RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

The organic world is sustained by plants through the fundamental process of photosynthesis. The way in which plants influence life on earth is remarkable. Plants have provided all the basic needs of man ever since his birth and evolution. Plants are the source of food, medicine, fuel, fiber etc. Plants synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates) by simple substances such as water, carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids etc) that are used as drugs (Bukhsh *et al.*, 2007).

Higher plants survive in a constantly fluctuating environment as they develop a series of pathways at different levels that combat with environmental stress, which produces more ROS. Increase in ROS causes damage to the metabolities such as proteins, lipids and nucleic acids etc. Plant posses specific mechanism to detoxify the reactive oxygen species which include activation of antioxidant enzymes (Ahmad *et al.*, 2009). Herbal remedies have many traditional claims and are employed in the treatment of diseases of diverse origins. They contain active constituents with useful physiological and pharmacological activities. Consequently, attention is now focused in the exploration of herbal remedies as alternatives in the treatment of infectious diseases since pathogens have been found to develop multiple resistances to most of the currently used synthetic antibiotics (Enwuru *et al.*, 2008).

The present study was carried out to evaluate the antioxidant status, free radical scavenging efficacy, cytotoxic effect and antibacterial property of *Denolix elata*. Different parts of the plants namely leaves, flowers and bark were selected for the invstigation. The samples were shade dried, powdered and used for extraction. The powdered plant samples were screened for their phytochemicals qualitatively and quantitatively. The

nutritive content of the plant was determined in terms of carbohydrates and proteins. The enzymic antioxidants like catalase, glutathione peroxidase, glutathione s-transferase, peroxidase, polyphenol oxidase and superoxide dismutase were analysed. Nonenzymic antioxidants such as flavonoids, polyphenols, reduced glutathione, carotenoids, ascorbic acid and α-tocopherol were determined. The powdered samples were kept in orbital shaker and the residue was dissolved in their respective solvents and used for free radical scavenging assays. The plant samples were extracted with different with different solvents using Soxhlet apparatus and each extract was tested for their antibacterial activity. Methanolic extract was used to detect the cytotoxic effect of the samples by brine shrimp lethality assay.

4.1. Determining the percentage yield of the extract

10g of each powdered plant sample of leaf, flower and bark of *Denolix elata* was extracted with different solvents such as petroleum ether, chloroform, methanol and water by using soxhlet apparatus. The extracts were evaporated to dryness and their yield was calculated in percentage and mentioned in Table 1. The samples were also subjected to another extraction method by keeping in an orbital shaker for 72 hours. The extracts were evaporated to dryness and the yield was noted in percentage and presented in Table I.

TABLE I

THE PERCENTAGE YIELD OF *Denolix elata* BY SOXHLET AND SHAKER METHODS

Samples	Extracts	Percentage Yields	
		Soxhlet extraction	Shaker extraction
Leaf	Petroleum ether	11	13
	Chloroform	9.5	14.4
	Methanol	19.2	19.8
	Aqueous	9.0	11.2
Flower	Petroleum ether	2.3	6.5
	Chloroform	2.8	7.8
	Methanol	36.3	38.4
	Aqueous	7.0	12.4
Bark	Petroleum ether	1.9	2.0
	Chloroform	2.0	2.6
	Methanol	4.0	4.3
	Aqueous	5.0	6.6

The yield was found to be high in methanol extracts of all the three samples namely leaf, flower and bark when compared to all other solvents used in both soxhlet and shaker method. The yield of methanolic flower extract was found to be higher than leaf and bark. The yield was found to be very low in petroleum ether extract of bark and flower and the aqueous extract of leaves produced the least amount of residue. Comparison of the yield of various solvent extracts of leaf, flower and bark indicated that shaker extraction produced more yield. In both the methods yield was found to be drastically reduced in bark.

4.2. Qualitative screening of phytochemical constituents of *Denolix elata*

The phytochemical screening of plant materials to determine the presence of bioactive constituents is thus vital in the knowledge of therapeutic properties of plants. Such bioactive constituents analyzed in this study are indicated in Table II.

TABLE II

QUALITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF *Denolix elata*

S.No	Phytochemicals	Occurrence in		
		Leaf	Flower	Bark
1.	Alkaloids	+	+	+
2.	Anthraquinones	+	+	+
3.	Flavonoids	+	+	+
4.	Glycosides	+	+	+
5.	Phenols	+	+	+
6.	Phytosterols	+	+	-
7.	Reducing sugars	+	+	+
8.	Saponins	-	-	-
9.	Tannins	+	+	+
10	Terpenoids	+	+	-

+ → Presence of the compounds

- → Absence of the compounds

Phytochemicals such as alkaloids, anthraquinones, flavonoids, glycosides, phenols, reducing sugars and tannins were found to be present in leaves, flowers and bark of *Denolix elata*. Saponin was not detected in all parts of the plants whereas phytosterols and terpenoids were detected only in leaf and flower and not in bark.

Aqueous-methanolic extract of *Hibiscus sabdariffa* was investigated for its phytochemical constituents. The extract was found to contain cardiac glycosides, flavonoids, saponins and alkaloids (Tolulope, 2007). Qualitative analysis of six Malaysian medicinal plants was done for their phytochemical constituents. Each plant showed that tannins, saponins, flavonoids, terpenoids and alkaloids were present in all the plants tested (Krishnaiah *et al.*, 2009). Awoyinka *et al.* (2007) investigated the water and ethanolic extracts of leaves of *Cnidoscolus aconitifolius* for eight principle bioactive compounds, out of which phenols, saponins and cardiac glycosides were found to be positive.

4.3. Quantitative analysis of active compounds present in *Denolix elata*

A well organized quantitative estimation of the phytochemicals present in leaves, flowers and bark was simultaneously carried out with qualitative studies. The samples was found to contain alkaloids, phenols, reducing sugars, tannins and chlorophyll and are represented in Table III and Figure I.

The content of phenols, reducing sugars and tannins was found to be higher in flower when compared to leaf and bark of *Denolix elata*. Bark recorded the least amount of all these constituents. The estimation revealed that chlorophyll and alkaloid content in the leaf was found to be higher in amount followed by flower and bark.

TABLE III

CONTENT OF PHYTOCHEMICALS IN *Denolix elata*

S.No	Name of the Compounds	Content (mg/g)		
		Leaf	Flower	Bark
1.	Alkaloids	0.951 ±0.053	0.756 ±0.025	0.674 ±0.055
2.	Phenols	0.869 ±0.019	1.570 ±0.004	0.485 ±0.007
3.	Reducing sugars	1.036 ±0.019	1.301 ±0.003	0.577 ±0.010
4.	Tannins	1.514 ±0.100	1.645 ±0.470	0.992 ±0.108
5.	Chlorophyll	11.202 ±0.136	1.754 ±0.050	0.968 ±0.0567

Values are mean±SD of triplicates

The presence of major phytoconstituents were detected qualitatively and quantitatively in *Anethum graveolens* and the compounds were found to be alkaloids, flavonoids, tannins, saponins and cardiac glycosides (Kaur and Arora, 2009). Ali *et al.* (2008) analysed qualitatively and quantitatively the roots and leaves of *Withania somnifera* and indicated the presence of various phytochemicals like alkaloids, carbohydrates, proteins, saponins, flavonoids and phenols. Chlorophyll is a natural pigment that absorbs light energy for photosynthesis. Differences in leaf chlorophyll content can be an indicator of plant vigor and its capacity for photosynthesis, strongly dependent on chlorophyll content (Golkar *et al.*, 2009).

