

A Review on Phytochemical and Pharmacological Study of *Cucumis Dipsaceus* Ehrenb. (Fruit)

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Abstract: Fruit of *Cucumis dipsaceus* Ehrenb. belongs to the family-Cucurbitaceae, which commonly known as gourd family and members are commonly called as Cucurbits. Its common name is hedgehog cucumber. It comprises of 122 genera and 940 species, which is mostly distributed in the tropical and warm regions of the world. Shape of fruit is cylindrical with non-breakaway hairs and hispid. The fruit contains certain major bioactive constituents such as, glycosides, fixed oils, tannin, alkaloids, saponins, flavonoids, resins, steroids, essential amino acids and minerals, which are responsible for medicinal value. Methanolic extract screened for the presence of flavonoid glycosides by column chromatography and for molecular isolation of polyphenolic compounds TLC, HPTLC and LCMS methods were use. Traditionally, fruit extract was use for the treatment of diarrhoea, stomach pain, constipation, gastrointestinal disease, meningitis, anti-acne lotions, headache, dysentery, hemorrhoid for rabies etc. Fruit extract was evaluated for various types of pharmacological activities like Anti-inflammatory activity, Anti-nutritional factors, Antioxidant activity, cytotoxic activity, Antimicrobial activity, Analgesic, Anti-bacterial activity and Hepatoprotective activity by employing the animal models. This review brings the literature on Anti-diabetic activity of *Cucumis dipsaceus* Ehrenb. Therefore, that literature provides a direction for further clinical research to promote safe and effective herbal treatments to cure a number of diseases.

Keywords: *Cucumis dipsaceus* Ehrenb., Morphology, Phytochemical and Pharmacological Activities

1. Introduction

Cucumis dipsaceus Ehrenb. is a species of flowering fruit belonging to the family *Cucurbitaceae*, which commonly known as gourd family and members are commonly called as Cucurbits. It comprises 122 genera and 940 species which are mostly distributed in the tropical and warm regions of the world (Mabberley, 2008) [1]. The common name of this fruit is hedgehog cucumber, Teasel gourd, Arabian cucumber, pepinodiablito, concombreporc-epic, wild gourd etc. Sometimes this herb is cultivated in different tropical regions. It is found in Tanga region and in northern and western parts of Tanzania as well as in the southern highlands, Uganda, Kenya in countries of Africa [2], Ethiopia, Somalia, Sudan and Southern Egyptand also cultivated in other tropical regions but now found in forest of Maruthamalai, foothills (Western Ghats), Coimbatore (Tamil Nadu) and Mysore, (Karnataka), India [3].

- **Distribution:** Africa [4] (Ethiopia, Kenya, Somali, Tanzania, Uganda, Sudan, Southern Egypt) and Asia: India (Karnataka, Tamil Nadu, Kerala).
- **Flowering and Fruiting:** October–February [5].
- **Uses:** The tender leaves and young shoots are chopped, cooked, coconut milk or groundnut paste used as vegetable and then taken with staple food [6].
- **Traditional uses:** Fruit used as food in Nyasaland and Tanganyika. *Cucumis dipsaceus* fruit is use as anti-emetic. Its fruit used for diarrhoea, constipation, gastrointestinal diseases, meningitis, dysentery and stomach pain. Extract of fresh fruit are used haemorrhoid for rabies. All the uses of this herb are popular in different countries of East African. Fruit is use as fodder. Management of herbs is collecting from the wild plants, but it can easily propagate from seed. The status of this herb is easily and common accessible within its habitat from its native distribution [7].

- **Medicinal uses:** Fruit juice is use as an antidote for poisoning, but it has to be supplement by drinking fresh milk. *Cucumis dipsaceus* is use for headaches, dysentery etc. The seeds are cooling and diuretic. The fruit juice is use as a demulcent in anti-acne lotions. Its fruit used for gastrointestinal diseases, diarrhoea, stomach pain, constipation [8].
- **Morphological description of *Cucumis dipsaceus* Ehrenb. (Fruit)** is mono-colored, pale yellow. It is 3-6.5cm long and 2.5-4cm in diameter. It is densely aculeate and glabrous blunt at the apex. Seeds is elliptic, unwinged and 4-5 mm long× 2 mm diameter x 1mm thick. It is cylindrical 1-3(-4) cm long with non-breakaway hairs and hispid.

