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Preliminary phytochemical screening, in-vitro antibacterial and anthelmintic activities of ethanolic extract of peperomia pellucida

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Abstract: Antibacterial and anthelmintic activities of plants are being increasingly reported from different parts of the world. The main aim of this study is to investigate preliminary phytochemical screening, antibacterial and anthelmintic activity of ethanolic extract of *peperomia pellucida*. Antibacterial activity was evaluated by using agar cup plate method at different concentrations (100mg/ml, 300mg/ml and 500mg/ml) of extract and the results were compared with gentamycin (standard antibiotic). Zone of inhibition formed after 48hrs and proved that it has good antibacterial activity. Anthelmintic activity was evaluated by using Indian adult earth worms (*pheritima posthuma*) because they posses anatomical and physical resemblance of intestinal round worm. Earth worms were collected and washed in normal saline solution then released in different concentrations (100mg/ml, 200mg/ml, 500mg/ml and 1000mg/ml) of ethanolic extract of *peperomia pellucida* and in standard albendazole. Time taken for the paralysis and time taken for the death of the earth worms were noted. So the extract exhibited mild anthelmintic activity. Preliminary phytochemical analysis of the extract showed the presence of alkaloids, flavonoids and tannins. Tannins have been outlined as having anthelmintic activity. İt can bind to free proteins in the gut of the host or glycoprotein on the cuticle of the parasite and this leads to death of parasite.

***KEY WORDS - peperomia pellucida*, antibacterial activity, anthelmintic activity, preliminary phytochemical screening**

1. INTRODUCTION

Medicinal plants are discovered and utilized in traditional medicine practice since prehistoric times. Plants synthesize many functions including defense against like herbivores, bacteria, fungus, virus, helminths and for the diagnosis and treatment of diseases [1].

Plant chemicals or phytochemicals are the chemical substances which are produced by plants for their self-resist from other microorganisms like fungi, bacteria, virus etc., and consumption from other animals and insects [2]. Different types of phytochemicals are present in plants some will produce poisonous phytochemicals these chemical substances are harmful to the insects and humans.

Plants has different kinds of chemical substances that may be divided as two categories: primary metabolites and secondary metabolites. The primary metabolites are found in all plants within the type of sugar (carbohydrates), fats, oils and proteins, which are involved within the fundamental biochemical reactions. Secondary metabolites are formed for the special reasons. They will protect the plant from other predators, insects, etc., some chemicals will help to draw into other creatures so pollination are happened, and the plant can distribute their seeds [3]. Other metabolites will protect the plant from sun burn, some will act as chemical signal and that they have called as environmental clues. Remaining some are designed to discourage or kill the microorganisms like bacteria, fungus virus etc., Secondary metabolites are varying in plants, and that they will produce some medicinal properties for humans. Alkaloids, tannins, carbohydrates, cardiac glycosides, triglycerides, volatile oils, flavonoids, minerals, proteins etc., are the phytochemicals which present in plants. Phytochemicals possess various medicinal properties like wound healing, antibacterial activity, anthelminthic activity, ant inflammation, hallucinogen, cardiac treatment, etc. [4, 5, 6, 7].

Description of plant

Peperomia pellucida is a herb, has shallow roots and it is an annual plant. It grows in damp habitats with shades, in Asia, Africa and the Americas. It also thrives in humid soils, under the shades of trees. *Peperomia pellucida* belongs to the magnoliopsid family. This plant typically contains a small rooting system, which makes it ideal as an internal and dish garden. The leaves and stems are eaten raw in salads. After boiling the plant will be eaten by people, and therefore the plant provides a mild- mustard like flavor. *Peperomia pellucida* frequently cooked in soups. Pansit-pansitan (Indonesia) may be a tea prepared by boil with mixed leaves and stems of *peperomia pellucida*. This herb tea used for the treatment of rheumatism [8].

