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Antimicrobial Studies and GC-MS Analysis on Cucumber Peel (*Cucumis sativus* L.)

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Abstract
Globally, the application of medicinal plants in the management of acute and chronic wounds can be considered a common occurrence in most traditional medicine practices. In view of this, many plants in the tropical and subtropical regions have been screened for their wound healing activities. Consequently, plants having antimicrobial activity against multidrug-resistant (MDR) pathogens can be considered great assets. Therefore, this study evaluated ethanolic and aqueous extracts of medicinal plants (*Cucumis sativus* L.) for their antimicrobial activities against MDR bacterial pathogens isolated and GC-MS analysis. The maximum zone of inhibition observed in ethanol extract compared to aqueous extract for each bacterium was as follows *Bacillus subtilis* (43 mm), *Escherichia coli* (31 mm). The results of the present study will create a way for the invention of herbal medicines for several ailments by using *Cucumis sativus* L. plants, which may lead to the development of novel drugs. Presence of secondary metabolites has been identified from the phytochemical studies. Hence, this medicinal plant may be used as a source for treating many diseases.

Keywords
Antimicrobial activity, Ethanolic extract, *Bacillus subtilis* and *Cucumis sativus* L.

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INTRODUCTION
Plants are as the principal source of nutrients for animals, and sometimes as an unwelcome source of anti-nutritional factors that interfere with an animal's ability to maximize utilization of ingested nutrients. The present review considers the background of interest in the use of plants and their extracts are as alternative performance enhancers, and the non-nutrient bioactive compounds of plants (5). Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (11). According to (15) phytochemicals are bioactive non-nutrient plant compounds that have protective or disease preventive property. They confer plants with

odour (terpenoids), pigmentation (tannins and quinines) and flavour (capsacin). These bioactive components are said to be responsible for the antimicrobial effects of plant extracts *in vitro*. They are grouped as flavonoids, alkaloids, glycosides, saponins, tannins, terpenoids, carbohydrates and sterols. (20) have isolated the antimicrobial sphingolipids from cucumber stem. Cucumber (*Cucumis sativus* L.) belongs to family Cucurbitaceae, is most widely cultivated vegetable crop all over the world (18). Cucumber is the fourth most important vegetable crop after tomato, cabbage and onion (20). Although its calorie and nutritional value is very low, it is a primary source of vitamins and minerals in the human diet (14). Due to high content of potassium (50-80 mg/100g), cucumber can highly be useful for both high and low blood pressures (8). Cucumber is a widely cultivated plant of gourd family which is eaten in the unripe, green form. Its fruit extract has shown free radical scavenging and analgesic activities in mice (12), carminative and antacid property (19). A large number of medicinal plants are used as alternate medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical compounds present in the medicinal plants will provide some information on the different functional groups responsible for their medicinal properties.

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (7). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (4). Recently it is clearly known that they have roles in the protection of human health when their dietary intake is significant.

Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (9). Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals (2). These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent research demonstrate that many phytochemicals can also protect human against diseases (17).

The determination of phytoconstituents is largely performed by relatively expensive and often laborious techniques that are gas chromatography (GC) and liquid chromatography (LC) combined with specific detection schemes (21). Analysis of chemicals has become easier and more cost-effective owing to the development of hyphenated chromatographic techniques, which are GC or LC-MS. GC-MS analysis can identify nature of compounds even less than 1mg present in the crude plant extract (13). However, simple, cost-effective, and rapid tests for detecting phytocomponents are necessary. In recent years Gas Chromatography–Mass Spectrum (GC-MS) technique has been increasingly employed to analyze the secondary metabolites present in the medicinal plants, as this technique has been proved to be a best valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds (10).

Cucurbitaceae is a plant family, also known as gourd family, which includes crops like cucumbers, squashes, luffas and melons. Cucurbits form an important and a big group of vegetables crops cultivated extensively in the subtropical and tropics countries. The family consists of about 118 genera and 825 species. Plants of this family have many medicinal and nutritional benefits. Cucumber (*Cucumis sativus* L.) is one of the monoecious annual crops in the Cucurbitaceae family that has been cultivated by man for over 3, 000 years. With respect to economic importance, it ranks fourth after tomatoes, cabbage and onion in Asia.

