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PHYTOCHEMICAL INVESTIGATION AND *IN-VITRO* ANTHELMINTIC ACTIVITY OF THE LEAVES OF *GYNURA LYCOPERSICIFOLIA* LINN

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ABSTRACT

Introduction: The present study is aimed at phytochemical investigation and evaluation of the in-vitro anthelmintic activity of the leaves of the medicinal plant *Gynura lycopersicifolia* which was not reported earlier in an attempt to develop natural products as safe and effective alternatives for synthetic drugs that possess challenges like, antimicrobial resistance and side effects. **Methods:** Dried leaves of the plant was used for extraction with water, ethanol and ethylacetate as menstrum and the extracts thus obtained were used for phytochemical qualitative analysis for different phytopharmaceutical agents like alkaloids and flavanoids. The extracts which were found to possess maximum constituents were selected for biological evaluation for anthelmintic activity using Indian earth worms *in-vitro*. Albendazole was taken as reference standard. Time taken for paralysis and death of the worms was taken as criteria for the efficacy of standard and extracts. **Results:** Both the ethanolic and ethyl acetate extracts were found to contain various phytochemicals of pharmaceutical importance such as alkaloids and flavanoids. Both the extracts were also known to exhibit anthelmintic activity *in-vitro* against Indian earth worms at concentrations 30 mg/ml comparable to reference standard 10 mg/ml. **Conclusions:** Ethanolic and ethyl acetate extracts of the leaves of the plant *Gynura lycopersicifolia* contains significant phytochemicals of pharmaceutical importance can be used for the biological evaluation for different pharmacological activities. Both the extracts were found to possess significant anthelmintic activity against Indian earth worms *in-vitro.*

More study is required for isolation and characterization of chief chemical constituents responsible for the anthelmintic activity and its *in-vivo* evaluation.

KEYWORDS: Ethanolic, ethyl acetate extracts, Albendazole, Indian earth worms.

INTRODUCTION

Helminthiasis is one of the most important human as well as animal diseases worldwide that can cause heavy losses. The disease is prevalent all over the world especially in developing countries[3] and is always associated with poor management practices and inadequate and inappropriate control strategies. An integrated approach is required for the effective control of helminths which includes strategic and tactical use of anthelmintics which remains the corner stone to this end and careful management of grazing lands including control of stocking rates and appropriate rotation strategies.

Role of vaccinations is also vital for the control of various parasitic diseases as in the case of lungworms. However, various problems have emerged with the use of anthelmintics and among them; resistance against various species of helminthes is of utmost importance to different anthelmintic compounds and classes, as well as chemical residue and toxicity problems[18] In addition, recognition of the antigenic complexity of parasites has slowed vaccine development. For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific significance despite extensive use of synthetic chemicals in modern clinical practices all over the world. The plant kingdom is known to provide a rich source of botanical anthelmintics, antibacterials and insecticides.[10,16]

A number of medicinal plants have been used to treat parasitic infections in man and animals.[2,11,15] However, their scientific evaluation as compared to commercial anthelmintics is limited.

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.[19]

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethanomedical" plant sources.[17]

Plants are used medicinally in different countries and are a source of many potent and powerful drugs.[1]

A number of plants have been tested for their anthelmintic efficacy. *Momordica charantia* Linn. Known as Karela or bittergourd, Tobacco, Walnut, Wormwood, Clove, Kalonji seeds, Garlic, Malefern, Pineapple, Diatomaceous earth, Soya and other legumes, Honey, water and vinegar are mixed with warm water.[14]

The present study aims at the phytochemical investigation and study of the *in-vitro* anthelmintic activity of the medicinal plant *Gynura Lycopercisifolia* that was not reported earlier.

MATERIALS AND METHODS

Collection, identification and Authentication of plant

The plants *Gynura lycopersicifolia* are grown widely throughout India. Plant material was collected in Nalgonda region at Namapuram and prepared the herbarium. The plant was identified and authenticated by Prof. P. Suresh Govt. Degree College Ibrahimpatnam. A voucher herbarium (GP-PC-2015.) was stored in department of botany.

