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Evaluation of antidiabetic and antioxidant potential of custard apple (*Annona squamosa*) Leaf extracts: A compositional study

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Abstract
Since time immemorial custard apple is closely associated with humans. In Indian subcontinents it is popularly known for their delicious fleshy fruit and lesser for its antioxidant and antidiabetic activities. Because of its higher nutritional value and unique nutraceutical properties, custard apple is widely cultivated throughout the world. In view of this the present investigation was undertaken to study the methanolic leaf extracts of global custard apple germplasms to identify the genotypes with its best combination as having significant amount of antioxidant and antidiabetic. The results showed that wide variability was present among custard apple genotypes for both α-amylase inhibition and DPPH radical scavenging activity. It also evidenced that the fruit width was negatively correlated with both antioxidant activity and antidiabetic activities. Further the study showed that smaller the fruit size evidenced higher the antidiabetic and antioxidant activities with elevated level of total phenolic acids. Finally, the study revealed that the methanolic extract of genotypes evidenced lowest IC_{50} value *viz*., Aml-11 (2.41 µg/mL) followed by Aml-12 (3.07 µg/mL) and Aml-4 (6.73 µg/mL) showing higher inhibition of PPA could be considered as candidate material for future ethnomedicinal research.

Keywords: Custard apple, antioxidant, antidiabetic, methanolic extract, DPPH inhibition

Introduction
Custard apple (*Annona squamosa* L.), is a commonly known fruit crop of India mainly in tropical regions. It belongs to family Annonaceae and is a native of West Indies (Porwal *et al.*, 2011) [30]. In Indian subcontinents, custard apple is popularly known with several vernacular names such as sharifa, sitaphal, sweet sop and sugar apple (Ghawade *et al.*, 2018) [10]. It is commercially cultivated throughout the tropical, arid, and semi-arid region of the world for its fleshy fruit. The custard apple fruit enriched with high nutritional value such as carbohydrate contents, vitamin C, glycosides, phenolics, flavonoids, calories, dietary fiber, high amount of essential mineral (phosphorous, potassium and calcium), etc. (Pandey and Dushyant, 2011) [28]. Besides these nutritional properties, custard apple has some unique ethnomedicinal properties. Leaves and bark of the Custard apple plant is reported to contain flavonoids, glycosides, tannins, phenolic compounds, etc. (Pandey and Dushyant, 2011) [28]. Likewise, phytochemical characterization of fruits, seeds and other parts of custard apple revealed the presence of alkaloids, flavonoids, and acetogenins which exhibit lipid peroxidation, antidiabetic, antioxidative activity against various ROS, hypoglycemic, hypocholesterolemic, diaphoretic, anti-ulcerative, parasiticidal, insecticidal, and anti-cancer activities (Gajalakshmi *et al*. 2012) [8]. Although the plant produce these phytochemical to enhance their survival competency but due to their pharmaceutical activities these plant-derived compounds utilised in many ethno-medicinal preparations over the globe since ancient times (Mshana, 2000) [26]. Hence, these properties of custard apple could be explored for future Nutri-agricultural research and can make an effective contribution to alleviate the global malnutrition and healthcare concerns in nutrient insecure environment. Thus, In view of the above background the present investigation was conceptualized to (1) evaluate antidiabetic and antioxidant potential of methanolic leaf extracts, (2) identify individual phenolics present in the methanolic leaf extracts of various custard apple genotypes by high performance liquid chromatography (HPLC) estimation, and (3) correlate phenolics, DPPH free radical scavenging activity, and α-amylase inhibition potential of custard apple genotypes, henceforth the major goal of this study is to identify custard apple genotypes with its best combination as having significant amount of antioxidant and antidiabetic values.

Materials and methods

Plant material collection and preparations

The present study was conducted at research farm Madri Bag and Department of Biochemistry, Junagadh Agriculture University, Junagadh, Gujarat, India. For this, the material comprises thirty diverse Custard apple accessions representing world collections were collected from Horticulture Research Station, Junagadh Agriculture University, Junagadh (Table: 01). The experiment was conducted in completely randomized design with thirty selected genotypes as the treatments with three replications. The newly emerging leaves were randomly collected from healthy trees, washed in running water extracted with methanol and stored at -20ºc for further use.

Preparation of solvent extracts from Leaf extract

Fresh leaves of *A. squamosa* were used for making methanolic extract as method described by Siahpoosh and Javedani (2012) [35]. For this 10 g of leaves were powdered in liquid nitrogen and was extracted using 20 ml of methanol: distilled water (8:2). The extract was then centrifuged at 5000 rpm for 15 min and the supernatants were collected. The solvents in each sample were allowed to evaporate at room temperature and extract were lyophilized at 40^0c under reduced pressure. Finally, the leaf extract were prepared in respective solvent *viz*., methanol for further phytochemical studies.

