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TRACKING BIOACTIVE CONTENT IN BUTANOL EXTRACT OF *CITRULLUS COLOCYNTHIS*L. FRUIT PULP BY GC/MS

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ABSTRACT

Citrullus colocynthis (L.) a member of *Cucurbitaceae* family, was collected from the different areas of the Kachchh region. The fruit pulp of *Citrullus colocynthis* (L.) was subjected to continuous and successive soxhlet extraction with n-Butanol. The extract was dried in vacuum, antimicrobial activity was measured for the same. With the help of GC/MS analysis the compounds responsible for the biological activity were identified.

Keywords: Soxhlet extraction, *Citrulluscolocynthis*, GC/MS, Antimicrobial activity.

INTRODUCTION

Citrullus colocynthis (L.) a member of *Cucurbitaceae* family, is a desert plant with a rich history as an important medicinal plant and as a source of valuable oil. It is distributed in African and Arabian countries and India. It is commonly known as bitter apple, colosynth or wild gourd is used as an abortifacient, cathartic, purgative and vermifuse, and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, tumour and as an insect repellant [1] The fruit of *C. colocynthis* have been commonly used as an catharsis and antidiabetic agents in traditional Egyptian and Indian Ayurvedic medicines [2-5]. A number of plant secondary metabolites including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids have previously been reported from this plant [6-11].The search for bioactive metabolites and chemical constituents from *C. colocynthis*has been an ongoing project in our laboratory [12]. The *C. colocynthis* of the desert area of Kachchh has not been evaluated for the bioactive compounds. We therefore tested n-butanol extract of the fruit pulp of the plant for antibacterial activity. The activity of butanol extract is not known before [13]. Here in we have tracked the phytochemicals that are responsible for the biological activity in the n-butanol extract by GC/MS analysis.

MATERIALS AND METHOD

Plant Material

The samples were collected from the various part of the Kachchh district of Gujarat (India), during August 2009. The collected plant material was cleaned with tap water and pulp was separated from epicarp and seeds. The pulp was dried under the shade until complete removal of water from it. Such dried pulp was powdered using blender and stored in air tight container for the further use.

Extraction

The dried fruit pulp (60 g) of *C. colocynthis* fruit pulp was extracted with n-Butanol in a Soxhlet apparatus for 24hr. The extract was evaporated under vacuum. The residue (3.12 g) was dissolved in DMSO for the antimicrobial activity and in n-Butanol for the GC/MS analysis.

Antimicrobial Screening

The butanol extract of fruit pulp was tested for its antibacterial and antifungal activity (MIC) in vitro by broth dilution method [14-16] with two Gram-positive bacteria Staphylococcus aureus MTCC 96, Bacillus Cereus MTCC 10650, two Gram - negative bacteria Escherichia coli

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MTCC 442, Pseudomonas aeruginosa MTCC 441 and three fungal strains Candida albicans MTCC 227, Aspergillus niger MTCC 282, Aspergillus clavatus MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs.

Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations of 1.56, 3.12, 6.25, 10, 12.5, 25, 50, 62.5, 100, 125, 250, 500 and 1000 µg mL-1. The tubes were inoculated with 108 cfu mL-1 (colony forming unit mL-1) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied.

The GC/MS Analysis

The GC/MS analysis was performed on Shimadzu-GCMS-QP2010 model with FTD detector for the detection. In the separation and identification by GC/MS technique components were identified on thebasis of the retention time and spectral index from the NIST and WILEY library. The instrument specifications and analysis conditions adjusted are given below in Table 1.

RESULTS AND DISCUSSION

Antimicrobial Screening

As shown in Table 2, n-butanol extract of *C. colocynthis* pulp is effective against most of the microorganism assayed.

The GC/MS Analysis

The GC spectrum of the n-Butanol extract is shown in the Figure 1. Total of 21 compounds present in the n-Butanol extract were determined by the chromatographic method with the help of NIST and WILEY library as shown in Table 3.

Compound 1,6-Anhydro beta-D-glucopyranose was found to be in the highest concentration (23.31%) followed by Methyl palmitate (17.85%), Methyl linoleate (14.88%) and Methyl-9,12,15-octadecatrienoate (12.25%), other compounds were found in trace amount (Table 3). Either one or all the identified compounds may be responsible for the antimicrobial activity of the n-Butanol extract. Further separation of the identified compounds will be done in due course.

