Original Article

Estimation of Ascorbic Acid and Amylase & Lipase Inhibitory Effects in Fruit Extracts of Certain Courtyard Plants

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ABSTRACT

Objective: Fruits of are little sour in taste. Hereby making an attempt to quantify ascorbic acid content in them. Also aiming to screen Amylase and Lipase inhibitory effects of aqueous alcoholic extract of the same.

Methods: Aqueous alcoholic extract prepared and tested for various active constituents. Amount of ascorbic acid determined by spectrophotometric method. Lipase inhibitory effect determined by titrimetrically. Amylase inhibitory effect done based on the reduction reaction of amylase using calibration curve method.

Results: All the tested extracts were a rich source of alkaloids and glycosides. *Ribes rubrum* is also rich in flavonoid, tannin and terpenes. *P. edulis* and *R. rubrum* have very good lipase inhibitory effect and also have very good vitamin c content. All three found to have very good alpha amylase inhibitory activity.

Conclusion: Based on these results it could be concluded that these fruits are of great value for nutrition and treatment of diet related disease such as obesity and diabetics and consequently a new field of interest in the food industry regarding new bioactive ingredients would be considered.

Keywords: Phytochemical screening, Amylase inhibitory activity, Lipase inhibitory activity.

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INTRODUCTION

Passiflora edulis, Coccinia indica and *Ribes* rubrum are abundantly present in Kerala. They found to have sour taste, therefore thought to estimate ascorbic acid content. Since all are of edible we were eager to know how they influence amylase and lipase enzymes.

Now a days it is noticed that the increasing trend in obesity is accompanied by a growing incidence of diabetes. The inhibition of pancreatic lipase in the case of obesity, and of α-amylase in the case of diabetes, is the current therapeutic approach for the treatment of both diseases, since

these enzymes play an essential role in lipid and glucose metabolisms.

Vitamins are essential organic compounds which are required for growth and maintenance of normal life. Each and every vitamins are considered as regulatory substances and perform specific functions. In general the body cannot synthesis them at least in large amount to meet its need. Vitamin C or ascorbic acid is a water soluble vitamin which found in all living tissue of plants and animals. Many of the plants and animals can synthesize vitamin C from glucose but man, primates, guinea pigs, Indian fruit eating bats and red vented bulbuls(birds native to Indian) cannot make ascorbic acid due to lack of enzyme L-gluconolactone oxidase needed for the synthesis[2]. Davidson *et al* stated that ”Vitamin C is enriched in fresh fruits. Citrus fruit, black currants and guavas are particularly rich sources of vitamin C while green leafy vegetables are also good sources”[3]. The aim of this work is to estimate the quantity of vitamin C in some fruits named *Passiflora edulis, Coccinia gandis* and *Ribes rubrum* and also found out the inhibitory effect of α-amylase in the case of diabetes and pancreatic lipase in the case of obesity of this courtyard fruits.

MATERIALS AND METHODS

Extraction from dried fruits of *Passiflora edulis, Coccinia indica* and *Ribes rubrum*

The shade dried fruits of *Passiflora edulis, Coccinia indica* and *Ribes* rubrum used in the present study. Extraction from fruits (5g) were done with ethanol 50% in a microwave synthesizer for 30 minutes. The samples were concentrated and this extracts are used for further analysis.

Phytochemical screening

The prepared hydroalcoholic extract was subjected to preliminary Phytochemical analysis in order to detect the presence or absence of various Phyto constituents present in the extract by carrying out chemical test for Alkaloids, Flavonoids, Glycosides, Tannin, Saponin using various reagents. The phytochemicals may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents. The preliminary phytochemical screening reveal the presence of chemical constituents present in plants.

Determination of Vitamin c content by Titrimetric method[11,12]

The vitamin C content of the fruits of *Passiflora edulis, Coccinia grandis and Ribes. rubrum* were determined by Barakat (1993), and A.O.A.C (1980) method.

