EXPLORING THE PHYTOCHEMICALS OF *CUCUMIS DIPSACEUS* FRUIT FROM WESTERN GHATS

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Abstract

The present study is about the anatomical characters of Cucumis dipsaceus Ehrenb ex Spach and to find the phytochemical constituents present in both unripen and ripen fruits of C. dipsaceus through Preliminary phytochemical analysis and GC- MS spectrum analysis. The methanolic extract of C. dipsaceus shows the presence of carbohydrates, glycoside, flavanoids and terpenoids in ripen fruits and the methanolic extract of C. dipsaceus shows the presence of carbohydrates, protein, glycosides, flavanoids, terpenoids in unripen fruits of C. dipsaceus. In both ripen fruits and in unripen fruits of Cucumis dipsaceus, terpenoids are present. About 7 compounds such as Benzeneacetamide, n-heptyl-n-methyl; Nonadecanoic acid; Acetic acid, (aceteloxy); Acetohydroxamic acid; 2- Propanol, 1-methoxy; Propane, 1-(1- ethoxyethoxy); 1-octanesulfonyl chloride by GC-MS analysis of C. dipsaceus unripen fruits. About 5 compounds such as Butanoic acid, 2-hydroxy-3,3-dimethyl; Bicyclo [3.3.1] nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-; acetohydroxamic acid; 2,3-butanediol; Propanoic acid, 2-hydroxy-, ethyl ester, (s)- by GC- MS analysis of C. dipsaceus in ripen fruit. GC-MS analysis revealed that many compounds have the antimicrobial and anticancerous property.

Keywords: *Cucumis dipsaceus, ripen fruit, unripen fruit, Preliminary phytochemical analysis, GC-MS spectrum analysis*

1. Introduction

Cucurbitaceae members are extensively distributed in both the tropical [1] and subtropical areas. The family has about 130 genera and 800 species [2]. The crucial genera belonging to the family are *Trichosanthes, Lagenaria, Luffa, Benincasa, Momordica, Cucumis, Citrullus, Cucurbita, Bryonopsis* and *Corallocarpus* [3]. The family consist of numerous bioactive compounds which includes cucurbitacins, triterpenes, sterols and alkaloids [11].

The genus *Cucumis* consist of 17 species and they are found to be monoecious perennials, except *Cucumis Kala hariensis A. Meeuse* and *C. rigidus* [12]. The character of Cucumis was the evolution of smooth fruits with spiny fruits, and the mode of fruit opening are much more plastic. [12] About 13 species and 2 varieties of *Cucumis* were reported from India [4] and found to cosmopolitan in distribution [4]. However, Kirkbride, 1993 reported that the genus is represented by 32 [13]. Besides cucumber (*Cucumis sativus* L.) and melon (*C. melo* L.), the species *C. anguria* (West Indian gherkin) and *C. metuliferus* (African horned cucumber) are commercially

explored in several areas as well [13]. Other wild species originating mostly from arid and/or semi-arid regions of Africa, respectively, are cultivated as ornamental plants (*C. dipsaceus* – "hedgehog gourd", *C. myriocarpus* – "gooseberry gourd") [13]

Cucumis dipsaceus is a widely cultivated plant variety of gourd family it can be consumed in the unripe, green form. It is locally named as Khira or Sasha [11]. Phytochemical screening of the ethanolic extract of leaves and stems of *Cucumis sativus* which is one of the species from family Cucurbitaceae possessed phytoconstituents such as alkaloid, glycoside [7][11], steroid [11], saponin [7] [11] and tannin [7][11], flavonoid [7][11] and reducing sugars [11]. While alkaloid [11], glycoside [11], steroid [11], flavonoid [11], saponin [11] and tannin [11] were found in the crude chloroform extract [11]. *C. dipsaceus* Ehrenb. ex Spach., is originated in Ethiopia [5] and was distributed to nearby countries in Africa such as Kenya [5], Somali [6], Tanzania [6][7], Uganda [6][7], Sudan [6][7] and extended to Southern Egypt [6]. It is known by numerous common names like Arabian cucumber [5], hedgehog gourd [5], Pepino diablito [5], teasel gourd [5], wild gourd etc. The leaves of the species are consumed as leafy vegetables and the fruit juice is topically applied to prevent hair loss [5].

