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ANTI-URIOLITHIATIC, ANTI-OXIDANT AND ANTI-PANIC ACTIVITIES OF *CUCUMIS SATIVUS LEAVES*

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ABSTRACT

The *Cucumis sativus* fruit (CSLJ) was subjected to hydrogen peroxide radical scavenging assay in reference to butylated hydroxytoluene. This study was carried out to evaluate the anti-inflammatory and antioxidant activities of Cucumis sativus Linn. (Family: Cucurbitaceae) leaves. The methanolic extract of *Cucumis sativus* leaves (MCS) were investigated for anti-inflammatory activities at different doses of 150 and 250mg/kg body weight and the effects were compared with the standard, indomethacin (10mg/kg body weight). It was obtained by exhibited highest anti-inflammatory activity at the dose 250mg/kg. The extract doses at 150 and 250mg/kg body weight) significantly prevented the increase in volume of paw edema.

KEYWORDS

Cucumis Sativus leaves, Anti-oxidant and Anti-panic.

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INTRODUCTION

Cucumber

Cucumber (*Cucumis sativus*) are widely cultivated plant in the gourd, family. The cucumber is originally from South Asia, but now grows on most continents, it is found widely in the Himalayan region and also cultivated throughout India. Traditionally, It shows Anti-diarrhoeal, Anti-urolithiatic, Anti- inflammatory, Anti- hypertensive, proteolytic, Anti-fungal also Anti-oxidant and Anti-Panic activities[1,2]. And also this plant is used for headache, the seeds is cooling and diuretic.

Description

Annual, trailing or climbing herb. Stem angular, with scabridulous hairs. Leaves ovate, 14-19cm long, 2-9-lobed,-lobes acute, hispidulous or scabridulous on both surfaces; petiole elongated, 8-22mm long. Fruit oblong and obscurely trigonous or cylindric, when young sparsely tuberculated, otherwise smooth and glabro.

Medicinal action and uses

Cucumber *seeds* possess similar properties to those of the allied Pumpkin (*Cucurbita Pepo*, Linn.) which are distinctly diuretic, but mainly employed as a very efficient taeniacide[3].

MATERIAL AND METHODS

The plant

Cucumis sativus Leaves is a widely available plant, grown throughout the World, belonging to the family "Cucurbitaceae". The plant is unbranched tendrils up to 30cm long Leaves are alternate and simple, with 3-7 palmate lobes and serrated margins, Yellow serous flowers bearing either female or male organs Hairless cylindrical fruits are warty, yellow to green, and up to 50cm long.

Collection and processing of leaves

Identification and authentication

The collected plant part (leaves) of *Moringa oleifera. L* were identified and authenticated by Dr. K. Madhava Chetty, Botanist, Department of Botany, Sri Venkateswara University, Tirupati and a specimen (voucher no. 0935) was deposited in the herbarium for further reference.

Processing of leaves

The leaves collected were dried under shade and made in to coarse powder by using hand operated mill. The leaf powder was used for the extraction process and other evaluation methods.

Extraction Solid /liquid extraction

The extraction of soluble constituents from a solid by means of a solvent is commonly referred to as leaching.

Procedure

- The source material (*Cucumis sativus* leaf powder) to be extracted is placed in the thimble.
- The main chamber of soxhelet extractor.
- The extraction solvent, ethanol is placed in the distillation flask.
- The flask is placed on the heating mantel.
- The soxhelet extractor is placed at the top of the flask.
- The reflux condenser is placed at the top of extractor.
- The mantel heatens the flask containing ethanol, this gets evaporates, moves to condenser, get cool down and fall on the thimble, the crude drug get dissolved in ethanol and falls into flask.
- This process is continued until total amount of crude drug is get extracted[4].

Phytochemical Screening

Preliminary phytochemical screening[5,6]

Phytochemical screening of ethanolic extract of *Cucumis sativus leaf* was done by the following standard procedure. The ethanolic extract was concentrated under reduced pressure to 1/3 of the volume and screened for its various chemical constituents.

