



Phytochemical Analysis Using X-ray Diffraction Spectroscopy (XRD) and GC-MS Analysis of Bioactive Compounds in *Cucumis sativus* L. (Angiosperms; Cucurbitaceae)

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ABSTRACT

Cucumis sativus L. (Cucumber) is an important palatable member of the family Cucurbitaceae. Cucumber not only used as one of the popular vegetables but also it has been a source of conventional remedy since olden times. Since, Cucumber is rich in water content and low in calories hence one of the most preferred diet in many countries. There are several claims regarding its anti-diabetic impending, lipid lowering and antioxidant action. It has a purgative nature hence good for removing metabolic waste and toxins. Its juice is also known to nurture the skin with cooling effect. *Cucumis sativus* fruits are found useful in cases of thermoplegia and hyperdipsia. The seeds also have a preserving effect on the skin, and they are used as carminative. Numerous bioactive phytochemicals have been isolated from cucumber, viz., cucumegastigmanes I and II, cucurbitacins, cucumerin A and B, orienting, vitexin, etc. Regardless of vast investigation on cucumber in horticulture, a relatively less information available about its phytochemical profiling. This study has been done to get the phytochemical profile of this plant using X-ray diffraction spectroscopy (XRD) and GC-MS analysis.

Keywords: Angiosperms, Cucurbitaceae, *Cucumis sativus*, Phytochemical, GC-MS analysis, X-ray diffraction spectroscopy (XRD)

INTRODUCTION

Human and plants have mutualistic relationship since ancient time and they are in natural balance with each other for their existence (Borokini, 2012). Uses of plants as medicines are as old as human civilization and they are still worthwhile for remedial purposes; and an excellent source for future formulations of therapeutic uses (Masum et al., 2013). In the 21st century, allopathic medicines are increasingly in demand, but the associated side effects of these chemically created medicines are now a matter of concern and that is why the focus of populace has been shifted towards the herbal formulations in the last few decades (Qureshi, 2007; Savithramma et al., 2011; Alizadeh et al., 2018). Recently, Willis (2017) reported that almost 30,000 plant species are already been used for remedial purposes across the world. Furthermore, traditional understanding of the medicinal plants has been

playing vital role to produce various important drugs of modern day (Belayneh, 2014). Plants have been utilized as a source of medicine by folk and tribal peoples for decades to treat a variety of ailments (Uniyal, 2006). There are studies that provide evidences that the traditional knowledge was based on the practical experience of people and which has been transferred from one generation to another within the communities (Samar, 2015).

Based on the past knowledge and claims, many efforts have been made to find and validate the potential bioactive properties existing in several plants along with the isolation and characterization to develop herbal based strategies to prevent and control of a wide range of diseases (Farombi and Owoye, 2011). Among all plant groups, the angiosperms are the major contributors of bioactive compounds that are being used in the development of remedies against many ailments that

affect the human health. Various parts of plants produce specific phytochemical which is given in define doses and combinations to treat different diseases (Jima, 2018). Usually these phytochemicals are the derivatives of plant's specific metabolism and provide recognition of that plant as a remedy (Abifarin, 2019). The ranges of ailments that can be cured by these phytochemicals include many common diseases and few of the intricate diseases like cancer and tumors (Debbarma et al., 2017).

It is also evident that the phytochemical profiles of cooked, semi-cooked and fresh plants/plant parts show variations. Hence the plants/plant parts which can be consumed raw/fresh are more beneficial than cooked or semi-cooked ones. In this context the member of the family Cucurbitaceae have an advantage as most of the members are usually eaten as fresh. Keeping this in view this study has been done to get the phytochemicals profile of *C. sativus* (leaves and seeds) using X-ray diffraction spectroscopy (XRD) and GC-MS analysis to get its remedial impending.

MATERIALS AND METHODS

Collection of Plants and parts:

Leaves, fruits and seeds of *Cucumis sativus* were taken for this study. The leaves were collected fresh from field and seeds were procured from Krishi Vigyan Kendra (KVK) Banasthali Vidyapith. The plants parts and seeds were first wash properly and kept in shade to dry subsequently were kept in oven at 30-40°C for 24h (Mandey, 2019). Dried material was grounded with the help of automated grinder to get the powdered material for further steps.

Solvent extraction:

The Soxhlet extraction method was used. 20g of powdered form of plant parts and seeds were taken in filter paper and extract with 250 ml of six different solvent separately (Yadav, 2011; Abubakar, 2020). The extraction process was continued for about 24h till the extractor of the soxhlet become colorless (Kulczyński, 2020). The extracts were then placed on petri plates and dried until all the solvent had evaporated, after which the dried samples were stored in the refrigerator till future use.

