

Qualitative and Quantitative Phytochemical analysis of Fruits of *Cucumis dipsaceus* Ehrenb ex. Spach

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ABSTRACT

Plant compounds are non-nutritive, essential metabolites used to cure diseases and play a vital role in the preparation of natural drugs. Characterization of plant compounds in unexplored and less explored plant paves way for drug preparation and natural medicines. *Cucumis dipsaceus* (hedgehog cucumber) is a less explored plant in India though it possesses essential compounds. In the present study, fruits of *Cucumis dipsaceus* Ehrenb ex Spach belongs to Cucurbitaceae family were used to test for its phytochemical constituents. Solvents viz., Petroleum ether, Benzene, Acetone, Ethanol, and Water were used to extract the phytochemicals. Colour, consistency and yield percentage of the crude extracts were also recorded. Preliminary phytochemical screening was carried out to know the presence of secondary metabolites (alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, glycosides, gums & mucilage, fixed oils) in fruit extracts. Functional groups of the fruit extracts were analyzed by FT-IR spectral analysis. Quantitative phytochemical analysis was done to estimate the total content of alkaloids, flavonoids, triterpenoids, glycosides, and saponins. The results revealed the presence of secondary metabolites by preliminary phytochemical analysis. Crude extracts were ranging from light to dark brown in color. The consistency of the crude extracts was found to be semisolid, less sticky and sticky. Highest yield percentage was observed in aqueous fruit extract. FT-IR spectral analysis indicated the presence of alkanes, alkenes, esters, sulfones, and alcohols. Estimation of the total content of phytochemicals specifies that higher amount of alkaloid (62mg/g) was observed at aqueous extract and Saponin (370mg/g) in ethanol extract. Thus, the fruits of *Cucumis dipsaceus* found to have pharmacologically important compounds.

Key words: *Cucumis dipsaceus*, qualitative analysis, FT-IR, quantitative analysis.

1. INTRODUCTION

Phytochemicals are non-nutritive plant compounds present in various plant parts which play a vital role in drug preparation to treat various diseases without any side effects. Phytochemicals like Alkaloids, Flavonoids, Triterpenoids, and other compounds produce a physiological effect on the human body [1]. Plant compounds have a role in reducing the risk of chronic diseases such as cancer, diabetes, Alzheimer's disease and cardiac disease [2]. Current research focuses to evaluate and characterize plant constituents to establish its ability towards medicinal value.

Cucumis dipsaceus Ehrenb ex. Spach is an annual climbing herb belongs to the family Cucurbitaceae. Members of the Cucurbitaceae family (cucumber, pumpkin, zucchini, melon varieties, gourd varieties etc.) are well known as economically as well as medicinally important plants. The genus *Cucumis* mainly includes cucumber varieties and melon varieties, of which many of the species were originated in African countries [3]. *Cucumis dipsaceus* has originated in Ethiopia and commonly called Arabian cucumber, Hedgehog cucumber and teasel gourd [4]. Arabian cucumber is an unexplored plant in India and recent years, it is found distributed in some places of Western Ghats particularly around Marudhamalai foothills (Tamil Nadu).

Nutritional, antinutritional and antioxidant properties of the fruits and leaves of *Cucumis dipsaceus* were reported [5]. Preliminary phytochemical analysis, functional groups and phytochemicals present in crude leaf powder was previously studied [6] which revealed the presence various groups of fatty acids. Functional groups and bioactive phytochemicals of fruits of *Cucumis dipsaceus* were not yet

reported. Hence, the present study focused to study the phytochemicals of *Cucumis dipsaceus* fruits with the aid of preliminary phytochemical screening, FT-IR spectral analysis. Quantitative phytochemical analysis was also carried out to find the total alkaloid, flavonoid, Triterpenoid, glycosides and Saponin content.

II. MATERIALS AND METHODS

2.1 Collection and preparation of plant material

Fruits of *Cucumis dipsaceus* Ehrenb ex. Spach were collected in Coimbatore district, Tamil nadu during the month of October- November (2016). Small cut pieces of cleaned fruits were shade dried and milled into coarse powder by mortar and pestle. The powders were stored in sterilized air tight bottles and kept at room temperature for subsequent analysis.

