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COMPARATIVE STUDY ON PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY ON ETHANOLIC EXTRACT OF *ACTINIDIA DELICIOSA* AND *VITIS VINFERA*

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Abstract:
The present study was undertaken to investigate in vitro antioxidant activity of alcoholic extract of Actinidia Deliciosa And Vitis Vinfera.
Method and methodlogy: *The total Phenolic content was determined using folinciocalteau method while the total flavanoid content was determined using aluminum chloride method. In vitro antioxidant activity was evaluated using the Reducing power assay , Hydrogen peroxide scavenging assay, nitric oxide scavenging activity, and DPPH scavenging activity.*
Result: *In the present study we have conclude that Vitis Vinfera, has a significant activity than Actinidia Deliciosa*
Key words: *Actinidia Deliciosa, Vitis Vinfera, DPPH Scavenging Activity, Nitricoxide Radical Scavenging Activity, Hydrogen Peroxide Scavenging Activity.*

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INTRODUCTION:

Oxygen is an indispensible element for the sustenance of living beings and many biological systems. Cells reduce oxygen and generate adenosine triphosphate (ATP) in the mitochondria. Byproducts known as free radicals are created during this process. These free radicals are beneficial in moderate levels but at higher concentrations can damage tissues by oxidative stress. Since more than half a century the deleterious effects of these reactive species are known but in the last two decades a lot of work has been done in this area. The important role played by anti oxidants in providing protection cannot be underestimated. Antioxidants are increasingly being used to prevent and also repair the damage caused by these free radicals.

A free radical may be defined as a molecule or molecular fragment containing one or more unpaired electrons in its outermost atomic or molecular orbital. These when formed can be highly reactive and can start a chain reaction [1]. The sources of free radicals can be endogenous and exogenous in nature. Endogenous sources of free radicals are intracellularly generated from auto-oxidation or inactivation of small molecules. Exogenous sources of free radicals are tobacco smoke, certain pollutants, organic solvents, anesthetics and pesticides. The sites of free radical generation encompass all cellular constituents including mitochondria, lysosomes, peroxisomes, endoplasmic reticulum, plasma membrane and sites within the cytosol [2]. Apart from this, certain medications metabolized to free radical intermediate products also cause oxidative damage within the target tissues. Exposure to radiation results in the formation of free radicals within the target tissues [3].

In Ayurveda, it is used in a Rasayana formula sometimes with other mild sours and shatavari (Asparagus racemosus) and guduchi (Tinospora cordifolia). In this oriental system of traditional medicine, varied properties are attributed to different parts of the tree, both as food and medicine.

Extracts of the bark, leaves, stems, and unripe fruits have demonstrated antibiotic properties in vitro, and are used in traditional medicine tree is considered to be sacred by Hindus. All parts of plants such as root, bark, leaves flowers and fruit are used for medicinal and worship purpose. It is widely found in the forest the leaves of plant are being offered to Gods as a part of prayers in marriage ceremony so the plant is known as Kalpavraksha. The fruit is eaten raw or ripen fresh or dried, fresh juice drink as sharbat and mango fruity. Raw fruit used for pickle, chatni and making curry, gulamba, kairi, amsur. Powder of seeds used by rural for bread. Wood of tree used for furniture, building, agriculture tools and shadow of tree is very cool. The Mango leaves used in marriage ceremony and in Gaudi Padwa (New Marathi year) The young leaves can be eaten row and used in several diseases such as burning sensation, diarrhoea, dysentery haemorrhoids, hiccough hyperdipsia, ulcer, kidney stone and wound. Leaves pest used for hair blackening, piles, jaundice, vomiting, urinary diseases, liver disorder constipation, it is also used as anti-microbial, liver disorder and in bloody dysentery. Root of plant can be used against diarrhoea, leucorrhoea, pneumonia, rheumatism. Inner bark and young leaves used by tribals against diabetes. Flowers of plant used as anorexia, dyspepsia, ulcer and blood purification. Fruits raw as well as mature can be used in sunstroke, opthalmia, eruption, intestinal disorder, in fertility, night blindness, the oil used in eczema.Seed used in heart problem, amebiosis, carminative, nasal bleeding. It is also used in liver disorder, teeth diseases, acidity, uterus problem, and fistula it used against poisonous biting such as scorpion, makadi, honeybee etc. [4-6].

