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Original Article

Phytochemical and Pharmacological Activities of *Gynura lycopersicifolia* and *Gnaphalium polycaulon*

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ABSTRACT

The present study was focused on studying the anthelminthic activity of ethyl acetate plant extract of *Gynura lycopersicifolia*. The Anthelmintic Potential of the whole Plant of *Gynura lycopersicifolia* & *Gnaphalium polycaulon* using Pheretima posthuma as test worm was evaluated. Various concentrations (10 mg/ml, 20 mg/ml, and 30 mg/ml) of *Gynura lycopersicifolia* and *Gnaphalium polycaulon* were tested *in-vivo*, which involved the determination of Time of Paralysis (P) and Time of Death (D) of worms using Albendazole as standard. The present study indicated that the whole plant of *Gynura lycopersicifolia* and *Gnaphalium polycaulon* showed significant Paralysis and death of worms, especially at a lower concentration as compared to the standard reference of Albendazole.

Keywords: Anthelminthic activity, *Gynura lycopersicifolia, Gnaphalium polycaulon, Pheretima posthuman.*

1. INTRODUCTION

Natural products either in pure compounds or as standardized plant extracts provide unlimited opportunities for discovering new drugs. The majority of plant extracts are highly beneficial against parasitic and microbial infections. It is estimated that around 70,000 plant species from lichens to trees have been used at one time to others for medicinal purposes. The use of different parts of several medicinal parts to cure specific ailments has been in vogue from ancient times [1, 2]. The indigenous system of medicine such as ayurvedic, siddha, and unani, has been in existence for several centuries. This system of medicine caters to the

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needs of nearly 70% of the population residing in villages [3].

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious disease. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial in infection has led to the screening of several medicinal plants for their potential antimicrobial activity. The natural products play an important role in drug development programs in the pharmaceutical industry. However, the World Health Organization (WHO) also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines, and standards for botanical medicines [4].

Anthelmintic are the agents used for the treatment of infections with parasites. Those include flatworms, e.g., flukes, tapeworms and roundworms, i.e., nematodes. They are of huge benefit for tropical medicine in human and in veterinary medicine. The World Health Organization (WHO) estimates that a staggering 2 billion people are suffering from parasitic infections. Parasitic worms even more infect livestock and crops, affecting food production with resultant enormous economic impact. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programs [5, 6].

Herbal drugs are the huge increased demand in recent years because of its safety efficacy, less toxic effects and high therapeutic value, and also economical cost in contrast to the synthetic drug, which has several unwanted side effects. Herbal plants contain and produce a verity of the chemical substance used as a remedy for treating diseases. Anthelmintic has been treated with some medicinal plants or their extract based on folklore medicine [7].

Very few pharmacological activities have been reported on the whole plant of A. *Gynura lycopersicifolia*. B. *Gnaphalium polycaulon.* So, the author selected the plants to submit the project. Which involves phytochemical screening and Pharmacological (Anthelminthic activity) evaluation to provide scientific validation to its folklore claims.

2. MATERIALS AND METHODS

Collection identification and Authentication of plants

A. *Gynura lycopersicifolia*. B. *Gnaphalium polycaulon* are grown widely throughout India. The plant was isolated, identified, and authenticated by Prof. Vasthavya.S.Raju Dept. of taxonomy, a botanist in Kakathiya university, Warangal, was stored in the department of pharmacognosy.

Extraction and phytochemical investigation

Extraction procedure [8]:

Medicinal plants are the richest bioresource for traditional systems of medicine, allopathic medicines, pharmaceutical intermediates, nutraceuticals, food supplements, folk medicines and chemical entities for synthetic drugs .the first step in the value addition of the medicinal plants bioresource is the production of herbal drug preparation (i,e extracts), using a verity of methods from sample traditional technologies to advanced extraction techniques.

Extraction is the separation of medicinally active compounds of plant tissues selective solvent through the standard procedure. Such extraction techniques separate the soluble plant metabolites and leave behind the insoluble cellular marc. The product so obtained from the plant is a relative complex mixture of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and intended for oral or external use. These include classes of portions know as a decoction, infusion, fluid extract, or powder extracts. Such preparation has been popularly called galenicals, named after Galen, the second-century Greek physician.

