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EVALUATION OF CYTOTOXIC EFFECT OF TRICHOSANTHES DIOICA (LEAVES) COMPARED TO VINCRISTIN SULPHATE IN BANGLADESH

Md. Ariful Islam[1], Rashedul Hasan[1] and Md. Reyad-Ul-Ferdous[2,3,4*]

[1]Department of Pharmacy, Gono Bishwabidyalay, Savar, Dhaka-1344, Bangladesh.
[2]Department of Pharmacy, Progati Medical Institute, Dhaka-1216, Bangladesh.
[3]Department of Pharmacy, State University of Bangladesh, Dhaka-1207, Bangladesh.
[4]Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh.

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***Correspondence for Author**
Md. Reyad-Ul-Ferdous
Department of Pharmacy,
Progati Medical Institute,
Dhaka-1216, Bangladesh.

ABSTRACT

Trichosanthes, a genus of family Cucurbitaceae is an annual or perennial herb distributed in tropical Asia and Australia. Pointed gourd (Trichosanthes dioica Roxb.) is known by a common name of potol and cultivated mainly as a vegetable. Juice of leaves of *T. dioica* is used as tonic, febrifuge, in oedema, alopecia and in subacute cases of enlargement of liver. In Charaka Samhita leaves and fruits find mention for treating alcoholism and jaundice. A lot of pharmacological work has been scientifically carried out on various parts of *T. dioica* but some other traditionally important therapeutical uses are also remaining to proof till now scientifically. According to Ayurveda leaves of the plant are used as antipyretic, diuretic, cardiotonic, laxative, antiulcer, etc. It is also used in skin disorder by some communities of Asia traditionally. The various chemical constituents present in T. dioica are vitamin A, vitamin C, tannins, saponins, tetra and pentacyclic triterpenes etc. Cytotoxic activities of the alcohilic *T. dioica*. Leaves extracts of the root of *T. dioica* was subjected to Brine Shrimp lethality bioassay for possible cytotoxicity where, ethanol extract were found to be moderately cytotoxic showing LC_{50} of 26.89µg/ml while the LC_{50} of the reference anticancer drug vincristine sulphate was 0.98 µg/ml. Altogether, these result suggest that the ehtanolic extract could be usedas a potential antioxidant and anti-inflammatory agents.

KEYWORDS: Evaluation of Cytotoxic Effect, Trichosanthes Dioica, Leaves, Vincristin Sulphate. Bangladesh

1. BACKGROUND

Herbal medicine is the major stay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and few side effects. Bangladesh is sitting on a gold mine of well recorded and well practiced knowledge of traditional herbal medicine. In spite of tremendous development in the field of synthetic drugs during these days, they are found to have some side effects. Whereas herbal medicine still hold their own unique place, by showing no side effects (Sandhya, S. *et al*., 2010).

Rai *et al.* (2008) showed the glycemic attributes of an aqueous extract of *Trichosanthes dioica* leaves in normal as well as various diabetic models. The variable doses of 250, 500, and 750 mg kg- 1 body weight of the extract were administered orally to normal and streptozotocin (STZ) induced sub- and mild-diabetic rats in order to define its glycemic potential. This evidence clearly indicates that the aqueous extract of *Trichosanthes dioica*leaves has good hypoglycemic potential along with a high antidiabetic profile. **Rai *et al.* (2008)** showed that in rats with streptozotocin induced severe diabetes mellitus, aqueous extract of *T. dioica* fruits dose of 1000mg/kg body weight daily once for 28 days reduced the levels of fasting blood glucose, postprandial glucose, asparate amino transferase, alanine amino transferase, alkaline phosphatase, creatinine, urine sugar and urine protein where as total protein and body weight was increased. No toxic effect was observed during LD50. This study suggests that further detailed toxicity studies and mechanism of action of *T. dioica*would be useful for undertaking human trials. **Chandrasekar *et al.* (1988)** have reported that pointed gourd possesses the medicinal property of lowering blood sugar level in rats.