Determination of Antidiabetic activity by % α-amylase inhibition

The porcine pancreatic α-amylase (PPA) is a potent inhibitor, widely used for the inhibition of α-amylase from the sample extracts. The inhibition assay was performed using chromogenic di nitro salicylic acid (DNSA) method (Miller, 1959). In this method, the total assay mixture composed of 0.02M sodium phosphate buffer (pH-6.9), porcine pancreatic α-amylase (0.8 units) and sample extracts. 5mg per µl of starch solution (1% v/v) in the sodium phosphate buffer was added to each tube and kept for incubation for 15 minutes at 37ºc. Later on, the reaction was terminated using DNSA reagent (1.0 ml) by incubating it in water bath for 5 minutes at boiling temperature. Finally, the reaction mixture is kept at room temperature for cooling, diluted and the absorbance was measured using spectrophotometer (540 nm). The positive control having no sample added and the negative control with no enzyme *viz*, porcine pancreatic α-amylase added is prepared for the inhibition analysis at EC_{50}. The EC_{50} of each extracts were calculated using the given formula for those samples that inhibits 50% of the PPA activity (Karthic *et al*., 2008) [20].

$$\% \text{ PPA Inhibition} = \frac{\text{Absorbance of positive control} - \text{Absorbance of sample X 100}}{\text{Absorbance of positive control}}$$

Determination of total antioxidant by DPPH radical scavenging activity

The 1, 1-diphenyl-2-Picrylhydrazyl (DPPH) is a stable free radical and is widely used method to determine the radical scavenging activity of antioxidant compounds (McCune and Johns, 2002) [24]. The basic principle of DPPH method is the formation of DPPH-H (nonradical compound) after reduction of DPPH in the solvent (*viz*., methanol) in presence of a hydrogen donor (*viz*., antioxidant). In this method, the reaction mixture of total volume (3.0 ml) consists of DPPH (0.3 mM) in methanol (1.0 ml), 1.0 ml of the leaf extract and 1.0 ml of methanol is prepared. The sample preparation kept for incubation in dark for 10-15 minutes and then an aliquot of 0.1 ml sample extract were taken in 4 ml sized cuvette along with DPPH in methanol (3.9 ml). The absorbance was measured using spectrophotometer (517 nm) at time zero and every 10 second until it reached the steady state plateau. The dilutions of extracted samples and the control were made to analyse antiradical properties at EC_{50}. The EC_{50} of each extracts were calculated using the given formula for those samples that reduced more than 50% of DPPH activity at a given absorbance (Chanda and Dave, 2009) [5].

$$\% \text{ Inhibion DPPH radical} = \frac{\text{Absorbance control} - (\text{Sample with DPPH} - \text{Sample without DPPH})}{\text{Absorbance of control}} 100$$

Determination of Total phenolic contents (TPC)

The total phenolic content of *A. squamosa* leaves were estimated by Folin- Ciocateu reagent method (Singleton and Rossi, 1965) [36]. In this method, the reaction mixture was prepared by mixing sample extract (0.5 ml) with 0.5N Folin - Ciocalteu reagent (0.1 ml) and kept for incubation at room temperature for 15 minutes. Then 2.5 ml of sodium carbonate (7.5% w/v) was added and further incubated at room temperature for 30 minutes. Finally, the absorbance was measured using spectrophotometer at 765 nm. In this study, Tannic acid was used as positive controls. The Total phenolic content was articulated in terms of tannic acid equivalent to standard and was calculated using the given formula (Jayasinghe *et al*., 2003) [16].

$$\text{Total phenol (mg.g}^{-1}) = \frac{\text{Sample weight X Sample reading X Dilution factor}}{\text{Standard reading X Weight of sample (gram)}}$$