4.4. Determination of Nutritive values in plant samples

The leaves, flowers and bark of *Denolix elata* were assessed to determine the nutrients which are represented in Table IV and Figure II.

TABLE IV

NUTRIENTS CONTENT OF *Denolix elata*

S.No	Name of the Nutrients	Content (mg/g)		
		Leaf	Flower	Bark
1.	Carbohydrates	3.082 ±0.013	4.107 ±0.010	1.906 ±0.016
2.	Proteins	9.981 ±0.184	8.081 ±0.08	4.798 ±0.092

Values are mean±SD of triplicates

It is evident from the table that the amount of protein in the leaves of *Denolix elata* was found to be high, whereas flower has higher amount of carbohydrates. When compared to leaf and flower extracts bark registered lower amount of carbohydrates and proteins. Thus, *Denolix elata* has appreciable amount of nutrients such as carbohydrates and proteins.

The leaf powder of *Aquilaria agallocha* Roxb had the highest amount of carbohydrates (19.42 mg/g dry weight) and protein (24.37 mg/g dry weight) (Dash *et al.*, 2008). Adnan *et al.* (2010) assessed the proximate composition and nutrient contents of five medicinal plants and indicated that they are the good source of carbohydrates and proteins.

FIGURE I

CONTENTS OF PHYTOCHEMICALS IN *Denolix elata*

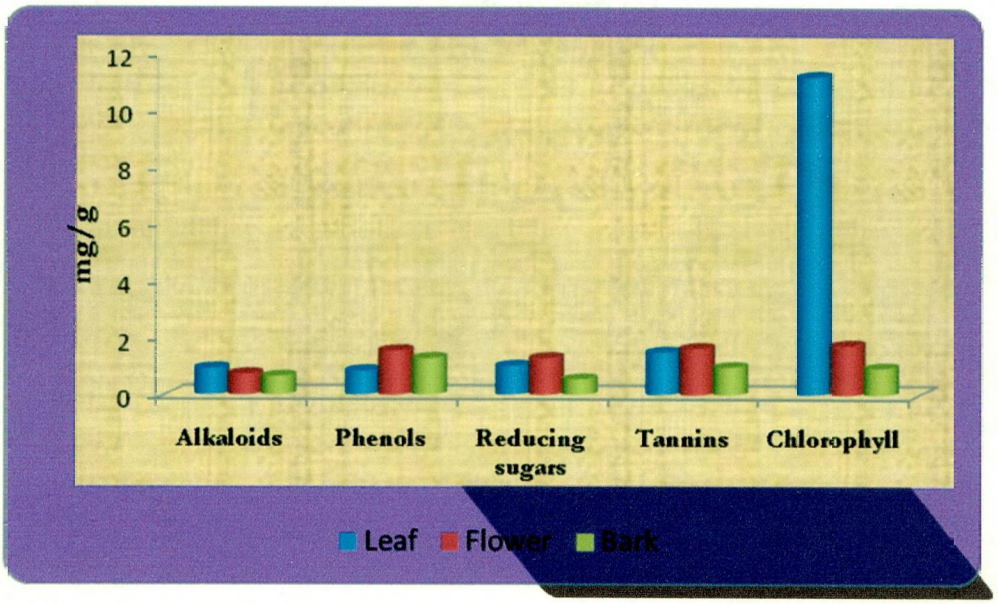
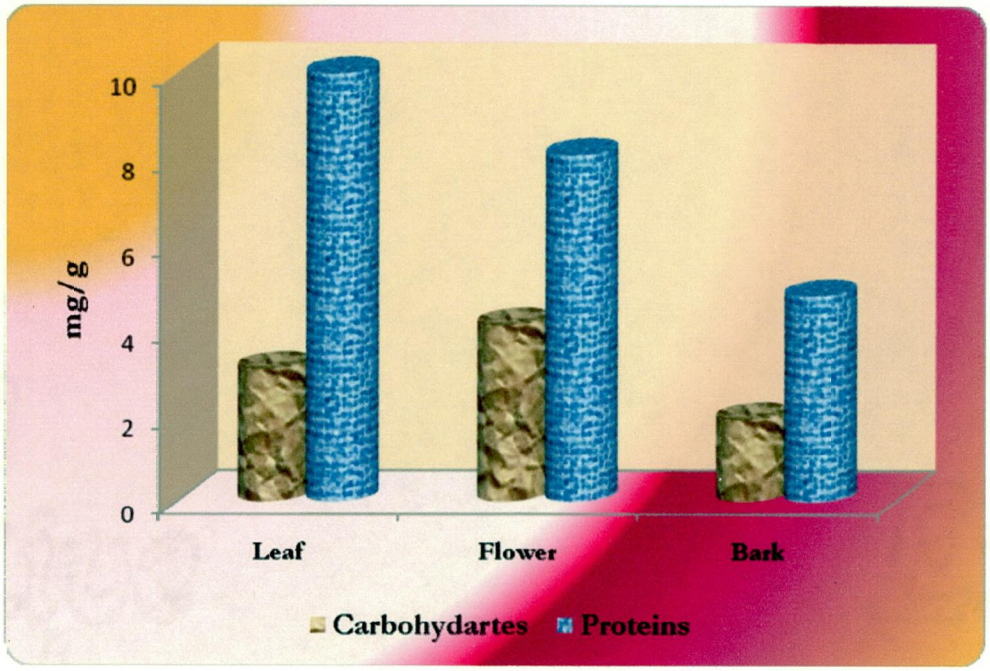


FIGURE II

NUTRITIVE CONTENT OF *Denolix elata*



4.5. Determination of Antioxidant Status

4.5.1. Enzymic antioxidants

The activity of catalase, glutathione peroxidase, glutathione s-transferase, peroxidase, polyphenol oxidase and superoxide dismutase in leaves, flowers and bark of *Denolix elata* was assayed and the values are presented in Table V, VI and Figure III.

TABLE V

THE LEVELS OF ENZYMIC ANTIOXIDANTS IN LEAF, FLOWER AND BARK OF *Denolix elata*

S.No	Samples	Enzymic antioxidants (U/g)		
		Catalase	Glutathione peroxidase	Glutathione s-transferase
1.	Leaf	675.36 ±54.02	0.0612 ±0.001	0.0580 ±0.0080
2.	Flower	756.36 ±70.46	0.1085 ±0.0002	0.0747 ±0.0085
3.	Bark	237.26 ±36.47	0.076 ±0.0005	0.0332 ±0.0083
C.D(0.05)		**135.616**	**0.00210**	**0.0165**

Values are mean±SD of triplicates.

ENZYMES	UNITS
Catalase	Amount of enzyme required to decrease the optical density by 0.05 units.
Glutathione peroxidase	µmoles of GSH consumed/min/g sample.
Glutathione s-transferase	µmoles of CDNB-GSH conjugates/min/g sample.

