BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
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Articulo Original / Original Article

Metabolites in *Conocarpus erectus* leaves attenuate α-amylase activity by modulating amino acid residues of α-amylase: an *in vitro* and docking study

[Los metabolitos en las hojas de *Conocarpus erectus* atenúan la actividad de la α-amilasa al modular los residuos de aminoácidos de la α-amilasa: un estudio *in vitro* y de acoplamiento]

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Section
Biological activity

Received: 24 October 2020
Accepted: 16 March 2021
Accepted corrected: 19 June 2021
Published: 30 May 2021

Citation:
Raza SA, Rashid A, Mumtaz MW, Bashir S, Ahmad M, Touqeer T, Mukhtar H, Akhtar MT, Ali S, William J Metabolites in *Conocarpus erectus* leaves attenuate α-amylase activity by modulating amino acid residues of α-amylase: an *in vitro* and docking study
Bol Latinoam Caribe Plant Med Aromat
21 (3): 352 - 364 (2022).
https://doi.org/10.37360/blacpma.22.21.3.21

Abstract: The antioxidant activity and the inhibitory potential of α-amylase of lyophilized hydroethanolic extracts of *Conocarpus erectus* leaves obtained by ultrasonication were determined. The most potent extract was subjected to ultra-high performance liquid chromatography system equipped with mass spectrometer for metabolite identification. The identified metabolites were docked in α-glucosidase to assess their binding mode. The results revealed that 60% ethanolic extract exhibited highest ferric reducing antioxidant power (4.08 ± 0.187 mg TE/g DE) and α-amylase inhibition (IC_{50} 58.20 ± 1.25 µg/mL. The metabolites like ellagic acid, 3-O-methyl ellagic acid, ferujol, 5, 2´-dihydroxy-6,7,8-trimethyl flavone and kaempferol glucoside were identified in the extract and subjected to molecular docking studies regarding α-amylase inhibition. The comparison of binding affinities revealed 3-O-methyl ellagic acid as most effective inhibitor of α-amylase with binding energy of -14.5911 kcal/mol comparable to that of acarbose (-15.7815 kcal/mol). The secondary metabolites identified in the study may be extended further for functional food development with antidiabetic properties.

Keywords: *Conocarpus erectus*; Metabolite profiling; Molecular docking; α-amylase inhibition; Antioxidant activity.

Resumen: Se determinó la actividad antioxidante y el potencial inhibidor de la α-amilasa de extractos hidroetanólicos liofilizados de hojas de *Conocarpus erectus* obtenidos por ultrasónicación. El extracto más potente se sometió a un sistema de cromatografía líquida de ultra alto rendimiento equipado con un espectrómetro de masas para la identificación de metabolitos. Los metabolitos identificados se acoplaron en α-glucosidasa para evaluar su modo de unión. Los resultados revelaron que el extracto etanólico al 60% exhibió el mayor poder antioxidante reductor férrico (4.08 ± 0.187 mg TE/g DE) e inhibición de la α-amilasa (IC_{50} 58.20 ± 1.25 µg/mL. Los metabolitos como el ácido elágico, 3-O-metil elágico ácido, ferujol, 5, 2´-dihidroxi-6,7,8-trimetil flavona y kaempferol glucósido se identificaron en el extracto y se sometieron a estudios de acoplamiento molecular con respecto a la inhibición de la α-amilasa. La comparación de las afinidades de unión reveló 3-O-metil El ácido elágico como inhibidor más eficaz de la α-amilasa con una energía de unión de -14,5911 kcal/mol comparable a la de la acarbosa (-15,7815 kcal/mol). Los metabolitos secundarios identificados en el estudio pueden ampliarse aún más para el desarrollo funcional de alimentos con propiedades antidiabéticas.

Palabras clave: *Conocarpus erectus*; Perfilado de metabolitos; Acoplamiento molecular; Inhibición de α-amilasa; Actividad antgioxidante.

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INTRODUCTION

The diabetes mellitus type II (DMT_2) is the major type of diabetes and rapidly spreading metabolic disorder throughout the globe. Synthetic drugs for treatment of DMT_2 involve acute drug reactions and high treatment costs making them less acceptable among consumers (Wang *et al*., 2013). The conventional antidiabetic drugs which include insulin, sulphonylureas, Meglitinides, biguanides, thiazolidinedions and acarbose are associated with adverse health impact. The serious side effects of these drugs are hypoglycemia, weight gain, lipodystrophy, cardiovascular problems, bone marrow damages, gastrointestinal problems, bladder cancer and hepatitis (Shah & Mudaliar, 2010; Osadebe *et al*., 2014). The acarbose is most effective and commercially available α-amylase inhibitor but has been reported to possess serious health complications including gastrointestinal issues (Floris, 2008). Therefore, need persists for alternate treatment of diabetes with high efficacy and safety, necessary for acceptance among patients. The inhibition of dietary enzymes is a workable tool to control postprandial blood glucose concentration to a significant level. The α-amylase enzyme hydrolyzes complex carbohydrates (starches) to convert them into simpler molecules for intestinal absorption. Resultantly, the intestinal absorption of glucose leads to high postprandial blood glucose level (Malunga & Eck, 2016). The inhibition of carbohydrate digesting enzymes is considered as an effective route to control postprandial blood glucose level for DMT_2 management (Saini *et al*., 2015).

Plants may contain phytochemicals with dietary enzyme inhibitory properties. The plant based polyphenols are advantageous over synthetic compounds and existing enzyme inhibitors like acarbose. A study indicated that acarbose mode of interaction with carbohydrate digesting enzymes was competitive whereas plant polyphenols followed non-competitive inhibition. The non-competitive inhibition was more advantageous due to chances of multiple site deactivations rather than occupying few active sites of enzyme (Zhang *et al*., 2015). Plant metabolites were reported to have significant α-glucosidase and α-amylase inhibitory properties. The inhibition of α-amylase by plant metabolites was based upon the compatibility of natural molecules to act with specific sites on enzymes. The molecular interactions among metabolites and functional sites of proteins are considered as key feature to study drug interaction and development (Amin *et al.*, 2019). Recent advances in *in-silico* molecular docking have oriented the scientific approaches more reliable, quick and productive (Saini *et al*., 2015).