Peperomia pellucida is employed in treating different types of diseases across the globe. Various studies (in- vivo and in-vitro) have revealed that this plant has some pharmacological actions. The presence of phytochemicals like Tannins, Glycosides, Saponins,

Flavonoids within the plant may be to blame for the pharmacological activities of the plant (Raghavendra et al, 2018). Leaves of *peperomia pellucida* abdominal pains, convulsions, headache, fever and eczema.
Pansit-pansitan could be a tea prepared by boil with mixed leaves and stems of *peperomia pellucida*. This herb tea used for the treatment of rheumatism.
The plant *peperomia pellucida* is also used as food and condiments in some places. Plants are utilized for the treatment of diseases like cold, fever, viral diseases, cough, Asthma, rheumatic pain, vaginal infections and kidney infections.
Studies have shown that *Peperomia pellucida* have biological activities like antimicrobial, hypotensive, analgesic, anti-inflammatory, healing, gastro protective and anti-diabetic activity [9].

Image 1. *Peperomia pellucida*

peperomia pellucida contain pellucidin B trimethoxy-substituent, 5-methoxy-2-methyl-2,3,8,9- tetrahydrofuro[2,3,h]chromen-4-one, pellucidin B, Isosachaftoside, N-methylcorydaline, liolide, vidarabine, 2',4',5'-trihydroxybutyrophenone, 2,6-di-tert-butyl-4-hydroxymethylphenol,velutin and brachystamide B etc., as chemical compositions. Crude fiber, proteins, ash, moisture, fat and carbohydrates as proximate composition. Calcium, magnesium, potassium, sodium, manganese and iron as mineral compositions [10].

2. RESEARCH METHODOLOGY

2.1.1. Collection of plant material

The aerial stem parts of *peperomia pellucida* were collected from the surroundings of Surempalem, Andhra Pradesh and validated by botanist. Collected plants were washed in running water and then shade dried for 30days.

2.1.2. Solvent extraction

Dried plant parts were crushed and finely powdered using pestle and motor. Dried powder was then exposed to maceration process with ethanol (95%) for 75hrs. The obtained solution was filtered using filter paper and subjected to extraction process by hot percolation method. The extraction was condensed and dried in desiccator with anhydrous chloride for further studies.

2.1.3. Evaluation of phenolic content

The phenolic content of ethanolic extract of *peperomia pellucida* were estimated by Folin-Ciocalteu method (Deshpande HA et al., 2013). To 0.5ml of crude extract, add 3ml of 1:10v/v phenolic reagent and kept aside for 5minutes, then add 4ml of 20% w/v NaCO3 solution. All the contents were kept aside for 15mnts at 30°C for color development and absorbance was observed at 765nm wavelength using spectrophotometer. From standard Gallic acid in a methanol calibration curve, phenolic content was estimated, and the results were given in units of Gallic acid equivalent mg/100mg dried weight extract.

2.1.4. Evaluation of flavonoids content

Flavonoid content of ethanolic extract of *peperomia pellucida* were estimated by Aluminum Chloride method. To the crude extract, 1.7ml of methanol, 0.1ml of 10% Aluminum Chloride, 0.1ml of 1M Sodium acetate and 3ml of distilled water are added and these contents were allowed to stand for 30 minutes under room temperature. The absorbance was measured at 415nm from the calibration curve, standard quercetin in methanol is used to determine the flavonoid content and the results are given as quercetin equivalent mg/100mg dry weight of extract.

2.1.5. Evaluation of alkaloid's content

The alkaloid content of ethanolic extract of *peperomia pellucida* was estimated by Fazel et al., (Mayavatis Patil et al., 2003). 1mg/ml of crude extract dissolved in 2N HCl and filtered. To filtrate, add bromocresol and 5ml pH-7 phosphate buffer. Above contents were diluted with chloroform and measured absorbance at 470nm.

From calibration curve, alkaloid content was estimated by standard atropine and the results were expressed atropine mg/100mg of extract.

2.2. Evaluation of antibacterial activity

Chemicals and reagents used:

Gentamycin, DMSO (Dimethyl Sulphoxide), sodium chloride, peptone, beef extract, and dextrose

Test bacterial strains used:
Gram positive bacteria:

Bacillus subtilis (ATCC11774)
Staphylococcus aureus (ATCCBAA1026)
Staphylococcus werneri (ATCC27836)

Gram Negative bacteria:

Escherichia coli (ATCC10536)
Pseudomonas aerogenosa (ATCC10662)
Pseudomonas putida (ATCC700007)

Reference standard used: Gentamycin 80mg/2ml.