Individual phenol profile by HPLC analysis

Total seven individual phenolic compounds *viz*., Gallic Acid, chlorogenic, salicylic acid, ferullic Acid, caffeic Acid, cinnamic acid and quercetin, responsible for antioxidant and antidiabetic activities were quantified from methanolic leaf extracts of custard apple genotypes by HPLC analysis (Gupta *et al*., 2012) [12]. The quaternary gradient prep HPLC (SHIMANDZU HPLC 10A series) equipped with 2996 photodiode array detector, multi solvent delivery system and reverse-phase chromatographic analysis was carried out using Phenomenx c 18 Preparative column having flow rate of 0.750 ml/min and pumped the sample sized 5 lm at P max-3000 psi. Samples were filtered through Whatman filter membrane (pore size- 0.45 µm) before injection in the sample port. 20µl mixture of Phenolic extracts of the sample were separated using a mobile phase, which included solvents system i.e, solvent-A (2% acetic acid in HPLC grade water) and solvent-B (80% methanol of HPLC grade) using linear gradient programme. The seven standards of phenolics were mixed in one vials and run with single injection. Gallic Acid, chlorogenic, salicylic acid, ferullic Acid, caffeic Acid, cinnamic acid and quercetin were used as standards. Phenolic compounds present in the methanolic leaf extracts were identified by comparing peak areas or retention time of standards with sample co-injected into it under the same experimental conditions (Fig. 1).

Statistical analysis of custard apple genotypes

The data collected on individual characters/traits were tabulated and subjected to statistical analysis by using completely randomized design (CRD) with thirty selected genotypes as the treatments with three replications. OPStat Excel Version software package were used for statistical

analysis and performed using a one-way analysis of variance (ANOVA) of individual characters/traits (Sheoran *et al*. 1998) [33] and results were considered statistically significant when p>0.05. Excel software of Microsoft was used for graphical representation.

Results and Discussion

Phytochemical studies of the different methanolic leaf extracts of *Annona squamosa* revealed the presence of a diversity of phytochemicals including phenolic compound, flavonoids, glycosides, alkaloids, polyphenols, etc, which may be responsible for the antioxidant and antidiabetic activities. For these thirty custard apple genotypes were examined for various fruit morphology and quality traits particularly for fruit width and total carbohydrate (Table: 1). In the present investigation, significant variation in fruit width (5.57 to 7.13 cm) with mean value of 6.48 cm of thirty genotypes were recorded. Ghosh *et al*. (2001) [11] observed that the genotype, Balanagar exhibited the highest fruit diameter (9.0 cm). Similar result was also revealed by Kad *et al*. (2016) [18] and Ghawade *et al*. (2018) [10]. They reported fruit width ranged from 5.90 to 17.61 cm which indicates the presence of sufficient variability of fruit width in custard apple genotypes. Similarly, Total carbohydrates were measured from fruit pulp of custard apple genotypes and observed significantly higher carbohydrate concentrations (4.41 %) in genotypes K-2, Lok-1, Lok-2, Aml-4, Aml-7, Aml-9, Aml-10 followed by Aml-3 (4.39 %) and DS-1 (4.31%) where as lowest content were recorded in B-1 (2.06 %) with mean value of 3.93 % among thirty genotypes. Similar result was also reported by Kad *et al*. (2016) [18] and Ghawade *et al*. (2018) [10]. Henceforth, due to the presence of sufficient variability in fruit morphology and fruit quality traits, the methanolic fraction of thirty custard apple leaves were utilized for evaluation of total phenolic constituents, and identify the best genotypes having significant amount of antioxidant and antidiabetic activities, which can be utilized in near future as a candidate material for ethnomedicinal research.

Antidiabetic activity by α-amylase inhibition

The porcine pancreatic α-amylase (PPA) is a key enzyme in our digestive system which catalyses the degradation of dietary starch *viz*., maltose to glucose in the small intestine. Degradation of this dietary starch results in rapid increase in glucose levels, leads to elevated post prandial hyperglycemia (PPHG). It has been also observed that dysfunction of hypothalamic-pituitary-adrenal (HPA) axis in the small intestine correlates to an increase in glucose levels, which is an important aspect in the treatment of diabetes (Eichler *et al*., 1984) [7]. Hence, retardation of starch digestion by inhibition of α-amylase enzyme would play a key role in the treatment of diabetes. In order to this, Antidiabetic activity of methanolic leaf extract of custard apple genotypes were measured as α- amylase inhibition and exhibited significant variations (Table 01). The significant highest activity was recorded in K-2 (43.64 %) followed by Lok-1 (43.20%), K-1 (42.62%), and Aml-6 (41.89 %). Similarly, the lowest antidiabetic activity was recorded in Aml-11 (2.41%) followed by Aml-12 (3.07%) and Aml-4 (6.73%) with mean activity of 23.81% among thirty genotypes. Earlier it was reported that the methanolic extracts of young leaves of the *Annona squamosa* has antidiabetic properties (Shirwaikar *et al*., 2004) [34]. Hence, in the present investigation methanolic leaf extract of custard apple genotypes were examined for IC_{50} values which indicates concentration of leaf extracts exhibiting more than 50% inhibition on porcine pancreatic α-amylase (PPA) activity. A lower IC_{50} value indicates a higher α-amylase inhibitory activity. Among the 30 custard apple genotypes, the methanolic leaf extract evident lowest in Aml-11 (2.41 µg/mL) followed by Aml-12 (3.07 µg/mL) and Aml-4 (6.73 µg/mL) and would, therefore, be excellent candidates for antidiabetic activity (Fig. 01). Basha and Subramanian, (2011) [4] reported that leaves extract of *Annona squasoma* has a significant role in reduced blood glucose level in rat. Likewise, Ponnusamy *et al*. (2010) [29] studied *in vitro* pancreatic amylase activity in human cell lines and found that leaf extracts of medicinal plants inhibits the pancreatic α-amylase activity and results in reduced levels of PPHG via reducing the rate of starch breakdown leading to lowered blood glucose levels. Further it was evident that the inhibitory compounds like glycosides, proteins, alkaloids, flavonoids, saponins, tannins, and steroids has inhibitory action on HPA (Gajera *et al*., 2017) [9].

