Original Research

Preliminary phytochemical analysis and cytotoxic potential of Cucumis trigonus Roxb

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Abstract
Aim: The present study involves in the investigation of various extracts of Cucumis trigonus Roxb. for its phytochemical constituents and also the cytotoxic potential of the Cucumis trigonus Roxb. ethanolic fruit extract.

Methods: The phytochemical screening with methanol, ethanol, petroleum ether, chloroform and aqueous extracts of Cucumis trigonus Roxb. was done by modern method of Peach and Tracey (1955) Cytotoxicity is estimated by staining technique using Trypan blue after the addition of drug, the dead cells are stained blue with Trypan blue. Confirmation studies were done by additional metabolic intervention experiments like (3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) MTT assays.

Results: Qualitative phytochemical analysis of the plant extracts were done and it was found that the presence of phytochemicals were maximum in the ethanolic extract when compared to other extracts. So the ethanolic extract of C. trigonus fruit was used for further investigations.

In the cytotoxic study the ethanolic fruit extract concentration at 1.25 mg / ml was found to be the effective dose because at this concentration, it exhibited 50% cytotoxicity against Hep2 cells.

Conclusion: These results conclude that the ethanolic fruit extract of Cucumis trigonus possess a good phytochemical strength and also an effective cytotoxic potential.

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INTRODUCTION
The uses of herbs to treat diseases are almost universal among non-industrialized societies. The World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspect of primary health care [1]. Herbalism is a traditional medicinal or folk medicinal practice based on the use of plants and plant extracts. Herbal medicines are popular remedies for diseases used by a vast majority of the world’s population [2].

Plants are utilized as therapeutic agents since time immemorial in both organized and unorganized form. The healing properties of many herbal medicines have been recognized in many ancient cultures [3]. Plants provide a variety of resources that contribute to the fundamental needs of food, clothing and shelter. Among plants of economic importance, medicinal and aromatic plants have played a vital role in alleviating human sufferings [4]. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts [5].

Numerous molecules have come out of Ayurvedic experiential base, examples include rauwolfia alkaloids for hypertension, holarrhena alkaloids in amoebiasis, baccosides in mental retention, picrosides in hepatic protection, phyllanthins as antivirals, many other
steroidal lactones and glycosides as immunomodulators [6]. Herbal drugs which are claimed to be safe are equally effective in comparison to allopathic drugs provide some answer to chronic diseases. However, these herbal drugs are marketed with innumerable pharmacological activities which are not mentioned in the text of various traditional systems of medicine [7].

Cucumis trigonus Roxburghii of family Cucurbitaceae is a perennial scabrid monoecious tendrillar herb with slender angled stem, leaves deep palmately five lobed, hispid on the nerves beneath and rounded at the apex. Male flowers are small and are found in clusters whereas female flowers are solitary. Fruits are ellipsoid or sub-global, yellow or yellow with green stripes, seeds are white and ellipsoidal. Cucumis trigonus is distributed throughout India, Sri Lanka, Afghanistan, Persia and Northern Australia. Roots, fruits and seeds are the medicinal parts of the plant. Roots are purgative and liver tonic. Fruits are used for stomachic, ascites, anemia and constipation and acts as a diuretic. Seeds have an unsaturated lipids as major constituents and acts as a coolant and astringent [8].

The present study is to analyze the phytochemical constituents and the cytotoxic activity of the Cucumis trigonus fruits against Hep2 cells with the help of MTT assay.

MATERIALS AND METHODS

Collection of the plant material

Cucumis trigonus Roxb. fruits were collected from Kovur foot hills of Coimbatore district, Tamil Nadu, India during the month of July to August, 2009. The plant was identified and authenticated by taxonomist Dr. K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimen was deposited herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore.

Qualitative phytochemical analysis of the fruit extract

The phytochemical screening with methanol, ethanol, petroleum ether, chloroform and aqueous extracts of Cucumis trigonus Roxb. was done by modern method [9] to identify the presence of alkaloids, flavonoids, tannins, saponins, triterpenes and glycosides.