In the present study, the estimated catalase, glutathione peroxidase and glutathione s-transferase levels in the different parts of samples ranged from (237.26 to 756 U/g), (0.0612 to 0.1085 U/g) and (0.0332 to 0.0747 U/g), respectively. The maximum catalase, glutathione peroxidase and glutathione s-transferase activity was exhibited by the flower extract and the minimum activity of catalase and glutathione s-transferase was observed in bark but the lowest level of glutathione peroxidase was found in leaf samples. In various parts of *Denolix elata* there was a significant difference in the activity of above enzymes except catalase activity of leaf and flower.

Pavana *et al.*, (2007) reported that enzymic antioxidants form the first line of antioxidant defence mechanism to protect the organism from ROS mediated oxidative damage. Rao *et al.*, (2009) stated that higher superoxide dismutase and catalase activities were detected in *Clitoria ternate* at concentration of 100mg/ml as compared to *Eclipta prostrata* (2.92 and 1.18 U/mg protein, respectively). Glutathione s-transferases are ubiquitous enzymes catalyzing the addition of reduced glutathione (GSH) to electrophilic substrates, which tags them for vascular sequestration. In addition to catalyzing GSH conjugation, GST_s also exhibits glutathione peroxidase (GSH-PX) activity, which suggests a role in protection against oxidative stress (Csiszar *et al.*, 2002).

TABLE VI

THE LEVELS OF ENZYMIC ANTIOXIDANTS IN LEAVES, FLOWER AND BARK OF *Denolix elata*

S.No	Samples	Enzymic antioxidants (U/g)		
		Peroxidase	Polyphenol oxidase	Superoxide dismutase
1.	Leaf	6.46 ±0.188	0.1436 ±0.006	11.76 ±0.188
2.	Flower	7.13 ±1.309	0.2786 ±0.0838	29.32 ±0.459
3.	Bark	2.8 ±0.282	0.0946 ±0.006	7.133 ±0.368
C.D(0.05)		**1.912**	**0.119**	**0.865**

Values are mean±SD of triplicates

ENZYMES	UNITS
Peroxidase	1µmoles of pyrogallol oxidised/min.
Polyphenol oxidase	Amount of enzyme that transforms 1µmole of dihydrophenol to 1 mole of quinone/min.
Superoxide dismutase	Amount that causes 50% reduction in the extent of NBT oxidation.

It is evident from the table that the highest activity of peroxidase, polyphenol oxidase and superoxide dismutase was shown by flower and the lowest value was recorded by bark samples. Moderate activity of peroxidase, polyphenol oxidase and superoxide dismutase was registered in leaf samples of *Denolix elata*. Among the three different samples of

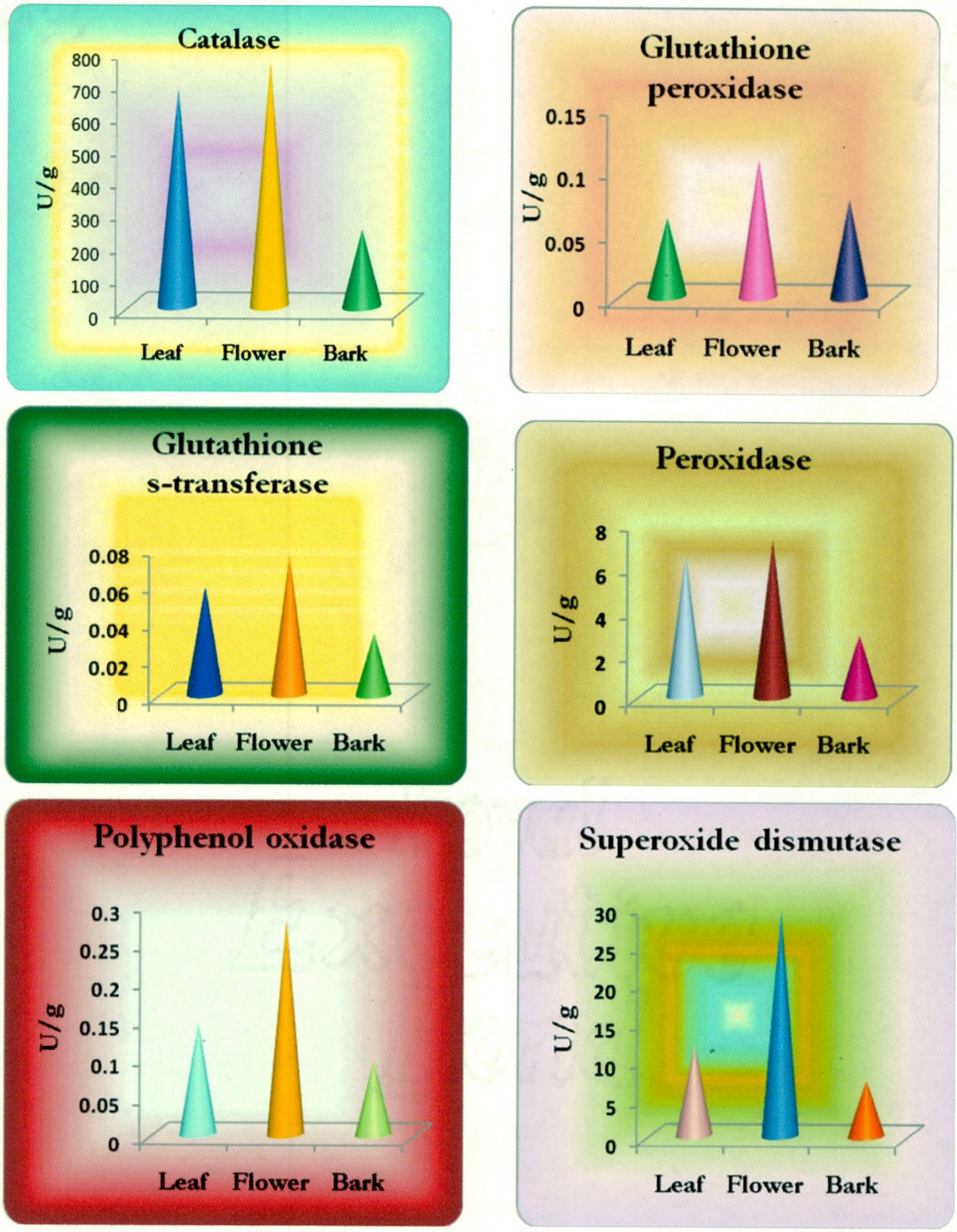
Denolix elata did not show any significant difference in the activity of peroxidase and in the case of polyphenol oxidase there was no significant difference in the activity of leaf and bark.

Polyphenol oxidase is expressed in many different tissues and organs including roots, leaves, flowers and vascular tissue. Polyphenol oxidase has often suggested to function as a defence against pests and pathogen (Constabel and Barbehenn, 2008). Grime *et al.* (2009) suggest that Polyphenol oxidase has been implicated in discoloration of fruits, vegetables and food products. The biological, industrial and economic significance of Polyphenol oxidase relates to its oxidation of phenolic compounds. Peroxidase is an oxidoreductase that is directly involved in many plant functions such as hormone regulation, defence mechanism, indolactic degradation and lignin biosynthesis. It catalyses a reaction in which hydrogen peroxide acts as the acceptor and another compound acts as the donor of hydrogen atom (Arnnok *et al.*, 2010).

