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Research

Effect of *Carissa macrocarpa* (Eckl.) A. DC. aerial parts on some non-communicable diseases: in vitro study and HPLC-QTOF/MS-MS analysis

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Abstract
Carissa species are evergreen plants that have long been employed in treating different diseases by traditional healers in many cultures. *Carissa macrocarpa* (Eckl.) A. DC. known as Natal plum is characterized by bright red, edible, plum-shaped fruit that tastes like cranberries. The pharmacological studies on *Carissa* species validated its use in indigenous medicine systems. The evidence-based modulatory potential of *C. macrocarpa* aerial parts (leaves and stems) on non-communicable diseases and hepato-protective activity is herein evaluated via testing its in vitro activity against key enzymes for metabolic disorders and support it with phytochemical study to identify the key metabolites responsible for the claimed activities. Potent antioxidant (DPPH, ABTS, and FRAP assays) and anti-inflammatory (iNOS, COX-1 and COX-2) potentials were observed along with significant inhibitory potential against *α*-amylase and *α*-glucosidase anti-diabetic enzymes. In addition, the hepato-protective activity (Annexin V apoptosis detection and evaluation of telomerase reverse transcriptase TERT) beside its beneficial effect on the neuropharmacological parameters (acetylcholinesterase and *β*-amyloid) were also proved. The HPLC-QTOF/MS-MS analysis allowed the identification of 10 fatty acids, 6 phenolics, 6 flavonoids, 4 triterpenoid saponins, and 3 miscellaneous metabolites. These findings support the notion that *C. macrocarpa* is a medicinal plant with multifactorial therapeutic potentials against some non-communicable diseases. Furthermore, this study supports the claim of traditional healers that *Carissa* species are promising hepato-protective and anti-diabetic medicines.

Article Highlights

1. *Carissa macrocarpa* aerial parts potential in management of some non-communicable diseases was evaluated.
2. Potent antioxidant, anti-inflammatory, anti-diabetic and hepato-protective potentials were observed.

Fatma A. Mokhtar and Mona M. Okba have contributed equally to this work.

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3. Phytochemical analysis led to identification of 29 compounds which are responsible for the claimed biological activities.

Keywords *Carissa macrocarpa* · Antioxidant · Anti-inflammatory · Hepato-protective · Neuropharmacological · HPLC-QTOF/MS-MS

Abbreviations

Aβ42	*β*-Amyloid 1-42
ABTS	2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
AChE	Acetylcholinesterase enzyme
CMME	*C. macrocarpa* methanol extract
COX	Cyclooxygenase enzyme
DPPH	1,1- Diphenyl-2-picryl hydrazyl
FRAP	Ferric reducing antioxidant power
GNPS	Global Natural Products Social Molecular Networking
HPLC-QTOF-MS/MS	High Performance Liquid chromatography-Quadrupole Time of Flight-mass spectrometry
IC_{50}	Inhibitory concentration leads to 50% inhibition of the measured activity
Indo	Indomethacin
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
MNs	Molecular networks
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NCDs	Non-communicable diseases
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
PGs	Prostaglandins
PI	Propidium iodide
R_t	Retention time
SI	Selectivity index
STZ	Streptozotocin
TERT	Telomerase reverse transcriptase

1 Introduction

Non-communicable diseases (NCDs) are a cluster of diseases such as liver illnesses, diabetes and neurodegenerative diseases. NCDs are chronic diseases having in common an underlying inflammation of low-grade [1]. According to the World Health Organization, non-communicable diseases cause 41 million mortalities per year (71% of deaths around the world) [2]. Plants are getting more popular because of their health benefits worldwide [3]. A comprehensive strategy for the treatment of non-communicable diseases contains radical scavenging activity, in addition to inhibition of pro-inflammatory conditions; inducible nitric oxide synthase (iNOS) and cyclooxygenases (COX-1 and COX-2), and key enzymes responsible for carbohydrate metabolism (*α*-amylase and *α*-glucosidase) [4].

Carissa, a genus of the Apocynaceae family, consists of more than 100 species of shrubs or small trees native to tropical and subtropical regions of Africa. Several *Carissa* species are traditionally used to treat edema, hepatitis, headaches, chest pain, rheumatism, and asthma. Several studies reported their antioxidant, anti-inflammatory and anti-diabetic potentials [5] as well as cytotoxic effect [6]. *Carissa macrocapa* (Eckl.) A. DC. (Figures S1, S2) is an edible fruit producing plant. It is characterized by simple, opposite decussate and petiolate leaf which is ovate in shape with entire margin and symmetric base. The leaf has mucronate apex and pinnately reticulate venation. The leaf length is about 1–3 cm while the width is about 1–2 cm at the middle parts. Stem has cylindrical to elliptical shape with slightly bitter astringent taste and no odor. The stem of the shrub measure up to 70 cm in length and its diameter is up to 1 cm [7, 8].