Alkaloids

a.Dragendorff’s test: (Kraut-potassium bismuth iodide) 8.0g of Bi (NO3)3. 5H2O was dissolved in 20ml of HNO3 and 2.72g of potassium iodide in 50ml of water. These were mixed and allowed to stand when KNO3 crystalizes out. The supernatant was decanted off and made upto 100ml with distilled water. The alkaloids were regenerated from the precipitate by treating with Na2CO3 followed by extraction of the liberated base with ether.

To 0.5ml of alcoholic, petroleum ether, chloroform and aqueous solution of plant sample added 2.0ml of HCl. To this acidic medium, 1.0ml of reagent was added. An orange red precipitate was produced immediately which indicates the presence of alkaloids.

b. Meyar’s reagent (potassium iodide)

1.3g of mercuric chloride was dissolved in 60ml distilled water and 5.0g of potassium iodide in 10ml of water. The two solutions were mixed and diluted to 100ml with distilled water.

To 1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of samples few drops of reagent was added. Formation of white or pale yellow precipitate showed the presence of alkaloids.

Flavanoids

In the test tubes containing 0.5ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, 5-10 drops of dilute HCl and small piece of zinc or magnesium were added and the solution was boiled for few min. In the presence of flavonoids, reddish pink or dirty brown color was produced.

Tannins

Ferric chloride test

To 1-2ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, few drops of 5% aqueous FeCl3 solution was added. A bluish black color, which disappears on addition of a few ml of dilute H2SO4 was followed by the formation of yellowish brown precipitate.

Saponins

In a test tube containing about 5.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins.

Steroids

Libermann-Burchard’s test

To 1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, 1.0ml of cone. H2SO4 was added followed by the addition of 2.0ml of acetic anhydride solution. A greennish color developed and turned blue indicates the presence of steroids.

Terpenoids

Salkwski reaction
5.0ml of the alcoholic, petroleum ether, chloroform and aqueous solution of the plant was mixed in 2.0ml of chloroform and concentrated H2SO4 (3.0ml) was carefully added to form a layer. A reddish brown coloration in the inter phase formed to show positive results for the presence of terpenoids.

**Resins**

To 2.0ml of alcoholic, petroleum ether, chloroform and aqueous solution the plant sample, 5-10ml of acetic anhydride was dissolved by gentle heating, cooled and then 0.5ml of H2SO4 was added. A bright purple color rapidly changing in to violet was produced, indicating the presence of resins.

**Glycosides**

A small amount of alcoholic, petroleum ether, chloroform and aqueous solution of the plant sample was dissolved in 1.0ml of water and then aqueous sodium hydroxide solution was added. Formation of a yellow color indicates the presence of glycosides.

**Phenols**

a. Ferric chloride test

To 1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant sample 2.0ml of distilled water followed by few drops of 10% aqueous FeCl3 solution were added. Formation of blue or green color indicates the presence of phenols.

b. Lead acetate test

1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant sample was diluted to 5.0ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate was formed to indicate the presence of phenols.

**Cytotoxic Assay**

**Minimal essential media preparation:**

Media is defined as a complex source of nutritional supplementation vital for the growth proliferation and maintenance of cells in vitro. The MEM vial was rinsed in the millipore distilled water, mixed well, closed and sterilized at 15lbs pressure at 121ºc for 15min. Add ingredients depending on the concentration of fetal calf serum (2% or 10%) and was mixed well and then shaken. Take care to avoid spills, pass CO2 using sterile pipette, shake the bottle, check pH and adjust to 7.2 to 7.4. The MEM bottles are kept for 2 days at 37ºc and checked for sterility, pH drop and floating particles. They are then transferred to the refrigerator.