The bioactive fractions and ingredients of numerous plants have been explored for their digestive enzyme inhibitory properties (Mahmood *et al.*, 2020). A report presented the α-glucosidase inhibitory potential of *Cycas revoluta* leaf extract and this inhibitory activity was due to apigenin and its derivatives identified in extract. The molecular binding studies revealed the six hydrogen bond interactions of apigenin with α-glucosidase at various residues (Arshad *et al.*, 2019). Another study reported the α-glucosidase inhibioty potential of 40 natural compounds and predicted rutin, quercetin, and myricetin as most effective inhibitors on the basis of binding energies (Hyun *et al*., 2014). A recent investigation highlighted the α-amylase inhibition by secondary metabolites of hydroethanolic leaf extract of *Calotropis procera*. The myrciacitrin IV was identified as the most potent inhibitor of α-amylase and found a close proximal place to standard drug acarbose on the basis of binding affinity. Myrciacitrin IV exhibited six hydrogen bondings at Asp408, His229, Glu304, Pro309 and His295 (Nadeem *et al*., 2019). The α-amylase inhibitory potential of *Syzygium sp* was attributed to luteolin which exhibited binding with catalytic triad of enzyme in same manner followed by acarbose (Freitas *et al*., 2019).

As the secondary metabolites of plants are considered as major driving aspect regarding therapeutic impacts of plants hence can be explored for novel α-amylase inhibitors to manage DMT_2 in naturopathic way. The dietary intake of polyphenol rich diets may be a helping tool to reduce the postprandial blood glucose level by inhibiting the action of α-amylase on starch (Hanhineva *et al*., 2010).

The *Conocarpus erectus* (*C. erectus*) of *Combretaceae* family was recently reported to have impressive hypoglycemic properties in diabetic mice, most probably due to polyphenols. The hydroethanolic extract of *C. erectus* showed excellent α-glucosidase inhibition. However, α-amylase inhibitory activity was not performed and compounds probably responsible for antidiabetic activity were not identified (Raza *et al*., 2018). The current study was designed to calculate the α-amylase inhibitory potential and identification of secondary metabolites in leaves of *C. erectus* along with molecular docking studies for α-amylase. The specific site compatible interactions between metabolites and α-amylase may provide a deep insight to search novel

pharmacological agents for drug development and food functionalization. This study was the probably the first study on *C. erectus* regarding α-amylase inhibition and molecular docking of active metabolites.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals and reagents were of analytical grade. The hydrochloric acid (HCl) from Merck (1003172500), ethanol (99.5%) from Sigma-Aldrich (459836), TPTZ (Sigma-Aldrich 298-96-4) butylated hydroxytoluene (BHT Sigma-Aldrich 128-37-0) and $FeCl_3.6H_2O$ (Sigma-Aldrich 236489). Trolox was procured from Aldrich (BCBS9713V).

Plant material and extraction

The plant material was collected from Lahore, Pakistan and coordinates of geological position were N 31º33'22" and E 74º21'49". The plant was identified as *C. erectus* from GC University Lahore vide voucher specimen GC. Herb.33.Bot. 3379. The harvested leaves of *C. erectus* were immediately quenched in liquid nitrogen, ground and extracted with pure water, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol and pure ethanol (at ambient conditions) in 1:10 by mass and sonicated. The extracts were shaken for 2 hours, filtered and freeze dried.

The ferric reducing antioxidant power assay (FRAP)

The little modified method was utilized for FRAP assay determination (Benzie & Strain, 1996). Acetate buffer stock solution of 3.6 pH was prepared by mixing 3.1 g of $CH_3COONa.3H_2O$ and 16 mL of CH_3COOH, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 Mm of HCl and 20 mM solution of $FeCl_3.6H_2O$. Fresh solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ and $FeCl_3.6H_2O$ followed by heating at 370 °C for further immediate use. Trolox and plant sample both were dissolved in methanol, 250 µg/mL each. After that 10 µL of sample solution and BHT solution were taken separately followed by addition of 3 mL of FRAP reagent solution both in trolox and plant sample. Reaction mixture was stayed for 30 minutes in dark and reading of complex was noted at 593 nm. Results were reported as mg trolox per gram dry extract (mg TE/DE).

The α-amylase inhibition assay

The α-amylase inhibitory assay was performed as per previously reported method (Kazeem *et al*., 2013). About 1.0-10 mg of plant extracts were dissolved in 1 mL of methanol and 250 µL each of these dilutions were added in 0.5% starch solution. Then 0.5 mg/mL of porcine α-amylase was prepared in 0.02M sodium phosphate buffer (pH 6.8) followed by mixing with extract solutions. The mixtures were incubated for a period of 10 min at 25°C. The reaction was stopped by adding 500 µL dinitrosalicylicacid (DNS). A further incubation of 5 min was carried out and samples were diluted with 5 mL distilled water to form final dilutions. The absorbance of the dilutions was measured at 540 nm. A control without plant extracts was also run. The standard compound acarbose was used as α-amylase inhibitor. The percentage enzyme inhibition was computed by using following formula.

$$\% \text{ inhibition} = \frac{Ab - As}{Ab} \times 100$$

The absorbance noted for blank was given as Ab and absorbance taken for sample was given as As. Results were reported in terms of IC_{50} (µg/mL) value.