METHODS AND MATERIALS:

Chemicals required:

Potassium ferric cyanide , trichloro acetic acid , ferric chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, hydrogen peroxide, Ascorbic Acid, acetic acid (glacial), pyridine, sodium nitropursside, sulfanilic acid, N-(1-Naphthyl)ethylenediaminedihydrochloride sodium hydroxide, Gallic acid, sodium carbonate, folinciocalteau reagent , Aluminium chloride, sodium nitrite, catechin, distilled water etc.

Essential instruments: UV- VISIBLE Spectrophotometer, pH meter, Incubator, homogenizer, water bath, heating mantle, centrifuge, refrigerator, weighing balance etc.

Glassware: Test tubes, conical flask, pipettes, beakers, stirrer, measuring cylinder, funnel, centrifuge tubes, Reagent bottles etc

Miscellaneous: Test tube stand, test tube holders, filter paper, butter paper, spatula, thermometers, stands, tissue paper, zip pouches, markers, gloves, labels, cotton swabs, disinfectant etc.

Collection and Authentication of Plant Material

The plant material *Actinidia Deliciosa* and *Vitis Vinfera.* were collected in the month of December - 2017 from local market,madinaguda in Hyderabad.

Preparation of Ethanolic Extract

Method: The Ethanolic extract of the plant was prepared using reflex condensation process. The fresh fruit about 200g was weighed and placed in a 500 ml round bottom flask with 200ml of ethanol and it's refluxed for 8 hrs at 40°c . Then suspension was filtered through a fine muslin cloth. The solvent was evaporated by heating until ¾ is reduced. The remaining solvent is evaporated under room temperature. A semisolid residue was obtained.

Phytochemical Evaluation: 500 mg of the dried extract were reconstituted in 10 ml of respective solvents and used for preliminary phytochemical testing for the presence of different chemical groups of compounds. Carbohydrates,Glycoside,Saponins, Alkaloids Phytosterols, Fixed Oils, Gums and Mucilage, Proteins, Phenolic compounds and Tannins, Flavonoids

Determination of Total Phenolic Content [7]:
Total Phenolic content of the extract was determined by Folin ciocalteau reagent according to Singleton and Rossi using Gallic acid as a standard. 0.1ml (100 µg) of sample solution was made up to 3ml using distilled water. About 0.5ml of Folin ciocalteau reagent was added and mixed thoroughly. Incubated for 3min at room temperature. After incubation 3ml of 20% Na_2CO_3 was added and mixed thoroughly, incubated in boiling water bath for 1 min. the absorbance was measured at 650nm. The concentration of total phenols was expressed in terms of mg of Gallic Acid equivalents per gram of extract.

Determination of Total Flavanoid Content [8]:
Total Flavanoid assay was measured by the aluminum chloride colorimetric assay. An Aliquot (1ml) of extracts or standard solution of catechin (20, 40, 60, 80 and 100µg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To the flask was added 0.3ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6th min, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled H2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/ g of extract. Samples were analyzed in duplicates.

***In vitro* antioxidant activity:**
Ferric Reducing Power: [9]
The reducing power was determined according to the method of Oyaizu. Different concentrations of the extract (50, 100,150, 200, 250 µg/ml) prepared in methanol were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferric cyanide {$K3Fe(CN)_6$} (2.5ml, 1%) . The mixture was incubated at 50°C for 20 min and 2.5ml of tricholoroaceticacid (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10min. the upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased Absorbance of the reaction mixture indicated increased reducing power. Ascorbic Acid was used as Standard.

