from whole plant not only paralyzed but also killed the earth worms. It was observed that both ethyl acetate, pet ether, chloroform and methanol extracts showed a remarkable Anthelmintic potential against intestinal parasitism. Further, there is scope to evaluate those active principles of *Acmella paniculata* whole plant for their Anthelmintic activity to open the new door for natural Anthelmintic.

Key words : *Acmella paniculata*, anthelmintic, Phytochemical, *Pheretima posthuma.*

INTRODUCTION

Helminthes are the most common infections in man, affecting a large proportion of the world's population. Parasitic diseases may cause several morbidities including lymphatic filariasis (a cause of elephantiasis), onchocerciasis, and schistosomiasis. Development of resistant to most of commercially available anthelmintic became a severe problem worldwide.

Sharma *et al* (2010) reported that the therapeutic use of medicinal plant has gained considerable momentum in the world during the past decade. The over use of synthetic drugs with impurities, resulting in higher incidence of adverse reaction, has motivated mankind to go back to nature for safer remedies about one third of all pharmaceuticals are derived from plants and over 60% of the pharmaceutical preparations are plant based (Lingaiah, 2013). Among the estimated 2, 50,000-4, 00,000 plant species only 6% have been studied for biological activity, and about 15% have been investigated phytochemical constituents. A side effect of plant derived drugs is less than synthetic substance. This article intends to provide an overview of history, pharmacognosy, phytochemistry, pharmacological activities and available marketed preparation of *Acmella paniculat.*

Acmella Paniculata is an annual hairy herb up to 32-60 cm tall, with numerous stems and

marigold eye flowers. Stem is glandular and hairy with pungent taste. The whole plant is acrid in taste. Leaves are opposite, broadly ovate lanceolate, 2.5-5cm by 1.3-3.8 cm subobtus, irregularly crenate-serrate or sometime entire, glabrous, or nearly so base usually acute petioles 0.6-1.6 cm long, pubescent.

Acmella paniculata whole plants are rich for phytoconstituents viz. alkaloids, saponins, flavonoids, and tannins. These drugs are widely used in the treatment of different ailments Indian system of medicine. Therefore the aim of this study is to evaluate anthelmintic activity and phytochemicals present in the Acmella paniculata plant extracts.

Fig1: *Acmella paniculata* plant with leaves stem and flowers

The dried air plant material is powdered by using grinder and stored in air tight sealed plastic container at room temperature and till the time of extraction

MATERIAL AND METHODS

Plant material collection:

Fresh whole plant parts of *Acmella Paniculata* were collected from Prof. Vastsavaya.S.Raju, Plant systematic laboratory, Kakatiya University, Warangal. The plant voucher specimens identification was done with the help of Prof. Vastsavaya.S.Raju Professor Department of Botany Kakatiya University, Warangal and the same was deposited at S.R. College of Pharmacy.

Preparation of the plant material and extraction:

The plant materials taken out of the respective collections were washed with water and chopped into small pieces then kept on news paper and shade dried at room temperature for two weeks .The dried air plant material is powdered by using grinder and stored in air tight sealed plastic container at room temperature and till the time of extraction. After placing the cotton plug the powdered plant material of 2kg each was placed in the soxhlet apparatus sufficient quantity of different type of solvents as the base of polarity was poured into the soxhlet till the powder is submerged. A cotton plug is placed over it before fixing the soxhlet over the mantle. Then the power plug was made on to heat the mantle for about 8hrs per day for 7days. Through vaporization & condensation, the process of extraction of chemicals was carried out. When the soxhlet was drained colorless, the extraction process of that material was stopped. The solvent was then removed and using rotary flash evaporator a semi-solid mass is obtained for analytical study.

Photochemical studies:

The following are the general procedure for identification of phytochemical constituents

1) Carbohydrates tests

i) *Iodine test:* Take 2ml of plant extract in a test tube and add pinch of iodine it gives blue or purpule, reddish brown colour.

ii) *Molish test:* Take 2ml of plant extracts in a test tube and add few drops of alcoholic α-naphthal and few drops of Hcl the side of the test tube it gives purpule to violet ring appeared at the junction of the two liquids.

iii) *Benedict test*: Take 2ml of plant extract in a test tube and add Bendic reagent break red or yellow colour is observed.

iv) *Fehling's test:* Take 2ml of plant extract in a test tube and add Fehling's reagent break red or yellow colour is observed.

