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PHYTOCHEMICAL EVALUATION, STANDARDIZATION AND ANTIOXIDANT ACTIVITY OF *Punica granatum* AND *Psidium guajava*

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Abstract
In current study, we accepted out a systematic record of the relative antioxidant activity in selected medicinal plant species extracts. The total ash value, loss on drying, water insoluble ash, acid insoluble ash, water extractive value and alcoholic extractive value was found to 5.37%, 5.08%, 1.394%, 3.98%, 9.94% and 4.76 respectively. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging result of the extracts was determined spectrophotometrically. The maximum radical scavenging consequence was observed in ethyl acetate extract of *Punica granatum* with IC_{50} = 49.45 mg ml^{-1}. The strength of radical scavenging power of *Punica granatum* extract was found to be 45 mg ml^{-}1. better than synthetic antioxidant butylated hydroxy toluene (BHT). The superior amount of phytoconstituent compounds leads to further potent radical scavenging result as shown by *Punica granatum* leaves extract.

Introduction
Free radicals donate to more than one hundred disorders in humans counting atherosclerosis, arthritis, ischemia and reperfusion damage of numerous tissues, central nervous system injury, gastritis, cancer and AIDS. Free radicals due to ecological pollutants, radiation, chemicals, toxins, profound fried and spicy foods as well as corporeal stress, cause exhaustion of immune system antioxidants, modify in gene expression and persuade abnormal proteins. Oxidation development is one of the most imperative routs for producing free radicals in food, drugs and still living systems. Catalase and hydroperoxidase enzymes change hydrogen peroxide and hydroperoxides to nonradical forms and purpose as natural antioxidants in human body. Owing to depletion of immune system natural antioxidants in dissimilar maladies, overwhelming antioxidants as free radical scavengers may be essential. At present available synthetic antioxidants similar to butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been supposed to cause or punctual negative health effects. Consequently, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and reasonable antioxidant activity (Barlow, 1990; Branen, 1975). Recently there has been an increase of interest in the therapeutic potentials of medicinal plants as antioxidants in dropping such free radical induced tissue injury. Polyphenolic compounds with known properties which include free radical scavenging, inhibition of

hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). A number of confirmations suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al.,* 1987). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the occurrence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al.,* 2002). In particular, despite extensive use of wild plants as medicines in Iran, the prose contains few reports of antioxidant activity and chemical composition of these plants. In current study, we carried out a systematic record of the relative free radical scavenging activity in selected medicinal plant species, which are being used traditionally: The leaves of *Metha Pulegiam* (Lamiacea) and seeds of *Pargularia deamia* (Apocynaceae). We have also found the relationship of total flavonoid and phenol contents with antioxidant activity. In the longer term, plant species (or their active constituents) recognized as having high levels of antioxidant activity *in vitro* may be of value in the design of additional studies to unravel novel treatment strategy for disorders connected with free radicals induced tissue damage. Besides well-known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements (Schuler, 1990). Also many other plant species have been investigated in the search for novel antioxidants (Chu, 2000; Koleva *et al.,* 2002; Mantle *et al.,* 2000; Oke and Hamburger, 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996).

Materials and Methods

Cold Maceration:

Plant material was extracted by using cold maceration method; plant samples were collected, washed, rinsed and dried properly. Powder form of plant sample was extracted with different organic solvents (petroleum ether, ethyl acetate, and methanol) and allow standing for 4-5 days each. The extract was filtered using filter paper to remove all non-extractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated; excessive moisture was removed and extract was collected in air tight container **(Kokate *et al.,* 2006)**. Extraction yield of all extracts were calculated using the following equation below:

$$\textbf{Percentage Yield} = \frac{\textbf{Actual yield}}{\textbf{Theoretical yield}} \times 100$$

Pharmacognostical evaluation

Total ash value:

About 5 g each of powdered parts were accurately weighed and taken separately in silica cruc ible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder **(Gami and Parabia, 2010).**

$$\textbf{\% Ash content} = \frac{\textbf{Weight of crucible + ash – Weight of crucible}}{\textbf{Weight of crucible + sample - Weight of crucible}} \times 100$$

Loss on drying

Place 2 to 6 g of the sample into a weighing bottle which has been accurately weighed, and weigh it accurately. Then, dry it at 105 o C for 5 - 6 hours and cool it in desiccators with silica gel. When the material is dried to a constant weight, the percent of loss on drying is determined **(Shinners et al., 1991).**

$$\text{LOD \%} = \frac{\text{Wt. of petridish + crude drug - After drying Wt. of petridish + sample} \times 100}{\text{Weight of crude drug}}$$

Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water . The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted form the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively **(Gami and Parabia, 2010).**

$$\text{\% Water soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible} \times 100}{\text{Crude drug weight}}$$

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug **(Gami and Parabia, 2010).**

$$\text{\% Acid soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible} \times 100}{\text{Crude drug weight}}$$

Alcoholic extractive value

5g of powdered material was weighed into 250mL stopper conical flask containing 100mL of 90% ethanol and the stopper replaced. The flask and content was placed in a mechanical shaker for 6hrs and then allowed to stand for 18hrs. The mixture was filtered and 20mL of the filtrate was measured into an evaporating dish with a known weight, and evaporated to dryness. The constant weight of the residue was gotten after drying in the oven at 1050C for about 3 minutes **(Egharevba and Kunle et al., 2010).** The extractive value was calculated.

Water soluble extractive value = Weight of reside /Weight of the drug ×100

Water extractive value

The procedure was the same as above except that water used in place of 90% ethanol **(Egharevba and Kunle et al., 2010).**

$$\text{Water soluble extractive value} = \frac{\text{Weight of residue}}{\text{Weight of the drug}} \times 100$$

Qualitative phytochemical analysis of plant extract

Following standard methods by **Kokate, 1986** the *Punica granatum and Psidium guajava* extract obtained was subjected to the preliminary phytochemical analysis. The extract was screened to spot the presence or absence of many active constituents like carbohydrates, glycosides, phenolic compounds, alkaloids, flavonoids, saponins, fats or fixed oils, protein, amino acid and tannins.

