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Evaluation of *In vitro* antioxidant, antidiabetic and anti-lipase activities of selected fruits (Amla, Grape, Jamun and Kiwi)

K Sudha, D Baskaran and Rita Narayanan

Abstract
The present study was undertaken to evaluate *in vitro* antioxidant, antidiabetic and anti-lipase activities of selected fruits *viz*., Amla, Grape, Jamun and Kiwi. Antioxidant activity of fruit extracts were assessed by DPPH and SOD methods. In DPPH method, the highest antioxidant activity was observed in aqueous extract of jamun (92.333±0.71), followed by kiwi, grape and amla (86.500±0.34) whereas in SOD method, the highest antioxidant activity was found in aqueous extract of amla (94.886±0.49), followed by grape, jamun and kiwi fruit (81.451±0.70). *In vitro* antidiabetic activity of different fruit extract was observed by starch-agar gel diffusion method, it was found that the highest inhibitory activity of alpha amylase was observed in jamun (98.333±1.05), followed by grape, kiwi and amla (70.965±0.14) whereas, the highest inhibitory activity of alpha glucosidase was found in amla (94.420±0.10) followed by grape, jamun, and kiwi fruit (50.778±0.17). Anti-lipase activity of aqueous fruit extract was assessed by rhodamine agar plate method, the highest inhibitory activity was exhibited by aqueous extract of jamun (94.675±0.11), followed by kiwi, amla and grape (51.511±0.38). In pancreatic lipase inhibition method, maximum inhibitory activity was found in jamun (67.751±0.09), followed by kiwi, grape, and amla (31.760±0.35). In the study, fruit extracts proved to have therapeutic effects as antioxidant, antidiabetic and anti-lipase properties were found in fruit extracts.

Keywords: Fruits, antioxidant, antidiabetic and anti-lipase

1. Introduction
Fruits are botanically diverse, perishable, seasonal and regional commodities. They come in many forms, shapes and sizes, colors, flavors and textures; and are an important part of a healthy diet. Low intake of fruits and vegetables has been suggested by the World Health Organization (WHO) as one of the risk factors for non-communicable diseases (NCDs) such as various forms of cancers, cardiovascular diseases, diabetes, etc. Besides vitamins, minerals, fibres, and other nutrients, fruits contain various phytochemicals like phenolic compounds having pharmacological potentials.

The antioxidants in fruits and vegetables might confer these health protective benefits (Lako *et al*., 2007, Naczk and Shahidi, 2006) [26, 35] by alleviating oxidative stress, i.e. preventing free radicals from damaging proteins, DNA and lipids. Through additive and synergistic effects, the complex mixture of phytochemicals in fruits and vegetables may provide better protection than a single phytochemical (Liu, 2003) [28]. Organisms defend themselves against the destructive actions of ROS by using enzymatic and non-enzymatic mechanisms. The enzymatic mechanism makes use of the enzyme superoxide dismutase (SOD) and the hydrogen peroxidase enzyme (PO) (Bray, 2000; Breusegem and Dat, 2006; Dasgupta and De, 2006) [10, 11, 13]. The non-enzymatic antioxidant pathway includes the actions of vitamins, phenolic compounds, tannins and Gallic acid (Breusegem and Dat, 2006; Wu and Ng, 2008; Harr and Ishmail, 2012) [22, 54, 17].

Diabetes mellitus (DM) is the most common metabolic disorder characterized by persistent hyperglycemia, which is due to carbohydrate, protein and lipid metabolism disturbance caused by relative or absolute deficient in insulin secretion and/or insulin action in the peripheral tissues (American Diabetes Association, 2017) [3]. The estimates in 2019 showed that 77 million individuals had diabetes in India, which is expected to rise to over 134 million by 2045 (Pradeepa and Mohan 2021) [41]. α-amylase and α-glucosidase digest the carbohydrates and increase the postprandial glucose level in diabetic patients. Inhibiting the activity of these two enzymes can control postprandial hyperglycemia, and reduce the risk of developing diabetes.

Obesity is frequently associated with the intake of a lipid-rich diet.