Ehrenbergerova *et al.* (2009) reported Green biomass of young barely plants exhibited statistically higher activity of superoxide dismutase and catalase activities. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical (Palanivel *et al.*,2008).

FIGURE III

THE LEVELS OF ENZYMIC ANTIOXIDANTS IN *Denolix elata*



4.5.2. Non-Enzymic antioxidants

The levels of various nonenzymic antioxidants present in the selected parts of the plant were analysed and discussed in Table VII, VIII and Figure IV.

TABLE VII

THE LEVELS OF NON-ENZYMIC ANTIOXIDANTS IN LEAVES, FLOWER AND BARK OF *Denolix elata*

S.No	Samples	Non-enzymic antioxidants (mg/g)		
		Carotenoids	Ascorbic acid	α-Tocopherol
1.	Leaf	108.68 ±4.023	0.728 ±0.060	0.010 ±0.503
2.	Flower	56.05 ±0.308	0.905 ±0.013	0.012 ±1.108
3.	Bark	30.945 ±0.294	0.414 ±0.013	0.005 ±0.570
C.D(0.05)		**5.706**	**0.0569**	**0.00216**

Values are mean±SD of triplicates

The present study reveals that the leaves, flowers and bark of *Denolix elata* have 108.68±4.023, 56.05±0.308 and 30.945±0.294 mg/g of carotenoid content respectively. The leaves were found to have higher levels of carotenoids than flower and bark samples. The flower of *Denolix elata* recorded high levels of ascorbic acid and α-tocopherol followed by leaves. Bark was found to contain the least amount in all the mentioned nonenzymic antioxidants.

Ahmed and Beigh *et al.* (2009) reported green leafy vegetables belonging to *Brassica oleracea* is promising group with rich antioxidant

composition such as carotenoids, ascorbic acid and phenolic content. Selvi *et al.* (2007) suggested Vitamin C is regarded as the first line natural antioxidant defense in plasma and a powerful inhibitor of LPO. α- tocopherol, the most active form in human and reported to be a powerful biological antioxidant with highest vitamin bioactivity in *Cnidoscolus aconitifolis* (Ogunlade *et al.*, 2009). A significant difference in the levels of α- tocopherol, carotenoids, flavonoids and reduced glutathione was seen between the leaves and stem of *Aristolochia indica* (Devi *et al.*, 2010).

TABLE VIII

THE LEVELS OF NONENZYMIC ANTIOXIDANTS IN LEAVES, FLOWER AND BARK OF *Denolix elata*

S.No	Samples	Nonenzymic antioxidants (mg/g)		
		Flavonoids	Polyphenols	Reduced glutathione
1.	Leaf	0.869 ±0.111	2.26 ±0.010	1.324 ±0.018
2.	Flower	1.504 ±0.005	2.605 ±0.006	1.667 ±0.014
3.	Bark	0.485 ±0.078	1.322 ±0.007	0.4282 ±0.026
C.D(0.05)		**0.157**	**0.0191**	**0.0499**

Values are mean±SD of triplicates

It is clear from table VIII that the highest values of flavonoids, polyphenols and reduced glutathiones were observed in the flower of *Denolix elata*. Bark of *Denolix elata* was noted to be the poorest source of flavonoids, polyphenols and reduced glutathione. Moderate amount of the

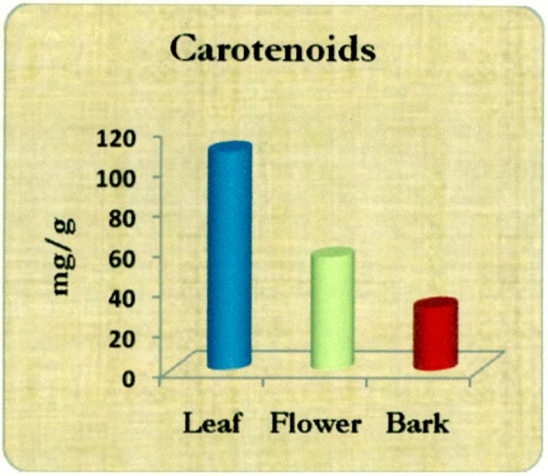
above constituents was observed in the leaves of *Denolix elata*. Analysis of variance showed significant difference among the tested samples for the above nonenzymic antioxidants.

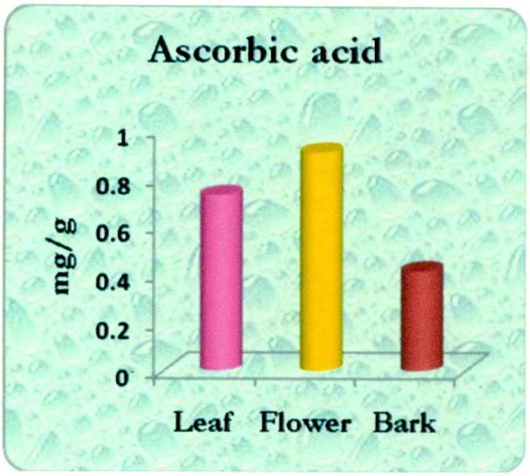
Hydroxyl functional groups in flavonoids are responsible for antioxidant effect in plants (Ebrahimzaden *et al.*, 2008). Pourmord *et al.* (2006) showed high content of phenols and flavonoids in *Melliotus officinalis* responsible for its radical scavenging activity. Antioxidant studies of *Commiphora caudate* and *Commiphora var pubescens* revealed that high antioxidant activity of the plant extract can be correlated with the phenolic and flavonoid content of the plant extract (Deepa *et al.*, 2009).

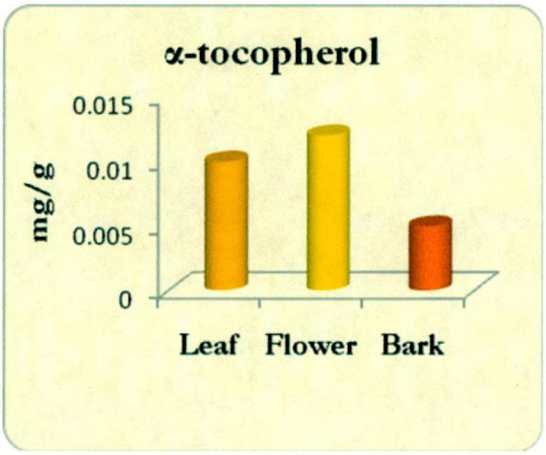
Polyphenols are the major plant compounds with high level of antioxidant activity. This activity could be due to their ability to absorb, neutralize and to quench free radicals (Oyedemi *et al.*, 2010). Leonards *et al.* (2008) suggested that the polyphenol content in the olive leaf range from 1.5 to 7.0g per 100g in fresh leaves. Patil (2007) suggested that reduced glutathione is an effective reductant and plays an important role in a variety of detoxification process.