MATERIALS AND METHODS

Materials

The experimental material selected for the present study is Cucumber (*Cucumis sativus* L.) belongs to the family Cucurbitaceae. The plant material was collected from Puliyankulam village of Kovilpatti taluk of Tuticorin district of Tamil Nadu, India. It is situated 8km away from sub-district headquarter kovilpatti and 60km away from district headquarter Tuticorin. The taxonomic features of collected species have been

confirmed with the 'Flora of Presidency of Madras' (3) and the 'Flora of the Tamilnadu Carnatic' (16). (Figure-1)

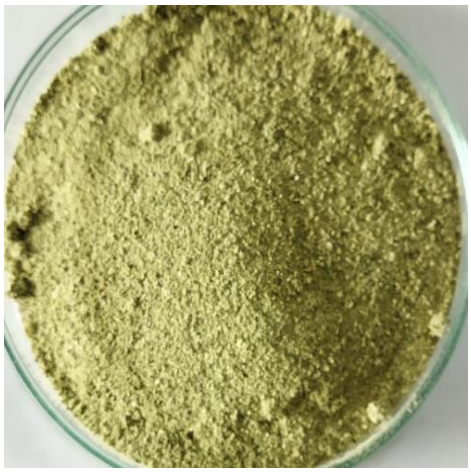
Figure. 1. Natural Habit of Cucumber (*Cucumis sativus* L.)

Fruit

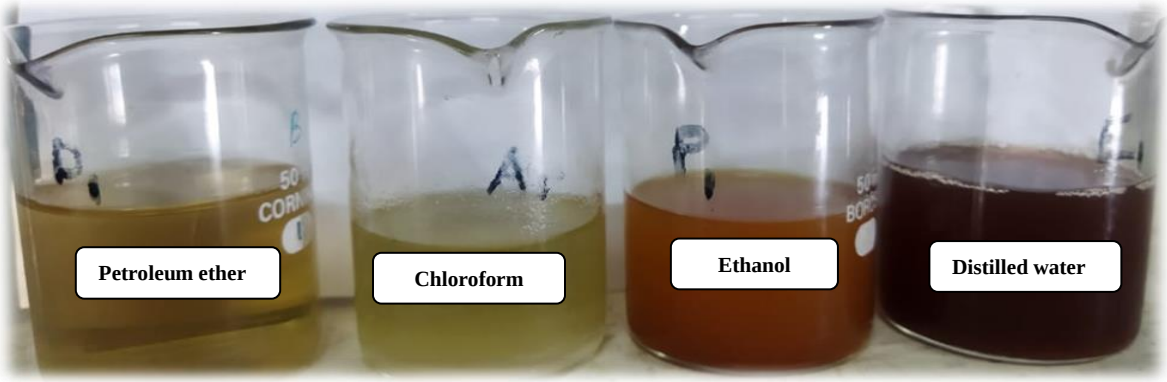
Peel of Cucumber

Dry peel

Powder



Different solvent extracts of dry peel of Cucumber (*Cucumis sativus* L.)



Phytochemical analysis

Extraction

Mature and healthy plants were collected and washed thoroughly, and the plant material was cut into small pieces, dried at room temperature. The dried plant was ground to fine powder. About 30 gm of plant powder was taken in a digestion flask fitted to the Soxhlet apparatus and extracts were obtained with different solvents such as, petroleum ether, chloroform, and ethanol. The aqueous extract was prepared directly by boiling the powder with distilled H_2O. These extracts were concentrated and were kept in brown bottles and used for further analysis.

The extracts were evaporated to dryness on a water-bath. The plant extracts were off with distillation apparatus and yielded quantities of extracts in ethanol solvents were obtained and were further taken to evaluate the Phytochemical studies (6).

Gas Chromatography-Mass Spectrometry Analysis

The GC-MS analyses were carried out in a shimadzu GC-MS-QP 2010 gas chromatograph fitted with a DBI (Methylphenyl siloxane, 30 m x0.25 mm id. d) capillary column. Carrier gas, helium with a flow rate of 0.7 ml/min; column oven temperature 70^0C, 5 min in 180^0C, 180-260^0C at 3^0C/min, 5 min in 60^0C, 260-280^0C at 0.2^0C/min and finally 5 min in 280^0C; injector temperature, 280^0C detector temperature 290^0C, volume injected, 1µL of TMS ether derivatives in *n*-hexane (2%); split ratio, 3:0. The MS operating parameters were as follows: ionization potential 70 eV; ion source temperature 200^0C; quadrupole 100^0C amu, eV voltage 3000 volts.