Like the plant growing in wild from the foothills of Maruthamalai hills of Coimbatore district in Tamil Nadu [8], the species was reported from the different states of India [8], Attappadi hills of Palakkad district, Kerala [8]and Karnataka states [8]. The wide distribution of the species was confirmed in India after the present observations of the species in wild state at three locations of two districts of Maharashtra state [8]. However, despite the extensive utilisation of C. dipsaceus in traditional medicine to manage, in Kenya, C. dipsaceus is used as traditional medicine to manage microbial infections and diarrhoeal, there is scanty empirical proof to support the claimed pharmacologic efficacy and safety [14].

The aqueous and methanolic leaf and fruit extracts of *Cucumis dipsaceus* have significant antidiarrheal efficacy and varying degrees of antimicrobial activity *against S. enteritidis, E. coli, B. subtilis, P. aeruginosa*. *C. albicans* was only susceptible to the aqueous leaf extract of the plant. (Kimathi *et al.,* 2022). Cucurbit plants were used in traditional treatment of different diseases. They have demonstrated anti-inflammatory [7], antitumor, hepatoprotective [7], cardiovascular and immunoregulatory activities. They are rich source of proteins, with many biological activities like anti-fungal, anti-bacterial, anti-viral, anti-diabetic, anti-tumour and anti-AIDS. It also has carminative and antacid property [11]. The Fruit of the plant was used for constipation and aid indigestion [11]. Seeds has the properties of tonic, diuretic and anthelmintic [11]. Leaf extract or juice taken from leaves induces vomiting and aid digestion [11]. Mature uncooked cucumbers help individuals suffering from celiac disease, malnutrition due to poor absorption inner intestine and also promote skin health [11]. To treat dysentery immature cucumbers are cooked and consumed [11]. A poultice made from fresh cucumbers can be applied to burns [11].

2. Materials and Method

2.1 Collection of plant material

The fruits of *Cucumis dipsaceus* from various places of Coimbatore were collected. Freshly collected fruits were cleaned to remove adhering dust and then are dried under the shade in room temperature. The dried sample was powdered by pulverizer and used for further studies.

2.2 Preparation of plant extract

The powdered fruit was extracted successively with organic solvent, methanol. This extract is taken for further studies.

2.3 Preliminary phytochemical analysis [20]

The preliminary screening of selected plants with methanolic extract were analysed by standard method. The test for carbohydrates, protein, amino acid, glycosides, steroids, flavanoids, alkaloids, saponnins, tannins, terpenoids were analysed.

2.4 Gas Chromatography- Mass Spectrometer (GC-MS) analysis [21]

Gas Chromatography- Mass Spectrophotometer (GC-MS) analysis was executed out on fruit extracts (Petroleum ether, Acetone, Ethanol and Water) to identify the extracted phytocompounds. The Clarus 680 GC was taken for the analysis a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) Clarus 680 GC was taken. Helium was used as carrier gas at a constant flow of 1 ml/min to separate these components. The injector temperature adjusted to 260°C during the overall process. extract sample of 1μL was inserted into the instrument the oven temperature was taken at: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, it was then kept for 6 minutes. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. Later database spectrum of known components stored in the GC-MS NIST (2008) library. were compared to the obtained spectrum

3. Result

Cucumis dipsaceus is widely cultivated plant variety of gourd family it can be consumed in the unripe, green, form. The species is found in well aerated soil and scattered populations of a few individuals on gravelly. The thick root stock of plant remains dormant during dry period of the time, surviving the plant under xeric conditions. Plants are annual, scabrous and climbing herbs. Stems are weak, quadrangular, grooved, branched, hispid. Leaves are ovate in structure, shallowly trilobed, densely hairy on both surfaces, base cordate, apex acute to obtuse, serrate margin; petioles and simple tendrils are present. Inflorescence monoecious; small yellow flowers are present. Fruits are oblong, when unripen it's green and yellow when matured, many-seeded, densely aculeate. The fruit smell like cucumber but tastes bitter [8] (figure 1 A and B).