Taxonomy of *Cucumis dipsaceus* [9]

Item	Name
Taxonomic classification	
Kingdom	Plantae
Subkindom	Viridaeplantae
Viridaeplantae	Streptophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Infradivision	Angiospermae
Class	Magnoliopsida
Superoder	Rosanae
Order	Cucurbitales
Family	Cucurbitaceae
Genus	<i>Cucumis</i> L.
<i>Cucumis</i> L.	<i>Cucumis dipsaceus</i>

2. Phytochemical Analysis

The fruit extract act as nutraceuticals supplement to the human diet, which contains large amount of all essential amino acids and minerals. Different amount of amino acids like threonine 15 mg, cysteine 4 mg, methionine 10 mg, valine 26 mg, isoleucine 20 mg, leucine 39 mg, tyrosine 15 mg, histidine 10 mg, phenylalanine 25 mg, and lysine 30 mg

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per kg/day of body weight are required for healthy human diet. Amount of alanine, leucine, valine, and isoleucine was enhanced. It also stimulates metabolic signals and produce energy to the muscles. Fruit extracts were analysed for the presence of saponins, phenolic compounds, carbohydrates, proteins, amino acids, alkaloids, cardiac glycosides, phytosterols, glycosides, flavanol, glycosides, tannins, flavonoids, gums and mucilages and fixed oils and fats according to standard methods [10].

Phytochemical parameter and extractive values [11]: The Phytochemical parameters give very informative knowledge about secondary metabolites. According to standard procedures as per WHO guidelines and they gave some results which was shows by these phytochemical parameters. Moreover, these parameters were studied like-

- 1) Swelling index
- 2) Foaming index
- 3) Loss on drying
- 4) Foreign organic matter
- 5) Ash values
- 6) Extractive values

Thin Layer Chromatography: By performing TLC finger printing, the solvent system (chloroform: ethyl acetate) was used for separation of flavonoids and glycosides. The sample were prepared in methanol solvent and then spotted on precoated silica gel G aluminum plates with the glass capillary tubes. The spotted plates were place in mobile phase of solvent system (Ethyl acetate: Formic acid: Glacial Acetic Acid: Water 100:11:11:26 v/v/v/v solvent). The spotted plate was take out from solvent system after proper separation of chemical constituents and then dried at room temperature. The color of spots was seen in visible and UV light (254nm and 366nm) and R_f values of each spots were calculated [12]. The developed pre-coated TLC plates were derivatized with 0.5% solution of anisaldehyde - H_2SO_4 acid and visualized under visible light and UV (366nm). TLC, HPTLC and LCMS methods used to estimate and found the polyphenolic compounds. Spectroscopic methods (IR, 1D and 2D NMR and Mass spectrometry) used to elucidate and confirm the structure. The confirmed structure of flavonoid glycosides was quercetin- 3-rutinoside-7-rhamnoside (M Wt: 756.663g/mol) and checked with literature data. It also provides protection for hepatic damage or hepatitis. The structures of isolated polyphenolic compounds were elucidate from IR, 1D and 2D NMR and LCMS spectra.

High performance thin layer chromatography (HPTLC) profile: The fruit extract was dissolve in methanol (HPTLC grade) and the solution was centrifuge. Then these test solution was further use for HPTLC estimation. Ethyl acetate: Formic acid and Glacial Acetic Acid: Water solvent system was used for developing HPTLC finger print profile of flavonoids and its glycosides [13].

Development of Chromatogram: Chromatogram was developing in Ethyl acetate: Formic acid: Glacial Acetic Acid: Water solvent system (mobile phase). The developed chromatogram was dried at room temperature and then placed in CAMAG REPROSTAR 3 a photo - documentation chamber and photographs were capture in visible and UV

light. The chromatogram was scan by the using densitometer after using detecting/derivatizing reagent and drying in hot air oven. The finger print data as peak number with area and R_f values of were recorded by WINCATS software [14].

Identification of polyphenolic or flavonoid glycosides by HPLC- UV- ESI-MS: The chemical compositions of fruit extract were identifying by using HPLC-UV-ESI-MS method. By this method, the individual polyphenolic or flavonoid glycosides were identifying. This present efforts also exercised for the knowledge of how many types of flavonoid glycosides were present in methanol extract of fruit of *Cucumis dipsaceus*. These flavonoids glycosides were act as strong antioxidant and hepatoprotective agent [15].

