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PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF ARKA KIRAN VAR. OF GUAVA

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Abstract

In the present study the presence of phytochemicals, and *in vitro* antioxidant activities of ethanolic leaves extract of *Psidium guajava* Linn variety, namely Arka kiran was investigated. The ethanolic leaf extract of Arka kiran was found to processes secondary metabolites of alkaloids, flavonoids, saponin, phenols and glycosides. Gas Chromatography and Mass Spectroscopy (GCMS) study revealed the presence of therapeutic compounds like octadecenoic acid methyl ester, deoxyspergualin and eicosatrienoicacid. The extract assessed for its α–amylase and α–glucosidase inhibition inhibition at a dose of 20 - 100 µg/ml, at 5% level of significance on both antidiabetic activities were observed. Further the results of *in vitro* antioxidants (DPPH radical, superoxide and nitricoxide radical) scavenging activities confirmed their antioxidant potentials with the IC_{50} values of 326, 345 and 248 µg/ml, respectively.

Introduction

Medicinal plants play an important role in maintaining the health of individuals and communities. The medicinal value of these plants lies in some bioactive compounds that produce a definite physiological action on the human body. The bioactive compounds that are present in the plants are referred to as phytochemicals which are derived from different parts of the plant such as leaves, flowers, fruits, barks and roots are used as therapeutic purpose therapeutic purpose (Hemavathy *et al* 2019).

Psidium guajava L. known as guava is a medicinal plant, which possesses antidiabetic and anticancer properties. Guava leaves contain a variety of compounds such as polyphenols, terpenoids, flavonoids and tannins. The reason might be due to guava leaves which have antidiabetic effects directly and indirectly by controlling glucose and carbohydrate hydrolyzing enzyme activities (Roh *et al.*2009.) The 'Arka kiran' guava was developed by the Indian Institute of Horticultural Research, Bengaluru whichwhich has a potential to yield medium sized round fruits with deep red, firm pulp with high lycopene content (Sanjana *et al.* 2022).

In recent years guava leaf tea is available in several shops, because of its health benefits. Due to its health beneficial effects, used for the maintenance of modulation of blood sugar and protection of the cells, organs of the body against reactive oxygen species. The role of medicinal plants in disease prevention isattributed to its antioxidant properties due to the presence of bioactive constituents. Antioxidants are capable of stabilizing the deactivating free radicals (Valko *et al*. 2007). In the present study, the bioactive compounds, anti-diabetic and antioxidant activities of ethanolic leaf extract of *P.* (Arka kiran) were investigated.

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Materials and Methods

The leaves of *Psidium guajava* variety, namely Arka kiranwas collected from orchard of Horticultural College and Research Institute for Women, TNAU, Trichy, Tamil Nadu. The leaf sample was washed with distilled water then shade dried and the dried leaves were milled to fine powder, sieved and then stored in sterile airtight containers at for further use. Fine chemicals were purchased from Sigma Chemical Co., USA. All other chemicals used including the solvents, were of analytical grade. Hundred grams of powdered leaf samples of Arka kiranwas extracted with 250 ml of three different solvents *viz.*, hexane, ethanol and water. The contents of the flasks were kept in mechanical shaker over night. This suspension was filtered and the residue was resuspended with an equal volume of solvents for 48 hrs extraction and then filtered again. The two filtrates were pooled, and the solvents (hexane and ethanol) were evaporated. The dried residue was used for further analysis.

The phytochemical screening of hexane, ethanol and water extracts of leaves of Arka kiranwas carried out with standard procedures for determining the presence of phytochemicals (Peach and Tracey 1955).GC-MS analysis was performed with the GC-MS instrument (Perkin Elmer Clarus SQ8C make) and a non polar capillary and a non polar capillary column DB-5 MS. One micro liter of ethanolic leaf extract sample was injected into the column and helium was used as the carrier gas. The chromatogram and spectrum of the peaks were obtained and visualized. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in CAS database.

For the purpose of α-amylase inhibitor assay (1% w/v) starch solution was prepared by stirring 1 g starch in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7 mM of sodium chloride. The enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic amylase α-amylase in 100 ml of 20 mM of phosphate buffer (PBS, pH 6.9) containing 6.7mM of sodium chloride. To 100 µl of (20, 40, 60, 80,100 µg/ml) plant extracts, 200 µl porcine pancreatic amylase was added and the mixture was incubated at 37°C for 20 min. To the reaction mixture 100 µl (1%) starch solution was added and incubated at 37ºC for 10 min. The reaction was stopped by adding 200 µl DNSA (1g of 3,5 di nitro salicylic acid, 30 g of sodium potassium tartarate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 min. There action mixture diluted with 2.2 ml of water and the absorbance was read at 540 nm. For each concentration, blank tubes were preparedby replacing the enzyme solution with 200 µl of distilled water. Control (representing 100% enzyme activity) was prepared in a similar manner, without extract (Ali *et al.* 2006).

