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GC-MS analysis and phytochemical screening of methanolic fruit extract of *Citrullus colocynthis* (L.) Schrad

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Abstract
Citrullus colocynthis (L) Schrad commonly called indrayan or ghorumba is distributed in the desert areas globally. It is a valuable cucurbit having many pharmacological properties *viz.* anti-inflammatory, anti-diabetic, anti-analgesic, anti-epileptic, hair growth-promoting and abortifacient. In the present study, methanolic fruit extract was screened to identify the presence of active phytoconstituents. Photochemical screening of the extract was carried out for the presence of various phytochemicals as per standard methods. Phytoconstituents analysis was carried out by GC-MS (Thermo scientific) and spectrum of unknown phytoconstituents was compared with the phytoconstituents stored in NIST library of GC-MS apparatus. Phytochemical screening of fruit extract of CC has shown the presence of phenols, flavonoids, steroids and glycosides. GC-MS chromatogram of methanolic fruit extract of CC revealed 14 different peaks which confirmed the presence of 14 different phytoconstituents with their respective retention time (RT). Some of the identified components possess pharmacological actions as per information available in the literature. From the results, it is evident that CC contains various phytocomponents of phytopharmaceutical importance. The isolation of particular phytoconstituent and identifying their biological activities will definitely open new areas of investigation of their pharmacological potential.

Keywords: Phytoconstituents, *Citrullus colocynthis*, GC-MS, pharmacology

Introduction
Plants have been used for treatment of various human ailments due to the presence of active compounds with specific therapeutic potential. Many drugs have been isolated from plants based on their traditional uses. Presently about eighty percent of the world population uses herbal medicine for of primary health care aspect as they are cost effective and have few side effects [1-2]. Therefore, plants are continuously screened for the presence of novel phytocompounds to meet out the challenges that mankind is facing today in healthcare [2]. Hence, identification of active phytocompounds is essential for biological and pharmacological studies.
GC-MS is an important technique for such studies and has been adapted precisely to evaluate various phytoconstituents present in different plant extracts along with their structures. It has superior separation ability and can produce a chemical fingerprint with high accuracy and precision. Moreover, it gives quantitative data along with the coupled mass spectral database which is of enormous value for investigating the correlation between phytocompounds and their applications in pharmacology [3].
Citrullus colocythis (L.) Schrad (Cucurbitaceae) is a valuable plant that is used for the treatment of asthma, diabetes and jaundice and many other ailments [4-8]. The fruits (commonly called colocynth or bitter apple) are used as blood purifier and as a remedy for the treatment of enlarged spleen, diabetes and tumors [7-11]. Literature studies has shown the presence of coumarins, tannins, terpenoids and flavonoids in the whole plant extract of CC [11]. The present study was planned for detailed analysis of phytoconstituents in the methanolic fruit extract of CC for bioactive biomolecules using GC-MS and their pharmacological actions for understanding their medicinal properties.

Materials and methods
Collection of plant material
Fresh plants of CC along with fruits were collected from roadsides and fields in district Jhajjar, Haryana. The collected plants were identified by Dr Bhoo Dev Vashistha; plant taxonomist Kurukshetra University, Kurukshetra. Fruits were washed with double distilled water and then dried in shade.

Sample preparation
The fruits of CC were dried in shade at room temperature and then grinded to obtain a coarse powder. 50 grams powdered fruit material (1:5W/V) was used for extraction in a Soxhlet apparatus using methanol solvent (250ml, Himedia). Prepared fruit extract was stored in refrigerator at low temperature.

Preliminary phytochemical screening
Methanolic fruits extract of CC was qualitatively screened for the presence of different phytochemicals as per standard methods [12-13].

GC-MS Analysis
Methanolic fruit extract of CC was analyzed for the presence of various phytoconstituents by using GC-MS apparatus (Thermo Scientific Co. Trace 1300). The temperature programme was set at 70°C for 2 min. and then increased at 7°C/min. upto 200°C. It was further accelerated at 5°C/min upto 220°C with 5 minute hold. Injector temperature was 220°C. The scanning of mass range was from 35 to 400 (m/z). Xcalibur software was used for processing data peaks in GC-MS system.

Phytoconstituent identification
Phytoconstituents identification was based on the relative retention time and their peak area with the NIST library of the GC-MS system. The unknown phytoconstituents spectrum was compared with the spectrum of known phytoconstituents preloaded in the NIST library. Spectrum was also compared with the available literature. The compounds name, retention time, similarity index, reverses similarity index, peak area percentage, molecular weight, molecular structure, nature and reported bioactivity of the methanolic fruit extract were ascertained.

