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Original Article

Free radical scavenging activities of crude methanolic extract of *Psidium Guajava* leaves and its different fractions

Sharmin S[1*], Bolai P[2], Jimam NS [3, 4]

[1]Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University, Bangladesh;
[2]Department of Dosage Form Design, Faculty of Pharmacy, [3]Department of Clinical Pharmacy, Faculty of Pharmacy, MAHSA University, Selangor, Malaysia
[4]Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria

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ABSTRACT
Psidium guajava leaves and its fruits are widely used as a source of food and for ethnomedicinal purposes in Boshundhara Baridhara of Bangladesh, but not many studies have been conducted on the locally grown species of the plant in the area. This study investigated the phytochemical, and antioxidant activities of crude methanolic extract of locally grown *Psidium guajava* leaves and its different fractions. A qualitative phytochemical analysis was carried out on the crude extract using standard scientific procedures. Furthermore, antioxidant activities of both the crude methanolic extract and the various fractions were assessed using DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) test, with ascorbic acid as standard. The results showed the presence of saponins, alkaloids, flavonoid, and carbohydrate, and tannin was absent. The DPPH results showed that methanolic crude extract had the highest free radical scavenging activity with an IC50 value of 12.25 µg/ml, followed by the aqueous soluble fraction with an IC50 value of 14.45 µg/ml. Similarly, n-hexane fraction of the extract had a better activity (IC50 = 50.25 µg/ml) compared to those of carbon tetrachloride, dichloromethane soluble fraction whose IC50 values were recorded as 92.50.0 and 97.75 µg/ml respectively. These observations showed that polar fractions of the locally grown *Psidium guajava* leave could be good sources of antioxidants of natural origin.

Keywords: Psidium guajava, DPPH, methanol, antioxidant, phytochemical

Address for Correspondence: Sharmin S, Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University, Bangladesh; Email: sharmin_204@yahoo.com

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INTRODUCTION

Studies have linked the pathogenesis of many ailments including cancer, atherosclerosis, and aging, to an imbalance between free radicals and the anti-oxidative systems of the body. [1] These free radicals have the ability to multiply very rapidly, high levels of which results in cellular damage of the body leading to several diseases; hence the need for antioxidants to reduce the oxidative stress by neutralizing or scavenging the reactive species through hydrogen donations. [2] Once an antioxidant molecule neutralizes a free radical, the antioxidant loses its neutralizing ability; hence the need for a continuous supply of antioxidants in the body more than its ability to produce it in order to help protect against the cellular damage caused by oxidative stress and free radicals. [3]

The use of herbs as sources of natural antioxidants for the treatment of ailments is as old as the existence of mankind on earth, and it has further been encouraged in this present age due to health professionals' continuous struggles to meet up with the increased challenges of the emergence of new diseases without a definite cure. [4, 5] *Psidium guajava* has been widely used as food supplements and for its ethnomedicinal properties. [6] It belongs to the myrtle family (Myrtaceae), a dicotyledonous shrub, or small evergreen tree about 33 ft (10 in) high, with spreading branches, with smooth, thin, copper-colored bark that flakes off, showing the greenish layer beneath with attractive, "bony" trunk which may in time attain a diameter of 10 in (25 cm). [7] *Psidium guajava* leaves is widely used in some rural areas of Bangladesh for the treatment of many ailments, including diabetes, wound dressing, ulcers, skin disease, gastroenteritis, nausea and vomiting, toothache, coughs, sore throat, diarrhea and dysentery, and inflamed gums. Although many studies have been reported on the antioxidant activities of different extracts of the plant using many solvents [8 -10], the present study was aimed at assessing the free radical scavenging properties of the crude methanolic extract, and the various fractions of the *Psidium guajava* leaves locally grown in Boshundhara Baridhara using ascorbic acid as the positive standard.

MATERIALS AND METHODS

Collection and preparation of plant materials

The plant under investigation was collected from Boshundhara Baridhara, Dhaka in March 2014. The leaves were then harvested from the plants' stuck, separated, and air-dried under the shade for several days; after which it was pounded to a powder using pestle and mortar, and stored at room temperature in an airtight container prior to use.

Extraction of plant materials

Forty-one (41) grams of the powdered material was transferred into a round bottom flasks and sufficient amount of methanol was added until it was soaked. The container with its content was sealed by aluminum foil and kept for 15 days with occasional shaking and stirring; after which it was filtered using Whatman no.1 filter paper and concentrated at room temperature before it was air-dried to a solid residue and weighed using analytical balance.

