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# Isolation and Structural Elucidation of Pancreatic A-Amylase Polyphenolic Inhibitors from *Prunus Domestica* and *Phoenix Dactylifera* Seeds

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**ABSTRACT**

*In the present study, fruit seeds such as Mangifera indica (mango), Phoenix dactylifera (dates), Annona reticulata (custard apple), Citrullus lanatus (water melon), Artocarpus heterophyllus (jack fruit) and Prunus domestica (plum) were screened for the pancreatic α-amylase inhibitory activity. The HWEs of Prunus domestica and Phoenix dactylifera seeds possess highest (98.09 ± 1.3%) and lowest (51.20 ± 3.8%) inhibitory activities, respectively. RSM mediated studies of Phoenix dactylifera showed a significant improvement (99.24 ± 0.5%) in the amylase inhibitory activity at an optimal condition of 70°C, 150 rpm, 7.5 minutes and 1:15 material ratio. The HPLC/DAD/MS analysis of PTLC eluate of Prunus domestica has revealed the presence of polyphenols such as quercetin, flavonoid glycoside melonate, genistein, chalcone derivative, 3,8'-diprenyl,5,7,4'-trihydroxy flavonone and isosakurametin. Similar analysis recorded for Phoenix dactylifera has showed the presence of O- hexosyltricin and 3,4 - dicaffeoylquinic acid. The studies concluded the significant pancreatic α-amylase inhibitory effect of Prunus domestica and Phoenix dactylifera seeds.*

**Keywords:** *Phoenix dactylifera; Prunus domestica;* Response surface methodology; Quercetin; Genistein; 3,4 - dicaffeoylquinic acid

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## INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by relative or absolute deficiency of insulin or insulin resistance, marked by hyperglycemia and characterized by alterations in carbohydrate, lipid and protein metabolism that leads to long term complications like ketoacidosis, nephropathy, neuropathy, blindness, foot ulcer and cardiac arrest [1]. The International Diabetes Federation (IDF) has estimated that the total number of people in India with diabetes is 50.8 million in 2010 and this may rise to 87.0 million by 2030. Recently, herbal based medicines have attracted the human population as a potent source of hypoglycemic agents and several research reports have been documented that plants as a whole or their parts like leaves, rhizome, bulb, root bark, stem bark, seeds, husk, fruit and root possess significant antidiabetic effect [2-4]. In humans, pancreatic amylase is chiefly responsible for the hydrolysis of starch and malto-oligosaccharides to smaller oligosaccharides and maltose. Hence, control of pancreatic amylase activity is a classical regulatory mechanism for the maintenance of blood glucose and thereby, can act as an indicator for monitoring post prandial hyperglycemia. Recent therapeutic measure suggests the utilization of sulphonylureas, α-amylase inhibitors, α-glucosidic inhibitors, lipase inhibitors and insulin for the treatment of diabetes mellitus. Especially, inhibitors of amylase (saliva and pancreas) have been successfully used for treating diabetes and obesity [5]. Broadly, amylase inhibitors can be classified as proteinecous or non-proteinecous (polyphenols) and many plant based bioactive principles like allicin, caffeine, nimbin, catechins, cinnamaldehyde, eugenol, quercetin, ursolic acid, terpernoids, sitosterol, stigmasterol, myricetin etc., have been exploited as hypoglycemic agents [3]. *Prunus domestica* Linn. (English name: plum; Tamil name: Aluppukkarappalam) belongs to Rosaceae family is rich in vital nutrients like carbohydrates, amino acids, vitamins, minerals, dietary fibers and polyphenolic components [6]. Previous reports have suggested the presence of phenolics like rutin, chlorogenic acid, caffeic acid, coumaric acid, anthocyanins, proanthocyanidins [7,8], benzaldehyde, 2-furancarboxyaldehyde and ethyl cinnamate [9] in dry