Extraction of leaves of Gynura lycopersicifolia

The collected leaves of *Gynura lycopersicifolia* was dried under shade 10 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.

Ethyl acetate extraction

The dried of leaves (50 gm) was first extracted with Ethyl acetate by maceration process done 2 days then after extract was collected by distillation for remove 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 Aqueous by maceration process done 2 days then after extract was collected by distillation for remove of solvent. The concentrated solution was evaporated to dryness. The yield of Aqueous was collected.

Ethanolic extraction

The dried of leaves (50 gm) was first extracted with Ethanol by maceration process done 2 days then after extract was collected by distillation for remove of solvent. The concentrated solution was evaporated to dryness. The yield of Ethanol was collected.

Qualitative phytochemical screening

The following tests were performed according to the procedure mentioned in textbook of Pharmacognosy by CK Kokate et. al.,[12]

Chemical tests

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

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

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

Mayer's test: 1 ml of filtrate was taken into a test tube and added two drops of Mayer's reagent (Potassium mercuric iodide solution) along the sides of the test tube 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 solution) along the sides of the test tube 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 precipitate, which indicates positive test for the presence of alkaloids

Detection of Carbohydrates

Molish's test: 1 ml of the test solution was taken and two drops of alcoholic solution of α–naphthol (Molish's reagent) was added. The mixture was shaken and 1 ml of conc. H_2SO_4

was added slowly from the sides of the test tube. The test tube were cooled in ice water and allowed to stand. Then the test tubes were observed for violet ring formation at the junction which indicates the presence of carbohydrates.

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 and allowed to b oil for 1min and observed for the formation of red precipitate, which indicates the presence of reducing sugar.

Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes and observed for the formation of yellow, green or red colored precipitate, which indicates the presence of reducing sugar.

Barfoed's test: To 1 ml of filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes and observed for the formation of red precipitate which indicates the presence of non-reducing sugar.

Detection of Proteins and Amino acids

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

Ninhydrin test: To 3 ml of filtrate, three drops of 5% Ninhydrin reagent was added and heated in boiling water bath for 10 minutes. Formation of a characteristic purple color indicates the presence of amino acids.

Detection of Steroids and Terpenoids

Salkowski test: To the filtrate, 2 ml of chloroform and 2 ml of concentrated sulphuric acid were added, shaken well and observed the coloration of chloroform and acid layers. Appearance of chloroform layer as red in color and acid layer as greenish yellow fluorescence indicates the presence of steroids.

Liebermann – *Burchard's test:* To the filtrate, 2 ml of acetic anhydride, 2 ml of chloroform were added and heated to boiling and cooled. Then 1 ml of concentrated sulphuric acid was added along the sides of the test tube and observed for the formation of color at the junction. Formation of red, pink or violet color at the junction of the liquids indicates the presence of steroidal triterpenoids.

Detection of Phenolic compounds and Tannins

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 few drops of ferric chloride solution gives dark color

Lead acetate test: The filtrates were taken and to this 3 ml of 10% lead acetate solution was added. Formation of bulky white precipitate indicates the presence of phenolic compounds.

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

Detection of Glycosides

Test for cardiac glycosides

Legal test: The filtrates were taken and added few drops of pyridine and 1 drop of 2% sodium nitroprusside and a drop of 20% sodium hydroxide solution was added. Formation of deep red color indicates the presence of cardiac glycosides.

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 appear at the junction of the two liquid layers and upper layer appear bluish green colour 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, chloroform layer was separated and 10% ammonia solution was added. 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.

Anthelmintic activity

The Ethyl acetate and ethanolic extracts of *Gynura lycopersiciifolia*, were tested in various doses in each group. Normal saline water was used as control. Albendazole was used as standard drug.

Procedure

The method of Ghosh et.al.,[6] was followed for screening of anthelmintic activity. Anthelmintic activity was evaluated on adult *pheretima posthuma*. Earthworms were devided into eight groups (5 each).