It has been established that crude extracts, fractions, and pure metabolites isolated from various *Carissa* species are particularly efficient treatments for liver illness. Their extracts effectively treat serious liver illnesses brought on by viruses,

harmful chemicals, and drinking too much alcohol [5]. *Carissa* species were also reported to decrease blood glucose level in streptozotocin (STZ) induced diabetes in rats [9, 10]. Their hypoglycemic effect is probably due to initiating the release of insulin from the pancreatic *B*-cells.

This prompted us to investigate the antioxidant activity of *C. macrocarpa* methanol extract (CMME) of the aerial parts (leaves and stems) as well as its ability to inhibit key enzymes involved in many disorders like diabetes mellitus, Alzheimer's and liver illnesses. Furthermore, its phytochemical profile was investigated using HPLC-QTOF-MS/MS analysis to correlate the newly proved biological potentials to its secondary metabolites. The newly explored biological potentials, if any, may contribute to incorporating *C. macrocarpa* crude extract or its secondary metabolites as part of the strategy against diabetes-related metabolic disorders.

2 Materials and methods

The materials and methods section is presented as Supplementary Material.

3 Results and discussion

3.1 Preparation of the methanol extract

The crude extract (CMME) yield was 154.47 gm which representing 15.45%.

3.2 Antioxidant activity

The pathogenesis of metabolic, inflammatory, and cardiovascular diseases is largely attributed to oxidative stress. Exogenous antioxidants are required to offer synergistic activity with the endogenous antioxidant defense system in order to improve this pathological situation [11]. Three distinct assay models; DPPH, radical scavenging activity (ABTS), and redox potential (FRAP); were used to evaluate the antioxidant capacity of CMME.

3.2.1 DPPH free radical scavenging activity

The antioxidant activity of CMME was 45.27 ± 1.56% at concentration of 100 µg/mL. The IC_{50} was 99.13 ± 1.01 µg/mL and the IC_{90} was 159.54 ± 0.79 µg/mL. Vitamin C was used as a positive control and has an IC_{50} value of 4.80 ± 0.61 µg/mL. Results are shown in supporting information (Table S1).

3.2.2 ABTS assay

The IC_{50} of CMME was 79.64 ± 6.12 µg/mL while that of the standard Trolox was 5.559 ± 0.10 µg/mL. Results are shown in Fig. 1.

3.2.3 FRAP assay

The sample ability to reduce Ferric is displayed as µM TE/mg sample. The average reading of CMME at 593 nm was 0.27 ± 3.50 and the micro molar Trolox equivalent per mg sample (µM TE/mg sample) was 99.14.

Several *Carissa* species were previously reported to have powerful antioxidant capabilities in scavenging DPPH, superoxides, hydrogen peroxide, hydroxyl, and ABTS radicals as well as having strong iron chelating activity due to their high levels of total phenolic and flavonoid contents [5, 12]. Souilem et al. studied the antioxidant activity of the hydroethanoilic extracts of *C. macrocarpa* leaves and stems using DPPH radical-scavenging activity, reducing power, inhibition of *β*-carotene bleaching, and thiobarbituric acid reactive substance (TBARS) assay techniques [13]. Regarding DPPH assay, EC_{50} values were found to be 26 ± 1 µg/mL and 281 ± 1 µg/mL for leaves and stems respectively. EC_{50} values for reducing power were 36 ± 1 µg/mL and 33 ± 1 µg/mL while that of *β*-carotene bleaching inhibition were 300 ± 1 µg/mL and 270 ± 10 µg/mL for leaves and stems respectively. For TBARS inhibition, EC_{50} values were 15.4 ± 0.1 µg/mL and 12.1 ± 0.1 µg/mL for leaves and stems respectively. Trolox EC_{50} values were 43.03 ± 1.71 µg/mL for DDPH, 29.62 ± 3.15 µg/

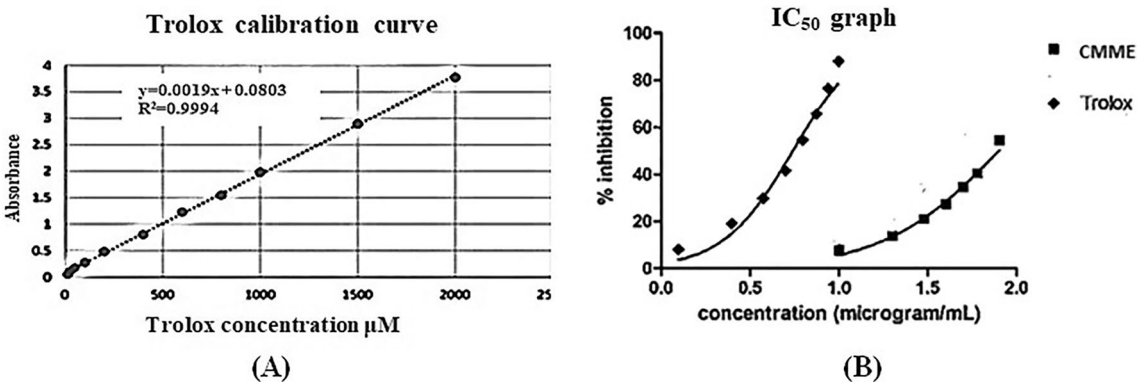

Fig. 1 Effect of CMME on oxidative parameters. **a** Dose response line for Trolox. **b** Percentage of inhibition of different concentrations of CMME. CMME: *C. macrocarpa* methanol extract; IC_{50}: the concentration of the test sample leading to 50% radical scavenging activity of ABTS

mL for reducing power, 2.63 ± 0.14 µg/mL for *β*-carotene bleaching inhibition, and 3.73 ± 1.9 µg/mL for TBARS inhibition [13].