Fat degradation is carried out by lipase, a key enzyme that plays a role in breaking down triglycerides into monoacylglycerols and fatty acids in the gastrointestinal tract (Pirahanchi & Sharma, 2020) [40]. In the absence of lipase, fat cannot be absorbed by the body (Ko *et al*., 2020) [23]. Thus, strategies to prevent and treat obesity can be pursued by reducing fat absorption through inhibition of lipase activity (Birari & Bhutani, 2007; Yun, 2010; Liu *et al*., 2020) [7, 56, 29]. Consumed as part of a regular diet these naturally occurring plant constituents are believed to provide a wide range of physiological benefits as antioxidants, antidiabetic. Anti-lipase, antiallergic, anticarcinogenic, anti-inflammatory, etc. Thus, in view of the above aspects the objectives of this study were to evaluate in-vitro antioxidant, antidiabetic and anti-lipase activities of selected fruits *viz*., Amla, Grape, Jamun and Kiwi fruits.

2. Materials and Methods

2.1 Fruit extract preparation

The fruits were thoroughly rinsedin tap water and then distilled water separately. The whole fruits were pureed well using a sterilized juicerand then filtered through a muslin cloth to obtain a clear aqueous fraction of fruit free from pulp (Waterhouse *et al*., 2009) [53]. Fruit extracts were cooled at 4 ˚C for further analysis.

2.2 Estimation of Antioxidant activity

2.2.1 Determination of antioxidant activity with 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The total antioxidant activity of fruit extract was determined as per the DPPH **(**1,1-diphenyl-2-picrilhydrazyl) method adopted by Brand-Williams *et al*., (1995) [9]. The DPPH radical scavenging activity was estimated by measuring the decrease in the absorbance of the methanolic solution of DPPH. Different volumes of fruit (50-200µl) extract were allowed to react with DPPH solution (3.3 mg of DPPH in 100 ml methanol), incubated for 30 minutes in the dark and the absorbance (A_1) was read at 517 nm. The absorbance (A_0) of a reaction control (methanol instead of fruit extract) was also recorded at the same wavelength. Ascorbic acid (5-50 µg/ml) was used as a standard. Scavenging ability (%) was calculated by using the formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[A_0 - A_1]}{A_0} \text{X } 100$$

Where, A_0 was the absorbance of reaction control and A_1 was the absorbance of fruit extracts or standards.

2.2.2 Determination of antioxidant activity-Superoxide dismutase activity

Superoxide Dismutase activity (SOD) activity was determined according to the method of Marklund and Marklund (1974) [31] and is based on the inhibition of pyrogallol autoxidation by SOD. An aliquot of different volumes of fruit extract (50-200 µl) was mixed with 2.5ml of Tris-HCl buffer, 0.1ml of EDTA, pH 8.2, followed by the addition of 0.5ml of 0.5 mMpyrogallol. The reactions were carried out at a temperature of 25 ˚C and absorbance of the mixtures was recorded at 420 nm immediately, and then every 30 sec for 3 min using a spectrophotometer. Changes in the rates of absorbance were calculated and converted into units of SOD activity per mg protein, where one unit is equivalent to the quantity of SOD that is needed to produce 50% inhibition of pyrogallol autoxidation.

$$\text{Rate (R)} = \frac{\text{Final OD-Initial OD}}{\text{3 minutes}}$$

$$\text{\% of inhibition} = \frac{\text{Blank OD-R}}{\text{Blank OD}} \text{X } 100$$

2.3 Estimation of Anti diabetic activity

2.3.1 Starch-agar gel diffusion assay for α-Amylase inhibition activity

For screening of α-amylase inhibition activity, the method developed by Fossum and Whitaker (1974) [14]. A starch substratemedia containing 5 g agar and 5 g starch in 500 mL distilled water was prepared, autoclaved and poured inpetriplates. For determination of α-amylase inhibition activity wells of 10 mm in diameter were made in the starch agar gel with cork borer. A fixed volume (10µl) of enzyme and different volume of fruit extract (25-100µl) were added into the wells. The plates were allowed to incubate for 24 hours at 37 °C. At the end of incubation, the starch agar plate was flooded with Gram's iodine solution and excess solution poured off. The presence of inhibitory activity was indicated by blue colour around the wells because of non-hydrolysis of starch. It was compared with control, containing α-amylase (10µl) solution. The presence of α-amylase activity was indicated by clear zone around the well because of hydrolysis of starch.

