



Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com



BIOLOGICAL EVALUATION OF *CUCUMIS SATIVUS* LEAF EXTRACT USING ALBINO MICE

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ABSTRACT

Cucumis sativus (Cucumber) is a widely cultivated plant (Family: Cucurbitaceae). It is a creeping vine that bears cucumiform, fruits that are used as vegetables. Traditionally, *Cucumis sativus* possesses anti-diarrhoeal, anti-urolithiatic, anti-inflammatory, anti-hypertensive, proteolytic, anti-fungal, antioxidant and anti panic activities. The leaves of this plant is used for headache, seeds as diuretic, the fruit juice of this plant is used as nutritive and as a demulcent in anti-acne lotions. In the present study the ethanolic leaf extract of *Cucumis sativus* is screened for its phytochemical and biological activity (anti-panic activity) as per the traditional value by scientific manner. The results concluded that the ethanolic leaf extract of *Cucumis sativus* possessed significant anti-panicactivity when compared with the standard diazepam.

KEYWORDS

Cucumis sativus, Urolithiatic, panic, Phytoconstituents and Diazepam.

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INTRODUCTION

Depressive anxiety is one of the most common emotional disorders and treatment of phobias or panic attacks is still not trivial. Pharmacological therapies play an important role in the therapeutic concept¹. Benzodiazepines have been the most widely used anxiolytics in various practices for many year and are relatively safe drugs for a shorten treatment of anxiety disorder despite their drug dependence potential and side effects. Diazepam and Buspirone are standard anxiolytic and also employed in behavioral pharmacology as the reference compound for an anxiolytic like effect

even if the screened drug is not acting via benzodiazepine receptors².

In the conventional form of the test, anxiety is generally assessed by measures of open arm avoidance while locomotor activity is most reliably measured by the frequency of closed arm entries. The elevated plus maze, with two adjacent closed arms perpendicular to two open arms, has been validated by use of pharmacological and behavioral manipulations in rodents. Time spent in open areas is frequently reported as spent time of treated mice in open arm compared to control mice, time spent in the closed and open areas, there by significantly high in the central region from anxiety analysis³.

AIM AND OBJECTIVE

Aim

The present study was taken up for performing the antipanic activity of *Cucumis sativus*, leaf.

Objective

The main object of the study is,

1. To screen the phytochemical and antipanic activity of leaves of *Cucumis sativus*.
2. To establish pharmacological profile of the flowers of *Cucumis sativus*.
3. To evaluate the antipanic activity in mice by Elevated plus maze model.

Plant Profile

Cucumber (*Cucumis sativus*) is a widely cultivated plant in the gourd, family Cucurbitaceae. It is a creeping vine that bears cucumiform, fruits that are used as vegetables. There are three main varieties of cucumber. *slicing*, *pickling* and *seedless*. Within these varieties, several cultivars have been created¹. In North America, the term "wild cucumber" refers to plants in the genera *Echinocystis* and *Marah*, but these are not closely related. 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. Many different types of cucumber are traded on the global market. Traditionally, It shows Anti-diarrhoeal, Anti-urolithiatic, Anti-inflammatory, Anti-hypertensive, proteolytic, Anti-fungal also Antioxidant and Anti

Panic activities². And also this plant is used for headache, the seeds are used for cooling and diuretic, the fruit juice of this plant is used as nutritive and as a demulcent in anti-acne lotions⁴⁻⁶.

Scientific classification

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Rosids
Order : Cucurbitales
Family : Cucurbitaceae
Genus : *Cucumis*
Species : *C. sativus*
Binomial name : *Cucumis sativus* L^{10,11}.

Common Names

Cucumber, garden cucumber, apple cucumber, gherkin, *concombre*, *cornichon* (French), *pepino* (Spanish and Portuguese), *huang gu* (pinyin, China), *khira* (Pakistan)⁷⁻⁹.

MATERIAL AND METHODS

Collection and processing of leaves

The leaves of plant *Cucumis sativus* was used for the present study were primarily collected from the Petlurivaripalem fields, Narasaraopet, Guntur (Dist). 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.

Preparation of extract

The extracts obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents. Alcohol is an ideal solvent for extraction of various chemicals like tannins, resins, etc. Extractive values of crude drug are useful for their evaluation, especially when the constituents of the drug cannot be readily estimated by any other means. Further these values indicate the nature of constituents present in the crude drug¹².

Phytochemical screening

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 constituents¹³⁻¹⁵.

Test for Alkaloidal Bases

5 ml of extract was taken and evaporated to give a residue. The resultant residue was dissolved in 5 ml of 2 % of HCL. To 2 ml of the resulting solution, 2 to 3 drops of Mayer's reagent was added and appearance of whitish yellow precipitate indicates the presence of alkaloidal bases.

Test for Tannins

The extract was taken in to a test tube and then 2 ml of water was added, mixed thoroughly and filtered. The filtrate was then subjected to the following tests.

Ferric chloride test

To 1 ml of filtrate, 5 drops of ferric chloride was added, and observed blackish precipitate, which indicates the presence of tannins.

Gelatin test

To 1 ml of filtrate, 0.5 ml of pyridine and few drops of sodium nitroprusside solution was added and white precipitate was observed due to presence of tannins.

Iodine test

To 1 ml of filtrate added few drops of iodine solution and observed red color, this indicates the presence of tannins.