Anti-urolithiatic activity

Spectrophotometry estimation of calcium phosphate by using dissolution model

Chemicals used

Cacl2dihydrate, Na oxalate, p-phenylene, diamine Potassium permanganate, sodium meta-bisulfite, tris-buffer and Cystone tablets.

Preparation of the semi-permeable membrane from eggs

Apex of eggs were punctured by a glass rod in order to squeeze out the entire content. Empty eggs were washed thoroughly with distilled water and placed in a beaker consisting 4ml concentrated HCl in 200ml distilled water. The semi permeable membranes was removed carefully from egg shells; washed thoroughly with distilled water and placed it in ammonia solution for neutralization of acid traces, and then rinsed it with distilled water. It was stored in refrigerator at a pH of 7-7.4 in the moistened condition.

Preparation of calcium phosphate crystals

1.47gm of calcium chloride dihydrate was dissolved in 100ml distilled water and 1.42gm of disodium hydrogen phosphate was dissolved in 100ml of 2N H_2SO_4. The resultant calcium phosphate was freed from traces of sulfuric acid by ammonia solution; washed with distilled water and dried at a temperature 60°C for 2 hours.

Preparation of molybdate-sulphuric acid reagent

Molybdate-sulphuric acid reagent was prepared by 5% w/v of sodium molybdate solution, 13ml of conc. H_2SO_4 in 80ml of distilled water. Finally, volume was adjusted to 100ml with distilled water.

Preparation of reducing solution

1gm of p-phenylene diamine was dissolved in 100ml of 3%w/v of sodium meta- bisulfite solution.

Method

Group I: 1ml of calcium phosphate (1mg/ml) + 1ml of distilled water

Group II: 1ml of calcium phosphate (1mg/ml) + 1ml of Cystone solution (400mg/ml)

Group III: 1ml of calcium phosphate (1mg/ml) + 1ml of extract of *Cucumis sativus* leaves (20mg/ml)

All groups were packed it together in egg semi permeable membrane tied with thread at one end and were suspended in a conical flask containing 150ml 0.1M Tris buffer each. At another end of thread tied by a stick placed on the mouth of conical flask and covered with aluminum foil. Then add 4ml of 1N H_2SO_4 and 3ml of molybdate-sulphuric acid reagent, 1ml of reducing solution were added and kept aside for 2 hours. Colour change from dark pinksh to colourless were observed after 2 hours. Change of colour intensity was measured against 620nm spectrophotometrically. Concentration of undissolved calcium was determined from standard calibration curve of calcium phosphate by using the measured absorbance readings.

Nucleation assay (Turbidity method)

The inhibitory activity of the extracts on the nucleation of calcium oxalate crystals was determined by a spectrophotometric assay12. Crystallization was initiated by adding 100µl of 4M calcium chloride and 100µl of 0.5M sodium oxalate solutions to 0.5ml of human normal urine, both prepared in a buffer containing 0.5ml of 0.05M Tris buffer and 0.5ml of 0.15M NaCl solution at pH 6.5 and 37°C and adjusted to volume by adding 1.5 ml of distilled water.The optical density (OD) was recorded at 620nm and the percentage inhibition calculated as (1-OD (experimental)/OD (control))/100.

Anti-oxidant activity hydrogen peroxide scavenging method

Among many methods employed for evaluating antioxidant activity of phytoconstituents, hydrogen peroxide scavenging method was followed.

Principle

The hydrogen peroxide present in various endothelial cells which may damage the cells or tissues due to hydrogen peroxide undergoes homolytic cleavage to generate free radical whichis a toxic intermediate and act as a oxidative reagent. The principle involved in the present in- vitro model is perfect. Hence, intracellular conditions have been maintained. Here the reference standard as well as ethanolic extract of *cucumis sativus* was evaluated for their hydrogen peroxide scavenging property.

Preparation of reagents

Phosphate buffer pH 7.5

Dissolve 6.8gm of potassium dihydrogen orthophosphate and 1.56gm of NAOH in 900ml of water. Adjust the pH 7.5 with NaoH solution and dilute with water to produce 1000ml.

Preparation of 0.002% of H_2O_2

Take 0.4ml hydrogen peroxide in 1000ml of water from that 1ml of hydrogen peroxide added with 1000ml of water from that take 3.2ml and dissolves in 100ml distilled water.