$$\text{Amylase inhibition (\%)} = \frac{\text{Dia. of zone of Control (mm)-Dia. of zone of fruit extract (mm)}}{\text{Diameter of zone of Control (mm)}} \text{X100}$$

2.3.2 α-Glucosidase inhibitory assay

This assay was carried out to investigate the *in vitro* inhibitory activity of fruit extract on sucrase and maltase (α-glucosidase). The inhibitory effect was measured by the method used by Dahlqvist (1970) [12]. One milligram of commercially available α-glucosidase was dissolved in 12 mL of maleate buffer (100 mM, pH 6). The homogenate was used as α-glucosidase solution. The assay mixture consisted of 100 mM maleate buffer (pH 6), 2% (w/v) of each sugar substrate solution (100 *m*l) and the sample extract. The mixture was pre incubated for 5 minutes at 37 °C, and the reaction was initiated by adding crude α-glucosidase solution (50 *m*l), followed by incubating the mixture again for 10 minutes at 37 °C. The amount of glucose released in this reaction was determined by a commercially available glucose estimation kit. The rate of carbohydrate decomposition was calculated as a percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. Acarbose was used as reference drug as α-glucosidase inhibitor. The rate of inhibition was calculated by the following formula:

$$\text{Inhibition rate (\%) } Z = \frac{\begin{array}{c}\text{(Amount of glucose produced by the positive control)-}\\ \text{(Amount of glucose produced by the addition of fruit extract)-}\\ \text{(Glucose production value in blank)}\end{array}}{\text{Amount of glucose produced by the positive control}} \text{ X } 100$$

2.4 Estimation of anti-lipase activity

2.4.1 Rhodamine agar plate assay

The lipase inhibition activity was assayed using rhodamine agar plate assay according to the method of Kouker and Jaeger (1987) [24]. Rhodamine B-Olive oil Agar medium containing Olive oil 3% (v/v), Agar 2% (w/v), rhodamine B 1% (v/v), Tris-HCl buffer (pH 7), 50 mM CaCl21% was used for the study. Rhodamine agar medium was prepared in distilled water, autoclaved and cooled to 60 °C. The cooled medium was added with 3% of olive oil previously sterilized at 160 °C for 2 h in hot air oven and 1% filter sterilized rhodamine B (1 mg/ml). The contents were mixed well to dissolve and the medium was poured into petri dishes. Circular wel1s of 10 mm were punched in the agar plates and 10µl of the commercially available lipase enzyme solution and different levels of fruit extract was dispensed into each well. The plates were allowed to incubate for 48 hours at 37 °C. The hydrolysis of substrate causes the formation of orange fluorescent halos around the well visible upon UV irradiation. The rate of inhibition was calculated by the following formula:

$$\text{\% of inhibition} = \frac{\text{Dia. of zone of Control (mm)-Dia. of zone of fruit extract (mm)}}{\text{Diameter of zone of Control (mm)}} \text{X } 100$$

2.4.2 Pancreatic Lipase Inhibition Assay

Porcine pancreatic lipase (PPL, type II) activity was measured using p-nitrophenyl butyrate (p-NPB) as a substrate. The method used for measuring the pancreatic lipase activity previously described by Slanc *et al*., (2004) [48]. PPL stock solution (1 mg/mL) was prepared in a 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions were stored at -20 °C. To determine the lipase inhibitory activity, the extracts or Orlistat (at same concentrations) as a positive control were pre-incubated with PPL for 1 h in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30 °C before assaying the PPL activity. The reaction was then started by adding 0.1 µL NPB as a substrate, all in a final volume of 100 µL. After incubation at 30 ˚C for 5 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer. The activity of the negative control was also examined with and without an inhibitor. The inhibitory activity was calculated according to the following formula:

$$\text{Inhibitory activity (\%)} = \frac{100 - ((B - b)}{(A - a)} \text{X } 100$$

Where, A is the activity without inhibitor; a -is the negative control without inhibitor; B is the activity with inhibitor and b is the negative control with inhibitor. DMSO was used as negative control and its activity was also examined.

