



**PHYTOCHEMICAL EVALUATION, GAS CHROMATOGRAPHY-MASS
SPECTROSCOPY AND ANTI DIABETIC, ANTI OXIDANT, ANTI BACTERIAL
ACTIVITY OF *CUCUMIS SATIVUS* SEED EXTRACT**

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ABSTRACT

Cucumis sativus seed extract are traditionally used to treat diabetes and its complications (inflammation and formation of reactive oxygen species) around the world. The present study investigates the antidiabetic, anti oxidant and anti microbial activity. Gas chromatography equipped with mass spectrometry (GC-MS) is deployed for the identification of various compounds in crude extracts. On activity level, its ethyl acetate extract showed the highest inhibition towards α -amylase and α -glucosidase with an IC 50 of 70.9 μ g/ml and 69.1 μ g/ml respectively, inspected through the substrate-based method. GC-MS result revealed the presence of 19 compounds in ethyl acetate extract belonging to flavonoid and phenolic categories. Identification of these compounds from *Cucumis sativus* seed extract and its biological implication also support the community-based usage of this plant and its medicinal value. The ethyl acetate extract of (at a concentration μ g/ml) exhibited DPPH and Hydrogen peroxide good free radical scavenging activity respectively. Ethyl acetate extract of Seeds of *Cucumis sativus*, with an IC50 value of 62.8 μ g/ml and 64.0 μ g/ml (DPPH and Hydrogen peroxide). The results of the antimicrobial activity by Disc diffusion method of *Cucumis sativus* seed ethyl acetate extract. The ethyl acetate extract was inhibited the growth of selected Gram positive bacterial strain *Bacillus subtilis* 20 mm and comparing standard Gentamycin 34 mm. Gram negative bacterial strain *Escherichia coli* in the range of 22 mm and comparing standard Ciproflaxacin 36 mm in the bacterial concentration of 30 μ g/ml. The *Cucumis sativus* seed extract in all gram positive and gram negative microorganism showed maximum good inhibition. So it is proved that the ethyl acetate extract of *Cucumis sativus* seed has good anti bacterial activity. This modest work indicates that the *Cucumis sativus* seed extract have an important antidiabetic, anti oxidant and microbial effect that can be well established treatment of diabetes and its complications.

KEYWORDS: *Cucumis sativus* seed extract, antidiabetic, anti oxidant, GCMS, anti microbial activity.

INTRODUCTION

Plants are basic therapy springs for diseases that can be traced back to time immemorial. Having little or no harm effects of plant materials, therefore herbs based medicines flourished now a days. Plants possess specific biochemical components which exerts curative effects on living organism to treat various diseases.^[1] The concrete significance of the medicinal plants is because of antioxidants and antimicrobials properties.^[2,3] Microbes become resistant to the available sources of drugs, novel

plant sources are needed to handle these resistant strains. It is needed to comprehend the genetics of the resistant microbes for developing new drugs better than the existing ones to reduce threat of infectious diseases.

Plant sources possess chemicals like alkaloids, glycosides, flavonoids, terpenoids etc. in plenty which are potential sources of biological properties.^[4] There is an inborn antioxidative mechanism in the human body and many natural activities such as the anticarcinogenic

and anti-aging responses, initiate due to antioxidants. Antioxidants soothes or disable free radicals, earlier their attack to the target cells. Disease occurred due to production of free radicals in living system which damage it causing serious and long lasting diseases like diabetes, ageing, low immune system neuro-degeneration etc. The diseased body needed antioxidants in the form of proper nutritional and medicinal supplements to overcome the threat.

Plant based research showed that antioxidants are there in the form of chemical components like phenols, flavonoids, proanthocyanidine etc. Plants based antioxidants enhances the defense against diseases and slows down the aging process. Natural antioxidants reduces the risks of infectious and chronic disease.^[5] Medicinal plants are continuously investigated for the therapeutic properties especially for antioxidants. Natural antioxidants either in fresh form or its pure chemical ingredients are active to check the critical progressions produced by reactive oxygen species (ROS)].