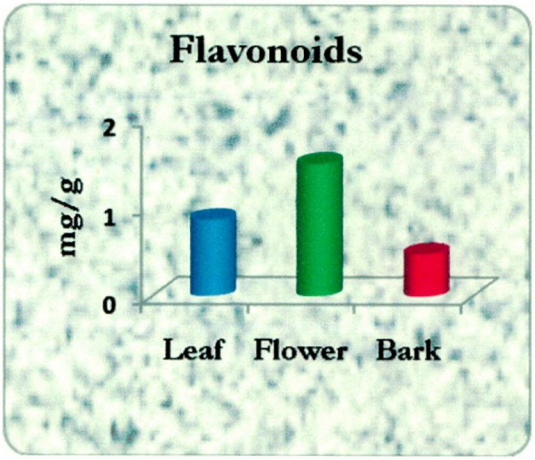
FIGURE IV

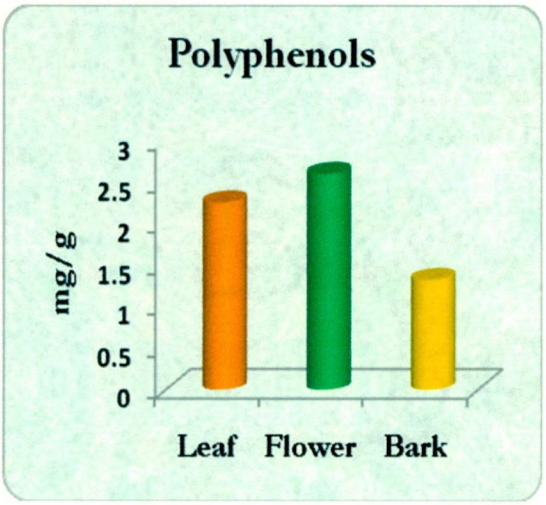
THE LEVELS OF NONENZYMIC ANTIOXIDANTS OF *Denolix elata*

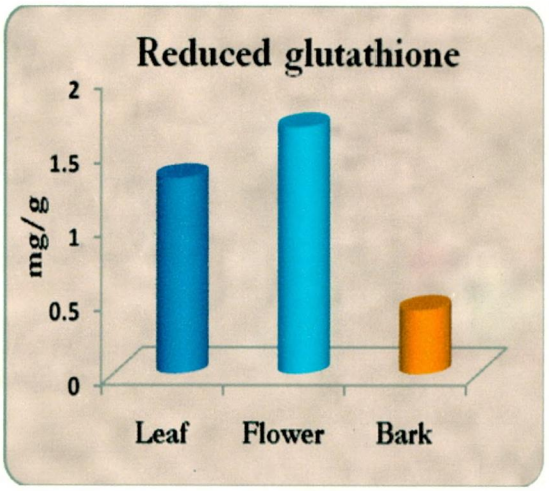












4.6. Assessment of Free radical scavenging effect of *Denolix elata*

Percentage inhibition of ABTS, DPPH, H_2O_2 and hydroxyl radical scavenging were assessed in leaf, flower and bark of different solvents in *Denolix elata* are presented in Table IX and Figure V.

The methanol extract of the leaf of *Denolix elata* exhibited maximum inhibition for the formation of ABTS radical when compared to all other extracts. The flower and bark also showed considerable free radical scavenging activity in all the extracts tested. All the three parts of different extracts tested showed that the methanolic extracts had the maximum DPPH scavenging activity. Among them, the methanolic flower extracts showed the highest percentage followed by leaf.

The four different extracts of leaf, flower and bark of *Denolix elata* were effective in scavenging H_2O_2. The methanolic extract of flower showed better scavenging of H_2O_2 than the other extracts. The hydroxyl radical scavenging effect of the leaf, flower and bark extracts of *Denolix elata* was assayed using deoxyribose. The scavenging effects of different extracts of various parts of the plant were compared. It was observed that the methanolic extract of flower had greater scavenging effect than leaf and bark extracts. Compared to leaf extracts, the scavenging effect of bark was lower.

TABLE IX

FREE RADICAL SCAVENGING POTENTIAL OF *Denolix elata*

S.NO	Samples	Extracts	Percentage Inhibition			
			ABTS	DPPH	H_2O_2	OH
1.	Leaf	Petroleum ether	87.89 ±0.17	61.40 ±0.05	54.47 ±0.32	48.23 ±1.97
		Chloroform	47.91 ±0.08	69.44 ±0.10	59.65 ±0.18	52.56 ±4.63
		Methanol	95.33 ±0.02	88.83 ±0.06	66.82 ±0.18	71.79 ±0.98
		Aqueous	94.36 ±0.06	64.78 ±0.08	52.57 ±0.17	33.01 ±1.37
2.	Flower	Petroleum ether	64.00 ±0.08	55.52 ±0.10	64.99 ±0.15	40.86 ±1.56
		Chloroform	75.58 ±0.12	79.33 ±0.08	62.58 ±0.13	84.61 ±1.41
		Methanol	94.11 ±0.10	88.93 ±0.06	80.49 ±0.15	88.77 ±1.58
		Aqueous	93.54 ±0.07	49.84 ±0.10	46.89 ±0.37	39.26 ±1.19
3.	Bark	Petroleum ether	34.43 ±0.08	47.74 ±0.05	60.98 ±0.12	37.65 ±1.77
		Chloroform	61.20 ±0.11	69.97 ±1.56	57.91 ±0.22	51.91 ±4.43
		Methanol	92.43 ±0.12	79.88 ±0.10	58.33 ±0.09	64.25 ±1.77
		Aqueous	88.67 ±0.08	70.72 ±0.08	54.89 ±0.22	23.39 ±0.98

Values are mean±SD of triplicates

Ethanolic extract of *Canthium parviflorum* Lam. leaves was analysed for ABTS and metal chelating activity. The extract at 500µg/ml showed maximum scavenging activity (51.60%) of ABTS radical cation followed by the iron chelation (45.12%) at the same concentration (Kumar *et al.*, 2008). The methanolic extracts of leaves and flowers of *Lippia alba* had shown very significant DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity of the extract was increased with the increasing concentration (Ara and Nur, 2009).

Plant extract of *Psidium guajava* scavenged H_2O_2 in concentration dependent manner and more effectively than Butylated Hydroxyl Anisole (Ogunlana and Ogunlana, 2008). Extracts of *Lagenaria siceraria* scavenged hydrogen peroxide in concentration-dependent manner (Deore *et al.*, 2009). Gupta *et al.* (2004) have also shows that methanolic extract of *Ervatamia coronaria stapf.* leaves inhibited the hydroxyl radical generated by Fentons reaction indicating that the methanolic extract of *Ervatamia coronaria stapf* can be a potential source of natural antioxidants.

According to Lavhale and Mishra, (2007) methanol extract of *Butea monosperma* along with its ethyl acetate and butanol fractions showed potent free radical scavenging activity, whereas aqueous fraction was found to be devoid of any radical scavenging properties.