Antioxidant activity by DPPH free radical scavenging assay

The phenolic compounds like polyphenols, flavonoids, tannins, and terpenes presence in plants extract showed significant antioxidant effect due to their free radical scavenging activity (Rahman and Moon, 2007) [31]. Free radicals such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and superoxide anion radical are often generated by various biological oxidation reactions. These oxidative mediators can lead to the damage of important biomolecules such as proteins, nucleic acid, and lipids. The antioxidant activities of methanolic leaf extract of thirty custard apple genotypes were measured as DPPH free radical scavenging activity and exhibited significant variations (Table 01; Fig. 01). Significantly highest antioxidant activity as percent DPPH inhibition was measured in S-3 (31.10 %) followed by S-2 (25.25 %), KT-1 (25.25 %), and Lok-1 (25.00 %) and the lowest value was measured in Aml-6 (11.30%) followed by Aml-12 (14.38%) and Aml-7 (14.33%) with mean activity of 19.16% among thirty custard apple genotypes. Chandrashekar *et al*., (2011) [6] studied DPPH free radical scavenging activity of methanolic extract and reported significant antioxidant activity of 9.62, 24.28, and 45.62% in custard apple. Karadeniz *et al*., (2015) [19] studied different extracts of fruits of *Annona cherimola* and showed good radical scavenging activity by methanolic fruits extract (52.7%). Almost similar result was observed by Mariod *et al*., (2012) [22] using bark, leaves, roots and seedcake methanolic extract and reported significant antioxidant activities with IC_{50} values from 7.81 to 125.0 µg/ml. The results are encouraging and indicating the utilization of the leaf of custard apple as a significant source of natural antioxidants. Thus, in the present investigation antioxidant potency of custard apple is more evident from its capacity to scavenge DPPH free radicals. Different extract of *Annona squasoma* leaf extracts possess concentration dependent radical scavenging activity as observed in fruits extract of *A. cherimola* (Barreca *et al*., 2011) [3]. The DPPH free radical scavenging capacity was found to be proportional to total phenolic compounds present in the methanolic extract of leaf, as observed in fruit (Banerjee *et al*. 2005; Hasan *et al*. 2009; Gajera *et al*., 2017) [2, 13, 9].

Determination of total phenolics in methanolic leaf extract

Total phenolics from methanolic leaf extracts of custard apple genotypes were estimated by Folin- Ciocateu reagent method. Total phenol content was found to be significant in variations

in different genotypes of custard apple and their leaves (Table 01). Significantly highest amount of Total phenol content was measured in DS-1 (1478.4 $mg.g^{-1}$) followed by Sindhan (1377.6 $mg.g^{-1}$), M-1 (1366.4 $mg.g^{-1}$) and Aml-9 (1332.8 $mg.g^{-1}$) and the lowest value was measured in D-1 (212.8 $mg.g^{-1}$) followed by KT-1 (246.4 $mg.g^{-1}$) and Aml-6 (593.6 $mg.g^{-1}$) with mean value of 943.79 $mg.g^{-1}$ among thirty custard apple genotypes. Almost similar result was observed in previous studies indicating that methanolic extract of leaves contains maximum amount of Total phenolics than other parts of plants (Zhi *et al*. 2008; Atale *et al*. 2011; Gajera *et al*., 2017) [38, 1, 9]. In general plants contain phenolic compounds of various chemical natures, such flavonoids, astocopherols, lignins, lignans, carotenoids, tannins and phenolic acids (Matthäus, 2002). Thus, the total phenolics may play a promising role in the antioxidant activity. The fraction of phenolics like alkaloids, flavonoids, glycosides and anthocyanins were also measured from methanolic leaf extract of custard apple genotypes and they significantly varied with genotypes (Kumar *et al*., 2018) [21].