The first group (I) serviced the standard drug albendazole at a dose level of 10mg/ml groups (VI) to (IX) received doses of Ethyl acetate, Ethanol extracts of 10 mg/ml, 15mg/ml, 20mg/ml, 25mg/ml, 30mg/ml, and 35mg/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 of their body of their body color.

RESULTS AND DISCUSSION

Percentage yield of extraction

Table 1: Percentage yield of Gynura lycopersicifolia leaves.

Extract	Practical yield	Percentage yield
Ethyl acetate extract	3.3	7.2
Ethanol extract	5.8	9.6
Aqueous extract	2.9	6.4

High yield was obtained by ethanolic and ethyl acetate extracts of *Gynura lycopersicifolia* taken for the study.

Phytochemical studies

Qualitative phytochemical analysis of extracts

Table 2: Phytochemical analysis of *Gynura lycopersicifolia.*

Test	Ethyl acetate extract	Ethanolic extract	Aqueous extract
Alkaloids	+	+	-
Carbohydrates	-	-	-
Proteins & Amino acids	+	+	-
Steroids and Terpenoids	-	-	-
Phenolic compounds	-	+	+
Tannins			
Glycosides	+	-	-
Flavanoids	+	+	-
Saponins	+	+	+

Gynura lycopersiciifolia shows the presence of various phytochemical constituents which are analyzed by qualitative phytochemical test. The qualitative tests of ethanol extraction (*Gynura lycopercisifolia*) was given positive tests for, alkaloids, Proteins & amino acids, Phenolic compounds, Tannins, Glycosides, Flavanoides and saponins.

Anthelmintic activity

Table 3: Anthelmintic activity of Ethanol extract (EE) of *Gynura lycopersicifolia.*

Groups	Treatment	Concentration Used (mg/ml)	Time taken For paralysis (min) (X=S.D)	Time taken For death (min)(X=S.D)
1	Vehicle normal saline	-	-	-
2	Standard(albendazole)	10	0.9±0.25*	35.6±0.2
3	EE 1	10	92.6±0.5	125.21±0.5
4	EE 2	15	76±0.5*	108.3±.4
5	EE3	20	63±0.2*	96±0.1
6	EE4	25	51±0.3*	72.2±0.4
7	EE5	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 Ethanol extract of Gynura lycopersicifolia, the death time of worms was increases. The Ethanol extract of Gynura lycopersicifolia, showed significant paralysis and also caused death of worms at concentration comparable to standard reference of Albendazole.

Table 4: Anthelmintic activity of Ethyl acetate extract (EAE) of *Gynura lycopersicifolia.*

Groups	Treatment	Concentration Used (mg/ml)	Time taken For paralysis (min) (X=S.D)	Time taken For death (min)(X=S.D)
1	Vehicle normal saline	-	-	-
2	Standard(albendazole)	10	9 ±0.25*	35.6±0.2
3	EAE 1	10	98.6±0.2	112±0.5
4	EAE 2	15	72.3±0.2**	98.5±0.2
5	EAE3	20	68±0.1	86±0.1
6	EAE4	25	48±0.3*	68.6±0.4
7	EAE5	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. P value was considered significant.

When decreasing the concentration of Ethyl acetate extract of *Gynura lycopersicifolia*, the death time of worms was increases. The Ethyl acetate extract of plant showed significant paralysis and also caused death of worms at concentration as comparable to standard reference of Albendazole.

The results are comparable to the previous reports for Momordica and Tobacco [7 , 13] for anthelmintics activity.

CONCLUSION

Both the ethanolic and ethyl acetate extracts of the leaves of the plant *Gynura lycopersicifolia* were shown to possess several secondary metabolites of Pharmaceutical importance such as alkaloids as chemical constituents.

Both the extracts were found to possess significant anthelmintic activity at concentrations comparable to the standard Albendazole.

However, more study is required for isolation and characterization of the chief chemical constituents responsible for the activity and its *in-vivo* evaluation.

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