

THE COMPARATIVE ALPHA-AMYLASE INHIBITORY AND ANTIOXIDANT POTENTIALS OF DIFFERENT EXTRACTS OF *CAPPARIS DECIDUA* FRUIT OF PAKISTANI ORIGIN

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Abstract

Use of plant materials, extracts and bioactive compounds termed traditional practice. This type of exercise provided less expensive remedies and better health care. Therapeutic characteristics of natural herbs are usually joined to the secondary metabolites. Traditional medicinal plants have a significant role to manage diabetes mellitus. Consequently, our study is mainly focused on the evaluation of phytochemicals, antioxidant and anti-diabetic properties of *Capparis decidua* fruit as well as comparative study of its different extracts, such as; n-hexane (NCD), chloroform (CCD) and methanolic (MCD). The phytochemical investigation of *C. decidua* confirmed the presence of carbohydrates, alkaloids, proteins, glycosides tannins and fixed oils. In addition, total phenolic and flavonoids contents were also assessed and found chief quantity in NCD extract. DPPH protocol was employed to evaluate the anti-oxidant activity with all extracts. The percentage-inhibitions 60.58%, 56.88% and 47.62% exhibited with NCD, CCD and MCD respectively. Values of IC₅₀ were observed, 977.4µg/mL (NCD), 1214µg/mL (CCD) and 3011µg/mL (MCD). Anti-diabetic activity (*In-vitro*) was calculated through α-amylase inhibition protocols. The percentage suppressions with MCD, CCD and NCD were observed 80.56, 83.18 and 86.11% respectively. The IC₅₀ values with all said extracts 605, 456 and 167 µg/mL were analyzed. The comparative antioxidant and anti-diabetic aptitudes were also examined and concluded NCD found significant potentials.

Key words: *Capparis decidua*; Alpha amylase inhibition; Phytochemicals; Antioxidant.

Introduction

Because of traditional use and therapeutic significance of medicinal plants, researchers have been interested to explore the identification and isolation of the novel active medicinal compounds from natural flora (Gossell-Williams *et al.*, 2006). In line with WHO, more than 80 % of the world population relied on traditional mode of treatments due to their safety and financial benefits (Azaizah *et al.*, 2003). Different phytochemicals obtained from seeds, fruits, roots, flowers, leaves and barks, such as; carbohydrates, tannins, alkaloids steroids, flavonoids and terpenes have been employed to manage veterinary, agriculture and human diseases (Cragg & Newman, 2001; Yadav & Agarwala, 2011; Cragg & Newman, 2013). The existence of phytochemicals in medicinal plants guaranteed the diagnosis, prevention and treatment of several illnesses (Nostro *et al.*, 2000). Unani and Folk remedies have been a part of medication since ancient times and exhibited antipyretic, anthelmintic, antibacterial, anti-fungal, anti-diabetic, antihypertensive, anti-protozoa, anti-rheumatic and anti-flatulent potentials as well as to treat eczema and dyspepsia. Plants which were frequently used, such as; *Malia azedarach*, *Caesalpinia crista*, *Trachelsospermum jasminoides*, *Saussura lappa* and *Butea fronsosa* (Akhtar *et al.*, 2000; Nostro *et al.*, 2000).

Globally, the most commonly known ailment is the Diabetes mellitus which is effecting millions (200 million) of population and around three hundred million people at

the risk of with diabetes (Alwin Robert & Al Dawish, 2019). Two types of diabetes have been recognized namely; insulin dependent (Type-1) and non-insulin dependent (Type-2) diabetes mellitus. In Type-1 diabetic patients, deficiency of insulin is associated to the almost suppression of pancreatic β-cells' function. While Type-2 diabetes is more common and it is generally seen in obese and sedentary style population. In addition, suppression of antioxidants in the body is also lined to diabetes (Ighodaro, 2018). Type-2 diabetes is not restricted to the insulin use and it is well managed by the change of lifestyle and control of body weight (Hogan *et al.*, 2010). Oral anti-diabetic therapy usually recommended in case of inadequate management of metabolic system with physical measurements (Ranjit *et al.*, 2011).