The concentrated extract was injected into the GC-MS instrument (Hewlett Packard 5890 GC-MS with Mass Selective Detector with an HP-1 glass capillary column). The sample is volatilized at the injection port and eluted through a capillary column under increasing temperature. As the sample moved through the column, various components were separated due to their affinity for the stationary phase of the column and could be identified by retention time (the time it takes for a compound to pass through the column and gas chromatograph system). Each chemical component in a sample had a distinct retention time measured in minutes, shown in a peak on a graph which measured abundance on the ordinate against retention time on the abscissa. The integrated peak was correlated to the concentration of the chemical. A mass selective detector breaks up each chromatographic component into fragment ions, which were shown by their abundance, with each ion represented as a vertical line in increasing molecular weight. The height of each line corresponds to the abundance of that ion. The resulting mass spectrum was unique to that chemical. This mass spectrum formed a "Fingerprint" that could identify the compound by a computer search of mass spectra. A computer search of the mass spectra corresponding to all the chromatographic peaks for a sample should yield a statistical match for nicotine at a 12.9 min retention time value if they were present two modes of GC-MS were possible with this instrumental method. First, there was a "Scan" mode which observed at all the constituents of a sample, listed whatever chemical components were present.

Compound Identification

Components of the ethanolic extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST '98 MS computer library (Wiley).

Antimicrobial Activity

The antibacterial activities of different solvents of dry peel of cucumber (*Cucumis sativus* L.) extract were tested by agar disc diffusion method (1). The antibacterial activity of isolated plant extraction pellets was tested by Kirby-Bauer method. The bacteria were collected from Tirunelveli. The test organisms used for assay were *E. coli*, *Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antibacterial activities of different solvent extracts were evaluated by measuring the zone of inhibition method. The samples for each bacterial strain were subcultured in individual agar slants. The name, gram reaction, nature and distribution of the bacterial strains used for the purpose of bioassay (Table 2).

a. Preparation of plant extract

Extracts were made from air dried samples. 30 g of the dry peel powder of Cucumber (*Cucumis sativus* L.) (powdered test materials) was separately extracted successively with 250 ml of petroleum ether (60-80º C), ethanol and distilled water. This sequence of solvents allowed for leaching all compounds based on their polarity. The individual fractions were collected and concentrated to obtain crude extracts. Water extract was prepared separately. For the purpose of experimental use, each extract sample was dissolved in respective solvent so as get 1/10 solution.

b. Microbial strains

The antibacterial activity of isolated plant extraction pellets was tested by the agar disc diffusion method. The bacterium was collected from Liberty Microbiological Diagnostic Laboratory, Vannarpettai, Tirunelveli. The bacterial strains were grown on Muller

Hinton (MH) agar plates at 37º C and maintained on nutrient agar slants, while fungi were grown at 30˚ C and maintained in potato dextrose agar slants. Each organism was maintained in a separate culture medium and was recovered for testing by sub-culturing on a fresh medium. Inoculums of each bacterial strain were transferred in 10 ml of Muller Hinton agar broth and incubated overnight at 37° C.

c. Preparation of Muller Hinton Agar Medium

Muller Hinton Agar - 35 gm.
Distilled water - 100 ml.

35 gm of Muller Hinton Agar was dissolved in distilled water, boiled the content for complete dissolution of ingredients. The media along with petri dishes, pipette and metallic borer were sterilized in an autoclave for 15 minutes at 121º C and 15 psi pressure. The media were poured into petri dishes under aseptic condition.

d. Muller Hinton Broth

Muller Hinton Agar - 120 gm.
Distilled water - 100 ml

Muller Hinton Agar was dissolved in distilled water. The medium was filtered through cheese cloth and then autoclaved it.

e. Preparation of sterile antibiotic discs

Antimicrobial activity was assayed by filter paper disc diffusion method. Whatman No. 1 filter paper of 5 mm diameter was used. These discs were sterilized before use. The extracts of the medicinal plants were added to the sterile disc. Each sterile disc was incorporated individually with 200-500 µl of an extract of the medicinal plants using micropipette. Precautions were taken to prevent the flow of the solvent extract of the outer surface of the disc. The condensed extracts were applied to the disc.

f. Antibacterial assay

Antibacterial assay was conducted by the method described by (13) with some modification. 0.5 ml of the dilute microbial culture was spread on sterile Muller Hinton agar plates. The presoaked and dried discs were placed on the seeded plates and gently pressed down to assure contact.

Amikacin (10 µg/ml) was used as negative control. The plates were incubated at room temperature for 24 hrs. After the incubation period the inhibition zone around the discs were measured and recorded. Three replicates for each concentration were maintained.