Metabolite profiling

Identification of bioactive molecules in 60% ethanolic extract was carried out by UHPLC-Q-TOF-MS/MS. Extract was filtered with polytetraflouroethylene (PTFE) filter having 0.45 µm pore size. The sample was subjected to UHPLC-Ion Trap-Hybrid Quadrupole-MS/MS (AB Sciex 5600-1 equipped with Eksigent UHPLC). Analysis was characterized by scanning range from 50 to 1200 m/z for MS-MS with negative ionization mode. Thermo Hypersil Gold Column (100 mm × 2.1 mm × 3 µm) was used and gradient mobile phase composed of water and acetonitrile (each having 0.1% formic acid and 5 mM ammonium formate). Gradient programming started from 10% acetonitrile to 90% acetonitrile. Mobile phase flow rate was 0.25 mL/min with sample injection volume of 20 mL. Instrument was equipped with duo spray ion source with N_2 as curtain gas, ion source gas (GS1) and turbo gas (GS2) at 25, 40 and 40 psi respectively. Ion spray voltage was -4500 V and desolvation temperature was 500 °C. The Sciexpeak views 2.1 software, ACD labs MS fragmenter and Chemspider database was used for data interpretation. Peaks resolving also involved the account of values reported in literature.

Docking studies

Docking studies were carried out by using Molecular

Operating Environment (MOE 2016.08). Docking studies were carried out on homology modelled α-amylase. Preparation of ligands downloaded enzymes (3D protonation, energy minimization and determination of binding site was carried out. The docking result analysis of surface with graphical representations was done using MOE and discovery studio visualizer.

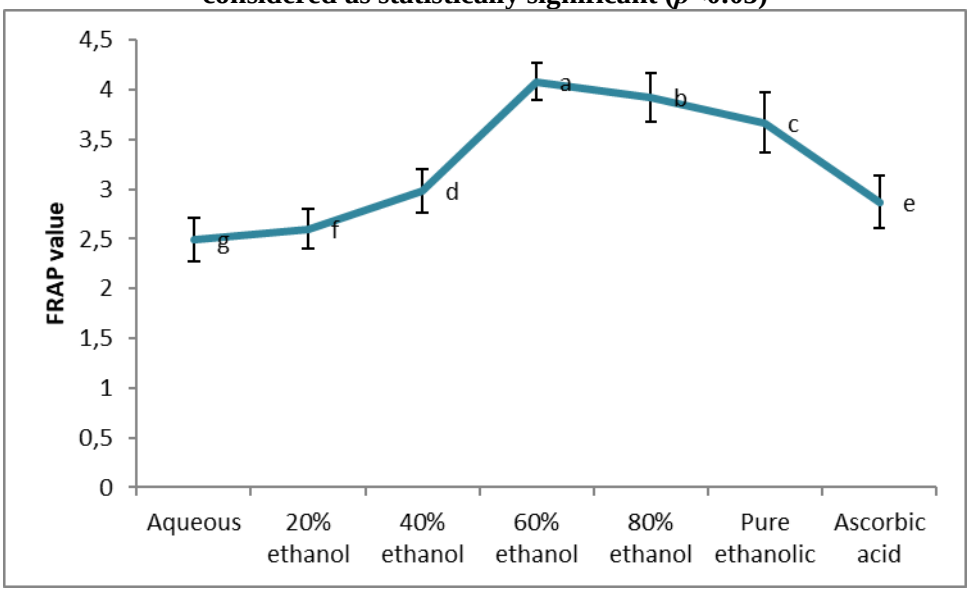
RESULTS

FRAP assay

The FRAP assay was used for antioxidant activity of hydroethanolic leaf extracts. The results of FRAP assay are given as Figure No. 1. The results indicated that 60% ethanolic extract showed highest antioxidant activity. The FRAP value of 60% hydroethanolic extract was found significantly higher when compared statistically with other extracts and ascorbic acid (ρ<0.05).

Figure No. 1

The FRAP values of extracts in mg trolox equivalent per gram dry extract. Values with different letters were considered as statistically significant (ρ<0.05)



The α-amylase inhibition

The results of α-amylase inhibition are given as Figure No. 2. The results depicted that 60% ethanolic leaf extract of *C. erectus* exhibited IC_{50} value of 58.20 ± 1.25 µg/mL and acrbose exhibited IC_{50} value of 35.50 ± 0.50 µg/mL for α-amylase inhibition. The aqueous extract exhibited least inhibition of α-amylase. The statistical analysis (Table No. 1) revealed 60% ethanolic extract inhibited α-amylase significantly as compared to other extracts (ρ<0.05).

The identified secondary metabolites by UHPLC-QTOF-MS/MS were ellagic acid, 3-O-methyl ellagic acid, ferujol, 5,2´-dihydroxy-6,7,8-trimethyl flavone and kaempferol glucoside, respectively. The detail of identified compounds with their characteristic parent and daughter peaks (m/z) are given in Table No. 2.

The mass fragmentation patterns of compounds were also studied for structure elucidation and confirmation. The possible fragmentation is summarized in Figure No. 4. The peak recorded at Rt 9.668 with m/z 301 was the ellagic acid. The fragment ions at m/z 284 [M-OH-H]-, m/z 229 [M-CO2-CO-H]-, m/z 185 [M-2CO2-CO]- corresponded to typical fragmentation pattern for ellagic acid [214-216]. The kaempferol glucoside appeared with parent ion peak at 447.09 m/z at Rt 10.314. Further fragmentation produced daughter ion peak at 285 m/z was generated due to hexose removal having mass 162 amu [M-Glucoside- H] – which confirmed the compound as kaempferol glucoside.