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra.

Cucumber (*Cucumis sativus* L.): The Cucurbitaceae family includes the annual herbaceous climbing vegetable *Cucumis sativus* L., which is said to have originated in the tropical foothills of the Himalayas. Cucumbers are divided into four groups according to their geographic distribution: Indian group, Xishuangbanna group, Eurasian group, and East Asian group. Gas chromatography–mass spectrometry (GC–MS) is a potent analytical method that separates, identifies, and characterizes chemical compounds in complicated materials by combining gas chromatography and mass spectrometry.

MATERIALS AND METHODS

COLLECTION OF MATERIALS

The seeds of *Cucumis sativus* is obtained from local area, Thirupuvanam, Madurai, Tamilnadu. The seeds were cleaned under running tap water and dried at room temperature.

PROCESSING OF EXTRACT PREPARATION

200 g of *Cucumis sativus* seeds were shade-dried and ground using and grind to coarse make fine powder and sieved. Powdered sample was extracted with 500 ml of ethyl acetate by cold maceration method at room temperature for 7 days. The beaker was covered with aluminum foil. After the extraction period, the aluminum foil was removed, and the extract was filtered through Whatman No.1 filter paper. The ethyl acetate was concentrated using hot plate to get yellowish liquid extract.

Table 1: Percentage Yield and the Colour of the Extract of *Cucumis Sativus* Seed Extract.

S.NO	EXTRACT	PERCENTAGE YIELD	COLOUR
1	Ethyl acetate extract	14.3%	Yellow

GAS CHROMATOGRAPHY -MASS SPECTROSCOPY (GC-MS)

GC-MS analysis of ethyl acetate extract was carried out using the equipment GC Clarus 500 Perkin-Elmer system comprising a AOC–20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following condition: Equipped with a column Elite-1, fused silica capillary column (30 m × 0.25 mm ID × 1µm df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium gas (99.999%) was used as carrier gas at a constant flow rate of 1.20 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The injector temperature is set at 250°C, and the ion-source temperature is 280 °C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase for 10 °C/min, to 200°C/ min, then 5°C/ min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan-interval of 0.5 seconds, and fragments from 45 to 450 Da. Total GC running time was 36 min 6.7.

ANTI OXIDANT ACTIVITY^[8,9]

DPPH Assay Reagent preparation

- 0.1 M DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working procedure

- Extract the food samples using a suitable solvent like methanol/ethanol. Filter or centrifuge the extract and create a dilution series.
- Different volumes of sample extracts were made upto 40 µl with DMSO and 2.96 ml DPPH (0.1mM) solution was added.
- The reaction mixture was incubated in dark condition at room temperature for 20min. After 20 min, the absorbance of the mixture was read at 517 nm by UV-Visible Spectrophotometer. 3ml of DPPH was taken as control.

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

HYDROXYLRADICAL SCAVENGING ASSAY

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Klein et al. The reaction mixture contained 1.0 ml of different concentration of extracts (5-100 µg/mL), 1.0 ml of iron-

EDTA solution (0.13% ferrous ammoniumsulphate 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1.0 ml of DMSO (0.85% in 0.1M phosphate buffer pH 7.4) and 0.5 ml of 0.22% ascorbic acid. The tubes were capped tightly and heated in a water bath at 80-90°C for 15 minutes, the reaction was terminated by adding 1.0 ml of ice-cold TCA (17.5%).

To the above reaction mixture 3.0 ml of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid and 2.0 ml of acetyl acetone) were mixed and distilled water was added to a total volume of 1 L was added and incubated at room temperature for 15 minutes for color development.