To conduct α-glucosidase inhibitior assay the inhibition of α-glucosidase activity was determined using the modified method of Kim *et al.* (2011).

To conduct DPPH radical scavenging assay the free radical scavenging activity of the plant extract with ascorbic acid as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) (Bolis 1958).

To conduct nitric oxide radical ($NO^{\cdot}$) scavenging assay nitric oxide generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.* (1994).

Superoxide radical ($O_2^{\cdot-}$)-scavenging assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) as described by (Awah *et al.* 2010).

Statistical data are presented as the mean ± standard deviation (SD) of three independent triplicate experiments. Statistical analysis was performed by using statistical package SPSS version 21.0 software. The differences between treated and untreated groups were assessed by

one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests. The result was considered significant when $p < 0.05$.

Results and Discussion

Results of preliminary phytochemical screening of the leaf extracts (hexane, ethanol and water) of *P. guajava* variety Arka kiran presented in Table 1 revealed the presence of phytochemicals such as alkaloids, flavonoids, saponin, phenols and glycosides in three different extracts. Among the extracts, ethanolic extract showed the maximum presence of phytochemicals when compared to hexane and water. So the ethanolic leaf extract of Arka kiran was used for further investigations.

Table 1. Photochemical screening of leaf extracts of Arka kiran.

Tests	Arka kiran leaf extract		
	Hexane	Ethanol	Water
Alkaloids	-	+	+
Flavonoids	-	+	+
Saponins	-	+	-
Phenols	+	+	+
Steroids	-	-	-
Glycosides	-	+	+
Tannins	+	+	+
Triterpnes	-	-	-
Resins	-	+	+

(+) presence, (-) absence

Spectrum is shown in the Fig. 1 and list of bioactive compound identified is presented in the Table 2.

Table 2. Bioactive compounds of Arka kiran extract in Gas Chromatography and mass spectroscopy (GC-MS).

RT	Hit	Compound name	Match	R. match	Prob	Cas	Library
3.69	1	6-Octadecenoic acid methyl ester	483	530	8.0	2777-58-4	Replib
	2	Deoxyspergualin	478	530	6.4		Mainlib
	3	Cyclopropane butanoic acid	475	527	5.7	56051-53-7	Mainlib
	4	8,11,14 Eicosatrienoic acid methyl ester	473	548	5.2	17364-32-8	Mainlib
	5	2H Benzo Oxieno benzofuran	471	538	4.8		Mainlib
	6	1,1'-Bicyclopropyl-2-octanoic acid 2'-hexyl- methyl ester	468	552	4.3		Mainlib
	7	2 Octanoic acid 2 hexyl methyl ester	461	549	3.3	56687-68-4	Mainlib
	8	17 Octadecadiynoic acid methyl ester	461	515	3.3		Mainlib
	9	11 Octadecadiynoic acid methyl ester	461	513	3.3	52380-33-3	Mainlib
	10	13,16 Octadecadiynoic acid methyl ester	459	568	3.0	56846-98-1	maInlib

The GC-MS analysis (Fig. 1 and Table 2) revealed the presence of 41peaks. Peaks represent the bioactive compounds from the ethanolic leaf extract of *P. guajava*. Out of ten matches in the CAS database, six major bioactive compounds have been identified and they are namely 6-Octadecenoic acid methyl ester, Deoxyspergualin, Eicosatrienoic acid methyl ester, 1,1'-Bicyclopropyl-2-octanoic acid 2'-hexyl- methyl ester, 17-Octadecynoic acid methyl ester and 13,16-Octadecadiynoic acid methyl ester.