plum fruits. It possess antioxidant [10], anticancer [11], antihyperlipidemic [12], antihypertensive [13] and antidiabetic [14] activities. Similarly, *Phoenix dactylifera*, commonly known as date palm (Tamil name:karchuram, perichchankay) is one of the ancient edible sweet fruit and a low cost nutritious food because of the presence of energy rich carbohydrate, dietary fibers and minerals [15,16]. It exhibits antidiabetic [17], antibacterial [18], antifungal [19], antiviral [20], antioxidant [21], inhibition of lipid peroxidation [22] and hepato and nephroprotective effect [15,23]. Several procyanidin oligomers and flavonol glycosides that includes quercetin, luteolin and apigenin conjugates, and phenolic acids like ferulic, sinapic and p-coumaric acids have been isolated from the fruits [16,24]. The leaves were reported to contain several phytochemicals like alkaloids, flavonoids, tannins, saponins, steroids and phenols. The ethanolic seed extracts of *Citrullus lanatus* has been recorded to control the blood glucose level and also to prevent the organs from organic damage [25]. *Mangifera indica* (English name: Mango; Tamil name: Maangaai) is rich in dietary fibres, minerals and vitamin B6, A and C. The seed of *Mangifera indica* was reported to exhibit a significant anti-diabetic activity in streptozotocin induced diabetic rats [26]. Similarly antidiabetic activity of the methanol and ethanol extracts of seeds of *Anona squamosa* [27] and *Artocarpus heterophyllus* [28] has been reported. So far, limited published document is available about the antidiabetic activity of fruits and other parts of *Prunusdomestica* and *Phoenix dactylifera*. Moreover, no literature details were available for the pancreatic α-amylase inhibitory property of these plant species. So, our laboratory has focused to identify and isolate the possible polyphenols (flavonoid and phenolic acids) that may be responsible for amylase inhibition from the seeds of *Prunus domestica* and *Phoenix dactylifera*.

## MATERIALS AND METHODS

### Chemicals

Porcine pancreatic amylase, 3,5-dinitro salicyclic acid (DNS), ethanol, ethyl acetate, aluminium chloride ($AlCl_3$), HPLC grade acetonitrile and ammonium hydroxide were obtained from S.D. Fine chem. Ltd., India.Starch, formic acid and silica gel $G_{60}$(TLC analysis)were obtained from Merck, Darmstadt, Germany.

### Collection of Fruits and Preparation of Hot Water Extract (HWE)

Six different fruits like Mango (*Mangifera indica)*, Jack fruit (*Atrocarpus heterophyllus*), Dates (*Phoenix dactylifera*), Water melon (*Citrullus lanatus*), Plum (*Prunus domestica*) and Custard apple (*Annona squamosa*) were purchased from the local market at Coimbatore, Tamil Nadu, India. The seeds were removed, air dried and finely powdered. About 0.5 g of the powdered material was weighed and extracted with 25 ml of distilled water by placing in a water bath at 90˚C for 5 minutes. The extractant was filtered using Whatman No.1 filter paper, the filtrate obtained was precipitated by adding 10% ammonium sulphate with slow constant stirring and the contents were centrifuged at 5000 rpm for 10 minutes. The resultant supernatant (non-protein part) was used for experimental analysis.

### Optimization of Polyphenol (Flavonoids and Phenolic Acid) Extraction and Investigation of Amylase Inhibitory Activity by Central Composite Design Based Response Surface Methodology

Central composite design (CCD), a second order design of response surface methodology (RSM) was adopted to optimize the extraction of polyphenol content and investigation of amylase inhibitory activity from *Phoenix dactylifera* seeds. Four different variables like time (5-10 minutes), temperature (60°C – 80°C), solid to liquid ratio (1:10-1:20) and agitation rate (100-200 rpm) were investigated using a full factorial CCD with 16 factorial cube points, 8 axial points and 7 system recommended centre points (Table 1). The response variable can be fitted by a second order model in order to correlate with other independent variables. The following is the equation of the second degree polynomial equation:

$$Y_i = \beta_o + \sum_{j=1}^{3} \beta_j x_j + \sum_{i<j} \beta_{ij} x_i x_j + \sum_{j=1}^{3} b_{jj} x_j^2$$

Where, $Y_i$ is the predicted response, $x_i x_j$ are input variables that influence the response variable $Y$. $\beta_o$ is the offset term; $\beta_i$ is the $i^{th}$ linear coefficient; $\beta ii$ the $i^{th}$ quadratic coefficient and $\beta ij$ is the $_{ij}th$ interaction coefficient. A single factor analysis of variance was adopted to investigate the effect of each factor on the extraction of flavonoid (Results expressed in terms as amylase inhibitory activity). MINITAB 15 trial version software was used to create and analyze CCD design.