Hydrogen Peroxide Scavenging Activity: [10]
The H_2O_2 scavenging ability of the extract was determined according to the method of Ruch et al. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). 100, 200,300,400,500 µg/ml concentrations of extract in 3.4ml Phosphate buffer were added to H_2O_2 solution (0.6ml, 40mM). The absorbance value of the reaction mixture was recorded at 230nm. The percent of scavenging of H_2O_2 was calculated by using the following equation.
% of scavenging = [(A of control – A of sample) / A of Control] X 100
Where A of control is the absorbance of the control reaction (containing all reagents except test compound) and a sample is the absorbance of the test compound. Test was carried out in triplicate.

Nitric Oxide Scavenging Activity: [11]
Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 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°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% glacial acetic 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 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

The nitric oxide radicals scavenging activity was calculated according to the following equation:
% Inhibition = $[(A_0-A_1) / A_0] \times 100)$
Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

DPPH free radical scavenging activity:
The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity (Braca et al., 2002). Ethanolic solution of DPPH (0.05 mM) (300 l) was added to 40 l of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation (Yen and Duh, 1994)

RESULTS AND DISCUSSIONS:

1. PERCENTAGE YIELD OF THE EXTRACT:

S.No	Name of The Plant	Percentage Yield (%)
1	*Actinidia Deliciosa*	13.1%
2	*Vitis Vinfera.*	10.6%

2. PHYTOCHEMICAL SCREENING:

s.no	Name of the plant	Alk	Carb	Gly	Tan	Phytos	Flav	sapo	Pro	muci
1	*Actinidia Deliciosa*	+	+	+	+	+	+	+	-	+
2	*Vitis Vinfera.*	+	+	+	+	+	+	-	+	+

The above table indicates the presence (+) or absence (-) of phytochemicals in ethanolic extract(Alk:Alkaloids , Carb:Carbohydrates , Gly:Glycosides, Tan:Tannins, Phtos:Phytosterol,Flav:Flavanoids , Sapo:Saponins , Pro:Proteins , Muci:Mucilages

3. TOTAL PHENOLIC CONTENT

Data showing absorbance of various concentration of Gallic acid

Standard (Gallic acid) Calibration curve	
Concentration (µg/ml)	Absorbance
10	**0.184**
20	**0.214**
30	**0.244**
40	**0.273**
50	**0.304**
60	**0.334**
70	**0.364**
80	**0.414**

Sample	
Concentration (100µg/ml)	Absorbance
Actinidia Deliciosa	0.166
Vitis Vinfera.	0.184

4.TOTAL FLAVANOID CONTENT

Data showing absorbance of various concentration of Catechin.

Catechin Standard curve	
Concentration (µg/ml)	Absorbance
10	**0.060**
20	**0.113**
30	**0.166**
40	**0.219**
50	**0.272**

Sample Solution	
Actinidia Deliciosa	0.138
Vitis Vinfera.	0.115

5. FERRIC REDUCING POWER

Data showing absorbance of various concentrations of extracts of Wheat grass and standard on ferric reducing power treatment

Standard (Ascorbic Acid)				
Concentration (µg/ml)	Absorbance at 700nm			Mean
50	0.329	0.287	0.310	0.309
100	0.388	0.378	0.245	0.337
150	0.391	0.398	0.400	0.396
200	0.578	0.585	0.587	0.583
250	0.822	0.820	0.828	0.823
Actinidia Deliciosa				
Concentration (µg/ml)	Absorbance at 700nm			Mean
50	0.421	0.424	0.423	0.423
100	0.485	0.496	0.500	0.495
150	0.508	0.533	0.522	0.519
200	0.556	0.561	0.562	0.561
250	0.578	0.598	0.595	0.590
Vitis Vinfera.				
Concentration (µg/ml)	Absorbance at 700nm			Mean
50	0.280	0.280	0.290	0.283
100	0.427	0.432	0.434	0.431
150	0.334	0.335	0.334	0.334
200	0.605	0.603	0.605	0.605
250	0.760	0.766	0.763	0.763

