Research Article

Phytochemical Evaluation and Gas Chromatography-Mass Spectrometric Analysis of Column Fractions of *Carissa edulis* Leaf Extract

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Abstract

The research aims at evaluating the phytoconstituents of methanolic leaf extract of *Carissa edulis*. The air-dried, ground leaf of *Carissa edulis* was extracted with methanol by maceration. The crude extract was defatted with n-hexane and partitioned with chloroform, ethyl acetate and n-butanol. The methanol crude, n-hexane, chloroform, ethyl acetate and n-butanol were screened for phytochemicals. The n-butanol partitioned portion was subjected to column chromatographic (CC) analysis using ethyl acetate and n-butanol as the mobile phase at different ratios and silica gel 60-120 mesh as the stationary phase. The methanol crude extract, n-hexane, chloroform, ethyl acetate and n-butanol yielded yield of 28.03% $^{w}/_{w}$, 36.04%, 11.24%, 3.08% and 15.94% respectively. The phytochemicals analysis of the methanol leaf extract of *Carissa edulis* revealed the presence of soluble starch, tannins, flavonoids, terpenoids, cardenolides, saponins and cardiac glycosides. Fractions CEBa and CEBb showed one (1) visible spot on TLC. Gas Chromatography-Mass spectrometry (GC-MS) analysis revealed that fraction CEBa and CEBb had seven (7) and (4) compounds namely; tetradecanal, 1-hexadecanol, 3-heptene, 4-ethyl, benzothiazole- 2-methyl, 17-pentatriacontene, tridecane, 2-pentanone 3-methylene; and 9-oxobicyclo [6,1,0] nonane, phenol 2,5-bis (1,1-dimethyl ethyl), dibutyl phthalate, pentalene octahydro 1-(2-octyldecyl) respectively. The presence of these phyto constituents in the leaf of *Carissa edulis* may be responsible for the folkloric use of leaves by local people for the treatment of various ailments. Isolation of each chemical component could lead to establishment of novel drug.

Keywords: Chromatography, *Carissa edulis*, Phytochemicals, Gas Chromatography-Mass Spectrometry

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Introduction

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects [1]. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs [1]. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences [1].

The therapeutic value of medicinal plants is due to substances found in the plant tissues that produce a definite physiological action on the human body *Carissa edulis* Vahl is widespread in many parts of Africa. It belongs to the family Apocynaceae and grows at forest edges, in forests and woodlands [2].

Carissa edulis (**Figure 1**) is a well-known African medicinal plant widely used in traditional treatment of headaches, chest pains, rheumatism, gonorrhoea, syphilis and rabies. The plant roots have been used in Africa for a variety of medicinal purposes. The vapour from a hot aqueous root bark infusion is inhaled as treatment for chest congestion and the root powder is applied to toothache to relieve pain. The roots are also used to treat gastric ulcers and the decoction is used to treat malaria [3, 4]. Phytochemicals such as sesquiterpenes, benzonoids lignans, propanoids, cumarins phenolic [5, 6], steroids, terpenes, tannins, flavonoids and cardiac glycosides [7] have been isolated from *Carissa edulis*.

Pharmacological studies revealed that extract of the leaves of *Carissa edulis* reduces blood glucose level in streptozotocin diabetic rats, indicating the presence of compounds with hypoglycaemic activity [8]. Aqueous extracts of roots of *Carissa edulis* have exhibited significant activity against the *Herpes simplex* virus (HSV) *in vitro* and *in vivo* for both wild type and resistant strains of HSV [9].

Chemical constituents isolated from *Carissa edulis* include 2- hydroxyacetophenone [6], phenolic compounds, insoluble proanthocyanidins, lignans; sesquiterpenes of the eudesmane and germacrane derivatives, sterols, tannins,

cardiac glycosides and flavonoids have been isolated [2, 5]. In addition to these six, volatile compounds from the root of *Carissa edulis* have been analyzed by GC/MS [10].

Figure 1 *Carissa edulis* plant in its natural habitat

Materials and Methods

Equipment used for sample collection, preparation and chemical analysis (extraction and phytochemical analysis, column and thin chromatographic analysis) were: cellophane bags, wooden mortar and piston, beakers, separating funnels, conical flasks, spatula, steel trays, test tubes, measuring cylinders, Whatmann No. 1 filter papers, Muslin cloth, columns glass tube (90x2.8cm), thin layer chromatographic plates, capillary tubes, developing tanks, retort stands wash bottles, glass mortar and piston, desiccators, bunsen burner and steam bath.