### *In Vitro* α-amylase Inhibitory Assay

A modified method of Bernfeld proposed by [29] was adopted to investigate the *in vitro* α-amylase inhibitory activity. Pipetted out 0.1 ml of the sample in the "Blank" and "Test" tubes and 0.1 ml of amylase enzyme in the "Test" and "Control"tubes,respectively. Added 1ml of 50 mM phosphate buffer (pH 7.0) and 0.5 ml of 1%

starch into all the tubes, and incubated at 37°C for 10 minutes. Added 1 ml of 3,5- DNSA reagent into all the tubes, mixed well and incubated in a boiling water bath for 10 minutes. Cooled the tubes and read the absorbance at 540 nm against blank. The maltose liberated was determined against a constructed standard maltose curve and the inhibitory activity was calculated using the following formula.

(%) inhibition = [(Absorbance of control – Absorbance of test) / Absorbance of control] × 100.

**Table 1: Central composite design (CCD) based response surface methodology (RSM) with different variables**

| Runs | Sol: liq | Time | Temperature | Rpm |
|---|---|---|---|---|
| 1 | 01:15 | 7.5 | 70 | 150 |
| 2 | 01:20 | 10 | 60 | 200 |
| 3 | 01:10 | 10 | 80 | 100 |
| 4 | 01:20 | 5 | 60 | 200 |
| 5 | 01:05 | 7.5 | 70 | 150 |
| 6 | 01:10 | 5 | 80 | 200 |
| 7 | 01:20 | 5 | 60 | 100 |
| 8 | 01:20 | 5 | 80 | 200 |
| 9 | 01:20 | 10 | 60 | 100 |
| 10 | 01:10 | 10 | 80 | 200 |
| 11 | 01:15 | 7.5 | 70 | 150 |
| 12 | 01:15 | 7.5 | 70 | 150 |
| 13 | 01:10 | 5 | 60 | 100 |
| 14 | 01:20 | 10 | 80 | 200 |
| 15 | 01:25 | 7.5 | 70 | 150 |
| 16 | 01:15 | 12.5 | 70 | 150 |
| 17 | 01:15 | 2.5 | 70 | 150 |
| 18 | 01:15 | 7.5 | 70 | 150 |
| 19 | 01:10 | 10 | 60 | 200 |
| 20 | 01:15 | 7.5 | 70 | 150 |
| 21 | 01:15 | 7.5 | 50 | 150 |
| 22 | 01:10 | 5 | 80 | 100 |
| 23 | 01:10 | 5 | 60 | 200 |
| 24 | 01:20 | 5 | 80 | 100 |
| 25 | 01:10 | 10 | 60 | 100 |
| 26 | 01:10 | 10 | 80 | 100 |
| 27 | 01:05 | 10 | 60 | 200 |
| 28 | 01:15 | 7.5 | 90 | 150 |
| 29 | 01:20 | 10 | 80 | 100 |
| 30 | 01:15 | 7.5 | 70 | 150 |
| 31 | 01:15 | 7.5 | 70 | 250 |

**Identification and Isolation of Flavonoid by Thin Layer Chromatography (TLC) and Preparative Thin Layer Chromatography (PTLC)**

The glass plates (20 × 10 cm) were coated with silica gel (0.1 – 0.2 mm thickness) and dried at room temperature for few minutes. The dried plates were then activated at 100°C for about 30 minutes in an oven. About 25 µl of the optimal aqueous extract (*Prunus domestica* and *Phoenix dactylifera*) was spotted 1.5 cm far from the edge of the plate along with standard markers (rutin and quercetin) and chromatogram was developed one dimensionally in an air tight chamber in the presence of mobile phase solvent mixture consists of ethyl acetate - ethanol - water (5:1:5, v/ v/ v). The developed plates were air dried, sprayed with liquid ammonia and visualized under far UV light at 365 nm [30]. The PTLC technique for the isolation of flavonoid was adopted according to the method proposed by Meena and Patni [31].