**Preparation of ingredients:**

a. Penicillin and Streptomycin: (Concentration 100 IU of Penicillin and 100 µg of Streptomycin)

Dissolved both antibiotics in sterile millipore distilled water, so as to give a final concentration of 100 IU of penicillin and 100µg of streptomycin/ml. Mix well and distribute in 1ml aliquots. Stored at -20º C.

b. Amphotericin B (Fungizone),20µg/ml

Dissolved in sterile millipore distilled water so as to give a final concentration of 20µg/ml and distribute in 1ml aliquots in vials. Store at - 20ºC.

c. L-glutamine( 3%)

Weighed 3.0g of l-glutamine accurately and dissolved in 100ml sterile millipore distilled water and mixed well. Filtered through millipore membrane filter 0.22µ and distributed in 5ml aliquots in vials. Store at - 20ºC.

d. 7.5% Sodiumbicarbonate

Weighed requisite quantity of sodium bicarbonate (to give 7.5% solution) accurately and dissolve in 100ml of sterile millipore distilled water. Filtered through what mann filter paper No.4, distributed into bottles and sterilized at 121ºC, 15lbs pressure for 15min. Cooled and stored at - 4ºc.

e. Foetal calf serum

Brought FCS to room temperature. Inactivated at 56ºC in water bath for 30min. and cooled at room temperature. If floating particles were seen filtered through Seitz filter. Distributed in 100ml, 50ml, and 20ml quantities in sterile bottles and Stored at - 20ºC.

f. Trypsin, PBS, versene, glucose solution: (TPVG)

i. 2% Trypsin

Weighed 2.0g of trypsin accurately; dissolved in 100 ml sterile millipore distilled water with magnetic stirrer for 30min. Filtered through membrane filter. Stored at -20ºC.

ii. 0.2%EDTA (versene)

Weigh 200mg of EDTA accurately. Dissolved in 100 ml of sterile millipore distilled water. Autoclaved at 15lbs/15min.

iii. 10% Glucose

Weighed 1.0g of glucose accurately. Dissolved in 100 ml of sterile millipore distilled water and filtered through What Mann filter paper and autoclaved at 15lbs / 15 min.

iv. TPVG: 100ml

For the preparation of 100ml of TPVG solution, 840 ml of PBS solution is mixed with 50 ml of 0.2% trypsin, 100 ml of 0.2% EDTA, 5 ml of 10% glucose and 5 ml
of penicillin and streptomycin and mix all the ingredients and adjusted the pH to 7.4 with 0.1 N HCl or 0.1 N NaOH. Distributed in 100 ml aliquots. Stored at - 20°C.

MAINTENANCE OF CELL LINE

Thawing was done by bringing the medium and TPVG to room temperature. The tissue culture bottles were observed for growth, cell degeneration, pH and turbidity by seeing in an inverted microscope. Wipe the mouth of the bottle with cotton soaked in spirit to remove the adhering particles. Discard the growth medium in a discarding jar and keep distance between the jar and the flask. Then add 4 - 5 ml of MEM without FCS and gently rinsed with tilting. The dead cells and excess FCS are washed out and then discard the medium. TPVG was added over the cells and incubate at 37°C for 5 min. for disaggregation. The cells become individual and it became a suspension. Add 5ml of 10% MEM with FCS by using serological pipette, if any clumps were present then repeat the process. After passing, split the cells into 1:2, 1:3 ratio for cytotoxicity studies for plating method

MTT Assay

MTT assay is called as (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide was first proposed by Mossman in 1982. After incubation, remove the medium from the wells carefully for MTT assay. In each well wash with MEM (w/o) FCS for 2 - 3 times. Added 200µl of MTT (5mg/ml). Incubate for 6-7hr. in 5% CO2 incubator for cytotoxicity. After incubation added 1.0ml of DMSO in each well and mixed by pipette and left for 45sec. If any viable cells present formazan crystals after adding solubilizing reagent (DMSO) it shows the purple color formation. The suspension was transferred in to the cuvette of spectrophotometer and the O.D values are read at 595nm by taking DMSO as a blank.