Antifungal study

The standard strain used for the study is *Aspergillus flavus* and *Candida albicans*. This was grown on Sabourauds dextrose agar (SDA) (Hi Media Laboratories Pvt. Ltd; Mumbai, India) overnight at 37° C for 24 hours and 48 hours. 3-5colonies of standard strain of *Aspergillus flavus* and *Candida albicans* were suspended in 2 ml of sterile normal saline and vortexed. The turbidity of the homogenous suspension was adjusted to approximately 0.5 McFarland standards.

a. Preparation of Sabourauds dextrose agar (SDA) medium

Dextrose - 40 g
Peptone - 10 g
Agar - 15 g

Suspend 65 g of the medium in one liter of distilled water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121° C for 15 minutes. Cool to 45 to 50°C and pour into petri dishes or tubes for slants. Final pH (at 25° C) 5.6 ± 0.2.

b. Preparation of Potato dextrose agar (PDA) medium

Potato - 200 g.
Dextrose agar and distilled water

To prepare potato infusion, boil 200 g sliced, unpeeled potatoes in 1-liter distilled water for 30 min. Filter through cheesecloth, saving effluent, which is a potato infusion (or use commercial dehydrated form). Mix with Dextrose, Agar and Water and boil to dissolve. Autoclave 15 min at 121° C. Dispense 20-25 ml portions into sterile 15 × 100 mm petri dishes. Final pH, 5.6 ± 0.2. The sterile swab was dipped in suspension and swabbed on dried plates of Sabouraud's dextrose agar to get lawn culture. 6 mm sterile filter paper discs were purchased and sterilized. These were placed and inoculated on dried SDA plates. 10 µl of the extraction was placed on the disc. These plates were incubated at 37° C. Zone of inhibition was noted around the disc at 24 and 48 hrs. These experiments were repeated for three times and average diameter was recorded. The negative control used in the study was the respective solvents and the positive control was Clotrimazole.

RESULTS AND DISCUSSION

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus, in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacogenetic studies is still more reliable, accurate and inexpensive means. According to World Health Organization (20) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be

carried out before any tests are undertaken (Anonymous, 2002).

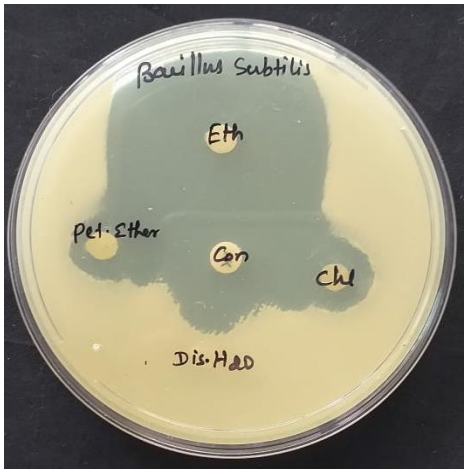
Antibacterial activity Screening

A number of synthetic drugs have been used to cure various diseases caused by pathogenic microbes in man. These drugs produced side effect due to overdose. Several medicinal plants have been identified and used to cure bacterial infection to human beings. In the present investigation antibacterial activity of the dry peel of Cucumber (*Cucumis sativus* L.) was reported with six pathogens and their zone of the inhibition was tabulated. The results indicated that the plant extracts have good antimicrobial activity against different microorganisms. Recently, the use of plant extracts against bacteria has increased because of the gradual increase in drug resistance among microorganisms. A number of synthetic drugs have been used to cure various diseases caused by pathogenic microbes in human beings. Several medicinal plants have been identified and used to cure bacterial infection to human beings.

In the present study the antibacterial activities of different solvent extracts of dry peel of Cucumber (*Cucumis sativus* L.) were investigated and showed good activity for the zone of inhibition against four human pathogens (Table 2 and Figure 3). The ethanol solvent extract exhibited different zones of inhibition against various microorganisms. Among the different extract of dry peel of Cucumber (*Cucumis sativus* L.) showed maximum level of zone of inhibition. *Bacillus subtilis* showed maximum level of inhibition against ethanol extract (43mm) and *E. coli* showed maximum zone of inhibition in ethanol extract (31mm). But compare with antibiotic there has more in activity. *Staphylococcus aureus* exhibited minimum level of inhibition against aqueous extracts (Figure 3).