3.3 Anti-inflammatory activity

3.3.1 Nitric oxide inhibition assay on RAW264.7 macrophages

Non-communicable diseases (NCDs) have an etiopathology that is associated with pro-inflammatory conditions. This is demonstrated by an increase in the production of nitric oxide (NO) by the inducible isoform of the nitric oxide synthase (iNOS) enzyme. Because NO accelerates the synthesis of reactive nitrogen species, it has been thought to exacerbate tissue damage. Therefore, preventing iNOS from being induced by pro-inflammatory cytokines may enhance changes linked to NCDs [14]. As a result, blocking these enzymatic pathways is thought to be a useful therapeutic approach for diseases associated with NCDs [15]. As displayed in Fig. 2A, The NO inhibition assay revealed the activity of CMME to significantly inhibit the LPS-induced NO production in RAW264.7 macrophages at 30 µg/mL and the activity was comparable to the indomethacin-treated cells. Therefore, we employed Western blotting to reveal this activity on the cellular pathways through the analysis of iNOS in cell lysates of RAW264.7. As displayed in Fig. 2B, treatment of RAW264.7 cells caused concentration–dependent decrease of the iNOS expression compared to the LPS only treated cells. Maximum

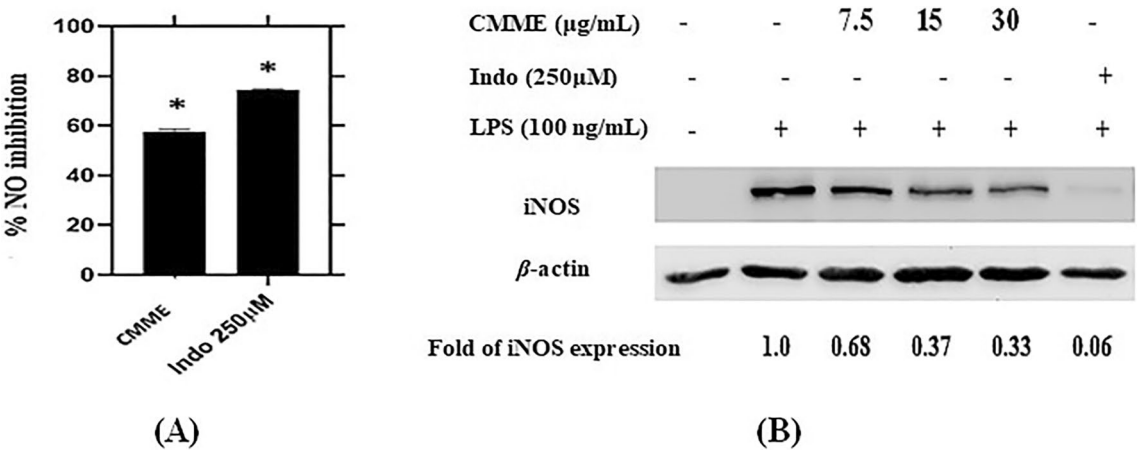

Fig. 2 Anti-inflammatory potential of CMME. RAW 264.7 macrophages were cultured and treated as described in the materials and methods section. **a** shows the preliminary screening of NO inhibition by the CMME, data are mean ± SEM of 4 determinations. Data were analyzed using one way ANOVA, significance level * refers to $P < 0.05$. **b** shows the Western immunoblot of iNOS inhibition of expression by CMME and densitometric analysis of iNOS normalized to *β*-actin. CMME: *C. macrocarpa* methanol extract; Indo: indomethacin; LPS: lipopolysaccharide

iNOS inhibition of expression was achieved at 30 µg/mL (67.0% inhibition) as revealed with densitometric analysis of obtained iNOS bands, normalized to the house keeping protein *β*-actin (Figure S3).