6. HYDROGEN PEROXIDE:

Data showing absorbance of various concentrations of extract and standard on HYDROGEN PEROXIDE treatment

Standard (Ascorbic Acid)				
Concentration (µg/ml)	Absorbance at 700nm			Mean
100	0.225	0.220	0.212	0.219
200	0.222	0.224	0.223	0.223
300	0.314	0.314	0.314	0.314
400	0.391	0.380	0.390	0.387
500	0.452	0.445	0.429	0.442
Actinidia Deliciosa				
Concentration (µg/ml)	Absorbance at 700nm			Mean
100	0.220	0.217	0.215	0.217
200	0.337	0.341	0.331	0.336
300	0.384	0.373	0.371	0.376
400	0.406	0.400	0.404	0.403
500	0.477	0.483	0.491	0.484
Vitis Vinfera.				
Concentration (µg/ml)	Absorbance at 700nm			Mean
100	0.014	0.012	0.017	0.015
200	0.056	0.062	0.056	0.058
300	0.083	0.091	0.087	0.087
400	0.107	0.111	0.111	0.110
500	0.124	0.119	0.120	0.121

Data showing absorbance of various concentrations of extract and standard on HYDROGEN PEROXIDE treatment

PERCENTAGE INHIBITION:

CONC	**Ascorbic Acid**	*Actinidia Deliciosa*	*Vitis Vinfera.*
100	94.70	72.63	98.10
200	89.65	57.62	92.68
300	84.48	52.58	89.02
400	62.42	49.18	86.12
500	59.14	38.96	84.74

7.NITRIC OXIDE:

Data showing absorbance of various concentrations of extracts on nitric oxide reducing power treatment

Standard (Ascorbic Acid)				
Concentration (µg/ml)	Absorbance at 700nm			Mean
25	0.036	0.027	0.032	0.032
50	0.089	0.083	0.079	0.084
75	0.142	0.138	0.143	0.141
100	0.302	0.305	0.309	0.305
125	0.486	0.487	0.482	0.485
Actinidia Deliciosa				
Concentration (µg/ml)	Absorbance at 700nm			Mean
25	0.306	0.297	0.294	0.295
50	0.315	0.312	0.309	0.311
75	0.346	0.343	0.341	0.343
100	0.378	0.378	0.376	0.377
125	0.393	0.395	0.396	0.395
Vitis Vinfera.				
Concentration (µg/ml)	Absorbance at 700nm			Mean
25	0.092	0.091	0.090	0.091
50	0.100	0.085	0.076	0.087
75	0.185	0.181	0.189	0.185
100	0.253	0.253	0.254	0.254
125	0.352	0.348	0.343	0.348

8.DPPH:

Data showing absorbance of various concentrations of extracts

Standard (Ascorbic Acid)				
Concentration (µg/ml)	Absorbance at 700nm			Mean
100	0.034	0.034	0.034	0.034
200	0.371	0.368	0.365	0.368
300	0.465	0.478	0.480	0.474
400	0.569	0.581	0.571	0.574
500	0.671	0.672	0.673	0.672
Actinidia Deliciosa				
Concentration (µg/ml)	Absorbance at 700nm			Mean
100	0.085	0.085	0.085	0.085
200	0.085	0.085	0.084	0.085
300	0.132	0.142	0.134	0.136
400	0.146	0.157	0.152	0.151
500	0.340	0.254	0.249	0.281
Vitis Vinfera.				
Concentration (µg/ml)	Absorbance at 700nm			Mean
100	0.120	0.138	0.143	0.134
200	0.328	0.326	0.327	0.327
300	0.436	0.431	0.428	0.432
400	0.577	0.576	0.576	0.576
500	0.524	0.527	0.527	0.526

DISCUSSIONS:

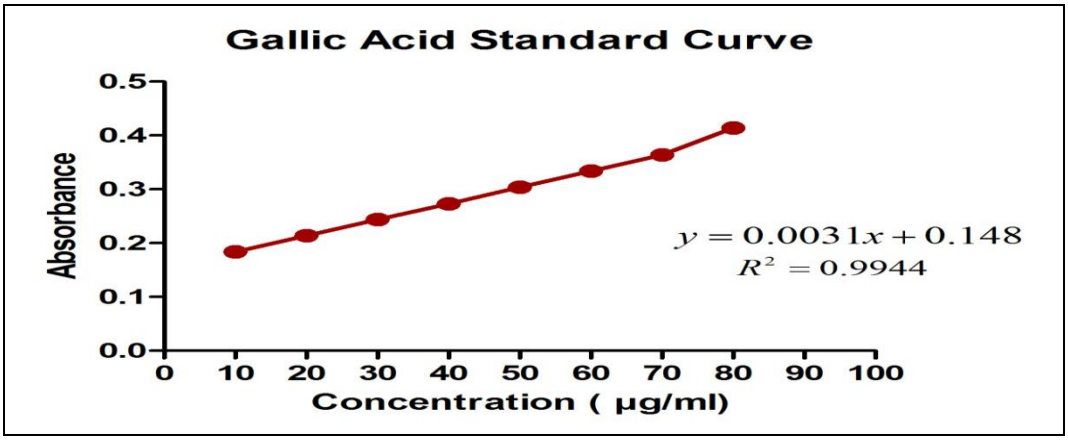
From the table -1 we have come to know the percentage yield of the ethanolic herbal extract were obtained in which the *Actinidia Deliciosa* is having highest yield is about 13.1 % and the lowest is *Vitis Vinfera*. is about 10.6%.

The above table 2 indicates the presence of phytochemicals in ethanolic extract:Alkaloids , Carbohydrates , Glycosides, Tannins, Phytosterol,Flavanoids ,Proteins , Mucilages but absent of Saponins in *Vitis Vinfera*.

The above table 2 indicates the presence of phytochemicals in ethanolic extract:Alkaloids , Carbohydrates , Glycosides, Tannins, Phytosterol,Flavanoids , Saponins , Mucilages but absent of Proteins in *Actinidia Deliciosa*

TOTAL PHENOLIC CONTENT:

Graph 1



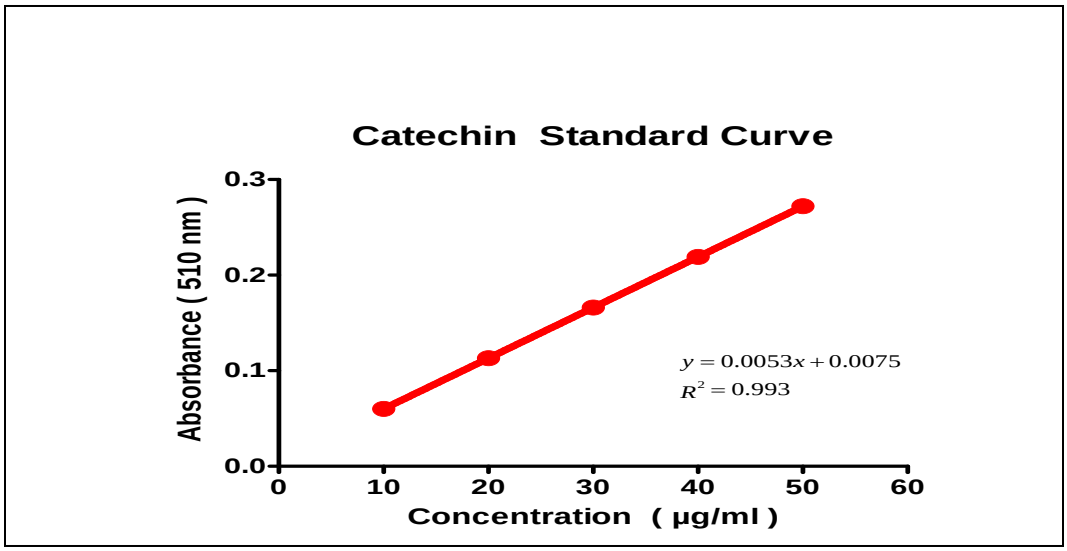
From the Standard Graph of Gallic Acid, The total phenol concentration present in the *Actinidia Deliciosa and Vitis Vinfera* was found to be:

Actinidia Deliciosa: **58 mg GAE/ g of extract**

*Vitis Vinfera***: 116.1 mg GAE / g of extract**

TOTAL FLAVANOID CONTENT:

Graph 2



From the Standard Graph of Catechin, The total flavanoid concentration present in the *Actinidia Deliciosa and Vitis Vinfera* extract was found to be:
Actinidia Deliciosa: **202.8 mg of CE/ g of extract**
Vitis Vinfera: **242.6 mg of CE / g of extract**

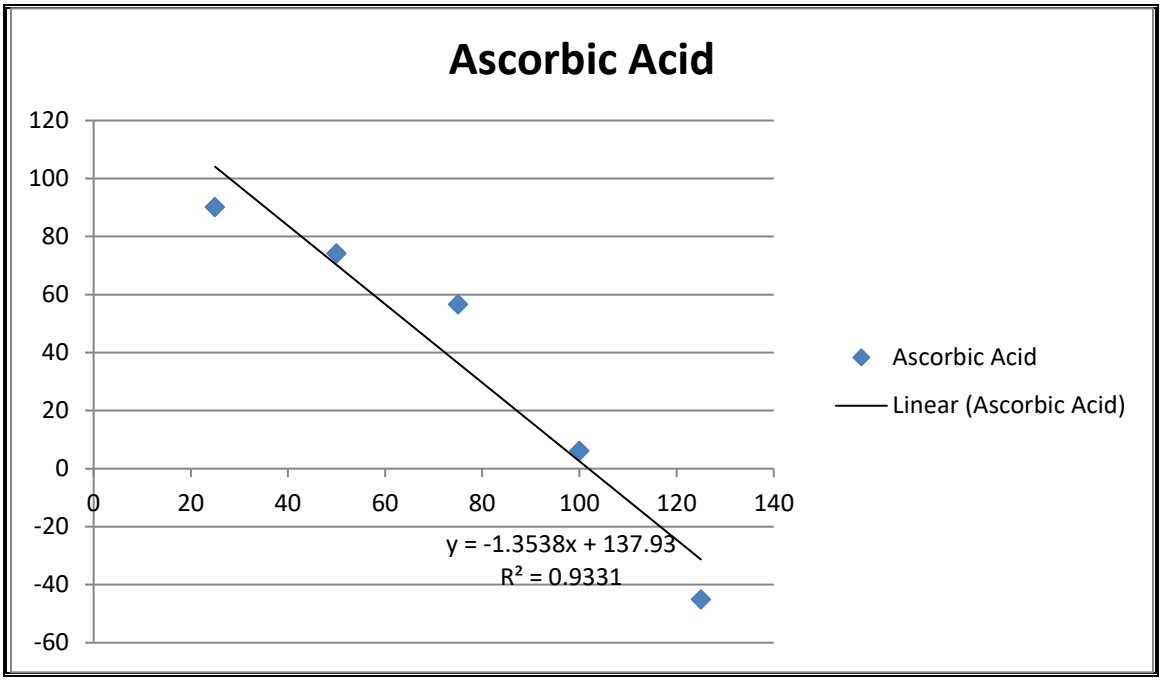
Ferric Reducing Activity:
The reducing power has been used as one of the important antioxidant capabilities for medicinal herbs. The reducing power of ACTINIDIA DELICIOSA and VITIS VINFERA of alcoholic extract of was dose-dependent. The absorbance increases with increase in the concentration. From the above graph it can be inferred that the increase in ferric reducing activity was more for VITIS VINFERA alcoholic extract then the ACTINIDIA DELICIOSA extract.