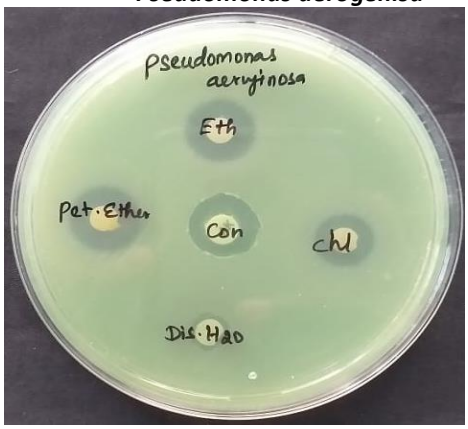
Figure No. 3. Antimicrobial activity of dry peel extracts of Cucumber (*Cucumis sativus* L.) using disc diffusion assay

Bacillus subtilis

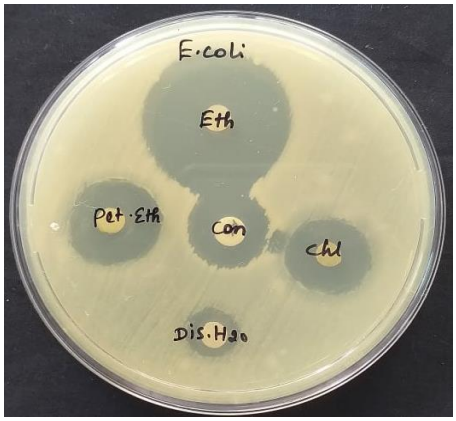


Staphylococcus aureus

Pseudomonas aerogenisa



E. coli



Aspergillus flaves ***Candida albicans***

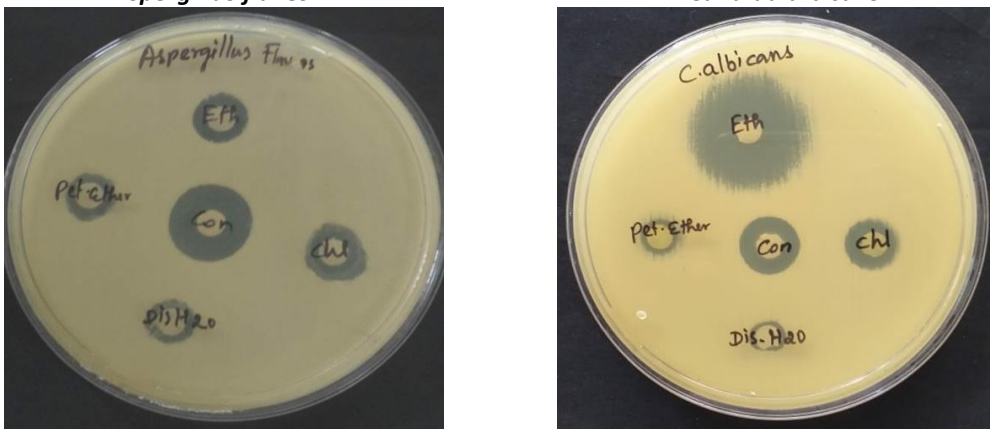
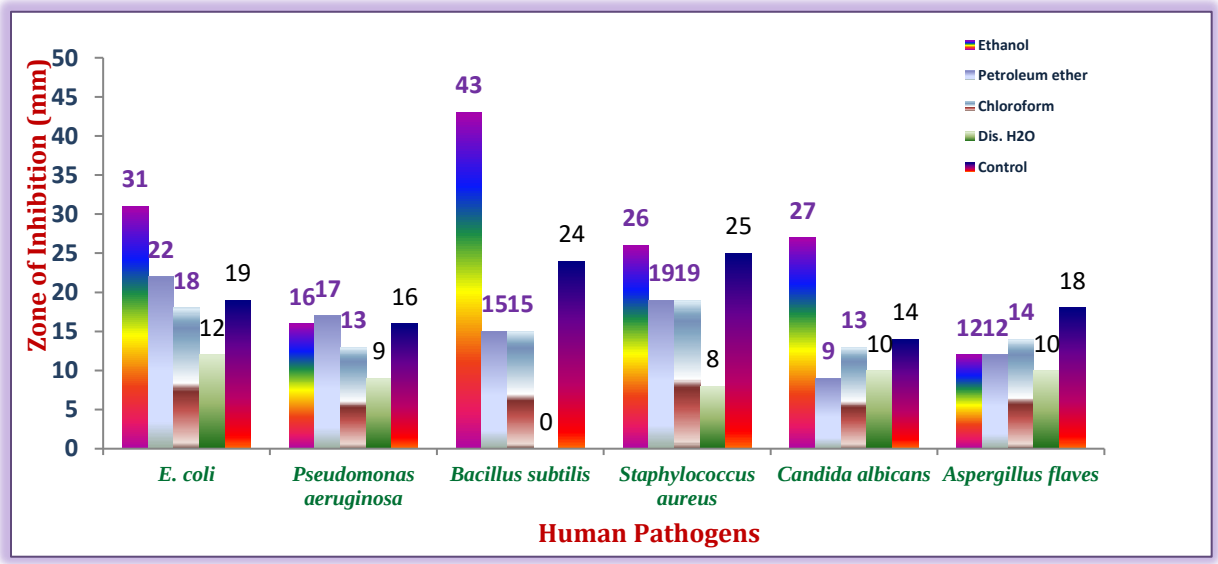