Tests for Carbohydrates

Molish Test

In a test tube 2 ml of aqueous extract was added with 2 drops of alcoholic α-naphthol solution and then 1 ml of concentrated sulphuric acid was mixed carefully along the sides of the test tube. The presence of carbohydrates is indicated by the formation of violet ring at the junction.

Fehling's Test

1 ml of Fehling's A and 1 ml of Fehling's B solutions were added to 1 ml of aqueous extract in a test tube and for 10 minutes heated in the water bath. The presence of reducing sugar is indicated by formation of red precipitate.

Benedict's test

In a test tube equal volume of Benedict's reagent and extract were mixed and for 5-10 minutes heated in the water bath. Depending on the amount of reducing sugar present in the test solution solution turns green, yellow or red which depicts the presence of reducing sugar.

Tests for Alkaloids

Dilute hydrochloric acid was added to the extract, shake it well and filtered. The following tests were performed in the filtrate.

Mayer's Test

Few drops of Mayer's reagent were added to 2-3 ml of filtrate along the sides of test tube. Formation of white or creamy precipitate suggests the presence of alkaloids.

Hager's Test

Few drops of Hager's reagent were added to 1-2 ml of filtrate in a test tube. The presence of alkaloids is indicated by the formation of yellow color precipitate.

Wagner's Test

In a test tube added 1-2 ml of filtrate with few drops of Wagner's reagent. Formation of reddish-brown precipitate shows the presence of alkaloids.

Tests for Triterpenoids and Steroids:

Salkowski's Test

The extract was added with chloroform and filtered. The filtrate was then treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Sterol is present if the lower layers turn red. The presence of triterpenes is indicated by the presence of golden yellow layer at bottom.

Tests for Flavonoids

Lead Acetate Test

Few drops of lead acetate solution were added to the extract. Development of yellow precipitate may indicate the presence of flavonoids.

Alkaline Reagent Test

In a separate test tube the extract was treated with few drops of sodium hydroxide. Development of intense yellow colour, which becomes colourless on addition of few drops of dilute acid, shows the presence of flavonoids.

Tests for Tannin and Phenolic compounds

Ferric Chloride Test

Little amount of extract was dissolved in distilled water. 2 ml of 5% ferric chloride solution was added to this solution. Presence of phenolic compounds indicates the formation of blue, green or violet color.

Lead Acetate Test
In distilled water little amount of extract was dissolved. To this solution few drops of lead acetate solution was add on. Formation of white precipitate shows the presence of phenolic compounds.

Gelatine Test
Into the distilled water some quantity of extra
ct was dissolved. To this solution 2 ml of 1% gelatine solution containing 10% sodium chloride was added. Development of white precipitate depicts the presence of phenolic compounds.

Tests for Saponins
Froth Test
With the help of distilled water the extract was diluted and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for Fats and Oils
Solubility test
Add few ml of chloroform to 1-2 ml of the alcoholic solution of extract, and solubility was observed. Add few ml of 90% ethanol to 2-3 ml of the alcoholic solution of extract, and solubility was observed.

Tests for Protein and Amino acids
Biuret's Test
In a test tube the extract was added to 1 ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution was mixed to the above mixture. The production of violet or pink colour specifies the presence of proteins.

Ninhydrin Test
3 drops of 5% Ninhydrin solution was added to 3 ml of the test solution and heated in a water bath for 10 minutes. The presence of amino acids is indicated by the formation of blue colour.

Tests for Glycosides
Borntrager's Test
Dilute sulphuric acid was added to 3 ml of test solution, boiled for 5 minutes and filtered. Equal volume of benzene or chloroform was added to the cold filtrate and mixed it well. The organic solvent layer was isolated and ammonia was added to it. Presence of anthraquinone glycosides is confirmed by the formation of pink to red color in ammonical layer.

Legal's Test
In pyridine 1 ml of test solution was dissolved. To it 1 ml of sodium nitropruside solution was added and using 10% sodium hydroxide solution the solution was made alkaline. Formation of pink to blood red color shows the presence of Cardiac glycosides.

Keller-Killiani Test
In a test tube added 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride. By the side of the test tube mix carefully 0.5 ml of concentrated sulphuric acid. The presence of Cardiac glycosides is depicted by formation of blue color in the acetic acid layer.

Activity (In-vitro Anti-oxidant Activity)
DPPH Radical Scavenging Activity
a) Preparation of DPPH reagent
0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

b) Preparation of Sample/Standard

Freshly 1 mg/ml methanol solution of all extracts of *Punica granatum* and *Psidium guajava* standard was prepared. 1 mg of extracts/standard was taken with methanol to make 1mg/ml stock solution. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature.

C) Preparation of control

For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm **(Athavale *et al.*, 2012).**

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Superoxide anion radical scavenging activity

1 ml of nitroblue tetrazolium (NBT) (100 µl of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH (468 µl in 100 mM phosphate buffer, pH 7.4), solution as well as varying volumes of extracts of *Punica granatum* and *Psidium guajava* (*sample*) (20, 40, 60, 80 and 100 µg/ml), were mixed well with methanol. The reaction was started by the addition of 1 ml of phenazine methosulfate (PMS) (60 µl/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30°C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the sample (extract) was used as a blank sample. Ascorbic acid was used as the standard in comparing the different sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity **(Nishikimi *et al.*, 1972).**The percentage scavenging was calculated by using the formula shown below:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100 µg/ml.