**HPLC-PDA-MS (ESI+) Analysis**

The liquid chromatography electron spray mass spectrometry (LC-MS) analysis was performed on Varian Inc, (USA) 410 Prostar Binary LC with 500 MS IT PDA Detectors. The column was $C_{18}$, 250 × 4.6 mm, i.d. 5 µm. The mobile phase A was made up of acetonitrile; while B was made of 0.1% formic acid (pH 4.0, adjusted with ammonium hydroxide). The gradient elution was performed at 1 ml/min with an initial condition of 12% of mobile phase A and 88% of mobile phase B for 10 min. The mobile phase A was linearly increased from 15% to 100% and analysis was performed from 20 minutesto 95 minutes. The eluates were monitored by PDA (Multi wavelength) detector at 260 nm. About 20 µl of the PTLC *Prunus domestica* and *Phoenix dactylifera* seed eluates were introduced into the ESI source separately and the mass spectra were scanned in the range 100-1000 amu. The maximum ion injection time was set to 200 nS, ion spray voltage at 5.3 KV and capillary voltage at 34 V. The MS scan ran upto 25 minutes [32].

**Statistical Analysis**

The results expressed as Mean ± S.D were calculated by MS Excel Program (version 2007). One way analysis of variance (ANOVA) post hoc multiple comparison (Turkey) tests for the comparison studies at 5% level of significance, response surface and contour plots were generated and analyzed by MINITAB 15 (trial version).

## RESULTS

***In vitro* Pancreatic α-amylase Inhibitory Activity of HWEs of Different Fruit Seeds**

The hot water extract obtained from various plant seeds were screened for the presence of pancreatic amylase inhibitory activity. The results were depicted in the Table 2. The results of the assay implied the significant (98.09 ± 1.3%) and poor (51.20 ± 3.8%) inhibitory activities by *Prunus domestica* and *Phoenix dactylifera*, respectively. The order of the amylase inhibitory activity recorded by the plant species were as follows: *Prunus domestica* (98.09 ± 1.3%) > *Artocarpus heterophyllus* (96.82 ± 1.6%) > *Annona squamosa* (95.55 ± 2.2%) > *Citrullus lantus* (94.92 ± 2.3%) > *Mangifera indica* (88.59 ± 1.5%) > *Phoenix dactylifera* (51.20 ± 3.8%).

**Table 2: Pancreatic α-amylase inhibitory activity (%) of various hot water extracts**

| HWEs of fruit seed | Inhibition (%) |
|---|---|
| *Mangifera indica* | 88.59 ± 1.5 |
| *Annona squamosa* | 95.55 ± 2.2 |
| *Prunus domestica* | 98.09 ± 1.3 |
| *Artocarpus heterophyllus* | 96.82 ± 1.6 |
| *Phoenix dactylifera* | 51.20 ± 3.8 |
| *Citrullus lantus* | 94.92 ± 2.3 |

**Optimization of Amylase Inhibitory Activity of *Phoenix dactylifera* Seeds by Response Surface Methodology**

The optimization studies of amylase inhibitory activity of *Phoenix dactylifera* was designed according to CCD based RSM. Experimental runs were performed by adopting different variables (temperature, time, agitation rate and solid: liquid ratio) with different levels in order to select the relevant/ significant factors that affect the amylase inhibitory activity. The optimal condition for the inhibitory activity was found to be 70˚C (temperature), 7.5 minutes (time), 1:15 (solid: liquid ratio) and 150 rpm (Agitation rate), and maximum amylase inhibitory activity was found to be 99.24 ± 0.5%. The detailed results were depicted in Table 3.

