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Evaluation of In-Vitro Antioxidant activity of Averrhoa carambola Stem Ethanolic Extract

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Abstract: *Averrhoa carambola* of family Oxalidaceae is commonly known as star fruit, carambola and in local name Kamrakh (hindi),Ambanamkaya (telugu). Various parts of tree has been used in traditional folkloric medicine. The present study was concentrated on the *in-vitro* antioxidant methods like DPPH free radical, nitric oxide radical, hydrogen peroxide radical scavenging and reducing power assays. The ethanolic extract of *Averrhoa carambola* stem was subjected to the above methods. The results of anti oxidant activity revealed that, the ethanolic extract shows good IC_{50} values. The results were compared with the standard ascorbic acid. The plant contains flavonoids, alkaloids, saponins and tannins. These active constituents alone or in combination may be responsible for the observed antioxidant activity.
Keywords: *Averrhoa carambola,* antioxidant activity, ascorbic acid, ethanolic extract.

INTRODUCTION:

Averrhoa carambola of family Oxalidaceae is commonly known as star fruit, carambola and in local name Kamrakh (hindi), Ambanamkaya (telugu). Its nutritive values are also useful to the human health. Various parts of tree has been used in traditional folkloric medicine.[1] It also reported having anti inflammatory activity of leaves, antimicrobial activity of fruit extract, hypoglycaemic of fruits, antioxidant of fruit and residue extracts, hypocholesterolaemic and hypolipidaemic activity of fruits, metabolic effects of enzymes of fruits, antiulcer activity.[2]

Antioxidants are the substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance.[3] Plants are the potential source of natural antioxidants. The scientific reports and experimental studies have shown that plants contain a large variety of phytochemicals that have antioxidant property.[4]

Antioxidants may exert their activity by several mechanisms,[5-6] like by suppressing the production of active species by reducing hydroperoxides and hydrogen peroxide; by sequestering metal ions, termination of chain reaction by scavenging active free radicals and also caused repairing and/or clearing damage of cell, biosynthesis of other antioxidants or defense enzymes also induced by some antioxidants.

Materials And Methods:

Plant Material:

The stem of *Averrhoa carambola* were procured from Gowhati, Assam. The authentification of the plant was done by Prof. Dr.K.Madhva Chetty, Dept. of Botany, Sri Venkateswara University, Tirupathi. The Voucher specimen was deposited in the herbarium of our department.

Preparation of Extract:

Freshly collected plant material was shade dried at room temperature and coarsely powdered in Wiely mill. The powdered stem (450g) was extracted with ethanol using Soxhlet apparatus. The crude extract was evaporated to dryness in a rotary film evaporator (Roteava, Equitron Medica instrument, India) and was found to be 6 gms respectively.

***Physiochemical studies of Extract* :**

The ethanolic stem extract of *Averrhoa carambola* was subjected for evaluation of oraganoleptic characters, solubility and phytochemical tests.

***Chemicals*:**

All the chemicals used were of analytical grade obtained from Aman Scientific Products, Vijayawada.

***In-Vitro* antioxidant study:**

The ethanolic extract of *Averrhoa carambola* stem tested for its free radical scavenging property using different in-vitro models. All experiments were performed thrice and the results were averaged.

DPPH Free Radical Scavenging Activity:

The ethanolic plant extract was tested for the DPPH free radical scavenging activity according to the method of Pan *et al*.[7] with minor modification. 0.2 mL of the extract solution in ethanol (95 %) at different concentrations was added to 8 mL of 0.004 % (w/v) stock solution of DPPH in ethanol (95 %). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517nm until the reaction reached the steady state, using a UV–Visible spectrophotometer. As a positive control, synthetic antioxidant gallic acid was used. All determinations were performed in triplicate. The DPPH radical scavenging activity (*S%*) was calculated using the following equation:

$$S\% = [\,(A_{control} - A_{sample})\,/A_{control}\,] \times 100$$

where $A_{control}$ = absorbance of the blank control(containing all reagents except the extract solution) and A_{sample} = absorbance of the test sample.

Nitric Oxide Free Radical Scavenging Activity:[8]

2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25^{o}C for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacialacetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated as:

$$S\% = [\ (A_{control} - A_{sample})\ /A_{control}\ ] \times 100$$

Where $A_{control}$ = absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} = absorbance of the test sample.