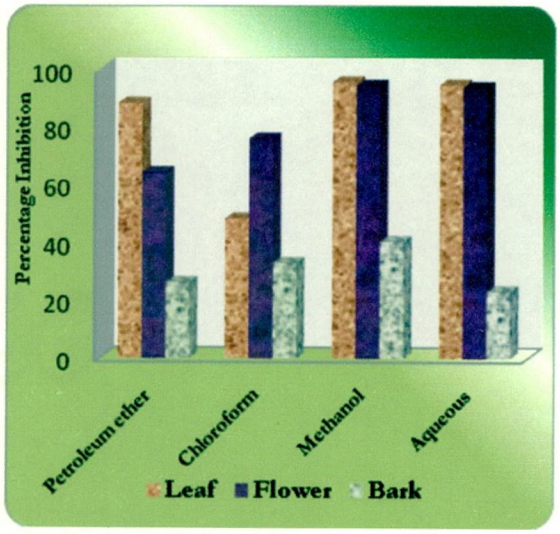
Percentage inhibition of ferrous ion chelating, *invitro* lipid peroxidation, superoxide generation and nitric oxide generation were assessed in leaf, flower and bark of *Denolix elata* on different extracts were recorded in Table X and Figure VI.

FIGURE V

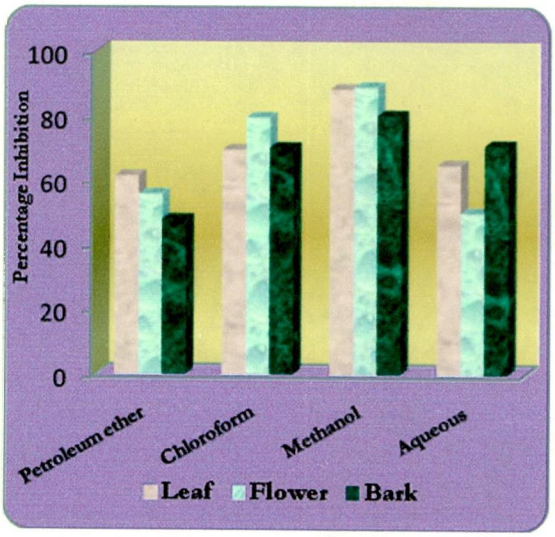
FREE RADICAL SCAVENGING POTENTIAL OF

Denolix elata

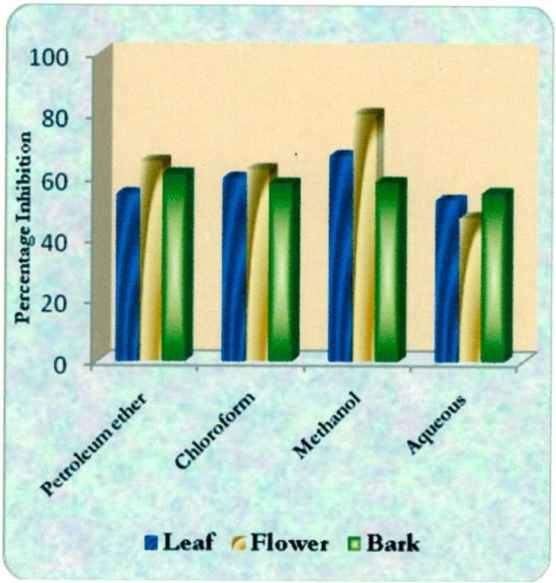
ABTS



DPPH



HYDROGEN PEROXIDE



HYDROXYL RADICAL

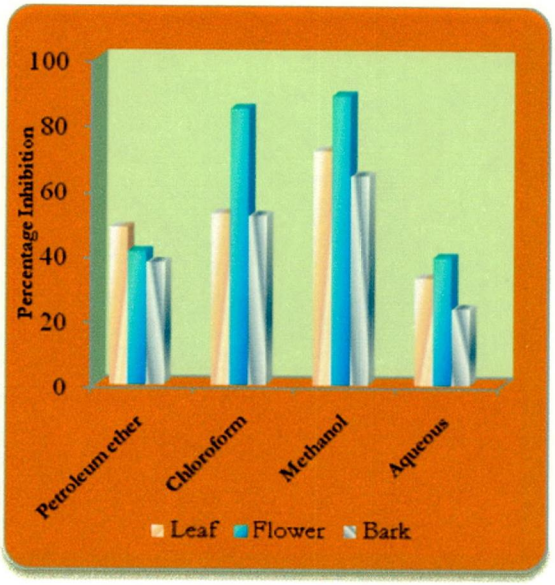


TABLE X

FREE RADICAL SCAVENGING POTENTIAL OF *Denolix elata*

S. NO	Samples	Extracts	Percentage Inhibition			
			Ferrous ion generation	*Invitro* lipid peroxidation	Superoxide generation	Nitric oxide generation
1.	Leaf	Petroleum ether	52.50 ±0.49	49.06 ±0.25	56.05 ±5.66	45.19 ±0.76
		Chlorform	54.93 ±0.75	64.50 ±0.34	56.06 ±9.34	56.64 ±1.53
		Methanol	68.93 ±0.29	77.33 ±0.50	65.14 ±5.66	76.68 ±1.502
		Aqueous	41.76 ±0.53	38.49 ±0.37	62.11 ±7.72	56.02 ±1.53
2.	Flower	Petroleum ether	57.75 ±0.17	50.89 ±0.48	49.99 ±3.17	47.64 ±0.76
		Chlorform	65.67 ±0.22	66.29 ±0.37	59.08 ±3.71	59.09 ±0.76
		Methanol	81.25 ±0.67	85.54 ±0.49	77.26 ±9.82	81.79 ±0.76
		Aqueous	49.82 ±0.27	43.02 ±0.37	49.99 ±7.42	58.27 ±2.64
3.	Bark	Petroleum ether	25.80 ±0.34	39.84 ±0.33	48.48 ±5.67	37.82 ±1.25
		Chlorform	32.71 ±0.20	60.14 ±0.62	56.05 ±5.66	46.21 ±1.26
		Methanol	40.07 ±0.35	68.31 ±1.18	54.54 ±3.71	68.70 ±1.00
		Aqueous	22.88 ±0.56	28.87 ±0.33	42.42 ±5.67	49.69 ±1.00

Values are mean±SD of triplicates

Methanolic extract of leaf and flower inhibited the generation of ferrous ion, lipid peroxidation, superoxide and nitricoxide to a greater extent when compared to other solvents. In the case of bark, methanolic extracts maximally inhibited in the generation of ferrous ion, lipid peroxidation and nitricoxide generation whereas chloroform extract caused maximum inhibition of superoxide generation. A study of antioxidant potential of leaves of three different species of *Annona* by Baskar *et al.,* (2007) suggests that the extract of *Annona muricata* possess potent *invitro* antioxidants activity as compared to leaves of *Annona squamosa* and *Annona reticulata* revealing its role as an effective free radical scavenger.

Aqueous extracts of three different parts of the plant showed the least inhibition towards the generation of ferrous ion and lipid peroxidation and petroleum ether exhibited minimum inhibition for the generation of nitricoxide. The least inhibition of superoxide generation was exerted by both petroleum ether and aqueous extract. The present study reveals that different extracts of leaf, flower and bark of *Denolix elata* showed potential scavenging efficacy. Out of which methanol extracts possess good scavenging effect. Our result shows that these parts of *Denolix elata* possess strong radical scavenging activity, which attributed to its medicinal properties.