Table No. 2. Antimicrobial activity of dry peel extracts of Cucumber (*Cucumis sativus* L.) using disc diffusion assay

S. No	Pathogens	Antibacterial activity - Zone of Inhibition (mm)				
		Ethanol	Petroleum ether	Chloroform	Dis. H_2O	Control (Amikacin)
1.	*Escherichia coli*	31 ± 0.5	22 ± 0.3	18 ± 0.4	12 ± 0.6	19 ± 0.4
2.	*Pseudomonas aeruginosa*	16 ± 0.2	17 ± 0.4	13 ± 0.5	09 ± 0.3	16 ± 0.5
3.	*Bacillus subtilis*	43 ± 0.3	15 ± 0.2	15 ± 0.6	-	24 ± 0.4
4.	*Staphylococcus aureus*	26 ± 0.4	19± 0.5	19 ± 0.4	08 ± 0.5	25 ± 0.3
	Antifungal activity - Zone of Inhibition (mm)					
5.	*Candida albicans*	27 ± 0.7	09 ± 0.5	13 ± 0.4	10 ± 0.5	14 ± 0.6
6.	*Aspergillus flaves*	12 ± 0.2	12 ± 0.4	14 ± 0.3	10 ± 0.4	18 ± 0.5

± Standard Error, + Present, – Absent

Figure. 4. Antimicrobial potential of different extracts of dry peel of Cucumber (*Cucumis sativus* L.)



Antifungal activity

The antifungal activity of various solvent extracts of dry peel of Cucumber (*Cucumis sativus* L.) were carried out against two strains *viz. Aspergillus flaves* and *Candida albicans*. The results of the antifungal extract of *Cucumis sativus* L. and the minimum zone of inhibition (9mm) in the petroleum ether extract. In *Aspergillus flaves*, ethanol extract has a maximum level of zone of inhibition (12mm) and minimum zone of inhibition (10mm) in aqueous extract of dry peel of Cucumber (*Cucumis sativus* L.).

Gas chromatography–mass spectroscopic spectra analysis

Gas chromatography-mass spectroscopy (GC-MS) is the premium method to scrutinize the biologically active constituents, namely alcohols, long chain hydrocarbons, branched chain hydrocarbons, esters, etc. GC is the preferable tool on account of its sensitivity, effective in separating the constituents from the mixture and also used for the qualitative and quantitative study of the mixture. 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 (Hites, 1997). This technique (GC-MS) has proved to be a valuable and important method in analyzing fatty acids, non-polar compounds, lipids, volatile essential oil and alkaloids.

activity of different extracts of Cucumber (*Cucumis sativus* L.) were presented in Table 2. Candida albicans growth is controlled by a maximum zone of inhibition (27mm) in the ethanol dry

The compounds present in the ethanol extracts of dry peel of Cumber (*Cucumis sativus* L.) were identified after the comparison of the mass spectra with NIST library by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight and structure in various extracts of Cucumber (*Cucumis sativus* L.) were presented. GC-MS spectrums of ethanol extract of dry peel of *Cucumis sativus* L. showed 8 different major peaks which indicated the presence of 8 compounds. The prevailing compounds in ethanol extract were Silane, dimethyl- dimethylsilicon (0.55%), Ethyl acridine, 1-ethylacridine Acridine (14.44%), Phthalic acid 1,2-benzenedicarboxylic acid (7.52%), 3-Hydroxydodecanoic acid beta-Hydroxylauric acid (45.60%), Benzo[h] quinoline 7,8-Benzoquinoline (1.93%), [1-(tert-Butyl dimethylsiloxy)-2,2,2-trichloroethyl]benzene tert-butyl dimethyl(2,2,2,-trichloro-1-phenylethoxy)silane (3.28%), Trimethyl(2,6 ditert.-butylphenoxy)silane (4.0%), 3,5-Heptanedione, 2,2,6,6-tetramethyl- 45.32%). The spectrum profile of GC-MS confirmed the presence of eight major components with retention time 2.365 min, 16.546 min, 16.730 min, 16.980 min, 17.871 min, 18.604 min, 20.998 min and 21.673 min respectively (Figure 2 and Table 1).