As concerned the treatment of diabetes, traditional herbal therapy is equally appreciated with allopathic method of remedy. Universally, more than eight-hundred medicinal plants have been explored to control the diabetes mellitus (anti-diabetic) successfully (Naghbi *et al.*, 2014). It was investigated through *In-vivo* studies that anti-diabetic potential has found in around 37 European medicinal plants, out of these, eleven have demonstrated a significant anti-hypoglycemic threshold. Studies also showed that hydrophilic compounds of herbal drugs directly involved in the inhibition of the intestinal absorption of glucose, boosted transportation in muscular tissues that augmented the metabolic process which eventually endorsed the secretion of insulin (Cai *et al.*,

2004). As lot of medicinal plants and herbs have influential role in the achievement of novel medicinal compound to manage diabetes. Hence, *Capparis decidua* (*C. decidua*) was selected for the evaluation of comparative anti-diabetic activity of its n-hexane, chloroform and methanol extracts. The origination of *C. decidua* is Pakistan, Kingdom of Saudi Arabia, Deccan, Egypt and India (Ruggles & Sinha, 2009).

In Pakistan mostly April-May is the flowering season and fruit ripen in October-November. Fruits are spherically small and green in color, then converted to pink and finally black when completely dried (Verma *et al.*, 2011). Various parts of *C. decidua* have been employed in several therapeutic situations such as; its fruit is effectively managed the cardiac issues, paralysis, spleen enlargement and in worm infestation (Singh & Singh, 2011). Roots' powder and its extract used for the treatment of jaundice and hemorrhoids (Sharma & Patni, 2012; Yeung & D'Souza, 2013). Due to the medical importance of this plant it was used to control rheumatism, diabetes, hypertension and indigestion (Madhu & Sharma, 2009). As it contains hemicellulose, it significantly facilitated the excretion of excess bile acid and cholesterol from the body and posed potential hypocholesterolemia response (Subramoniam, 2003). The presence of isothiocyanate aglycone and glucocapparin exhibited the antibacterial activity (Singh & Singh, 2011; Verma *et al.*, 2011).

Materials and Methods

Collection, identification and processing of Crude – Drug: Collection of *Capparis decidua* fruit was made in November from Mian Channu Morr beside railway line, Borewala, District Vehari, Punjab, Pakistan. For the future reference, plant specimen was preserved in Herbarium after authentication and identification from Department of Botany, Government College University, Lahore with ID# GC-Herb-Bot-3413. Fruit was dried under shade for fortnight; fine powder was attained with mechanical chopper and stored in well closed glass vial.

Extraction of crude powder: The extraction of fruit powder of *C. decidua* (270 g) was acquired with n-hexane using Soxhlet apparatus, till the discoloration of solvent seemed in thimble area. Extract was filtered through porcelain cloth first then with Whatman filter paper (Grade 01). Filtrate was concentrated with rotaryevaporator and extract was allowed to dry to semi-solid mass (dark brown) in an oven at 40°C. The residue was used to extract with chloroform and methanol by the same protocol successively. All three extracts were weighed and their percentage yields were determined. These extracts were filled in amber colored bottles after assigning the specific code and stored at 2-8°C for the further investigation. The n-hexane, chloroform and methanol extracts were tagged as NCD, CCD and MCD respectively.

Instruments: Soxhlet Evaporator (GLHMP-F100), Rotary Evaporator (RV10BS99), Incubator, Hot-Air Oven (RL10-03465), UV/Visible Spectrophotometer (PG Instruments Ltd T-80), Analytical Balance (DHAUS corporation USA), Electric-Corbolite furnace Sheffield, UVGL-58 Handled UV Lamp, Centrifuge 2-16 KC, Sonicator (ROHS DSA 100-Sk-2.8 L), Water-bath (Jisico korea), Desiccator (Made in China).

Plant sample preparation: Accurately weighed (10mg) of each extracts of *C. decidua* were poured in tarred glass vessel and sonicated with 10 ml methanol. The resulting stock solutions were attained 1mg/ml of plant extracts. Several dilutions like 125µg, 250µg, 500µg and 1000µg were prepared from the stock solution with methanol. The procedure was repeated in triplicates. The positive control group contained 01ml methanol and 02ml DPPH.