Individual phenol profile by HPLC analysis

Individual phenolics from methanolic leaf extract of custard apple genotypes were detected by HPLC analysis with identified corresponding retention time of standard phenolic compounds (Table: 03; Figure: 02). Total seven phenolic compounds *viz*., Gallic Acid, chlorogenic, salicylic acid, ferullic Acid, caffeic Acid, cinnamic acid and quercetin, responsible for antidiabetic and antioxidant activities were identified and quantified from methanolic leaf extract of custard apple genotypes (Table: 01). However, kampeferol was not identified in any of the methanolic extract of leaves. Among the leaf extracts, Gallic acid was only detected in two custard apple genotypes of which maximum were quantified in Aml-3 (0.89 $\mu g.g^{-1}$) genotypes followed by Aml-4 (0.45 $\mu g.g^{-1}$). A diet containing gallic acid as antioxidant may be beneficial to type II diabetic mellitus patients (Rizvi *et al*., 2005) [32]. The highest chlorogenic acid were quantified in Aml-5 (5.00 $\mu g.g^{-1}$) genotypes followed by K-2 (2.21$\mu g.g^{-1}$) and K-1 (1.84 $\mu g.g^{-1}$). The chlorogenic acid reduces apical glucose transporter in the intestine which results in inhibition of glucose-6-phosphate translocase 1 activity which ultimately hamper glucose absorption across intestine in animals (Van Dam, 2006) [37]. Salicylic acid was only detected in K-2 (0.64 $\mu g.g^{-1}$) whereas Ferulic acid was detected in 14 custard apple genotypes of which maximum 2.82 $\mu g.g^{-1}$ were quantified in B-1 genotype followed by Aml-9 (2.42 $\mu g.g^{-1}$) and S-3 (2.41 $\mu g.g^{-1}$). Similarly, Caffeic acid was found in seven genotypes exhibiting highest in Aml-11 (2.57 $\mu g.g^{-1}$) followed by B-3 (2.06 $\mu g.g^{-1}$) and H-1 (1.29 $\mu g.g^{-1}$). Finally, phenolic compounds like Cinnamic acid and Quercetin was also detected in some custard apple genotypes which exhibited significantly for antidiabetic and antioxidant activities. Much earlier, Jung *et al*. (2006) [17] studied the antioxidant activity of caffeic acid and concluded that the caffeic acid has a important role in lowering of blood glucose in mice. Similarly, Zhi *et al*. (2008) [38] studied the antioxidant activity of leaf extracts in black jamun and revealed that leaf extracts exhibits antioxidant activity due to major phenolic compounds *viz*., ferulic acid and catechin. Likewise because of inhibitory action on intestinal α-glucosidase to reduce blood glucose level, quercetin can be considered as a potential candidate for designing drug against type 2 diabetes (Hussain *et al.*, 2012) [15]. Phenolic by-product from leaf extracts may also exhibits cellular injuries and acute anti-inflammatory response induced by various oxidative damage or stresses (Hossain *et al*., 2016; Gajera *et al*., 2017) [14, 9].

Correlations between antidiabetic, antioxidant and phenolic compounds

Fruit size measured as fruit width was negatively correlated with both antioxidant activity and antidiabetic activities but statistically non significant for antidiabetic activity at $P<0.001$ level of significances (Table: 02). It indicate that smaller the fruit size evidenced higher the antidiabetic and antioxidant activities with elevated level of total phenolic acids as reported in black jamun (Gajera *et al*., 2017) [9]. The DPPH free radical scavenging activities of custard apple genotypes was negatively correlated with total carbohydrate which is proportional to the total phenolic acids and individual phenolic constituents present in the methanolic extract of leaves across thirty genotypes. Likewise α-amylase inhibition activities of custard apple genotypes was positively correlated with total carbohydrate which is proportional to the total phenolic acids and individual phenolic constituents present in the methanolic extract of leaves across thirty genotypes. In most cases, total phenolics are positively correlated with DPPH antiradical activities but negatively correlated with individual phenolics showing great deal of variation with different level of significances. Similarly, total phenolics are negatively correlated with α-amylase inhibition (PPA) activities but positively correlated with individual phenolics showing great deal of variation with different level of significances. Similar to present study, Muniappan *et al*. (2012) [27] and Gajera *et al*. (2017) [9] reported significantly correlation of fruit parts with total phenolics and individual phenolics of black jamun landraces for antidiabetic and antioxidant activity.