The common anatomical character of plant *C. dipsaceus* in family Cucurbitaceae have the presence of hollow pith at the centre of the stem and presence of multicellular trichomes in both leaf and stem. The stem is hollow with 5 ridges and furrows. Uniseriate epidermis passing over ridges and furrows. Trichomes originate from the epidermis, (figure 2-D) shows the presence of trichomes in stem. The Collenchyma that is present below the epidermis. Endodermis as the innermost layer of cortex. Sclerenchyma represents the 4-5 layers of pericycle made of lignified cells. Vascular bundles are 6 in number and are arranged in 2 rows where, Outer row corresponds to the ridges and Inner row corresponds to the furrows. Xylem occupies the centre of bundle. Xylem consist of protoxylem and metaxylem. Protoxylem, has smaller vessels on inner side and Metaxylem, is the bigger vessels a little higher up. pith is present in the centre (figure 2-C).

In leaf, multicellular trichomes are present, (fig- B) shows the presence of trichomes. Uniseriate epidermis with thick cuticle. Presence of upper and lower

epidermis. Lower epidermis is single layered with thin cuticle. Mesophyll cells are present below the epidermis. It contains 2- 3 layers of elongated, cylindrical cells, closely packed together palisade parenchyma. Numerous chloroplasts are present. The midrib region consists of bicollateral vascular bundles, lies towards upper epidermis and phloem which lies towards the lower epidermis. Vascular bundles are surrounded by the border parenchyma or bundle sheath. The result of the anatomical structure of *Cucumis dipsaceus* leaf was presented in figure 2 -A.

Medicinal plants contain some organic compounds that has physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids etc that are synthesise by primary or rather secondary metabolism of living organisms [15]. Secondary metabolites are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [15]. Plant products have been part of phytomedicines that can be derived from barks, leaves, flowers, roots, fruits, seeds [15].

In the present work, preliminary phytochemical analysis was carried out in ripen and unripen fruits of *C. dipsaceus.* By comparing the ripen and unripen fruits, Carbohydrates was present in both ripen and unripen fruits (Table 2) shows the formation of purple colour. Protein content was found to be present only in unripen fruits, it forms a yellow colour which indicates its presence (Table- 2) and protein content was found to be absent in ripen fruits. This may due to the increase in the content of amino acid level (Martin *et al.*, 2011). In contrast, Amino acid was found in unripen fruits (Table 2) and are absent in ripen fruits. glycosides (Table 2) and flavanoids (Table 2) are present and steroids, alkaloids, saponins and tannins are absent in both types of fruits. According to Saxena, *et al.* (2013), flavonoids have been reported to exert multiple biological property including antimicrobial, antioxidant, cytotoxicity, anti-inflammatory, as well as antitumor activity [16]. It is mainly present as colouring pigments in plants also function as potent antioxidants at various levels. Terpenoids are present more in ripen fruits and lesser in unripen fruits. In fruits, terpenoids are present more ripened fruits, it gives aroma and colour to fruits [17]. While comparing *C. dipsaceus* with that of *Cucumis sativus* of same family. Flavonoids, mainly present as colouring pigments in both *C. sativus*. This was argument with our results where flavonoid is present in *C. dipsaceus*. Tannins, saponin, steroids are absent in in *C. dipsaceus* but tannins, saponins and steroids are present in *C. sativus* [16].

The cornerstone for qualitative and quantitative analysis of food contaminants and residues is the Gas chromatography-mass spectrometry (GC-MS). It is fast and sensitive, which provides high peak capacity that enables the determination of thermally stable and volatile compounds. Latest detection/separation solutions, such as fast chromatography, full two-dimensional GC (GC × GC), triple quadrupole. The principles, recent developments and future prospects of these new food determination approaches [18].