Methods

Hydrogen peroxide scavenging activity was measured by the modified method. The reaction mixture containing 100µl fraction of different concentration, 100µl of 0.0002% and Dissolve 6.8gm of potassium dihydrogen orthophosphate and 1.56gm of NAOH in 900mlof water. Adjust the pH

7.5 with NaoH solution and dilute with water to produce 1000ml.

Preparation of 0.002% of H_2O_2

Take 0.4ml hydrogen peroxide in 1000ml of water from that 1ml of hydrogen peroxide added with 1000ml of water from that take 3.2ml and dissolves in 100ml distilled water.

Methods

Hydrogen peroxide scavenging activity was measured by the modified method. The reaction mixture containing 100µl fraction of different concentration, 100 µl of 0.0002 % and 0.8ml of phosphate buffer were pre-incubated for 10 min at 37 degree centigrade ,to these reaction mixtures, 1ml of phenol red dye containing horseradish peroxidise solution was added. After 15 min 50µl of 1M NaoH was added and absorbence was measured at 766nm immediately. A control was prepared using 0.1ml of respective vehicle in the place of phenol red crude fraction/ascorbic acid. The % inhibition of peroxide scavenging activity was measured by comparing the absorbence value of the control.

% inhibition of H_2O_2= Absorbence of control-absorbence of test/absorbence of control x 100.

Anti-panic activityelevated plus maze method

Elevated plus maze (EPM) is one of the commonly used animal model for testing anti-anxiety activity. Some drugs was employed to assess anxiolytic activity of sample under study.

EPM is based on the apparent natural a version of rodents to open and high spaces animals have tendency to spend more time in enclosed arms than in open arms.

Chemicals and apparatus used

Diazepam
Test sample (extract of *Cucumis sativus)*
2% acacia suspension
Elevated plus maze apparatus
Beaker
Measuring cylinder
Weighing balance
Syringes
Droppers and pipettes

Procedure

Albino mice of either sex weighing between 18-25gm were divided in to four experimentalgroups of four animals.

Group I - Control (2% gum acacia)
Group II - Standard (Diazepam 2mg/kg)
Group III - Ethanolic extract of *Cucumis sativus* EECS1 (200mg/kg p.o)
Group IV -Ethanolic extract of *Cucumis sativus* EECS2 (400mg/kg p.o)

Standard drug diazepam was administered 45 min prior to testing and extracts were administered p.o 45 min prior to testing. Anxiolytic activity was measured using the elevated plus maze test. The maze consisted of two open (28cm × 5cm) and two closed (28cm × 5cm ×14cm) arms, extending from the central platform (5cm ×5cm) and elevated up to the height of 40 cm above the floor. The entire maze was made of clear Plexi glass. Mice were individually placed on the centre of the maze facing an open arm, and the number of entries and the time spend in closed and open arm were recorded during a 5 min observation period. Arm entries were defined as entry of four paws in to the arm. The percentage of open arm entries (100× open/total entries) was calculated for each animal.

RESULTS AND DISCUSSION

Extraction

The coarse powder of leaves of *Cucumis sativus* was extracted by soxhelet extraction method by using ethanol as solvent. The extract obtained was green in colour, soft in nature and shows 20.5% w/w of extractive value.

Phytochemical screening

The alcoholic extract of *Cucumis sativus* was screened for its various phytoconstituents by standard chemical tests. It was found to contain carbohydrates, glycosides, alkaloids, tannins, steroids, saponins, volatile oils, amino acids, etc.

Anti-urolithiatic activity

The alcoholic extract of *Cucumis sativus* was screened by spectrophotometric estimation of calcium phosphate by using dissolution model at 620nm. The dissolution percentage of test was

60.57% and reference standard cystone was 47.42%. Therefore the alcoholic extract of *Cucumis sativus* was shown potent Anti urolithiatic activity when compared to standard cystone tablets.