Figure No. 2
The α-amylase inhibitions by hydroethanolic leaf extracts of *C. erectus*.
Values with different letters were statistically significant (ρ<0.05)

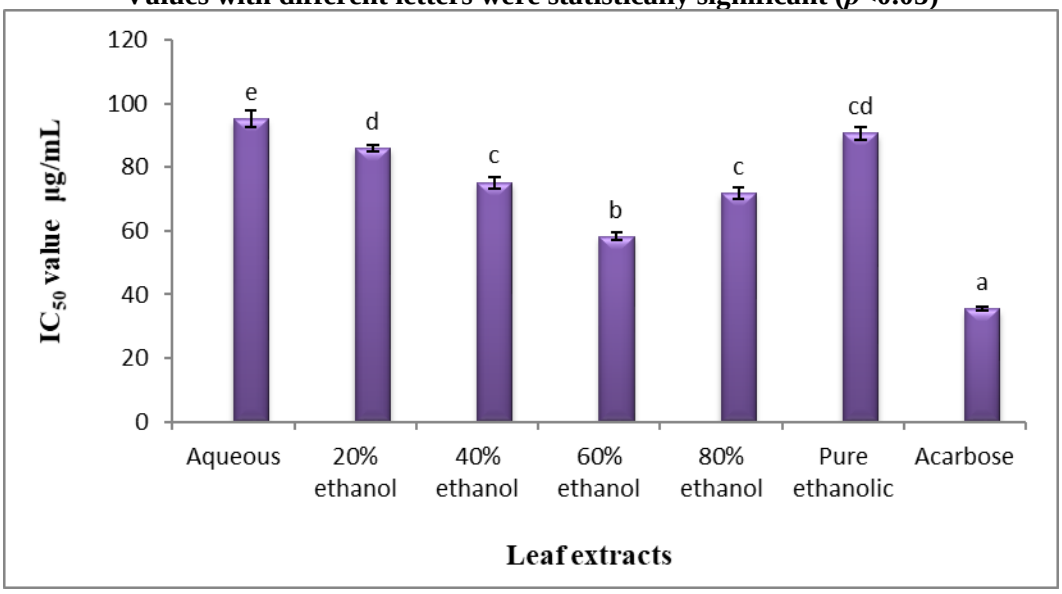


Table No. 1
Results of statistical analysis (Tukey's pair wise comparison at 95% confidence interval) for α-amylase inhibition

Solvent Composition	Mean values (IC50)	Standard deviation (±)	Degree of freedom	Adjusted sum of square	Adjusted mean square	F-value/ρ-value	Grouping
Aqueous	95.22	2.74	6	7809.15	1301.52	446.41/0.000	E
20% ethanol	85.97	1.16					D
40% ethanol	75.19	1.83					C
60% ethanol	58.2	1.25					B
80% ethanol	71.9	1.74					C
Pure ethanol	90.61	1.83					CD
Acarbose	35.5	0.5					A

Metabolite Profiling

The UHPLC chromatogram is given as Figure No. 3 and identified compounds were assigned the numbers.