2.2 Preparation of Fruit extracts

Extract preparation of the present study was carried out by hot percolation (soxhlet extraction) method. Solvents like Petroleum ether, Benzene, Acetone, Ethanol and Water ranging from non-polar to polar were taken to extract the phytochemicals. 20g of fruit powder and 150mL of the solvent were used for the extraction process. Concentrated crude extract was stored in sterile container at room temperature for further analysis. Colour, consistency and yield percentage of crude extracts were recorded for the fruits of *Cucumis dipsaceus*.

2.3 Qualitative Analysis

2.3.1 Preliminary Phytochemical screening

Qualitative phytochemical examination for plant metabolites (alkaloid, flavonoid, tannin, steroids, triterpenoids, saponins, glycosides, gums & mucilages and fixed oils) was carried out for different fruit extracts of *Cucumis dipsaceus*.

Detection of Alkaloids

i) Mayer's test: Few mL of extract was added with few drops of Mayer's reagent by the side of the test tube. A white or creamy precipitate indicates the test as positive^[7].

ii) Wagner's test: Few mL of extract was added with 1 or 2 mL of Wagner's reagent by the side of the test tube. A reddish-brown precipitate confirms the test as positive^[8].

Detection of Flavonoids

10% HCl and 5% NaOH test: 1 mL of extract was treated with few drops of 5% NaOH solution which turns into yellow colour then it becomes colourless by addition of few drops of 10% HCl solution, which indicates the presence of flavonoids^[9].

Detection of Tannins

Ferric chloride test: About 0.5 g of plant extract was boiled in 20 mL of distilled water in a test tube and then filtered, 1 mL of 0.1% FeCl₃ was added to the filtrate. Appearance of brownish green or blue black colour indicates the presence of tannins^[10].

Detection of Steroids and Triterpenoids

i) Libermann- Burchard's test: 1 mL of extract treated with few mL of acetic anhydride followed by 1 mL concentrated sulphuric acid by side of the test tube, brown ring is formed at the junction two layers. The upper layer turned to green colour indicates the presence of steroids and formation of deep red colour in lower layer indicates the presence of triterpenoids^[11].

ii) Salkowski's test: 1 mL of extract treated with 1 mL of chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenoids^[12].

Detection of Saponins

Foam test: Few mL of extract was diluted with the same amount of distilled water. The suspension is shaken for 15 to 30 seconds and allowed to stand for minimum 10 seconds. Formation of one or two centimetre layer of foam at the end indicates the presence of saponins^[13].

Detection of Glycosides

Keller - Kiliani test: One mL glacial acetic acid, three drops of Iron (III) chloride and few drops concentrated sulphuric acid added with two mL of extract. Appearance of Green-blue colour indicated the presence of cardiac glycosides^[14].

Detection of Gum and Mucilages

Whistler and BeMiller test: The extract (100 mg) was dissolved in 10 mL of distilled water and 25 mL of absolute alcohol was added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages^[15].

Detection of Fixed oils and Fats

Spot test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil^[13].

2.3.2 FT-IR analysis

Fourier Transform Infrared (FT-IR) Spectrophotometer is used to identify various functional groups present in various plant extracts. In the present study, FT-IR analysis was carried out for the fruit extracts of *Cucumis dipsaceus*. The infrared spectra were documented between the range of 500-4000 cm^{-1} using KBr pellet in Thermo Electron Scientific equipment.

2.4 Quantitative Analysis

Determination of Total Alkaloid content

Alkaloid content was determined for fruit extracts by spectrophotometric method using atropine as standard. One mg of plant extract was dissolved in dimethyl sulphoxide (DMSO) and then 1 mL of 2N HCl was added and filtered. To the filtrate, 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. Then the mixture was diluted with chloroform and standard solutions of atropine (20, 40, 60, 80 and 100 $\mu\text{g/mL}$) were also prepared. The absorbance for fruit extracts and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract^[16].