Qualitative Phytochemical analysis:

The qualitative analysis of phytochemicals was done using various established procedures of qualitative analysis. The extracts of *C. sativus* were examined for the presence of bioactive chemicals accordingly.

Reagents:

Mayer's reagent:

For Mayer's reagent in Solvent A 0.355g of mercuric

chloride was taken and dissolved in 60 ml of distilled water. In Solvent B 5g of potassium iodide was taken and dissolved in 20 ml of distilled water, both the solutions A and B were mixed and total volume of 100 ml was prepared by adding distilled water.

Wagner's reagent:

For Wagner's reagent 2g of iodine and 6g of potassium iodide dissolved in 100 ml of distilled water.

Benedict's reagent:

For Benedict's reagent preparation, Solution A, 17.3g of Sodium citrate and 10g of Sodium carbonate were dissolved in 80 ml of distilled water and Solution B, 1.73g of Copper sulphate pent hydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was mixed 10 ml of distilled water.

Millions reagent:

In this 0.1g of mercury was dissolved in 9 ml of nitric acid and incubated for 10-15 min and equal volume of distilled water was added.

Alkaloids Test:

Mayer's test:

The presence of alkaloids in the crude samples was confirmed by adding 2ml of Mayer's reagent to the test tube, the resultant white precipitate indicated the presence of alkaloids.

Wagner's test:

To check the presence of alkaloids in the plant sample, 5ml of Wagner's reagent was added to the test tube, resulting red brown precipitate means confirmed presence of alkaloids.

Protein Test:

Ninhydrin test:

In the crude extract, 2ml of 0.2% Ninhydrin solution mixed and boiled properly.

Test of Carbohydrates:

Benedict's test:

The crude extract was mixed with 2ml of Benedict's reagent properly and heated till boiling, the reddish-brown precipitate was appeared which indicated the presence of carbohydrates.

Test for fixed oil and fat:

This test was conducted by adding few drops of alcoholic potassium hydroxide solution (KOH) in crude plant sample then few drops of phenolphthalein were added and boiled on water bath for 2h for formation of soap or partial neutralization of alkali to confirm the presence of fixed oil and fats.

Test for Glycosides:**Liebermann's test:**

In this test, 0.5 ml of chloroform added to crude plant sample, then 0.5 ml acetic acid was added in resulting the violet to blue to green coloration showed the presence of glycosides.

Test for Phenolic compounds and tannin:**Ferric chloride acid test:**

In this test, the crude sample of plant was added to 5% ferric chloride solution, the dark green color as appeared which indicated the existence of phenolic compounds

Lead acetate test:

In this test in the crude extract of plant sample was mixed with few drops of 10% lead acetate the white precipitate confirmed the existence of tannins in the solution.

Test for Flavonoids:**Alkaline reagent test:**

In the plant extract 10% ammonium hydroxide solution was added and the appearance of yellow color indicated the presence flavonoids.

Test for phytosterols:**Liebermann-burchard's test:**

In this test, about 0.5 ml of plant extract as added acetic anhydride afterward 2 drops of concentration sulphuric acid were added to the solution to check the changes of color.

Test for saponins:

In this test, about 2ml of crude plants extract as added 20 ml of distilled water and mixed well for 10 to 15 min the resulting layer of foam indicated the presence of saponins.

Gas Chromatography and Mass Spectroscopy (GC-MS) Analysis:

The methanolic plant sample of *Cucumis sativus* was analyzed using Thermo Scientific TSQ 8000 triple quadrupole Gas Chromatography- MS (trace 1300 GC) which was facilitated with TG 5 MS (30 m × 0.25mm, 0.25µm) column 217. Carrier gas was used as 99.999% Helium gas at constant flow rate of 1 µl/min and a volume of 1.0 µl (Muflihunna, 2021). The temperature was kept at 25°C and ion source temperature was fixed at 230°C. Mass range was kept between 50-500 m/z and run time was 2.48 min. The oven temperature was held at 50°C.

X-Ray Diffraction Analysis:

The plant sample (*C. sativus*) was used to identify the presence of crystalline phase in the powdered form of leaf and fruit using a technique known as X-ray diffraction (XRD). A Soxhlet apparatus method was used for extraction of plant sample after that syringe filtration for the fine filtration of sample. The fine filtered sample was shade dried and then transferred to nano sized fine powder by adding methanol solvent (Guadalupe de la Rosa et al., 2013). The XRD pattern of prepared powder was recorded by Bruker Advance X- Ray diffraction Cu-Kα radiation and Ni filter in the angular range $2\theta = 10-80^\circ$ with angular step size $\Delta 2\theta = 0.05^\circ$.

RESULTS**Qualitative analysis:**

The phytochemical characteristics of *Cucumis sativus* plant leaf and seed tested and summarized in the table-1 and 2. Alkaloids, Protein, Carbohydrate, Glycosides, Phenols, Flavonoids, Tannins and saponins were present in the leaf and seeds of *Cucumis sativus*.