Preparation of extracts of Punica granatum and Psidium guajava seeds

Stock solutions of extracts of *Punica granatum* and *Psidium guajava* were prepared by dissolving 10 mg of dried extracts in 10 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the standard and extracts of *Punica granatum* and *Psidium guajava* (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2201). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard **(Quisumbing, 1978).**

Results

Pharmacognostical evalution

Total ash value

$$\text{\% Ash content} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Weight of crucible + sample - Weight of crucible}} \times 100$$

$$= \frac{22.10 - 21.83}{26.85 - 21.83} \times 100 = 5.37\ \%$$

Loss on drying

$$\text{LOD \%} = \frac{\text{Weight of petri dish + crude drug - After drying Weight of petridish + sample}}{\text{Weight of crude drug}} \times 100$$

$$= \frac{31.640 - 31.509}{2.578} \times 100$$

LOD%= 5.081

Water soluble ash

$$\text{\% Water soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

$$= \frac{21.99 - 21.83}{5.02} \times 100$$

$$= 1.394\ \%$$

Acid insoluble ash

$$\text{\% Acid soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

$$= \frac{22.03 - 21.83}{5.04} \times 100$$

$$= 3.98\ \%$$

Water extractive value

Water extractive value = Weight of reside /Weight of the drug × 100

= 0.487/5 × 100 = 9.74 %

Alcoholic extractive value

Alcohol extractive value = Weight of reside /Weight of the drug ×100

= 0.238/5 × 100 = 4.76 %

Table 1: Pharmacognostical evaluation of *Psidium guajava*:

S. No.	Parameters	Result
1.	Total ash value	5.37 %
2.	Loss on drying	5. 081 5
3.	Water soluble ash	1.394 %
4.	Acid soluble ash	3.98 %
5.	Water extractive value	9.74 %
6.	Alcoholic extractive value	4.76 %

Total ash value

$$\text{\% Ash content} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Weight of crucible + sample - Weight of crucible}} \times 100$$

$$= \frac{22.001 - 21.83}{26.85 - 21.83} \times 100 = 3.40\ \%$$

Loss on drying

$$\text{LOD \%} = \frac{\text{Weight of petridish + crude drug - After drying Weight of petridish + sample}}{\text{Weight of crude drug}} \times 100$$

$$= \frac{28.782 - 28.59}{3.14} \times 100$$

LOD%= 6.11

Water soluble ash

$$\text{\% Water soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

$$= \frac{21.99 - 21.83}{5.02} \times 100$$

$$= 3.18\ \%$$

Acid insoluble ash

$$\text{\% Acid soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

$$= \frac{21.854 - 21.83}{5.02} \times 100$$

$$= 0.478\ \%$$

Water extractive value

Water extractive value = Weight of reside /Weight of the drug × 100

= 0.558/5 × 100 = 11.16 %

Alcoholic extractive value

Alcohol extractive value = Weight of reside /Weight of the drug ×100

= 0.358/5 × 100 = 7.16 %

Table 2: Pharmacognostical evaluation of *Punica granatum*:

S. No.	Parameters	Result
1.	Total ash value	3.40 %
2.	Loss on drying	6.11 %
3.	Water soluble ash	3.18 %
4.	Acid soluble ash	0.47 %
5.	Water extractive value	11.16 %
6.	Alcoholic extractive value	7.16 %

Table 3: Percentage yield of *Psidium guajava* extract

S. No	Solvent	Color of extract	Theoretical Yield	Actual Yield	% Yield
1	N-hexane	Green	526	2.030	0.385
2	Ethyl acetate	Green	517.33	1.28	0.247
3	Methanol	Brown	504.39	6.92	1.371

Table 4: Solubility determination of *Psidium guajava* extract

S. No.	Solvent	N- hexane	Ethyl acetate	Methanol
1	Water	Insoluble	Insoluble	Soluble
2	Methanol	Insoluble	Slightly Soluble	Soluble
3	Ethanol	Insoluble	Slightly Soluble	Soluble
4	Ethyl acetate	Slightly Soluble	Soluble	Slightly Soluble
5	N- hexane	Soluble	Slightly Soluble	Insoluble
6.	DMSO	Soluble	Soluble	Soluble

Phytochemical Analysis of *Psidium guajava* extract:

Phytochemical Analysis

Table 5: Qualitative Phytochemical Analysis

S. No.	Experiment	Results		
		N-hexane	Ethyl aetate	Methanol
Test for Carbohydrates				
1.	Molisch's Test	-	-	+
2.	Fehling's Test	-	-	+
3.	Benedict's Test	-	-	+
4.	Bareford's Test	-	-	+
Test for Alkaloids				
1.	Mayer's Test	-	+	+
2.	Hager's Test	-	+	+
3.	Wagner's Test	-	+	+
4.	Dragendroff's Test	-	+	+
Test for Terpenoids				
1.	Salkowski Test	-	+	+
2.	Libermann-Burchard's Test	-	+	+
Test for Flavonoids				
1.	Lead Acetate Test	-	+	+
2.	Alkaline Reagent Test	-	+	+
3.	Shinoda Test	-	+	+
Test for Tannins and Phenolic Compounds				
1.	$FeCl_3$ Test	-	+	+
2.	Lead Acetate Test	-	+	+
3.	Gelatine Test	-	+	+
4.	Dilute Iodine Solution Test	-	+	+
Test for Saponins				
1.	Froth Test	+	+	-
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	-	+
2.	Biuret's Test	-	-	+
3.	Million's Test	-	-	+
Test for Glycosides				
1.	Legal's Test	-	-	+
2.	Keller Killani Test	-	-	+

3.	Borntrager's Test	-	-	+

Table 6: Percentage yield of *Punica granatum* extract

S. No	Solvent	Color of extract	Theoretical Yield	Actual Yield	% Yield
1	N-hexane	Green	503.14	1.57	0.312
2	Ethyl acetate	Green	490.36	0.82	0.167
3	Methanol	Brown	472.59	4.71	0.996