Chemicals and Reagents

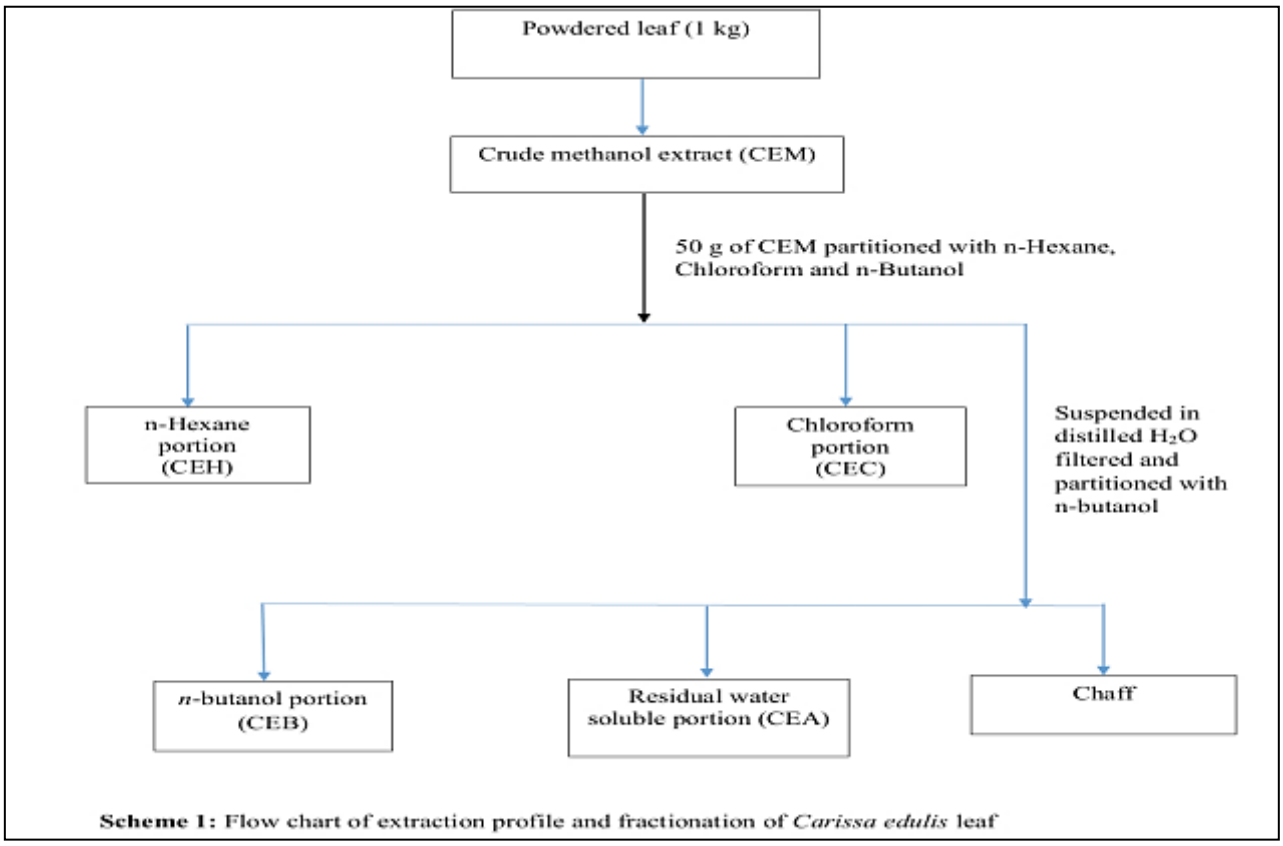
Chemicals and reagents used for chemical analyses: 95% methanol, Molisch;s reagent, concentrated sulphuric acid (H_2SO_4), distilled water, Fehling's solutions A and B, Dilute hydrochloric acid (HCl), resorcinol, 5% potassium hydroxide (KOH), 1% ferric chloride, 10% lead ethanoate, benzene, ammonia, chloroform, acetic anhydride, ethanol, glacial acetic acid, ferric chloride, Dragendorff's reagent, Mayer's reagent, 40% calcium hydroxide, silica gel 60-120 mesh (Loba Chemie, India), n-butanol, n-hexane, ethyl acetate.

Sample Collection and Identification

Fresh leaves of *Carissa edulis* were collected from the Faculty of Pharmacy Herbarium, University of Maiduguri Borno State, Nigeria and authenticated by a plant Taxonomist in the Department of Biological Sciences, University of Maiduguri, Nigeria. The plant was given a herbarium voucher number 663C. It was air dried under shade, rendered free of foreign material through manual picking, ground with a wooden mortar and pestle to a powder.

Plant Extraction and Partitioning

Five hundred gram (500 g) of the pulverized dried leaves of *Carissa edulis* was extracted by cold extraction method (maceration) with 2.5 L of 85% methanol at room temperature for seventy-two hours (72 hr) in a round bottom flask with occasional shaking. The soaked sample was passed through a muslin cloth to remove the vegetative debris and the liquid was filtered through Whatmann No. 1 filter paper. The crude extract was concentrated to dryness. The extract was weighed, labelled and subjected to further analysis. Fifty gram (50 g) of the crude extract was subjected to defatting with n-hexane and then partitioned with chloroform, ethyl acetate and n-butanol based on their polarities. The Schematic of the extraction and partitioning profile is shown in Scheme 1.