V) *Selivanoffs test*: Take 2ml of plant extract in a test tube and add resorcinol, equal volume of Hcl and heat on water both rose colour is produced.

2) *Amino acid test*: Take 2ml of plant extract in a test tube and add Ninhydrine keep in water

both for 2min and cool yellow colour is observed.

3) ***Protein test***

i) ***Biuret tes*****t**: Take 2ml of plant extract in a test tube and add Biuret reagent yellow colour is observed.

4*)* **Flavonoids test**:

i) Shinoda test: Take 2ml of plant extract in a test tube and add magnesium turners and Hcl drop wise pink colour is observed.

ii) $FeCl_3$ test: Take 2ml of plant extract in a test tube and add $Fecl_3$ solution green colour is observed.

iii) ***Zinc hydrochloride test*****:** Take 2ml of plant extract in a test tube and add zinc dust and conc. Hcl red colour is observed.

5) Tannins test:

i) ***Gelatin test:*** Take 2ml of plant extract in a test tube and add small amount of gelatin precipitation is observed.

ii) ***Metal test:*** Take 2ml of plant extract in a test tube and add small amount of copper, tin and lead precipitation is observed.

iii) ***Potassium dichromate solution test*****:** Take 2ml of plant extract in a test tube and add small amount of potassium dichromate solution precipitation is observed.

6) Phenolic test:

i) ***$FeCl_3$ test:*** Take 2ml of plant extract in a test tube and add small amount of $Fecl_3$ solution gives bluish black colour.

7) Alkaloid test:

i) ***Mayer's test: (Potassium mercuric iodide solution)*** Take 2ml of plant extract in a test tube and add small amount Mayers reagent it gives cream colour precipitation.

ii) ***Dragendraff reagent****: (Potassium bismuth iodide solution)* Take 2ml of plant extract in a test tube and add small amount Mayers reagent it gives reddish brown colour precipitation.

iii) ***Wagner's reagent: (Iodine potassium iodide solution*****)** Take 2ml of plant extract in a test tube and add small amount Wagner's reagent it gives reddish brown colour precipitation.

iv) ***Hager's reagent*****:** Take 2ml of plant extract in a test tube and add small amount Wagner's reagent it gives yellow colour precipitation.

8) Saponins test: Take 2ml of plant extract in a test tube and add small amount water and shake well it form a colloidal solution or foam.

9) Steroidal tests:

i) ***Libermann butchered test*****:** Take 2ml of plant extract in a test tube and add small amount of chloroform, acetic anhydride boil the solution add H_2SO_4 then cool and add absolute alcohol. Organic layer shows red colour, aqueous layer shows green colour.

ii) ***Salkowski test***: Take 2ml of plant extract in a test tube and add small amount of chloroform add H_2SO_4 cool the solution organic solution shows red or pink colour.

10) Proteins test:

i*)* ***Xanthoprotein test*****:** To the test solution 5ml add 1ml of conc. HNO_3 and boil the solution yellow precipitate is formed. After cooling it, add 40% NaoH solution orange layer is formed.

ii) ***Starch test*****:** To The plant extract add weak aqueous iodine solution, blue colour indicate the presence of starch. This disappears on heating and reappears on cooling

TLC profile:

Thin layer chromatography is an important tool in the separation, identification and estimation of different components. Here the principles of separation are adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phases, its preparation and use with different solvents can be achieved on the basis of partition and adsorption. The plant extracts showed good resolution in solvent system by trial and error method Generally Toluene: Acetone, Benzen: Ethyl acetate, n-Hexane: Acetone etc solvents are used.

Commercially available precoated aluminum sheets silicagel-G60 F254 (E.Merek), 10×10cm plates were used for this study, the plant extracts was used for analysis. TLC plates are prepared with silica gel-G (activated) as the stationary phase having a thickness of about 0.5mm. 20µl each of test solution was applied on silica gel-G plates (5×15cm). The TLC plates was developed in the saturated chamber containing Toluene: Ethyl acetate (1:0.5ml) solvent system.

The plates were developed in Camag Twin Trought Chamber using the solvent system as used in TLC. After developing, the plates were air dried and observed under U.V chamber or Iodine chamber and 5% H_2SO_4 contains (5ml of H_2SO_4 in 95ml of methanol), 1% Vanillin solution (1gm of vanillin in 100ml of methanol) used as a spraying reagent. This reagent is very important for identification of spots in case of plant extracts only. It gives clear sports.