Hydrogen Peroxide Free Radical Scavenging Activity:

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the method of Ruch.[9] Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following equation.

$$S\% = [\ (A_{control} - A_{sample})\ /A_{control}\ ] \times 100$$

where $A_{control}$ = absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} = absorbance of the test sample.

Reducing Power Assay:[10]

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu. The extract (0.75 mL) at various concentrations was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mLof potassium hexacyanoferrate $[K_3Fe(CN)_6]$ (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction wasstopped by adding 0.75 mLof trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min.The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical Analysis:

The statistical analysis was carried out by using software Graph Pad Prism5. Linear regression analysis was used to calculate IC50 values.

Results And Discussion:

Preliminary Physicochemical Screening:

The ACSEE was screened forvarious physicochemical test as per the reported methods and found the ethanolic extract as deep brown colour, having pungent smell and soluble in ethanoland water. The phytochemical tests confirm the presence of saponins, alkaloids, flavanoids and tannins.

DPPH Free Radical Scavenging Activity:

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. DPPH radicals were widely used to investigate the scavenging activity of some natural compounds.[11] When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavengedand the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate

thescavenging activity of some natural compounds. The mean IC_{50} values for DPPH radical with ethanolic extract of *Averrhoa carambola* stem was found to be 878.06 µg/ml, and ascorbic acid was found to be 38.36 µg/ml respectively. The lower the IC_{50}, the higher the free radical scavenging ability. The DPPH radical scavenging activity of ACSEE is shown in Table 1, Fig 1.

Table 1: Percentage Inhibition and IC_{50} Values of DPPH Free Radical Scavenging Activity *In-Vitro* by *Averrhoa carambola* stem ethanolic extract.

Sl. No.	Concentration (ug/ml)	Absorbance			% Inhibition (Mean±SD)	IC_{50} (µg/ml)
		1	2	3		
Control (DPPH Sol.)						-
1.	0.004% in ethanol	0.62			-	
Std (Ascorbic Acid)						
1	12.5	0.43	0.45	0.46	27.95± 2.46	38.36
2	25	0.34	0.33	0.35	45.16±1.61	
3	50	0.25	0.21	0.24	62.36±1.93	
4	100	0.13	0.10	0.11	81.72±2.46	
ACSEE						878.06
1	200	0.50	0.51	0.50	18.81±0.93	
2	500	0.47	0.45	0.43	27.40± 3.21	
3	800	0.28	0.30	0.31	52.15± 2.45	
4	1200	0.20	0.23	0.21	64.51±0.92	

ACSEE=*Averrhoa carambola* stem ethanolic extract.

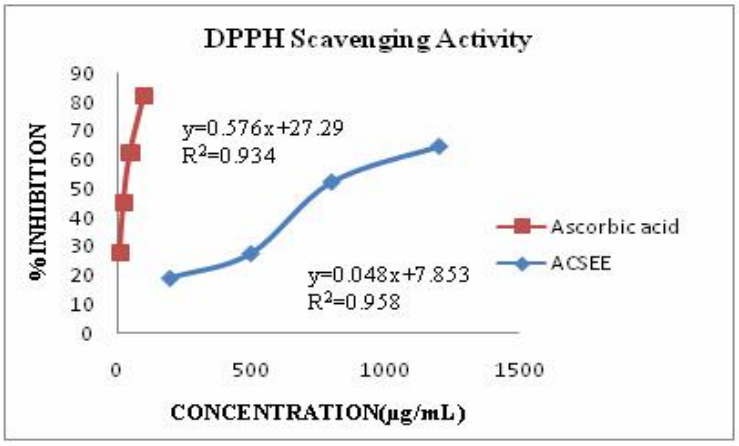

Figure 1: *In Vitro* Concentration Dependent Percentage Inhibition of DPPH Radical Scavenging Activity by Ethanolic extract of *Averrhoa carambola* stem and Ascorbic acid.

Nitric Oxide Free Radical Scavenging Activity:

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities.[12]Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases.[13] Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases. ACSEE significantly

inhibited nitric oxide in a dose dependent manner.The result indicated that the extract might contain compounds able to inhibit nitric oxide and offers scientific evidence for the use of the rhizomes in the indigenous system in inflammatory condition. The IC_{50} values for ACSEE and ascorbic acid were found to be 391.67 and 47.26 µg/ml respectively. The scavenging activity of ACSEE was shown in Table 2, Fig 2.

