Comprehensive GC-MS profiling of methanolic and chloroform extracts of *Combretum indicum* leaf for therapeutic potential use

Kunwar Sarvendra [1, 2#], Shiwa Chaubey[1], Vijay Kumar Shukla[3], Ajad Patel[4] and S.C Sati[2]

[1] Department of phytochemistry CSIR-National Botanical Research Institute, Lucknow, India

[2]Department of Botany, DSB Campus, Kumaun University, Nainital, Uttarakhand, India

[3]Department of Biochemistry Dr. R.M.L Avadh University Ayodhya Uttar Pradesh

[4]Department of Microbiology Dr. R.M.L Avadh University Ayodhya Uttar Pradesh

[#] Corresponding author

E-mail: sarvendrakr88@gmail.com

Abstract

This study presents a detailed gas chromatography-mass spectrometry (GC-MS) analysis of methanolic and chloroform extracts of *Combretum indicum*, a medicinal plant known for its traditional use in treating various ailments. The phytochemical profiles of both extracts were evaluated, revealing a diverse range of bioactive compounds with potential pharmacological properties. Major constituents identified include alkaloids, terpenoids, and flavonoids. The findings highlight the presence of metabolites having antioxidant, antimicrobial, and anti-inflammatory potentials, contributing to the growing evidence supporting its use in traditional medicine. The results of this study provide a scientific basis for further pharmacological investigations and the development of therapeutic agents from *C. indicum*.

Keywords: *Combretum indicum*, GC-MS, methanolic extract, chloroform extract, phytochemical analysis, bioactive compounds, therapeutic potential

1. Introduction

Medicinal plants have been a cornerstone of traditional healing systems and are increasingly gaining recognition in modern pharmacology.The growing global interest in plant-based therapeutics has led to an intensified search for bioactive compounds from medicinal plants. *Combretum indicum* (syn. Quisqualis indica), commonly known as Rangoon creeper or Chinese honeysuckle, belongs to the Combretaceae family and is widely recognized for its ornamental and medicinal properties [1]. Native to tropical Asia and Africa, this plant has been traditionally used in folk medicine for its anthelmintic, antipyretic, and antimicrobial activities [2]. Recent advancements in phytochemical studies and analytical techniques like Gas Chromatography-Mass Spectrometry (GC-MS) have provided new opportunities to explore its chemical diversity and therapeutic potential [3]. *Combretum indicum* is known to contain a broad range of phytochemicals, including flavonoids, alkaloids, saponins, and tannins, which contribute to its pharmacological properties [4]. The therapeutic efficacy of these compounds in combating oxidative stress, microbial infections, and inflammation has been documented [5]. However, comprehensive chemical profiling of its bioactive compounds using GC-MS remains limited, necessitating further investigation to identify and characterize both polar and nonpolar metabolites. The methanolic and chloroform extracts of *C. indicum* offer unique matrices for isolating a diverse array of bioactive constituents, providing insights into its multifaceted medicinal value.GC-MS is a powerful analytical tool for the identification of volatile and semi-volatile compounds in complex plant matrices [6]. By coupling gas chromatography's separation capabilities with the mass spectrometer precise molecular identification, this technique enables comprehensive profiling of phytochemicals. Applying this technique to *C. indicum* extracts could elucidate the chemical composition responsible for its pharmacological properties and facilitate its application in developing new therapeutic agents. The present study aims to perform a comprehensive GC-MS-based phytochemical profiling of methanolic and chloroform extracts of *Combretum indicum* to identify its polar and nonpolar metabolites. This research seeks to bridge the gap between traditional medicinal knowledge and modern pharmacological applications.

2. Materials and Methods

2.1 Plant Material Collection and Preparation

Fresh *Combretum indicum* leaves were collected from garden of CSIR National Botanical Research Institute Lucknow. The plant material was washed, shade-dried, and ground into a fine powder using electric grinder.

2.2 Extraction Procedure

The 5gm powdered plant material was subjected to solvent extraction using methanol and chloroform. Soxhlet extraction was performed for 48 hours. The extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4°C for further analysis.

2.3 Identification of metabolites using GC–MS

For the GC-MS analysis of non-volatile extract converted into volatile nature by the preparation of trimethyl silyl derivative (TMS). TMS Derivative prepared using methods of Bhati et al., 2015 [7].In brief 5 mg of extract dissolve in 40 µl of methoxyamine hydrochloride in pyridine reagent (20 mg/ ml) and, shacked on thermo mixture for 2 hours at 37 ºC. Now add 70 µl MSTFA, and again shake for 30 min. at 37 ºC.