Figure No. 3
Base peak chromatogram of 60% ethanolic extract of *C. erectus*

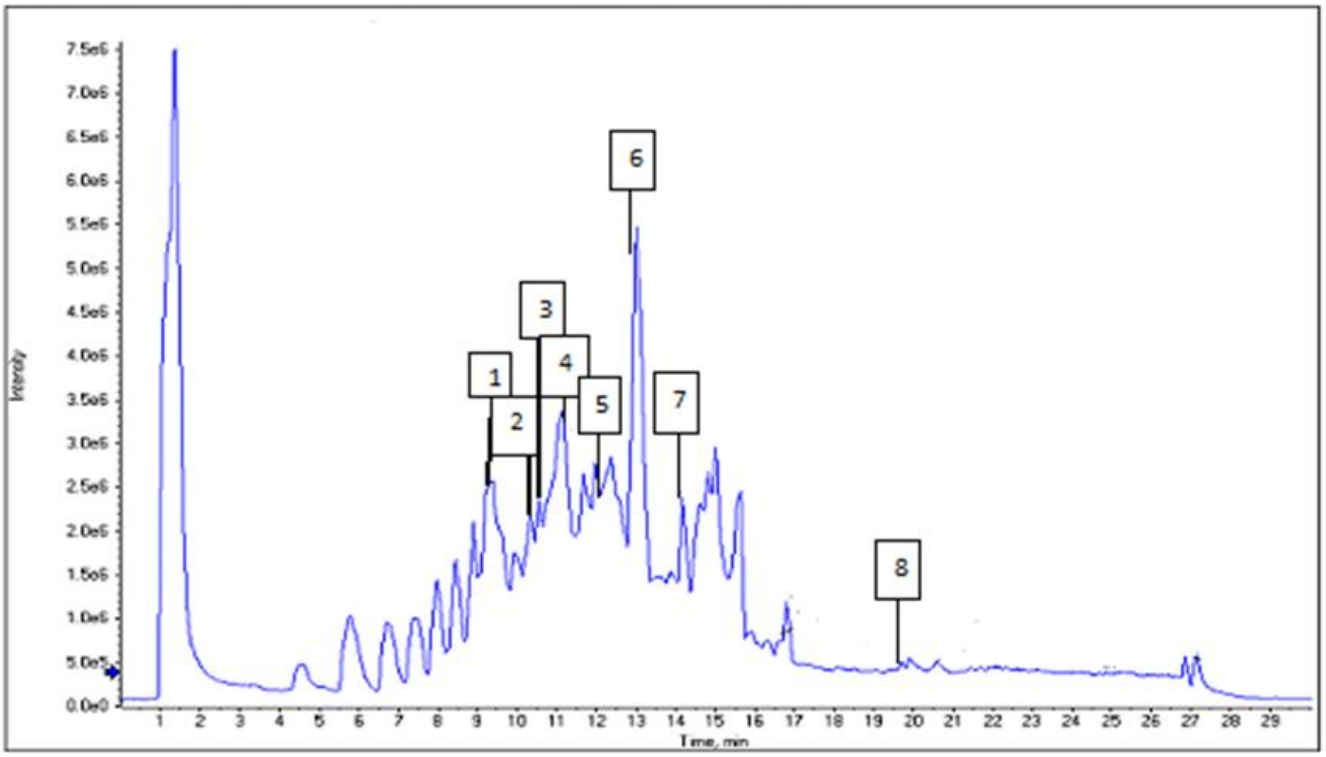
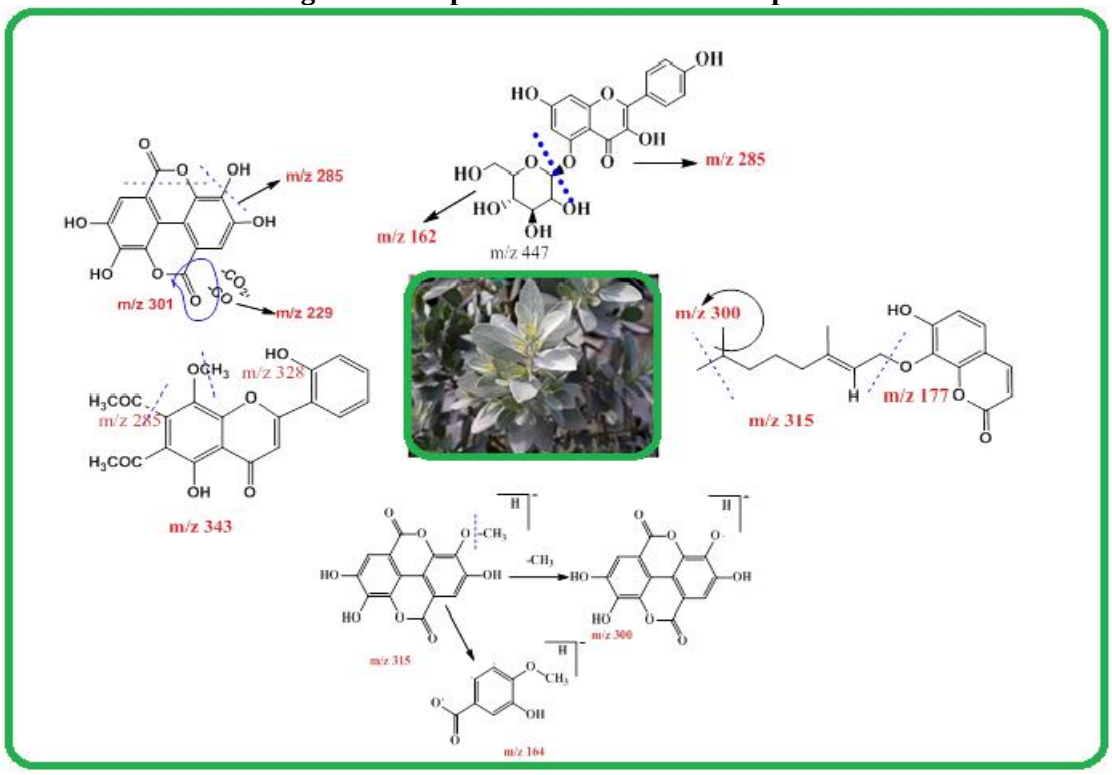


Table No. 2
Chromatographic and spectrometric data of identified compounds

Sr. No	Compound	Retention time (Rt) in min	Molecular ion peak (m/z)	Main fragments ion (m/z)	Molecular formula
1	Ellagic acid	9.668	301	300, 284, 229, 185, 145	$C_{14}H_6O_8$
2	Kaempferol glucoside	10.314	447	327, 285, 284, 255, 227, 151, 97	$C_{21}H_{20}O_{11}$
3	3-O-methyl ellagic acid	10.470	315	300, 284, 235, 164, 149, 121	$C_{15}H_8O_8$
4	Ellagic acid derivative	11.103	425	345, 330, 315, 287, 271, 243	$C_{24}H_{10}O_8$
5	Ellagic acid derivative	11.647	409	329, 314, 299, 271, 247	$C_{19}H_6O_{11}$
6	5,2´-dihydroxy-6,7,8-trimethyl flavones	12.920	343	423, 343, 328, 313, 298, 285	$C_{18}H_{15}O_7$
7	Ferujol	14.182	315	314, 300, 273, 255, 241, 191, 177, 149, 127, 123, 108	$C_{19}H_{24}O_4$
8	Kaempferol glucoside derivative	19.781	653	447, 285, 79	$C_{36}H_{46}O_{11}$

Figure No. 4
Fragmentation patterns of identified compounds



The peak at 255 [m/z 285-M-CH_2O- H] $^-$, 227 [m/z 255-CO- H] $^-$ were recorded. A signal at 151 m/z was due to cleavage of heterocyclic C-ring. The 3-O-methyl ellagic acid appeared at Rt 10.470 with m/z 315. The fragmentation pattern of 3-O-methyl ellagic acid is given in Figure No. 4. The fragment ion peak at 300 m/z was produced due to removal of methyl group [M-CH_3-H]$^-$. The obtained molecule of ellagic acid exhibited typical ellagic acid fragment ions at m/z 284 [M-OH-H]$^-$, m/z 235 [m/z 300-65Da]$^-$, m/z 164 instead of typical m/z 151 due to methyl group, 149 [m/z 164-15Da]$^-$.