Isolation of Flavonoid Glycosides by Column Chromatography from Methanolic Extract: The fruit extract of dried powder was prepared by using methanol as solvent with the help of soxhlet apparatus at room temperature. The methanol filtrate was concentrated and dried with rotary vacuum distillation apparatus [10]. Fruit extract was dissolving in methanol and mixed in silica gel powder. The isolation of phytoconstituents with chloroform, ethyl acetate, and methanol in different ratios Petroleum ether (100%), Chloroform (100%), Petroleum ether: Chloroform (95:5), Petroleum ether: Chloroform (90:10), Petroleum ether: Chloroform (5:95); Chloroform: Ethyl acetate (95:5); Ethyl acetate (100%), Methanol (100%), Ethyl acetate: Methanol (95:5) can be done by column chromatography. Each 20 ml elute was collected. By using precoated TLC plates, the presence of phytoconstituents were analyzed and to know similar spots. R_f values of isolated phytoconstituents further same elutes were pooled and concentrated by using vacuum distillation and then stored in vacuum desiccators to keep it free from moisture. Then the solvent system (Chloroform: Ethyl Acetate) was sticky dark brown mass were again dissolved in methanol. The solvent system of Chloroform: Ethyl acetate was repeat for Column chromatography for isolation and purity with different ratios of Chloroform (100%), Ethyl acetate (100%), Chloroform: Ethyl acetate (95:5) was obtained. It was isolate by column chromatography with increasing polarity like methanol, petroleum ether, and chloroform, ethyl acetate solvents of different ratios. Each 20 ml elute was collected, analyzed with precoated Merck F254 TLC plates to compare similar spots and R_f values for same category of contents [16].

Liquid Chromatography Mass Spectroscopy: The crystals of chloroform: ethyl acetate fraction (10mg CEF2) was dissolved in 10ml methanol (HPLC grade) and used for the separation of polyphenolic compounds (phenolic and flavonoids). The injection (10 μ l vol.) were filled and injected to the Waters 2795 HPLC (Waters Micromass Q-ToF Micro) which having 0.05- 5.0 ml/Min flow rate and configured with quaternary pumping. This equipment was auto sampler for next injection refilled with capacity 100 μ l volume. The acetonitrile and acidified water (0.5% Acetic acid, v/v) used for mobile phase A and B respectively. When introduced to mass spectrometer the effluent was splitted by using T- type splitter (split = 1:3) from the HPLC column and temperature was maintained at 25°C. Thus, the flow rate

0.2% was reach to the ESI-Q-TOF-MS detector. Maintained the temperature of Q-TOF provide calibration curve which provided accurate masses values for complete run. This method not needed any type of dual spray requirement for internal mass calibration and all vales were record [17].

Pharmacology of *Cucumis dipsaceus* Ehrenb. (Fruit): A number of pharmacological activities of fruit extracts of *Cucumis dipsaceus* such as hepatoprotective activity [18] analgesic, anti-inflammatory activity [19] and cytotoxic activity [20], antioxidant activity (leave and fruit) [21], antimicrobial activity and Antibacterial activity [22], [23] antiallergic [24], [25], anticarcinogenic [26] and anti-obesity activities [27] was reported on fruit as shown in figure 1. Fruit extracts of *Cucumis dipsaceus* was reported as methanol extract which was further used to test their antibacterial activity by using *E. coli* (-ve), *Bacillus subtilis* (+ve), *Staphylococcus aureus* (+ve) [28].

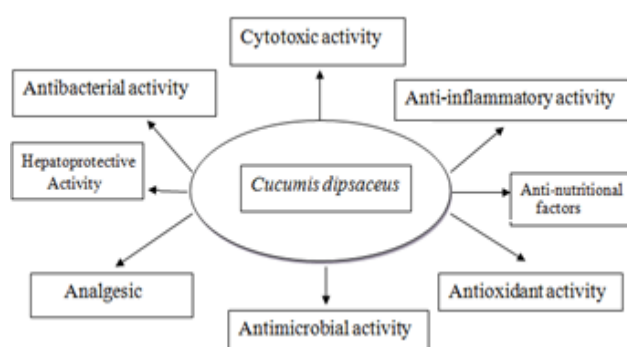


Figure 1: Pharmacological activities of *Cucumis dipsaceus* Ehrenb. (Fruit)

Anti-nutritional factors: Anti-nutritional factor is also known as “Secondary metabolites”, which are formed as side products in process synthesis of primary metabolites [29]. These are highly biologically active. The literature study of *Cucumis dipsaceus* shows strong antioxidant activity due to presence of flavonoids that are very good research material for further exploring pharmacological evolutions. Not only this, but the antioxidant and nutritional property of this fruit together could encourage its use as a nutraceutical supplement [30]. Anti-nutritional factors show strong antioxidant activities, analgesic, cytotoxic activities, antibacterial activities and anti-inflammatory activities.