Procedure: DPPH solution (02 ml) was added to each standard, control and sample test tube. All tubes were incubated for 30 min in darkness and then absorptions were measured at 517nm using UV Spectrophotometer. By employing given formula, Radical-scavenging activity was measured.

$$\% \text{ INHB} = \frac{\text{Positive control (standard)}}{\text{Positive control}} \times 100$$

Evaluation of *In-vitro* α-amylase inhibitory assay: The α-amylase inhibitory (*In-vitro*) protocol was implemented with little modifications (Subramanian *et al.*, 2008).

Procedure: Different concentrations of the extracts of *C. decidua* were prepared with phosphate buffer (20mM, pH-6.9) in Eppendorf safe-lock tubes. For the standard or positive control, the stock solutions of various strengths of acarbose were prepared with phosphate buffer such as; 200, 400, 600 800 and 1000µg/ml. One milliliter of α-amylase was added to all experimental test tubes and incubated for 15min at 37°C at room temperature. Then 01ml of DNSA reagent (3,5-dinitro salicylic acid) was poured to all incubated test tubes and heated in water-bath for 05 min by maintaining the temperature up-to 85°C. Cooled under tap water and made the volume with distilled water up-to 10ml in each test tube. The negative control tubes were contained only buffer and distilled water. Measurement of absorbance was determined at 540 nm using Spectrophotometer.

The percentage inhibition of α-amylase was determined using given formula:

$$\alpha\text{-amylase inhibitory activity} = \frac{(Ac_+) - (Ac) - (As - Ab)}{(Ac_+) - (Ac)} \times 100$$

Here

Ac₊: absorption of the 100% enzyme activity (solvent with enzyme only)

Ac: absorption of the 0% enzyme activity (solvent without enzyme)

As: absorption of the test sample with enzyme

Ab: absorption of the test sample without enzyme

How calculated the IC₅₀, is mandatory report the mathematical method

The half-maximal inhibitory concentration (IC₅₀) against α-amylase activities was determined from the inhibitions obtained from a range of concentrations of extracts. The IC₅₀ value was derived from the least-squares regression line of the plot of inhibition % versus log₁₀ concentration previously described method ((Xiong *et al.*, 2020)).

Results

Extracts' yield: The percentage yield of extracts resulted from the fruit of *Capparis decidua* showed maximum in methanolic and least in CCD extracts presented in Table 1.

Table 1. Percentage yield of *Capparis decidua* fruit extracts.

Plant name	Extract	Percentage yield (%)
<i>Capparis decidua</i>	n-Hexane (NCD)	13.55
	Chloroform (CCD)	10.04
	Methanolic (MCD)	30.13

Phytochemical investigation: The *In-vitro* qualitative phytochemical study was performed on plant extracts and results were shown in Table 2.

Antioxidant activity: The radical scavenging activity (*In-vitro*) of *C. decidua* was determined with different concentration of extracts (125, 250, 500 and 1000 µg/ml) using DPPH assay technique. The significant inhibitory response observed with 1000 µg/mL of n-hexane (63.33 ± 0.02), chloroform (56.88 ± 0.02) methanolic (47.62 ± 0.01) extracts on comparison with standard drug (Fig. 1).

α -amylase suppression potential and IC₅₀ outcomes: The *In-vitro* alpha-amylase suppressive response of *C. decidua* was evaluated with different concentrations of extracts and found inhibitory actions. The significant inhibitory response was observed with 1mg/mL of NCD (86.11 ± 0.01), MCD (80.56 ± 0.01) and CCD (86.11 ± 0.01) on comparison to reference drug (88.25 ± 0.01) as shown in (Fig. 3). The IC₅₀ grades of alpha amylase inhibitions were NCD (167 ± 7.02), CCD (456 ± 6.65), MCD (606 ± 4.50) and standard drug 65.58 ± 5.01 shown in (Fig. 4).