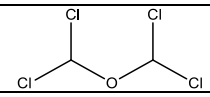
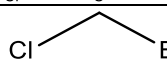
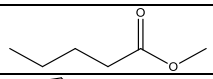
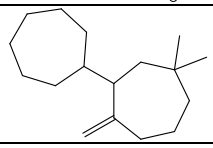
Results and Discussion
In present study, methanolic fruits extract of CC was used for phytochemical screening and GC-MS analysis. The study revealed that methanolic fruits extract of CC contains phenols, flavonoids, glycosides and steroids whereas Alkaloids, Terpenoids and Saponins were absent as summarized in Table 1. GC-MS analysis of the fruit extract of CC has shown the presence of fourteen different peaks with their respective retention time that confirmed the presence of fourteen different phytocompounds (Figure1). The spectra of these phytocompounds was matched with NIST library's Xcalibur software of GC-MS apparatus.
The identified compounds were presented with their retention time (RT), similarity index (SI), reverses similarity index (RSI), peak area percentage, molecular weight, molecular structure, nature and reported bioactivity (Table-2). The phytocompounds *viz.* Methane and oxybisdichloro (82.55%), trans-13-Octadecenoic acid (4.38%), 2-Ethyl-1-hexanol (2.63%), Adipic acid, isohexyl methyl ester (2.04%), Hexanedioic acid, dimethyl ester (1.87%) and Methyl stearate (0.59%) were present abundantly. Along with these compounds Hexadecane, 11,13-Dihydroxy-tetradec-5-ynoic acid, Methyl ester, Bromochloro, Methyl valerate, Bicyclo heptane, 6,6-dimethy l-2-methylene Dodecane, Acetic acid were also present but in minute quantities. The total peak area (%) is 95.86% of whole GC-MS chromatogram.
The presence of various bio-active phytoconstituents in the methanolic fruit extract of CC justifies its ethno medicinal use. GC-MS analysis of the fruit extract revealed the presence of alkane, Chloro alkyl ethers, hydrocarbons, alcohol, methyl donor carboxylic acids and organic compounds for various pharmaceutical applications. Chandra *et al*. (2018) detected palmitic and Linoleic acid as the major metabolites of hexane extracts in seeds extract of CC [11]. Twelve chemically diverse metabolites comprising sugars, sugar alcohols, organic acid glycoside, amino acids, cucurbitane and inositol were characterized in methanolic extracts of roots, stem, fruit pulp, seeds and leaves of CC [11]. Fructose, glucose and sucrose were found to be the dominant metabolites in semi-polar extracts of CC [11]. This present study determined formula and structure of fourteen biomolecules present in methanolic fruits extract of CC that may lead to screening of their further pharmacological importance for new drug development.

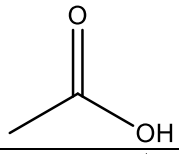
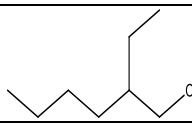
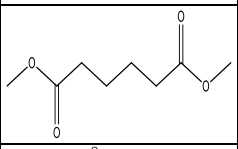
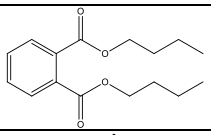
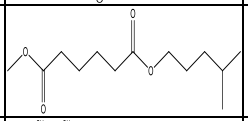
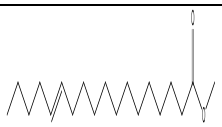
Table 1: Phytochemical analysis of methanolic fruit extract of CC

Phytochemicals	Alkaloids	Flavonoids	Glycosides	Phenols	Saponins	Steroids	Terpenoids
Results	-	+	+	+	-	+	-

(Where (+) indicate the presence of phytochemicals, (-) indicate the absence of phytochemicals

Table 2: Phytocompounds exhibited by the methanolic extract of CC by GC-MS

RT	Phyto compounds	SI/RSI	Area (%)	MF	MW	Molecular structure	Nature and reported bioactivity*
3.25	Methane, oxybisdichloro	842/866	82.55	C_2H_2ClO	182		Chloroalkyl ethers Not reported.
3.60	Methane, bromochloro	577/694	0.16	CH_2BrCl	128		Not reported.
4.12	Methyl valerate	854/884	0.20	$C_6H_{12}O_2$	116		Methyl ester of valeric acid Fragrances, Cosmetics, Plasticiser etc.
5.26	Bicyclo heptane, 6,6-dimethyl-2-methylene	792/825	0.12	$C_{10}H_{16}$	136		Not reported.
5.65	Dodecane	911/913	0.58	$C_{12}H_{26}$	170		Alkane hydrocarbon Not reported.