3. Results and Discussion

3.1 Antioxidant activity of aqueous extract of amla, grape, jamun and kiwi-DPPH method

From figure 1, the antioxidant activity of fruit extracts at various level were found to be in the range of 71.833±0.54 to 92.333±0.71 per cent. The antioxidant activity of aqueous jamun extract (200 µl) was exhibited a significantly ($p \leq 0.01$) higher value followed by kiwi, grape and amla in descending order.

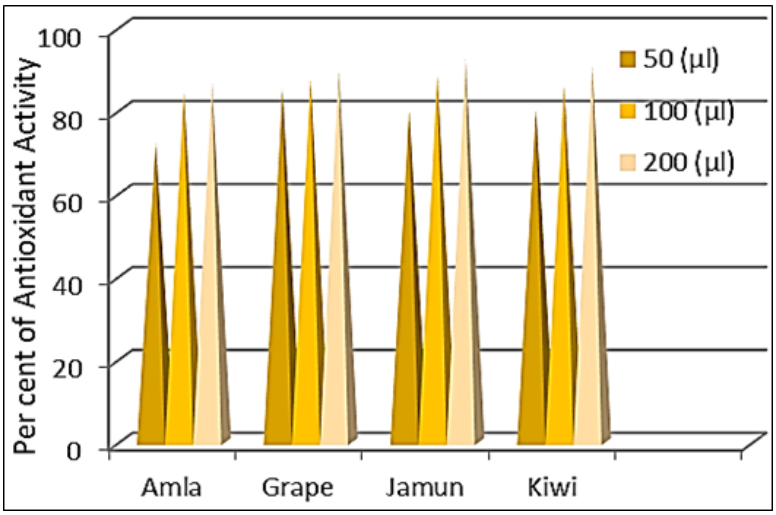

Fig 1: Antioxidant activity of aqueous extract of amla, grape, jamun and kiwi-DPPH method

The present study were in agreement with the results of Adelia *et al.* (2011) [1] who observed that the free radical scavenging capacity of jamun was increased mainly due to the presence of carotenoids and non-anthocyanic phenolics present in jamun. The results of the present study were in concurrence with the findings recorded by Sravanthi and Gangadhar (2015) [51] who indicated that the antioxidant activity of grape was invariably due to the presence of

phenolics and flavonoid compounds in grape fruits (Sagar *et al.* 2011) [44].

The result of the present study on antioxidant activity of kiwi was found to be in coincidence with the findings observed by Bekhradnia *et al.* (2011) [6]. Most phenolic and flavonoid compounds were described to possess anti-oxidative action inliving systems, as they acted as scavengers of free radicals (Rice-Evans *et al.* 1997) [43]. The results of the present study were coincided with the findings of Suresh *et al.* (2011) [52] who reported that antioxidant activity of amla was mainly due to the presence of phytochemicals.

3.2 Antioxidant activity of aqueous extract of amla, grape, jamun and kiwi-SOD method

The antioxidant activity of selected fruits was observed by SOD method varied in the wide range of 63.140±0.64to 94.886±0.49 per cent. The antioxidant activity of aqueous amla extract (200 µl) was exhibited a significantly ($p \leq 0.01$) higher value followed by grape, jamun, and kiwi fruit in descending order (Figure. 2).

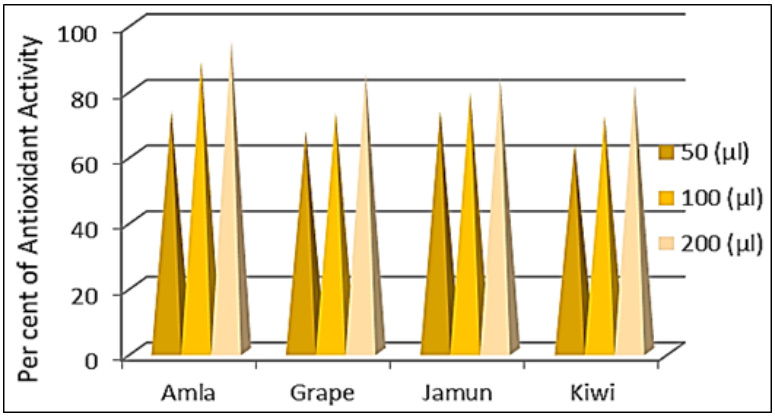

Fig 2: Antioxidant activity of aqueous extract of amla, grape, jamun and kiwi-SOD method