Antioxidant activity: Relationship between rates of oxidant generation, antioxidant activity, oxidative stress, and oxidative damage in diabetes. $[O_2]^*$ represent in various forms of reactive oxygen species [ROS]. The overall rate of formation of oxidative products causes oxidative tissue damage which is dependent on ambient levels of both substrate and $[O_2]^*$ [31]. Increased generation of $[O_2]^*$ depends on several sources including glucose autoxidation, increased mitochondrial superoxide production [32] and as a result of the receptor for advanced glycosylation end products activation. Antioxidant defenses are compromise in diabetes due to this $[O_2]^*$ Deactivation is reducing. Note that oxidative stress which also promotes other hyperglycemia-induced mechanisms of tissue damage. Oxidative stress activates protein kinase C (PKC) and accelerates the formation of advanced glycosylation end products (AGEs) [33].

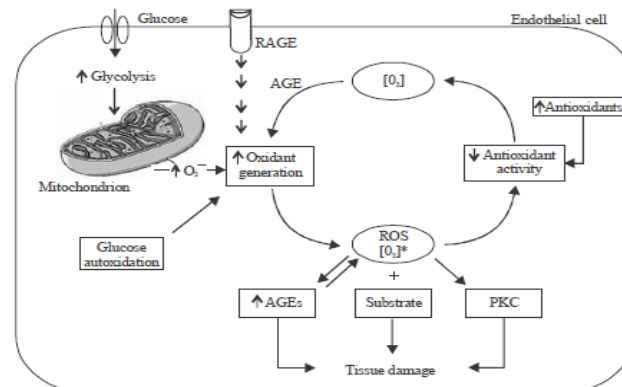


Figure 2: Different Pathways of Oxidant Generation and Antioxidant Activity in Endothelial Cell.

All the biomolecules mostly lipids, carbohydrates and proteins can be affected by degradation of oxidative reactions. Due to the toxicity of synthetic anti-oxidants like BHA (Butylated Hydroxyl Anisole) and BHT (Butylated Hydroxyl Toluene) the interest is highly focused on searching plant based antioxidants because of their therapeutic effects and low toxicity. Antioxidants protect the cellular structures and macromolecules from damage due to free radicals [34]. Carotenoids and phenolic compounds are dietary antioxidants. Response of *Cucumis dipsaceus* extracts towards various antioxidant assays was appreciable in ABTS+, metal chelating, nitric oxide and DPPH assays. Methanol extract of *Cucumis dipsaceus* fruit showed the highest activity (4907.22 $\mu\text{g TE/g}$) to stabilize the ABTS radical. *Cucumis dipsaceus* fruit of methanol extract (12.4 g EDTA Eqv / 100g extract) exhibited metal chelating activity. DPPH (IC₅₀ = 10.37 $\mu\text{g/mL}$) assay also revealed higher free radical inhibition of fruit [35].

Cytotoxic and antitumor Activity of *Cucumis dipsaceus* (fruit): The ethanolic extract of *Cucumis dipsaceus* showed high percentage of cytotoxic effect against K562 and Hep-2 that are human tumoral cellular line. Anti-tumor effect was performed against the microorganism *Agrobacterium tumefaciens* that produced tumor due to presence of sterol, triterpenes and flavonoids [36].

Analgesic, Anti-Inflammatory Activity of *Cucumis dipsaceus* (fruit): Extracts of petroleum ether, dichloromethane, methanolic and ethanolic *Cucumis dipsaceus* (fruit) showed high analgesic and anti-inflammatory activity, but dichloromethane and methanolic extract was showed highest analgesic effect and highest anti-inflammatory activity of dichloromethane extract [37].

Antibacterial activity: The extracts of *Cucumis dipsaceus* fruit with solvent methanol and used to test their antibacterial activity. Studies reported using bacteria such as *E. coli* (-ve), *Staphylococcus aureus* (+ve) and *Bacillus subtilis* (+ve) [38].

