Bioactive compounds from *Carissa opaca* roots and xanthine oxidase and alpha-amylase inhibitory activities of their methanolic extract and its fractions in different solvents

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Submitted: 17-01-2015 Revised: 09-02-2015 Published: 21-10-2015

ABSTRACT

Background: *Carissa opaca* is known for its many ethnomedicinal uses. There was a need to study its bioactivities and identify its phytochemicals. **Objective:** The objective was to isolate and identify phytochemicals from roots of *C. opaca* and to evaluate xanthine oxidase (XO) and alpha-amylase inhibitory activities of their methanolic extract and its fractions. **Materials and Methods:** Methanolic extract of finely divided powder of roots of *C. opaca* was obtained by cold maceration, followed by its fractionation to obtain hexane, chloroform, ethyl acetate, *n*-butanolic, and aqueous fractions. Phytochemicals screening was done by standard protocols. XO and alpha-amylase inhibitory activities of the methanolic extract and its fractions were studied. The most active ethyl acetate fraction was subjected to the column and thin layer chromatography to isolate its compounds, which were identified by gas chromatography-mass spectrometry and high-performance liquid chromatography comparison. **Results:** Methanolic extract displayed significant activity against both the enzymes with IC_{50} of 156.0 µg/mL and 5.6 mg/mL for XO and alpha-amylase, respectively. Ethyl acetate fraction showed highest activity against both the enzymes with IC_{50} of 129 µg/mL and 4.9 mg/mL for XO and alpha-amylase, respectively. Chloroform fraction had IC_{50} of 154.2 µg/mL and 5.5 mg/mL for XO and alpha-amylase, respectively. Aqueous fraction exhibited significant efficacy against alpha-amylase (IC_{50} 5.0 mg/mL). Hexane fraction showed good activity against alpha-amylase in a dose-dependent manner but exhibited opposite trend against XO. The compounds isolated from ethyl acetate fraction included limonene, vanillin, lupeol, rutin, quercetin, β-sitosterol, Vitamin E, 2-hydroxyacetophenone, naphthalenone, 2,3,3-trimethyl-2-(3-methylbuta-1,3-dienyl)-6-methylenecyclohexanone, and 2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester. **Conclusions:** Moderately polar phytochemicals of *C. opaca* roots possess exploitable inhibitory activity against both the enzymes.

Key words: Alpha-amylase, *Carissa opaca* roots, Inhibitory activity, Phytochemicals, Xanthine oxidase

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.158440

Quick Response Code:

INTRODUCTION

Inhibition of enzymes provides a valid strategy to cure different diseases. This necessitates constant quest for safe, effective, and affordable enzyme inhibitors. The enzyme xanthine oxidase (XO) catalyzes the conversion of hypoxanthine into xanthine and of xanthine into uric acid along with superoxide.[1] Patients with hyperactivity of this enzyme may suffer from conditions known as hyperuricemia as well as oxidative stress. Hyperuricemia or elevation of uric acid in blood plasma can eventually cause gout.[2] Oxidative stress can lead to serious health risks including cancer and cardiovascular disorders.[3] XO inhibitors have the potential to cure gout and reduce oxidative stress. Synthetic drugs are available but they have serious side effects.[4] This demands search for safer alternatives for XO inhibition.

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Alpha-amylase converts starch into maltose, which ultimately changes into glucose.[5] Substances with alpha-amylase inhibitory activity can play a role in the control of diabetes type 2 by controlling blood glucose level.[6] Type 1 diabetes is the outcome of inability of pancreas to produce insulin, while type 2 is caused by abnormal secretion of insulin or inability of the body to use the insulin it produces.[7] Type 2 diabetes appears to be one of the major human health concerns in the 21st century and according to International Diabetes Federation 382 million people were suffering from diabetes in 2013, which is estimated to rise to 592 million by 2035.[8] As the synthetic alpha-amylase inhibitors may cause severe side effects natural alpha-amylase inhibitors from plants are a better option to control postprandial hyperglycemia with no or very little side effects.[9-11] Alpha-amylase inhibitors can also help in weight control by decreasing conversion of food starch into bioavailable glucose in our body.