By GC-MS analysis, volatile compounds present in both ripen and unripen fruits of *C. dipsaceus.* About 7 compounds are present in unripe fruits of *C. dipsaceus* (Table- 4 & figure 4-A). Testosterone, oxime has antimicrobial, antitumor, antifungal properties. Nonadecanoic acid, are certain insects as pheromones [19]. Acetic acid, aceteloxy is effective in preventing cataract development in severely galactosemic rats when administered as an eyedrop solution [19]. Acetohydroxamic acid inhibits the hydrolysis of urea and production of ammonia in urine infected with urea splitting organisms. 2-propanol, 1-methoxy Propane, 1-(1- ethoxyethoxy) compounds are present. 1-octanesulfonyl chloride has antimicrobial activity.

About 5 compounds are present in ripen fruits of *C. dipsaceus* (Table- 4 & figure 4-B). Butanoic acid, 2-hydroxy- 3,3-dimethyl and 2,3-butananediol has Anticancerous activity [19]. Bicyclo [3.3.1] nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)- (Gopinath *et al.,* 2015) and propanoic acid, 2-hydroxy-, ethyl ester, (s)-. Acetohydroxamic acid is the urease inhibitor in plants and bacteria (Goldstein *et al.,* 2015). GC-MS analysis shows that Acetohydroxamic acid is common in both ripen and unripen fruits of *C. dipsaceus*. The major compound present in the unripen fruit is testosterone, oxime and the major compound present in ripen fruit is butanoic acid, 2- hydroxy-3,3-dimethyl. Majority of the compounds are present in ripe and unripe fruits has the property of antimicrobial and anticancerous activity (Table- 4) Further studies such as animal studies and nutritional analysis of both ripe and unripe fruit of *C. dipsaceus* to be studied in future.

3.1 Table 1

ANATOMICAL FEATURE OF *C. DIPSACEUS* STEM AND *LEAF*

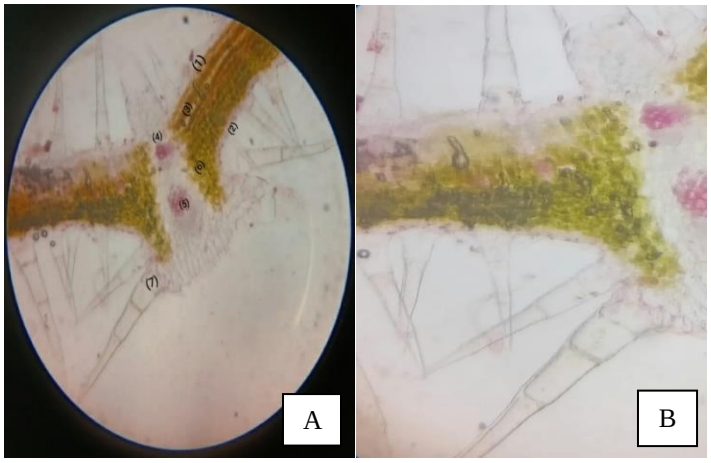
Sl. No.	Plant Part	Characteristic Features
1.	**T. S. of stem.**	**Epidermis:** Uniseriate epidermis passing over ridges and furrows
		Collenchyma: Collenchyma present below epidermis.
		Parenchyma: 2 to 3 layers are present.
		Sclerenchyma: Sclerenchyma represents the 4-5 layers of pericycle made of lignified cells
		Vascular bundles: Vascular bundles are 6 in number and are arranged in 2 rows. Outer row corresponds to the ridges Inner row corresponds to the furrows. Hollow with 5 ridges and furrows.
		Xylem: Xylem occupies the centre of bundle. Protoxylem, smaller vessels on inner side. Metaxylem, bigger vessels a little higher up.
		Pith: pith is present in the centre.
2.	**L.S. of Leaf**	**Epidermis**: Uniseriate epidermis with thick cuticle. Lower epidermis is single layered with thin cuticle
		Mesophyll: Mesophyll cells are present below the epidermis. 2- 3 layers of elongated, cylindrical cells, closely packed together palisade parenchyma. Contain numerous chloroplasts are present.