Hydrogen peroxide:
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 H_2O_2 that is allowed to accumulate. As shown in the above graph, the ACTINIDIA DELICIOSA and VITIS VINFERA has demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. The decomposition of H_2O_2 by the extract may at least partly result from its antioxidant and free radical scavenging activity. The activity was higher for VITIS VINFERA when compared to ACTINIDIA DELICIOSA and was comparable to that of standard i.e. ascorbic acid.

Nictric oxide:
PERCENTAGE INHIBITION:

CONC	**Ascorbic Acid**	ACTINIDIA DELICIOSA	*Vitis vinfera*
25	90.15	9.23	72
50	74.15	4.30	73.23
75	56.61	-5.53	43.07
100	6.15	-16	21.84
125	-45.07	-21.53	-7.07

IC 50:
ASCROBIC ACID:



NAME OF EXTRACT	**IC50 µg/ml**
Ascorbic Acid	64.96
ACTINIDIA DELICIOSA	-95.90
Vitis vinfera	63.72

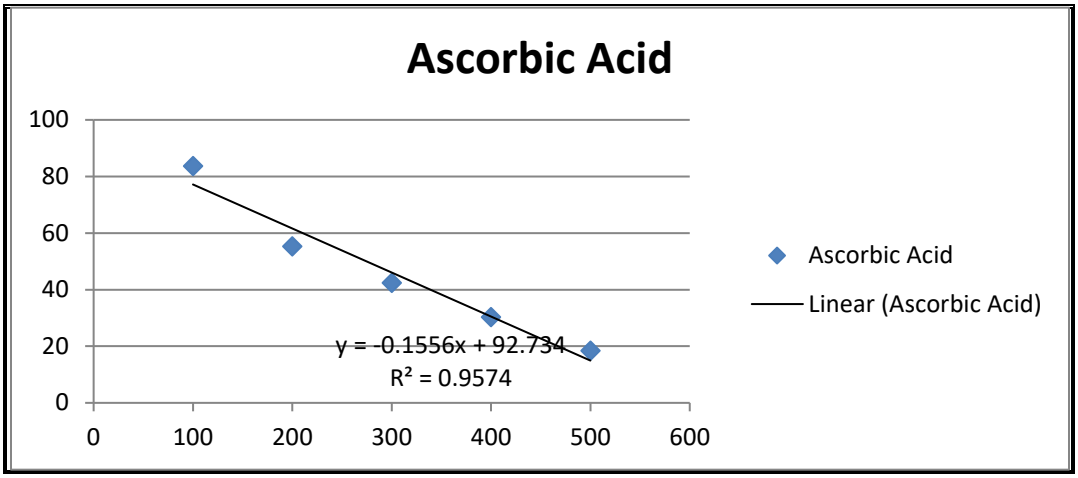
Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO_2, N_2O_4, N_3O_4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage. VITIS VINFERA have good activity which was near to the standard ascorbic acid where as actinidia deliciosa doesn't show any activity.

DPPH:

PERCENTAGE INHIBITION:

CONC	**Ascorbic Acid**	actinidia deliciosa	*Vitis vinfera*
100	83.73	89.68	83.73
200	55.33	89.68	60.31
300	42.47	83.49	47.57
400	30.33	81.67	30.09
500	18.44	65.89	36.16

Ic 50 : Ascorbic acid:



NAME OF EXTRACT	**IC50 µg/ml**
Ascorbic Acid	275.67
actinidia deliciosa	886.36
Vitis vinfera	313.44

DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole (Fig. 1), so that the molecules do not dimerise, like most other free radicals. The delocalisation also gives rise to the deep violet colour, with an absorption in ethanol solution at around 520 nm. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour. By the results we can say that the actinidia deliciosa have more activity than vitis vinfera.

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