Carissa opaca (family *Apocynaceae*) is an evergreen, thorny shrub distributed in Himalayan mountainous regions of Pakistan and India.[12,13] The plant is well-known for its rich ethnomedicinal value. It is used to cure asthma, jaundice, and hepatitis.[14,15] A paste of roots of *C. opaca* is applied on wounds and injuries for healing.[16] Phytochemical research on the plant has shown it to contain flavonoids, tannins, terpenoids, and glycosides.[17]

The objective of the present study was to determine XO and alpha-amylase inhibitory activities of the roots of *C. opaca*, and isolate and identify the chemical constituents of the most active fraction. The methanolic extract of the roots and its fractions in hexane, chloroform, ethyl acetate, *n*-butanol, and water were, therefore, investigated. This study is being reported here for the 1st time.

MATERIALS AND METHODS

General

The solvents used for extraction and fractionation of plant material were of high-performance liquid chromatography (HPLC) grade. XO, xanthine, allopurinol, acarbose, and starch were purchased from Sigma-Aldrich, alpha-amylase from MP Biomedicals, sodium potassium tartrate from Merck and 3,5-dinitrosalisylic acid (DNS) from Fisher Scientific. All other reagents were of analytical grades.

Collection of plant material

The roots of *C. opaca* were collected from the hills near Abbottabad, Pakistan, in March 2013. The identification of the plant was confirmed by the taxonomist Mr. Ajaib Khan of GC University, Lahore, Pakistan (voucher specimen: GC-Herb Bot 2271).

Preparation of samples

The roots were washed with distilled water to remove soil particles and slightly dried under shade for 2 days. They were crushed and ground to get a fine powder. The powder (7 kg) was then macerated in pure methanol for 15 days. The extraction was repeated for 3 times and the extracts were combined. The solvent methanol was evaporated on rotary evaporator under reduced pressure to obtain dried methanolic extract (600 g) of the roots. A portion of the methanolic extract (500 g) was suspended in distilled water and fractionated into solvents with successively increasing polarity. As a result, hexane (40.1 g), chloroform (20.9 g), ethyl acetate (30.55 g), *n*-butanolic (90.6 g), and residual aqueous fractions were obtained.

Determination of xanthine oxidase inhibitory activities

Xanthine oxidase inhibitory activities of methanolic extract of roots of *C. opaca* and its fractions in different solvents were determined according to a reported assay.[18] Sodium phosphate buffer (100 mL, 7.0 mM, pH 7.5) was prepared by dissolving 0.1817 g sodium dihydrogen phosphate monohydrate and 1.5231 g disodium hydrogen phosphate heptahydrate in distilled water, and its pH was adjusted by adding $Na_2HPO_4.7H_2O$. To prepare enzyme solution (0.01 units/mL), 10 mg XO was added into 100 mL sodium phosphate buffer (pH 7.5). Xanthine solution (150 μM) was used as a substrate, which was prepared by mixing 1.14 mg xanthine into 50 mL buffer. Stock solution of each plant sample was prepared by adding 5 mg of plant material in dimethyl sulfoxide (DMSO) to obtain a concentration of 1 mg/mL. Different dilutions (50–650 μg/mL) were then prepared in distilled water. In a test tube, 750 μL test sample was taken. To it, 525 μL buffer and 450 μL enzyme solution were added. The mixture was shaken and incubated for 15 min at 25°C. After incubation, 900 μL substrate solutions were added into the mixture. The mixture so obtained was incubated for 30 min at 25°C. The reaction was stopped by adding 375 μL HCl solution (1 N). Absorbance was measured at 290 nm against a blank. The blank contained the equal volume of buffer in place of enzyme solution. Remaining procedure was same. The control was prepared by using DMSO in place of the test sample. Rest of the procedure was same. Allopurinol was used as positive control. The enzyme inhibitory activity was calculated using the following formula:

$$\% \text{ Inhibitory activity} = 100 ([Ac - As]/Ac).$$

Here, Ac and As are absorbances of control and sample, respectively. Based on the graph obtained by plotting concentration versus absorbance, IC_{50} values were also calculated.