Table 1: Phytochemical Analysis of *Cucumis sativus* leaves and fruits

S.N	Variable	Petroleum Ether	Benzene	Chloroform	Methanol	Isopropanol	Acetone
1	Alkaloids						
	Mayer's test	-	-	-	+	+	+
	Wagner's test	-	-	+	+	-	+
2	Amino acid						
	Ninhydrin test	-	-	-	+	-	-
3	Carbohydrate						
	Benedict's test	+	+	+	++	+	-
4	Fixed oil and Fats						
	Saponification test	-	+	+	++	++	+
5	Glycosides						
	Liebermann's test	+	+	+	+	++	-
	Phenolic compound						
6	And Tannins						

	Ferric chloride test	-	-	-	+	+	+
	Lead acetate test	-	+	-	+	+	+
7	Flavonoids						
	Alkaline reagent test	+	-	-	+	+	-
8	Liebermann Burchard test	-	-	-	++	+	-
9	Protein						
	Million test	+	+	+	++	+	-
	Biuret test	-	+	+	+	+	-
10	Saponin	-	-	+	+	+	-

++ (Highly Presence), + (Moderately Presence), - (Absence)

Table 2: Phytochemical Analysis of *Cucumis sativus* seed

S.N	Variable	Petroleum Ether	Benzene	Chloroform	Methanol	Isopropanol	Acetone
1	Alkaloids						
	Mayer's test	+	-	-	++	+	+
	Wagner's test	-	+	+	+	-	+
2	Amino acid						
	Ninhydrin test	-	-	-	+	-	-
3	Carbohydrate						
	Benedict's test	+	+	+	+	+	+
4	Fixed oil and Fats						
	Saponification test	-	-	+	++	++	+
5	Glycosides						
	Liebermann's test	+	+	+	+	+	-
6	Phenolic compound And Tannins						
	Ferric chloride test	-	-	-	++	+	+
	Lead acetate test	-	+	-	+	+	+
7	Flavonoids						
	Alkaline reagent test	-	-	-	+	++	-
8	Liebermann Burchard test	-	-	-	+	+	+
9	Protein						
	Million test	-	-	+	++	+	-
	Biuret test	-	-	+	+	++	-
10	Saponin	-	-	+	+	+	-

++ (Highly Presence), + (Moderately Presence), - (Absence)

Table 3 represents bioactive compounds of *Cucumis sativus* from GC-MS

S.N.	Area (%)	Reaction time (min)	Compound Name	Molecular Formula	Molecular Weight	Biological Activity
1.	71.36	32.17	Cycloheptasiloxane, tetradecamethyl-	C ₁₄ H ₄₂ O ₇	519	Antibacterial, Antifungal and inhibit tumor growth

2.	92.97	39.13	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298	Antimicrobial
3.	83.23	49.48	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Antibacterial, Anti-inflammatory, anti-malarial
4.	36.91	54.89	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{18}H_{32}O_2$	280	Anticancer, Anti-inflammatory
5.	14.16	55.03	9-Octadecenoic acid, methyl ester, (E)	$C_{19}H_{36}O_2$	296	Antioxidant activity
6.	8.41	55.15	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Antioxidant, Antimicrobial activity, decrease blood cholesterol etc.
7.	69.89	55.79	Methyl stearate	$C_{19}H_{38}O_2$	298	Anti-inflammatory, Lipid metabolism regulator, Gastrin inhibitor etc.
8.	52.15	56.03	Fluconazole	$C_{13}H_{12}F_2N_6O$	306	Inhibit Antifungal activity, Yeast infection, increase immune system.
9.	19.97	56.76	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	Antioxidant activity
10.	10.47	59.91	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$	374	Anti-cholesterol compound.

Gas Chromatography and Mass Spectroscopy (GC-MS) Analysis:

The bio active components in the methanol extract of plant leaf of *C. sativus* was identified by Gas Chromatography and Mass Spectroscopy techniques (Shettima, 2013). Table 2 depicts the existence of 10 bioactive phytochemical compounds presence in the methanol extract of the plant (Marsili, 2000). The bioactive compounds and the Retention time (RT), compound name, molecular structure, molecular weight and their biological activity are present in table 2. The extraction and analysis of plant play a vital role in the pharmaceutical, modernization, development and quality control of herbal formulations (Yusuf, 2021). Figure 2 represents GC-MS analysis of plant sample which shown the presence of compounds, namely two compounds 9,12-Octadecadienoic acid (Z,Z)-, methyl ester and other one as Hexadecanoic acid, methyl ester were found to be considered as major in this fraction with 36.91% and 83.23% peak area respectively. Many minor constituents were also identified such as Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl] methyl]cyclopropyl]methyl]-, methyl ester (10.47%), Cycloheptasiloxane, tetradecamethyl (71.36%), Octadecanoic acid methyl ester (92.97%) etc. The recognized compounds have been reported to possess beneficial biological activity (Table 3).