Ghaisas *et al.* (2008) showed hepatoprotective activity of aqueous and ethanolic extract of Trichosanthes dioica (whole plant) in ferrous sulphate-induced liver injury. Ethanolic and Aqueous extracts of *Trichosanthes dioica*at different doses (100, 200 and 400 mg/kg) and silymarin (100 mg/kg) were administered orally for 10 days. The groups treated with 400 mg/kg aqueous and ethanolic extract showed significant reduction in AST, ALT, ALP level. The pretreatment with *Trichosanthes dioica* extracts showed profound histopathological

protection to liver cells as evident from histopathological studies. Hence it can be concluded that *T. dioica*has significant hepatoprotective activity.

Sharmila *et al.* (2007) observed cholesterol lowering activity of the aqueous fruit extract of *Trichosanthes dioica* Roxb. in normal and streptozotocin diabetic rats. **Sharma & Pant *et al.* (1992)** showed influence of alcoholic extract of whole fruit of *T.dioica*on blood sugar, serum lipids, lipoproteins and faecal sterols in normal albino rabbits. Effect of oral administration of 2 ml per day of suspension (in water) of alcoholic extract of whole fruit of *Trichosanthes dioica* (2%) with basal diet for four weeks was studied in the normal albino rabbits. It was observed that this extract lowered the blood sugar, total cholesterol, low density lipoprotein cholesterol and triglyceride levels and increased the high density lipoprotein cholesterol, phospholipid and faecal sterol levels.

Fulzul *et al.* (2001) found anti-inflammatory activity of polyherbal formulation "*Jatyadi Ghrita*", the ingredients of *Jatyadi Ghrita* are *Jasmine officinale, Azadirachta indica, Berberisaristata, Curcuma longa, Picrorrhiza kurroa, Rubia cordifolia, Trichosanthes dioica, Aristolochia indica, Hemidesmus indicus, Randio spinosa, Glycyrrhiza glabra & Cow'sghee.*

Bhujbal (1999) showed that polyherbal formulation including *T. dioica*is useful in skin disorder. Fifty cases of various skin diseases were treated with decoction of a mixture of *Trichosanthes* & ohter herbal crude drugs in a dose of 20ml to 40 ml empty stomach with hot water & honey for 4 to 6 weeks. The drug was found to be useful and no side effect was observed.

Hariti & Rathee *et al.* (1996) stated that the fixed oil of seeds of *Trichosanthes species* including *T. dioica*have antifungal property. **Hariti & Rathee *et al.* (1995)** showed antibacterial activity of the unsaponifiable fraction of the fixed oil of *T. dioica*seeds against Bacilus anthracis & Xanthomonas malracearum **Rai *et al*. (2010)** reported the in vitro assessment of antimicrobial activity of different concentration of extract of different part of *Trichosanthes dioica*. Five clinical isolates of different bacterial strains were used and the disc diffusion method was opted. The results revealed that leaves fruits and seeds of *Trichosanthes dioica*plant may be used as antibacterial agents. Though the leaves extract was active against all five strains, the highest inhibition was observed against Mycobacteriumsmegmatis. Thus the leaves extract could be used for tuberculosis treatment

Extent of Antimicrobial activity of *Trichosanthes dioica* against certain pathogens Leaves extract: *M.smegmatis >S. aureus > E. coli >K. pneumonia & P. aeruginosa***; Fruits extract:** *S. aureus > K. pneumonia > E. coli, P. aeruginosa & M. smegmatis (Nil)***; Seeds extract:** *S. aureus > E. coli > K. pneumonia, P. aeruginosa & M. smegmatis (Nil)***; Streptomycin:** *E. coli &P. aeruginosa >S. aureus > K. pneumonia & M. smegmatis (Nil).*

Shivhare *et al.* (2010) evaluate the antioxidant activity of fruits of *Trichosanthes dioica* (Cucurbitaceae) and compared with ascorbic acid (Standard). Materials and Methods: Anti-oxidant activity of aqueous extract of *Trichosanthes dioica* (TSD) fruits was studied for its free radical scavenging property in different in vitro methods as 1, 1 diphenyl-2- picryl hydrazyl, nitric oxide, reducing power assay and hydrogen peroxide radical method. The findings could justify the inclusion of this plant in the management of antioxidant activity.