Determination of alpha-amylase inhibitory activities

Alpha-amylase inhibitory activities of methanolic extract of roots of *C. opaca* and its fractions in different solvents were determined according to a reported assay.[19] Stock solution (15 mL) of the plant sample was prepared by dissolving 0.6 g of the extract into DMSO. Dilutions of this solution (1, 2, 3,…, 12 mg/mL) were prepared by adding appropriate volume of DMSO. The coloring reagent DNS (96 mM 3,5-dinitrosalicylic acid) solution was prepared by dissolving 0.438 g 3,5-dinitrosalicylic acid into 20 mL distilled water. It was warmed and stirred until complete dissolution. To prepare sodium potassium tartrate solution, 12 g sodium potassium tartrate tetrahydrate was dissolved in 8 mL NaOH solution (2 M). It was heated and stirred until a clear solution was obtained. The 96 mM 3,5-dinitrosalicylic acid and sodium potassium tartrate solutions were mixed and diluted with 12 mL distilled water. The solution was kept in dark until used. To prepare 0.5% starch solution, 0.5 g of starch was suspended in 100 mL buffer solution and boiled for 15 min. The solution was cooled to room temperature and the volume was maintained by adding distilled water. In a test tube, 0.5 mL sample solution was mixed with 0.5 mL enzyme solution. The test mixture was incubated for 30 min at 25°C. After preincubation, 1 mL 0.5% starch solution was added. The mixture was again incubated for 3 min, and 1 mL DNS reagent was added into it. This test mixture was then heated for 15 min at 85°C. It was cooled to room temperature and 9 mL cold water was added. Absorbance was measured at 540 nm against a blank. For blank, 1 mL DNS reagent was added before 3 min incubation, and 1 mL starch solution was added after it. Remaining procedure was the same as for sample. For control preparation, the enzyme solution was incubated with DMSO instead of sample solution. Remaining procedure was same as used to prepare the sample. Percentage of inhibitory activity was calculated using the following formula:

% Inhibitory activity = 100 ([Ac − As]/Ac).

Here, Ac and As are absorbances of control and sample, respectively. IC_{50} values were calculated from the straight line equation obtained by plotting concentration versus absorbance.

Phytochemical screening

Standard tests were performed for detection of carbohydrates, flavonoids, phenols, steroids, saponins, oils and fatty acids, tannins, glycosides, gums and mucilage, and alkaloids.[20]

Isolation of compounds

The ethyl acetate fraction, which exhibited highest anti-enzymatic activity, was selected for isolation and identification of its chemical constituents. Column and thin layer chromatographic (TLC) techniques were used for isolation of compounds.

Column chromatography

Silica gel (200–400 mesh) soaked in hexane was filled in a glass column (diameter 1.5 inch; height 3.5 feet). Dried ethyl acetate fraction (25 g) was loaded on the column, and elution was carried out with solvent systems with increasing polarity, starting with hexane-ethyl acetate followed by ethyl acetate-methanol. The eluates were analyzed on precoated silica gel TLC and the ones showing similar patterns were combined. In this way, five eluates or sub-fractions were obtained which were designated as EA1–EA5 Table 1.

The eluted fraction EA1 that was obtained from the column upon elution with hexane-ethyl acetate (4:1) showed five major spots on TLC with mobile phase hexane-ethyl acetate (7:3). They were separated using silica gel preparative TLC (PTLC) and recovered by dissolving in methanol. They were designated as F1–F5, and their Rf values on PTLC were, 0.24, 0.34, 0.37, 0.65, and 0.71, respectively.