Anti-oxidant activity

The alcoholic extract of *Cucumis sativus* was screened by using Hydrogen per oxide scavenging method for various concentrations. At a concentration of 125µg/ml of alcoholic extract was found to be 94.28% and reference standard ascorbic acid at the same concentration was found to be 85.71%, therefore the Alcoholic extract of *Cucumis sativus* was shown potent Anti-oxidant activity when compared to the standard ascorbic acid.

Anti-panic activity

The alcoholic extract of *Cucumis sativus* leaf was screened by Elevated plus maze method, Diazepam was used as standard. The sample of alcoholic extract of *Cucumis sativus* gives a better results when compared with standard Diazepum, by this we confoirms that the ethanolic extract of *Cucumis sativus* is effective aganist anxiolytic activity when compared with standard Diazepum.

Table No.1: Phytochemical screening report of ethanolic extract of *Cucumis* leaf

S.No	Plant constituents	Inference
1	Test for alkaloids	+
2	Test for tannins	+
3	Test for glycosides	+
4	Test for steroids	+
5	Test for volatile oils	+
6	Test for amino acids	+
7	Test for carbohydrates	+
8	Test for phenols	+
9	Test for saponins	+
10	Test for flavonoids	+
11	Test for resins	-
12	Test for fixed oils	-

Presence --- (+)

Absence --- (-)

Table No.2: Anti-urolithiatic activity of ethanolic extract of *Cucumis* leaf

Group	Mean ± SD	Weight of calciumreduced	Dissolution percentage
Group - 1 [control]	0.175	--	--
Group - II [Standard]	0.092	0.083	47.42
Group - III [Test]	0.069	0.106	60.57

Table No.3: Anti-oxidant activity of ethanolic extract of *Cucumis* leaf by H2O2 method

S.No	Group	Conc µg/ml	Absorbence (766nm)	%inhibition
1	Control		0.079	
2	Test	25	0.094	47.14
3	Test	50	0.097	72.85
4	Test	75	0.09	78.57
5	Test	100	0.086	87.14
6	Test	125	0.068	94.28
7	Standard	25	0.079	27.24
8	Standard	50	0.088	34.28
9	Standard	75	0.099	55.71
10	Standard	100	0.067	67.14
11	Standard	125	0.01	85.71

Table No.4: Anti panic effect of ethanolic extracts of *Cucumis sativus* leaves on mice by elevated plus maze method

S.No	Treatment	% open arm time	% closed arm time	% open arm entries
1	Control	52.6±3.04	216±3.94	4.03±3.02
2	Diazepam (2mg/kg)[standard]	145.3±0.52	52±1.571	8.8±3.04
3	ECS1 (200mg/kg) [test-1]	113.6±3.853	76±1.22	6.9±3.76
4	EECS2 (400mg/kg) [test-2]	134±4.587	54±3.040	7.1±3.16