Solvent-solvent partition of crude extract

The solvent-solvent partition was performed using the protocol designed by Kuchpan and modified by Wegnen. [11, 12] Five (5) grams of the crude extract was triturated with 45 ml of 10% methanol and dissolved completely; after which it was partitioned with solvents of different polarities such as n-hexane, carbon tetrachloride, and dichloromethane respectively and the different fractions separately collected and evaporated. The different fractions in addition to the aqueous methanolic fraction were subsequently analyzed for detection of DPPH free radical activity.

Phytochemical investigations

Phytochemical analyses of the crude extract were carried out to identify the presence or absence of secondary metabolites using standard methods. [13, 14] Antioxidant Activities of fractions of *Psidium guajava* leaves extracts by DPPH Method
The free radical scavenging activity of various fractions of *Psidium guajava* leaves extracts were investigated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method, using ascorbic acid as standard. [15]

The inhibitory concentration providing 50% inhibition (IC50) values were calculated from the plotted graph of percent inhibition against the concentration of each of the extracts and ascorbic acid (positive control) with concentrations between 0.977 to 500 µg/ml as follows: (I%) = (1 – A sample/Ablank) X 100.

Where A blank is the absorbance of the control reaction (containing all reagents except the test material); and the tests were carried out in triplicate and average values were taken.

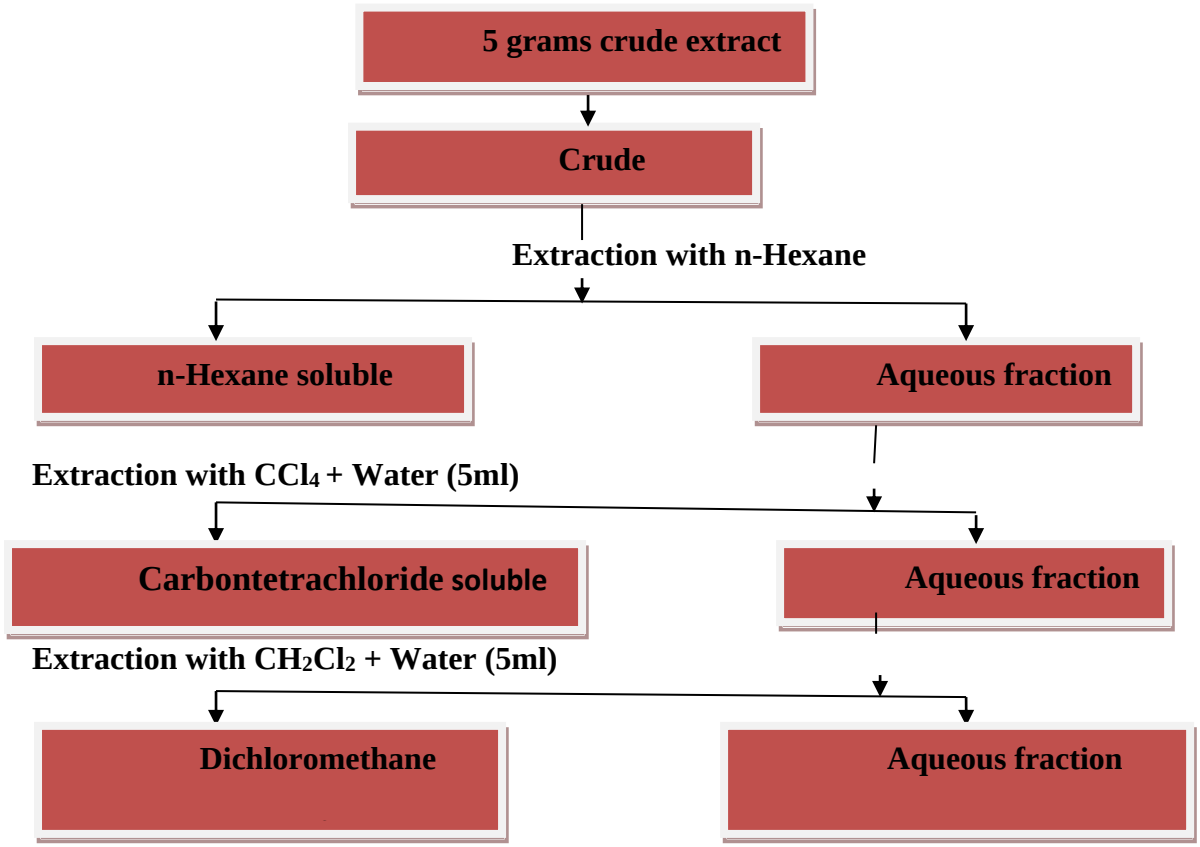

Figure 1: Schematic presentation of *Psidium guajava* leaves crude methanolic extract partitioning