Table 1: Compounds identified in ethyl acetate fraction of methanolic extract of *Carissa opaca* roots and their anti-enzymatic activities

Eluate	Compound isolated	RT on GC-MS/HPLC	XO inhibitory activity	Alpha-amylase inhibitory activity
EA1	F1, Limonene	7.22	ND	ND
	F2, 2-HAP	10.4	+++	-
	F3, Vanillin	11.36	-	-
	F4, Naphthalenone	19.72	-	-
	F5, 2,3,3-Trimethyl-2-(3-methylbuta-1, 3-dienyl)-6-methylenecyclohexanone	21.485	ND	ND
EA2	F6, BCA	24.64	-	++++
EA3	F7, β-sitosterol	22.12	-	+++
	F8, Vitamin E	23.67	-	-
EA4	F9, Rutin	6.29	+++	-
	F10, Quercetin	6.53	++++	-
EA5	F11, Lupeol	26.16		++

ND=Not determined due to less quantity; -=Not active; ++= moderately active; +++=Very active; ++++=Highly active; RT=Retention time; XO=Xanthine oxidase; BCA=1,2-benzenedicarboxylic acid, mono (2-ethyhexyl) ester; 2-HAP=2-hydroxyacetophenone; HPLC=High performance liquid chromatography; MS=Mass spectrometry; GC=Gas chromatography

The eluate EA2, obtained upon elution with hexane-ethyl acetate (5:5), showed one major compound on TLC, which was separated as F6 (Rf, 0.82) through PTLC using mobile phase hexane-ethyl acetate (4:6). F6 was recrystallized from ethyl acetate. The sub-fraction EA3, obtained when hexane-ethyl acetate (3:7) was used as eluent, yielded two compounds, F7 (light yellow oily material) and F8 (colorless powder) upon PTLC (mobile phase, hexane-ethyl acetate, 2.5:7.5) with Rf values 0.5 and 0.67. F8 was crystallized from ethyl acetate.

The sub-fraction fraction EA4, obtained on elution with ethyl acetate-methanol (9:1), produced two compounds F9 (Rf, 0.73) and F10 (Rf, 0.80) as yellow crystals upon PTLC (mobile phase, *n*-butanol-water-acetic acid, 6:3.9:0.1).

The sub-fraction EA5, which was obtained upon elution with ethyl acetate-methanol (7:3) showed one major spot on TLC with a mobile phase consisting of *n*-butanol-acetic acid-water (5:0.1:4.9). This was isolated and named as F11 (Rf 0.88).

Statistical analysis

To ensure reproducibility, each experiment was performed at least in triplicate and mean was calculated with ± standard deviation (n = 3) using Excel 2013 Microsoft Corporation(Redmond, WA, USA).

RESULTS AND DISCUSSION

Xanthine oxidase inhibitory activities

Xanthine oxidase catalyzes the oxidation of hypoxanthine into xanthine and of xanthine into uric acid. Superoxide radical is also produced during the reaction. Inhibition of this enzyme will reduce the concentration of uric acid in blood plasma. The strategy is used to cure gout, which is caused by elevated concentration of uric acid in serum. XO inhibitory activities of methanolic extract of *C. opaca* roots and its fractions in various solvents were determined according to standard protocol and results are shown in Table 2 and Figure 1. The methanolic extract displayed dose-dependent activity with IC_{50} of 156.0 µg/mL. The activity of its fractions was also dose-dependent except for hexane fraction, which exhibited a reverse trend. It showed very high activity at low concentration, which decreased with the increase in concentration. Ethyl acetate fraction showed highest XO inhibitory potential (IC_{50} 129 µg/mL), which means most of the compounds in methanolic extract that were active against this enzyme had appeared in this fraction. Chloroform fraction also showed significant inhibitory potential (IC_{50} of 154.2 µg/mL), which was only slightly less than that of ethyl acetate fraction. The *n*-butanolic fraction was less potent, while aqueous fraction showed no appreciable activity. The chemical compounds of this plant that are active against XO are, thus, of moderately polar nature. The order of total phenolic content and total flavonoid content in these solvents was also same, that is, ethyl acetate > chloroform > *n*-butanolic. It can, therefore, be postulated that phenolics and flavonoids play a significant role in inhibition of XO.[21,22] The ability of unsaturated fatty acids and their esters to scavenge reactive oxygen species might explain the inverse behavior of hexane fraction against XO, which produces superoxide during its catalytic action.