Determination of Total flavonoid content

Total flavonoid content of fruit extracts was measured by the aluminium chloride colorimetric assay using quercetin as standard. To the one mL of extract, four mL of distilled water was added followed by addition of 0.30 mL of 5% sodium nitrite and 0.3 mL of 10% aluminium chloride with interval of 5 minutes. Then 2 mL of 1M NaOH was treated and diluted to 10 mL with distilled water. Standard Quercetin solutions (20, 40, 60, 80 and 100 $\mu\text{g/mL}$) were prepared and absorbance recorded at 510 nm using UV-Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract^[17].

Determination of Triterpenoid content

Triterpenoid content of fruit extracts was determined using oleanolic acid as standard. 10 mg of oleanolic acid diluted with ethyl acetate to prepare 1.0 mg/mL standard solution. Fruit extracts were also diluted with ethyl acetate to prepare sample solutions and colour developing agent (Vanillin solution) also prepared using Chromogenic method. The absorbance of sample and standard solutions was measured at 210 nm wavelength, a blank solution as the control reference^[18-20].

Determination of Glycosides content

Glycosides content was determined by the method as described by Solich *et al*^[21] with some modifications using securidaside as standard. For determination of glycoside content, Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH) was mixed with few mL of fruit extract and after an hour, the mixture was diluted with distilled water. Same procedure repeated for different concentration of securidaside standard solutions. The absorbance was measured at 495 nm by UV-Visible Spectrophotometer. Total glycosides were expressed as mg of securidaside/g of extracts.

Determination of Saponin content

Total Saponin content determination was done using diosgenin as standard. Fruit extracts were diluted with water and made into sample solution. Standard solution was prepared by dissolving 10 mg of diosgenin in 16 mL of methanol and 4 mL of distilled water. Standard solutions of diosgenin (20, 40, 60, 80 and 100 $\mu\text{g/mL}$) were prepared with 80% aqueous methanol. For total saponins estimation, 500 μL of sample, 500 μL of 0.5% anisaldehyde reagent, were mixed and kept aside for 10 min. Later, 2 mL of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature at 60°C. After 10 min tubes were cooled and absorbance was taken at 435 nm. Same procedure was performed for standard solutions to record values. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard^[22].

III. RESULTS AND DISCUSSION

3.1 Yield and Nature of Crude fruit extracts

Cucumis dipsaceus (hedgehog cucumber) fruits were used in the present study and crude extracts were prepared using different solvents like Petroleum ether, Benzene, Acetone, Ethanol and Water by soxhlet extraction method. The colour, consistency and yield percentage of crude extracts were noted (Table-1). The maximum percentage of yield was observed in water extract (C5) of 9.45% and

Petroleum ether extract (C1) of 4.35%. Fruit extracts were semisolid in nature and variation of colour from pale yellow to dark brown also observed.

Table-1 Yield percentage and consistency of fruit extracts of *Cucumis dipsaceus*

Fruit extracts	Solvents used in soxhlet extraction	Colour of crude extract	Consistency of crude extracts	% of yield (w/w)
C1	Petroleum ether	Pale yellow	Oily, semisolid	4.35
C2	Benzene	Pale brown	Oily, semisolid	1.35
C3	Acetone	Brown	Semisolid	1.1
C4	Ethanol	Dark brown	Less sticky, semisolid	2
C5	Water	Dark brown	Sticky, semisolid	9.45

C1- Petroleum ether extract; C2- Benzene extract; C3- Acetone extract; C4- Ethanol extract; C5- Aqueous extract

3.2 Preliminary Phytochemical analysis

Phytochemical analysis is useful method primarily to know the presence of major compounds using different tests and reagents. Preliminary qualitative phytochemical analysis of fruit extracts of *Cucumis dipsaceus* revealed the presence of alkaloids and Flavonoids in C3, C4 and C5 extracts. Triterpenoids, Gums and mucilages were present in all fruit extracts whereas saponins, glycosides and steroids showed presence in C4 and C5 extracts. Fixed oils were present C1 and C2 extracts (Table-2). The studies on leaves of *Cucumis dipsaceus* indicates the presence of alkaloids, Flavonoids, tannins, saponins, terpenoids and glycosides^[23-24]. Other species of the same genus, *Cucumis sativus* fruit extracts showed the presence of Alkaloids, Glycosides, steroids, saponins, tannins, Flavonoids, terpenoids and resins^[25]. *Cucumis melo* var. *agrestis* fruits indicated the presence of tannins, saponins, alkaloids, glycosides^[26] whereas seeds showed presence of cardiac glycosides, terpenoids, steroids and quinines^[27].