Preparation of Nutrient agar Media

Beef extract (0.6gm), Sodium chloride (0.10gm), Peptone (0.10gm), Agar (3gm) and Distilled Water (200ml) are placed in a conical flask and added 200ml of distilled water. The ingredients are dissolved by heating on a water bath with stirring until the clear solution is obtained. PH was adjusted up to 7.6 and then filled by putting cotton plug into funnel immediately. When the solution is hot, distributed around 10ml into each test tube highly close the test tube with non-absorbent cotton and sterilized it by allowed autoclaving at 15lbs pressure 120°C for 30 minutes. After removing from the autoclave, allowed it to cool but not solidify. The nutrient agar was poured into each Petri plate and leave the plates on the sterile surface until the agar has solidified.

Preparation of test and standard

The stock solution of test compound was prepared by dissolving the ethanolic extract of *peperomia pellucida* in DMSO (100mg/ml, 300mg/ml, and 500mg/ml). The stock solution of reference standard Gentamycin was prepared at a concentration of 25µg/ml in sterile water.

Sterilization
Sterilization was carried out in autoclave at 15lbs for 20minutes. Agar media, water, etc., were sterilized in autoclave.

2.2.1. Antibacterial Assay by Cup Plate method

30ml of sterile nutrient agar medium was poured into sterile Petri dishes by spread plate technique and allowed to solidify. The petri plates were incubated at 37°C for 24 hours to check for sterility. The medium is seeded with the organism by pour plate method using sterile top agar (4ml) contained 1ml culture. Borers were made on the medium using sterile borer. Dried ethanolic extract of *peperomia pellucida* was dissolved in DMSO to obtain different concentrations i.e.: 100mg/ml, 300mg/ml and 500mg/ml. From these concentrations 0.05ml of each concentration was added to respective bores. 0.05ml of Gentamycin at a concentration of 25µg/ml was taken as reference. All the plates were kept in a refrigerator at 2°C to 8°C for a period of 2 hours for effective diffusion of test compounds and standard. Later they were incubated at 37°C 24hours. Zone of inhibition around the cups indicated antibacterial activity. It was measured by using graph paper.

2.3. Evaluation of anthelmintic activity

Chemicals and reference drug

For the anthelmintic test, the ethanolic extract of *peperomia pellucida* was tested using different concentrations. Distilled water was used as control and albendazole was used as the standard drug for evaluation of anthelmintic activity**.**

Collection of worms

Healthy adult Indian earthworm (*pheritima posthuma)* about 3-5cm in length and 0.1-0.2cm in width & weighing about 0.8-3.04g were collected.

Reference standard: Albendazole 20mg/ml

Experimental model

For this investigation, Indian earthworm *pheritima posthuma*, were used to study the anthelmintic activity were collected from the waterlogged areas of soil at surempalem, east Godavari district about 3-5cm in length and 0.1-0.2cm in width & weighing about 0.8-3.04g. Worms were authenticated by parasitologist, Kakinada, Andhra Pradesh, India. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the anthelmintic activity.

Anthelmintic Assay

The test was carried out by using the adult earthworm (*pheritima posthuma*) because of its anatomical and physiological similarly with the intestinal round worm parasite of human beings. All the worms were washed with normal saline water to remove all the fecal matters present on their bodies. Extract was weighed and dissolved in 10ml of DMSO to obtain the concentrations of 100mg/ml, 200mg/ml, 500mg/ml and 1000mg/ml. Earth worms were divided into five groups (each group containing five worms) in a Petri dish. The ethanolic extract of *peperomia pellucida* was added to the Petri dishes and the time of paralysis was determined. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining those worms neither moved when shaken vigorously nor when dipped in warm water (50ºC) followed with their fading away of their body colors.

2.4. Statistical analysis

All results are expressed as mean ± SEM groups of data was compared with analysis of variance (ANOVA) values would be considered statistically significant, when $p<0.05$.

3. RESULTS

3.1. Phytochemical analysis

3.1.1. Qualitative Analysis of ethanolic extract of *peperomia pellucida*.

The preliminary qualitative phytochemical screening of aerial stem ethanolic extract of *peperomia pellucida* showed the presence of Tannins, Flavonoids and Alkaloids.