Alpha-amylase inhibitory activities

Inhibition of alpha-amylase during digestion of food can be used as a strategy to control the blood glucose level and hence as a remedy for diabetes and obesity. Plants, with a great diversity of natural products, have the potential to provide safe and affordable inhibitors of alpha-amylase. Alpha-amylase inhibitory activities of *C. opaca* roots and its fractions in various solvents were determined in this study and the results are displayed

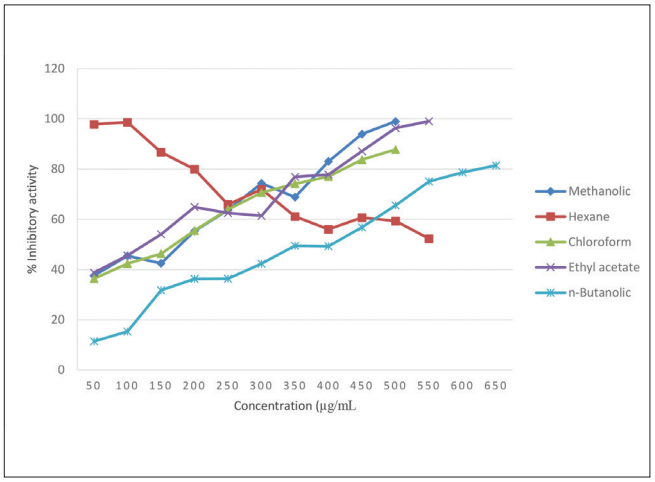

Figure 1: Xanthine oxidase inhibitory activities of methanolic extract of *Carissa opaca* roots and its factions in different solvents. Concentrations are in µg/mL

Table 2: EC_{50} of methanolic extract of *Carissa opaca* roots and its fractions against XO (in µg/mL) and alpha-amylase (in mg/mL) as compared to the standard drugs

Enzyme	Methanolic	Hexane	Chloroform	Ethyl acetate	*n*-butanolic	Standard drug (µg/mL)
XO	156.00	ND	154.25	129.00	371.00	2.74
Alpha-amylase	5.63	5.64	5.54	4.92	7.59	105.32

ND=Not determined due to inverse trend. Allopurinol and acarbose were standard drugs for XO and alpha-amylase, respectively. XO=Xanthine oxidase

in Table 2 and Figure 2. The activities of the extract and its fractions were determined as a function of concentration, and IC_{50} values were calculated. The methanolic extract and its fractions displayed parallel trends, which were dose-dependent. Ethyl acetate fraction was most potent against alpha-amylase activity with IC_{50} of 4.92 mg/mL followed by an aqueous fraction with IC_{50} of 5.02 mg/mL. Chloroform fraction also displayed significant activity (5.54 mg/mL). On the other hand, *n*-butanolic fraction exhibited lowest potential against alpha-amylase. The trend signified that moderately polar natural products and highly polar water-soluble phytochemicals should be most potent against alpha-amylase.[23] The fractions with high phenolic and flavonoid contents exhibited high alpha-amylase activity although the correlation was not that much strong as was in the case of XO. It seems a broad range of phytochemicals influences the activity of alpha-amylase.

Identification of chemical constituents

The five major compounds, F1–F5 Table 1, separated from sub-fraction EA1 were evaluated on gas chromatography-mass spectrometry (GC-MS).[24] F1 was identified to be the terpenoid limonene (retention time [RT], 7.222 min), F2 was identified as 2-hydroxyacetophenone (2-HAP) (RT, 10.4 min), and F3 as vanillin (RT, 11.36 min), which was isolated in good quantity (375 mg/25 g of ethyl acetate fraction). Its melting point was 80°C, and that of pure synthetic vanillin is 82°C. Infrared (IR) spectrum of F3 expressed prominent band at 3139.89 cm,$^{-1}$ which was due to the presence of O-H group, an intense peak at 1659.49 cm^{-1} was due to C=O group. Other peaks were at 1582.22 cm^{-1} (aromatic C=C) and 1262.12 cm^{-1} (C-O). F4 was detected as naphthalenone (RT 19.724 min), and F5 as 2,3,3-trimethyl-2-(3-methylbuta-1,3-dienyl)-6-methylenecyclohexanone (RT 21.485 min).