3.3.2 COX-1 and COX-2 Inhibitory potential

Non-steroidal anti-inflammatory drugs (NSAIDs) are used worldwide in treating inflammation and pain. High levels of prostaglandins (PGs) are produced through inflammation. NSAIDs act as anti-inflammatory by decreasing or preventing PGs production by direct inhibition of the cyclooxygenase (COX) enzymes. NSAIDs are known to inhibit both COX-1 and COX-2, suggesting that, along with their beneficial therapeutic effect of inhibiting COX-2, they also have undesired side effect which is inhibition of COX-1. Selective inhibition of COX-2 obviously confirmed its role in inflammation [16]. The IC_{50} of CMME was 67.27 ± 4.01 µg/mL and 11.66 ± 0.59 µg/mL for COX-1 and COX-2 respectively while the IC_{50} of ibuprofen (a well-known NSAID) was 8.07 ± 0.48 µg/mL and 6.58 ± 0.33 µg/mL against COX-1 and COX-2 respectively as shown in Fig. 3. Selectivity index was 5.77 and 1.23 for CMME and ibuprofen respectively as shown in supporting information (Table S2). It is obviously clear that CMME is a potent anti-inflammatory with greater selectivity index than that of ibuprofen. The extract demonstrated superior inhibitory efficacy against COX-2 compared to COX-1, with an excellent COX-2 selectivity index of 5.77. In addition to being expressed constitutively in a variety of tissues, COX-2 is primarily an induced enzyme form. It is mostly expressed at sites of inflammation, infection, and cancer and creates prostanoids that are responsible for disease pathogenesis. COX-1 is a maintenance enzyme that is typically found in most tissues. Search for anti-inflammatory drugs with an improved selectivity index is therefore required. The generation of COX-2 selective inhibitors is crucial because COX-1 inhibition is known to have side effects such as bleeding, gastrointestinal problems, and an increased risk of cardiovascular disease [17].

Our observed anti-inflammatory activity of *C. macrocarpa* aerial parts extract is in accordance with the previously published anti-inflammatory potential of other *Carissa* species roots, stems, and leaves. The observed anti-inflammatory activity of several *Carissa* species was attributed to the presence of antioxidants. *Carissa macrocarpa* leaves and stems ethyl acetate and dichloromethane fractions were previously reported to exhibit potent anti-inflammatory activity [18]. Souilem et al. studied the anti-inflammatory activity of the hydroethanoilic extracts of *C. macrocarpa* leaves and stems using NO inhibition assay on RAW264.7 machrophages [13]. They stated that, IC_{50} values were found to be 179 ± 6 µg/mL and 208 ± 9 µg/mL for leaves and stems respectively while that of dexamethasone (positive control) was 16 ± 1 µg/mL.

3.4 Hepato-protective activity

Liver problems are considered as a worldwide concern, and conventional medicinal therapies are useless. Hence, protecting the healthy liver is important for good health and well-being. Some causes of liver ailments are immune problems, cancer, infections due to virus, an overdose of drugs, and alcohol abuse. Medicinal plants derived antioxidants are able to prevent liver damage caused by oxidative stress system and different chemicals. Plants and their metabolites are attractive hepato-protective agents with fewer side effects and still there is a lot of awareness shown in consuming herbal tonics for the treatment of liver diseases [19].

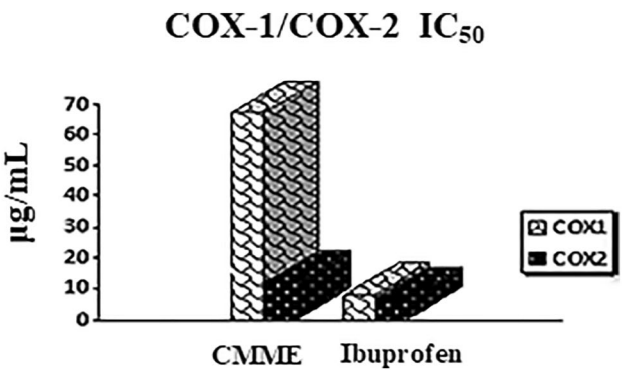

Fig. 3 Effect of CMME on inflammatory parameters: COX-1 and COX-2. CMME: *C. macrocarpa* methanol extract

3.4.1 Annexin V apoptosis detection

By using a double-labeling for annexin V and propidium iodide (PI), it is possible to distinguish between living, apoptotic, and dead cells, which may then be examined using either flow cytometry or fluorescence microscopy. It is ideal to use a single cell suspension made from the cells or tissue under examination when applying flow cytometry for the measurement of annexin V-positive apoptotic cells. An illustration of the flow cytometric analysis of annexin V labelling experiment to illustrate apoptosis in hepatic cells is shown in Fig. 4. The cytograms of the bivariate annexin V/PI analysis of the control, silymarin treated and CMME cell suspensions are illustrated in Fig. 4A. The percentage of cells in early apoptosis stage for negative control group was 0.41% compared to 3.28% for silymarin treated cells and 1.88% for CMME treated cells. The percentage of cells in late apoptosis stage for negative control group was 0.15% compared to 1.64% for silymarin treated cells and 0.73% for CMME treated cells. The percentage of cells in necrosis stage for negative control group was 2.20% compared to 22.41% for silymarin treated cells and 6% for CMME treated cells. CMME do not cause apoptosis so its use is safe. Its hepato-protective activity exceeds that of silymarin. It is worthy to note that the hepato-protective effect of other *Carissa* species was reported before. Several *Carissa* species cause significant hepatoprotection by reducing lipid peroxidation, alkaline phosphate, serum transaminase, and bilirubin, while elevating the serum and liver glutathione levels [5].