Test for Glycosides

The extract was hydrolysed with 5 ml of conc. HCL and boiled for 2-3 hrs on water bath. The resultant hydrolysates were subjected to the following tests.

Legal's test

To 1 ml of hydrolysate, 1 ml of pyridine and few drops of sodium nitroprusside solution was added and made alkaline with sodium hydroxide solution and observed dark color which indicates the presence of glycosides.

Borntragger's test

To 1 ml of sample solution, chloroform was added and chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added and observed no color change.

Test for carbohydrates

The extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests.

Molisch test

2-3 ml of filtrate was taken, to that few drops of alpha naphthol solution was added, shaken and add conc. sulphuric acid through the sides of the test tube, violet ring was formed at the junction of two liquids, this indicates the presence of carbohydrates.

Barfoed test

Few ml of filtrate was added with 2-3 ml of Barfoed's reagent, heated for 1-2 min in boiling water bath and cooled. Red precipitate was observed, this indicates the presence of carbohydrates.

Test for Steroids: Salkowski reaction

2 ml of extract was added with 2 ml of chloroform and 2 ml of sulphuric acid. Shake well. Chloroform layer appears red and acid layer appears greenish yellow fluorescence, indicates the presence of steroids.

Test for Volatile oils

The extract was taken in a test tube and added with few ml of 90 % alcohol and mixed well, until the extract was completely soluble in alcohol. This indicates the presence of volatile oils.

Test for Flavanoids: Shinoda test

The extract was taken and added with 5 ml of 95 % ethanol, few drops of conc. HCL and 0.5gms of magnesium-turnings. Pink color is observed, indicates the presence of flavanoids^{16,17}.

Test for Amino acids: Ninhydrine test

Few ml of extract was taken and added with 3 drops of 5 % ninhydrine solution in boiling water bath and heated for 10 min, purplish blue color was appeared indicates the presence of amino acids.

Test for Saponins

To the extract, 20 ml of water was added and agitated in the graduated cylinder for 15 min and observed the formation of foam, which is an indication for the presence of saponins.

Test for phenols

The extract was taken in to a test tube and dissolved in alcohol and heated with 3 ml of neutral ferric chloride solution, blue black color was observed, which indicates the presence of phenols¹²⁻¹⁵.

Animals

Albino mice of either sex weighing between 20-25 gm were used for this study. The animals were kept in solid-bottomed polypropylene cages and acclimatized for laboratory conditions. The mice were fed with standard pellet diet and water ad libitum. All the experimental protocols were prior approved Institutional Animal Ethical Committee (IAEC)

Drug Treatment

Albino mice's of either sex were divided into four groups and each group contains four animals.

Group I - Control (2 % gum acacia)

Group II - Standard (Diazepam 2mg/kg)

Group III - Test (low dose) - Ethanolic extract of *Cucumis sativus* EECS1 (200mg/kg p.o)

Group IV - Test (high dose) - Ethanolic extract of *Cucumis sativus* EECS2 (400mg/kg p.o)

Acute toxicity studies

Acute toxicity test was performed in mice. All animals were fasted overnight before treatment and were given food 1h after EECS treatment. A single high dose (400 mg/kg), as per OECD guidelines was administered orally to mice. General behavior was also observed at 1, 3 and 24 h after administration. The number of animals that died after administration was recorded daily for 14days.

Determination of Antipanic activity

Elevated plus maze

The plus-maze consists of two open arms, two enclosed arms, 50x10x40cm, with an open roof, arranged so that the two open arms are opposite to each other. The maze is elevated to a height of 50cm¹⁸. 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^{19,20}.

RESULTS AND DISCUSSION

Extraction

The coarse powder of leaves of *Cucumis sativus* was extracted by soxhlet extraction method 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 and the results were described in Table No.1.

Anti panic activity

The alcoholic extract of *Cucumis sativus* leaf was screened by Elevated plus maze method, using Diazepam as standard. The percentage of open arm entries (100× open/total entries) was calculated for each animal. From this study it was shown that the sample of alcoholic extract of *Cucumis sativus* had given a better result when compared with standard Diazepam. By this it can be confirmed that the ethanolic extract of *Cucumis sativus* is effective against antipanic activity when compared with standard Diazepam and the results were described in Table No.2.

Table No.1: Phyto Chemical Screening Report of Ethanolic Extract of *Cucumis sativus* 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 flavanoids	+
11	Test for resins	-
12	Test for fixed oils	-

Presence --- (+)

Absence --- (-)

Table No.2: 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	EECS1 (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: *Cucumis Sativus* Leaf



Figure No.3: *C. Sativus* Leaf Powder



Figure No.4: Soxhlet Apparatus



Figure No.5: Elevated Plus Maze

CONCLUSION

The phytochemical study was proved that the extract contains alkaloids, glycosides, carbohydrates, tannins, steroids, volatile oils, amino acids, phenols, saponins, flavanoids and showed the positive result against antipanic activity. In pharmacological screening method, the *Cucumis sativus* leaf extraction when administered in mice had shown the effective response when compared with the standard drug Diazepam using elevated plus maze. Hence the scientific manner proved its traditional claim.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Supriya J et al. Biological evaluation of *cucumis sativus* leaf extract using albino mice, *Asian Journal of Phytomedicine and Clinical Research*, 6(2), 2018, 55-61.