Shivhare *et al.* (2010) reported a scientific evaluation for the wound healing potential of methanolic (MeOH) extract of *T. dioica*fruits. **Shivhare *et al.* (2010)** studied methanolicextract of the plant *T. dioica*for assessment of healing potential in the form of simple ointment using full thickness burn wound model in rats. The effect produced by the extract ointment provides significant healing when compared with the control and standard groups. Despite, the various claims on *Trichosanthes dioica Roxb* medicinal uses, particularly its potential as analgesic, anti-inflammatory, no attempt has been made to our best knowledge, to scientifically confirm on this matter using alcoholic extract of leaves. Thus, the aim of the present study was to evaluate the analgesic, anti-inflammatory activity of alcoholic extract of *Trichosanthes dioica* leaves using various types of animal models.

2. MATERIALS AND METHODS

2.1 Plant collection and Extraction

The leaves of ***Trichosanthes dioica*** were collected in the month of March 2013 from Dhamrai area in Dhaka, Bangladesh. The collected materials were shed dried at 35º – 40ºC for a week and crushed into moderately coarse powder.

1 kg of ***Trichosanthes dioica***leaves powder was taken in soxhlet apparatus with 4000 ml 80% ethanol (250 gm powder with 1000 ml 80% ethanol in each time) and heated at 78^0c (bp of ethanol) on a heating mantle and procedure was carried out for 6 hours each time. Then the solvent washing the constituents of *Trichosanthes dioica* leaves powder was collected in a container.

2.2 Drying

The collected mixture of active constituents with ethanol was dried with a Rotary evaporator (EYELA Rotary Vacuum Evaporator, N-N Series with digital water bath, SB-100. Rikakikai Co. Ltd. Tokyo, Japan) under reduced pressure to get viscous substance. Then it was transferred to a beaker and taken on a water bathe for further drying at room temperature. Finally a solid mass was obtained and the crude ethanolic extract was dried by freeze drier and preserved in a Petridis in the refrigerator.

2.4 Cytotoxic effect evaluation of *Trichosanthes dioica*

2.4.1 Brine Shrimp Lethality Bioassay

Brine Shrimp lethality bioassay **(Luo*et al.*, 2000; Mclaughlin *et al.*, 1998; Meyer *et al.*, 1982)** is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. The method utilizes *in vivo* lethality in a simple zoological organism (Brine nauplii) as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. Brine toxicity is closely correlated with 9KB (human nasopharyngeal carcinoma) cytotoxicity (p=0.036 and kappa = 0.56). ED_{50} values for cytotoxicities are generally about one-tenth the LC_{50} values found in the Brine Shrimp test. Thus, it is possible to detect and then monitor the fractionation of cytotoxic, as well as 3PS (P_{388}) *(in vivo* murine leukaemia) active extracts using the Brine lethality bioassay **(Alkofahi *et al.*, 1988; Mclaughlin et al., 1998; Meyer *et al.*, 1982)**. The Brine Shrimp assay has advantages of being rapid (24 hours), inexpensive, and simple (e.g., no aseptic techniques are required). It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (2-20 mg or less). Furthermore it does not require animal serum as is needed for cytotoxicities **(Mclaughlin *et al.*, 1998).**

2.4.2 Preparation of seawater

38 gm sea salt (without iodine) was weighed, dissolved in one liter of distilled water and filtered off to get clear solution.

2.4.3 Hatching of Brine Shrimp

Artemia salina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The

hatched shrimps are attracted to the light (phototaxis) and so nauplii free from egg shell was collected from the illuminated part of the tank. The nauplii was taken from the fish tank by a pipette and diluted in fresh clear sea water to increase visibility and 10 nauplii were taken carefully by micropipette.