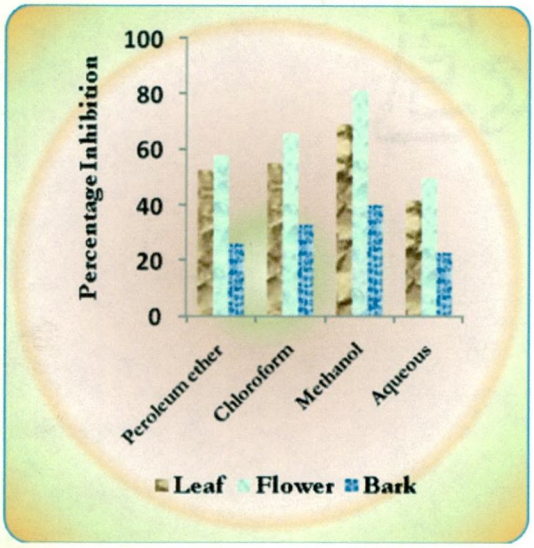
Methanolic extract and ethanolic extract of fringe tree were more effective in chelation of ferrous ion (Glucin *et al.*, 2007). Kaur and Arora (2009) have indicated that the methanolic extract of bark and leaves of *Cassia siamens* and *Cassia javanica* showed the strong antioxidant potential. According to Jelili *et al.* (2010) the leaf extract of *Holarrhena floribunda* showed significant inhibition of NO with IC_{50} value of 244.00µg/ml. Investigation of *Thuja occidentalis* Linn showed maximum inhibition of 61.516± 0.131 (Dubey and Batra, 2009) of lipid peroxidation at 300µg/ml. The results demonstrated that the plant has the potential in scavenging free radicals in appreciable level and can be a vital source of antioxidant phytochemicals.

FIGURE VI

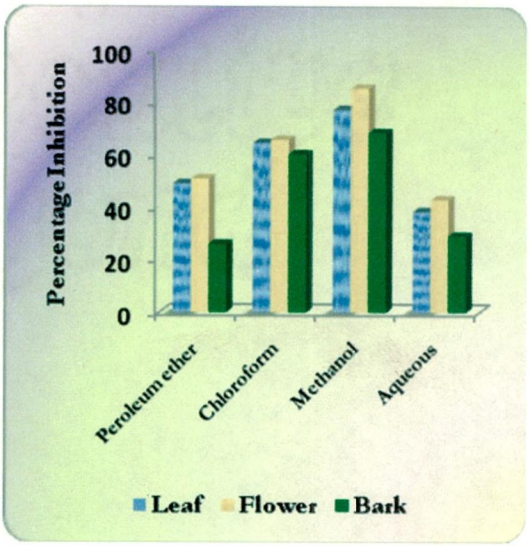
FREE RADICAL SCAVENGING POTENTIAL OF

Denolix elata

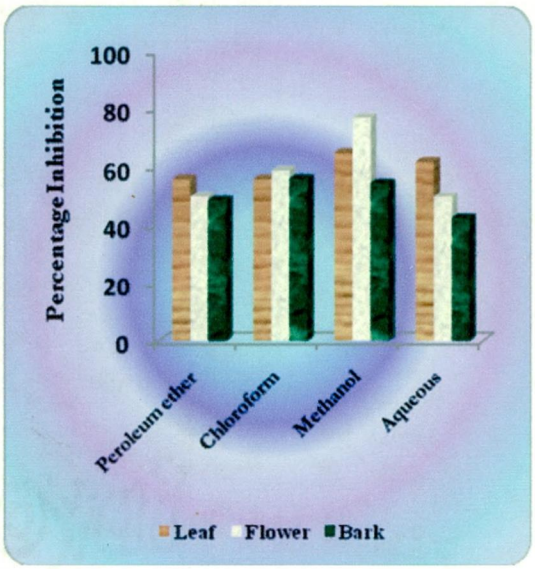
FERROUS ION CHELATION



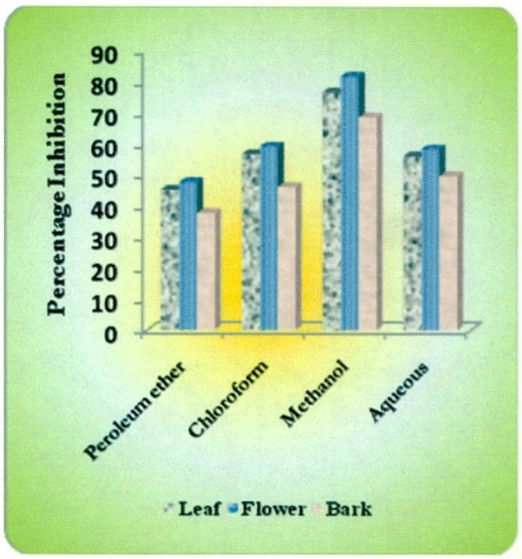
***INVITRO* LIPID PEROXIDATION**



SUPEROXIDE GENERATION



NITRICOXIDE GENERATION



4.7. Biosafety screening of *Denolix elata*

Cytotoxicity study was carried out with brine shrimp following the procedure of Zakaria *et al.* (2007). The lethality of the methanolic extract of leaf, flower and bark of *Denolix elata* to brine shrimp, *Artemia salina* was determined after 24 hour of exposure and the findings are depicted in the Table XI and Figure VII.

TABLE XI
CYTOTOXICITY EFFECT OF METHANOLIC EXTRACTS OF LEAF, FLOWER AND BARK OF *Denolix elata*

S.No	Concentration (µg/ml)	Percentage Lethality			Potassium dichromate
		Leaf	Flower	Bark	
1.	100	0	0	10	40
2.	200	20	20	20	50
3.	300	30	30	50	60
4.	400	50	40	70	80
5.	500	70	50	100	80
6.	1000	100	100	100	100
LC_{50}(µg/ml)		**399.94**	**498.88**	**299.92**	**199.98**

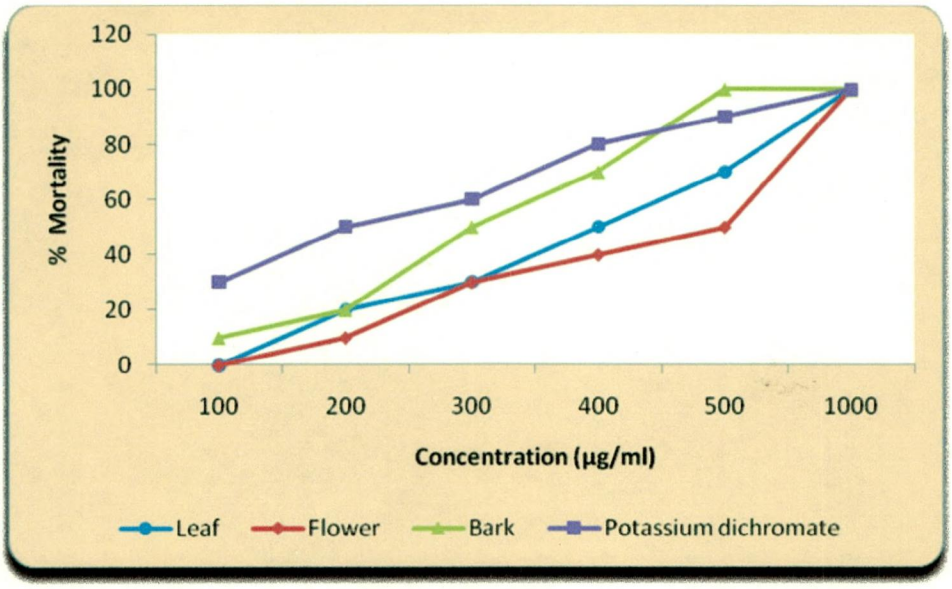
Values are mean±SD of triplicates

The results of brine shrimp lethality assay are expressed in percentage lethality LC_{50} values of the plant extracts were obtained by a plot of percentage of the shrimp naupli killed against the log concentration of the extract. The percentage mortality increased with increase in