**Table 3: Optimisation of pancreatic α-amylase inhibitory activity of *Phoenix dactylifera* seed using CCD based RSM**

| Experimental run | Agitation rate (rpm) | Solid:liquid ratio | Time (mins) | Temperature(˚C) | Amylase Inhibition (%) |
|---|---|---|---|---|---|
| 1 | 150 | 15 | 7.5 | 70 | 94.49 ± 1.5 |
| 2 | 200 | 20 | 10 | 60 | 93.92 ± 3.8 |
| 4 | 200 | 20 | 5 | 60 | 88.21 ± 0.7 |
| 5 | 150 | 5 | 7.5 | 70 | 94.30 ± 2.1 |
| 6 | 200 | 10 | 5 | 80 | 86.31 ± 1.8 |
| 7 | 100 | 20 | 5 | 60 | 96.77 |
| 8 | 200 | 20 | 5 | 80 | 93.92 ± 3.8 |
| 9 | 100 | 20 | 10 | 60 | 96.39 |
| 10 | 200 | 10 | 10 | 80 | 86.31 ± 2.1 |
| 11 | 150 | 15 | 7.5 | 70 | 93.16 ± 1.5 |
| 12 | 150 | 15 | 7.5 | 70 | 95.63 ± 0.8 |
| 13 | 100 | 10 | 5 | 60 | 97.91 ± 1.2 |
| 14 | 200 | 20 | 10 | 80 | 93.54 ± 0.8 |
| 15 | 150 | 25 | 7.5 | 70 | 91.44 ± 2.5 |
| 16 | 150 | 15 | 12.5 | 70 | 89.92 ± 2.2 |
| 17 | 150 | 15 | 2.5 | 70 | 88.40 ± 1.2 |
| **18** | 150 | 15 | 7.5 | 70 | 99.24 ± 0.5 |
| 19 | 200 | 10 | 10 | 60 | 86.31 ± 1.9 |
| **20** | 150 | 15 | 7.5 | 70 | 99.24 ± 0.5 |
| 21 | 150 | 15 | 7.5 | 50 | 79.66 ± 1.9 |
| 22 | 100 | 10 | 5 | 80 | 97.53 ± 0.5 |
| 23 | 200 | 10 | 5 | 60 | 93.54 ± 0.5 |
| 24 | 100 | 20 | 5 | 80 | 98.48 ± 0.4 |
| 25 | 100 | 10 | 10 | 60 | 97.53 ± 0.5 |
| 26 | 100 | 10 | 10 | 80 | 95.63 ± 0.5 |
| 28 | 150 | 15 | 7.5 | 90 | 85.74 ± 1.1 |
| 29 | 100 | 20 | 10 | 80 | 96.38 ± 1.1 |
| 30 | 150 | 15 | 7.5 | 70 | 85.17 ± 3.2 |
| 31 | 250 | 15 | 7.5 | 70 | 91.16 ± 1.2 |

The predicted multiple regression equation was as follows: Amylase inhibition (%) = 95.0+0.0403 Temperature˚C - 0.060 Time (mins) + 0.090 Solid: liquid ratio (g/ml)- 0.0421 Agitation rate (rpm) – 1.91 Temperature˚C × Temperature˚C – 0.354 Time (mins) × Time (mins) + 0.573 Solid: liquid ratio (g/ml) × Solid: liquid ratio (g/ml) - 0.34 Agitation rate (rpm) × Agitation rate (rpm) - 0.131 Temperature˚C × Time (mins) + 1.034 Temperature˚C × Solid: liquid ratio (g/ml) - 0.083 Temperature˚C × Agitation rate (rpm) + 0.773 Time (mins) × Solid: liquid ratio (g/ml) + 0.179 Time (mins) × Agitation rate (rpm)+ 1.106 Solid: liquid ratio (g/ml) × Agitation rate (rpm). Where, 95.0, 0.0403, 0.060, 0.090 and 0.0421 were the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and temperature, time, agitation rate and solid: liquid ratio were the independent variables. Out of the selected variables, agitation rate was found to be the significant factor (p=0.044) that greatly affects the amylase inhibition, and temperature and solid: liquid ratio was found to be directly proportional, while, time and agitation rate was inversely proportional to the amylase inhibitory property. Moreover, the predicted second order polynomial equation implies that the model is not significant relative to the noise and there was 10.20% (lack of fit) chance that the model can generate error/ noise in the selected experimental runs. The results of surface and contour plots, and interaction plots were depicted in Figures 1and 2.