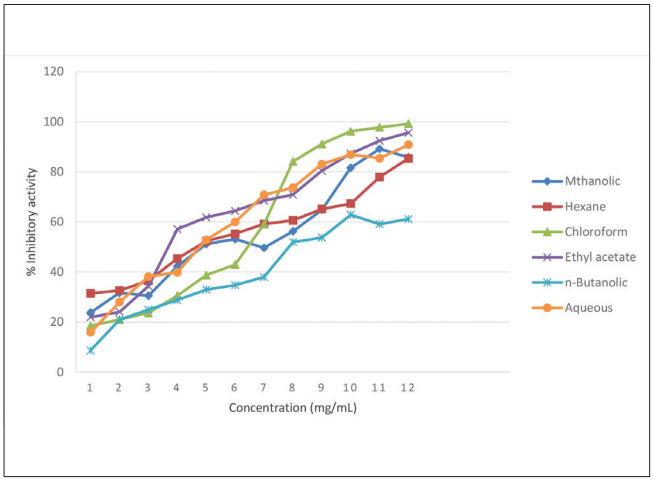

Figure 2: Alpha-amylase inhibitory activities of methanolic extract of *Carissa opaca* roots and its factions in different solvents as a function of concentration (n = 3)

F6, which was isolated from EA2, was found to be 1,2-benzenedicarboxylic acid, mono (2-ethyhexyl) ester (BCA) (RT, 24.636 min). It formed colorless crystals (m.p., 29°C).

The compound F7 was detected to be β-sitosterol (RT, 22.12 min) on GC-M. The IR spectrum of F7 indicated peaks at 3422 cm^{-1} (O-H), a broad peak at 1627.99 cm^{-1} (C=O). Other prominent peaks were at 2937, 2850, 2291 cm^{-1}. F8, which was a light yellow viscous material, identified as alpha-tocopherol (Vitamin E) through GC-MS, showing a peak at RT 23.67 min.

The yellow colored compounds F9 and F10 were not detected by GC-MS. The IR spectrum of F9 showed broad absorption at 3495-3373 cm^{-1} (O-H), 1617 cm^{-1} (C=C), 1513.33 cm^{-1} (C=C, aromatic), and 1244-1094 cm^{-1} (C-O). The IR spectrum of F10 indicated the peaks at 3314.00 cm^{-1} (O-H), 1655.15 cm^{-1} (C=O conjugated with C=C), 2941.18 cm^{-1} (C-H), and 1019 cm^{-1} (C-O). The color and IR spectra of F9 and F10 indicated them probably to be flavonoids. Comparison of these compounds with different standards on HPLC using mobile phase methanol-water (3:2) revealed them to be rutin and quercetin, respectively. Under the same conditions used, the RT of F9 was 6.29 min against 6.35 min of rutin, while RT of F10 was 6.52 min against 6.54 min of quercetin. The melting point of F9 was 240°C, and that of F10 317°C.

The compound F11 was shown to be lupeol (RT, 26.16 min) by GC-MS analysis. It was in the form of colorless crystals (m.p., 217°C). Its IR spectrum indicated peaks at 3395.83 cm^{-1} (O-H), 1617 cm^{-1} (C=C), 1453 cm^{-1} (C=C), and 1019 cm^{-1} (C-O).

Of the compounds isolated from an ethyl acetate fraction, three were more active against alpha-amylase as compared to the parent fraction Table 3. They included BCA, lupeol, and β-sitosterol, BCA being most

Table 3: EC_{50} of ethyl acetate fraction of methanolic extract of *Carissa opaca* roots and BCA*, lupeol and β-sitosterol against alpha-amylase (in mg/mL) as compared to the standard drug

Enzyme	Ethyl acetate	BCA*	Lupeol	β-sitosterol	Standard drug (mg/mL)
Alpha-amylase	4.92	0.133	0.138	0.175	0.105

*BCA=1,2-benzenedicarboxylic acid, mono (2-ethyhexyl) ester; Acarbose was standard drug used as alpha-amylase inhibitor

active. BCA is a known plasticizer, and it is subject to debate if it is metabolite or a contamination. However, irrespective of its origin, it showed significant alpha-amylase inhibitory activity, which, in our opinion, must not be ignored.