Table 2: Percentage Inhibition and IC_{50} Values of Nitric Oxide Free Radical Scavenging Activity *In-Vitro* by *Averrhoa carambola* stem ethanolic extract.

Sl. No.	Concentration (µg/ml)	Absorbance			% Inhibition (mean ±SD)	IC_{50} (µg/ml)
		1	2	3		
Control Blank						
1.	**Control Blank**	1.52			-	-
Std (Ascorbic Acid)						
1	12.5	1.12	1.16	1.07	26.53±2.96	47.26
2	25	0.87	0.90	0.89	41.66±1.00	
3	50	0.64	0.63	0.67	57.45±1.36	
4	100	0.40	0.43	0.37	73.68±1.97	
ACSEE						
1	200	1.13	1.10	1.06	27.85±2.31	391.67
2	300	0.97	0.94	0.90	38.37±2.30	
3	400	0.75	0.77	0.74	50.44±0.99	
4	500	0.57	0.55	0.50	64.47±2.37	

ACSEE=*Averrhoa carambola* stem ethanolic extract.

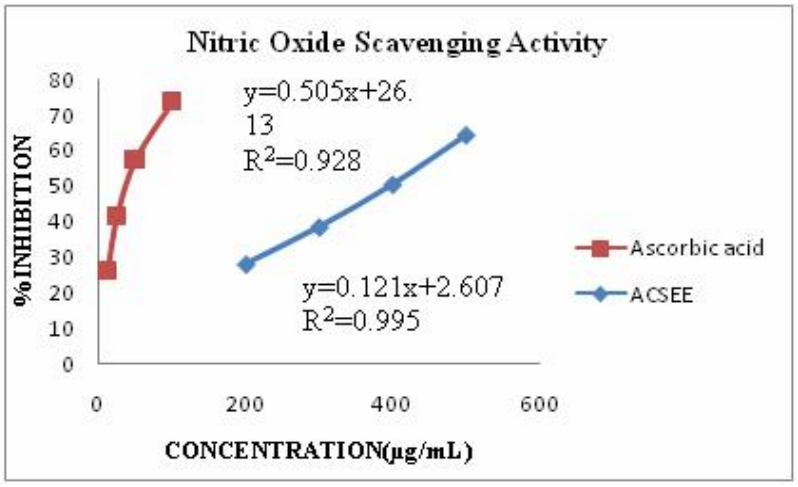

Figure 2: *In Vitro* Concentration Dependent Percentage Inhibition of Nitric Oxide Radical Scavenging Activity by Ethanolic extract of *Averrhoa carambola* stem and Ascorbic acid.

Hydrogen peroxide scavenging activity assay:

As shown in Table 3, Figure 3, ACSEE also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner with an IC_{50} of 83.29 µg/ml. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.[14]

The decomposition of H_2O_2 by ACSEE may at least partly result from its antioxidant and free radical scavenging activity. The ascorbic acid having IC_{50} value of 41.88 µg/ml.

Reucing Power Assay:

For the measurements of the reducing ability, the Fe^{3+}–$^{Fe2+}$ transformation was investigated in the presence of ACSEE. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[15] However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.[16-17] Similar to the antioxidant activity, the reducing power of ACSEE increased with increasing dosage. The result shows that ACSEE consist of hydrophilic poly phenolic compounds that cause the greater reducing power. The reducing power of ACSEE was shown in Table 4, Figure 4.

Table 3: Percentage Inhibition and IC_{50} Values of Hydrogen Peroxide Radical Scavenging Activity *In-Vitro* by *Averrhoa carambola* stem ethanolic extract.

Sl. No.	Concentration (µg/ml)	Absorbance			% Inhibition (mean ±SD)	IC_{50} (µg/ml)
		1	2	3		
Control Blank						
1.	Control Blank	1.32				-
Std (Ascorbic Acid)						
1	12.5	0.97	0.98	0.95	26.51±0.28	41.88
2	25	0.78	0.77	0.80	40.90±1.21	
3	50	0.47	0.53	0.44	64.39±1.23	
4	100	0.26	0.27	0.26	80.05±0.25	
ACSEE						
1	50	0.79	0.76	0.80	40.65±1.57	83.29
2	100	0.58	0.59	0.58	55.81±0.25	
3	250	0.43	0.41	0.38	69.19±1.90	
	500	0.24	0.23	0.20	83.08±1.58	

ACSEE= Averrhoa Carambola stem ethanolic extract.