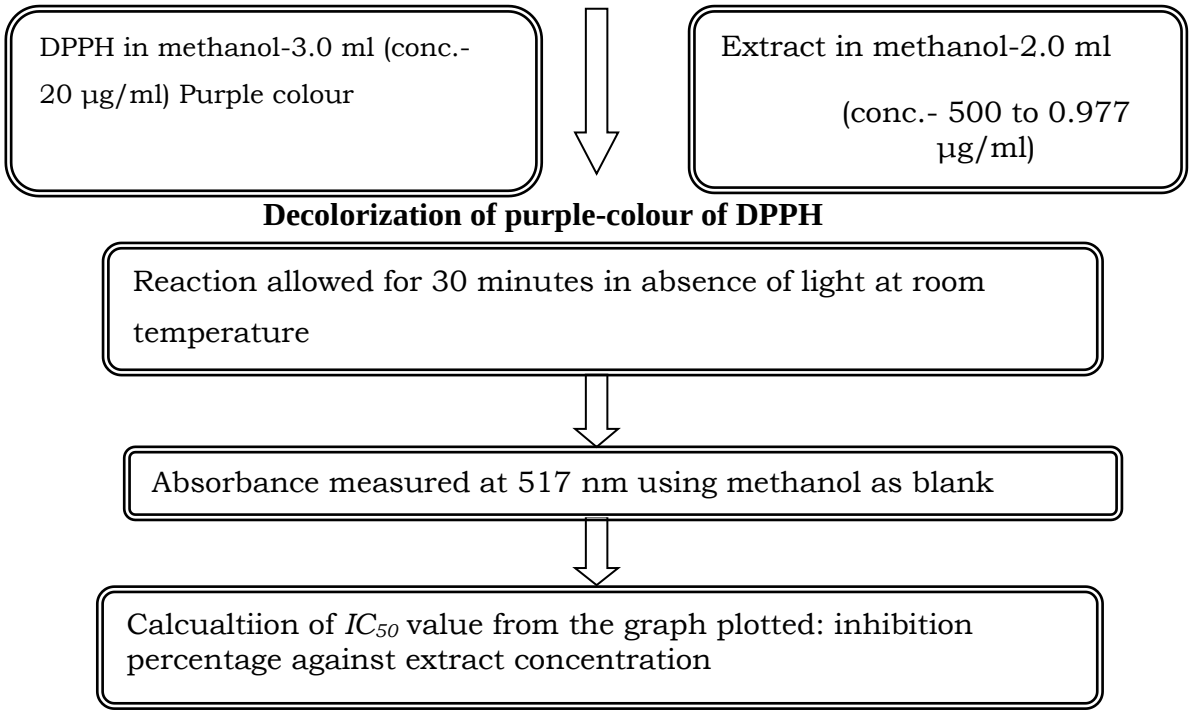

Figure 2: Schematic presentation of the method of assaying free radical scavenging activity

Mechanisms of free radical scavenging

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and was purple in color. The color turns from purple to yellow as the molar absorptive of the DPPH radical at 517 nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. DPPH radical scavenging activity was described as IC50 which was the concentration of samples to produce 50% reduction of the DPPH.