2.4 GC-MS Analysis

GC-MS analysis of the fungal extracts was carried out using Thermo Trace GC Ultra coupled with Thermo fisher DSQ II mass spectrometers as selective detector. The chromatographic separation was performed through polysiloxane column (30 m x 0.25 mm Thermo TR50), coated with 50% methyl and 50% phenyl groups. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The injector temperature was set at 250°C, and the oven temperature was programmed as follows: initial temperature 50°C (held for 2 minutes), increased to 280°C at 10°C min^{-1}, and held for 10 minutes. 1 µL aliquot of the extract was injected in split mode (10:1). The characterization of chromatographic and mass spectrum data was carried out with the help of Xcalibur 1.5 software. Mass spectra were recorded in the electron impact mode at 70 eV. Compounds were identified by comparing their mass spectra with the data base National institute standard and technology (NIST) MS data libraries.

3.0 Results and Discussion

3.1 Methanolic Extract Metabolites

The GC-MS analysis of the methanolic extract of *Combretum indicum* revealed the presence of 23 metabolites, including sugars, sugar alcohols, and organic acids. The most abundant metabolites were D-(-)-Fructose anti (15.43%), D-Glucose (E) (15.23%), and D-(-)-Fructose (12.72%), highlighting the dominance of monosaccharides in the extract. Myo-Inositol (8.23%) and D-Glucose (Z) (7.2%) were also present in significant amounts, indicating their potential contribution to the bioactivity of the extract. Sugars such as sucrose (9.81%) and D-(+)-Turanose (6.19%) were also detected, suggesting the presence of disaccharides with nutritional and therapeutic relevance.Among sugar alcohols, D-Mannitol (1.29%) and D-(+)-Glucuronic acid γ-lactone (3.67%) were identified, both of which have been reported in previous studies for their roles in osmotic regulation and antioxidant activity [8]. Glycerol (0.94%) and L-Threitol (0.32%)

were also detected, though in smaller amounts, indicating their minor contribution to the chemical profile.

Table 1. Metabolites of methanolic extract of *Combretum indicum*

RT (min.)	Metabolites	Formula	Molecular weight (g/mol)	% Area
10.82	Glycerol	$C_3H_8O_3$	92.09	0.94
13.43	Phosphoric acid	H_3PO_4	97.99	0.28
16.26	L-Threitol	$C_4H_{10}O_4$	122.12	0.32
20.61	Ribitol	$C_5H_{12}O_5$	152.15	0.16
24.09	D-Mannitol	$C_6H_{14}O_6$	182.17	1.29
24.45	D-(-)-Fructose anti	$C_6H_{12}O_6$	180.16	15.43
24.81	D-(-)-Fructose	$C_6H_{12}O_6$	180.16	12.72
25.07	d-Glucose (E)	$C_6H_{12}O_6$	180.16	15.23
25.29	d-Glucose (Z)	$C_6H_{12}O_6$	180.16	7.2
25.55	d-Galactose	$C_6H_{12}O_6$	180.16	0.63
25.92	D-(-)-Tagatose	$C_6H_{12}O_6$	180.16	1.25
26.27	D-(+)-Glucuronic acid γ-lactone	$C_6H_8O_6$	176.12	3.67
26.61	D-(+)-Xylose	$C_5H_{10}O_5$	150.13	0.42
27.63	Myo-Inositol	$C_6H_{12}O_6$	180.16	8.23
30.88	Maltose	$C_{12}H_{22}O_{11}$	342.3	0.49
31.3	β-D-Galactopyranoside	$C_6H_{12}O_6$	180.16	0.14
32.07	D-(+)-Cellobiose	$C_{12}H_{22}O_{11}$	342.3	0.92
36.87	Sucrose	$C_{12}H_{22}O_{11}$	342.3	9.81
37.6	Maltose	$C_{12}H_{22}O_{11}$	342.3	0.57
37.82	D-(+)-Turanose	$C_{12}H_{22}O_{11}$	342.3	6.19
38.72	D-Trehalose	$C_{12}H_{22}O_{11}$	342.3	0.71
40.65	Lactulose	$C_{12}H_{22}O_{11}$	342.3	0.56
41.47	Galactinol	$C_6H_{12}O_7$	196.16	0.57

The detection of ribitol (0.16%) and galactinol (0.57%) aligns with earlier findings on the sugar alcohol profiles of methanolic extracts in medicinal plants, which contribute to their osmoprotectant properties. Additionally, rare sugars such as D-(-)-Tagatose (1.25%) and lactulose (0.56%) were identified, which are known for their prebiotic and therapeutic applications [9].