The purpose of standardized extraction procedure for crude drug (medicinal plant parts) is to attain the therapeutically desired portions, and to eliminate material by treatment with selective solvent known as menstrum the extracted thus obtained, after standardization, may be used as medicinal agent as such in the form of tincture or fluidextracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products all contain a complex mixture of many medicinal plant metabolites, such as glycosides, terpenoids, flavonoids, and lignans. In order to be used as a modern drug, an extract may be further processed through various techniques of fractionation to isolate individual chemical entities such as vincristine, vinblastine from vinca.

Extraction of leaves of *Gynura lycopersicifolia* and*Gnaphalium polycaulon*:

The collected whole plant of A. *Gynura lycopersicifolia*. B. *Gnaphalium polycaulon* was dried under shade ten days and then made into a coarse powder with a mechanical grinder for further use. Extracted with Ethyl acetate, Ethanol, Water. All solvents were distilled before use [9].

Ethyl acetate extraction [10]:

The dried of leaves (50 gm) was first extracted with Ethyl acetate by maceration process done two days then after extract was collected by distillation for removal of solvent. The concentrated solution was evaporated to dryness. The yield of ethyl acetate extract was collected.

Aqueous extraction:

The dried of leaves (50 gm) was first extracted with the Aqueousby maceration process done two days then after extract was collected by distillation for removal of solvent. The concentrated solution was evaporated to dryness. The yield of Aqueouswas collected.

Ethanol extraction:

The dried of leaves (50 gm) was first extracted with Ethanol by maceration process done two days then after extract was collected by distillation for removal of solvent. The concentrated solution was evaporated to dryness. The yield of Ethanol was collected.

Qualitative phytochemical studies [11]:

Chemical Tests:

The Ethyl acetate, Ethanol, Aqueous extracts of *Gynura lycopersiciifolia*, and *Gnaphalium polycaulon* were subjected to different qualitative phytochemical screening tests for detection and establishment of the nature of the chemical composition.

Detection of alkaloids: The filtrate was tested for the presence of alkaloids using the following tests.

- **Dragendorff's test:**

To 1 ml of filtrate, two drops of dragendorff's reagent (Potassium bismuth iodide solution) was added and observed for the observation of precipitate. The formation of a prominent reddish-brown precipitate indicates a positive test for the presence of alkaloids.

- **Mayer's test:**

1 ml of filtrate was taken into a test tube, and two drops of Mayer's reagent (Potassium mercuric iodide) were added and observed for white or creamy precipitate, which indicates the presence of alkaloids in the extract.

- **Wagner's test:**

1 ml of filtrate was taken into a test tube, added two drops of Wagner's reagent (Iodine-Potassium iodide) was added and observed for reddish-brown precipitate, which indicates the presence of alkaloids in the extract.

- **Hager's test:**

To 1 ml of filtrate, two drops of Hager's reagent (picric acid) was added and observed for prominent yellow color precipitate, which indicates a positive test for the presence of alkaloids.

Detection of carbohydrates [12, 13].

a) Molish's test:

One ml of the test solution was taken, and two drops of alcoholic solution α– naphthol (Molish's reagent) was added. The mixture was shaken and one ml of conc. H_2SO_4 was added slowly. The test tube was cooled in ice and allowed to stand. Followed by, the test tubes were observed for violet ring formation at the junction, which indicates the presence of carbohydrates.

b) Fehling's test:

1 ml of filtrate was boiled on a water bath with a mixture of 1 ml each of Fehling's solutions A and B were added and allowed to boil for 1min and observed for the formation of a red color precipitate, which indicates the presence of reducing sugar.

c) Benedict's test:

To 0.5 ml of filtrate, 0.5 ml of benedict's reagent was poured. The mixture was heated on a boiling water bath for 2 minutes and observed for yellow, green, or red colored precipitate, which indicate the presence of reducing sugar.

d) Barfoed's test:

To 1 ml of filtrate, 1 ml of Barfoed's reagent was added and heated on a water bath for two minutes and observed for the formation of red precipitate, which indicates the presence of non-reducing sugar.