Scheme 1: Flow chart of extraction profile and fractionation of *Carissa edulis* leaf

Phytochemical Screening of Carissa edulis

Preliminary phytochemical screening was carried out on the dried powdered leaf, methanol extract and partitioned portions of *Carissa edulis* to test for the presence or absence of tannins, terpenes, steroids, flavonoids, anthraquinone, cardenolides, saponins, phlobatanin, cardiac glycoside, alkaloids, soluble starch and resins using standard procedures [11-16] as adopted by Yakubu *et al*. [17]

Chromatographic Separations of Phytoconstituents from the Methanol Leaf Extract of Carissa edulis
Column and Thin Layer Chromatography Analysis

Two hundred and fifty (250 g) of silica gel 60-120 mesh was used to prepare a slurry. The silica gel was mixed with chloroform and stirred with a clean glass rod until a uniform mixture was obtained then it was packed cautiously and manually to about two third the size of the column using a glass funnel. The gel was then allowed to settle and pack for 24 hrs to pre-swell and activate the silica gel. The pre-absorbed sample (3 g) was mounted on the already equilibrated silica gel. This was topped with a small layer of cotton, then sand to protect the shape of the sample from the velocity of newly added eluent (stationary phase). The eluting solvent initially was 100% ethyl acetate and the polarity was gradually increased by 80:20, ethyl acetate: n-butanol ratio until 0:100 ethyl acetate: n-butanol ratio was used. The column chromatographic setup and separation is shown in Plate 1.

Commercially prepared aluminium TLC plates of 20 x 20 cm x 0.25 mm activated silica gel of 60 F_{254} (Merck, Germany) was cut to size of 5 x 5 cm. n-butanol partitioned portion of *Carissa edulis* extract which contained the most phytochemicals and had the largest quantity was preferred for the chromatographic analysis. Methanol was used to dissolve the partition portion and allowed to stand for 30 minutes and then it was spotted at the bottom of the TLC plate (about 2 cm from the base). The partition portion which was dissolved in a few drops of methanol was dried in air for 30 minutes and was used for the spotting by the aid of a capillary tube. The spotted plate was kept in a previously saturated developing chamber containing the chosen solvent system as mobile phase and was covered with a watch glass and allowed to run $3/4^{th}$ of the height of the prepared plates [18].

Solvent combination of n-hexane, ethyl acetate and methanol was used in increasing polarity, with the ratio combination of 7:4:1.4 respectively. The fractions collected were monitored by TLC and similar fractions were pooled together. Solvent was then evaporated from the bulked fraction, allowed to dry and weighed. TLC analysis was carried out on the semi dry bulked fraction using various polar solvents to ensure the purity of the fraction. The Thin Layer Chromatographic process in shown in Plate 2. Fraction CEBa and CEBb (Plate 3 and Plate 4) which gave

a single spot when developed using chromatographic tank, sprayed with 10% sulphuric acid in methanol was allowed to dry and viewed under ultra-violet light of 254 nm wavelength. A crystalline solid was obtained which was soluble in methanol and ethyl acetate. The melting point was determined (uncorrected) and was further analysed using GC-MS technique.

Plate 1: column chromatography in progress

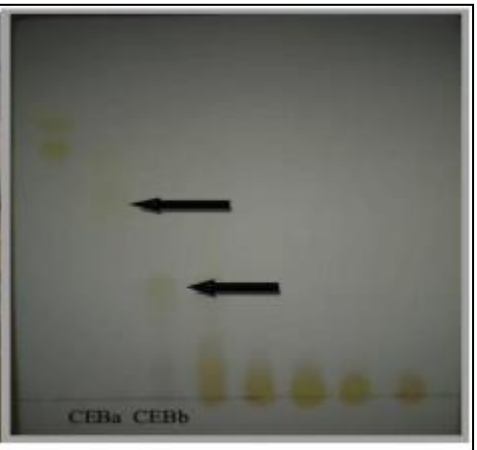


Plate 2: Thin Layer chromatographic plate showing isolated spots by Preparative TLC technique

Plate 3: Isolated fraction CEBa

Plate 4: Isolated fraction CEBb

Gas Chromatography- Mass Spectrometry (GC-MS)

A SHIMADZU, QP-2010 plus (Japan) GC-MS was used to analyse the samples at Research Laboratory of the Department of Chemistry, American university, Yola, Adamawa State, Nigeria. The GC-MS was equipped with a split injector and an ion-trap mass spectrometer detector, together with a fused-silica capillary column having a thickness of 1.00 µm, dimensions of 30 m x 0.25 mm and temperature limits of 60 ºC to 325 ºC. The column temperature was programmed between 80 ºC and 250 ºC at a rate of 3.0 ml/min. The temperature of the injector and detector were at 250 ºC and 200 ºC respectively. Helium gas was used as a carrier gas at a flow rate of 46.3 cm/sec. Mass spectra was recorded across the range of 40 to 600 m/z for the duration of 25 min.