The fragmentation pattern represented the typical ellagic acid mass spectrum. The 5,2´-dihydroxy-6,7,8-trimethyl flavone peak appeared at Rt 12.920 min with m/z 343. The fragmentation pattern of 5,2´-dihydroxy-6,7,8-trimethyl flavone peak at m/z 423 was due to conjugated sulphonate group with flavone molecule. The peak with m/z 343 [M-SO_3-H]$^-$ was generated due to removal of sulphonate group leaving behind flavone moiety. Consecutive removals of three methyl groups produced fragment ions with m/z value of 328, 313 and 298, respectively. A typical characteristic peak for flavonoids was noted with m/z 285 [M-$COCH_3$-H]$^-$[218]. Ferujol peak was identified at Rt 14.182 with m/z 315. The fragment ion peak at m/z 300 [M-CH_3-H] $^-$, m/z 273 [M-300-CO-H] $^-$, m/z 255 [M-273-H_2O-H] $^-$, m/z 241 [M-255-CH_2-H] $^-$ and m/z 177 were due to removal of side chain [M-$C_{10}H_9$-H] $^-$.

Docking studies

Figure No. 5 demonstrated the superimposing of standard drug acarbose into binding sites of homology model of α-amylase. The synergistic effect of identified metabolites in most potent leaf extract of *C. erectus* was evaluated by subjecting the metabolites to docking simulation. For this purpose, the identified compounds were docked into the active site of the homology modelled α-amylase. Three-dimensional (3D) energy binding poses of all the compounds are superposed in Figure No. 6.

Figure No. 5
The 3D depiction of acarbose docked at active sites of α-amylase

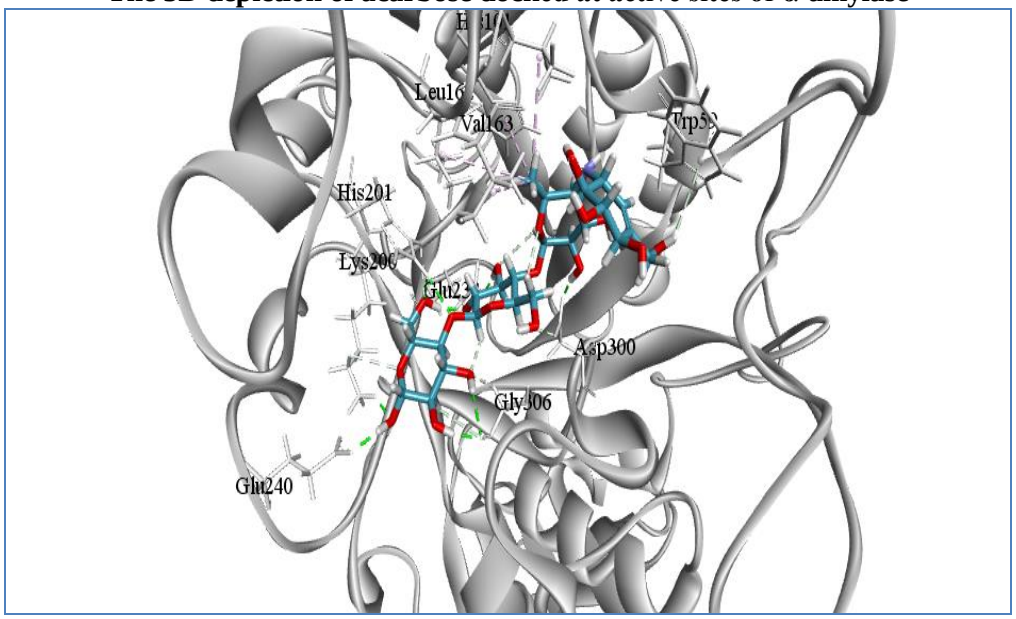
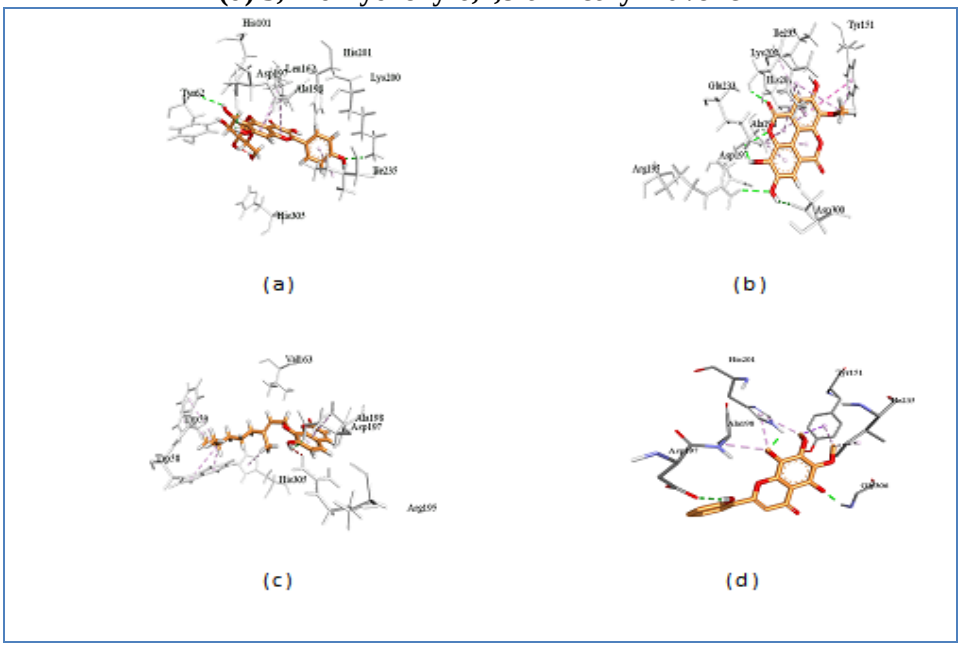


Figure No. 6
The 3D superimposed pose on α-amylase (a) kaempferolglucoside (b) 3-O-methyl ellagic acid (c) ferujol (d) 5,2´-dihydroxy-6,7,8-trimethyl flavone



The binding affinities in terms of energy calculations were determined to evaluate the efficacy of compounds to inhibit the active sites of dietary enzyme (Table No. 3). The docking analysis indicated that kaempferol glucoside interacted with Lys 200, Asp 197, Tyr 62 residues of α-amylase through hydrogen bonding. It also interacted with Ala 198, Leu 162 and Ile 235 amino acid residues through π-alkyl bonding. The C-H-bonding at His 101 and His 305 were also observed. The binding affinity value of -14.3268 kcal/mol of kaempferol glucoside was calculated. The 3-O-methyl ellagic

acid made hydrogen bonding with Lys 200, Ala 198, Glu 233, Arg 195and Asp 300 residues of α-amylase. A π-alkyl interaction was observed for Ile 235 and Tyr 151.