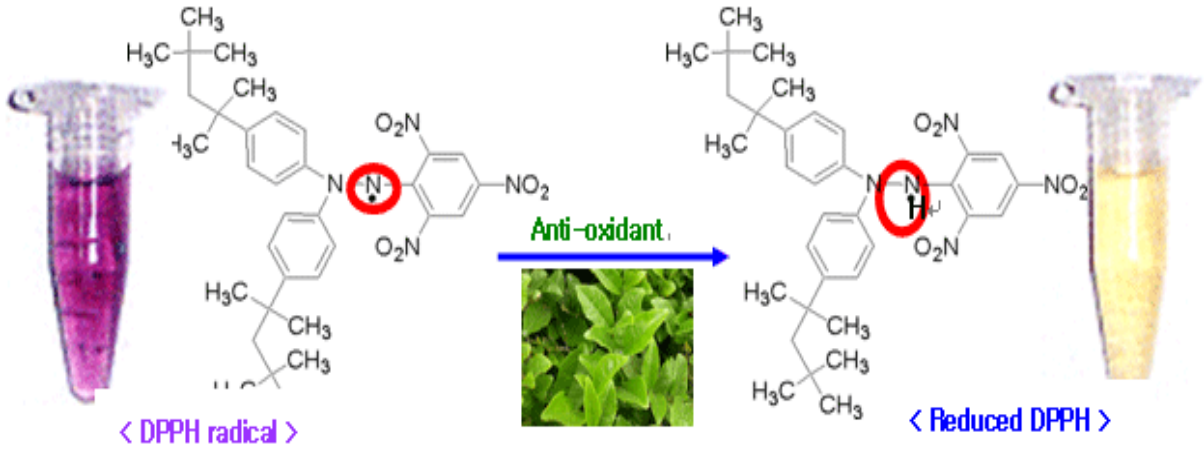

Figure 3: Mechanism of free radical scavenging activity of *Psidium guajava* leaves extracts by DPPH method

RESULTS

Table 1 below shows the result of the preliminary phytochemical investigation on the crude methanolic extract of psidium guajava leaves, indicating the presence of saponins, alkaioids, flavonoid and carbohydrate, with the absence tannins constituents.

Table 1: Phytochemical Constituents of Crude Methanolic Extract of *Psidium guajava* Leaves

Constituents	Reactions
Carbohydrates	Present
Saponins	Present
Flavonoids	Present
Tannins	Absent
Alkaloids	Present

Anti-oxidant Activities of *Psidium guajava* leaves

The results of DPPH scavenging activity of the crude methanolic extract, the various fractions, and ascorbic acid (standard) are shown below. The free radical scavenging activities were estimated by comparing the percentage of inhibition of DPPH radicals by the tested extracts and the ascorbic acid. [15]

The inhibitory concentration providing 50% inhibition (IC50) values (Table 2a-f) were calculated from the plotted graph of percent inhibition against the concentration of each of the extracts and ascorbic acid (positive control) with concentrations between 0.977 to 500 µg/ml (Figure 4a-f).

Table 2a: IC_{50} value of ascorbic acid

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of extract	% Inhibition	IC_{50} (µg/ml)
1	0.325	500	0.005	98.461	3.25
2		250	0.006	98.153	
3		125	0.011	96.615	
4		62.5	0.012	96.307	
5		31.25	0.015	95.384	
6		15.625	0.038	88.307	
7		7.813	0.098	69.846	
8		3.906	0.139	57.230	
9		1.953	0.175	46.153	
10		0.977	0.186	42.769	

Table 2b: IC_{50} value of crude methanolic extract of *Psidium guajava* leaves

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of extract	% Inhibition	IC_{50} (µg/ml)
1	0.345	500	0.033	90.434	12.25
2		250	0.049	85.797	
3		125	0.079	77.101	
4		62.5	0.097	71.884	
5		31.25	0.130	62.318	
6		15.625	0.152	55.942	
7		7.813	.201	41.739	
8		3.906	0.241	30.145	
9		1.953	0.273	20.869	
10		0.977	0.305	11.594	

Table 2c: IC_{50} value of aqueous extract of *Psidium guajava* leaves

SL	Absorbance of Blank	Concentration (µg/ml)	Absorbance of Extract	% Inhibition	IC_{50} (µg/ml)
1	0.347	500	0.028	91.930	14.45
2		250	0.035	89.913	
3		125	0.062	82.132	
4		62.5	0.090	74.063	
5		31.25	0.124	64.265	
6		15.625	0.167	51.873	
7		7.813	0.223	35.734	
8		3.906	0.268	22.766	
9		1.953	0.301	13.256	
10		0.977	0.307	11.527	

Table 2d: IC_{50} value of *n*-hexane soluble fraction of *Psidium guajava* leaves

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of extract	% Inhibition	IC_{50} (µg/ml)
1	0.352	500	0.076	78.409	50.25
2		250	0.098	72.159	
3		125	0.144	59.490	
4		62.5	0.163	53.693	
5		31.25	0.195	44.602	
6		15.625	0.231	34.375	
7		7.813	0.249	29.261	
8		3.906	0.289	17.897	
9		1.953	0.301	14.488	
10		0.977	0.311	11.627	