Table 1.**The instrument specifications and analysis conditions**

GC Program	
Column	BP x 5 (1.0µm x 30 meter x 0.25 mm)
Oven Temperature	80 °C (1.5 min) ---10 °C / min ---290 °C (15 min)
Injection Temperature	250 °C
Injection Mode	Split
Flow Control Mode	Pressure
Pressure	67.3 kPa
Total Flow	34.0 mL/min
Column Flow	1.00 mL/min
Linear Velocity	36.9 cm/sec
Split Ratio	30.0
Detector Temperature	250 °C
Injection Volume	1 mL
Syringe Volume	10 mL
Sample preparation	The extracted components were dissolved in HPLC grade methanol
Ion Source Temprature	200 °C
Interface Temprature	200 °C
Solvent Cut time	2.50 min
Detector Gain	0.90 kV
Threshold	100
MS Program	
Start time	3.00 min
End time	37.50 min
Interval	0.50 Sec
Start m/z	25.00
End m/z	1000.00

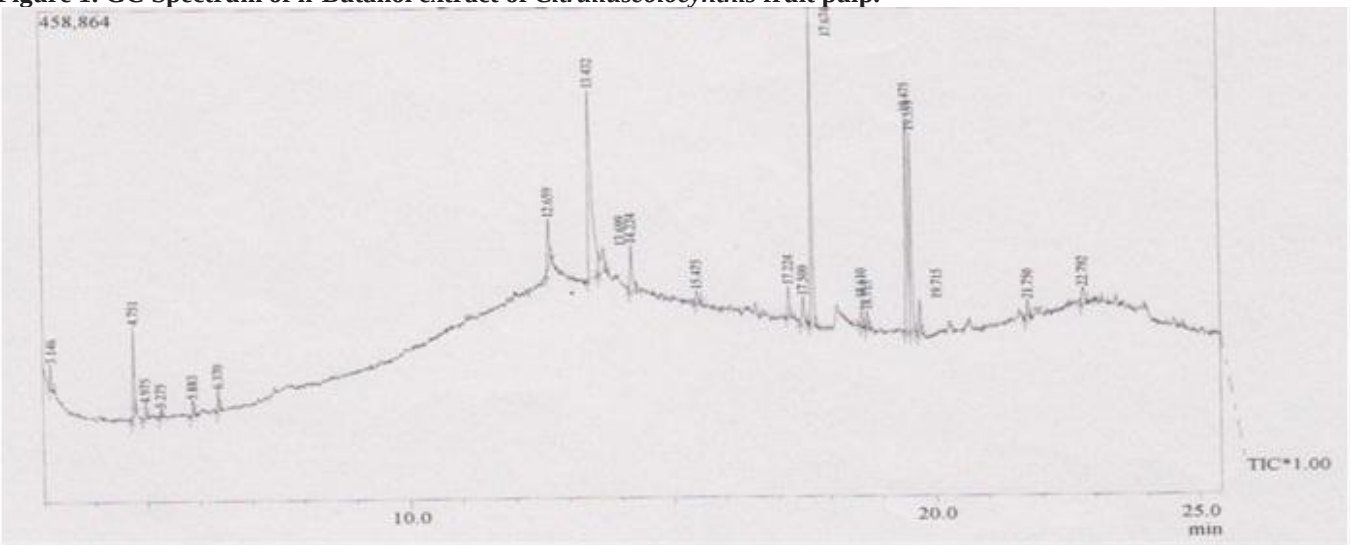
Table 2. **Antibacterial and antifungal activity of eugenol**

Name of the compounds	Minimal inhibition concentration (µg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a	***B.c.***	***E.c.***	***P.a.***	***C.a.***	***A.n.***	***A.c.***
n-Butanol extract	50	10	25	100	250	500	100
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

Table 3. Results of the GC/MS analysis of n-Butanol extract

S.No	Compound	RT	%Area
1	Solvent peak (n-Butanol)	3.146	1.07
2	n-Butyl ether	4.753	4.13
3	Dihydrosimplexindiacetate	4.975	0.89
4	Neopentyl-methyl ketone	5.275	0.27
5	L-Fucose-perbenzoyl o-benzyloxime	5.883	0.74
6	n-Butyl butanoate	6.370	1.06
7	1,2-Benzene dicarboxylic acid dimethyl ester	12.659	3.40
8	1,6-Anhydro beta-D-glucopyranose	13.432	23.31
9	Nerolidol	13.699	3.52
10	1,2-Benzene dicarboxylic acid diethyl ester	14.224	4.08
11	2-Octadecyloxy-1-cis-9-octadecenyloxy ethane	15.475	0.85
12	Isobutyl phthalate	17.224	1.74
13	Methyl palmitoleate	17.500	2.08
14	Methyl palmitate	17.674	17.85
15	Methyl-9,10-methylene hexadecanoate	18.610	1.61
16	Methyl heptadecanoate	18.715	1.04
17	Methyl linoleate	19.425	14.88
18	Methyl-9,12,15-octadecatrienoate	19.595	12.25
19	Methyl stearate	19.715	2.16
20	Eicosamethylcyclodecasiloxane	21.750	1.25
21	Octadeca methyl cyclononasiloxane	22.792	1.82

Figure 1. GC Spectrum of n-Butanol extract of *Citrulluscolocynthis* fruit pulp.



CONCLUSION

Present study of n-Butanolic extract of *Citrullus colocynthis* fruit pulp indicated that it contains biologically active compounds. The properties of these compounds probably contribute, at least to some extent, to the pharmacological and traditional medicinal uses of the *Citrullus colocynthis*. Further separation and identification of compound present in it may give new biologically active compounds, which can be used as lead compounds in future.

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