The methanolic extract of *C. opaca* roots and its fractions were comparatively much more effective against XO than alpha-amylase Figure 1 and 2. However, their dependence on concentration followed similar pattern except for hexane fraction, which followed opposite trend against XO.

Three compounds were found active against XO enzyme including quercetin, rutin, and 2-HAP Table 4. Quercetin showed highest inhibitory activity against the enzyme. Activities of the other two compounds were also significant and were quite close to that of the standard drug.

The crude plant extracts were less potent against both the enzymes as compared to their standard drugs used in the study. Therefore, higher doses of plant samples would be needed to cause efficacy comparable to that of the drugs. Some of the compounds isolated from the plant extracts, however, showed much better efficacy.

Phytochemical screening

Standard tests were performed to detect different types of phytochemicals in the methanolic extract of roots of *C. opaca* and their distribution in solvents of different polarity.[25] The results are displayed in Table 5. All the tested types of compounds were found to be present in methanolic extract except gums and mucilage and alkaloids. Significantly, the roots of this plant indicated the presence of flavonoids, phenolics, steroids, saponins, tannins, and glycosides. Distribution of these phytochemicals in various fractions was interesting. Ethyl acetate fraction, which showed highest inhibitory activity against both the enzyme, contained all the phytochemicals except saponins. Oils and fatty acids were not detected in polar fractions of *n*-butanol and water, while hexane fraction did not indicate the presence of carbohydrates. Flavonoids, phenolics, and steroids were present in all the fractions. The presence of these bioactive natural products may explain the efficacy of the roots of *C. opaca* against different ailments for which they are used in traditional medicine. Quantitative estimation of phenolic and flavonoid contents showed that they were highest in ethyl acetate followed by chloroform and then *n*-butanolic fractions.

Table 4: EC_{50} of ethyl acetate fraction of methanolic extract of *Carissa opaca* roots and quercetin, 2-HAP and rutin against XO (in µg/mL) as compared to the standard drugs

Enzyme	Ethyl acetate	Quercetin	2-HAP	Rutin	Standard drug (µg/mL)
XO	129.00	8.40±0.56	11.66±0.27	10.66±0.21	2.74

*2-HAP=2-hydroxyacetophenone; allopurinol was standard drug used as XO inhibitor. XO=Xanthine oxidase

CONCLUSIONS

Carissa opaca roots exhibited significant inhibitory activity against XO and alpha-amylase. Methanolic extract and its fractions were more active against XO than alpha-amylase. Ethyl acetate fraction, which had the highest contents of phenolics and flavonoids was most potent against both the enzymes. The 11 compounds isolated from this fraction were identified by GC-MS and HPLC comparison. BCA, lupeol, and β-sitosterol were found active against alpha-amylase, while 2-HAP, quercetin and rutin were active against XO enzyme.

It can, therefore, be reasonably anticipated that ethyl acetate fraction of roots of this plant should yield exploitable candidates for future drugs.[26]

Table 5: Results of qualitative screening of phytochemicals in methanolic extract of roots of *Carissa opaca* and its fractions in various solvents

Test	Methanolic	Hexane	Chloroform	Ethyl acetate	*n*-butanolic	Aqueous
Carbohydrates	+	−	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Saponins	+	+	−	−	+	+
Oils and fatty acids	+	+	+	+	−	−
Tannins	+	+	−	+	+	−
Glycosides	+	+	+	+	+	+
Gums and mucilage	−	−	−	−	−	−
Alkaloids	−	−	−	−	−	−

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Cite this article as: Saeed R, Ahmed D. Bioactive compounds from *Carissa opaca* roots and xanthine oxidase and alpha-amylase inhibitory activities of their methanolic extract and its fractions in different solvents. Phcog Res 2015;7:295-301.

Source of Support: Nil, **Conflict of Interest:** None declared.