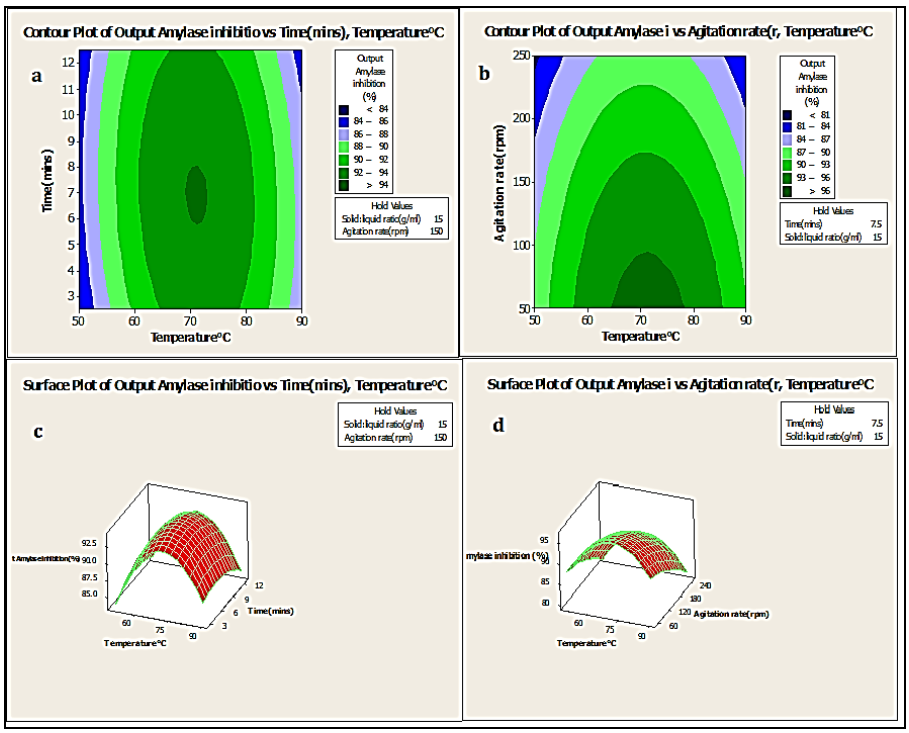


**Figure 1: The contour and surface plots of *Phoenix dactylifera*: (a) time vs. temperature; (b) agitation rate vs. temperature; (c time vs. temperature; (d) agitation rate vs. temperature**

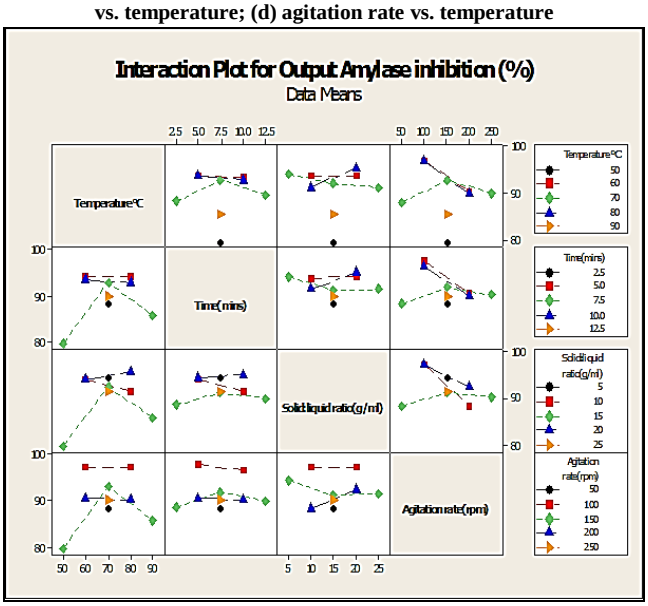


**Figure 2: The interaction plot between the variables in the effect of amylase inhibitory property of *Phoenix dactylifer***

**Identification and Isolation of Flavonoid by TLC and PTLC Techniques from *Prunus domestica* and *Phoenix dactylifera* Seeds**

TLC is a simple technique used for the identification various polyphenols. In the current study, the $R_f$ values of flavonoid standards such as quercetin and rutin were found to be 0.856 and 0.871, respectively. The $R_f$ values of *Prunus domestica* and *Phoenix dactylifera* seed extracts were recorded as 0.678 and 0.878, respectively, which, proved the presence of flavonols and their glycosides. The UV detection of the thin layer chromatogram of both the extracts has proved the presence of phenolic acids (Figure 3). The PTLC analysis has revealed the presence of single strong spot with bluish yellow color in both the extracts that has proved the high distribution of flavonoid and phenolic acids, and was eluated for further analysis.