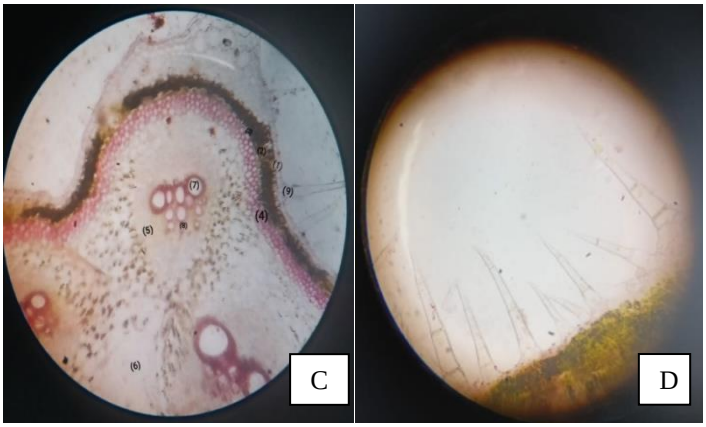
		Vascular bundles: The midrib region consists of bicollateral vascular bundles, lies towards upper epidermis and phloem which lies towards the lower epidermis. Vascular bundles are surrounded by the border parenchyma or bundle sheath.

3.2. Figure 1

Habit of *Cucumis dipsaceus*

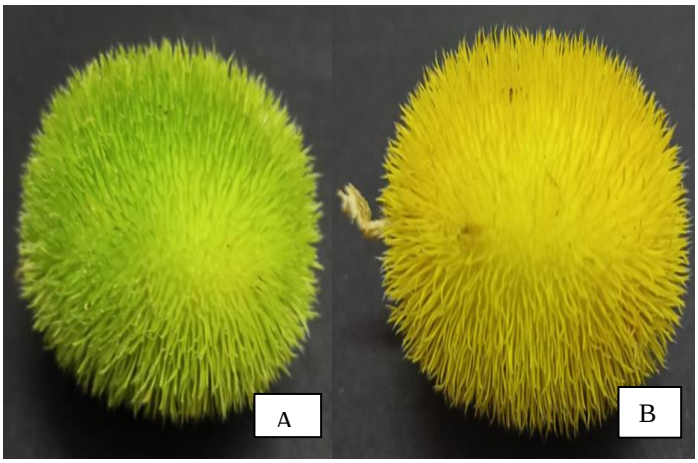
3.3 Figure 2





(A) 1- Upper epidermis, 2- lower epidermis, 3- palisade tissue, 4- Phloem, 5- Xylem, 6- Mesophyll cells, 7- multicellular trichomes. **(B)** Multicellular trichomes of leaf. **(C)** 1- epidermis, 2- collenchyma, 4- parenchyma, 5- phloem, 6- pith, 7- metaxylem, 8- protoxylem, 9- multicellular trichomes. **(D)** Multicellular trichomes of stem.

3.4 Figure 3



(A) Unripen fruit **(B)** Ripen fruit

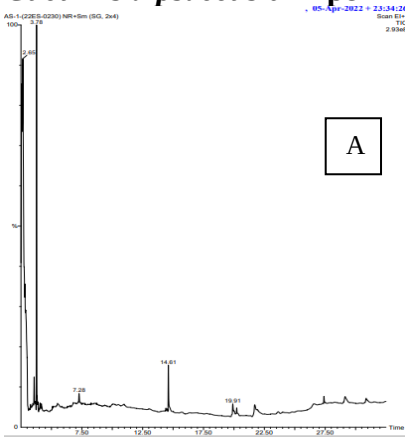
Table 2.
Preliminary Phytochemical analysis of *Cucumis dipsaceus Ehrenb. ex. Spach.*

Sl.No.	Phytoconstituents	Ripen	Unripen
1.	Carbohydrates	++	++
2.	Protein	-	+
3.	Amino acid	-	+
4.	Glycosides	++	++
5.	Steroids	-	-
6.	Flavanoids	++	++
7.	Alkaloids	-	-
8.	Saponnins	-	-
9.	Tannins	-	-
10.	Terpenoids	+++	++

+++ indicates high content; ++ and + indicates low content; - indicates absence of phytochemicals

3.5 Figure 4

(A) GC- MS Spectrum of *Cucumis dipsaceus* unripen fruit.