Table 1: Fruit quality, phenolics constituents, Antidiabetic and antioxidant activity of thirty custard apple genotypes

S. No.	Genotype	Fruit width (cm)	Total Carbohydrate (%)	Total Phenols ($mg.g^{-1}$)	Individual phenolics ($\mu g.g^{-1}$)							DPPH (µg/mL)	PPA (µg/mL)
					Gallic acid	Chlorogenic acid	Salicylic acid	Ferulic acid	Caffeic acid	Cinnamic acid	Quercetin		
1.	K-1	5.70	4.26	851.2	ND	1.84	ND	0.72	ND	ND	1.52	22.78	42.62
2.	K-2	5.93	4.41	649.6	ND	2.21	0.64	ND	ND	ND	ND	17.83	43.64
3.	N-1	6.27	4.40	907.2	ND	ND	ND	ND	ND	ND	ND	17.00	35.96
4.	Lok-1	5.77	4.41	918.4	ND	ND	ND	ND	ND	ND	ND	25.00	43.20
5.	Lok-2	6.23	4.41	806.4	ND	ND	ND	ND	ND	ND	ND	22.78	32.46
6.	H-1	6.43	3.89	672.0	ND	ND	ND	ND	1.29	0.05	ND	21.50	38.38
7.	Aml-1	6.27	3.64	884.8	ND	ND	ND	1.45	ND	0.02	ND	14.80	29.61
8.	Aml-2	6.33	4.23	996.8	ND	ND	ND	ND	ND	ND	ND	19.38	16.08
9.	Aml-3	6.37	4.39	1254.4	0.89	ND	ND	1.31	ND	ND	1.60	16.20	28.22
10.	Aml-4	6.73	4.41	884.8	0.45	ND	ND	1.56	ND	ND	0.79	16.40	6.73
11.	Aml-5	6.73	4.35	884.8	ND	5.00	ND	2.04	ND	0.05	ND	15.65	27.78
12.	Aml-6	6.67	4.30	593.6	ND	ND	ND	ND	0.99	ND	ND	11.30	41.89
13.	Aml-7	6.47	4.41	806.4	ND	ND	ND	ND	0.94	ND	ND	14.33	34.06

14.	Aml-8	7.13	3.36	1142.4	ND	ND	ND	ND	0.94	ND	ND	14.55	33.41
15.	Aml-9	7.07	4.41	1332.8	ND	ND	ND	2.42	ND	ND	ND	21.10	25.80
16.	Aml-10	6.67	4.41	739.2	ND	ND	ND	ND	ND	ND	ND	18.38	34.50
17.	Aml-11	6.80	3.53	1176.0	ND	ND	ND	ND	2.57	ND	0.19	19.38	2.41
18.	Aml-12	7.13	4.25	963.2	ND	ND	ND	ND	ND	ND	ND	14.38	3.07
19.	DS-1	6.73	4.31	1478.4	ND	ND	ND	1.68	ND	ND	ND	23.78	24.49
20.	B-1	6.90	2.06	795.2	ND	ND	ND	2.82	ND	ND	ND	14.80	11.11
21.	B-2	6.93	3.27	1008.0	ND	ND	ND	1.62	ND	ND	ND	19.38	21.64
22.	B-3	6.47	3.62	795.2	ND	ND	ND	ND	2.06	ND	ND	18.35	24.42
23.	D-1	7.13	3.91	212.8	ND	ND	ND	ND	ND	ND	ND	20.28	19.08
24.	KT-1	6.43	3.76	246.4	ND	ND	ND	0.85	ND	ND	ND	25.25	7.02
25.	S-1	6.37	3.46	974.4	ND	ND	ND	1.06	ND	ND	ND	18.78	21.71
26.	S-2	6.13	4.15	1232.0	ND	ND	ND	1.15	ND	ND	ND	25.25	14.18
27.	S-3	5.67	2.66	1187.2	ND	ND	ND	2.41	ND	ND	ND	31.10	10.31
28.	M-1	6.57	3.30	1366.4	ND	ND	ND	ND	ND	ND	ND	19.78	22.30
29.	M-2	6.80	4.28	1176.0	ND	ND	ND	0.84	ND	ND	ND	16.13	8.63
30.	Sindh an	5.57	3.51	1377.6	ND	ND	ND	ND	0.07	ND	ND	19.38	9.50
Grand Mean		6.48	3.93	943.79	-	-	-	-	-	-	-	19.16	23.81
S.E± m		0.3465	0.0961	19.399	-	-	-	-	-	-	-	0.477	2.131
C.D at 5%		NS	0.2718	54.8687	-	-	-	-	-	-	-	0.168	0.752
C.V. %		9.26	4.24	3.56	-	-	-	-	-	-	-	1.52	5.468

Note: Values representing mean of three independent replications and each replication consisted of five leaf extract per one tree, ND: Not detected

Table 2: Correlation between Agro-morpho-biochemical characteristics of thirty custard apple genotypes.