Table 2- Preliminary Phytochemical analysis of Different Fruit extracts of *Cucumis dipsaceus*

Compounds	Tests	Fruit Extracts				
		C1	C2	C3	C4	C5
Alkaloids	Mayer's test	-	-	+	+	-
	Wagner's test	-	-	+	+	+
Flavonoids	10% HCl & 5% NaOH test	-	-	+	+	+
Tannins	5% FeCl ₃ Test	-	-	-	-	+
Steroids	Liebermann's Burchard test	-	-	-	+	+
Triterpenoids	Liebermann's Burchard test	+	+	+	+	+
	Salkowski's test	+	+	+	+	-
Saponins	Foam test	-	-	-	+	+
Glycosides	Killer & Killian Test	-	-	-	+	+
Gums & Mucilages	Whistler & Bemiller test	++	++	+	+	+
Fixed Oils	Spot Test	+	+	-	-	-

+ = presence; - = absence; C1- Petroleum ether extract; C2- Benzene extract; C3- Acetone extract; C4- Ethanol extract; C5- Aqueous extract

3.3 FT-IR analysis of Fruit extracts

The presence of functional groups in different fruit extracts of *Cucumis dipsaceus* were identified by Fourier Transform Infrared (FT-IR) spectroscopic analysis. The absorption frequency, functional groups and compounds of each fruit extract identified were illustrated in Table-3 and Fig.1-5. The absorption frequency 3008.95cm⁻¹ responsible for the functional group C-H stretch, alkene compound was identified in C1, C2, and C3 extracts. C-H stretch alkane groups were found in all fruit extracts. S=O stretch functional group of sulfone compound has absorption frequency 1159.22 cm⁻¹ in C1; 1161.15cm⁻¹ in C2 and C3; 1348.24 cm⁻¹ in C4 extracts was identified. S=O stretch of Sulfoxide (1070.49cm⁻¹, 1049.28 cm⁻¹) in C5 extract was identified. C=O stretch functional group responsible for ester compounds were identified in C1, C2, C3 and C5 fruit extracts. In C5 extract, functional group O-H bend of carboxylic acid was identified at 1390.68 cm⁻¹ frequency and C-H bend functional group (1573.91 cm⁻¹ and 1539.20 cm⁻¹) responsible for aromatic compounds was identified. Halogen compounds were identified in C1, C2 and C5 extracts. C-I stretch at 580.57 cm⁻¹ in C1; C-Cl stretch at 570.93 cm⁻¹ in C2; 584.43cm⁻¹, 509.21 cm⁻¹ in C5 extracts. Presence of alcohols was also recorded in fruit extracts. Hence, FT-IR analysis of fruit extracts of *Cucumis dipsaceus* found to have alkenes,

alkanes, esters, sulphates and halo compounds. FT-IR spectral analysis of *Cucumis dipsaceus* leaves^[6] revealed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics and nitrocompounds present in crude leaf powder.