Discussion

With increasing polarity, n-hexane, chloroform and methanol were used to extract fruit of *Capparis decidua*. The percentage yield resulted from the methanolic (MCD), n-

hexane (NCD) and chloroform (CCD) extracts were 30.13, 13.55 and 10.04% respectively. This yield difference of *C. decidua* extracts is associated to the compatibility of components with appropriate polarity of the solvent (Hsu *et al.*, 2006). The therapeutic activities of the plants are mainly exhibited due the presence of phytochemicals such as; glycosides, phenols, saponins, flavonoids, carbohydrates, alkaloids and proteins. Alkaloids found in natural plants, mostly allied to the class of CNS, diuretics, analgesics, antimicrobial and antispasmodics (--). Glycosides effectively control CVS related diseases. Saponins are commonly endorsed as anti-cancer related compounds (Yadav & Agarwala, 2011). In addition, glycosides and carbohydrates have beneficial roles in food supplements and strengthening immune system (Theis & Lerda, 2003). The anti-oxidant potential is generally perceived in plants containing phenolic compounds like; tocopherols, phenolic acids and flavonoids (Ali *et al.*, 2008). Anti-inflammatory, anti-carcinogenic, anti-aging, anti-apoptosis and cardio protective properties also found with phenolic constituents (Mendoza-Espinoza *et al.*, 2020; Han *et al.*, 2007).

By using the DPPH scavenging activity, extracts of the fruit of *C. decidua* were assessed and showed that extracts (CCD, NCD, MCD) considerably reduced the stable; 1,1-diphenyl-2-picrylhydrazyl radical. Current study demonstrated the inhibitory responses are directly interconnected to the doses of extracts. The maximum inhibition was noticed with NCD ($82.92 \pm 0.02\%$) with a significant potential on comparison to other extracts and control drug (Fig. 1). The appropriate antioxidant activity in our tested extracts, might be due the presence of higher concentration of flavonoids counts (Bonina *et al.*, 2002). The lower IC₅₀ values of extracts presented the higher antioxidant potentials given in Fig. 2. The imperative order of IC₅₀ of all extracts presented as NCD > CCD > MCD.

IC₅₀ value of extracts of *Capparis decidua*'s: Free radical scavenging potential of NCD, CCD and MCD were perceived 977.5 ± 7.50 , 1214 ± 13.50 and 3011 ± 11.50 respectively (Fig. 2).

Table 2. Phytochemical characteristics of *C. decidua* extract.

Phytochemicals	Test name	<i>C. decidua</i>		
		n-hexane	Chloroform	Methanolic
Alkaloids	Mayer's	++	+	+
	Hager's	+	++	+
	Wagner's	++	+	+
Proteins	Biurette	+	-	-
	Ninhydrine	+	-	-
	Million's	+	+	+
Carbohydrates	Molish's	+	+	+
	Fehling's	+	++	-
	Barford's	+	+	+
Glycosides	Legal's	+	+	+
	Borntrager's	+	+	+
	Alkaline reagent	++	+	++
Phenolic	Ferric chloride	++	+	+
	Gelatin	++	+	+
	Lead acetate	+	++	+
Fixed oil	Spot test	+	-	+