The results of the present study were in concurrence with the findings recorded by Khopde *et al.* (2001) [22] who stated that amla extract acted as an efficacious antioxidant by scavenging the reactive oxygen species and protected the SOD antioxidant enzymes utilized in this study. These findings were well correlated with the observation of Spanou *et al.* (2011) [50] in which grape extracts could induce SOD activity, thereby implying a possible antioxidant action. These results were in concurrence with the findings recorded by Prince *et al.* (1998) [42] indicating that the aqueous extract of jamun seeds increased the SOD activity and clearly revealed the antioxidant property of the jamun.

Antioxidant activity of kiwi was found to be in correlation with the findings of Spada *et al.* (2008) [49]. Kiwi fruit is a wealthy source of vitamins E, isoflavones and flavonoids which are significantly occurring phytochemicals in kiwi extract (Shehata and Soltan, 2013) [45] that protected the cells from oxidative degeneration (Nagib, 2013) [36].

3.3 Antidiabetic activity of aqueous extracts of amla, grape, jamun and kiwi-α-Amylase inhibition assay

The antidiabetic activity of selected fruits was observed by starch agar gel diffusion method varied in the wide range of 29.221±0.12 to 98.333±1.05 per cent.

α-Amylase inhibitory activity of aqueous jamun extract (200 µl) was exhibited a significantly ($p \leq 0.01$) higher value followed by grape, kiwi and amla in descending order (Figure. 3). α-Amylase inhibitors acted through two mechanisms either form a complex with enzyme and limit its activity or reduce the diffusion rate of glucose from the active site.

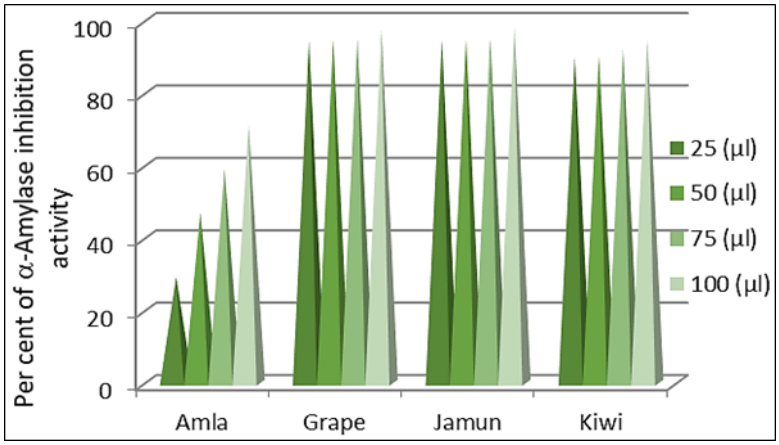

Fig 3: Antidiabetic activity of aqueous extract of amla, grape, jamun and kiwi-α-Amylase inhibition assay

The results of the present study were in correlation with the findings by Singh and Marar (2011) [47] in which aqueous and methanolic extracts of jamun showed significant inhibition of α-amylase. Jamun fruits were richest sources of polyphenol and these compounds had the ability to inhibit α- amylase activity (McDougall *et al.* 2005) [32]. Tannic acid in jamun

fruit inhibited the salivary amylase action and thereby delaying the starch degradation process (Kandra *et al*. 2004) [21].

The results obtained were correlated with the studies carried out by Patel *et al.* (2015) [38] who determined the inhibition of α- amylase activity in kiwi extract which had broken starch into glucose and described the antidiabetic potential of extracts from the kiwifruit. The result of the present study on antidiabetic activity of grape was found to be in correlation with the results furnished by Balasubramanian *et al.* (2015) [5]. The antidiabetic activity of grape fruits was mainly due to the presence of various bioactive compounds (Hogan *et al*., 2010) [18]. α-Amylase inhibition activity of amla was found to be in correlation with the results observed by Kumari *et al.* (2015) [25]. Presence of ellagic acid and ascorbic acid in the extract was reported to be responsible for the antidiabetic activity of amla (Nampoothiri *et al*., 2011) [37].