Detection of proteins and amino acids [14]

a) Biuret test:

To 3 ml of filtrate, two drops of 4% NaOH was added and treated with two drops of 1% $CuSO_4$ solution. The observation of pink color indicates the presence of proteins.

b) Ninhydrin test:

To 3 ml of filtrate, three drops of 5% ninhydrin reagent were added and heated in a boiling water bath for ten minutes. The appearance of a characteristic purple color indicates the presence of amino acids.

Detection of steroids and terpenoids

a) Salkowski test:

To the filtrate, two ml of chloroform and two ml of concentrated sulphuric acid were added, shaken well, and observed the color formation in both chloroform and acid layers. The appearance of the chloroform layer as red in color and acid layer as greenish yellow fluorescence indicates the presence of steroids.

b) Liebermann – Burchard's test:

To the filtrate, two ml of acetic anhydride, two ml of chloroform were added and heated to boil, followed by cooling. One ml of concentrated sulphuric acid was added and observed for the appearance of color at the junction. Formation of red, pink, or violet color at the junction of the liquids indicate the presence of steroidal triterpenoids.

Detection of phenolic compounds and tannins

a) Ferric chloride test:

The filtrates were taken and added two drops of neutral 5% ferric chloride solution and observed for blue, green, or violet color, which indicates the presence of phenolic compounds. Test solution treated with a few drops of ferric chloride solution gives a dark color

b) Lead acetate test:

The filtrates were taken, and to this, 3 ml of 10% lead, acetate solution were added. The formation of a bulky white precipitate indicates the presence of phenolic compounds.

c) Bromine water test:

The filtrates were taken, and 1ml of bromine water was added and observed for the discoloration of bromine water. The discoloration of bromine water indicates the presence of phenolic compounds.

Detection of glycosides

Test for cardiac glycosides

a) Legal test:

The filtrates were taken and added a few drops of pyridine and one drop of 2% sodium nitroprusside, and a drop of 20% sodium hydroxide solution was added. The formation of deep red color indicates the presence of cardiac glycosides.

b) Keller - Killiani test:

The filtrates were taken and added 2 ml of glacial acetic acid and two drops of 5% ferric chloride solution and mixed. Then 1 ml of sulphuric acid was added. Reddish-brown color

appears at the junction of the two liquid layers, and the upper layer appear bluish-green color indicates the presence of steroidal glycosides.

Test for anthraquinone glycosides:

Borntrager's test:

To 2 ml of filtrate, 3 ml of chloroform was added and shaken, the chloroform layer was separated, and a 10% ammonia solution was added. The formation of pink color indicates the presence of anthraquinone glycosides.

Test for saponin glycosides:

Foam test: Filtrates were taken, and 20 ml of distilled water was added and shaken for 15 min in a graduated cylinder. A layer of stable foam indicates the presence of saponin glycosides. Pharmacological studies of A. Gynura lycopersiciifolia. B. Gnaphalium polycaulon.

a. *IN VITRO* antioxidant activity [15]

Chemicals required: 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid.

Procedure:

Antioxidant activity has been performed by the DPPH method. The scavenging activity of antioxidants was studied by using DPPH (1.1-diphenyl-2-picrylhydrazyl free radical). Various of concentration of as 0, 10, 25, 50, 75, 100 and 100µg/ml in 0.1ml was added to 0.9ml of a solution of DPPH in Ethyl acetate, Ethanol. Ethyl acetate, Ethanol only (0.1ml), was used as an experimental control. After 30 min of incubation at room temperature, the reduction in the number of free radicals was measured by reading the absorbance at 517nm. Reference Standard (Ascorbic acid). The scavenging activity of the sample corresponded to the intensity of quenching DPPH.

The percent inhibition was calculated from the following equation:

% Inhibition = (Absorbance of control – absorbance of the test sample) / absorbance of control ×100.

b. Anthelmintic activity [15]

Drugs: The Ethyl acetate extract of *Gynura lycopersiciifolia* and Ethanol extract of *Gnaphalium polycaulon* Was tested in various doses in each group. Normal saline water was used as control. Albendazole was used as a standard drug.