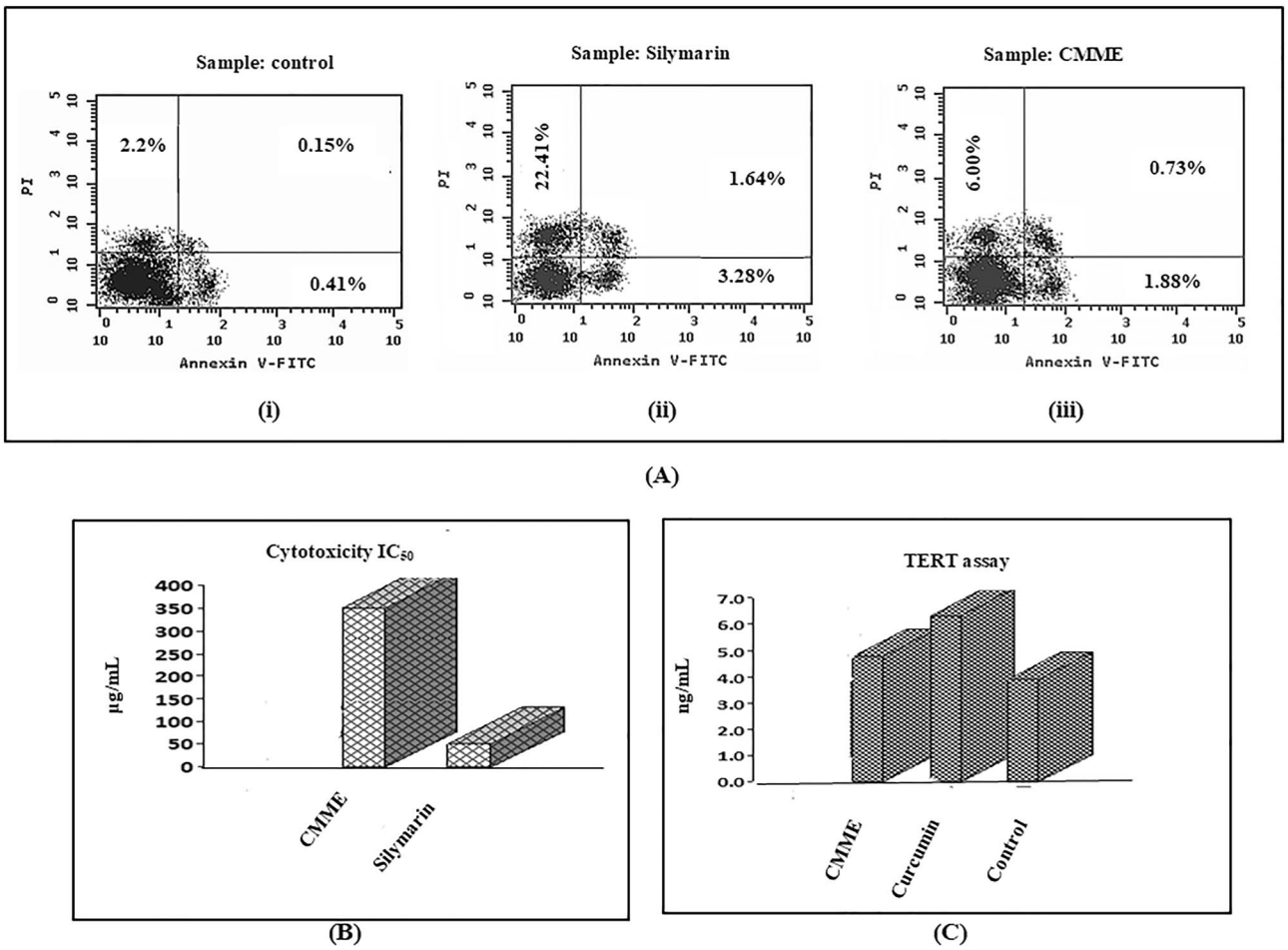

Fig. 4 Effect of CMME on hepatic parameters. **a** the bivariate annexin V/PI analysis of: (i) Control cell suspension, (ii) silymarin treated cell suspension, (iii) CMME treated cell suspension. **b** MTT assay. **c** TERT enzyme assay. CMME: *C. macrocarpa* methanol extract; IC_{50}: inhibition concentration that causes the death of 50% of cells in 48 h, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; PI: propidium iodide; TERT: telomerase reverse transcriptase

3.4.2 Cytotoxicity determination using MTT assay

IC_{50} using BJ normal cells (which are fibroblasts established from skin taken from normal foreskin from a neonatal male) was determined. IC_{50} of silymarin was 51.42 ± 2.09 µg/mL compared to 350.70 ± 14.20 µg/mL of CMME (Fig. 4B). It is noted that the plant extract is highly safe on normal cells as it has IC_{50} about seven times as that of silymarin which is a well-known hepato-protective drug. The extract can be used safely as a potent hepato-protective drug due to its low cytotoxic effect on normal cells.