**HPLC-PDA-MS (ESI+) Analysis of *Prunus domestica* and *Phoenix dactylifera* Seeds**

The HPLC/DAD/MS analysis of PTLC eluate of *Prunus domestica* has recorded the presence of compounds such as quercetin, genistein, chalcone derivative, flavonoid glycoside melonate, 3,8'-diprenyl-5,7,4'-trihydroxy flavonone and isosakurametin. Likewise, *Phoenix dactylifera* has revealed the presence of O- hexosyltricin and 3,4 - dicaffeoylquinic acid. The MS/ MS spectrum of *Prunus domestica* has recorded two fragment peaks at *m/z* 134.1 and *m/z* 155, and base peaks at *m/z* 241.2 and *m/z* 201.2 that has proved the presence of quercetin (Figure 4).

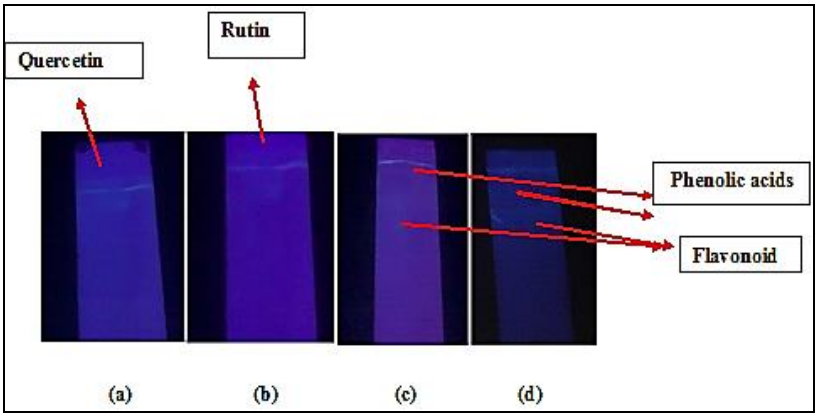


**Figure 3: Thin layer chromatogram of: (a) quercetin; (b) rutin; (c) flavonoid and phenolic acid of *Prunus domestica* and (d) *Phoenix dactylifera* under far UV light**

A base peak recorded at *m/z* 225.4 has proved the presence of isosakurametin and recorded peak at *m/z* 409.5 was found to be 8, 3'-diprenyl 5, 7, 4' trihydroxyflavonone. Similarly, the predicted MS/ MS spectrum at *m/z* 491/ 473/ 329 in *Phoenix dactylifera* has showed the presence of O- hexosyltricin and spectrum recorded at *m/z* 513/ 353 has revealed the abundance of 3,4 - dicaffeoylquinic acid.