Table 7: Solubility determination of *Punica granatum* extract

S. No.	Solvent	N-hexane	Ethyl acetate	Methanol
1	Water	Insoluble	Insoluble	Soluble
2	Methanol	Insoluble	Slightly Soluble	Soluble
3	Ethanol	Insoluble	Slightly Soluble	Soluble
4	Ethyl acetate	Slightly Soluble	Soluble	Slightly Soluble
5	N-hexane	Soluble	Slightly soluble	Insoluble
6	DMSO	Soluble	Soluble	Soluble

Phytochemical Analysis of *Punica granatum* extract:

Table 8: Qualitative Phytochemical analysis

S. No.	Experiment	Results		
		N-hexane	Ethyl acetate	Methanol
Test for Carbohydrates				
1.	Molisch's Test	-	-	+
2.	Fehling's Test	-	-	+
3.	Benedict's Test	-	-	+
4.	Bareford's Test	-	-	+
Test for Alkaloids				
1.	Mayer's Test	-	+	+
2.	Hager's Test	-	+	+
3.	Wagner's Test	-	+	+
4.	Dragendroff's Test	-	+	+
Test for Terpenoids				
1.	Salkowski Test	-	+	+
2.	Libermann-Burchard's Test	-	+	+
Test for Flavonoids				
1.	Lead Acetate Test	-	+	+
2.	Alkaline Reagent Test	-	+	+
3.	Shinoda Test	-	+	+
Test for Tannins and Phenolic Compounds				
1.	$FeCl_3$ Test	-	+	+
2.	Lead Acetate Test	-	+	+
3.	Gelatine Test	-	+	+
4.	Dilute Iodine Solution Test	-	+	+
Test for Saponins				

1.	Froth Test	+	+	-
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	-	+
2.	Biuret's Test	-	-	+
3.	Million's Test	-	-	+
Test for Glycosides				
1.	Legal's Test	-	+	+
2.	Keller Killani Test	-	+	+
3.	Borntrager's Test	-	+	+

Anti-oxidant activity

DPPH asssay

Table 9: DPPH activity of Ascorbic acid

Concentration	Absorbance	% Inhibition
20	0.461	52.12
40	0.400	58.46
60	0.357	62.92
80	0.273	71.65
100	0.134	86.08
Control	0.963	
IC50	**19.95**	

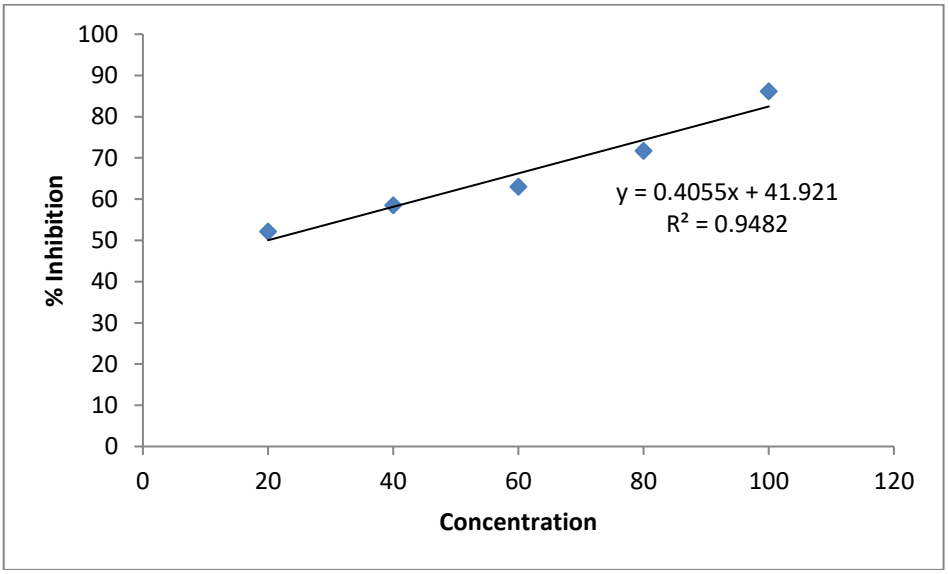

Fig 1: Graph represents the Percentage Inhibition *Vs* Concentration of ascorbic acid

Table 10: DPPH activity of *Punica granatum* Ethyl acetate extract

Concentration	Absorbance	% Inhibition
20	0.464	34.95
40	0.374	45.12
60	0.299	53.74
80	0.166	68.85
100	0.115	74.62
Control	0.878	
IC50	**49.45**	

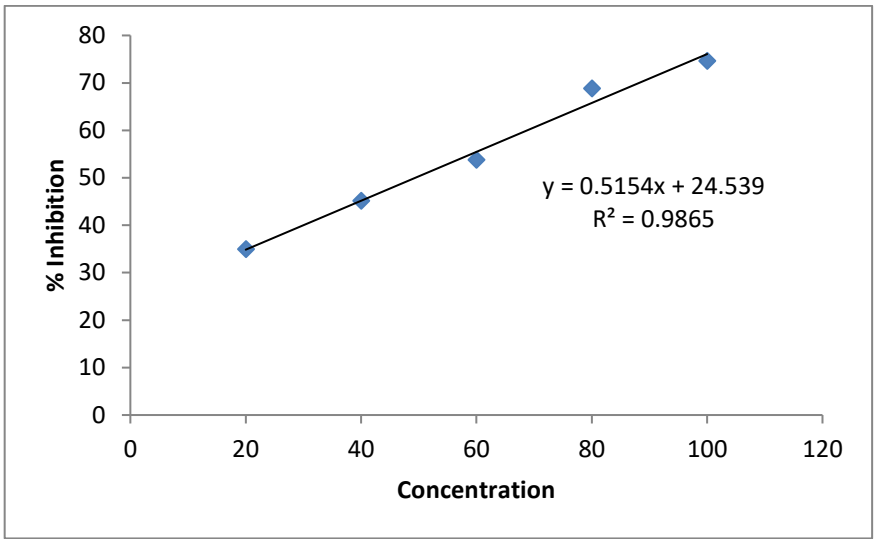

Fig. 2: Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 11: DPPH activity of *Punica granatum* Methanol extract