Components were identified by computer-aided matching of their spectra with spectra of known compounds from the Library of spectra of the National Institute of Standards and Technology (NIST), formerly National Bureau of Standards, Washington, USA. The fragmentation patterns of the identified compounds were then examined for consistency with known data from literature [19]. In addition, the hit quality which indicated how closely matched the compound is with the Library data was used to further verify the identity of the compounds.

Results

Extraction Profile of Powdered Leaf of Carissa edulis

Table 1 showed the result of the extraction carried out on the leaf of *Carissa edulis*. Extraction of the leaf of *Carissa edulis* using methanol give a percentage yield 28.03% $^{w}/_{w}$.

Table 2 showed the partitioning of the 50 g of the crude methanol extract using n-hexane, chloroform, ethyl acetate and n-butanol which gave a percentage yield 36.04% $^{w}/_{w}$, 11.24% $^{w}/_{w}$, 3.08% and 15.94% $^{w}/_{w}$ respectively. The colour of the methanol extract was black while the partitioned portion of n-hexane was oily black, the chloroform portion was a greenish powder, the ethyl acetate portion was a light green powder, and the n-butanol portion was brown and gummy in colour.

Table 1 The extraction profile of the crude methanol and partitioned portions of the leaf of *Carissa edulis*

S. No.	Fraction	Weight (g)	%Yield ($^{w}/_{w}$)	Colour	Texture
1	Methanol	140.16	28.03	black	gummy

Table 2 The extraction profile of the partitioned portions of the leaf of *Carissa edulis*

S. No.	Fraction	Weight (g)	%Yield ($^{w}/_{w}$)	Colour	Texture
1	n-hexane	18.02	36.04	black	Oily
2	Chloroform	5.62	11.24	greenish	powdery
3	Ethyl acetate	1.54	3.08	light green	powdery
4	n-Butanol	7.97	15.94	brown	gummy
5	Aqueous	55.34	16.92	brown	gummy

Table 3 Phytochemical Constituents of leaf, Methanol, N-hexane, Chloroform, Ethyl acetate and N-butanol extract of *Carissa edulis*

S. No.	Active Component/Test	PCE	MCE	HCE	CCE	ECE	BCE	WCE
1. Carbohydrates								
i	Molish test	+	+	□	□	□	□	+
ii	Barfoed test	□	□	□	□	□	□	□
iii	Fehling test	+	+	□	□	□	□	+
iv	Combined reducing sugar	+	+	□	□	□	□	+
v	Test for pentoses	+	+	□	□	□	□	+
vi	Test for ketoses	+	□	□	□	□	□	□
2	**Soluble Starch**	+	+	□	□	□	□	+
3.	**Tannins**	□	□	□	□	□	□	□
i	Ferric chloride	+	+	□	□	□	□	+
ii	Lead acetate	+	□	□	□	□	□	□
4.	**Flavonoids**	□	□	□	□	□	□	□
i	Shinodas test	+	+	□	+	+	+	+
ii	Ferric chloride	+	+	□	□	□	□	+
iii	Lead acetate	+	□	□	□	□	□	□
iv	Sodium hydroxide	□	□	□	□	□	□	□
5.	**Anthraquinones**	□	□	□	□	□	□	□
i	Free anthraquinone	□	□	□	□	□	□	□
ii	combined anthraquinone	□	□	□	□	□	□	□
6.	**Terpenoids**	+	+	+	+	+	+	+
7.	**Test for Cardenolides**	□	□	□	□	□	□	□
i	Keller Killiani's test	+	+	□	□	□	□	+
8.	**Saponins**	+	+	□	□	□	□	+
9.	**Phlobatannins**	□	□	□	□	□	□	□
10.	**Cardiac Glycoside**	□	□	□	□	□	□	□
i	Salkowski test	□	□	□	□	□	□	□
ii	Lieberman Burchard test	+	+	□	+	+	+	□
11.	**Alkaloids**	□	□	□	□	□	□	□

i	Dragendorff reagent	□	□	□	□	□	□	□
ii	Mayer reagent	□	□	□	□	□	□	□
iii	Wagner reagent	□	□	□	□	□	□	□

Key: (+)= Present, (-)= Absent, PCE = Powder *Carissa edulis*, **MCE =** Methanol crude extract of *Carissa edulis*, **HCE =** n-hexane portion of *Carissa edulis*, **ECE =** Ethyl acetate portion of *Carissa edulis*, **BCE =** n-butanol portion of *Carissa edulis* and **WCE =** water extract of *Carissa edulis*.

Phytochemical Screening of Powdered Leaf, Crude Methanol and Partitioned Portions of Carissa edulis

The results show the presence of carbohydrate, soluble starch, tannins, saponins and flavonoids, in powdered leaf, crude methanol and aqueous extracts. Terpenoids, cardiac glycosides and flavonoids were obtainable in all the extracts undergo study even though some of the chromagenic test gave negative results as can be seen from **Table 3**. There was conspicuous absence of glycosides and alkaloids.