(B) (B) GC- MS Spectrum of *Cucumis dipsaceus* ripen fruit.

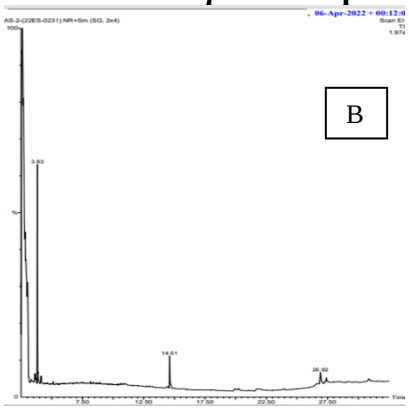
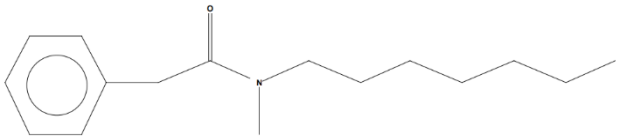
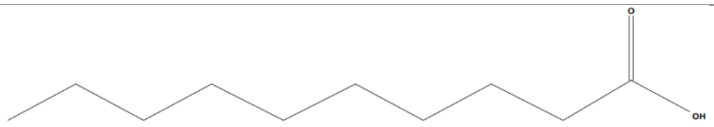
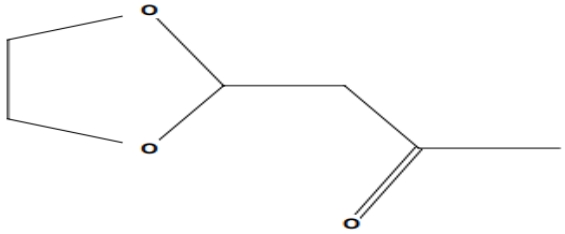
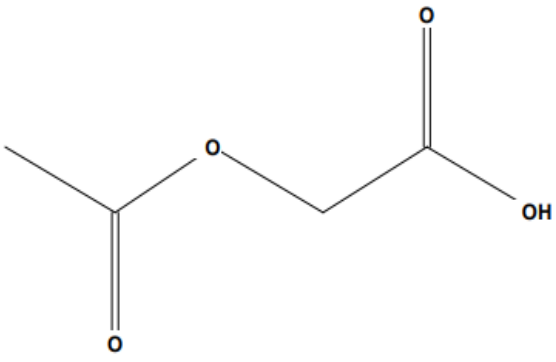


Table – 3 GC-MS ANALYSIS OF UNRIPEN FRUIT IN *CUCUMIS DIPSACEUS*

COMPOUND NAME	STRUCTURE	USES
Testosterone, Oxime		Antimicrobial, antitumor, antifungal properties. [19]
Nonadecanoic acid		It is certain insects as pheromones. [19]
Acetic acid, (aceteloxy)		Effective in preventing cataract development in severely galactosemic rats when administered as an eyedrop solution. [19]
Acetohydroxamic acid		urinary infections caused by urea-splitting bacteria may reduce pathogenicity of the infecting organism and may lead to prevention and/or dissolution of stones commonly associated with it. (Griffith *et al.*, 1975)