Concentration	**Absorbance**	**% Inhibition**
20	0.418	46.22
40	0.373	51.095
60	0.286	60.64
80	0.204	69.50
100	0.152	75.20
Control	0.916	
IC50	**32.49**	

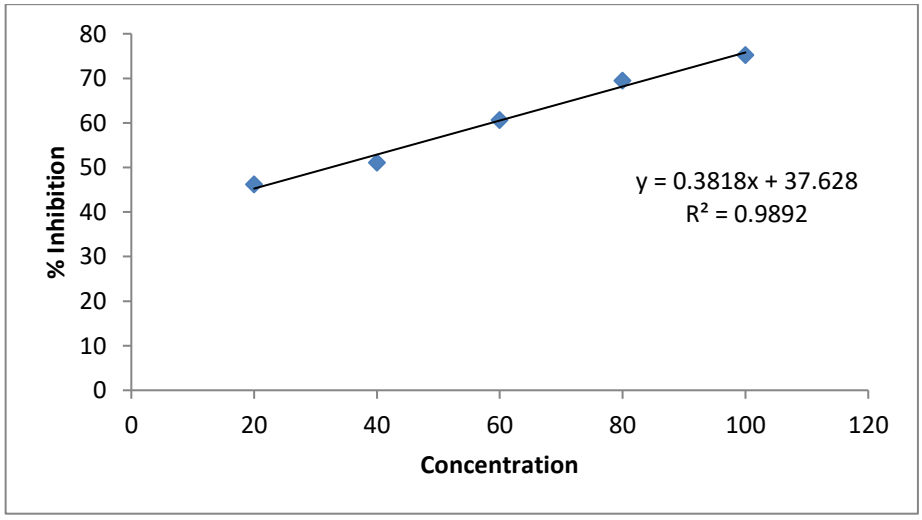

Fig. 3: Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract

Table 12: DPPH activity of *Psidium guajava* Ethyl acetate extract

Conc.	Absorbance	% Inhibition
20	0.585	41.08
40	0.517	47.93
60	0.403	59.41
80	0.334	66.36
100	0.258	74.01

Control	0.993	
IC50	**41.63**	

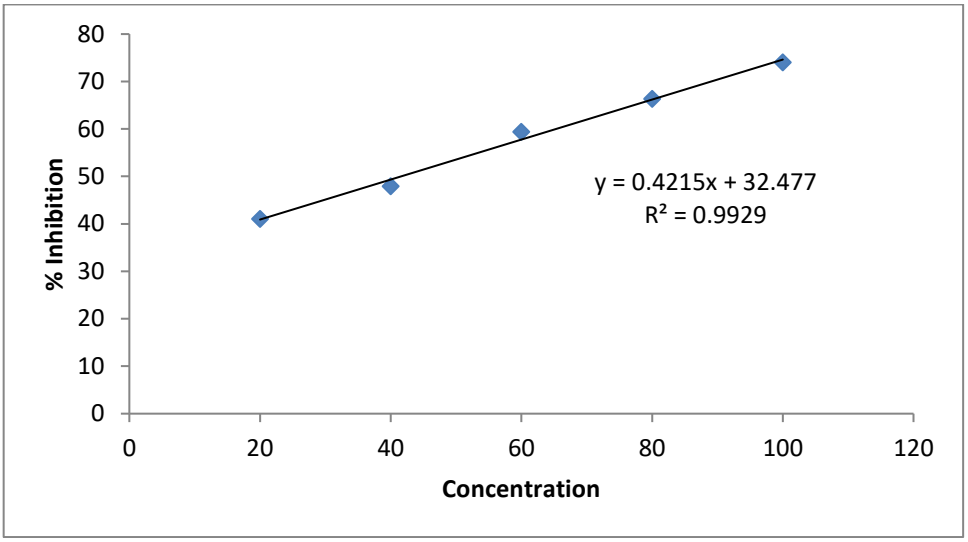

Fig. 4: Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 13: DPPH activity of *Psidium guajava* Methanol extract

Concentration	Absorbance	% Inhibition
20	0.343	51.17597765
40	0.286	57.47020484
60	0.177	69.72346369
80	0.134	74.49068901
100	0.100	78.25232775
Control	0.895	
IC50	**22.11**	