5 gm of the sample were extracted with100 ml of EDTA/TCA (2:1), the extracting solution were mixed by stirring for half an hour. The resultant solution centrifuged at 3000 rpm for 20 minutes. Then transferred to a100 ml volumetric flask and volume made up to 100 ml with extract. 20 ml of the solution was withdrawn from this and add 1% indicator and titrate it against 20% $CuSO_4$ solution.

Estimation of vitamin c or ascorbic acid (Spectrometric method)[9]

The estimation of ascorbic acid in the sample was done using the method of Roe and Keuther. 1g of the sample taken and homogenized in 4% TCA and made up to 10 ml. The supernatant liquid obtained were treated with a pinch of activated charcoal. Shaken well and kept for 10 minutes. Centrifuged once again to remove the charcoal residue. Noted the volume of the clear supernatants obtained and this supernatant were used for assay. The assay volumes were made up 2.0ml with 4% TCA. The working standard solution 0.2 to 1.0 ml containing 20-100μg of ascorbate respectively were taken in clean dry test

tube, the volume of which were also made up to 2.0ml with 4% TCA. 0.5ml of DNPH reagent is added to all the test tubes, followed by 2 drops of 10% thiourea solution. Incubated at 37ºC for 3 hours. The osazones formed were dissolved in 2.5 ml of 85% sulphuric acid, in cold, drop by drop, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of H_2SO_4. The tubes were incubated for 30 minutes at room temperature, and the absorbance was read spectrophotometrically at 540nm.

Determination of lipase activity[1,4-8]

The activity of lipase was determined by incubating an emulsion containing 8 ml of olive oil, 0.4 ml of phosphate buffer and 1ml of chicken pancreatic lipase for an hour in rotary shaker, followed by stopping the reaction by addition of 1.5 ml of a mixture solution containing acetone and 95%ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as an indicator.

Lipase inhibitory activity

Lipase inhibitory activity of the extracts were tested by mixing 1ml of each extract, 8 ml of oil emulsion and 1 ml of lipase enzyme followed by incubation of 60 minutes. The reaction was stopped by adding 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1).The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as an indicator.

Percentage inhibition of lipase activity was calculated using the formula:

Lipase inhibition = A-B/A 100, Where A is lipase activity, B is activity of lipase when incubated with the extract.

Amylase Inhibitory activity[10]

Alpha amylase hydrolyses alpha 1, 4-linkages of starch molecules in a random manner. The reducing sugars (mainly maltose) produced by the action of alpha-amylase react with dinitrosalicylic acid and reduce it to a brown/orange red coloured product, nitro aminosalicylic acid. The starch hydrolyzed product concentration under a specified level of alpha-amylase enzyme, with and without inhibitor is used to express the alpha amylase inhibitory activity. The percentage inhibition was calculated by the equation given below.

$$\%\ \alpha\text{-amylase Inhibitory activity} = 100 - \left[\frac{[\text{Maltose}]_{sample} \times 100}{[\text{Maltose}]_{control}}\right]$$

Method

Preparation of maltose calibration curve

Pipette aliquots of 0.1 to 1.0 ml of maltose (100-1000μg) solution into test tubes and make up the volume to 1ml with suitable addition of distilled water. To each tube add 2ml of dinitrosalicylic acid reagent. Cover tubes with marbles. Keep the tubes in water bath for 10 minutes. Cool the tubes and add 10 ml of distilled water to each test tube. The orange red colour formed is measured at 540nm against a reagent blank.

Determination of α-amylase inhibitory activity

Pre incubate the entire reagents for 15 minutes at 37^0 c in a water bath. Pipette 0.5 ml of 1% starch solution adds it to 0.25 ml of phosphate buffer (0.2m, p h 7) and 0.25 ml of α amylase enzyme solution. Similarly a second set of test tubes (blank) by using phosphate buffer in place of enzyme solution. prepare a third set of test tubes containing 0.5 ml of starch solution,2ml of dinitrosalicylic acid reagent.0.25 ml of α-amylase enzyme solution; this set is called the zero time control. Incubate all the tubes at 37^0c for three minutes. At the end of the incubation add 2 ml of dinitrosalicylic acid reagent to first and

second set of tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes. After cooling under cold water, add 10 ml of distilled water, mix thoroughly and take absorbance at 540nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve. One unit of enzyme activity is defined as that amount which liberate1μmol of reducing sugars (calculated as maltose) /min from soluble starch at 37^0C, pH 7, and under the specified experimental condition.