Column Chromatographic Analysis of N-butanol Leaf Portion of Carissa edulis

Table 4 Shows the result of the column chromatographic analysis of the n- butanol partitioned portion of *Carissa edulis* leaf extract. Fractions collected from the column were combined based on the similarities of their retardation factor (R_f) and encoded accordingly. The profile of the combined fractions was determined and the pooled fractions coded CEBa, with a weight of 0.19 g while CEBb had a weight of 0.07 g.

Phytochemical Screening of Column Fraction of N-butanol Portion of Carissa edulis Leaf

Column fractions collected from the n- butanol portion of *Carissa edulis* leaf extract were screened for phytochemicals. The pooled column fraction coded CEBa showed the presence of terpenoids, cardenolides and cardiac glycosides while CEBb contained terpenoids, and cardiac glycosides as shown in **Table 5**.

Table 4 The profile of the combined fraction of column chromatographic analysis

Recombined Fractions	**Weight of dried pooled Fraction in (g)**	**%($^{w}/_{w}$) yield of pooled fraction**	**Colour**
CEBa (1-2)	0.19	6.3	Brown
CEBb (3-6)	0.07	2.3	light yellow

Solvent front = 8 cm; Solvent system = ethyl acetate, methanol, water = 7:4:1.4

Table 5 Phytochemical Screenings of column fraction of n- butanol partitioned leaf extract of *Carissa edulis*

Active component/Test		**CEBa**	**CEBb**
1.	**Test for tannins**		
i.	Ferric chloride	□	□
ii.	Lead acetate	□	□
2.	**Test for flavonoids**		
i.	Shinoda's test	□	□
ii.	Ferric chloride	□	□
iii.	Lead acetate	□	□
3.	**Test for terpenoids**	+	+
4.	**Test for cardenolides**		
i.	Keller Killiani's test	+	□
5.	**Test for cardiac glycoside**		
i.	Salkowski test	+	+
ii.	Liberman Burchard's test	□	□

Key (+) = Present, (-) = absent, CEB = *Carissa edulis* n-butanol leaf extract

Table 6 Summary Fraction CEBa and CEBb

Fraction	Colour of fractions	No. of spot	Distance (cm)	Rf
CEBa	Brown	1	5.00	0.63
CEBb	Light yellow	1	2.50	0.31
Solvent front=8cm Solvent system = ethyl acetate, methanol and water 7:4:1.4				

Thin Layer Chromatographic Analysis of Column Fraction of n-butanol Leaf Portion of Carissa edulis

The fractions collected from column chromatography were monitored by using TLC technique. Fraction CEBa and CEBb were brown and light yellow in colour which gave one (1) visible spots each respectively when n-hexane, ethyl acetate and methanol are used as solvent system at ratio of 4:4:2. The R_f value of the two spots were 0.63 and 0.31 respectively as shown in **Table 6**.