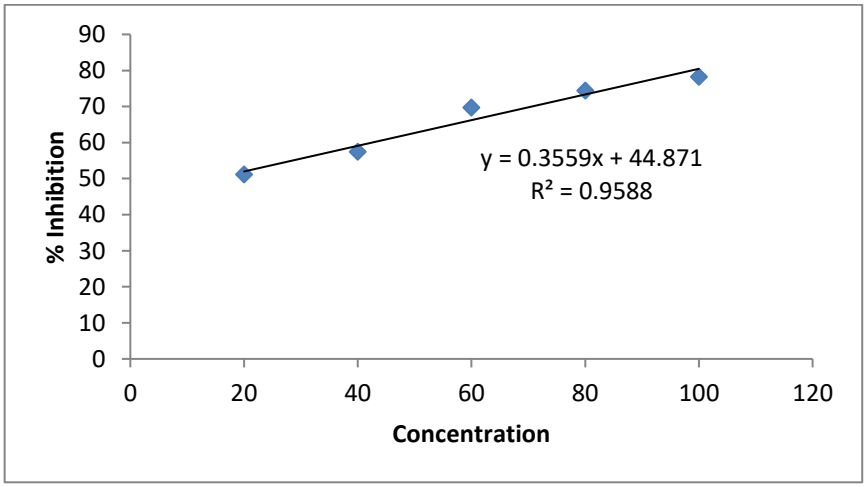

Fig. 5: Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract

Reducing power assay

Table 14: Reducing power activity of Ascorbic acid

Concentration	Absorbance
20	0.412
40	0.467
60	0.531

80	0.583
100	0.663

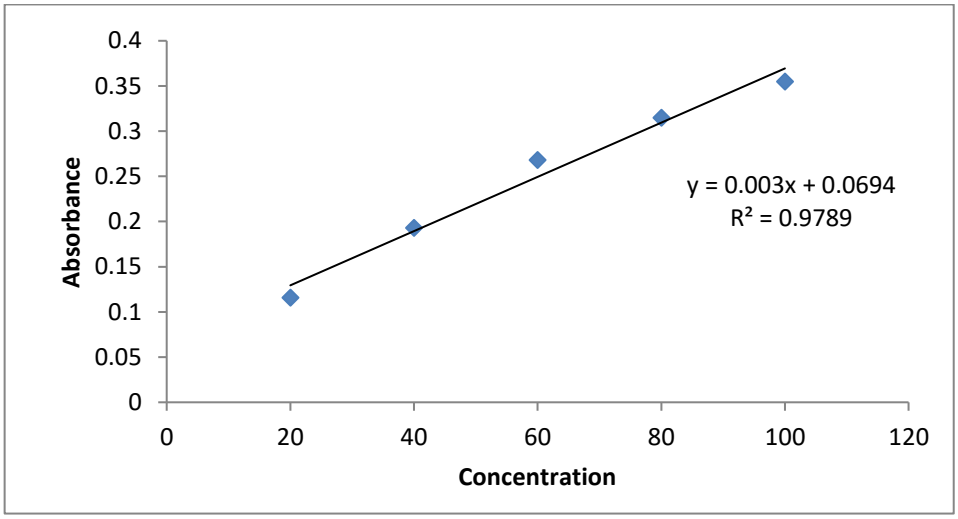

Fig. 6: Graph represents the Percentage Inhibition *Vs* Concentration of ascorbic acid

Table 15: Reducing power activity of *Punica granatum* Ethyl acetate extract

Concentration	Absorbance
20	0.042
40	0.094
60	0.123
80	0.179
100	0.214

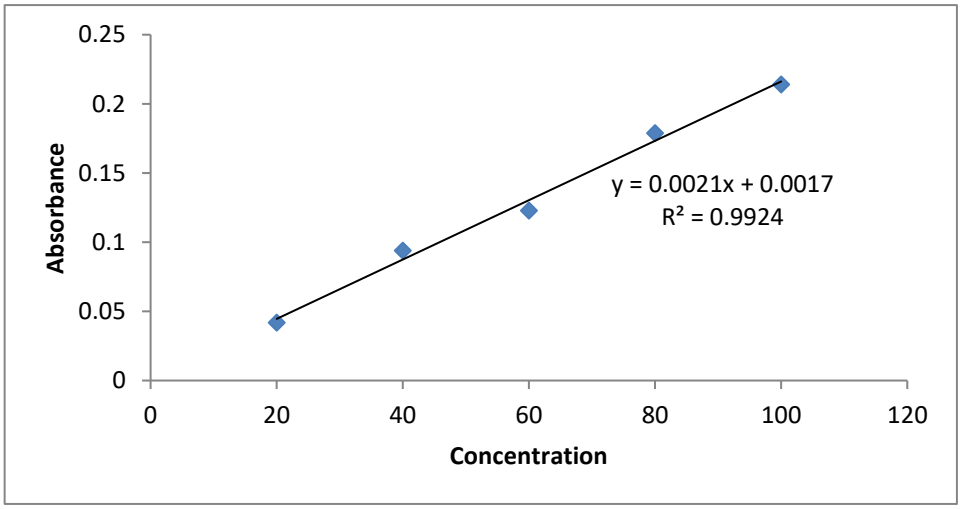

Fig. 7: Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 16: Reducing power activity of *Punica granatum* Methanol extract

