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GC-MS ANALYSIS OF *Citrullus colocynthis* SCHRADER FRUIT EXTRACT IN BENZENE AND ETHYL ACETATE

¹Patil, U. S. and ²Kutemate, O. G.

¹Associate Professor and Head, Department of Botany, Bharatiya Mahavidyalaya, Amravati ²Research student, Department of Botany, Bharatiya Mahavidyalaya, Amravati.

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ABSTRACT

Citrullus colocynthis Schrader belongs to family Cucurbitaceae. It grows widely in Melghat, Maharastra India and it has been used in folk medicine by tribals. To evaluate the different medicinal properties and compound identification GC-MS is helpful. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. The Benzene extract of *Citrullus colocynthis* Schrader seeds analyzed by GC-MS shows the presence of compounds like N-Hexadecanoic acid, 17-Octadecynoic acid, Octadecynoic acid, Oleic acid, 2-Acetyltetradecanoic acid, Ethyl ester, Hexatriacontane, 2R-Acetoxymethyn-1,3,3-Trimethyl-4T-(3-Methyl-2-Betun-1-YL)-1T-Cyclohexanol, 1-1':3',1''-Tercyclopentane,2'-Docecyl- and Ethyl acetate extract revealed existence of N-Hexadecanoic acid, 9,12 octadecadedienoic acid (Z,Z)-, 1- Hexyl-2- Nitrocyclohexane, Tritetracontane, 2,3 Dehydro-4-oxo-Beta-Iononone, 2R-Acetoxymethyl-1,3,- Trimetyl 4T-(3 Metyl-2- Buten-1-YI)-1T-Cyclohexanol, 1,1':3',1''-

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INTRODUCTION

Plant based medicines are important curative agents with no side effects, this is important reason for revival of interest in use of plant medicine. Citrullus colocynthis Schrader is monoecious plant with perennial root, creeping stem, diffuse or slender angled branches, scabrid. Tendrils are simple, slender, and hairy. Leaves are variable, pale green above, ashy beneath, usually deltoid in outline, three lobed, lobed deeply pinnate. Male flowers: Peduncles long villous, Calyx tube broadly campanulate, hairy, long, pale yellow, segments obovate, apiculate. Stamens 3 short, free, anther cohering, one 1-celled, other 2-celled. Ovary is rudimentary, glanduliform. Female flowers: Calyx and corolla as in male. 3 legulate stamens, ovoid Ovary with 3-placentiferous and many compressed Ovules are present. Fruits globular, variegated green and white ripe filled with a dry spongy very bitter pulp, epicarp thin. Seed are off white or brown in colour. It is one of important ethnomedicinal plant used by local tribal medicinemen. Plant is traditionally used in treatment of wound healing.

Associate Professor and Head, Department of Botany, Bharatiya Mahavidyalaya, Amravati

The use of ethanopharmacological knowledge is one of the attractive ways to enhance the probability of success in new drug finding efforts (Cordell and Colvard, 2005; Patwardhan, 2005). Gas chromatography-mass spectroscopy (GC-MS) of Citrullus colocynthis Schrader is important for its use in pharmaceutical industry and so needs special consideration. The present study was undertaken to characterize and isolate phytochemicals from fruit of this plant (Salah Ali Idan, 2015). Citrullus colocynthis Schrader has been reported to possess therapeutic activities against various human ailments (Kim M.G. et al., 2014 and Gurudeeban S. et al., 2010). Medicinal significance of Citrullus colocynthis Schrader is due to the existence of colocynthin, cucurbitacin E-2-O -glucoside in fruit pulp of this plant (Yoshikawa M. et al. 2007 and Shekarchi M. et al., 2015). Chemical profiling of Citrullus colocynthis Schrader is limited to colocynthin and fatty acid contents (Pravin B. et al., 2013).

MATERIALS AND METHODS

Fresh fruits of *Citrullus colocynthis* Schrader were collected from Ambabarwa forest of Buldana District of Maharashtra, India. The fruits were shade dried and powdered.

^{*}Corresponding author: Patil, U. S.

S. N	Retention Time	Peak area %	Compound Analyzed	Molecular formula	Molecular weight	Probable Structural Formula	Activity reported
1	18.034	37.723	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	·····	Antioxident, Antiinflamatory, Nimaticidal
2	19.560	28.301	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	$\qquad \qquad $	
3	19.725	28.500	Octadecynoic acid	$C_{18}H_{36}O_2$	284		
4	20.160	1.887	Oleic acid	$C_{18}H_{34}O_2$	282	<u>ک</u> ر رو	Antibacterial
5	21.691	0.765	2-Acetyltetradecanoic acid, Ethyl ester	$C_{18}H_{34}O_3$	298		
6	25.802	0.944	Hexatriacontane	$C_{36}H_{74}$	506	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Analgesic, Antiinflammatory
7	29.854	0.676	2R-Acetoxymethyn-1,3,3- Trimethyl-4T-(3-Methyl-2- Betun-1-YL)-1T- Cyclohexanol	$C_{17}H_{30}O_3$	282	Å.	
8	30.984	1.206	1-1':3',1''-Tercyclopentane, 2'-Docecyl-	C ₂₇ H ₅₀	374	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	

 Table 1. GC-MS Analysis of Citrullus colocynthis Schrader (fruit) Benzene extract