3.4 Antidiabetic activity of aqueous extract of amla, grape, jamun and kiwi-α-Glucosidase inhibition assay

The antidiabetic activity of fruits extract was observed by spectrophotometer using alpha-glucosidase inhibition method varied in the wide range of 50.778±0.17 to 94.420±0.10 per cent. α-Glucosidase inhibitory activity of aqueous amla extract (200 µl) was exhibited a significantly ($p \leq 0.01$) higher value followed by dragon, grape, jamun, and kiwi in descending order (Figure. 4).

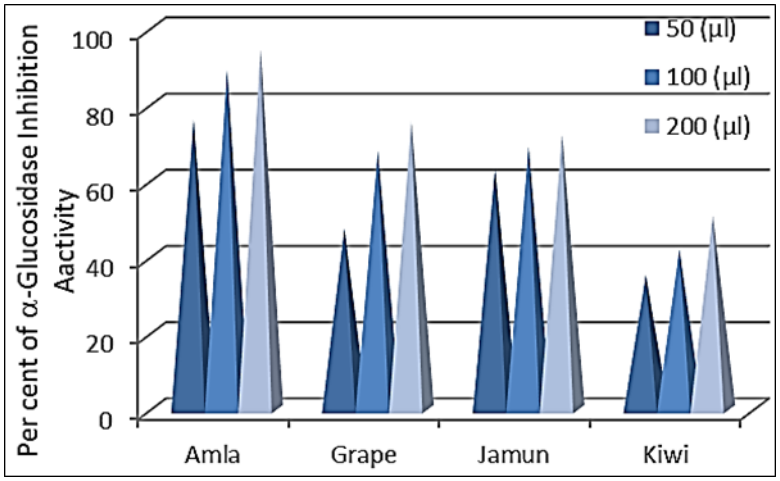

Fig 4: Antidiabetic activity of aqueous extract of amla, rape, jamun and kiwi-α-Glucosidase inhibition assay

The results obtained were correlated with the studies carried out by Francis and Sudha (2016) [15] & Gopinath *et al*., (2013) [16] in which the inhibitory activity of α-glucosidase in amla and jamun extracts were in concordance with the results of the present study. Similar results were found by Singh and Marar (2011) [47] who reported that the aqueous and methanolic extracts of jamun showed higher degree of inhibitory activity on pancreatic, liver and intestinal glucosidases.

The results furnished by Hogan *et al.* (2010) [19] were in coincidence with present study in which the red grape pomace extract contained significantly higher amounts of flavonoids and phenolic compounds and exerted significant inhibition on intestinal α-glucosidases. The result of the present study on inhibitory activity of α-glucosidase in kiwi was found to be in correlation with results furnished by Shirosaki *et al.* (2008) [46] in which triterpenoids and flavonoidsin kiwi significantly reduced the glucosidase enzyme activity.

3.5 Antilipase activity of aqueous extract of amla, grape, jamun and kiwi-Rhodamine Agar Plate method

The antilipase activity of fruits extract was observed by rhodamine agar plate method varied in the wide range of 46.938±0.10 to 94.675±0.11 per cent. The lipase inhibitory activity of aqueous jamun extract was exhibited a significantly ($p \leq 0.01$) higher value followed by kiwi, amla, grape and dragon fruit in descending order (Figure 5).

The anti-lipase activity of fruits might be due to the presence of polyphenols which has been reported to have anti-obesity property by inhibiting lipase activity (Lei *et al.* 2007; Yajima *et al.* 2005) [27, 55]. In addition, McDougall *et al.* (2009) [33] have established that the inhibitory lipase activity might be derived from the phenolic compounds found in some medicinal plants *viz*., gallic acid, catechin, epicatechin, ellagic acid myricetin, quercetin, kaempferol, resveratrol and anthocyanin (McDougall *et al.* 2009) [33].

Maqsood *et al.* (2017) [30] investigated that *L. siceraria* fruit possess more ability to inhibit action of pancreatic lipase. Joseph and George (2013) [20] determined that *Passifloraedulis* and *Ribesrubrum* fruits had best lipase inhibitory activity.