10.72	Acetic acid	776/907	0.13	$C_2H_4O_2$	60		Carboxylic acid Increase Aromatic Amino acid Decarboxylase Activity, Acidifier
11.17	2-Ethyl-1-hexanol	937/941	2.63	$C_8H_{18}O$	130		Alcohol Not reported
12.95	Hexadecane	833/866	0.13	$C_{16}H_{34}$	226		Alkane hydrocarban
16.95	Hexanedioic acid, dimethyl ester	945/946	1.87	$C_8H_{14}0_4$	174		Diester Used as plasticizer, Urinary Acidulant, Antioxidant,Hypocholesterolemic, pesticide, antiandrogenic nematicide [16] Antiinflammatory [15]
23.25	Dibutyl phthalate	854/922	0.28	$C_{16}H_{22}O_4$	278		Organic compound Used as Plasticiser. antibacterial properties [17]
24.25	Adipic acid, Isohexyl methyl ester	790/822	2.04	$C_{13}H_{24}O_4$	244		Ester Increase Aromatic Amino Acid Decarboxylase Activity, Acidulant
25.01	11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	660/695	0.20	$C_{15}H_{26}O_4$	270		Arachidonic acid inhibitor
25.51	Methyl stearate	829/844	0.59	$C_{19}H_{38}O_2$	298		Methyl-Donar
25.93	trans-13-Octadecenoic acid, methyl ester	918/920	4.38	$C_{19}H_{36}O_2$	296		Fatty acid ester, Anti-inflammatory, Cancer preventive, 5-alpha reductase inhibitor[15]

RT- Retention time, MF-molecular formula, MW- molecular weight, NIST match factor-similarity index (SI) and reverse similarity index (RSI); Area%-peak area%

* Source: Dr. Duke's online database on ethno botany and photochemistry.

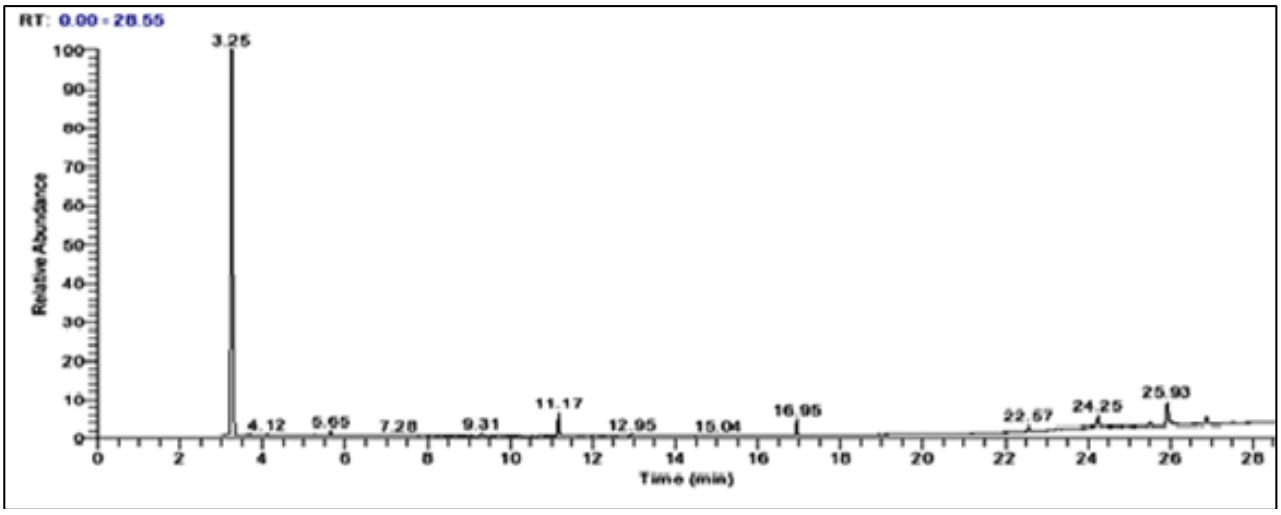


Fig 1: GC-MS analysis of methanolic fruit extract CC.

Abbreviations used: GC-MS: Gas chromatography mass spectrometry, CC: *Citrullus colocynthis*

Conflict of interest: None

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