++ Strongly present "+" Indicates presence & "--" Indicates absence

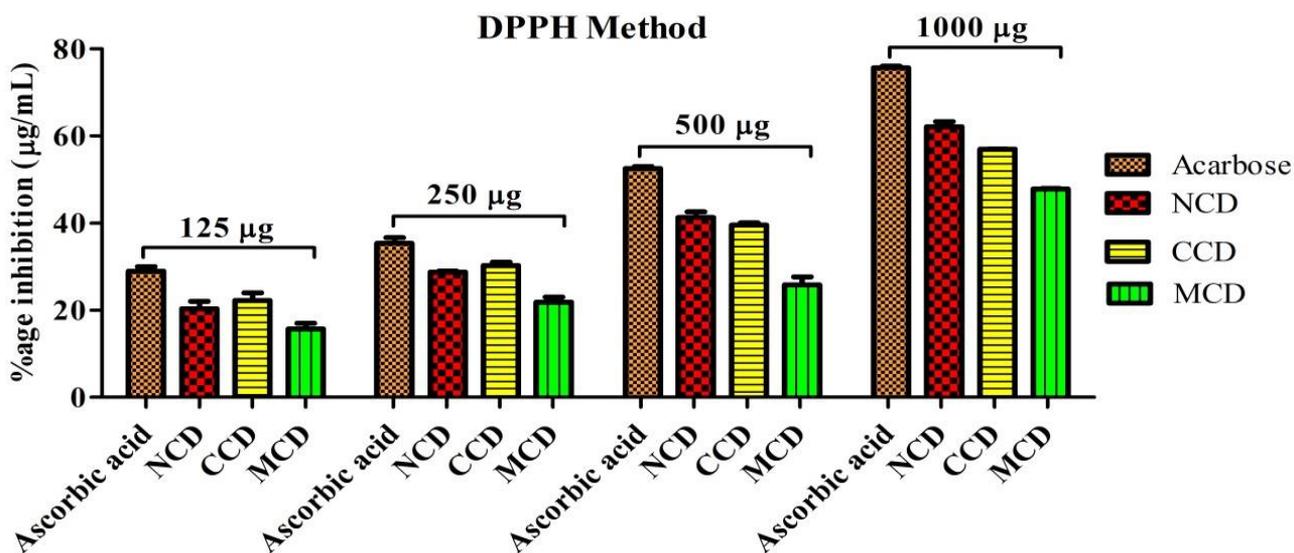


Fig. 1. Comparative percentage inhibition of extracts and Ascorbic acid.

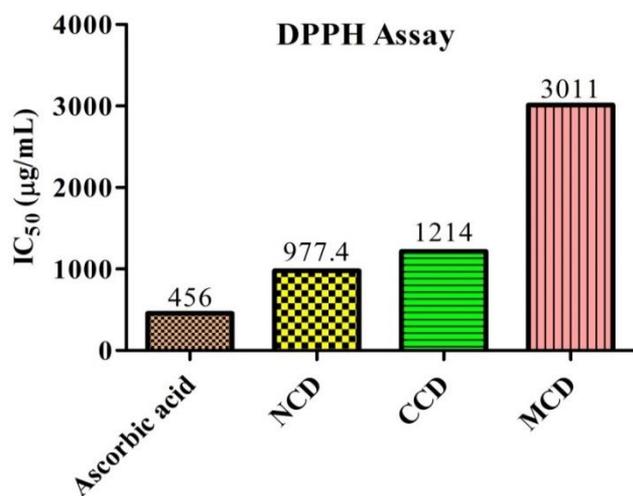


Fig. 2. Free radical scavenging abilities of extracts and standard by DPPH assay.

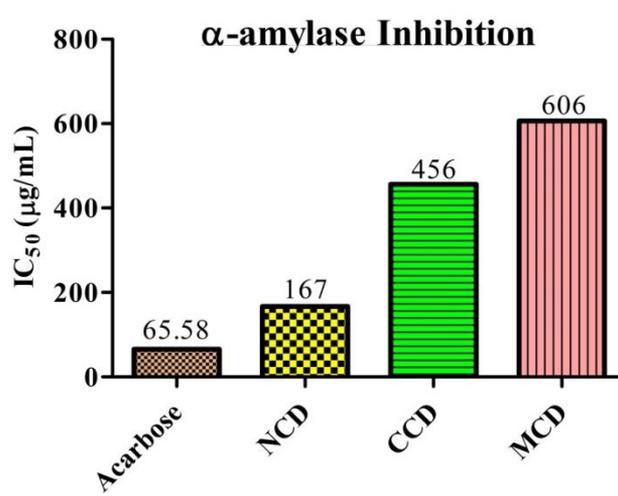


Fig. 4. Comparative IC₅₀ of extracts NCD (167±7.02), CCD (456±6.65), MCD (606±4.50) and standard drug 65.58±5.01.