3.4.3 Evaluation of telomerase reverse transcriptase (TERT)

Telomerase enzyme is active in cancer cells and inactive or has very low activity in normal somatic cells. Telomerase reverse transcriptase (TERT) assay is used for quantitative measurement of human TERT. The density of color is proportional to the amount of TERT captured from the samples. The concentration of TERT for CMME treated cells was 4.80 ± 0.23 ng/mL, while that of curcumin treated cells; a well-known anticancer compound [20, 21] was 6.30 ± 0.26 ng/mL and that for the negative control was 3.90 ± 0.34 ng/mL (Fig. 4C). Hence, CMME is effective and its activity is close to that of curcumin.

As far as we know, it is the first time to study the hepato-protective and cytotoxic effect of *Carissa* species by these methods. The results support the claim of traditional healers that *Carissa* species are promising hepato-protective medicines [5]. From our results we suggest using CMME as a hepato-protective remedy in liver dysfunctions due to its high safety as it does not cause any cytotoxicity or apoptosis for cells.

3.5 Anti-diabetic activity

Anti-diabetic potential was characterized for CMME through enzymology evaluation of *α*-amylase and *α*-glucosidase enzymes. *α*-Amylase inhibitor screening assay revealed that IC_{50} of CMME was 51.73 ± 2.57 µg/mL compared to 27.20 ± 1.35 µg/mL of acarbose (A known anti-diabetic drug). In addition, its *α*-glucosidase activity was evaluated. Its IC_{50} was 10.22 ± 0.51 mg/mL compared to 0.38 ± 0.02 mg/mL of acarbose (Fig. 5).

It was previously reported that *C. carandas* fruits aqueous extract showed potent inhibition of *β*-glucosidase activity [22]. *C. macrocarpa* flower extract exhibited a substantial *α*-amylase inhibitory potential with an IC_{50} of 65.40 µg/mL, suggesting its efficacy in slowing down conversion of polysaccharides like starch to glucose. On the other hand, acarbose showed IC_{50} of 39.6 µg/mL [23]. A well-known and effective anti-diabetic treatment is the inhibition of *α*-amylase and *α*-glucosidase enzymes which are responsible for starch breakdown and glucose absorption [15]**.** Our results support the traditional and practical anti-diabetic use of *Carissa* species [5]. Higher anti-diabetic potential could be observed after fractionation of CMME because fractionation increases segregation of biologically active metabolites. This was previously observed by Itankar et al. after fractionation of *C. carandas* crude methanol extract [10].

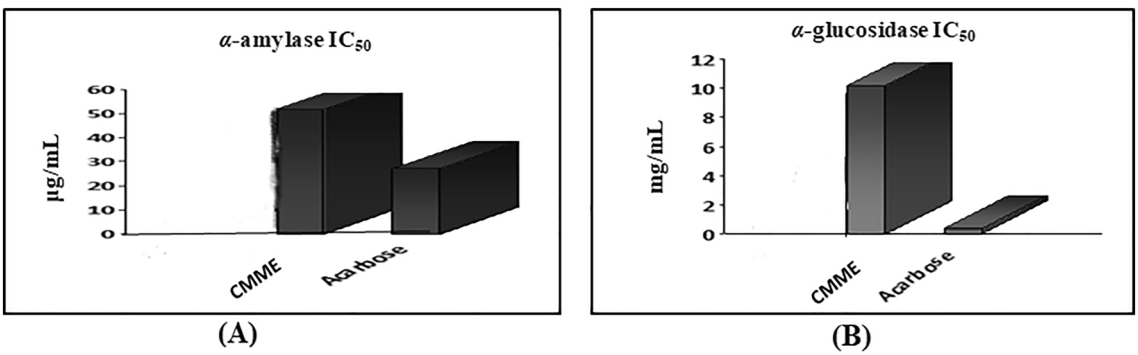

Fig. 5 Effect of CMME on diabetic parameters **a** *α*-amylase, **b** *α*-glucosidase inhibitory potentials. CMME: *C. macrocarpa* methanol extract; IC_{50}: inhibition concentration that causes reduction of the enzyme activity by 50%

3.6 Neuropharmacological parameters

Acetylcholinesterase inhibitor screening assay showed that IC_{50} of CMME was 4.00 ± 0.20 µg/mL compared to 0.074 ± 0.004 µg/mL of donepezil (A medicine used to treat dementia of the Alzheimer's type). While *β*-amyloid 1-42 (Aβ42) ligand screening assay showed the IC_{50} of the tested extract was 218.64 ± 10.23 µg/mL compared to 62.83 ± 2.95 µg/mL of donepezil (Fig. 6). Our findings match that of the previously reported on *C. carandas* and *C. edulis*. *Carissa carandas* revealed significant neuropharmacological activity on male albino rats [24]. The neuroprotective effect of the aqueous extract of *C. edulis* leaves was assessed using T-maze methods in mice to identify memory, the novel object recognition, open-field locomotion test, learning, and brain acetylcholinesterase enzyme (AChE) activity [25]. The results showed that oral administration of *C. edulis* improved the memory, object recognition and the locomotion of mice. On the other hand, mice administered the aqueous extract of *C. edulis* leaves decreased the AChE activity and brain oxidative stress. Hence by reducing AChE activity, *Carissa edulis* extract improved the memory of mice. Orabi et al. reported that *C. macrocarpa* polar extract of leaves exhibited a potential neuroprotective effect and improved doxorubicin-induced neurotoxicity in rats by downregulating the oxidative stress and inflammatory markers [26].