A π-donor hydrogen bond interaction was exhibited at His 201. Tyr 151 also showed π-π T shaped and C-H-bonding. The binding energy was -14.5911 kcal/mol. Ferujol made hydrogen bond with Asp 197. It also interacted with His 305, Val 163, Trp 59, Trp 58 through alkyl linkage. A π-alkyl interaction at Ala198 was also observed. The binding energy computed for ferujol was -10.7973 kcal/mol. The 5,2´-dihydroxy-6,7,8-trimethyl flavone exhibited prominent hydrogen bonding interactions with Asp68, Asp408 and Arg439. The two phenyl rings forms π- π stacking interactions with Tyr71, Phe177, Phe157 and Phe300. While a π-alkyl interaction was observed with Ala278. His239 residue was involved in C-H bonding. The binding energy computed for 5,2´-dihydroxy-6,7,8-trimethyl flavone as a result of these interactions was -13.6199 kcal/mol. The binding energy computed for acarbose against α-amylase was -15.7815 kcal/mol.

Table No. 3
Binding affinities of identified compounds and acarbose for α-amylase

No.	Compound Name	Binding affinity (kcal/mol)
1	Kampferol glucoside	-14.3268
2	3-O-methyl ellagic acid	-14.5911
3	Ferujol	-10.7973
4	5,2´-dihydroxy-6,7,8-trimethyl flavones	-13.6199
5	Acarbose (Standard)	-15.7815

DISCUSSION

The pharmacological properties of plant extracts are due to secondary metabolites. The secondary metabolites exert their impact through numerous biological activities including antioxidant potential. The antioxidant compounds play a pivotal role to maintain the general health. The aromatic hydroxyl groups of compounds have ability to encounter free radicals to mitigate oxidative stress and associated health disorders. The antioxidant activity of plant extracts is an essential feature to predict their phytomedicinal attributes. In current study all extract showed reasonable FRAP values however 60% ethanolic extract exhibited the highest FRAP value and aqueous extract possessed least value. The FRAP assay results revealed the antioxidant potential of leaf extracts of *C. erectus* which provided a lead to move for enzyme inhibitory properties because antioxidant potential was an indication of possible medicinal potential of extracts (Mettupalayam & Kilavan, 2020). The dietary intake of carbohydrate rich diet increases the postprandial blood glucose level. Theα-amylases catalyse the hydrolysis of starches to convert them into simpler molecules for intestinal absorption. The inhibition of α-amylase is considered as an effective approach to control postprandial glucose levels. The phytochemicals can delay the digestion of complex carbohydrates like starch by inhibiting the action of α-amylase on substrate (Uddin *et al*., 2014). However, it depends upon the nature of secondary metabolites of plants and their interaction with the protein. The difference of antioxidant and enzyme inhibitory properties among extracts was most probably due to polarity of solvent system used for extraction. Polarity of solvent is the most decisive factor to improve extraction of vital phytochemicals from complex plant matrix. Therefore, the nature of compounds present in a particular extract depends upon extraction strategy which is also reflected in better antioxidant and pharmacological properties (Raza *et al*., 2018; Raza *et al*., 2020). The antioxidant properties of extracts are considered as major contributor towards pharmacological potential of plants. Oxidative stress is a leading cause to disturb glucose homeostasis resulting in diabetes mellitus. Reduction in oxidative stress by using antioxidants may alleviate the disease intensity by improving the antioxidant defense system of living system (Arshad *et al*., 2020). The results of antioxidant activity and enzyme inhibitory properties projected 60% ethanolic extract of *C. erectus* as the most potent extract and therefore

subjected to metabolite profiling by UHPLC-QTOF-MS/MS. The ellagic acid, 3-O-methyl ellagic acid, ferujol, 5,2´-dihydroxy-6,7,8-trimethyl flavone, kaempferol glucoside and its derivative were identified in 60% ethanolic leaf extract. These compounds were reported to have substantial pharmacological properties. Ellagic acid, kaemferol and its derivatives were reported to have antidiabetic potential by inhibiting the α-glucosidase and identified as major contributor to antidiabetic potential of *Conocarpus lancifolius* and oak cups (Yin *et al.*, 2018; Raza *et al.*, 2020). The 5,2´-dihydroxy-6,7,8-trimethyl flavone, another important phytochemical was also identified in 60% ethanolic extract and a previous report supplemented its prodigious hypoglycaemic aspects under high fat induced obesity model (Song *et al*., 2012). A recent *in vivo* study to control hyperglycaemic blood physiology in obese mice proved the efficacy of *C. erectus* ethanolic extract. The hydroethanolic extract having high phenolics and flavonoids, significantly reduced the blood glucose level in diabetic mice. The extract also exhibited strong α-glucosidase inhibitory properties. However, the metabolites responsible for hypoglycaemic impact of *C. erectus* were not identified (Raza *et al*., 2018). A recent work reported the α-amylase inhibitory potential (IC_{50} 60.58 ± 3.24 µg/mL) of hydroethanolic extract of *Hyophorbe lagenicaulis* which was quite comparable with the IC_{50} value obtained for 60% ethanolic extract of *C. erectus* in current study. Some high values compounds including kaempferol, rutin, hesperetin 5-O-glucoside, isorhamnetin-3-O-rutinoside, trimethoxyflavone derivatives were identified in *Hyophorbe lagenicaulis* extract (William *et al*., 2019).