Sl No	Test	Results
1	**Test for Alkaloids** 1. Dragendroff's test 2. Mayer's test	 + +
2	**Test for Carbohydrates** 1.Molisch test 2.Benedict's test	 – –
3	**Test for Flavonoids** 1.Lead acetate test 2. NaOH test	 + +
4	**Test for Glycosides** 1. General Test 2. Legal' test	 – –
5	**Test for Proteins** 1. Xanthoproteic test 2. Millions test	 – –
6	**Test for Saponins** Foam and froth test	 –
7	**Test for Tannins** Ferric chloride test	 +
8	**Test for Steroids** 1. Salkowski test 2. Libermann-buchard test	 – –

Table 1. Phytochemical qualitative screening of ethanolic extract of *peperomia pellucida*

"+" sign of existence of phytochemicals

"-"sign of no existence of phytochemicals

3.1.2. Quantitative phytochemical screening

Ethanolic extract of *peperomia pellucida* were investigated for the determination of alkaloid, flavonoid and phenolic contents quantitatively using UV/visible spectrophotometer and the values were expressed as mean standard deviation.

Flavonoids (mg/ml)	Alkaloids (mg/ml)	Phenols (mg/ml)
1.38	0.654	177.9

Table 2. Result of quantitative analysis of ethanolic extract of *peperomia pellucida*

3.2. Antibacterial activity

Antibacterial activity was evaluated by using agar cup plate method. In-vitro screening of ethanolic extract of *peperomia pellucida* showed specific activity in inhibiting growth of bacteria in a concentration dependent manner and was comparable with standard drug.

Concentration used	Zone of inhibition of sample and standard drug (in mm)					
	Gram positive bacteria			Gram negative bacteria		
	Bacillus substilis	*Staphylococcus aureus*	*Staphylococcus westin*	*Pseudomonas putida*	*Pseudomonas aurogenosa*	*Escherichia coli*
100mg/ml	10.5±0.25	11.2±0.45	12.36±0.45	11.53±0.31	11.26±0.25	11.45±0.54
300mg/ml	12.46±0.55	13.6±0.42	13.27±0.26	14.43±0.58	13.56±0.62	13.67±0.29
500mg/ml	14.4±0.47	13.47±0.52	15.76±0.7	14.86±0.91	15.34±0.18	14.50±0.58
Gentamycin (25mg/ml)	17.2±0.25	17.2±0.6	17.41±0.55	16.30±0.15	18.1±0.12	17.43±0.4

Table 3. Result of antibacterial activity of ethanolic extract of *peperomia pellucida*