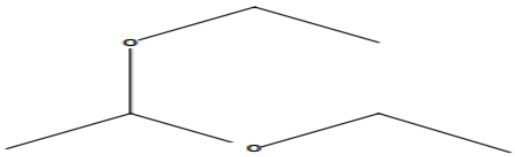
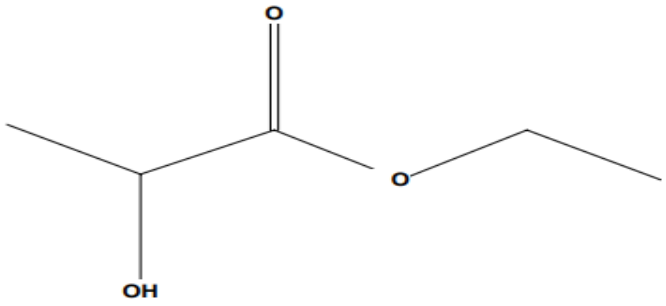
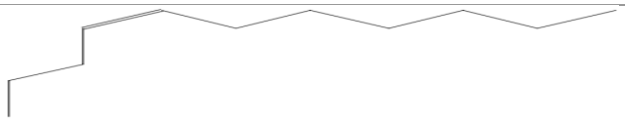
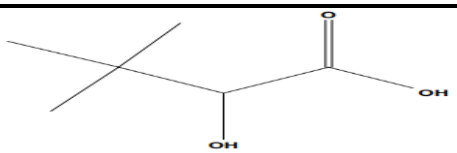
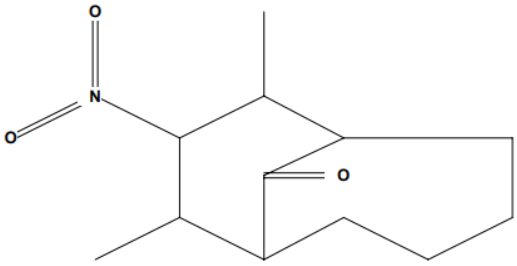
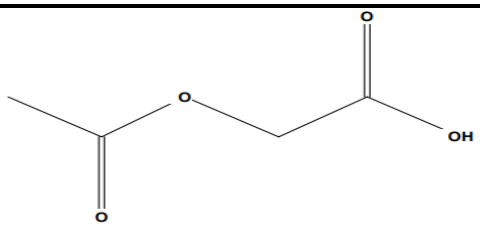
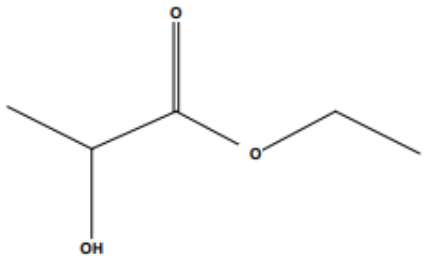
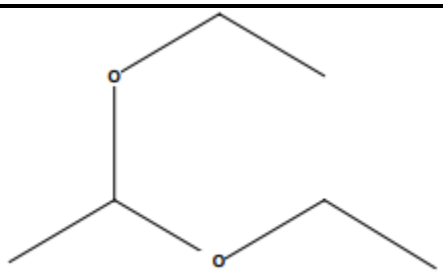
2- Propanol, 1-methoxy		
Propane, 1-(1-ethoxyethoxy)		
1-octanesulfonyl chloride		Antimicrobial activity (Robert and Robert,1954)

Table 4 GC-MS ANALYSIS OF *CUCUMIS DIPSACEUS* RIPEN FRUIT

COMPOUND NAME	STRUCTURE	USES
Butanoic acid, 2-hydroxy-3,3-dimethyl		Anticancerous activity. [19]
Bicyclo [3.3.1] nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-		Antimicrobial (Gopinath. *et al.*, 2012)

acetohydroxamic acid		urease inhibitor in plants and bacteria (Goldstein *et al.*, 2015)
2,3-butanediol		
Propanoic acid, 2-hydroxy-, ethyl ester, (s)-		

4 Conclusion

The present study concerns the anatomical study, preliminary phytochemical analysis and GC- MS analysis of *Cucumis dipsaceus* plant. It was concluded that several phytochemical compounds are present in the *Cucumis dipsaceus*. That reveals the presence of Carbohydrates, glycosides, flavonoids, terpenoids in ripen fruit of *C. dipsaceus*. In unripen fruits of *C. dipsaceus* shows the presence of carbohydrates, protein, aminoacid, glycosides, flavonoids, terpenoids. Terpenoid content was found to be high in ripened fruit compared to unripen fruit of *C. dipsaceus*. However, unripen fruit has moderate content of aminoacid compare to ripen fruit. in this study, we have concluded that ripe fruits appear to be the best choice for consumption of this edible wild fruit. GC-MS analysis of both fruits shows the possible activity such as anti-cancer activity, anti-microbial activity, etc.,

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