The presence of nutraceutically important metabolites was confirmed in current study which not only supported the ethnopharmacological use of *C. erectus* leaves but also provided the novel leads for functional food development. The molecular docking studies explored the possible site specific interactions of identified compounds with amino acid residues of α-amylase. The comparison of binding energies revealed that 3-O-methyl ellagic acid was the most effective inhibitor of α-amylase due to amino acid residues inactivation. The 3-O-methyl ellagic acid made hydrogen bonding with Lys 200, Ala 198, Glu 233, Arg 195and Asp 300 residues of α-amylase. A π-alkyl interaction was observed for Ile 235 and Tyr 151. A π-donor hydrogen bond interaction was exhibited at His 201. Tyr 151 also showed π-π T shaped and C-H-bonding. The binding energy computed as a result of these interactions was -14.5911 kcal/mol being, highest among all identified compounds. A report on the α-glucosidase inhibitory potential of *Cycas revoluta* leaf extract having apigenin derivatives as identified by UHPLC-QTOF-MS/MS was published previously. The molecular binding studies of the report revealed six hydrogen bond interactions of apigenin with α-glucosidase at Asp214, Glu276, Pro309, Arg312, His348 and Arg439, respectively (Arshad *et al*., 2019). A comparative study evaluated 4-methyl esculetin, genestein and herbacetin for α-amylase inhibition by in silico docking studies. All these flavonoids possessed binding energy comparable to acarbose (Madeswaran & Ashokkumar, 2015). A recent study performed molecular docking to see the site specific interaction of 3-oxolupenal and katononic acid isolated from *Nuxia oppositifolia* against α-amylase (Alqahtani *et al*., 2020).

Another investigation explored *Calotropis procera* and myriciacitrin IV, a flavanone glucoside was indentified along with hydroxybenzoic acid (phenolic acid), quinic acid and gluconic acid (organic acids) in hydroethanolic leaf extract. The molecular docking study showed that only myriciacitrin IV showed a reasonable binding energy when docked with α-amylase. No organic acid or phenolic acid could reach the considerable binding energy as compared to acarbose the study further revealed that myrciacitrin IV exhibited six hydrogen bonding at Asp408, His229, Glu304, Pro309, His295 (Nadeem *et al*., 2019) which were quite different from the hydrogen bonding observed for the compounds identified in current investigation. This very difference predicted the non-competitive mode of interaction between secondary metabolites and α-amylase. It was confirmed that binding of active sites of α-amylase by the phytochemicals was the leading cause behind the enzyme activity loss (Farooq *et al*., 2020). Hence, molecular docking provides a great deal of information on the molecular interactions among phytochemicals and enzymes to provide logical and essential information for pharmacological potential drug development.

The pharmacological properties like antioxidant and antidiabetic properties like α-glucosidase and α-amylase depends upon the electronic environment of functional molecules present in extracts. A recent study reported that the electron transfer potential of compounds is directly linked with antioxidant and other related properties (Nadeem *et al*., 2020). Ellagic acid, kaempferol and their derivatives are well reported for antidiabetic response due to modulation in dietary enzyme

activity. A study reported that ellagic acid and kaempferol derivatives from Mangolian oak cups inhibited the activity of α-amylase to a considerable extent as compared to acarbose (Yin *et al*., 2018). Similary 5,2´-dihydroxy-6,7,8-trimethyl flavones derivatives present in aerial parts *Achillea biebersteinii* Afan were also reported to exhibit as a major contributor towards α-amylase inhibition to stop postprandial hyperglycemia (Abd-Alla *et al*., 2016). The enzyme inhibitory potential of polyphenols truly depends on the structural viability to influence the enzyme activity. The comparative structure-function analysis revealed that hydroxyl groups on ellagic acid and flavones were said to be responsible for their α-amylase inhibition activity (Wu *et al*., 2019).

Ellagic acid was also reported to increase the insulin secretion by activating the pancreatic β-cells with improved antioxidant status in type-2 diabetic rats (Fatima *et al*., 2017) Plant extracts can adopt multi-target approach to reduce the blood glucose level. A study on 17 boreal forest medicinal plants reported the antidiabetic evaluation in Caco-2 human enterocytic cell line. The antidiabetic potential of plants might be due to regulation of SGLT-1 or GLUT-2 proteins (Baldea *et al*., 2010).

The antioxidant properties of identified compounds in addition to α-amylase inhibitory potential has been reported to mitigate the oxidative stress to improve the metabolic function leading to mitigate diabetes mellitus initiation and propagation.

The current study provided the deep insight about molecular modulations between α-amylase and functional metabolites of *C. erectus*. These computational investigations provided the possible reason for antidiabetic potential of *C. erectus* leaves. The intake of dietary nutraceuticals and antioxidants to prevent DMT_2 may be improvised by considering the *C. erectus* as natural candidate. The diet enrichment with *C. erectus* may be accomplished to enhance the food functionalities with antidiabetic attributes.

CONCLUSION

The current work evaluated the FRAP and *in vitro* α-amylase inhibitory potential of hydroethanolic leaf extracts of *C. erectus* to confirm its antioxidant and antidiabetic role. The 60% ethanolic extract exhibited highest α-amylase inhibitory properties among all extracts. The study also explored the presence of valuable secondary metabolites of functional nature in leaf extract of *C. erectus* and added novel information in phytochemical library. The findings of docking studies unveiled the molecular basis of α-amylase inhibitory properties of *C. erectus.* The outcomes may be used to move further into development of naturopathic treatment of diabetes mellitus and to enhance the food functionalities.

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