Calculation of R_f value:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent front}}$$

Anthelmintic activity:

Test organism:

Indian adult earthworms (pheretima posthuma) collected from the botanical garden of the department Kakathiya University and washed with normal saline. The earth worms of 3.5cm in length and 0.1-0.2cm in width were used for all the experimental protocol due to their anatomical and physiological resemblances with the intestinal roundworm parasites of human beings.

The anthelmintic activity was evaluated on adult Indian earthworms, *Pheritima postuma* (Annelida), due to its anatomical and physiological resemblance with intestinal round worm parasites of human beings. The worms were collected and identified at Vermi compost Division, Regional Agricultural Research Institute, Lam, Guntur.

Preparation of Standard compound:

Take Albendazole tablet crush the tablet by using motar and vessel. From the crushed powder take 0.07 w/v of Albendazole powder and dissolve it in 2ml of Dimethyl form amide and 8ml of normal saline solution (9% Nacl). This solution is taken in 10ml volumetric flask. This gives 0.07 w/v of standard concentration. From the crushed powder take 0.150 w/v of Albendazole powder and dissolve it in 2ml of Dimethyl form amide and 8ml of normal saline solution (9% Nacl). This solution is taken in 10ml volumetric flask. This gives 0.150 w/v of standard concentration. From the crushed powder take 0.220 w/v of Albendazole powder and dissolve it in 2ml of Dimethyl form amide (DMF) and 8ml of normal saline solution (9% Nacl). This solution is taken in 10ml volumetric flask. This gives 0. 220 w/v of standard concentration.

Pet ether, chloroform, ethyl acetate& methanol extracts of the whole plant of *Acmella paniculata* were investigated for their anthelmintic activity against albendazole. Various concentration of each extracts. Were tested in the bioassay, which involved determination of time of paralysis and time of death of worms. The anthelmintic assay was carried as per the method of Ajaryeoba et.al[7] .with minor modifications in the first set of experiment, six groups of six earthworms were released into 10ml of solution of dimethyl form amide & normal saline solution to prepare 7.5mg/ml, 15mg/ml, and 22.5mg/ml concentrations. Albendazole was used as the standard drug. All the extracts and drugs solutions were freshly prepared before starting the experiment. Six earthworms in each were placed into 10ml of desired formulations as following: vehicle (normal saline) Albendazole (20mg/ml) and three sets of three different groups were treated with extracts of respective concentrations. Observations were made for the time until the paralysis and death of an individual worm. The paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors.

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical analysis showed the presence of alkaloids, saponins, flavonoids and tannins in whole plant. The pet ether extracts contains alkaloids, flavonoids, tannins and saponins. The chloroform extracts contains alkaloids and saponins. The ethyl acetate extracts contains alkaloids, saponins and flavonoids. methanol extract contains saponins, flavonoids and tannins (Table-1).

The changes in the control feed fed fishes, agrimin or fishmin fed fishes muscle and liver tissues protease activity were presented in table 3. Protease activity was found to be more in the liver tissue. Agrimin or fishmin treatment enhanced the fish muscle and liver protease content and all the changes were found to be statistically significant over their corresponding control values.

Table 1: Preliminary Phytochemical screening of *Acmella paniculata*

S.No	Tests	Extracts			
		P.E	C.E	EA.E	M.E
1	Phenolic	-	-	-	-
2	Flavonoids	+	-	+	+
3	Alkaloids	+	+	+	+
4	Tannins	+	-	-	+
5	Amino acids	-	-	-	-
6	Saponins	+	+	+	+
7	Steroids	-	-	-	-
8	Carbohydrates	-	-	-	-
9	Proteins	-	-	-	-
10	Anthra-cyanosides	-	-	-	-

P.E = Pet ether extract; C.E = Chloroform extract ; EA.E =Ethyl acetate extract; M.E= Methanol extracts - shows absence of constituents; + shows presence of constituents.