*Activity Source: Dr. Duke's Phytochemical and ethnobotanical databases

The extraction was done by using Soxhlet's extraction method with analytical grade refluxing solvents benzene and Ethyl acetate. Benzene and Ethyl acetate extracts of fruit was subjected to GC-MS analysis from Sophisticated Instrumentation Facility (SIF), School of Advanced Science, Chemistry Division, VIT University, Vellore, Tamilnadu and results were obtained. The different extracts obtained from these plants were subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. GC-MS analysis of the samples were carried out using Perkin Elmerclarus 680 with mass spectrometer clarus 600 (EI) using TurboMass ver 5.4.2 Software with NIST -2008 Library ver. Helium was used as the carrier gas and the temperature of programming were set with initial oven temperature at 60°C and held for 2 min and final temperature of the oven was 300° C with the rate at 10° C per min. A 2-µL sample were injected with split 50:1. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 eV; a scan interval of 2 min and fragments from 50 to 600 Da. The chemical components form the fruit extracts of Citrullus colocynthis Schrader was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. A quantitative determination was made by relating respective peak areas to TIC areas from the GC-MS.

RESULTS AND DISCUSSION

GC-MS Analysis of *Citrullus colocynthis* **Schrader**GC-MS was carried out to determine the possible chemical components from fruits of *Citrullus colocynthis* Schrader.

The chromatogram of Benzene extract clearly shows the presence of eight peaks indicating presence of eight phytochemical compounds detected was shown in Fig. 1.



Fig. 1. GC-MS chromatogram of *Citrullus colocynthis* Schrader (fruit) Benzene extract

The eight phytoconstituents were characterized and identified on comparison of the mass spectra of the constituents provided by NIST library.



Fig. 2. GC-MS chromatogram of Citrullus colocynthis Schrader (fruit) Ethyl acetate extract

Sr. No.	Retention Time	Peak area %	Compound Analyzed	Molecular formula	Molecular weight	Probable Structural Formula	Activity reported
1	17.969	20.929	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	· · · · · · · · · · · · · · · · · · ·	Antioxident, Antiinflamatory, Nimaticidal
2	19.635	58.958	9,12 octadecadedienoic Acid (Z,Z)-	$C_{18}H_{32}O_2$	166	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
3	19.740	16.836	1- Hexyl-2- Nitrocyclohexane	$C_{12}H_{23}O_2N$	213	di L	Antimicrobial
4	25.807	0.636	Tritetracontane	$C_{43}H_{88}$	604		Antibacterial, Antihelmantic, Antiulcer, Diuretic
5	26.463	0.853	2,3 Dehydro-4-oxo-Beta- Iononone	$C_{13}H_{16}O_2$	204	X	
6	29.869	0.687	2R- Acetoxymethyl-1,3,- Trimetyl 4T-(3 Metyl-2- Buten- 1-YI)-1T-Cyclohexanol	$C_{17}H_{30}O_3$	282		
7	31.049	1.101	1,1':3',1"- Tetracyclopentane,2'- Dodecyl-	C ₂₇ H ₅₀	374		

Table 2. GC-MS Analysis of Citrullus colocynthis Schrader (fruit) Ethyl acetate extract

*Activity Source: Dr. Duke's Phytochemical and ethnobotanical databases

The Benzene extract of Citrullus colocynthis Schrader fruit analyzed by GC-MS shows the presence of compounds like N-Hexadecanoic acid, 17-Octadecynoic acid, Octadecynoic acid, Oleic 2-Acetyltetradecanoic acid, acid, Ethyl ester, Hexatriacontane, 2R-Acetoxymethyn-1,3,3-Trimethyl-4T-(3-Methyl-2-Betun-1-YL)-1T-Cyclohexanol, 1-1':3',1''-Tercyclopentane, 2'-Docecyl-. The active compound with their retention time (RT), % peak area, Compound analyzed, molecular formula, molecular weight (MW), functional group, probable structural formula and activity reported are presented in Table 1.

The determination of the possible chemical components from fruit of *Citrullus colocynthis* Schrader. was carried out by GC-MS. On the basis of data obtained by GC-MS of Ethyl acetate extract analysis revealed seven peaks. These seven peaks indicated the presence of seven phytochemical compounds. The GC-MS chromatogram of the seven peaks of the compound detected was shown in Fig.2. The seven phytoconstituents were characterized and identified on comparison of the mass spectra of the constituents provided by NIST library. The Ethyl acetate extract of *Citrullus colocynthis* Schrader. Fruit extract analyzed by GC-MS shows the presence of compounds like N-Hexadecanoic acid, 9,12 octadecadedienoic acid (Z,Z)-, 1- Hexyl-2- Nitrocyclohexane, Tritetracontane, 2,3 Dehydro-4-oxo-Beta-Iononone, 2R-Acetoxymethyl-1,3,- Trimetyl 4T-(3 Metyl-2- Buten-1-Yl)-1T-Cyclohexanol, 1,1':3',1"- Tetracyclopentane,2'- Dodecyl-. The active principles with their retention time (RT), % peak area, Compound analyzed, molecular formula, molecular weight (MW), functional group, probable structural formula and activity reported are presented in Table-2.

Conclusions

This study concludes that *Citrullus colocynthis* Schrader is highly effective as antibacterial, antiulcer, analgesic, antioxidant, anti-inflammatory and nimaticidal agent. Therefore, there has been a growing interest in the use of *Citrullus colocynthis* Schrader as a promising source for more efficient new therapeutic drugs. More investigations are needed on the location of active ingredients, their physical, chemical properties and antimicrobial potentialities. Further research is required at the molecular level.

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