The carbohydrate profile of *Combretum indicum* in this study corresponds with its potential bioactivity. Monosaccharides like D-(-)-Fructose and D-Glucose are essential for cellular energy and metabolic pathways. The presence of D-(+)-Xylose (0.42%) and β-D-Galactopyranoside (0.14%) highlights the complexity of the metabolite profile. Sucrose and maltose, identified at 9.81% and 0.57%, respectively, have been associated with their roles as energy reservoirs and stress response mediators in plants [10].

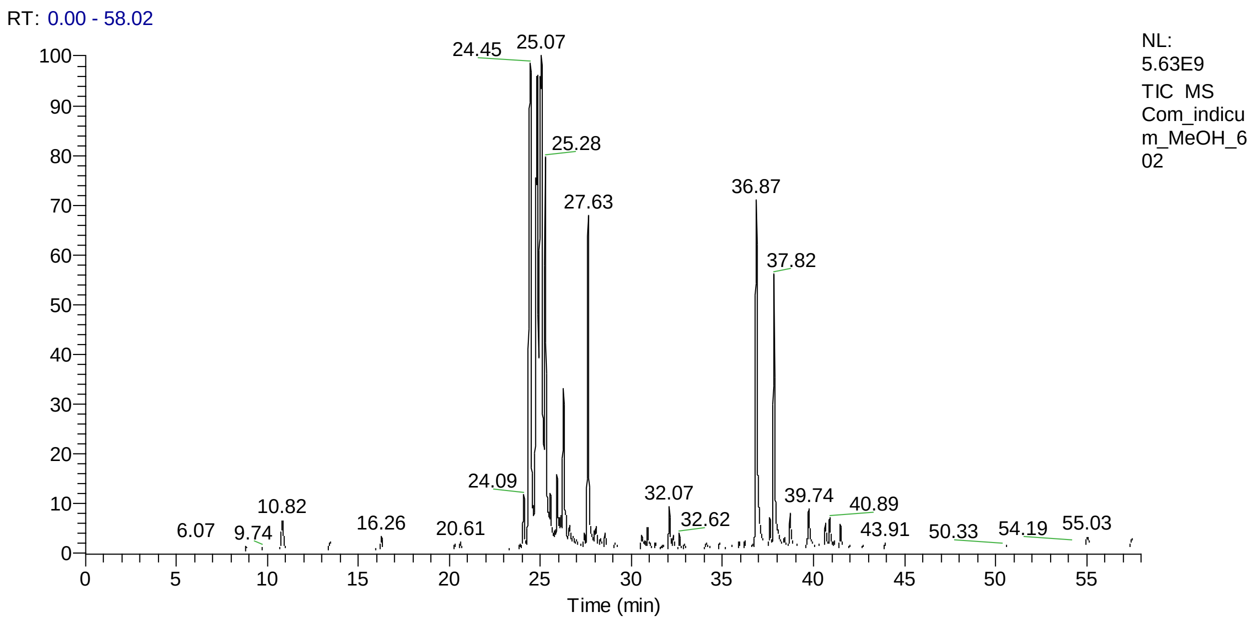

Figure 1. GC-MS chromatogram of methanolic extract

These findings demonstrate the diverse metabolic composition of *Combretum indicum*, aligning with its traditional uses in treating infections and inflammation. The abundance of sugars and sugar alcohols supports its potential as a source of antioxidants and osmoprotectants. Previous studies on *Combretum indicum* have highlighted its therapeutic properties, including antimicrobial and antioxidant activities [11]. The detection of metabolites such as myo-inositol and glucuronic acid γ-lactone in significant amounts provides further evidence of its functional properties.

Further investigations are recommended to isolate and evaluate the individual bioactivities of the identified metabolites. Additionally, exploring the synergistic effects of these compounds could offer insights into the medicinal value of *Combretum indicum*.

3.2 GC-MS analysis Chloroform Extract

The GC-MS analysis of the chloroform extract of *Combretum indicum* revealed a diverse profile of 32 metabolites, including fatty acids, hydrocarbons, sterols, alcohols, and vitamins. The most abundant compounds included tetratetracontane (7.73%), α-linolenic acid (7.07%), ursolic acid 2TMS (7.42%), and dotriacontane (6.1%), which collectively highlight the therapeutic and nutritional potential of the extract.

The presence of fatty acids such as palmitic acid (5.88%), stearic acid (3.35%), linoleic acid (2.62%), and α-linolenic acid (7.07%) indicates a rich profile of essential lipids. These compounds are well-documented for their roles in anti-inflammatory, antioxidant, and cardiovascular health [12].