3.6 Anti-lipase activity of aqueous extract amla, grape, jamun and kiwi-Pancreatic Lipase inhibition assay

The anti-lipase activity of fruits extract was observed by spectrophotometer using pancreatic lipase inhibition method varied in the wide range of 26.136±0.14 to 67.751±0.09 per cent. The lipase inhibitory activity of aqueous jamun extract was observed a significantly ($p \leq 0.01$) higher value followed by kiwi, grape and amla in descending order (Figure. 6).

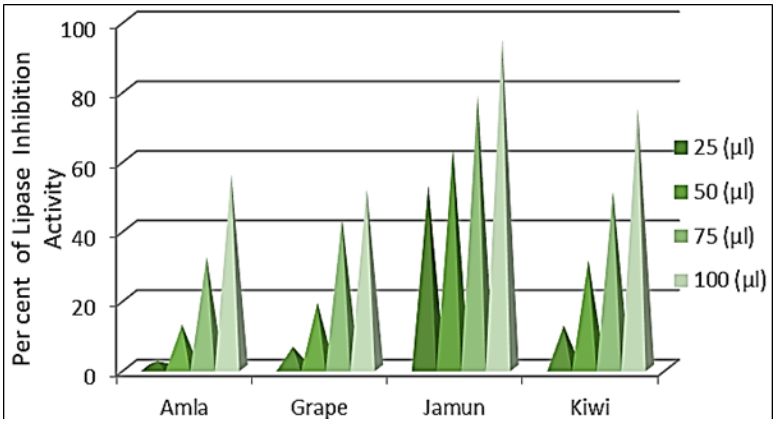

Fig 5: Anti-lipase activity of aqueous extract of amla, dragon, grape, jamun and kiwi-Rhodamine agar plate method

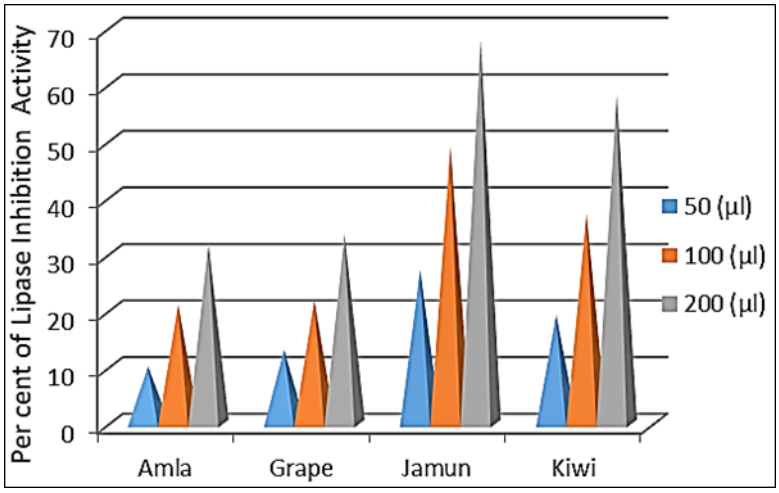

Fig 6: Anti-lipase activity of aqueous extract of amla, grape, jamun and kiwi-Pancreatic Lipase inhibition assay

Grape extract markedly inhibited pancreatic lipase with p-NPP which was used as a substrate. The results concurred with findings observed by Adisakwattana *et al*. (2010) [2] in which grape seed extract suppress pancreatic lipase activity. Furthermore, it also suppressed action on hormone sensitive lipase and thereby decreased free fatty acids releasing from adipose tissue (Moreno *et al.* 2003) [34]. The result of the present study on anti-lipase activity of jamun was found to be in correlation with results furnished by Borges *et al.* (2016) [8] in which the presence of proanthocyanidin, anthocyanins, phenolic acids and flavonoids present in jamun showed higher anti-lipase activity.

Flavonoids from *E*. *Officinalis* lowered level of cholesterol and significantly increased the excretion of faecal bile acids and neutral sterols also contributed to the hypocholesterolemic action (Anila and Vijayalakshmi, 2002) [4].

4. Conclusion

From the present results, it could be concluded that, the aqueous extract of selected fruits *viz*., Amla, Grape, Jamun and Kiwi fruit possesses significant antioxidant activity, antidiabetic and antilipase activity. The presence of several bioactive principles in this fruits extract might be responsible for these effects and could be used as an alternative therapeutic approach to managing postprandial hyperglycaemia, hyperlipidemia and associated oxidative stress.

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