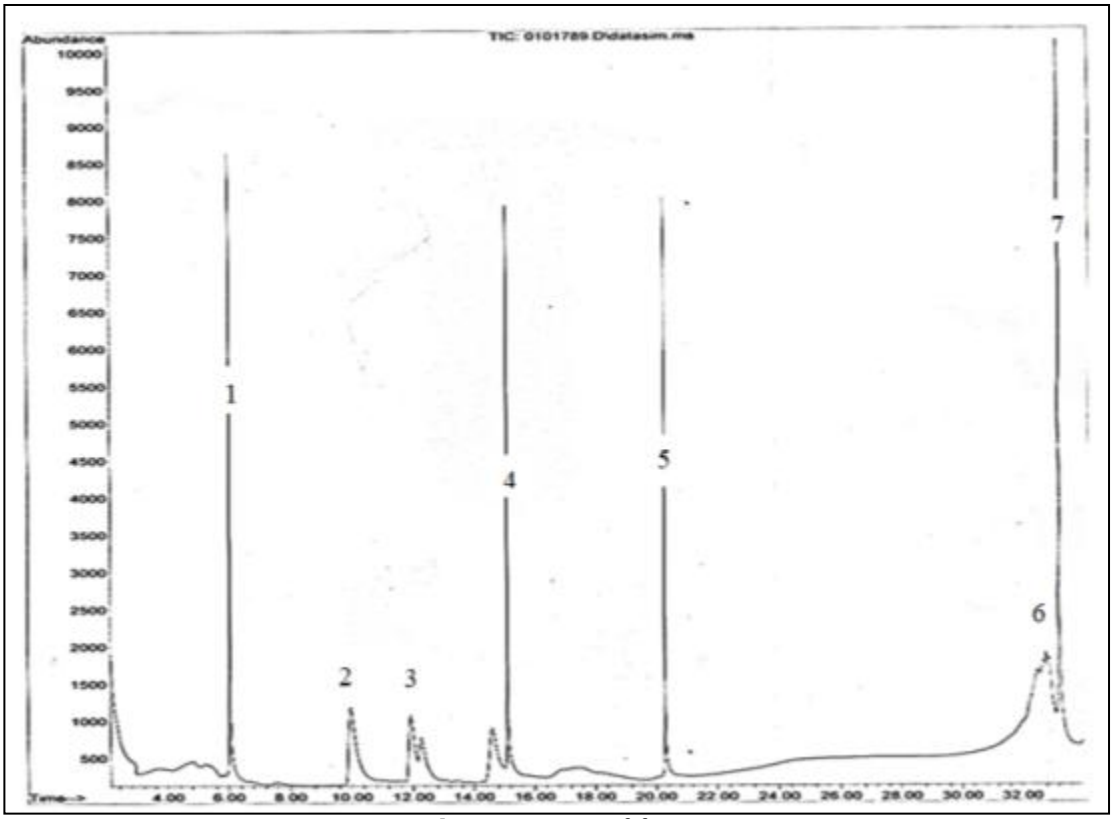


Figure 2 Chromatogram of fraction CEBa

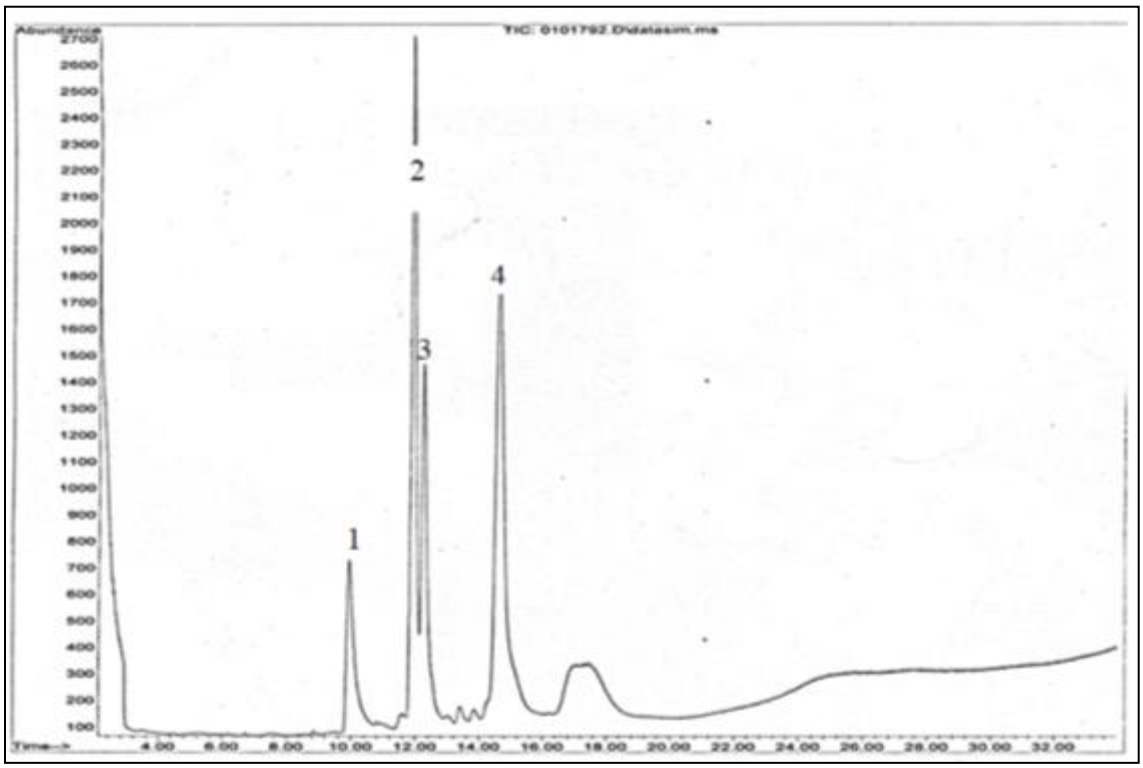


Figure 3 Chromatogram of fraction CEB_b

The Gas Chromatography-Mass Spectroscopy of fraction CEBa and CEBb

The chromatogram of the fraction CEBa and CEBb are shown in **Figures 2** and **3** respectively fraction CEBa had 7 prominent peaks and while that of CEBb had 4 peaks.

The profile of each of these peaks with the scan number, retention time, molecular formula and names of the compounds as given from the library are shown **Tables 7-8** respectively.