Antimicrobial activity: Undesirable side reactions may occur with oral administration of antibiotic substances and oral administration of penicillin may cause heartburn, nausea, vomiting, and diarrhea. Therefore, numerous trials were conduct on herbs and spices as antibiotic replacements. Various stains of gram positive and gram negative bacteria

were used for testing the antimicrobial activity of methanolic extract of *Cucumis dipsaceus*. For nutrient medium, Mueller Hinton agar medium was used for testing the antimicrobial activity [39]. It was sterilized by autoclaving at 120°C with pressure 15lb/inch 2.20 ml of agar medium inoculated with the respective strains of bacteria and then it was transferred aseptically into sterile Petri plates and the procedure was carried out under laminar airflow chamber under aseptic conditions. The extracts were freshly reconstituted with Dimethyl Sulfoxamide (DMSO) and tested at various concentrations. Four cavities were made in each petri plates. Alternative cavities were filling with sample solution. After prediffusion these plates were incubated at 37°C for 24 hrs.

Anti-inflammatory activity: Fruit of *Cucumis dipsaceus* possess secondary metabolites like tannin, alkaloids, saponin, flavonoids, resins, steroids and it was shows that flavonoids are responsible for treatment of inflammation of liver damage or hepatotoxicity [41].

Hepatoprotective activity: [42] Due to the Prophylactic effect and strong antioxidant of CDME and CDWE against CCl₄ induced hepatotoxicity in rat liver. The experimental hepatotoxin is CCl₄, which used for induction of hepatic damage in rat liver in various animal models. *In vivo* antioxidant enzyme was AST, ALT and ALP present normally in high concentrations in liver but hepatic damage occurs due to oxidative stress by CCl₄ induction [43], AST, ALT and ALP leaks into blood circulation and then increase serum levels in CCl₄ treated rats and thus predicting the status of liver. Any hepatocytic damage leaks out as intracellular enzymes to the blood circulation [44]. Significantly increase ($p < 0.05$) in levels of SGOT, SGPT, ALP, serum bilirubin but the concentration of total protein was decreased in CCl₄ treated group and thus show damage to membrane of hepatocytes, increased permeability and necrosis of hepatocytes. CCl₄ along with CDWE showed significantly decreased ($p < 0.05$) levels of SGOT, ALP, SGPT, serum bilirubin in liver which was very close to normal control group [45]. Patil *et al.* and Sharstry *et al.* reported that the *Amorphophallus paeoniifolius*, the flavonoids (Quercetin) is isolated and characterized, they show hepatoprotective activity by restoring elevated concentration of SGOT, ALP, SGPT, serum bilirubin and total protein in rat liver against CCl₄ induced hepatic damage. It shows the required repair and stabilization to injury caused to the biomembrane of hepatocytes of liver due to hepatotoxic agent, CCl₄ [46], [47]. *In vitro* hepatoprotective effect was studied on HepG2 cells line against H₂O₂ (Hepatotoxin used as inducing DNA damage which is dose dependent and its incubation time dependent for HepG2 cell line. These cell lines were attack to induction of DNA damage with low dose of hydrogen peroxide. Both the extracts (CDWE and CDME) at different concentrations like 0.01, 0.1, 1, 5, 10, 50, 100 and 500 µg ml⁻¹ shows non-cytotoxicity and significant cell viability (%) in the presence of hepatotoxic agent (H₂O₂) [48]. These extracts also indicate protection and restoration of hepatic cells from toxic effect due to hydroxyl free radical produced by hydrogen peroxide for oxidative stress. Due to strong antioxidant activity both the CDWE and CDME extracts possess hepatoprotective activity and by serum hepatic biomarker enzymes it shows scavenging effect on free radicals and isoenzymes or their

synergistic of phytophenolic active secondary metabolites present in both extracts and *in vivo* hepatic enzymes [49]. This study discovers the possible synergistic effects of carbohydrates, proteins, amino acid, vitamins, phenolic, minerals and flavonoids, fatty acid and saponin (*cucurbitacin*) from *Cucumis dipsaceus* (fruit). All of these are beneficial for scavenging the Free radicals which is produced by hepatotoxin (CCl₄), which is induces hepatic damage to hepatocytes of rat liver, which is similar to viral hepatitis disease [50].

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4. Author Contribution

Both the authors had contributed equally to the review of literature regarding this topic.

5. Conflict of Interests

We declare that we have no conflict of interest.

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