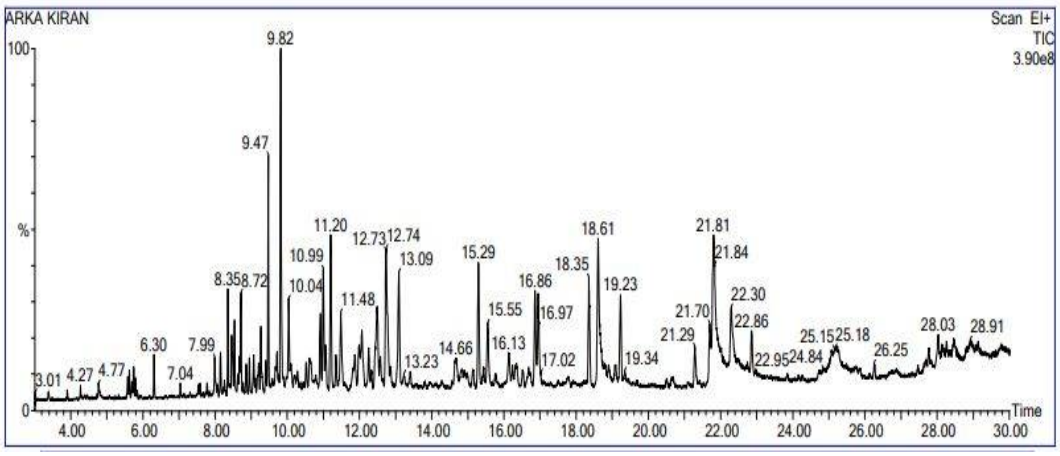

Fig. 1. Gas Chromatography and mass spectroscopy (GC-MS) analysis of ethanolic leaf extract of Arka kiran.

In the present study the *in vitro* α–amylase inhibitor and α– glucosidase inhibitor activities of the ethanolic leaf extract of Arka kiran was examined and the results are presented in Tables 3 and 4. With the increase of the sample concentration ranging from 20 to 100µg/ml, percentage inhibition increased by the extracts in dose-dependent manner. The α-glucosidase inhibitor activity was registered a maximum activity of 82.49% at 100 µg/ml than α-amylase inhibitor activity (maximum of 41.79 % at 100 µg/ml). Acarbose was used as standard drug for both α-amylase inhibitor and α–glucosidase inhibitor activities.

Table 3. α-amylase inhibitor activity of ethanolic leaf extract of Arka kiran and standard acarbose.

Sample concentration (µg/ml)	% inhibition	
	α–amylase inhibitor activity	*Std. Acarbose*
20	7.04 ± 0.02	10.63 ± 0.55
40	10.3 ± 0.37	36.31 ± 1.91
60	23.64 ± 0.47	45.69 ± 1.38
80	34.56 ± 0.90	54.37 ± 2.00
100	41.72 ± 0.70	72.77 ± 1.54

The plant extract might be used as starch blockers sinceit prevents or slows the absorption of starch into the body mainlyby blocking the hydrolysis of α1,4-glycosidic linkages of starch andother oligosaccharides into maltose. The secondary metabolites such as phenols, flavonoids, saponins, alkaloids and terpenoids present in the plant extract might be responsible for the effective inhibition of α-amylase and α-glucosidase inhibitor activities.

Table 4. *α*-glucosidase inhibitor activity of ethanolic leaf extract of Arka kiran and standard acarbose.

Sample concentration (µg/ml)	% inhibition	
	α–glucosidase activity	*Std. Acarbose*
20	17.64 ± 0.37	22.76 ± 0.67
40	58.57 ± 0.46	64.47 ± 0.74
60	66.97 ± 1.25	70.88 ± 0.41
80	69.32 ± 0.69	73.24 ± 0.25
100	82.11 ± 1.34	84.94 ± 0.45

DPPH radical scavenging activity is a widely used method to validate antioxidant activities of plant extracts (Gulcin *et al.* 2004). The antioxidant compounds in the extract and the standard neutralized the free radical character of DPPH by transferring electrons. (Naik *et al.* 2003), thereby changing the colour from purple to the yellow coloured stable diamagnetic molecule diphenylpicrylhydrazine. The degree of discoloration indicated the scavenging potential of the extracts (Mosquera *et al.* 2007).The percentage inhibition of DPPH radicals by the plant extract was determined and the results are represented in the Fig. 2. The scavenging effect of the extract was compared with standard ascorbic acid. The leaf extracts at different concentrations (100, 200, 300, 400 and 500 µg/ml) showed their DPPH radical scavenging activities ranging from 19.47 to 77.28 at 100 to 500 µg/ml, respectively. The standard ascorbic acid showed an inhibition of 73.21 at 50µg/ml. IC_{50} value for Arka kiran leaf extract was found to be 326 ± 10.17 µg/ml.