Values are expressed as mean±SEM; n=3

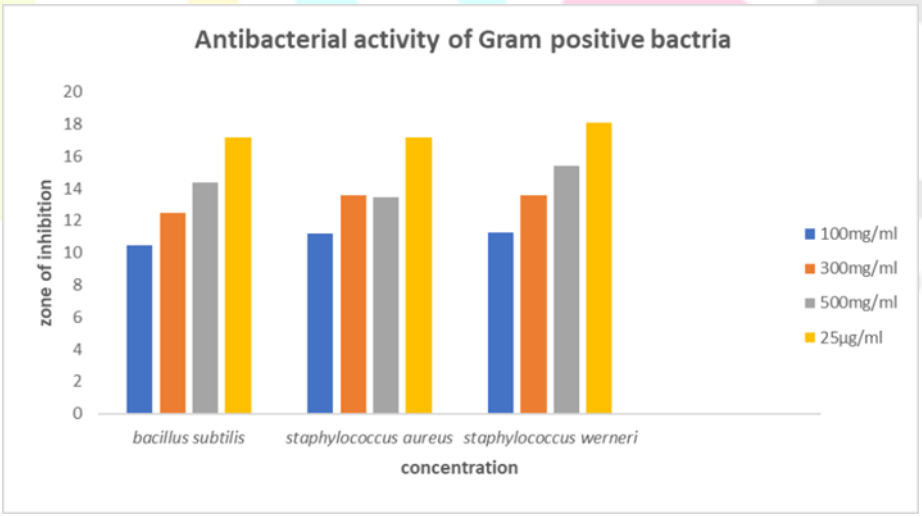

Figure 2. Result of antibacterial of gram positive bacteria

Values are expressed as mean±SEM; n=3

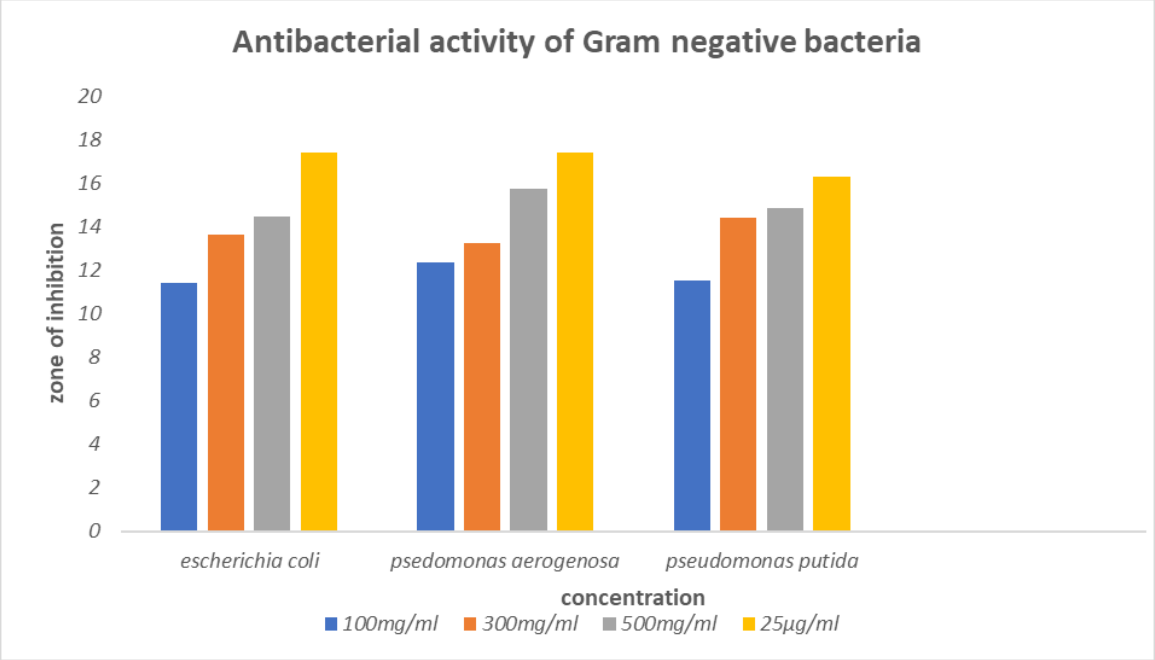

Figure 3. Result of antibacterial activity of gram negative bacteria

Values are expressed as mean±SEM; n=3

3.3. **Anthelmintic activity**

Type of extract	Dose in mg/ml	Time taken for paralysis (min)	Time taken for death (min)
Ethanolic extract	100	39.56	58.15
	200	26.56	47.23
	500	18.37	38.36
	1000	6.39	30.42
Standard albendazole	20	12.45	15.32
DMSO	═══	═══	═══

Table 4. Result of ethanolic extract of peperomia pellucida

Values are expressed as mean±SEM; n=3

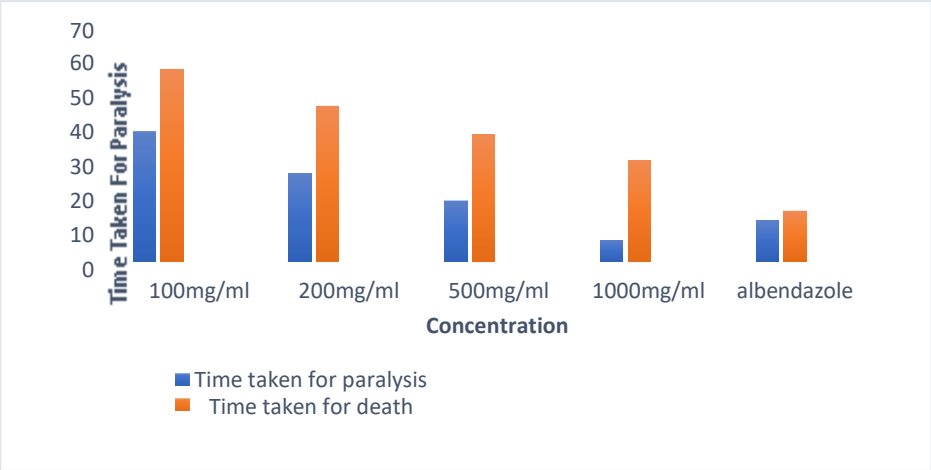

Figure 4. Result of after treated with ethanolic extract of *peperomia pellucida*