	FW	TC	TP	GA	Ch. A	SA	FA	Ca. A	Ci. A	Quercetin	DPPH	PPA
FW	1											
TC	-0.038^{NS}	1										
TP	-0.086^{NS}	-0.087^{NS}	1									
GA	0.006^{NS}	0.206^{NS}	0.159^{NS}	1								
Ch. A	-0.106^{NS}	0.218^{NS}	-0.124^{NS}	-0.076^{NS}	1							
SA	-0.234^{NS}	0.156^{NS}	-0.184^{NS}	-0.047^{NS}	0.351^{NS}	1						
FA	0.121^{NS}	-0.361^{NS}	0.267^{NS}	0.188^{NS}	0.182^{NS}	-0.152^{NS}	1					
Ca. A	0.172^{NS}	-0.127^{NS}	-0.055^{NS}	-0.115^{NS}	-0.136^{NS}	-0.084^{NS}	-0.373^{*}	1				
Ci. A	0.021^{NS}	0.047^{NS}	-0.154^{NS}	-0.082^{NS}	0.522^{**}	-0.060^{NS}	0.141^{NS}	0.100^{NS}	1			
Quercetin	-0.207^{NS}	0.221^{NS}	0.098^{NS}	0.741^{**}	0.127^{NS}	-0.062^{NS}	0.131^{NS}	-0.091^{NS}	-0.109^{NS}	1		
DPPH	-0.489^{**}	-0.121^{NS}	0.119^{NS}	-0.176^{NS}	-0.110^{NS}	-0.059^{NS}	0.173^{NS}	-0.182^{NS}	-0.114^{NS}	-0.027^{NS}	1	
PPA	-0.257^{NS}	0.396^{*}	-0.217^{NS}	-0.057^{NS}	0.260^{NS}	0.294^{NS}	-0.290^{NS}	0.003^{NS}	0.220^{NS}	0.118^{NS}	-0.119^{NS}	1

Note: FW- Fruit width, TC- Total carbohydrate, TP- Total Phenols, GA- Gallic acid, Ch.A- Chlorogenic acid, SA- Salicylic acid, FA- Ferulic acid, Ca. A- Caffeic acid, Ci. A- Cinnamic acid, DPPH- 1, 1-diphenyl-2-Picrylhydrazyl, and PPA- Porcine pancreatic α-amylase

Table 3: Standard of individual phenolics and their retention time observed through HPLC analysis

S. No.	Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1.	Gallic acid	3.242	4192984	7.61	163241
2.	Chlorogenic acid	6.625	9098180	16.51	42570
3.	Salicylic acid	22.060	10463014	18.99	70755
4.	Ferulic acid	39.775	6408514	11.63	132087
5.	Caffeic acid	40.224	8897095	16.15	127459
6.	Cinnamic acid	43.257	12975035	23.55	129623
7.	Quercetin	47.545	3064471	5.56	52125