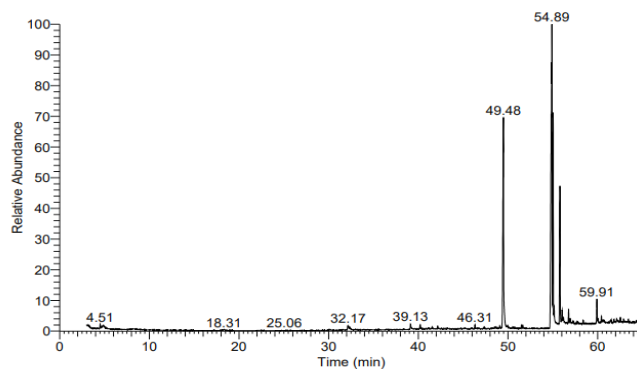


Figure 2. The GC-MS Analysis of *Cucumis sativus*

X-Ray Diffraction Method of *Cucumis sativus*:

X-Ray diffraction method has been used to show the crystalline size and nature of the sample plant of *Cucumis sativus*. The analysis of X-Ray diffraction pattern of *Cucumis sativus* has been shown in figure 2. The few intense peaks at 20.40° , 26.26° , 29.09° & 39.09° with finite width which indicates the presence of crystalline nature and few of them have small width peaks which reflect the semi crystalline nature of the plant sample. The peaks matched with XRD different pattern of different phases of carbon allotropes (Singh 2015).

The determination of FWHM or full width at half maxima has been set on using Gaussian fitting. Debye

Scherer method of X-ray diffraction says that the FWHM of the XRD peak is directly related with crystalline size.

$$D = 0.9 \lambda / \beta \cos \theta$$

Where, λ = wavelength, β = FWHM of peak, θ = angular position.

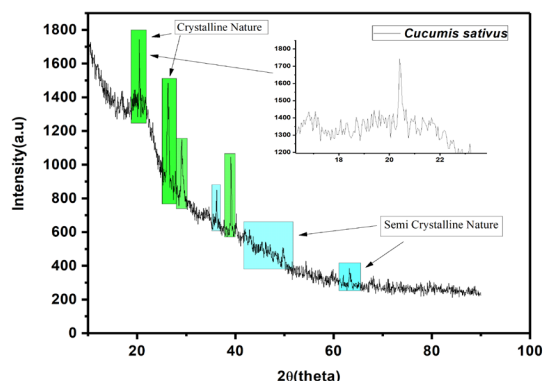


Figure 3. The X-Ray diffraction pattern of the sample plant of *Cucumis sativus*.

DISCUSSION

Plant utilization by human as medicine was a traditional practice which started from about 60,000 year back (Petrovska, 2012; Gomathi et al., 2015; Shanet al., 2020). In the piece of paper has been shown the extracts of the *C. sativus* potent which help in decrease the risk of many diseases like Cholesterol problem, skin problems, diabetic, antimicrobial, cancer (Malik et al., 2019). In general, the suitability of a medicinal plant for use is determined by associating phytochemicals with biological activity (Pachiappan, 2017). The present study reveals the existence of bio-active secondary metabolites in *C. sativus* based on the qualitative and quantitative analysis using standard procedures. The importance of this plant can be used as a medicinal plant (Nasser, 2020). In qualitative and quantitative analysis of *C. sativus* confirm the presence of alkaloids, carbohydrates, amino acids, flavonoids, glycosides.

Herbal formulations are regaining its popularity among the consumers worldwide, but the major drawback is the lack of quality profiling (Sharma, 2011). To tackle this problem, modern techniques like Chromatography should be used for analysis of the various components in many unexplored plants. Presently, Gas Chromatography (GC) is frequently used for the phytochemical analysis of the plants (Krishnamoorthy, 2014) whereas chromatography plays a fundamental role analytical technique for quality control and standardization of phytotherapeutics (Gomathi et al., 2015). The present analysis

of GC-MS bioactive compounds like, hexadecanoic acid, Cycloheptasiloxane, and methyl stearate in *C. sativus* (Belakhdar 2015) and confirmed the results obtained by antibacterial and antifungal and antitumor properties (Srivastava, 2015), likewise, 10-Octadecenoic acid, 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester showed anticancer and anti-inflammatory bioactivity (Begum et al., 2018) whereas, methyl ester acts as antioxidant and antimicrobial (Gupta, 2016).

CONCLUSIONS

All the identified phytochemicals in methanolic plant extracts have known for their considerable bioactivity hence would be used in future study to get new interesting source of herbal formulations.

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