Table 7 Gas Chromatogram Mass Spectroscopy (GC-MS) of Fraction CEBa

Peak No.	Scan No.	Retention time, (min.)	Mol. weight	Name of Compound	Mol. Formula
1	981	6.007	212	Tetradecanal	$C_{14}H_{28}O$
2	1985	10.018	242	1-Hexadecanol	$C_{16}H_{34}O$
3	2475	11.976	126	3-Heptene, 4-ethyl-	C_9H_{18}
4	3166	14.737	149	Benzothiazole, 2- methyl	C_8H_7NS
5	3254	15.089	490	17-pentatriacontene	$C_{35}H_{70}$
6	4556	20.291	184	Tridecane	$C_{13}H_{28}$
7	7785	33.192	100	2-pentanone 3-Methylene	$C_6H_{12}O$

Table 8 Gas Chromatogram Mass Spectroscopy (GC-MS) of Compound of Fraction CEBb

Peak No.	Scan No.	Retention time, (min.)	Mol. weigh t	Name of Compound	Mol. Formula
1	2451	11.880	126	9-oxabicyclo [6,1,0]	C8H14O
2	1969	9.954	206	phenol 2,4-bis (1,1- dimethyl ethyl	C14H22O
3	3102	14.48	278	dibutyl phthalate	C16H22O4
4	3768	17.142	362	pentalene, octahydro 1-(2-octyldecyl)-	C26H50

Discussion

The phytochemical screening of the methanolic extract of the leaves of *C. edulis* indicated the presence of carbohydrates, anthraquinones, saponins, tannins, flavonoids and terpenoids. The presence of these phytocompounds supports the findings of Ibrahim et al. [20]. The presence of these secondary metabolites is responsible for the plant's anti-inflammatory, analgesic, anti-oxidant, anticonvulsant and anti-microbial activities [2, 7, 21].

Chromatographic techniques (CC and TLC) showed significant effect in the purification process for the isolation of compounds. The successful separation of biomolecules by chromatographic technique depends upon a suitable solvent system which needs an ideal range of partition coefficient for each target compound. The TLC profiling of the extracts gave an impressive result that directing towards the presence of a number of phytochemicals which are present such as carbohydrates, cardenolides, saponins and glycoside. However, for efficient separation of metabolites, good selectivity and sensitivity of detection, together with the capability of providing on-line structural information will be needed.

The GC-MS analysis carried out on fractions CEBa and CEBb revealed the presence of some prominent compound such as tetradecanal, 1-hexadecanol, 3-heptene 4-ethyl, benzothiazole 2-methyl, 17-pentatriacontene, tridecane, phenol 2-pentanone 3-methylene, for CEBa and 9-oxabicyclo[6,1,0] nonane, phenol 2,5 bis (1,1-dimethyl ethyl, dibutyl phthalate, pentalene, octahydro 1-(2-octyldecy) for CEBb.

1-hexadecanol, an alcohol has been reported to posess antioxidant property [22]. Phenol, 2, 4-bis (1, 1-dimethylethyl)- derivative is present in various plants and is known for its antibacterial and anti-inflammatory activities [23, 24]. Tetradecanal, which was the first peak is an aldehyde, with a molecular formula $CH_3(CH_2)_{12}CHO$ [26]. It is a reduced form of mystric acid, naturally produced by bioluminescent bacteria of the vibrio genus [26]. Tetradecanal compounds have antibacterial activity [25]. Pentatriacontane with a formular of $C_{35}H_{70}$ also has antibacterial and antiviral properties. The identified compounds possess many biological properties such as antioxidant, antimicrobial, antidiarrhoeal, antispasmodic, antibacterial, and antiviral [26]. Dibutyl phthalate (DBP) is a phthalic acid ester and is widely used in polymeric products to make them more flexible.

Conclusion

This study revealed the presence of 7 and 4 bioactive chemical constituents in the methanol leaf extract of *Carissa edulis*. This was possible through the use of a hyphenated Gas chromatography- Mass spectroscopic system. Thus GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant. The presences of the identified phytochemicals may be the reason for the use of this plant for the treatment of various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Carissa edulis* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, isolation of individual phytochemical constituents might result in identification of a possible useful drug.

Acknowledgements

The authors would like to acknowledge the technical assistance of Mr. Fine Akawo, Department of Chemistry, University of Maiduguri, Research Laboratory of American University Yola, Adamawa State, Nigeria, for the GC-MS analysis.

References

[1] M. Ahvazi, F. Khalighi-Sigaroodi, M.C. Mahdi, F. Mojab, and H. Zakeri. Introduction of Medicinal Plants Species with the most Traditional Usage in Alamut Region. Iran. Journal of Pharmaceutical Research, 2012, 11(1):185-194.

[2] T. Nedi, N. Mekonnen, and K. Urga. Diuretic effect of the crude extracts of Carissa edulis in rats. Journal of Ethnopharmacology, 2004, 95: 57-61.

[3] J.O. Kokwaro. Medicinal Plants of East Africa. East African Literature Bureau, Nairobi, Kenya. 1976, pp.1-8: 25-26.

[4] [4] G. Addis, D. Abebe and K. Urga. A survey of traditional medicinal plants in Shirka district, Arsi Zone, Ethiopia. Ethiopian Pharmacy Journal, 2001, 19: 30-47.

[5] R. Achenbach, H. Weibal, and I. Addacmensah. Lignins from Carissa edulis. Phytochemistry, 1983. 24: 2325-2328.