2.4.4 Preparation of test solutions with samples of experimental plants

32 mg of each of the test samples were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) and finally the volume was made to 20 ml with sea water. Thus the concentration of the stock solution was 1600µg/ml. Then the solution was serial diluted to 800, 400, 200, 100, 50, 25, 12.5, 6.25 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml of sea water containing 10 nauplii.

Concentration (µg/ml)	Extract Solution	Sea water containing 10 nauplii	Final volume
800	2.5 ml (1600µg/ml)	2.5 ml	5 ml
400	2.5 ml (800µg/ml)	2.5 ml	5 ml
200	2.5 ml (400µg/ml)	2.5 ml	5 ml
100	2.5 ml (200µg/ml)	2.5 ml	5 ml
50	2.5 ml (100µg/ml)	2.5 ml	5 ml
25	2.5 ml (50µg/ml)	2.5 ml	5 ml
12.5	2.5 ml (25µg/ml)	2.5 ml	5 ml
6.25	2.5 ml (12.5µg/ml)	2.5 ml	5 ml

2.4.5 Preparation of control group

Control groups were used in cytotoxicity study to validate the test method and ensure that the results obtained were only due to the activity of the test agent and the effects of the other possible factors were nullified. Two types of control groups were used

i) Positive control

ii) Negative control

2.4.6 Preparation of the positive control group

Positive control in a cytotoxicity study is a widely accepted cytotoxic agent and the result of the test agent is compared with the result obtained for the positive control. In the present study vincristine sulfate was used. As vincristine is a very cytotoxic alkaloid it was evaluated at very low concentration (10, 5, 1, 0.5, 0.25, 0.125 and 0.06 µg/ml).

2.4.7 Preparation of the negative control group

50 µl of DMSO was added to each of three premarked test tubes containing 4.95 ml of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

2.4.8 Counting of nauplii

After 24 hours, the test tube were inspected using a magnifying glass against a black background and the number of survived nauplii in each tube was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The mortality was corrected using Abott's formula **(Abott W. S., 1925)**

$P_t = [(P_o - P_c)/ (100 - P_c)] \times 100$

Where, P_o= Observed mortality.

P_c= Control mortality.

The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}). This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure time and determined by linear regression method from plotting % mortality against correspondent log of concentration.

2.5 Statistical Analysis

Microsoft Office Excel (2007) was used as a statistical tool for inflammation and analgesia inhibition assay data. Statistical analysis for animal experiments was carried out using Independent-Sample T Test using SPSS 20 for windows. Data were presented as Mean ± SEM. The results obtained were compared with the vehicle control group. *p* values < 0.05, < 0.01 and < 0.001 were considered to be statistically significant, highly significant and very highly significant respectively.

3. RESULTS AND DISCUSSION

A scientific evaluation of herbs according to their traditional methods of use in various diseases management can incorporate into the complementary and alter-native medicine (CAM) system elsewhere. The plant ***Trichosanthes dioica*** Roxb belongs to family Cucurbitaceae and commonly known as "Sespadula" in English and "parwal" in Hindi, is

widely grown throughout India **(Chakravarthy, 1982).** The various parts of the plant like leaves, tender shoots have also been used in traditional system of medicine **(Sharma, 1988; Sharma, 1989; Singh, 1989)**. The chemical constituent present in Trichosanthes dioica includes vitamin A, vitamin C, tannins and saponins **(Ghaisas 2008)** and flavonoids, alkaloids **(Shivhare, 2010).** Several pharmacological studies have been carried out in different parts of *Trichosanthes dioica* Roxb. Generally, the plant exhibited anthelminitic **(Bhattacharya, 2009),** antihyperglycaemic **(Rai, 2009),** antioxidant **(Shivhare, 2010)**, antidiabetic **(Rai, 2009) 20**, antipyretic **(Bhargava, 2008),** cholesterol-lowering **(Sharmila, 2007)**, hepatoprotective **(Ghaisas 2008)** and wound healing activitiy **(Shivhare, 2010).** Despite, the various claims on *Trichosanthes dioica* Roxb medicinal uses, no attempt has been made to our best knowledge, to scientifically confirm medicinal use of this plan. Therefore in present study we try to evaluate analgesic, anti-inflammatory, antioxidant and cytotoxic potential of *Trichosanthes dioica using combination of in-vivo and in vitro model.*

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release and extravasations of fluid, cell migration, tissue breakdown and repair. It is also known that anti-inflammatory effects may be elicited by a variety of chemical agents and that there is no remarkable correlation between their pharmacological activity and chemical structure. This fact, associated with the complexity of the inflammatory process, makes the use of different experimental models essential when conducting pharmacological trials.