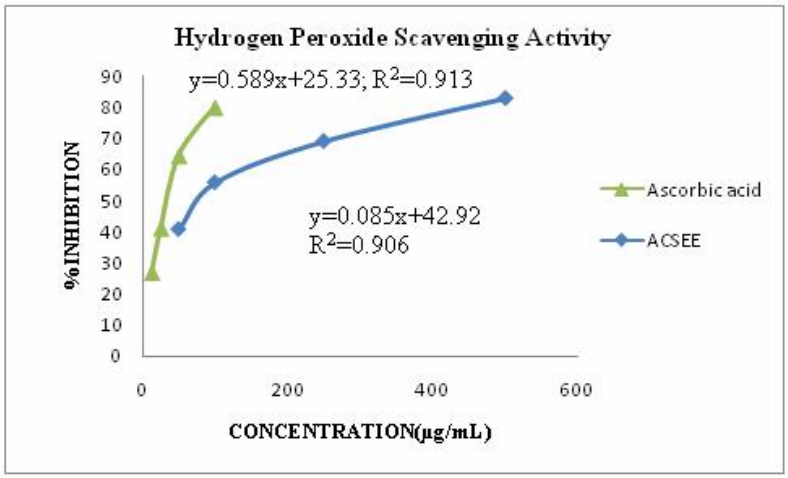

Figure 3: *In Vitro* Concentration Dependent Percentage Inhibition of Hydrogen PeroxideRadical Scavenging Activity by Ethanolic extract of *Averrhoa carambola* stem and Ascorbic acid.

Table 4: Percentage Inhibition and IC_{50} Values of Reducing Power Assay *In-Vitro* by*Averrhoa carambola* stem ethanolic extract.

Sl. No.	Concentration (µg/ml)	Absorbance			% Inhibition (mean ±SD)
		1	2	3	
Std (Ascorbic Acid)					
1	12.5	0.23	0.20	0.27	0.23±0.03
2	25	0.48	0.44	0.46	0.46±0.02
3	50	0.77	0.79	0.82	0.79±0.02
4	100	1.22	1.18	1.15	1.18±0.03
ACSEE					
1	50	0.50	0.47	0.48	0.48±0.01
2	100	0.65	0.67	0.62	0.65±0.02
3	250	0.91	0.96	0.93	0.93±0.02
4	500	1.04	1.06	1.12	1.07±0.02

ACSEE=*Averrhoa carambola* stem ethanolic extract.

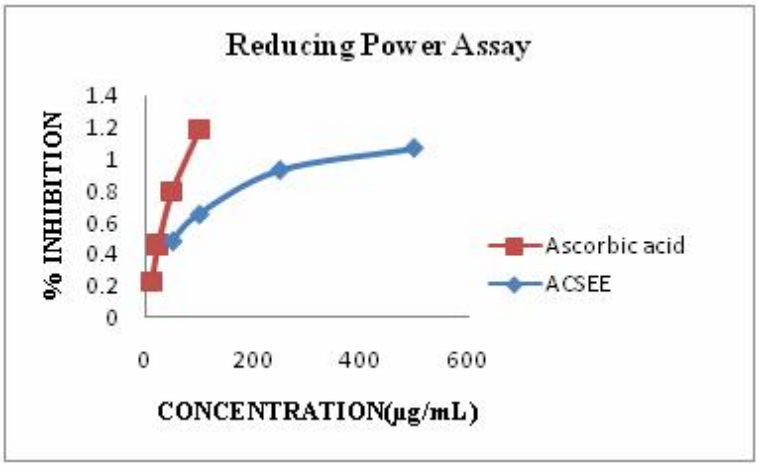

Figure 4: *In Vitro* Concentration Dependent Percentage Inhibition of Ethanolic extract of *Averrhoa carambola* stem and Ascorbic acid by Reducing power Assay.

Conclusion:

Presentstudy conclude that *Averrhoa carambola* stem ethanolic extract posses antioxidant activity and presence of phytoconstituents like saponins, alkaloids, flavanoids and tannins in extract as reported previously.The active constituents alone or in combination may be responsible for the antioxidant activity. Further systematic study of ethanolic extract of the stem of *Averrhoa carambola* might result in isolation of components, actually responsible for antioxidant activity.

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