Table 2e: IC_{50} value of carbon tetrachloride soluble fraction of *Psidium guajava* leaves

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of extract	% Inhibition	IC_{50} (µg/ml)
1	0.342	500	0.053	84.502	92.40
2		250	0.098	71.345	
3		125	0.154	54.838	
4		62.5	0.187	45.321	
5		31.25	0.203	40.643	
6		15.625	0.221	35.380	
7		7.813	0.254	25.730	
8		3.906	0.264	22.8040	
9		1.953	0.300	12.28	
10		0.977	0.304	11.111	

Table 2f: IC_{50} value of dichloromethane soluble fraction of *Psidium guajava* leaves

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of extract	% Inhibition	IC_{50} (µg/ml)
1	0.355	500	0.098	72.394	97.75
2		250	0.132	62.816	
3		125	0.163	54.084	
4		62.5	0.198	44.225	
5		31.25	0.256	27.887	
6		15.625	0.277	21.971	
7		7.813	0.288	18.873	
8		3.906	0.295	16.901	
9		1.953	0.305	14.085	
10		0.977	0.336	10.985	

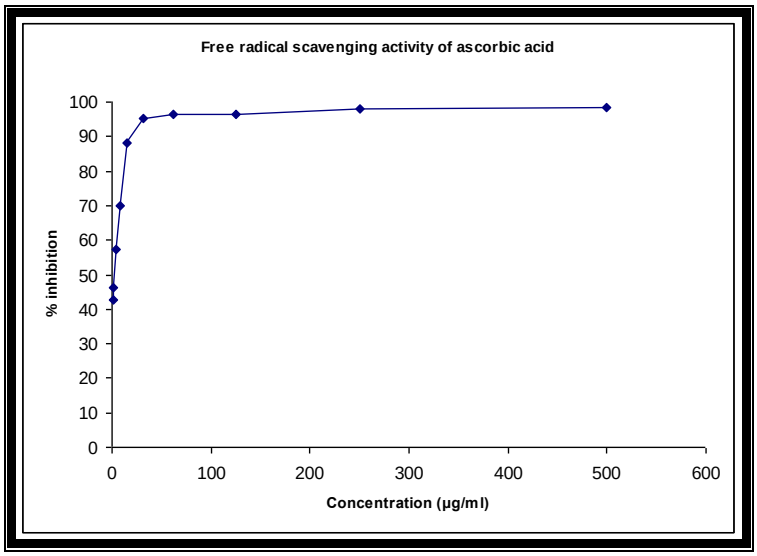

Figure 4a: Graphical presentation of Free radical scavenging activity of ascorbic acid

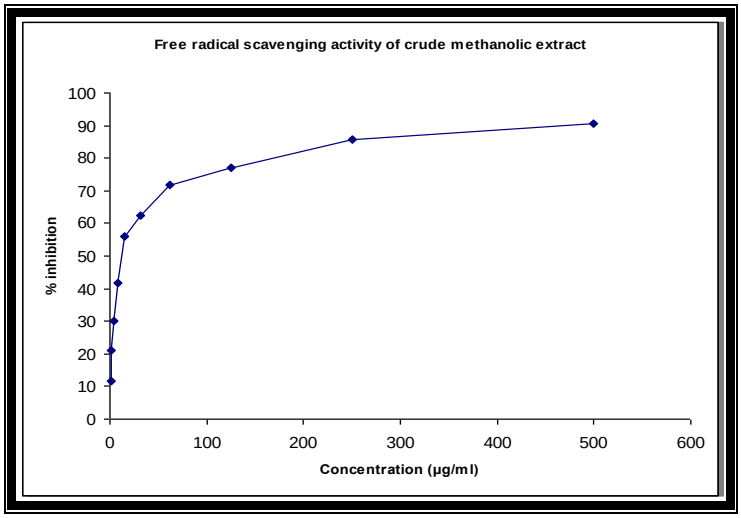

Figure 4b: Graphical Presentation of free radical scavenging activity of crude methanolic extract of *Psidium guajava* leaves