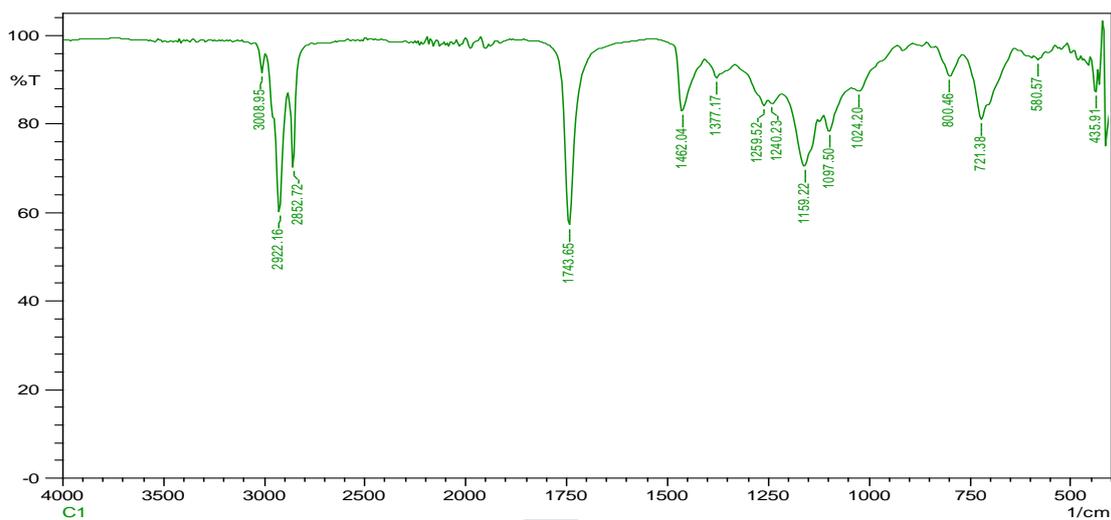


Fig.1- FTIR Spectrum of Petroleum ether extract (C1) of *Cucumis dipsaceus* fruits