3.7 HPLC-QTOF/MS-MS analysis

HPLC-QTOF/MS-MS analysis of CMME led to identification of twenty nine metabolites which were six phenolics, six flavonoids, four triterpenoid saponins, ten fatty acids, two polyols and one stilbene glycoside. The retention time (R_t), molecular formula, and the identity of each compound are represented in supporting information (Table S3). The total ion chromatogram is shown in supporting information (Figure S4). MS/MS spectra of the identified metabolites were represented in supporting information (Figures S5–S31).

Phenolics: Six phenolic compounds were identified as protocatechuic aldehyde, neochlorogenic acid, vanillic acid, feruloylquinic acid, isochlorogenic acid b (4, 5-dicaffeoylquinic acid) and isoferulic acid (hesperetic acid). protocatechuic aldehyde is a phenolic aldehyde while neochlorogenic acid is a cyclitol carboxylic acid and a cinnamate ester at the same time. Vanillic acid is a dihydroxybenzoic acid derivative while isoferulic acid is a hydroxycinnamic acid derivative. Neochlorogenic acid, feruloylquinic acid and isochlorogenic acid b can be considered as quinic acid derivatives.

Flavonoids: Six flavonoids were identified from HPLC-QTOF/MS-MS analysis. Three naringenin derivatives; naringin dihydrochalcone, naringin and prunin (naringenin-7-*o*-glucoside), one kaempferol derivative; robinin, one hesperitin derivative; neohesperidin, and one quercetin derivative; hyperoside were putatively identified. The identified flavonoids can be classified into four flavanones; naringin dihydrchalcone, neohesperidin, naringin and prunin, and two flavonols; robinin and hyperoside.

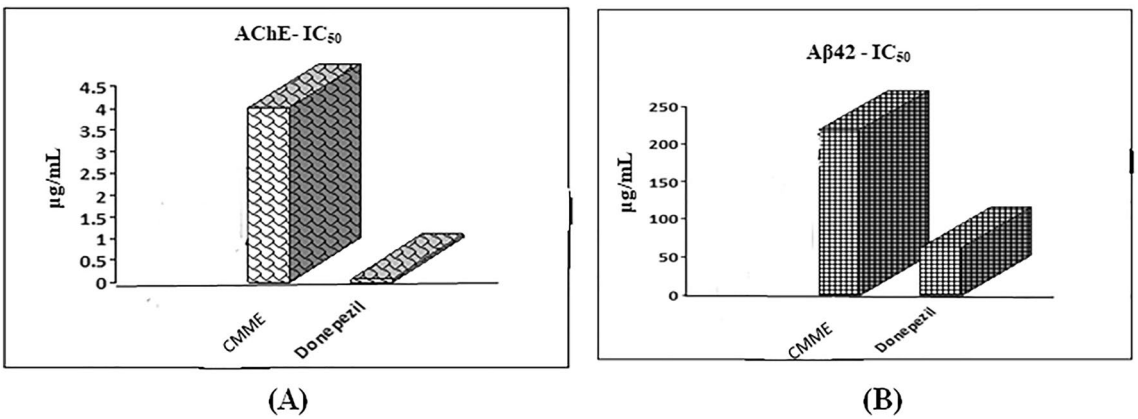

Fig. 6 Effect of CMME on neuropharmacological parameters **a** Acetylcholinesterase inhibitory, **b** *β*-Amyloid 1-42 (Aβ42) ligand screening assays. CMME: *C. macrocarpa* methanol extract; IC_{50}: inhibition concentration that causes reduction of the enzyme activity by 50%

Saponins: Four pentacyclic triterpenoid saponins; hederagenin, 18*β*-glycyrrhetinic acid, momordin Ic and oleanolic acid were identified.

Fatty acids: Ten fatty acids were putatively identified as octanoylglucuronide, 4,7,10,13,16,19-docosahexaenoic, pinellic, 12(13)-epoxy-9-octadecenoic, ricinoleic, 2-hydroxypalmitic, pinolenic, 10,12-octadecadienoic and 2-eicosenoic acids, and 1-stearoyl-2-arachidonoyl-glycero-3-phospho-(1'-myo-inositol).

Miscellaneous metabolites: Two polyols were identified which were quinic acid; a cyclitol, and rengyoside A; a dihydroxycyclohexyl glucoside. Only one stilbene glycoside was identified as tetrahydroxystilbene glucoside.