The intensity of the yellow colour formed was measured at 412 nm against a reagent blank.

$$\% \text{inhibition} = [(Control - Test) / Control] \times 100$$

ANTIDIABETIC ACTIVITY

α -Amylase inhibition assay^[10,11]

Different concentration of sample extract was added with 100 μ l of 0.02 M sodium phosphate buffer (pH 6.9) and 100 μ l of α -amylase solution (4.5 Units/ml/min) and pre incubated at 25°C for 10 min (Worthington, 1993). Then, 100 μ l of 1% starch solution was added and incubated at 25°C for 30 min and the reaction was stopped by the addition of 1.0 ml of dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was diluted (10-fold) with distilled water and the absorbance was measured at 540 nm. The readings were related with the control, which contains buffer instead of extract and the percentage of α -amylase enzyme inhibition was calculated.

$$\% \text{Inhibition} = [(Absorbance Control - Absorbance Sample) / Absorbance Control] \times 100$$

α -Glucosidase inhibition assay

Different concentration of the sample extract was taken with 100 μ l of 0.1 M phosphate buffer (pH 6.9) and 100 μ l of α -glucosidase solution (1 Unit/ml/min) and pre incubated at 25°C for 5 min (Worthington, 1993). Then,

100 μ l of *p*-nitro phenyl- α -D-glucopyranoside (5 mM) was added and incubated at 25°C for 10 min. After the incubation period, the absorbance readings were recorded at 405 nm and allegorized to a control that had 100 μ l of buffer in place of the sample. The results were calculated and expressed on percentage basis.

$$\% \text{Inhibition} = [(Absorbance Control - Absorbance Sample) / Absorbance Control] \times 100$$

ANTI MICROBIAL ACTIVITY

Microorganisms

The bacteria used as the test organisms in the study were *Bacillus subtilis* (Gram +) *Escherichia coli* (Gram-). The bacterial cultures were taken from Plant Secondary Metabolite Laboratory at Madurai. All bacterial cultures were maintained on Nutrient Agar/ Broth.

Antimicrobial activity^[12-16]

The antimicrobial activity of the *Cucumis sativus* seed ethyl acetate extract against the microbial pathogens was determined by Kirby and Bauer disc diffusion method. Mueller-Hinton agar plates inoculated with 1 ml of bacterial ml according to 0.5 McFarland standards was used for the antibacterial assay. Sterile filter paper discs of 6 mm dia were impregnated with 30 μ l of crude extracts of lichen of varying concentrations such as 1 mg/mL, 2 mg/mL and 5 mg/mL and after complete evaporation were placed on the surface of the inoculated agar plates. These plates were incubated at 37°C for 48 h. At the end of the incubation period, the antimicrobial activities were evaluated by measuring the zone of inhibition. In this assay, negative control was the respective pure solvent and the positive control was for *Bacillus subtilis* (Gentamycin), *Escherichia coli* (Ciprofloxacin).

RESULTS AND DISCUSSION

PRELIMINARY INVESTIGATION

Phytochemical analysis reveals the occurrence of alkaloids, anthracene derivatives, flavonoids, phenolics, phytosterols, saponins, tannins, triperpenoids and volatile oils. (Table 2).

PHYTOCHEMICAL

Table 2: Phytochemical analysis of Seeds Of *Cucumis Sativus*.

S.NO	PHYTO CONSTITUENTS	ETHYL ACETATE EXTRACT
1	Alkaloids	+
2	Carbohydrates	+
3	Tannins	+
4	Saponins	+
5	Triterpenoids	+
6	Proteins and amino acids	+
7	Phenolic compounds	+
8	Gums and mucilages	+
9	Fixed oil and fats	+
10	Glycosides	+
11	Triterpenes	+
12	Phytosterols	+

+ = presence of active constituents

GC-MS ANALYSIS OF SAMPLES

The given *Cucumis sativus* seed ethyl acetate extract was analysed through GC-MS for identification of different compounds.

principles with their retention time (RT), Molecular weight (MW) and concentration are presented in the compound of various extract is given below the table3.

IDENTIFICATION OF COMPOUND IN GC-MS ANALYSIS

19 Compounds were identified in *Cucumis sativus* seed ethyl acetate extract by GC-MS analysis. The active

Table 3: Compounds Identified In *Cucumis Sativus* Seed Ethyl Acetate Extract.