Values are expressed as mean±SEM; n=3

4. DISCUSSION

Peperomia pellucida is known for its varietyof pharmacological properties. Traditionally, it has been utilized in the folk medicine for medicine of several pantropical countries to treat a wide spectrum of ailments and diseases such as skin sores(Arrigoni-blankk et al., 2002), gastrointestinal sores(Mollik et al.,2010), dysentery, diarrhea, indigestion(Mollik et al.,2010), abscesses and injuries (Bojo et al.,1994). *Peperomia pellucida* essential oils and extracts are typically composed of phenylpropanoids followed by sesquiterpenes (De Diaz et al., 1998). However, the chemical composition can vary significantly depending on its place of origin[11]

The Piperaceae is a large family of angiosperms composed of around 3700species (Christenhusz and byng,2016)[12]. İt is among the oldest of the pantropical plants and its species are mainly distributed in two genera: piper with around 2000 species (Quijano-Abril et al.,2006) and peperomia with approximately 1600 (Frenzke et al., 2015)[13,14]. The group peperomia is one of the largest genera of basal angiosperms (Wanke et al.,2006) and is composed of herbs usually perennial (Shu,1999)[15,16], several peperomia species are cultivated as ornamentals because of the beauty of their foilage (Guimaraes and Carvalho-silva,2012)[17]. İt is an annual weed with a preference to humid places with reduced solar radiation and popularly employed in the treatment of variety of health conditions such as abscesses, abdominal pain, skin sores, conjunctivitis, measles and kidney troubles. Several studies have also described its antimicrobial, cytotoxic, antidiabetic and variety of other bioactivities.

Peperomia pellucida is an edible plant and is frequently used as a leafy vegetable or condiment in many parts of the tropics (Egwuche et al). Peperomia pellucida has high ash content (crude fiber content was higher and the carbohydrate content was highest) which reveals a high value mineral composition of manganese, potassium, calcium, and iron. *Peperomia pellucida* was rich in crude protein, carbohydrate, and protein, showing it has nutritional value [18].

İn this investigation, antibacterial activity of ethanolic extract of *peperomia pellucida* was investigated by using both gram positive and gram negative bacteria. The zone of inhibition against gram positive bacteria were found to be *staphylococcus aureus* (12.75mm), *staphylococcus westin* (14.04mm) and *Bacillus substillus* (12.45mm) which were shown in the figure 5.3. The zone of inhibition against gram positive bacteria were found to be *Escherichia coli* (13.20mm), *pseudomonas aerogenosa* (13.8mm) and *pseudomonas putida* (13.6mm) which were shown in the table 2 [19]. İn-vitro antibacterial activity was performed by cup plate method and concluded that it has good antibacterial activity against gram positive than gram negative bacteria. Prelminary phytochemical screening shows the presence of flavonoids, tannins and mild amount of alkaloids. Quantitative phytochemical screening gives the total phenolic contents present in the extract[20]. In-vitro anthelmintic activity was performed by parasitic motility test, the Anthelmintic activity was performed by parasitic motility test. The result of Anthelmintic activity was shown in Table 4[21]. Indian Earthworms (*Pheritima posthuma*) were used as test organisms. Time taken for the paralysis of worms and time taken for the death of the worms were reported in Table 4. Different concentrations such as 100mg/ml, 200mg/ml, 500mg/ml, and 1000mg/ml were taken. Albendazole 20mg/ml used as standard drug and the study proved that ethanolic extract of *peperomia pellucida* has mild anthelmintic activity [22].

5. CONCLUSION

The data obtained from this study has established the significant Antibacterial and Anthelmintic effects of *peperomia pellucida* aerial stem part extract. In further studies we can formulate a new methodology for the isolation and purification of the compounds present in aerial stem of peperomia pellucida which may act as a drug.

The presence of phytochemicals which may be responsible for these activities. Furthermore, clinical and pathological studies should be conducted to investigate the active potentials of bioactive compounds present in this study.

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