5.3 Cytotoxic effect evaluation of Trichosanthes dioica

5.3.1: Brine Shrimp Lethality Bioassay for Cytotoxic Activity

All the extracts were also subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, Ethanol extract of *Trichosanthes dioica* was found to be the toxic to Brine Shrimp nauplii, with LC_{50} of 26.896 µg/ml whereas anticancer drug vincristine sulphate showed LC_{50} value 0.98 µg/ml. On the other hand, all the other extracts showed moderate to low toxicity **(Table 4.4.1)** The order at which cytotoxic potential of the test samples decreased was as follows: Vincristine sulphate> TD (*Trichosanthes dioica*).

The lethality of a test sample in a simple zoological organism such as the shrimp **(Artemia salina)** has been utilized by **Meyer *et al.* (1982)** in the Brine Shrimp Cytotoxicity Test (BSCT). It is a very useful tool to screen a wide range of chemical compounds for their various bioactivities. It has been well utilized to screen and fractionation of physiologically

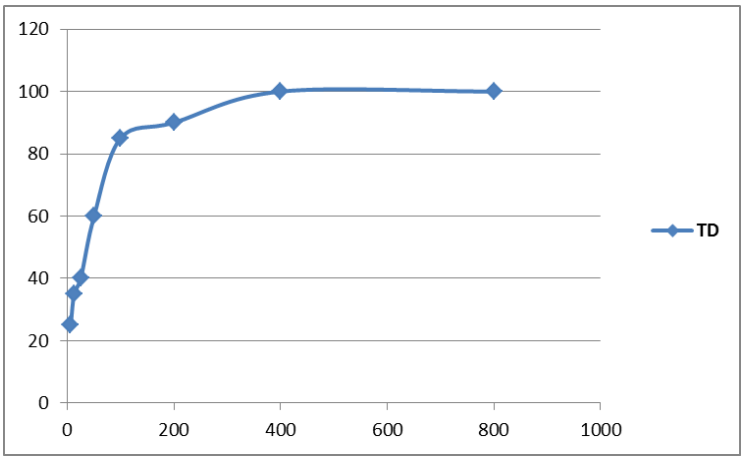
active plant extracts as well. It has been demonstrated that BSCT correlates reasonably well with cytotoxic and other biological properties **(Mclaughlin *et al.*, 1991).** The brine shrimp bioassay has been established as a safe, practical and economic method for determination of bioactivities of synthetic compound **(Almeida *et al.*, 2002)** as well as plant products **(Meyer*et al.*, 1982).** The significant correlation between the Brine shrimp assay and in vitro growth inhibition of human solid tumor cell lines demonstrated by the national Cancer Institute (NCI, USA) is significant because it shows the value of this bioassay as a pre-screening tool for antitumor drug research **(Anderson *et al.*, 1991).** In toxicity evaluation of plant extracts by Brine shrimp lethality bioassay LC_{50} values lower than 1000 µg/ml are considered bioactive **(Meyer *et al.*, 1982).** The Brine Shrimp Lethality Bioassay also indicates antifungal effects, pesticidal effects, teratogenic effects, toxicity to environment and many more **(Vanhaecke P. *et. al.*, 1981). (Table 4.4.1)** shows the lethality of extracts of *Trichosanthes dioica* to the Brine Shrimp nauplii. The degree of lethality shown by the extractives was foundto be directly proportional to the concentration of the extractives ranging from thelowest concentration (6.25 µg/ml) to the highest concentration (800 µg/ml). Thisconcentration dependent increment in percent mortality of Brine Shrimp naupliiproduced by the *Trichosanthes dioica* indicates the presence of cytotoxic principlesin these extractives. Preliminary phytochemical screening revealed the presence of alkaloids and steroids. So the observedcytotoxic action may be due to the presence of such compounds. Again, reports exist onthe role of alkaloids and steroids in cytotoxic activity of plant extracts **(Dhar *etal.*, 1973; Vijayan et al., 2004; Badami *et al.*, 2003).** However, phenolics and flavonoids arealso known to show cytotoxicity in Hoechst 33258 fluorescence assay by inhibitingcellular DNA in a concentration-dependent manner **(Chang *et al.*, 2002).**