Preparation of extract and quantification of α- amylase inhibitor activity

Take 1 gm of sample and extract with 75 ml of distilled water and 75 ml of ethanol for 2 hrs, at 40^0c.centrifuge the suspension at 5000rpm.Collect the supernatant. Take 0.25 ml and incubate with0.25 ml of enzyme solution for 15 minutes at 37^0C.Incubate all the reagents also at 37^0C for three minutes. At the end of the incubation add 2 ml of dinitrosalicylic acid reagent to first, second and sample tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes. After cooling under cold water, add 10 ml of distilled water mix thoroughly and take absorbance at 540nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve. One unit of enzyme activity is defined as that amount which liberate1μmol of reducing specified experimental condition.

Determination lipase inhibitory activity

Emulsion containing 8 ml of olive oil, 0.4 ml of phosphate buffer and 1ml of lipase for an hour in rotary shaker, followed by addition of 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1) in order to stop reaction. The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as an indicator.

Lipase inhibitory activity of methanol extract was tested by mixing 100 ml of each concentration of methanol extract, 8 ml of oil emulsion and1 ml of lipase followed by incubation for an hour. The reaction was stopped by adding 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M The oxalic acid) using phenolphthalein as an indicator.

According to following formula percentage inhibition of lipase activity was calculated:

Lipase inhibition = A-B/A × 100, Where A is lipase activity, B is the lipase activity when incubated with the extract.

RESULTS AND DISCUSSION

Phytochemical screening

The prepared hydroalcoholic extracts (Table 2) subjected to various chemical tests and revealed that phytoconstituents like alkaloids and glycosides are enriched in all fruits. Saponins were found to be absent in all fruit extracts. Other Phytochemical such as Flavonoids, tannins, terpenoids were absent in *Passiflora edulis* and *Coccinia grandis*, but these all constituents are present in the third one that is *Ribes rubrum*.

Ascorbic acid content, Amylase & Lipase inhibitory effects of fruit extracts

The maltose calibration curve (figure 1) was plotted, from the graph the concentration at which the sample absorbance value intercepts are taken as the appropriate sample concentration. The percentage α-amylase inhibition(in table6)of *Passiflora edulis* and *Coccinia grandis* and *Ribes rubrum* were found to be 52.96,96.12,and 40.25.and lipase inhibitory activity is 39.22 in *passiflora* and 35.69 in *Ribes rubrum*. Because of this high α-amylase inhibitory activity and lipase inhibitory activity this

compounds may have anti diabetic and antilipidemic activity.

CONCLUSION

Quantitative vitamin c content, lipase inhibitory, amylase inhibitory and phytochemical screening of courtyard fruits like *Passiflora edulis*, *Coccinia grandis* and *Ribes rubrum* were carried out. All of this fruits have good amylase inhibitory activity. *Passiflora edulis* and *Ribes rubrum* have good lipase inhibitory activity. *Passiflora edulis* and *Ribes rubrum* are rich in vitamin c also.

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Table 1. List of courtyard fruits used for this study

S. No.	Scientific name	Common name
1	Passiflora edulis	Passion fruit
2	Coccinia grandis	kovakkai
3	Ribes rubrum	Lubikka

Table 2. Phytochemical screening of the fruits

Botanical names	Alkaloids	Glycosides	Flavonoids	Tannins	Terpenoids	Saponins
Passiflora. edulis	++	++	--	-	-	--
Coccinia. grandis	++	++	--	-	-	--
Ribes. rubrum	++	++	++	+	+	--

Table 3. Estimation of ascorbic acid

Method	A. *Passiflora. edulis*	B. *Coccinia. grandis*	C. *Ribes. rubrum*
Titrimetry	7.9 mg/100g	5.1 mg/100g	1.329mg/100g
UV-spectrophotometry	7.66mg/100g	4.34mg/100g	0.96mg/100g