[6] M.D. Bentley, S.R. Brackeet, and A. Chapy. 2-hydroxyacetophenone principal root volatile of the east African medicinal plant, 1984; Carissa edulis. Journal Natural Product. 47: 1056-1067.
[7] H. Ibrahim. Pharmacognostic and biological (analgesic activity) studies of Carissa edulis Vahl. PhD. Thesis. Ahmadu Bello University, Zaria, Nigeria. 1997, 232 p.
[8] F.K. El-Fiky, M.A. Abou-Karam, and A. Afify. Effect of Luffa aegyptiaca (seeds) and Carisa edulis (leaves) extracts on blood glucose level of normal and streptozotocin diabetic rats. Journal Ethnopharmacology,1996, 60: 43–47.
[9] F.M. Tolo, G.M. Rukuga, F.W. Muli, E.N.M. Nijagi, W. Njue, K. Kumon, G.M. Mungai, C.N. Muthaura, J.M. Muli, L.K. Keter, E. Oishi, and M.W. Kofi- Tsekpo. Anti-viral activity of the extracts of a Kenyan medicinal plant Carissa edulis against herpes simplex virus. Journal of Ethnopharmacology. 2006, 104: 92-99.
[10] M. Moudachirou, K. Abel, M. Ayedoun, J.D. Gbenou, F.X. Garneau, H. Gagnon, and F.I. Jean. Essential oil of Carissa edulis Vahl. from Benin. Journal of Essential Oil Research. 1998, 10(2): 195-196.
[11] K.R. Brain, and T.D. Turner. The practical evaluation of pharmaceutical. Weight Sci. Tech. Bris. Britain. 1975, pp. 81-82.
[12] N.R. Vishnoi. Advanced Practical Identification. Academic Press New York, U.S.A. 1979. pp. 1-33.
[13] K.R. Markhan. Techniques of Flavonoids Identification. New York: Academic Press. 1982. pp. 1-133.
[14] G.L. Silva, I. Lee, and K. Douglas. Special problem with extraction of plants: In cannel JPR, editor. Natural Products Isolation. New Jersey: Humans press publisher. 1998. pp. 356-358.
[15] A. Sofowora. Medicinal Plants and Traditional Medicine in Africa. Ibadan: spectrum Books Ltd. 2008, pp. 26-100.
[16] W.C. Evans, Pharmacognosy. 16th Ed Sanders publishers. London. 2009, pp. 42-44; 221-229; 246-249; 304-306; 331-332; 391-393.
[17] J. Yakubu, Y.B. Usman, V.M. Balami, N. Jonathan, Y. Semiu, and D.M. Teri. Phytochemical and Elemental Analysis of some Organic Solvent Leaf Extracts of Piliostigma thonningii (Schumach) Milne-Redhead Ewemen Journal of Folklore Medicine, 2016, 2 (2): 49-57.
[18] E. Stahl. Thin Layer Chromatography: A Laboratory Handbook. 2nd ed., Springer (India); Pvt., Ltd., 2005, pp. 53-56.
[19] D.H. Williams, and I. Fleming. Spectroscopic Methods in Organic Chemistry. 4th Edition McGraw Hill Book Company, London, 1989. pp. 150-197.
[20] H. Ibrahim, F.E. Williams, K.M. Salawu, A.M. Usman (2015). Phytochemical screening and acute toxicity studies of crude ethanolic extract and flavonoid fraction of Carissa edulis leaves, Biokemistri: An International Journal of the Nigeria. 27: 39–43.
[21] J. Ya'u, A.H. Yaro, M.S. Abubakar, J.A. and Anuka, I.M. Hussaini. Anticonvulsant activity of Carissa edulis (Vahl) (Apocynaceae) root bark extract. Journal of Ethnopharmacology 2008. 120: 255–258.
[22] G. Kumaradevan, R. Damodaran, P. Mani, G. Dineshkumar, and T. Jayaseelan. Phytochemical screening and GC-MS analysis of bioactive components of ethanol leaves extract of Clerodendrum phlomidis (L.). 2015. American Journal Biology and Pharmaceutical Research, 2(3):142-148.
[23] L. Costantino, C. Parenti, M. di Bella, P. Zanoli, and M. Baraldi, (1993). Pharmacological Research, 1993, 27(4), 349-358.
[24] A.C. Amaral, L.A. Gomes, J.R. Sila, J. R., Ferreira, J. L., S Ramos, Ade, S.R. Mdo, A.B. Vermelho, and A. Rodrigues. Biomedical Research. 2014, doi: 10.1155/2014/694934.
[25] S. Soosairaj, and T. Dons. Bio-active compounds analysis and characterization in ethanolic plant extracts of Justicia tranquebariensis L. (Acanthaceae)-using GC-MS. International Journal Chemical Technology Research. 2016. 9: 260-265.
[26] B. Subbaiyan, P. Samydurai, P.M. Karthik, and V. Thangapandian, Gas chromatography and mass spectrum analysis of Catharanthus pusillus Murray (Apocyanaceae). International Research Journal of Pharmacy and Applied Science, 2014, 4(2): 48-52.

Publication History
Received 30.11.2020
Revised 07.01.2021
Accepted 15.01.2021
Online 30.01.2021