Anthelmintic activity:

Pet ether extracts the concentration of 0.07 w/v showed the time of paralysis and death at 20.30sec. and 60.32sec respectively for concentration of 0.15 w/v, the paralysis and death time was found 20.40 sec. and 60.12sec. respectively at the concentration 0.22 w/v, time was 22.30 sec. for paralysis and 90.02 sec. for death (Table 3). While in chloroform extracts at the concentration of 0.07 w/v the time of paralysis and death was found to be 30.39sec and 70.35sec. respectively at the concentration of 0.15 w/v time of paralysis and death was 30.43 sec and 80.45 sec. at the concentration of 0.22 w/v the time of paralysis and death was found to be 20.26 sec. and 60.55 sec. While in the ethyl acetate extracts at the concentration of 0.07 w/v the time of paralysis and death was found to be 20.16 sec and 50.03 sec. respectively at the concentration of 0. 15 w/v time of paralysis and death was found to be 20.00sec. and 30.04 sec. at the concentration of 0.22 w/v the time of paralysis and death was found to be 50.00 sec. and 70.00 sec. respectively in case of methanol extract at the concentration of 0.0 7 w/v the time of paralysis and death was found to be 20.58 sec. and 60.11 sec. concentration of time of incase of 0.15 w/v the time of paralysis and death was found to be 20.45sec. and 60.10 sec. at the concentration of 0.22 w/v the time of paralysis and death was found to be 30.16sec, 80.55 sec respectively all the concentration ethyl acetate extract at 0. 22 w/v concentration shows 50.00sec. paralysis time and pet ether extract shows death time 90.02 sec. at 0.22.5w/v.

It was observed that both ethyl acetate, pet ether, chloroform and methanol extracts showed a remarkable Anthelmintic potential against intestinal parasitism. The anthelmintic activity of extracts may be due to the synergetic effect of active phyto- constituents i.e. alkaloids, saponins, flavonoids and tannins etc. Present in the extract. Further, there is scope to evaluate those active principles of *Acmella paniculata* whole plant for their Anthelmintic activity to open the new door for natural Anthelmintic.

Table 2: Experimental results of Anthelmintic activity

S. No	Extracts	Concentration w/v	Time taken for paralysis in min	Time taken for death in min
1	P.E	0.075	40.30	60.32
		0.150	22.40	60.12
		0.220	19.30	50.02
2	C.E	0.075	30.39	70.35
		0.150	22.43	60.45
		0.220	20.26	39.55
3	EA.E	0.075	70.16	50.03
		0.150	20.00	38.04
		0.220	44.00	58.00
4	M.E	0.075	73.58	99.00
		0.150	20.45	60.10
		0.220	19.16	42.55
5	Albendazole	0.075	61.00	70.00
		0.150	55.00	59.00
		0.220	49.00	39.00

Fig 2: Anthelmintic activity paralysis time

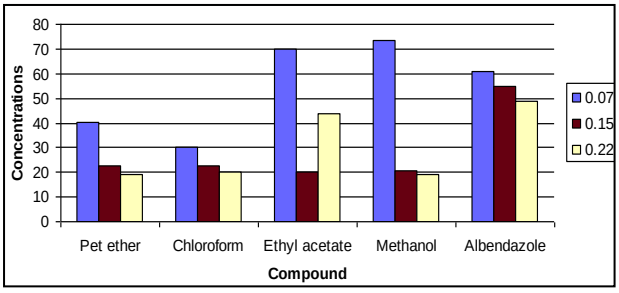
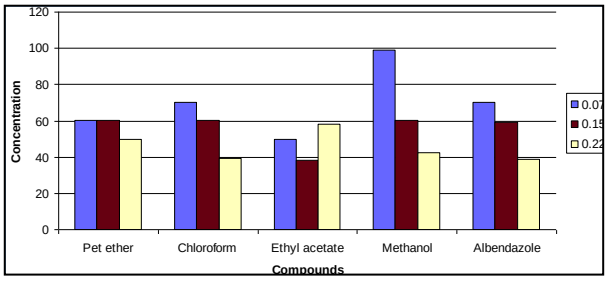


Fig 3: Anthelmintic activity death time



CONCLUSION

Anthelmintic activity it was observed that ethyl acetate, pet ether, chloroform and methanol extracts showed a remarkable Anthelmintic potential against intestinal parasitism. The Anthelmintic activity of extracts may be due to the synergetic effect of active phyto- constituents i.e. alkaloids, saponins, lavonoids and Tannins etc. present in the extract. As a result, the data support the use of an *Acmella paniculata* as a rich source of compounds with high therapeutic values for medicines and food supplement and as a health food.

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