S.NO.	PEAK #	R.TIME	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT
1	2	11.2267	2,4-Decadienal(E,E)	C ₁₀ H ₁₆ O	152
2	3	13.2987	1-(4-Ethoxyphenyl)propan-1-ol	C ₁₁ H ₁₆ O ₂	180
3	4	13.9069	Benzoic acid, 4-ethoxy-, ethylester	C ₁₁ H ₁₄ O ₃	194
4	5	14.3256	Dodecanoicacid	C ₁₂ H ₂₄ O ₂	200
5	6	16.6489	Tetradecanoicacid	C ₁₄ H ₂₈ O ₂	228
6	7	17.3044	Ethanedione, diphenyl-(Benzil)	C ₁₄ H ₁₀ O ₂	210
7	8	18.2948	Hexadecanoicacid, methyl ester	C ₁₇ H ₃₄ O ₂	270
8	9	18.8556	n-Hexadecanoicacid	C ₁₆ H ₃₂ O ₂	256
9	11	19.9444	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294
10	13	20.5526	9,12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280
11	17	21.7797	Heptacosane	C ₂₇ H ₅₆	380
12	18	22.6209	Heptacosane	C ₂₇ H ₅₆	380
13	22	23.4293	Heptacosane	C ₂₇ H ₅₆	380
14	25	23.8335	trans-9,12-Octadecadienoicacid, propyl ester	C ₂₁ H ₃₈ O ₂	322
15	26	24.2123	Tetracosane	C ₂₄ H ₅₀	338
16	27	24.5837	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₃₈ O ₄	354
17	28	24.9697	Heptacosane	C ₂₇ H ₅₆	380
18	31	25.8072	Squalene	C ₃₀ H ₅₀	410
19	32	26.4081	Pentacosane	C ₂₅ H ₅₂	352

ANTIOXIDANTACTIVITY**DPPH and H₂O₂ radical scavenging**

In the current study, seeds of *Cucumis sativus* ethyl acetate extract were evaluated for their radical scavenging activity on DPPH and Hydrogen peroxide enzymes by the *in-vitro* method.Extract significantly demonstrated inhibition to DPPH and Hydrogen

peroxide enzymes in a dose-dependent manner. The ethyl acetate extract of (at a concentration g/ml) exhibited DPPH and Hydrogen peroxide good free radical scavenging activity respectively. Ethylacetate extract of Seeds of *Cucumis sativus*, with an IC₅₀ value of 62.8µg/ml and64.0 µgm/ml (DPPH and Hydrogen peroxide.

Table 4: Dpph and Hydrogen Peroxide Free Radical Scavenging Activity of Ethylacetate Extract of Seeds of *Cucumis Sativus*.

ANTI-OXIDANTACTIVITY							
S.NO.	NAME OF THE TEST	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	1000 µg/ml	IC50
1	DPPH FREE RADICAL SCAVENGINGACTIVITY	17.2%	32.8%	47.6%	63.1%	78.5%	62.8 µg/ml
2	HYDROGEN PEROXIDE RADICALSCAVENGING ACTIVITY (H2O2)	17.0%	31.9%	46.8%	62.0%	77.3%	64.0 µg/ml

ANTIDIABETICACTIVITY**α-amylase and α-glucosidase inhibition assay**

In the current study, seeds of *Cucumis sativus* ethyl acetate extract were evaluated for their inhibitory effect on α-amylase and α glucosidase enzymes by the *in-vitro*

method. The extract significantly demonstrated inhibition to αamylase and α-glucosidase enzymes inadose-dependent manner. The ethylacetate extract of (at a concentration g/ml) exhibited α amylase and α-glucosidase good inhibitory activity respectively. Ethyl

acetate extract of *Cucumis sativus*, with an IC₅₀ value of 70.9 µg/ml and 69.1 µg/ml (α-amylase and α-glucosidase).

Table 5: Alpha Amylase And Alpha Glucosidase Activity Of Ethylacetate Extract of *Cucumis Sativus*.