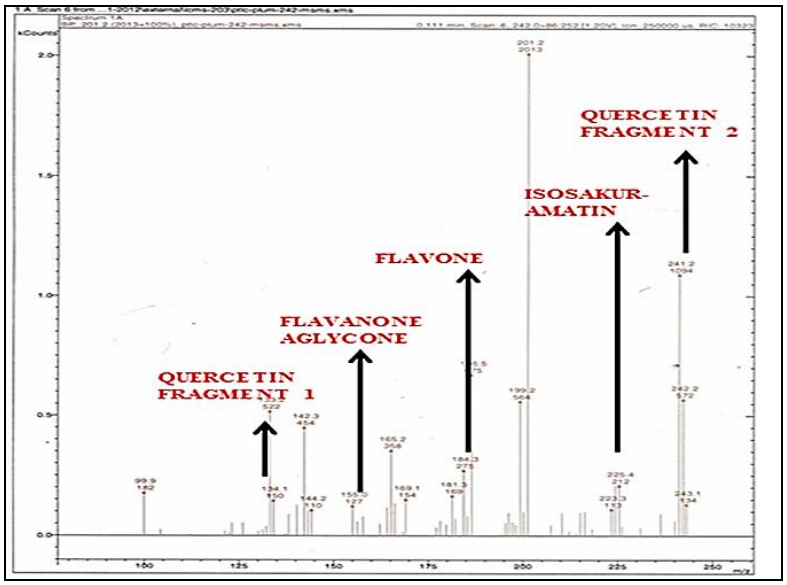


**Figure 4: MS/ MS fragmentation pattern of quercetin and isosakuramatin from the *Prunus domestica* seeds**

## DISCUSSION

Successful extraction of biologically active principles from plant samples is mainly based upon type of solvent used in the extraction procedure. Properties of a good solvent includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate [33]. During hot water extraction normally the temperature/ thermal energy of the water is increased which, leads to a steady state decrease in the permittivity, viscosity and surface tension, and an increase in the diffusivity. This results in the interaction of solute particles with the water molecules, and also creates a hypotonic condition (cells swell up), increased cell membrane fluidity and finally, cell rupture that directs the functional phytoconstituents to be released in the solvent [34]. Our current investigation has proved that simple hot water extraction, considered to be the safest green technology was the best method in the leaching the phytochemicals that includes polyphenols and thereby, an effective amylase inhibition. The process design of an experiment for the identification of an optimal response play a pivotal role in many large scale industries due to the ease and cost effectiveness of the technology. In general, efficiency of the extraction of a compound is influenced by the multiple parameters with various levels [35]. The current study has strongly proved the effectiveness of the design which has recorded an apparent 48% increase in the amylase inhibition by the *Phoenix dactylifera* seed extract. PTLC has been considered as the most basic equipment for the best separation and purification of polyphenols like flavonoids and phenolic acids. It is also used for monitoring the reactions in a large scale manner and is a method that requires the least financial outlay. PTLC in conjunction with open column chromatography has been widely used for the purification of natural products, although centrifugal TLC have found application in the separation of flavonoids [36]. In the current study, PTLC has proved as a simple and cost effective technique for the purification of polyphenols. According to Kayano *et al.,* [37] the MS/ MS spectrum of standard quercetin revealed two distinct peaks at *m/z* 137 and 153 and base peaks with *m/z* 241 and 201. A similar MS/ MS spectrum has been recorded in the present studies at *m/z* 134.1 and 155 and base peaks at *m/z* 241.2 and 201.2 has proved the presence of quercetin in the *Prunus domestica* sample. Similarly, an exhaustive investigation on the fragmentation of flavonol, flavone and flavonone aglycones has been performed by Fabre et al., [38]. According to their studies, a consecutive losses of CO and $CO_2$ has been observed in quercetin fragmentation that yielded a resonance-stabilized ion. Another different fragmentation mode was also observed i.e., loss of B ring (*m/z* 121) with subsequent retrocyclisation yielded a $^{1,2}A^-$ ion (*m/z* 179) and subsequent loss of –CO (*m/z* 151) and $CO_2$ (*m/z*107). The present investigation was well agreed with the above mentioned results with a loss of B ring (*m/z* 125) and subsequent retrocyclisation with the formation of $^{1,2}A^-$ ion (*m/z* 181.3). The loss of –CO has yielded (*m/z* 155) which proved the presence of quercetin. Previous studies on the fragmentation MS/ MS spectra of isosakurametin (flavonone) has revealed the base peaks at *m/z* 226 with a loss of $CO_2$ and $CH_3$ group. A similar documentation was also made in the present investigation i.e., a base peak at *m/z* 225.4 has been recorded which proved the presence of isosakurametin. The MS spectrum recorded at *m/z* 409.5 was found to be 8, 3'-diprenyl 5, 7, 4' trihydroxyflavonone and this has been previously documented as *m/z* 409 from the root of *F. Strobilifera* [39].

## CONCLUSION

From the studies, it was concluded that HWE of *Prunus domestica* possess significant pancreatic α-amylase inhibitory activity. The CCD based RSM has proved to significantly increase the amylase inhibition in the *Phoenix dactylifera.* The various polyphenols such as Quercetin, Genistein, Chalcone derivative, 3,8'-diprenyl,5,7,4'-trihydroxy flavonone, isosakurametin, O- hexosyltricin and 3,4 - dicaffeoylquinic acid from *Prunus domestica* and *Phoenix dactylifera* has identified as the causative molecules for the inhibition of amylase. Finally, it was concluded that the selected seeds of *Prunus domestica* and *Phoenix dactylifera* possess high pancreatic amylase inhibitory activity, and can be adopted by the healthcare industries for suitable herbal formulation to control the diabetes mellitus.

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