Cell viability (%) = Mean OD / Control OD x 100

RESULTS

Qualitative phytochemical analysis of the fruit extract

The qualitative analysis which produces a “fingerprint” chromatogram obtained under standard conditions is very useful for quality control of phytochemicals. The phytochemical screening with aqueous, ethanol, methanol petroleum ether and chloroform extracts of Cucumis trigonus fruit extract showed to possess secondary metabolites which are clearly depicted in table 1.

Table1. Qualitative analysis of phytochemicals in the fruit extracts of C.trigonus Roxb.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Chemical constituents</th>
<th>Water</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Pet. ether</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Resins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Phenolic constituents</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Cytotoxic assay
The ethanolic fruit extract was prepared from shade dried fruits of Cucumis trigonus to evaluate the cytotoxic efficacy using Hep2 cell line. The cytotoxic effect of varying concentrations of ethanolic fruit extract of Cucumis trigonus at the range of 10 mg / ml to 0.156 mg / ml on Hep2 cell lines was represented in Table 2 and Figure 1.

Table 2. Cytotoxic effect of C. trigonus fruit extract on Hep2 cells

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (mg / ml)</th>
<th>Absorbance</th>
<th>Percentage of cell viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.02</td>
<td>4.08</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.15</td>
<td>30.61</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>0.21</td>
<td>42.85</td>
</tr>
<tr>
<td>4</td>
<td>1.25</td>
<td>0.27</td>
<td>55.1</td>
</tr>
<tr>
<td>5</td>
<td>0.625</td>
<td>0.35</td>
<td>71.42</td>
</tr>
<tr>
<td>6</td>
<td>0.3125</td>
<td>0.41</td>
<td>83.67</td>
</tr>
<tr>
<td>7</td>
<td>0.156</td>
<td>0.47</td>
<td>95.91</td>
</tr>
<tr>
<td>8</td>
<td>Cell control</td>
<td>0.49</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1. MTT assay of C. trigonus fruit extract
The above results indicate that the cytotoxic effect of the ethanolic extract of Cucumis trigonus against Hep2 cells is dose dependent. At low concentrations the extract was found to be less toxic towards the Hep2 cells whereas at higher concentrations the toxicity was increased. Our results are similar to that of [10]. The concentration at 1.25 mg / ml was found to be effective dose because at this concentration, it exhibited 50% cytotoxicity against Hep2 cells.

The cytotoxicity study of C. trigonus Roxb. fruit extract on Hep2 cells is given in the Figure 2, which depicts the percentage of dead cells at different concentrations of the fruit extract. This clearly indicates that on increasing the concentration of the fruit extract the dead cells increase which clearly depicts the percentage of cell viability.

Fig. 2. Cytotoxicity effect of the fruit extract of Cucumis trigonus Roxb.

DISCUSSION
Qualitative phytochemical analysis of the plant extract was done and it was found that the presence of phytochemicals were maximum in the ethanolic extract when compared to other extracts. The phytochemical study with ethanolic fruit extract of selected medicinal plant showed the presence of alkaloids, flavanoids, tannins, saponins, steroids, terpenoids, resins, glycosides and phenolic constituents. So the ethanolic extract of C. trigonus R. fruit extract was used for further investigations.

The cytotoxic effect of ethanolic fruit extract of C. trigonus Roxb. might be due to the alkaloaid and glycoside components of the fruit possessing anticancer activity. Figure 1 represents the cytotoxicity effect of Cucumis trigonus R. fruit extract.
concentration at 1.25 mg/ml was found to be effective dose because at this concentration, it exhibited 50% cytotoxicity against Hep2 cells. The cytotoxicity effect of ethanolic fruit extract of C. trigonus Roxb. might be due to the alkaloid and glycoside components of the fruit possessing anticancer activity.

REFERENCES


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