Hypoglycemic activity

Table 4. Maltose calibration curve

Concentration	Absorbance	Concentration	Absorbance
0.1	0.0754	3	0.7315
0.2	0.1008	4	1.0210
0.3	0.1108	5	1.1660
0.4	0.1198	Blank	0.0333
0.5	0.1912	Control	0.1447
0.6	0.2319	Extract of A	0.4014
0.7	0.2569	Extract of B	1.1425
0.8	0.2777	Extract of C	0.3324
0.9	0.3281		
1	0.3491		
2	0.5252		

Table 5. Concentration of α-amylase enzyme of the three fruits from standard graph

S. No.	Plant name	Concentration of enzyme in mg/100g
1	*Passiflora indica*	1.25 mg/100g
2	*Coccinia grandis*	4.5 mg/100g
3	*Ribes rubrum*	0.95 mg/100g

Table 6. Concentration of α-amylase enzyme in percentage

S. No.	Plant name	Amylase inhibition in percentage
1	*Passiflora indica*	52.96%
2	*Coccinia grandis*	96.12%
3	*Ribes rubrum*	40.25%

Table 7. Lipase inhibitory activity

S. No.	Plant name	Lipase inhibitory activity in percentage
1	*Passiflora indica*	39.22%
2	*Coccinia grandis*	-15%
3	*Ribes rubrum*	35.69%

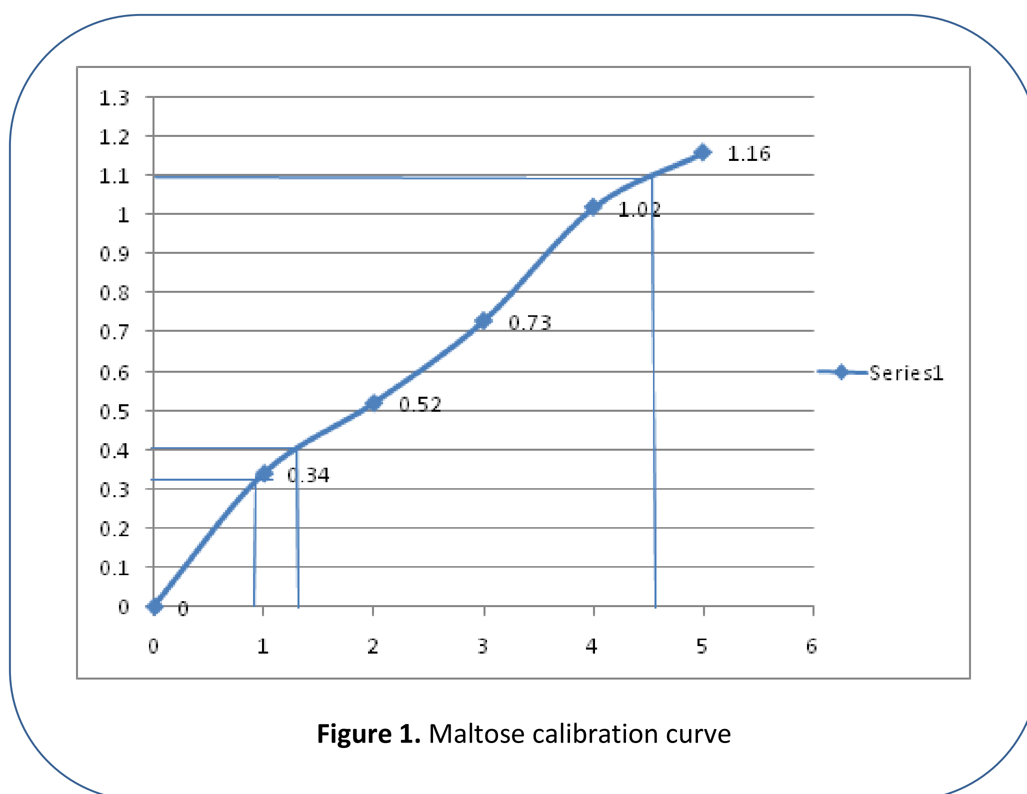

Figure 1. Maltose calibration curve