Figure 2: GC-MS analysis of ethanolic extract of dry peel of Cucumber (*Cucumis sativus* L.)

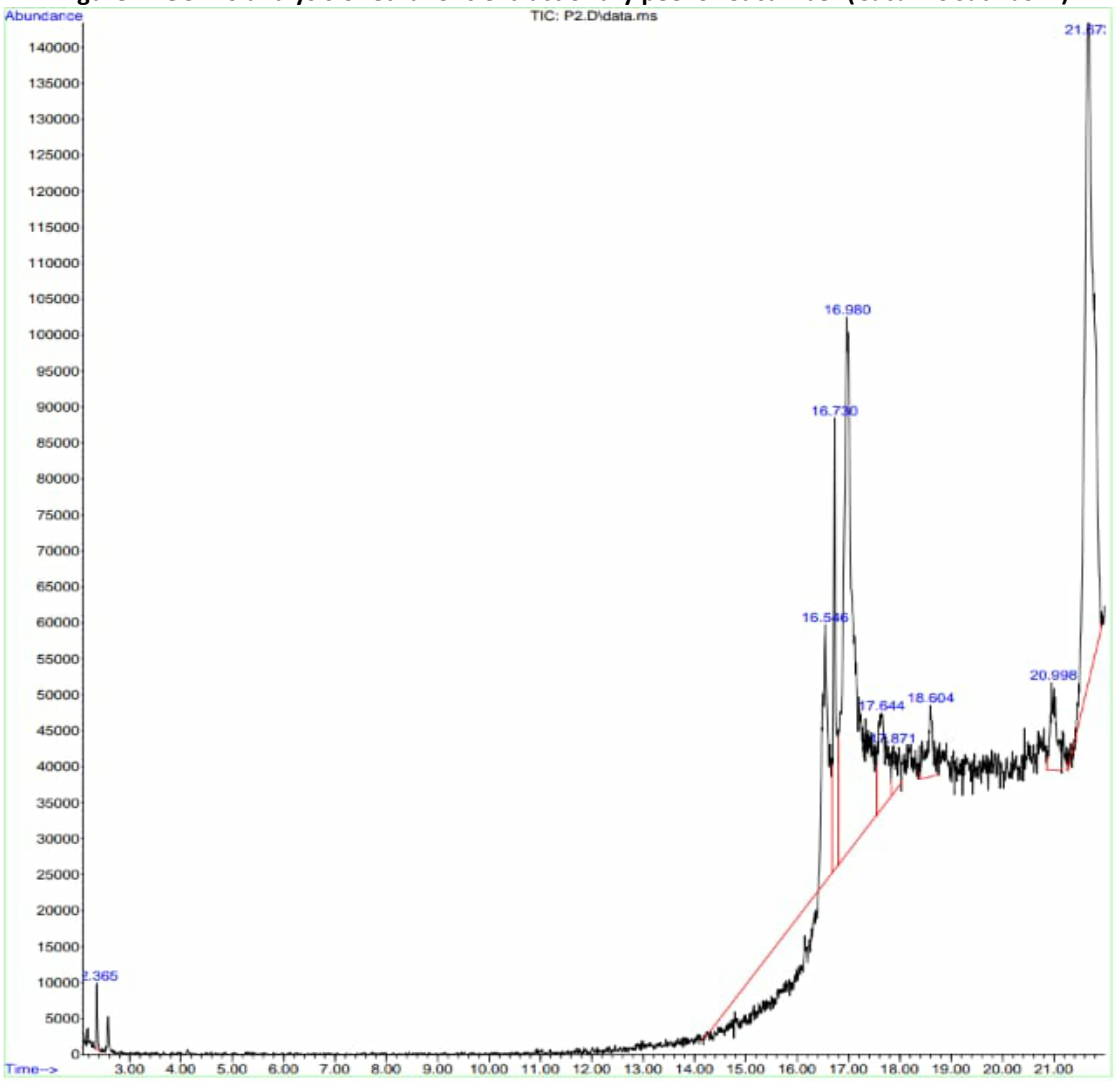
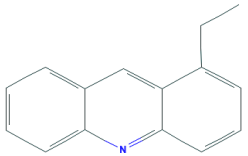
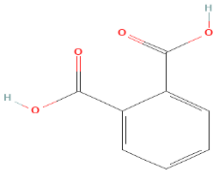
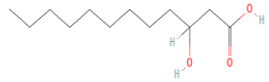
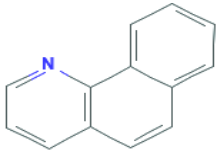
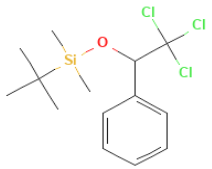
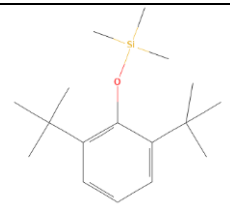
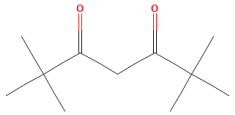


Table No 1: GC-MS analysis of ethanolic extract of dry peel of Cucumber (*Cucumis sativus* L.).