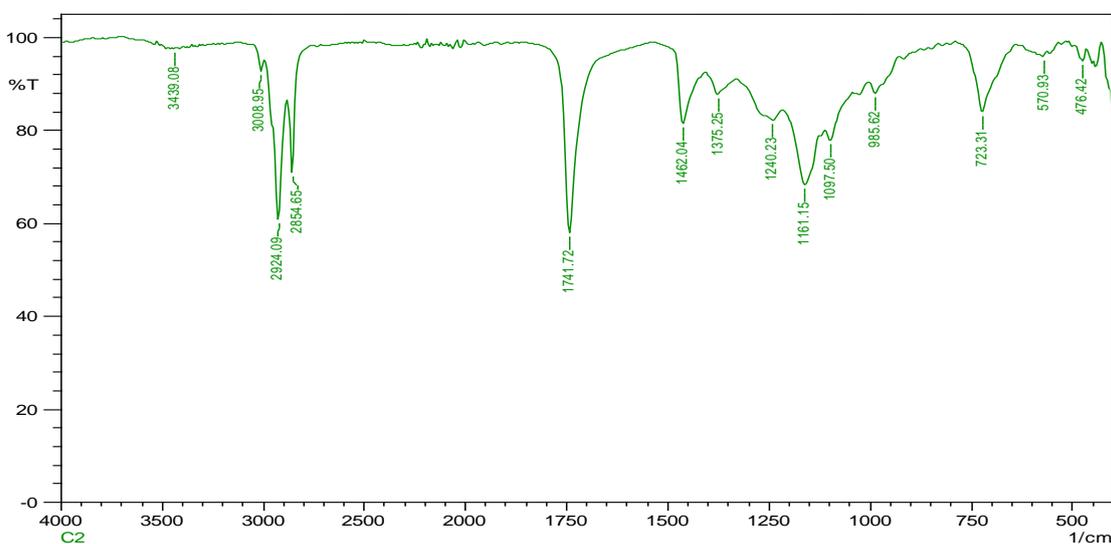


Fig.2- FTIR Spectrum of Benzene extract (C2) of *Cucumis dipsaceus* fruits

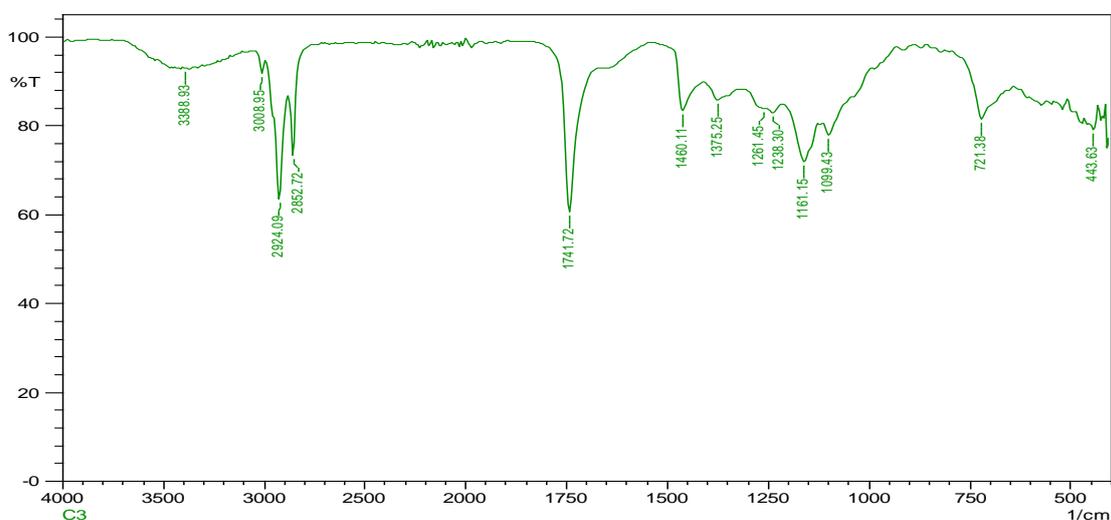


Fig.3- FTIR Spectrum of Acetone extract (C3) of *Cucumis dipsaceus* fruits

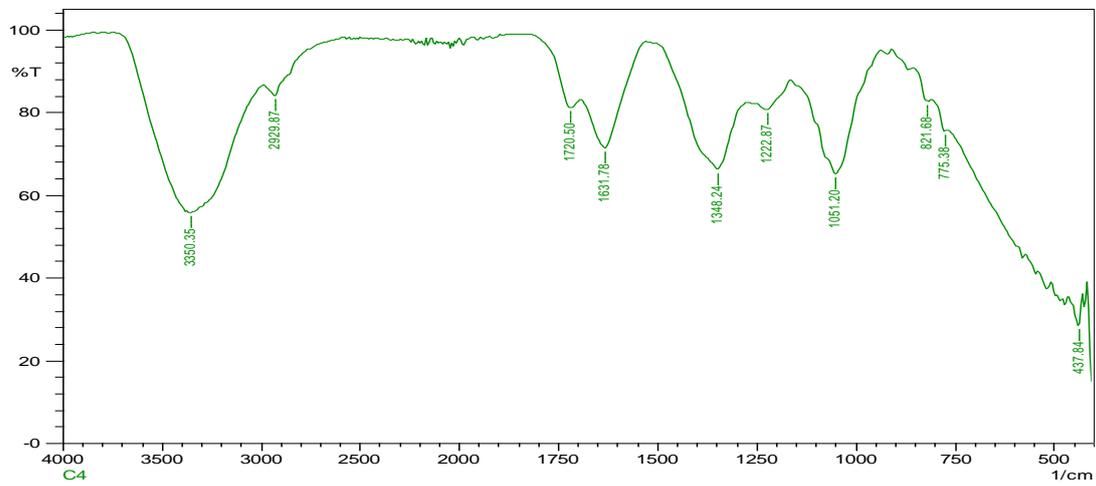


Fig.4- FTIR Spectrum of Ethanol extract (C4) of *Cucumis dipsaceus* fruits

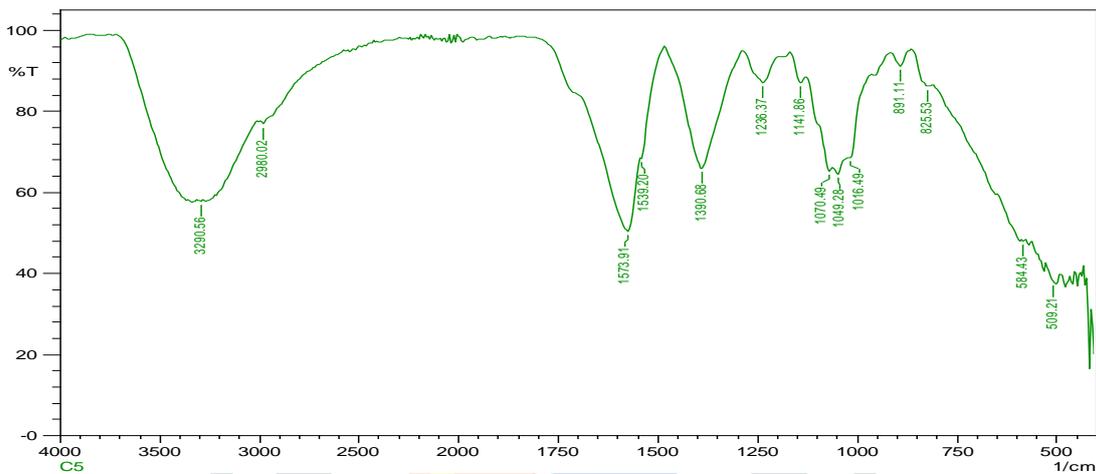


Fig.5- FTIR Spectrum of Water extract (C5) of *Cucumis dipsaceus* fruits

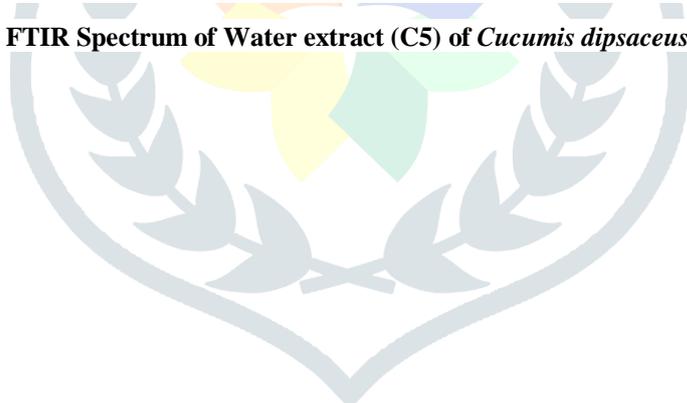


Table-3 FTIR Absorption frequencies of Fruit extracts of *Cucumis dipsaceus*

Fruit extract	Frequency Range (cm ⁻¹)	Absorption frequency (cm ⁻¹)	Functional groups and Compounds
C1	3000-2500	3008.95	C-H stretch, alkene
		2922.16	C-H stretch, alkane
		2852.72	C-H stretch, alkane
	2000-1650	1743.65	C=O stretch, esters
		1462.04	C-H stretch, alkane
	1400-1000	1377.17	S=O stretch, sulphate, sulfonate, O-H bend, carboxylic acid
		1259.52	C-O stretch, aromatic ester
		1240.23	C-O stretch, alkyl aryl ether
		1159.22	S=O stretch, sulfone
		1097.50	C-O stretch, secondary alcohol
1024.20		C-N stretch amine	
1000-650	800.46	C=C bend, alkene	
	721.38	C=C bend, alkene	
	580.57	C-I stretch, halo compound	
C2	4000-3000	3439.08	O-H stretch, alcohol
	3000-2500	3008.95	C-H stretch, alkene
		2924.09	C-H stretch, alkane
		2854.65	C-H stretch, alkane
	2000-1650	1741.72	C=O stretch, esters
	1600-1300	1462.04	C-H bend, alkane
		1375.25	S=O stretch, sulfonate, sulphate
	1400-1000	1240.23	C-O stretch, alkyl aryl ether
		1161.15	S=O stretch, sulfone