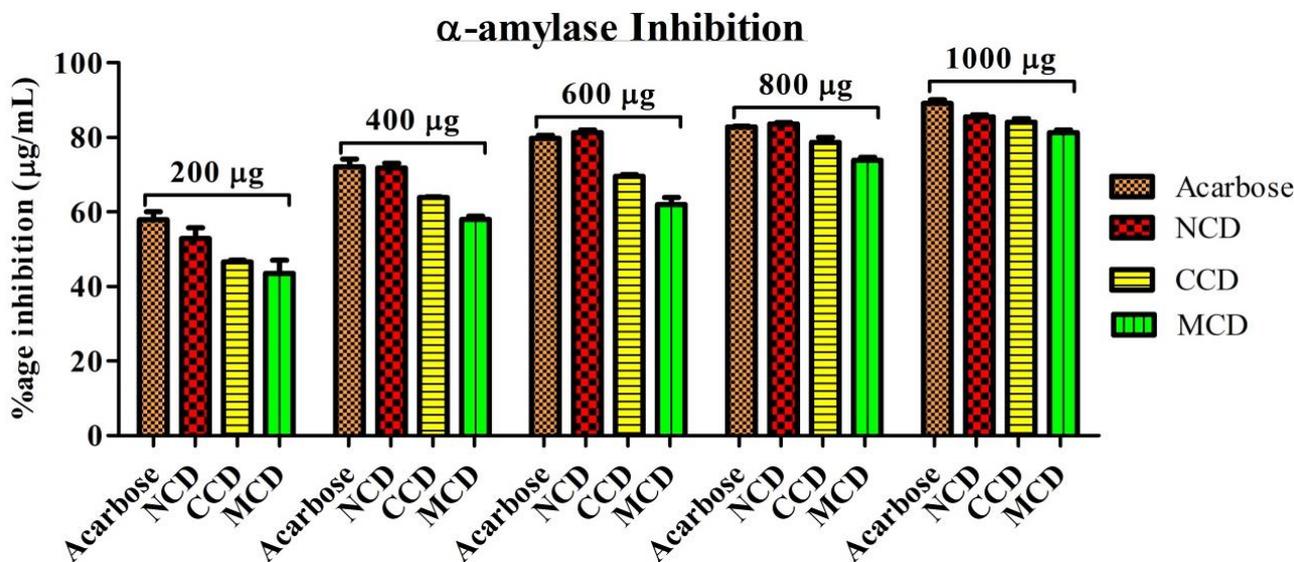


Fig. 3. Comparative α-amylase inhibition with extracts and standard drug.

The worldwide core disease is the diabetes mellitus, which affected around 347 mil adults in the form of neuropathy, retinopathy, nephropathy and angiopathy in un-controlled situations particularly (Danaei *et al.*, 2011). One of the imperative enzymes in the body is alpha-amylase which is associated to the breakdown of starch into individual components. Suppression of α -amylase, slow down the digestion of carbohydrates resulting reduced glucose absorption. Consequently, raised post-prandial sugar level dropped (Ali *et al.*, 2006). Carbohydrates metabolizing enzymes are formed from the reaction of alpha-glycosidase (intestine) and alpha-amylase (pancreatic) employed in the conversion of complex saccharides to absorbable monosaccharide° (Kwon *et al.*, 2006). The evaluation of α -amylase suppressive potential (*In-vitro*) of NCD, CCD and MCD was determined and significant response was perceived with 01mg/mL concentration of extracts (Fig. 3). Using non-linear regression analysis, we determined IC₅₀ of all experimental extracts and NCD exhibited 167 μ g/mL, indicated that suppression of alpha-amylase activity with NCD was more significant than MCD and CCD of *C. decidua* (Fig. 4). This anti-diabetic potential of our plant might be due the reality of high percentage of alkaloids in its fruit (Sharma *et al.*, 2010).

The order of activity (alpha amylase) of investigated extracts is given as; NCD (167 μ g/mL) > CCD (456 μ g/mL) > MCD (605 μ g/mL).

On comparison to the explored antidiabetic characteristics of *C. decidua* and our studies provided the superior outcomes than other work (Rathee *et al.*, 2010).

Conclusion

Our strong experimental data supported the recommendation of *Capparis decidua* as a dietetic supplement for the management of diabetes mellitus. The flavonoids and phenolic combinations in *C. decidua* are allied to the anti-diabetic and anti-oxidant deeds. Further exploration of Karir (*C. decidua*) regarding class characterization and structural elucidation is desperately required in future.

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