Molecular networking facilitated the classification and dereplication of metabolites identified in CMME by HPLC-QTOF/MS-MS analysis. GNPS platform (Global Natural Products Social Molecular Networking) was used to generate molecular networks (MNs) for the negative ionization mode, where MNs reflected the diversity of chemical scaffolds of metabolites. That was achieved by comparing the similarity of MS-MS fragmentation patterns, for correlating, grouping, sorting and dereplicating related metabolite [27]. The negative MN displayed 8 clusters (A–H) (Fig. 7). They were dereplicated as cluster A (flavanones), cluster B (oligosaccharides), cluster C (fatty acids), cluster D (quinic acid derivatives), cluster E (triterpenes), cluster F (alkylated sugars), cluster G (flavonols), and cluster H (stilbene). The triterpene oleanolic acid was the major compound identifed in CMME, followed by quinic acid then flavanone glycosides such as naringin and prunin (naringenin-7-*o*-glucoside). Phenolic acids and their derivatives were also observed.

HPLC-QTOF/MS-MS-identified compounds, for example phenolic acids, flavanones, and flavonols, are commonly regarded as potent antioxidants and promising remedies for the prevention and treatment of NCDs. They have been confirmed to have anti-diabetic, free radical scavenging, and inhibitory pro-inflammatory properties [28–32]. Concerning flavonoids, the presence of C3'–4' OH-groups is responsible for their antioxidant activity [33]. Thus, the observed potent antioxidant activity could be attributed to the presence of hyperoside in CMME. Dietary polyphenols have been shown to effectively inhibit enzymes related to NCDs. This function has been linked to their capability to hydrogen bond with proteins. The hydroxyl groups in the phenolic compounds were found to be involved in the mechanism of inhibition of *α*-amylase and *α*-glucosidase [15].

It was previously reported that, different plant parts of *C. macrocarpa* were found to have biological activities related to their phytoconstituents, such as naringin [5].

The results were consistent with earlier investigations relating the antioxidant capacities of phenolic and flavonoid compounds which led to the significant antioxidant activity [28–30]. Our results are supported by previous studies investigating the anti-diabetic properties of phenolic compounds present in edible plants [32].

Verma et al. [34] reported that *C. carandas* methanol extract has several metabolites that can efficiently protect the body against oxidative stress due to free radicals and therefore can be considered as a potent natural antioxidant drug. The herein observed powerful antioxidant capacity of CMME is likely due to the presence of phenolic and flavonoid compounds.

This study confirmed a considerable link between the phenolic and flavonoid contents of CMME and the NCDs-related enzymes' inhibitory action. Generally, Due to the strong attraction to proteins through hydrogen and hydrophobic interactions, phenolic compounds can inhibit enzyme activity. The phenolic compounds' functional groups enable interactions that lead to denaturation of the enzyme and a decrease in its catalytic activity [35].

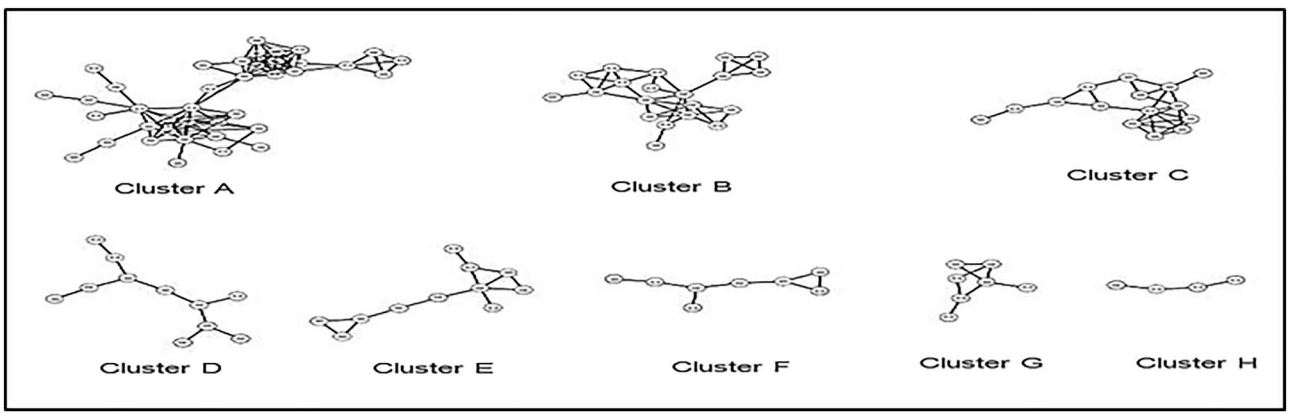

Fig. 7 The enlarged significantly dereplicated clusters of negative molecular network created using MS/MS data

4 Conclusions

Carissa macrocarpa (Eckl.) A. DC. aerial parts methanol extract has multifactorial therapeutic potentials against some non-communicable diseases. It acts as antioxidant, anti-inflammatory, hepato-protective, anti-diabetic and anti-Alzheimer's drug. Confirmatory detailed in vivo studies are recommended to evaluate the newly explored potent hepato-protective and hypoglycemic potentials. Furthermore, in vivo studies are required to validate the utilization of *C. macrocarpa* aerial parts positive neuropharmacological properties.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethical approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests The authors declare that they have no competing interests.

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