Table 2. Metabolites of chloroform extract of *Combretum indicum*

RT (min)	Metabolites	Formula	Molecular weight (g/mol)	% Area
7.05	Hexanoic acid	$C_6H_{12}O_2$	116.16	0.3
9.65	Monoamidoethylmalonic acid	$C_6H_{11}NO_5$	177.16	1.08
10.8	Glycerol	$C_3H_8O_3$	92.09	0.16
13.34	Phosphoric acid	H_3PO_4	97.99	0.43
18.48	Malic acid	$C_4H_6O_5$	134.09	0.4
19.56	Linalool oxide	$C_{10}H_{18}O_2$	170.25	0.1
23.86	Dodecadioic acid	$C_{12}H_{22}O_4$	230.3	0.09
25.56	Citric acid	$C_6H_8O_7$	192.12	0.18
26.09	Neophytadiene	$C_{20}H_{38}$	278.52	2.61
26.77	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	312.53	0.61
30.56	Palmitic	$C_{16}H_{32}O_2$	256.42	5.88
32.09	Phytol	$C_{20}H_{40}O$	296.53	2.66
32.36	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.45	0.12
34.09	Stearic acid	$C_{18}H_{36}O_2$	284.48	3.35
34.35	Linoleic acid	$C_{18}H_{32}O_2$	280.45	2.62
34.84	α-Linolenic acid	$C_{18}H_{30}O_2$	278.43	7.07
37.36	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280.45	0.65
37.67	Eicosapentaenoic Acid	$C_{20}H_{30}O_2$	302.46	0.33
39.29	1-Monopalmitin	$C_{16}H_{32}O_4$	288.42	0.46
40.42	Behenic acid	$C_{22}H_{44}O_2$	340.58	0.44
40.67	1,25-Dihydroxyvitamin D3	$C_{27}H_{44}O_3$	416.64	0.21
42.83	1-Linolenoylglycerol	$C_{21}H_{38}O_4$	354.52	0.72
43.27	10,12-Docosadiynedioic acid	$C_{22}H_{32}O_4$	360.49	0.26
43.52	Heptacosane	$C_{27}H_{56}$	380.74	1.17
44.45	Squalene	$C_{30}H_{50}$	410.72	2.31

46.21	Tetratetracontane	$C_{44}H_{90}$	618.19	7.73
46.52	Octacosane	$C_{28}H_{58}$	394.77	1.84
48.5	α-Tocopherol	$C_{29}H_{50}O_2$	430.71	1.67
48.73	Tetratetracontane	$C_{44}H_{90}$	618.19	4.84
48.97	1-Triacontanol	$C_{30}H_{62}O$	450.81	2.62
49.94	Stigmasterol	$C_{29}H_{48}O$	412.7	0.88
50.71	Sitosterol	$C_{29}H_{50}O$	414.71	5.4
51.3	Dotriacontane	$C_{32}H_{66}$	450.87	6.1
53.04	10,12-Docosadiynedioic acid	$C_{22}H_{32}O_4$	360.49	1.09
53.52	1-Tetratriacontanol	$C_{34}H_{70}O$	506.94	6.25
55.03	Ursolic acid 2tms	$C_{30}H_{48}O_4Si_2$	536.88	7.42

The, α-linolenic acid, a prominent omega-3 fatty acid, has been associated with reducing oxidative stress and promoting cellular health. Similarly, stearic acid and linoleic acid are precursors in metabolic and structural processes of cell membranes [13].

Sterols such as stigmasterol (0.88%) and sitosterol (5.4%) were also identified. These phytosterols are known to exhibit cholesterol-lowering and anti-inflammatory properties, contributing to the medicinal significance of *Combretum indicum*. Phytol (2.66%), a diterpene alcohol, was another notable compound detected, recognized for its antimicrobial and anti-inflammatory activities [14].