Figure No.1: *Cucumis sativus* plant

Figure No.2: Soxhlet extractor

Figure No.3: Soxhlet apparatus

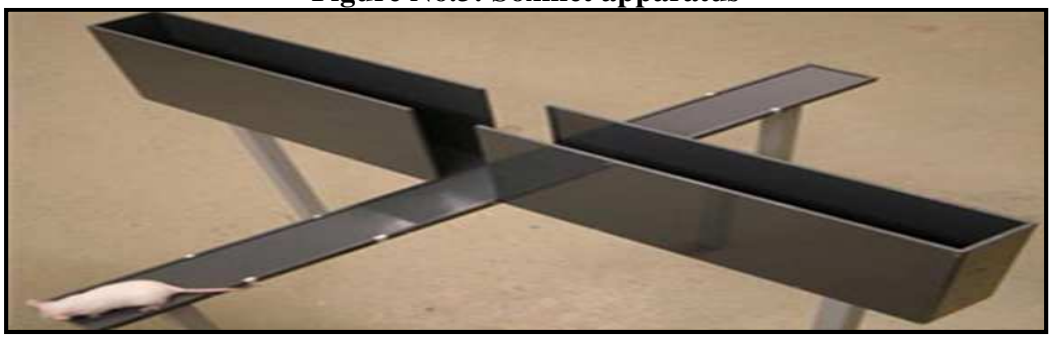

Figure No.4: Elevated plus maze apparatus

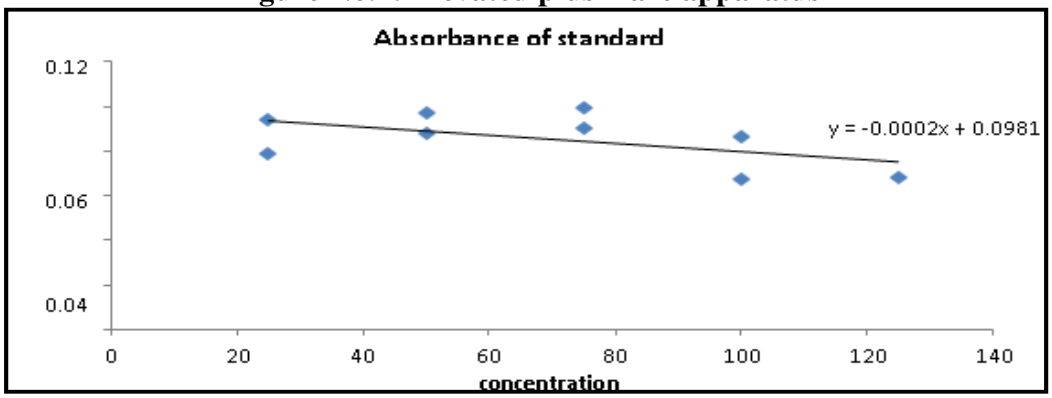

Figure No.5: Anti-oxidant activity of ethanolic extract of *Cucumis* leaf by H_2O_2 method

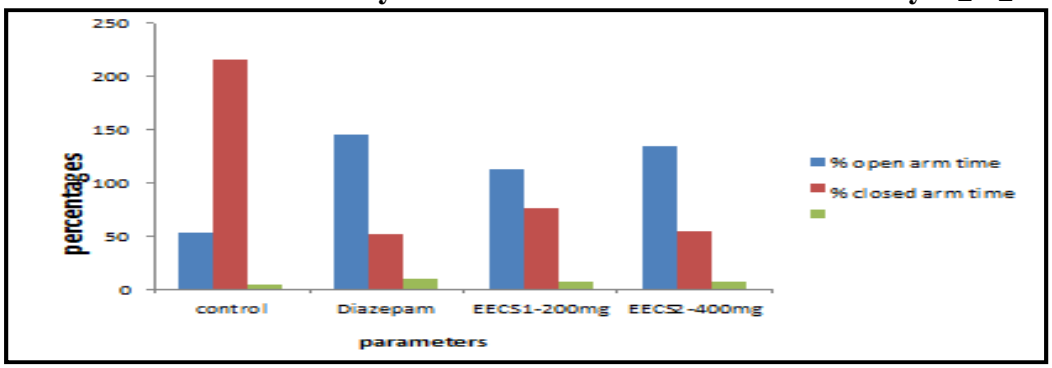

Figure No.6: Anti-panic effect of ethanolic extracts of *Cucumis sativus* leaves on mice by elevatedplus maze method

CONCLUSION

According to the literature review, traditionally the leaves of *Cucumis sativus* was used to treat kidney stones, also reduces anxiety. So we thought of proving the activity by the scientific approach. As per the traditional belief, we collected the leaves, identified by local flora, dried under shade, powdered and extracted by using ethanol. The ethanolic extract of leaves of *Cucumis sativus* was screened for its anti urolithiatic activity to reduce the kidney stones, by using cystone as reference standard. The ethanolic extract of leaves of *Cucumis sativus* shows significant anti urolithiatic activity aganist calcium phosphate crystals. Also by the phytochemical study we found to contain the presence of flavanoids, hence we made an attempt to study its anti-oxidant activity by using hydrogen peroxide scavenging method. The ethanolic extract showed significant anti-oxidant activity aganist the standard ascorbic acid. And finally we made an attempt to conduct experiment aganist the anti-panic activity by using elevated plus maze method. The ethanolic extract of leaves of *Cucumis sativa* shows positive results aganist antipanic activity. Hence the project work carried out by us under the scientific manner proved its traditional claim.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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