4.4 Cytotoxic effect evaluation of *Trichosanthes Dioica*

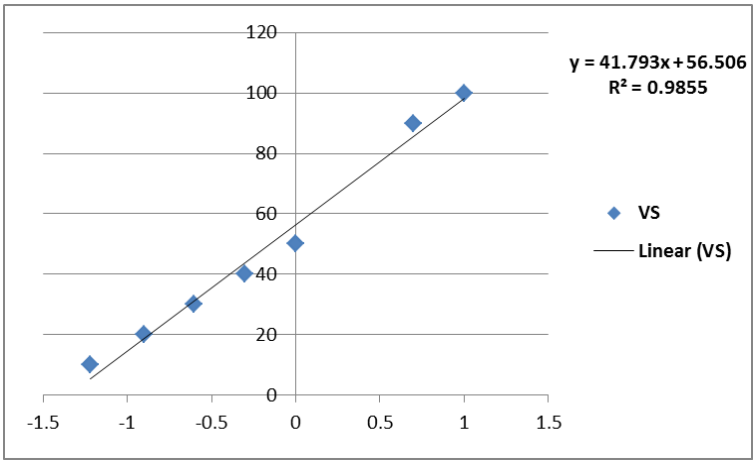
4.4.1 Brine Shrimp Lethality Bioassay

Concentration (µg/ml)	Log Concentration	%Mortality	Corrected %Mortality	LC_{50}	LC_{90}
6.25	0.796	25±7.07107	25±7.07107	26.896	207.488
12.5	1.097	35±7.07107	35±7.07107		
25	1.398	40±0.00	40±0.00		
50	1.699	60±14.1421	60±14.1421		
100	2.000	85±7.07107	85±7.07107		
200	2.301	90±0.00	90±0.00		
400	2.602	100±0.00	100±0.00		
800	2.903	100±0.00	100±0.00		

Concentration (µg/ml) Vincristin Sulfate	Log Concentration	%Mortality	Corrected %Mortality	LC_{50}	LC_{90}
0.06	-1.2218	10±0.00	0±0.00	0.98±0.08	7.27±0.82
0.125	-0.90309	20±0.00	11.11±0.00		
0.25	-0.60206	26.67±4.71	18.52±5.24		
0.5	-0.30103	40±0.00	33.33±0.00		
1	0.00	50±0.00	44.44±0.00		
5	0.6989	86.67±4.71	85.19±5.24		
10	1.00	100±0.00	100±0.00		



******TD Means *Tricosanthes dioica.***



*******VS Means Vincristin sulfat.**

CONCLUSION

The results obtained in this study indicate that *Trichosanthes dioica Roxb.* (Family: Cucurbitaceae),. Possesses analgesic and anti-inflammatory properties, which are mediated via peripheral and central inhibitory mechanisms. This could provide a rationale for the use of this plant in pain and inflammatory disorders in folk medicine. From the study of anti oxidant activity by different methods like DPPH, reducing power assay methods and total

antioxidant capacity test it can be asserted that the investigated plant materials are a viable source of natural antioxidants. The antioxidant activity of the methanolic extracts of root of *Trichosanthes dioica* might be due to the presence of phyto constituents like flavonoids and phenolics compounds. Further investigations are anticipated to identify the active components and lead to their further clinical use.

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