S. No	Retention Time (RT)	Name of the Compound	Molecular Formula (MF)	Molecular Weight (MW)	Peak Area (%)	Structure
1.	2.365	Silane, dimethyl- dimethylsilicon	C_2H_6Si	58.15 Da	0.55	
2.	16.546	Ethyl acridine, 1-ethylacridine Acridine,	$C_{15}H_{13}N$	207.27 Da	14.44	
3.	16.730	Phthalic acid 1,2-benzenedicarboxylic acid benzene-1,2-dicarboxylic acid o-phthalic acid	$C_8H_5O_4$	166.13 Da	7.52	
4.	16.980	3-Hydroxydodecanoic acid beta-Hydroxylauric acid Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216.32 Da	45.60	
5.	17.871	Benzo[h] quinoline 7,8-Benzoquinoline	$C_{13}H_9N$	179.22 Da	1.93	
6.	18.604	[1-(tert-Butyldimethylsiloxy)-2,2,2-trichloroethyl] benzene tert-butyldimethyl(2,2,2,-trichloro-1-phenylethoxy)silane	$C_{14}H_{21}Cl_3OSi$	3389.8 Da	3.28	

7.	20.998	Trimethyl (2,6 ditert.-butylphenoxy)silane (2,6-ditert-butylphenoxy)-trimethylsilane	$C_{17}H_3OSi$	278.5 Da	4.00	
8.	21.673	3,5-Heptanedione, 2,2,6,6-tetramethyl-	$C_{11}H_2OO_2$	184.26 Da	45.32	

Summary and Conclusion

Biodiversity, the variety of ecosystems, species, and genes, is recognized as one of the critical elements for human existence, as it provides vital goods and services such as food, carbon sequestration or wastewater purification. The rate of loss of biodiversity is considered to have passed its safe boundaries already. Notwithstanding large uncertainties linked with complexity of ecological systems and also lack of consensus on distinct cause-and-effect relationships and the true position of thresholds, it can be said with some confidence that earth cannot sustain the current loss of biodiversity without reduction of its capacity to provide useful services.

In the present investigation of GC-MS analysis and antimicrobial activity studies on selected plant was carried out. The selected plant is one of the important ethno medicinal plants used in different parts of the world. The plant is collected from Puliankulam village of Kovilpatti taluk of Tuticorin district of Tamil Nadu. Shade dried powdered and extracted with ethanol solvents for further analysis are the important phytochemical character for the identification drugs from adulterants in the pharmaceutical industries.

* Gas Chromatography-Mass Spectrum analysis was utilized as a part of this research to distinguish the compounds displayed in the plant extracts. Ethanolic extract of dry peel of cucumber (*Cucumis sativus* L.) contains 08 peaks. GC-MS analysis of ethanolic extracts of *Cucumis sativus* L. also exhibited numerous peaks is different retention time.
* Antimicrobial activity studies carried out with four human pathogenic bacteria. Maximum zone of inhibition observed in ethanol extract against *Bacillus subtilis,* and minimum zone of inhibition is *Staphylococcus aureus* is observed.
* The antimicrobial activities of the plant extracts obtained in petroleum ether, chloroform, ethanol and water are carried out against four human pathogenic bacteria and two fungi. The outcomes are accounted on the basis of the diameter of the zone of inhibition around each disc (in mm). In the present study generally all the selected plant extracts show moderate to good antimicrobial activity. Positive control Amikacin was used and showed remarkable. Ethanol extracts of showed maximum zone of inhibition against selected pathogenic bacteria and fungi compared to other solvent extracts. Each solvent extract of the plants had its own choice of inhibiting the microbial growth.

Scope for extension / integration research outcome with another field

- Further research is warranted to reveal the any direct action of this herbal medicine on metabolic pathways.

Further isolation and characterization studies on extracts and activity guided fractionation are needed to identify the specific compounds responsible for their therapeutic activities.

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