		1097.50	C-O stretch, secondary alcohol
985.62		C=C bend, alkene (monosubstituted)	
1000-650	723.31	C=C bend, alkene (cis)	
	570.93	C-Cl stretch, halo compound	
C3	4000-3000	3388.93	N-H stretch, aliphatic amine, O-H stretch, alcohol
	3000-2500	3008.95	C-H stretch, alkene
		2924.09	C-H stretch, alkane
		2852.72	C-H stretch, alkane
	2000-1650	1741.72	C=O stretch, esters
	1600-1300	1460.11	C-H bend, alkane
		1375.25	C-H bend, alkane
	1400-1000	1261.45	C-O stretch, aromatic ester
		1238.30	C-O stretch, alkyl aryl ether
		1161.15	S=O stretch, sulfone
1099.43		C-O stretch, secondary alcohol	
1000-650	721.38	C=C bend, alkene (cis)	
C4	4000-3000	3350.35	N-H stretch, secondary amine
	3000-2500	2929.87	C-H stretch, alkane
	2000-1650	1720.50	C=O stretch, carboxylic acid, aliphatic ketone
	1670-1600	1631.78	C=C stretch, alkene (monosubstituted)
	1400-1000	1348.24	S=O stretch, sulfone
		1222.87	C-O stretch, alkyl aryl ether
		1051.20	C-O stretch, primary alcohol
	1000-650	821.68	C=C bend, alkene (trisubstituted)
775.38		C=C bend, alkene	
C5	4000-3000	3290.56	O-H stretch, alcohol
	3000-2500	2980.02	C-H stretch, alkane
		1573.91	C-H bend, aromatic compound
	2000-1650	1539.20	C-H bend, aromatic compound
		1390.68	O-H bend, carboxylic acid
		1236.37	C-N stretch, amine
	1400-1000	1141.86	C-O stretch, ester; tertiary alcohol
		1070.49	S=O stretch, sulfoxide
		1049.28	S=O stretch, sulfoxide
		1016.49	S=O stretch, sulfoxide
		891.11	C=C bend, alkene
		825.53	C=C bend, alkene (trisubstituted)
	1000-650	584.43	C-I stretch, halo compound
509.21		C-I stretch, halo compound	