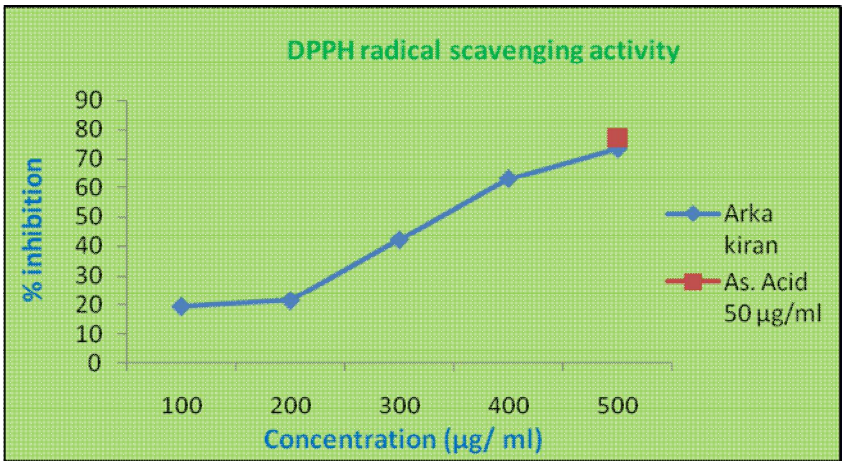

Fig. 2. DPPH radical scavenging activity of ethanolic leaf extract of Arka kiran and standard ascorbic acid.

Superoxide anions ($O_2^{\cdot-}$) are the type of free radicals, which increase in concentration under stress conditions. The ability of the extract to scavenge $O_2^{\cdot-}$radical generated from the photochemical reduction ofriboflavin resulted in a decrease in the absorbance of the blue formazan. The superoxide radical scavenging activity of the ethanolic leaf extract of Arka kiran variety was analysed and the results are depicted in Fig. 3. The plant extract had significant scavenging activities on superoxide radicals with highest percentage of inhibition (95.19%) at 500

µg/ml and lowest (22.52%) at 100 µg/ml. Standard ascorbic acid showed an inhibition percentage of 62.37 at50 µg/ml. IC_{50} value of the extract was found to be 345.77 ± 11.07 µg/ml.

The nitricoxide scavenging activity of the ethanolic leaf extract of Arka kiran was analysed and the results are given in Fig. 4. The nitric oxide scavenging activity increased with the increase of concentration of the plant extract from 100 to 500 µg/ml. The IC_{50} value was found to be 248.61 ± 22.04 µg/ml.

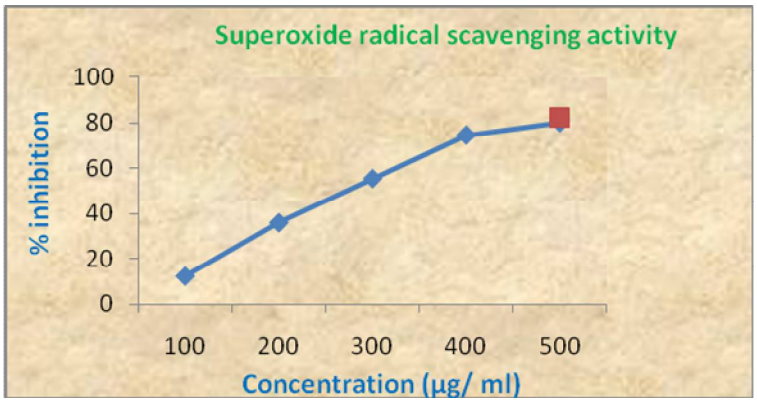

Fig. 3. Super oxide radical scavenging activity of ethanolic leaf extract of Arka kiran and standard ascorbic acid.

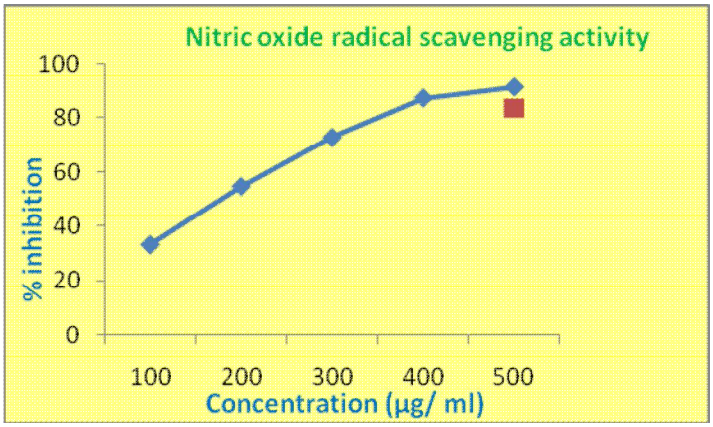

Fig. 4. Nitric oxide radical scavenging activity of ethanolic leaf extract of Arka kiran and standard ascorbic acid.

Results of the present study clearly revealed that the ethanolic leaf extract of Arka kiran have potent antidiabetic and antioxidant activities. The extract had significant α-amylase and α-glucosidase inhibitory activities that might be due to bioactive compounds present in the leaf of Arka kiran. Further DPPH, Superoxide radical and nitric oxide radical scavenging confirmed the antioxidant property of the plant.

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