Concentration	Absorbance
20	0.116
40	0.193
60	0.268
80	0.315
100	0.355

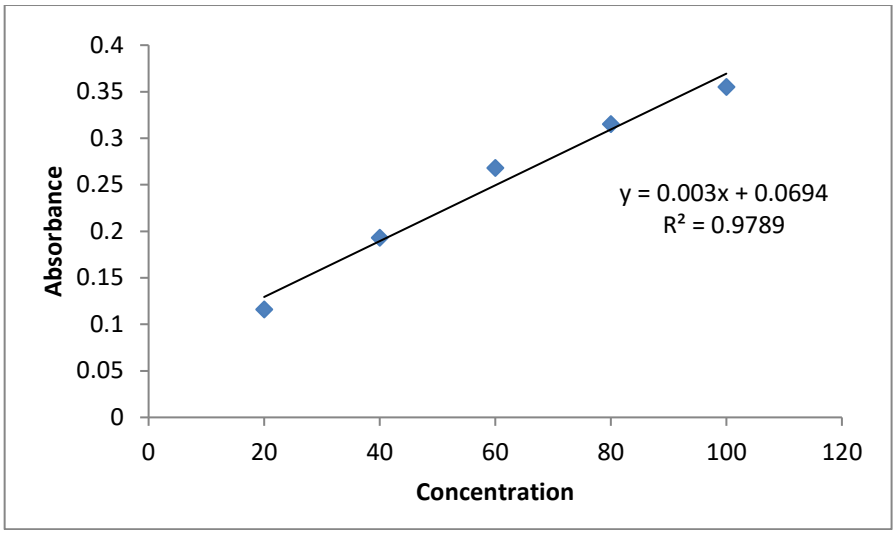

Fig. 8: Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract

Table 17: Reducing power activity of *Psidium guajava* Ethyl acetate extract

Concentration	Absorbance
20	0.074
40	0.126
60	0.158
80	0.191
100	0.236

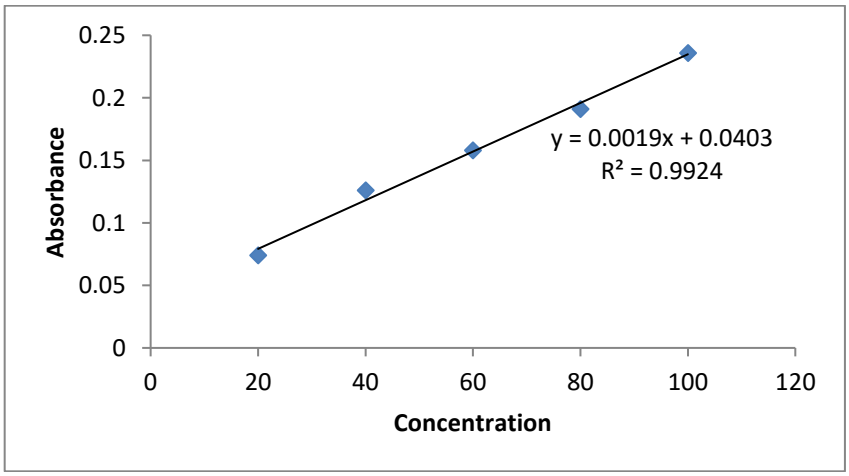

Fig. 9: Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 18: Reducing power activity of *Psidium guajava* Methanol extract

Concentration	Absorbance
20	0.19
40	0.22
60	0.273
80	0.321
100	0.356

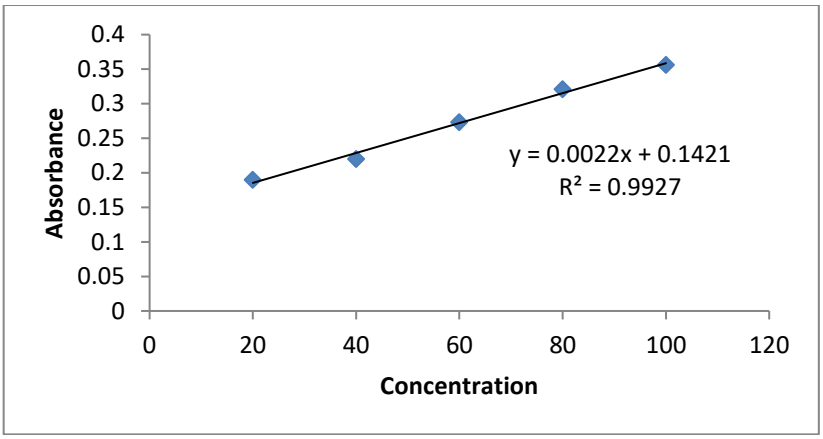

Fig. 10: Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract Super oxide scavenging activity

Table 19: SOS activity of Ascorbic acid

Concentration	Absorbance	% Inhibition
20	0.414	52.24
40	0.36	58.47
60	0.282	67.47
80	0.211	75.66
100	0.12	86.15
Control	0.867	
IC50	**17.64**	

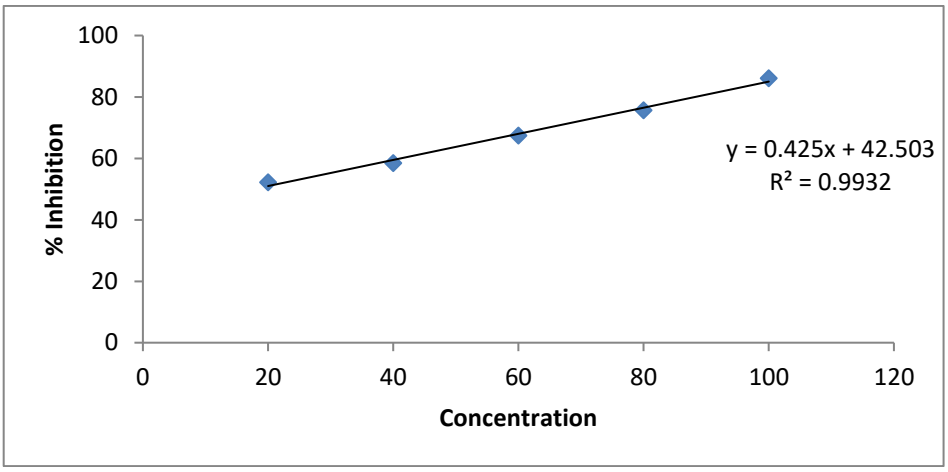

Fig. 11: Graph represents the Percentage Inhibition *Vs* Concentration of ascorbic acid

Table 20: SOS activity of *Punica granatum* Ethyl acetate extract

Conc.	Absorbance	% Inhibition
20	0.493	43.00
40	0.448	48.20
60	0.396	54.21
80	0.343	60.34
100	0.289	66.58
Control	0.865	
IC50	**45**	

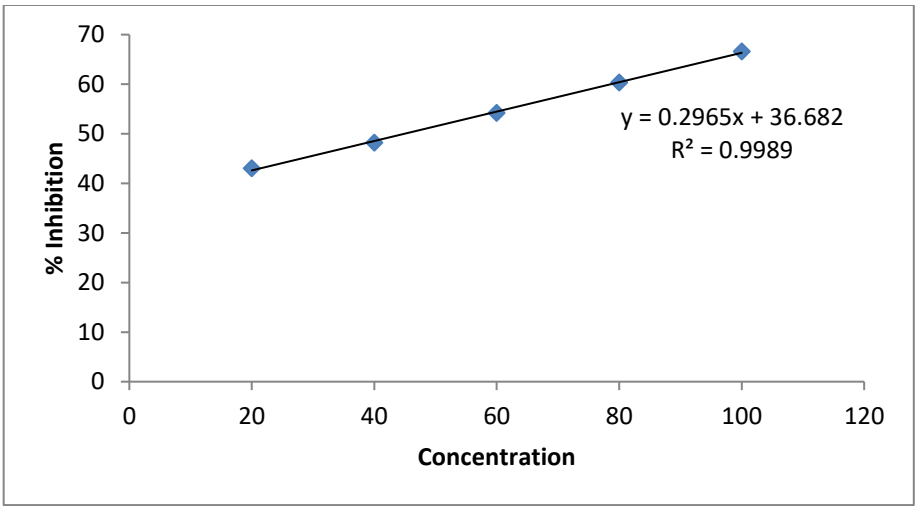

Fig. 12: Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 21: SOS activity of *Punica granatum* Methanol extract