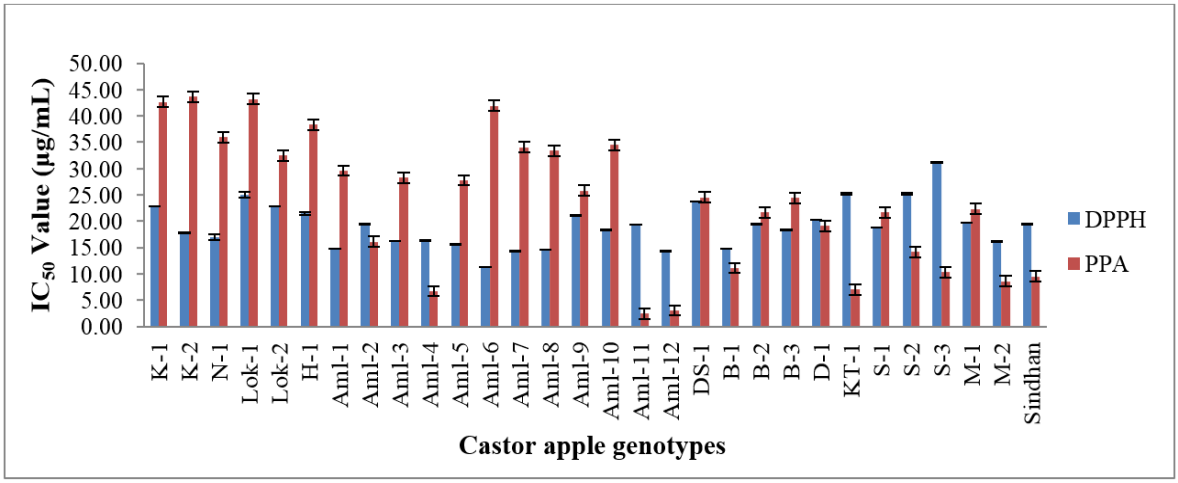

Fig 1: Antidiabetic and antioxidant activity of thirty custard apple genotypes

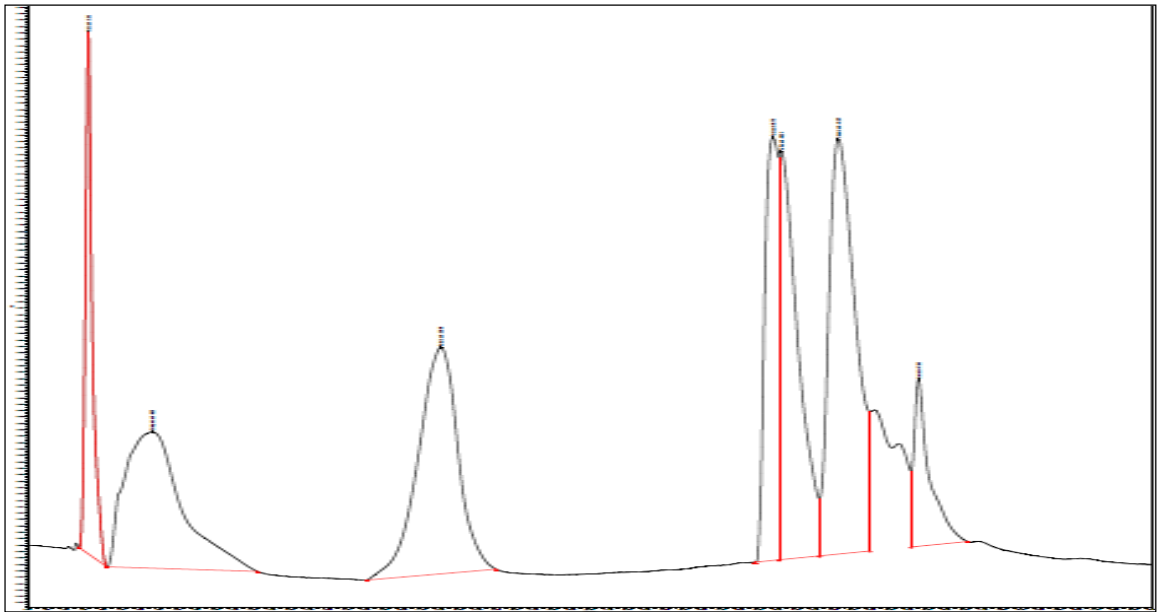

Fig 2: Chromatogram of seven phenol standards separated by HPLC analysis

Conclusion and future perspective

Thirty custard apple genotypes were investigated for various fruit morphology and quality traits particularly for fruit width, total carbohydrate, total phenolics and individual phenolic compounds to examine the antidiabetic and antioxidant potential of genotypes. The study evidenced that the fruit width was negatively correlated with both antioxidant activity and antidiabetic activities. It indicates that smaller the fruit size evidenced higher the antidiabetic and antioxidant activities with elevated level of total phenolic acids. Similarly, total phenolics are negatively correlated with antidiabetic activities but positively correlated with antioxidant and vice-versa for individual phenolics showing great deal of variation with different level of significances at $P<0.001$ across thirty genotypes. Henceforth, the methanolic extract of genotypes evidenced lowest IC_{50} value *viz*., Aml-11 (2.41 µg/mL) followed by Aml-12 (3.07 µg/mL) and Aml-4 (6.73 µg/mL) showing higher inhibition of PPA could be considered as candidate material for future ethnomedicinal research.

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Conflict of interest

This is to certify that there is no any conflict of interest. All co-authors are agreed for submission to the journal.

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