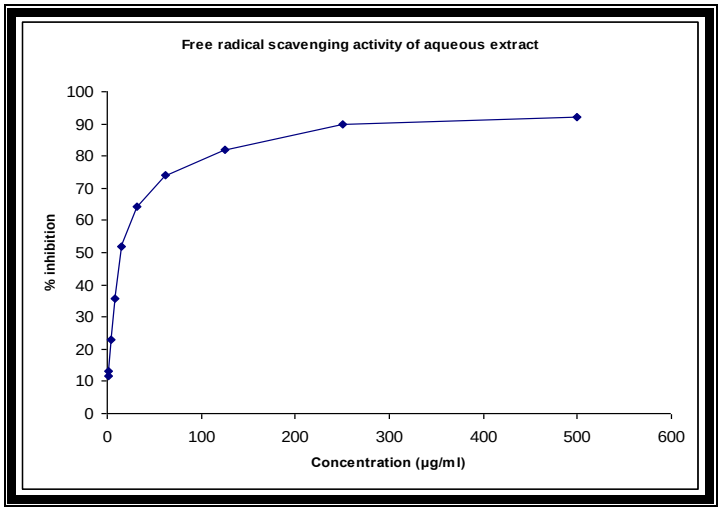

Figure 4c: Graphical Presentation of free radical scavenging activity of aqueous extract of *Psidium guajava* leaves

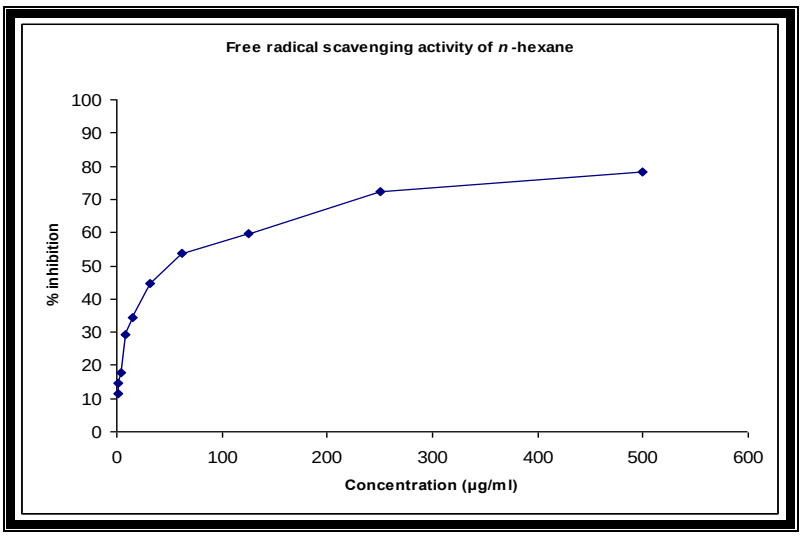

Figure 4d: Graphical Presentation of free radical scavenging activity of *n*-hexane soluble fraction of *Psidium guajava* leaves

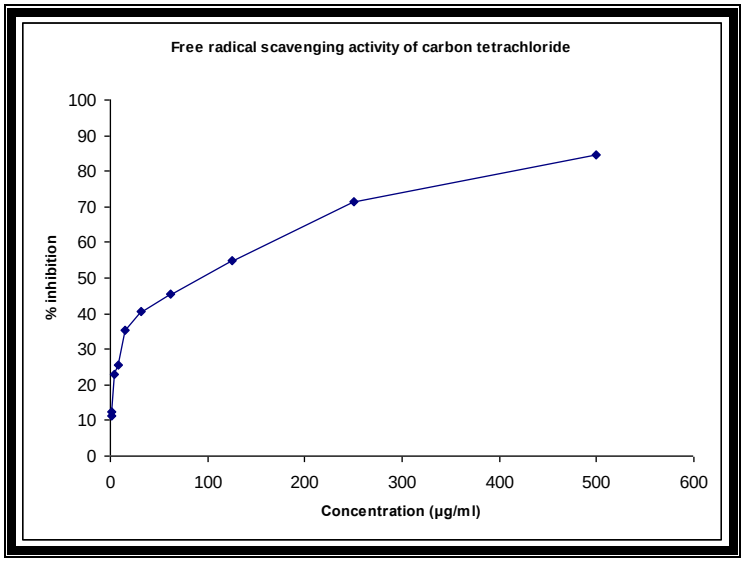

Figure 4e: Graphical Presentation of free radical scavenging activity of carbon tetrachloride soluble fraction of *Psidium guajava* leaves