Concentration	Absorbance	% Inhibition
20	0.475	45.21
40	0.401	53.74
60	0.343	60.43
80	0.28	67.70
100	0.21	75.77
Control	0.867	
IC50	**31.86**	

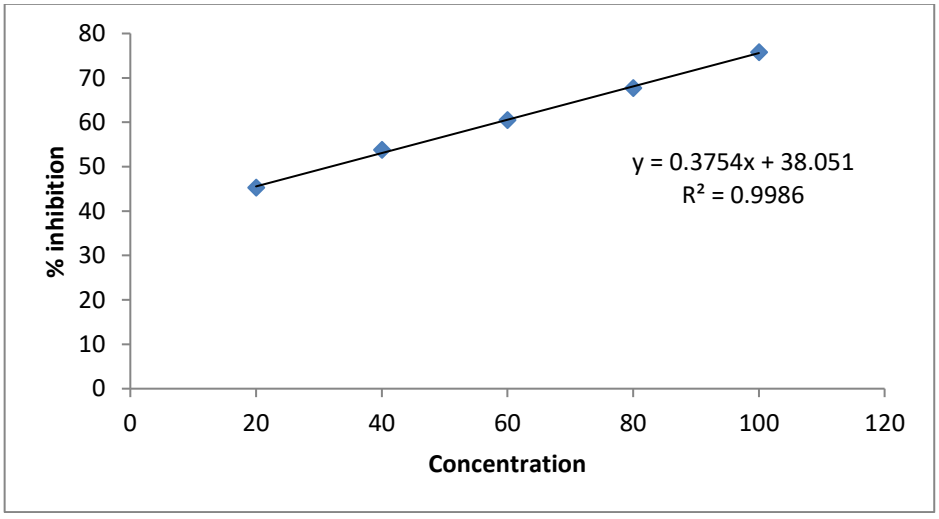

Fig. 13: Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract

Table 22: SOS activity of *Psidium guajava* ethyl acetate extract

Concentration	Absorbance	% Inhibition
20	0.487	43.82
40	0.436	49.71
60	0.359	58.59
80	0.295	65.97
100	0.244	71.85
Control	0.867	
IC50	**37.97**	

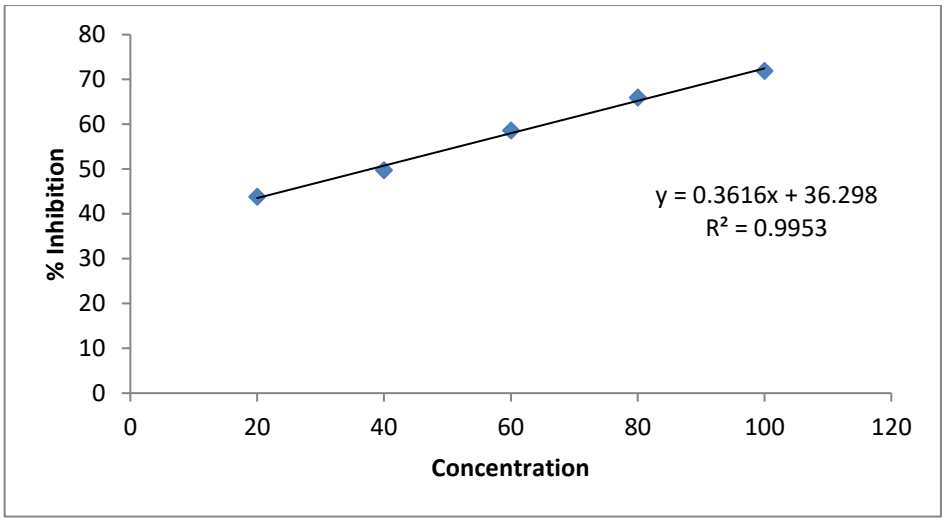

Fig. 14: Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 23: SOS activity of *Psidium guajava* Methanol extract

Concentration	**Absorbance**	**% Inhibition**
20	0.441	49.13
40	0.389	55.13
60	0.307	64.59
80	0.236	72.77
100	0.17	80.39
Control	0.867	
IC50	**24.125**	

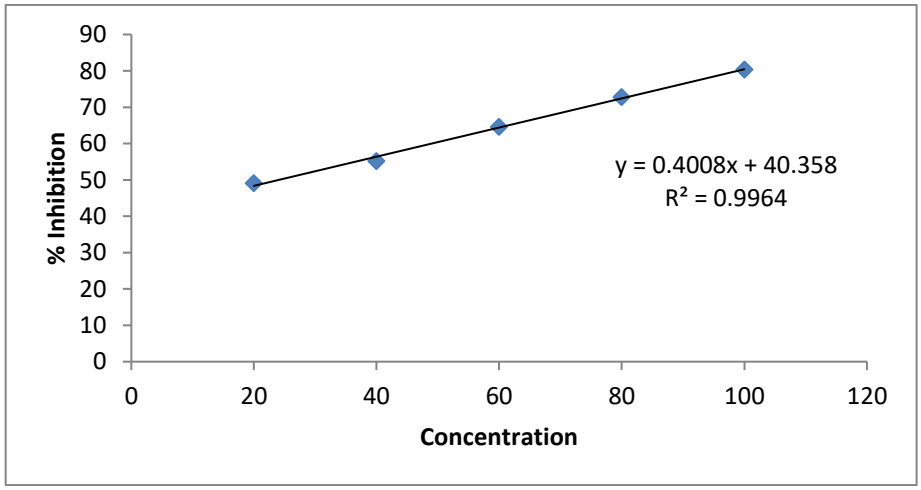

Fig. 15: Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract

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