Procedure:

The method of narrowed was followed for the screening of anthelmintic activity. Anthelmintic activity was evaluated on adult pheretima posthuma. Earthworms were divided into eight groups (5 each). The first group (I) served the standard drug albendazole at a dose level of 10 mg/ml groups (VI)to (IX) received doses of Ethyl acetate, Ethanol extracts of 10 mg/ml, 15 mg/ml, 20 mg/ml,25 mg/ml,30 mg/ml, and 35 mg/ml respectively. Observations were made for the time taken to cause paralysis and death of individual worm for two hours. Paralysis was confirmed when the worms did not revive even in normal saline water. Death was concluded when the warms lost their lost motility, followed by fading away from their body of their body color.

3. RESULTS AND DISCUSSION

The percentage yield of extraction

Table 1: Percentage yield of *Gynura lycopersicifolia*

S. No.	Type of extraction	Practical yield (gms)	Percentage yield (w/w)
1.	Ethyl acetate extract	3.3	7.2
2.	Ethanol extract	5.8	9.6
3.	Aqueous extract	2.9	6.4

Table 2: Percentage yield of *Gnaphalium polycaulon*

S. No.	Type of extraction	Practical yield (gms)	Percentage yield (w/w)
1.	Ethyl acetate extract	3.2	7
2.	Ethanol extract	5.5	10.1
3.	Aqueous extract	3.3	6.2

The powder leaves of *Gynura lycopersiciifolia* and *Gnaphalium polycaulon* plants were subjected to extraction with three solvents ethanol, ethyl acetate, and aqueous extract with increasing polarity. High yield was obtained by methanol extracts A. *Gynura lycopersiciifolia* plants (Table1) B. *Gnaphalium polycaulon* (Table 2).

- The *Gynura lycopersiciifolia* and *Gnaphalium polycaulon* plants were selected for thesis presentation, the plants were identified by based on literature survey, not much work was done on the leaves of this plants, so we selected leaves part for the project to investigated phytochemical and to evaluated pharmacological activity (antioxidant and Anthelmintic activity)
- **Preliminary phytochemical studies:**

Quantitative phytochemical analysis of extracts

Table 3: Phytochemical analysis of *Gynura lycopersicifolia*

S. No.	Test	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids	+	+	-
2.	Carbohydrates	-	-	-
3.	Proteins & Amino acids	+	+	-
4.	Steroids and Terpenoids	-	-	-
5.	Phenolic compounds	-	+	+
6.	Tannins	-	+	-
7.	Glycosides	+	-	-
8.	Saponins	+	+	+

Table 4: Phytochemical analysis of *Gnaphalium polycaulon*

S. No.	Test	Ethyl acetate extract	Ethanol extract	Aqueous
1.	Alkaloids	+	+	-
2.	Carbohydrates	+	+	+
3.	Proteins & Amino acids	+	+	-
4.	Steroids and Terpenoids	-	+	-
5.	Phenolic compounds	+	+	+
6.	Tannins	+	+	-
7.	Glycosides	+	+	+
8.	Saponins	-	+	+

Gynura lycopersiciifolia shows the presence of various phytochemical constituents, which are analyzed by the

qualitative phytochemical test. The qualitative tests of methanol extraction (*Gynura lycopercisifolia*) was given positive tests for alkaloids, Proteins & amino acids, phenolic compounds, tannins, glycosides, flavonoids, and saponins (Table 3) and methanolic extract of (*Gnaphalium polycaulon*) were given positive tests for alkaloids, carbohydrates, proteins, amino acids, Steroids & terpenoids, phenolic compounds, tannins, glycosides, flavanoids, and saponins (Table 4).