C1- Petroleum ether extract; C2- Benzene extract; C3- Acetone extract; C4- Ethanol extract; C5- Aqueous extract

3.4 Quantitative Phytochemical analysis

The results obtained from the quantitative analysis of fruit extracts indicated the presence of phytoconstituents from highest to lowest extent (Table-4). In Aqueous extract, alkaloid content (62mg/g) and flavonoid content (12mg/g) was observed higher when compared to other extracts. Higher amount of Saponin was noted (370mg/g) in ethanol extract. Glycosides (42mg/g) and Triterpenoids (72mg/g) found higher in acetone extract. Earlier study reported the amount of flavonoids in the fruits of *Cucumis trigonus* is found to be the maximum (1.59 mg/kg) and Saponin content (0.05mg/kg) found to be minimum^[28].

Table 4- Quantitative phytochemical analysis of fruit extracts of *Cucumis dipsaceus*

Fruit Extract	Alkaloids (mg/g)	Flavonoids (mg/g)	Triterpenoids (mg/g)	Glycosides (mg/g)	Saponin (mg/g)
C1	1.6	0.84	26	54	20
C2	1.2	2.5	18	97	4.1
C3	35	1.4	72	42	160
C4	57	3.2	68	33	370
C5	62	12	41	38	260

C1- Petroleum ether extract; C2- Benzene extract; C3- Acetone extract; C4- Ethanol extract; C5- Aqueous extract

IV. CONCLUSION

The present study was carried out to find out the phytochemicals present in fruits of *Cucumis dipsaceus* Ehrenb. ex spach using preliminary phytochemical screening, FT-IR analysis. Quantitative phytochemical analysis was also carried out to find out total content of Alkaloid, Flavonoid, Triterpenoid, Saponin and Glycosides. Qualitative phytochemical screening proved the presence of alkaloids, Flavonoids in Acetone, Ethanol and Aqueous extracts. Triterpenoids, Gums and mucilages were present in all fruit extracts whereas saponins, glycosides and steroids showed presence in Ethanol and Aqueous extracts. Fixed oils were present in Petroleum ether and Benzene extracts. FT-IR analysis indicated the presence of alkanes, alkenes, esters, carboxylic acids, sulphate and halo compounds. Among all the fruit extracts, aqueous extract of *Cucumis dipsaceus* have the higher amount of alkaloids and Flavonoids. Higher amount of Triterpenoids found in acetone extract, glycosides in benzene extract, Saponin in ethanol extract were noted. Thus, the fruits of *Cucumis dipsaceus* possess pharmacologically important bioactive phytochemicals.

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