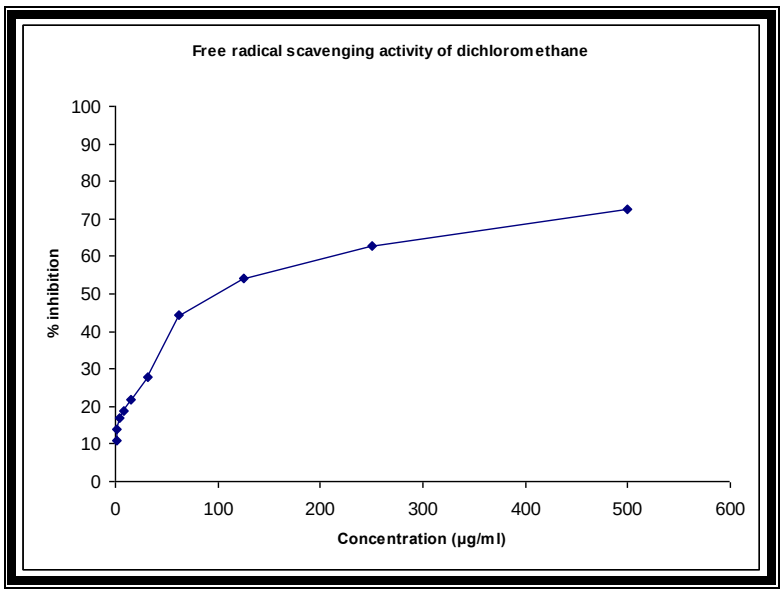

Figure 4f: Graphical Presentation of free radical scavenging activity of dichloromethane soluble fraction of *Psidium guajava* leaves

The summary of the results showed that the crude methanolic extract and aqueous soluble fraction had the highest free radical scavenging activity with IC_{50} value12.25 and 14.45 µg/ml, respectively. Similarly, *n*-hexane, carbon tetrachloride, and dichloromethane soluble fraction of methanolic extract showed free radical scavenging activity with IC_{50} value of 50.25, 92.50.0 and 97.75 µg/ml, respectively (Table 3).

Table 3: Summary of IC_{50} Values of crude methanolic extract of *Psidium guajava* leaves and its Fractions

Code	Sample	IC_{50} (µg/ml)
AA	Ascorbic acid	3.25
CME	Crude methanolic extract	12.25
AQF	Aqueous soluble fractions	14.45
HSF	*n*-Hexane soluble fractions	50.25
CTSF	Carbon tetrachloride soluble fractions	92.50
DMSF	Dichloromethane soluble fractions	97.75

DISCUSSION

Psidium guajava has many secondary metabolites which might be responsible for the plant's nutritional and ethnomedicinal activities. [16] For instance, the antiviral and antileukemic activities of alkaloids have been documented; [17, 18] while flavonoids are widely known for their anti-allergic, anti-inflammatory, antimicrobial and anti-cancer properties. [19, 20] Saponins are also known to have beneficial activities on the blood cholesterol levels, cancer, bone health, antioxidant and the immune system. [21]

The principle of antioxidant activity is the availability of electrons to neutralize any so-called free radicals. The DPPH scavenging assay which is an in-vitro technique for assessing antioxidant activities was used for the study. The free radical scavenging activities were observed to be significantly higher in the methanolic crude extract and aqueous extracts, with the former being higher than the latter; and this antioxidant activity was concentration-dependent of the extracts. Furthermore, n-hexane fraction had better antioxidant activities than those of carbon tetrachloride and the dichloromethane soluble fraction of methanolic extract.

The efficiency and efficacy of plants' extracts depend on the efficiency of the extraction method. [22] This is because studies have shown a linear relationship between extraction efficiency and activities of extracts. [23] The use of a solvent in extraction and isolation of bioactive plant constituents is a widely used extraction method, and the yield of the bioactive constituents is dependent on the conditions of extraction and the solvent polarity. [24] Hence, for appropriate choice of solvent for extraction, the principle of 'like dissolves like' is important and applicable; meaning that polar solvents will extract out polar substances, while non-polar components will be extracted out by non-polar solvents. [25] The above result of the present study shows that the *Psidium guajava* leaves have a high concentration of polar constituents compared to the non-polar counterparts, which was indicated by its antioxidants activities (Table 3). The high free radical scavenging activities of the polar components of the *Psidium guajava* leaves extract was similar to other documented plants' extract activities. [26]

CONCLUSION

The present study shows high free radical scavenging activities of polar fraction of the *Psidium guajava* leave extracts which could be a good source of antioxidants of natural origin.

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