ANTIDIABETICACTIVITY							
S.NO.	NAME OF THE TEST	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	1000 µg/ml	IC ₅₀
1	α-Amylase Inhibition Activity	15.2%	28.6%	42.3%	56.1%	70.3%	70.9 µg/ml
2	α – Glucosidase Inhibition Activity	15.8%	29.7%	43.6%	57.5%	71.6%	69.1 µg/ml

ANTIMICROBIALACTIVITY

The results of the antimicrobial activity by disc diffusion method of seeds of *Cucumis sativus* ethyl acetate extract. The ethyl acetate extract inhibited the growth of selected Gram-positive bacterial strain *Bacillus subtilis* with a zone of inhibition of 20 mm, compared with the standard Gentamicin (34 mm). Gram-negative bacterial strain *Escherichia coli* showed a zone of inhibition of 22 mm,

compared with the standard Ciprofloxacin (36 mm).

The seeds of *cucumis sativus* powder Ethyl acetate extract in all Gram positive and Gram-negative microorganisms showed moderate inhibition activity. So it is Proved that ethyl acetate of *Cucumis sativus* has good anti-microbial activity.

Table 6: Anti Microbial Activity of Seeds of *Cucumis Sativus*– Ethyl Acetate Extract.

S.NO	ORGANISMS	STANDARD DRUG (30 µg/ml)	ETHYL ACETATE (30 µg/ml)
1	Antibacterial Gram (+) <i>Bacillus subtilis</i>	Gentamicin 34mm	20mm
2	Gram (-) <i>Escherichia coli</i>	Ciprofloxacin 36mm	22mm

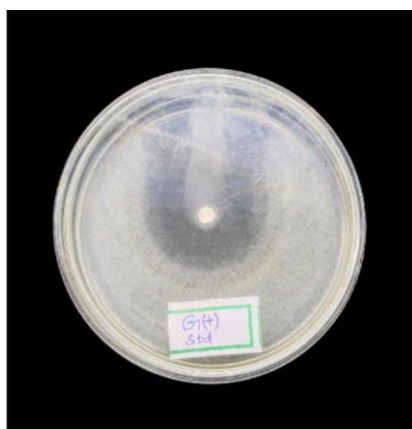


Fig. 1: *BacillusSubtilis*(Gram+ve)-Gentamicin (Standard).

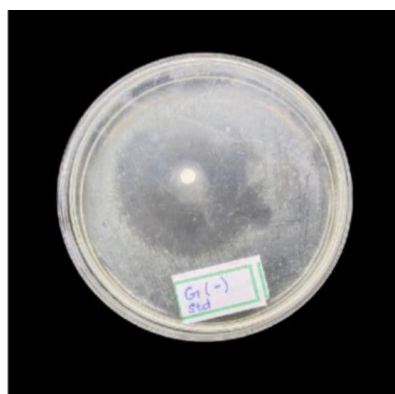


Fig. 2: *EscherichiaColi*(Gram-ve)-Ciprofloxacin (Standard).



Fig. 3: *Bacillus Subtilis* (Gram+ve)-*Cucumis sativus*.

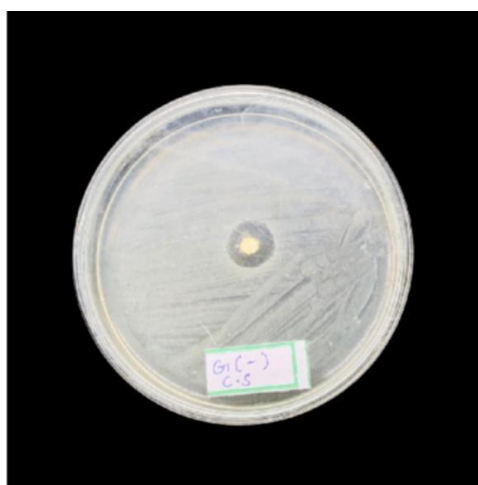


Fig. 4: *Escherichia Coli* (Gram-Ve)-*Cucumis sativus* (Sample).

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