Anthelmintic activity

Table 5: Anthelmintic activity of Ethyl acetate extract of *Gynura lycopersicifolia*

Groups	Treatment	Concentration Used (mg/ml)	Time is taken For paralysis (min) (X± S.D)	Time is taken for death (min) (X± S.D)
1.	Vehicle normal saline		-	-
2.	Standard (albendazole)	10	0.9±0.25*	35.6±0.2
3.	ETAE 1	10	92.6±0.5	125.21±0.5
4.	ETAE 2	15	76±0.5**	108.3±.4
5.	ETAE3	20	63±0.2*	96±0.1
6.	ETAE 4	25	51±0.3**	72.2±0.4
7.	ETAE 5	30	35.6±0.3	45±0.3

Significant difference from control by one-way ANOVA, followed by Dunnett's test (n=5), ***p<0.001

When decreasing the concentration of Ethyl acetate extract of *Gynura lycopersicifolia,* the death time of worms was increased. The Ethyl acetate extract of *Gynura lycopersicifolia*, showed significant paralysis & also caused the death of worms at a lower concentration as compared to the standard reference of Albendazole. The anthelmintic activity of Ethyl acetate extract of *Gynura lycopersicifolia* was showed in Table 5. Anthelmintic activity of Ethanol extract of *Gnaphalium polycaulon*was showed in Table 6.

Table 6: Anthelmintic activity of Ethanol extract of *Gnaphalium polycaulon*

Groups	Treatment	Concentration Used (mg/ml)	Time is taken for paralysis (min)	Time taken Death(min)
1.	Vehicle normal saline		-	-
2.	Standard (albendazole)	10	1 ±0.25*	35.6±0.2
3.	EOLC 1	10	98.6±0.2	112±0.5
4.	EOLC 2	15	72.3±0.2**	98.5±0.2
5.	EOLC 3	20	68±0.1	86±0.1
6.	EOLC 4	25	48±0.3*	68.6±0.4
7.	EOLC 5	30	36.2±0.3	43.3±0.3

Significant difference from control by one way ANOVA, followed by Dunnett's test(n=5), *** p<0.001.

Anthelmintic activity: the data on biological studies were reported as mean± standard deviation (n=5). For determining the statistical significance was employed. The "P" values were considered significant. When decreasing the concentration of Ethanolic extract of *Gnaphalium polycaulon*, the death time of worms was increased. The Ethanolic extract of Gnaphalium polycaulon showed significant paralysis & also caused the death of worms at a lower concentration as compared to the standard reference of Albendazole.

Determination of total phenolic content

Table7: Standard Graph of Ascorbic acid

Concentration (µg/ml)	Absorbance
0	0
20	0.015
40	0.029
60	0.049
80	0.057
100	0.071
1mg ethyl acetate extract of *Gynura lycopersicifolia* 1mg ethanol extract of *Gnaphalium polycaulon*	0.027
	0.023

Phenolics are the most wide-spread secondary metabolite in plants. These diverse groups of compounds have received much attention as a potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelators. Therefore, in the present study, total phenolic content present in the extracts was estimated using Folin- ciocalteau method. The standard graph was plotted by taking Ascorbic acid as the standard as shown in Table 7. In ethyl acetate extract of *Gynura lycopersicifolia* and ethanolic extract of *Gnaphalium polycaulon*(1 mg/ml), total phenolic compounds were found to be 47.4, 25.08 µg/ml respectively equivalent to Ascorbic acid was detected.

4. CONCLUSION

The whole plant of *Gynura lycopersiciifolia* and *Gnaphalium polycaulon* plants was observed for the phytochemical investigation and pharmacological evaluation. The percentage of yield and phytochemical was observed in ethyl acetate, Ethanolic, and Aqueous extraction, so these extractions were used for pharmacological activity. The Ethyl acetate extract of *Gynura lycopersiciifolia* and Ethanolic extract of *Gnaphalium polycaulon* plants shown anthelmintic activity. The anthelmintic activity of Ethyl acetate extract of *Gynura lycopersiciifolia* was better than the Ethanolic extract of *Gnaphalium polycaulon***.** Significant DPPH free radical scavenging activity was found in the Ethyl acetate extract of *Gynura lycopersicifolia* IC_{50} value 29.16, Ethanol extract of *Gnaphalium polycaulon* IC_{50} value is 32.26 Compare with reference standard Ascorbic acid IC_{50} value is 45.7. The Ethanolic extract of *Gnaphalium polycaulon* was better than Ethyl acetate extract of *Gynura lycopersiciifolia* for antioxidant activity.

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