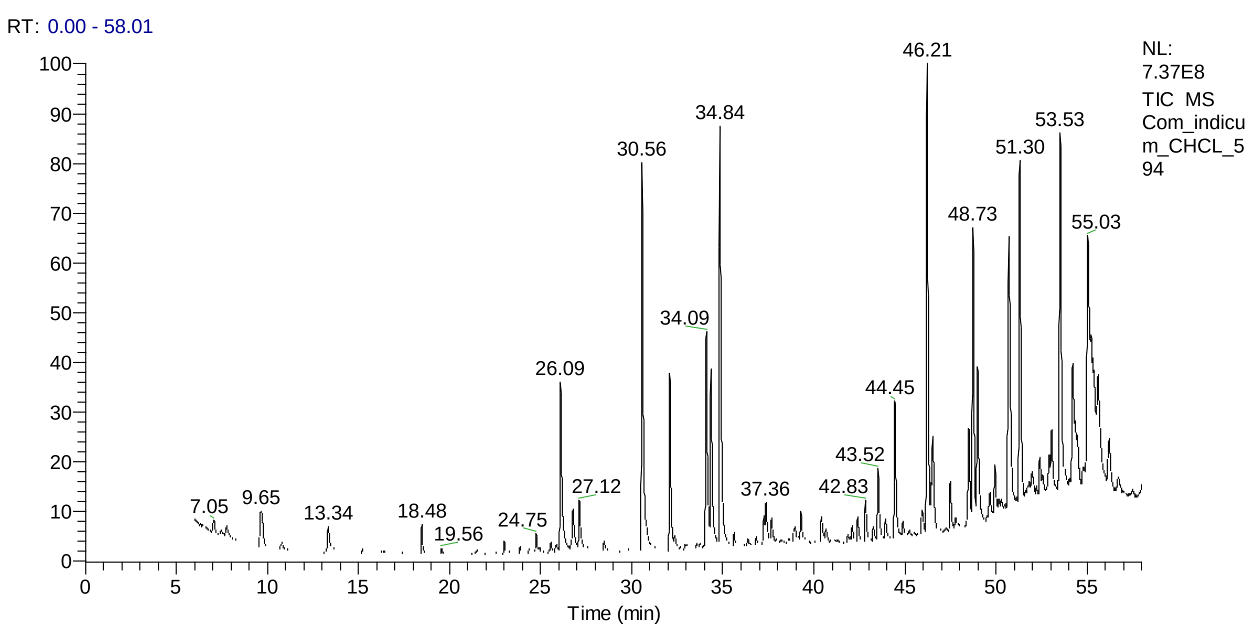

Figure 2. GC-MS chromatogram of chloroform extract

Hydrocarbons such as tetratetracontane (7.73%), dotriacontane (6.1%), and octacosane (1.84%) were identified in significant amounts. Moreover, the detection of squalene (2.31%), a precursor to sterols and triterpenes, highlights its importance in maintaining cellular integrity and its antioxidant properties.The extract also contained bioactive vitamins and alcohols, including α-tocopherol (1.67%) and 1-triacontanol (2.62%). α-Tocopherol, a form of vitamin E, is a potent antioxidant that protects against lipid peroxidation. 1-Triacontanol is a known plant growth regulator and has been reported for its antimicrobial properties [15]. The presence of ursolic acid (7.42%) further strengthens the therapeutic potential of the extract due to its antioxidant, anti-inflammatory, and anti-cancer activities [16]. Neophytadiene (2.61%) and 1-tetratriacontanol (6.25%) were detected in notable amounts and are associated with antimicrobial and anti-inflammatory properties, consistent with the traditional uses of *Combretum indicum*. The identification of 1,25-dihydroxyvitamin D3 (0.21%), though in smaller amounts, is particularly intriguing, as it highlights the nutritional and biofunctional value of the plant extract.

The detection of minor components such as hexanoic acid (0.3%), malic acid (0.4%), citric acid (0.18%), and behenic acid (0.44%) adds complexity to the metabolite profile and suggests potential contributions to the extract's therapeutic properties. These findings align with previous studies that have highlighted the diverse bioactive composition of *Combretum indicum* and its relevance in traditional medicine for treating infections, inflammation, and oxidative stress. The presence of high-value bioactive compounds such as ursolic acid, phytosterols, α-linolenic acid, and squalene underscores the extract's therapeutic potential. Future studies should focus on isolating these metabolites to explore their synergistic effects and validate their bioactivities through in vitro and in vivo studies.

4. Conclusion

GC-MS analysis of methanolic and chloroform extracts of *Combretum indicum* unveiled a wide spectrum of bioactive phytochemicals with promising therapeutic potential. This study establishes a foundation for further bioassay-guided isolation and pharmacological evaluations to explore novel drug leads.The major metabolites, particularly D-(-)-Fructose, d-Glucose, Myo-Inositol, α-Linolenic acid, Tetratetracontane, and Phytol, dominate the profile, suggesting significant roles in osmoregulatory functions, antioxidant, antimicrobial, anti-inflammatory, and lipid-regulating activities.This is the first GCMS based comparative study of Chloroform and methanolic extract of *Combretum indicum leaves.* There are the further need to evaluate bioactivity and toxicity analysis of *Combretum indicum* leaf.

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