concentration of leaves, flowers and bark samples. All the three parts showed 100% mortality to brine shrimp at 1000µg/ml. LC_{50} values ranged from 299.92 to 498.88. Bark exhibited greater cytotoxic effect when compared to leaf and flower. The lowest value was found to be the most potent. The result indicates the ability of the plant extract to kill cancer cells in cell cultures, kill pests and exert a wide range of pharmacological effects.

Figure VII

CYTOTOXIC EFFECT OF METHANOLIC EXTRACT OF LEAF, FLOWER AND BARK OF *Denolix elata*



Akbar *et al.* (2009) have reported moderate cytotoxic activity by methanolic fractions of leaves and bark of *Swietenia mahagoni.* According to Krishnaraju *et al.* (2006) aqueous extracts from118 Indian medicinal plants were screened by the brine shrimp lethality assay and found eleven out of the 118 extracts showed significant toxicity to the brine shrimp (<60 µg/ml).

Nigerian medicinal plants (*Gongronema latifolia, Besella alba linn, and Telfairia occidentalis*) were screened for cytotoxicity and anti oxidant activities using standard methods. The results revealed that all the selected plants were relatively non-toxic and the bioactivity study using brine-shrimps gave LC50 = 1175.16 µg/ml for the most inhibitory activity in *Gongronema latifolia* leaf (James, 2009).

The present study reveals cytoxicity of the plant samples which may have clinical and therapeutic proposition in most life threatening disease like tumour or cancer.

4.8. Evaluation of antibacterial activity

Petroleum ether, chloroform, methanol and aqueous extracts of leaf, flower and bark of *Denolix elata* were used to determine *invitro* antibacterial activity by agar well method. The different extracts of the plant parts were tested against the five bacterial isolates namely *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Shigella flexneri* and *Staphylococcus aureus.* Chloramphenicol disc (30µg) was used as positive control. Isolates were found to be susceptible, less susceptible and resistant to a particular antimicrobial agent on the basis of the diameter of the zone of inhibition. The zone of inhibition produced by leaf, flower and bark is represented in Table XII, XIII, XIV, Figure VIII and Plate II.

Of the various extracts, methanol exhibited greater antibacterial activity against all the microbes used in the study. But the zone of inhibition formed to be maximum only in the case of *Bacillus subtilis.* Followed by methanolic extract, chloroform inhibited microbial growth to a greater extent when compared to petroleum ether and water. Minimum zone of inhibition was caused by aqueous extract against all the microbes except *Staphylococcus aureus.*

TABLE XII

ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *Denolix elata*

Microorganism	Zone of Inhibition(mm)				
	Petroleum ether	Chloroform	Methanol	Aqueous	Control
Bacillus subtilis	9.33 ±1.24	12.66 ±4.10	19.66 ±2.05	7.1 ±0.29	17
Escherichia coli	9.66 ±1.25	11.66 ±2.49	17 ±0.82	7 ±0.81	14
Klebsiella pneumoniae	9 ±2.16	14.33 ±2.05	18.33 ±1.240	5.16 ±0.62	18
Shigella flexneri	7 ±0.81	9.1 ±0.84	13.9 ±0.78	4.6 ±0.53	16
Staphylococcus aureus	8.66 ±1.25	13 ±0.82	16.66 ±1.25	9.66 ±2.49	13

Values are mean±SD of triplicates

These findings are in agreement with the report of Anthony and Bukola, (2009) who have indicated that methanolic leaf extracts of *Lasienthera africanum* exhibited higher degree of antibacterial activities than aqueous extract. Joshi *et al.* (2008) observed largest zone of inhibition with *Xanthoxylum armatum* against *Bacillus subtilis* among tested gram positive bacteria. *Terminalia chebula Retz.* and *Syzygium cumini* showed the

promosing broad spectrum antibacterial properties, inhibiting all of the strains tested (Acharyya *et al.*, 2009).

TABLE XIII

ANTIBACTERIAL ACTIVITY OF FLOWER EXTRACT OF *Denolix elata*

Microorganism	Zone of Inhibition(mm)				
	Petroleum ether	Chloroform	Methanol	Aqueous	Control
Bacillus subtilis	15 ±1.63	19.66 ±2.05	24 ±0.82	7.96 ±0.82	19
Escherichia coli	14 ±0.52	20.66 ±1.25	21.66 ±1.70	8 ±0.82	14
Klebsiella pneumoniae	8 ±0.82	13.66 ±1.25	20.33 ±1.69	7 ±0.82	18
Shigella flexneri	10.66 ±1.24	12.66 ±2.49	19.66 ±2.06	9.66 ±1.25	15
Staphylococcus aureus	9.66 ±1.24	15.33 ±1.25	18 ±0.82	5 ±0.82	17

Values are mean±SD of triplicates

All the extracts of flower were found to be sensitive against all tested bacterial isolates. Methanolic extracts exhibited maximum zone of inhibition against *Bacillus subtilis* followed by *Escherichia coli, Klebsiella pneumoniae, Shigella flexneri* and *Staphylococcus aureus*. Chloroform extract also showed appreciable level of inhibition of bacterial growth. Aqueous extract caused the least inhibition among the tested extracts.

Leaf extracts of *Artemisia nilaggirica* were screened for the potential antimicrobial activity against phytophatogens and clinically important standard reference bacterial strains and the results revealed greater antimicrobial activity by methanol and hexane (Ahameethunsia and Hopper, 2010). Parekh and Chanda (2007) investegated thirty four medicinal plants for potential antibacterial activity and found that the ethanol/methanol were more effective than aqueous extracts against six bacterial strains.

TABLE XIV

ANTIBACTERIAL ACTIVITY OF BARK OF *Denolix elata*

Microorganism	Zone of Inhibition(mm)				
	Petroleum ether	Chloroform	Methanol	Aqueous	Control
Bacillus subtilis	8 ±0.82	9 ±1.63	11.66 ±2.05	6.66 ±1.24	16
Escherichia coli	R	7.66 ±1.24	10.33 ±1.24	2.66 ±0.42	13
Klebsiella pneumoniae	R	4.3 ±1.63	9 ±1.63	R	17
Shigella flexneri	R	5.3 ±1.25	10 ±2.66	4 ±0.82	15
Staphylococcus aureus	4 ±0.82	5 ±0.82	10 ±1.63	R	14

Values are mean±SD of triplicates

R → Resistant

Different extracts of bark were tested for their antibacterial activity and their maximum zone of inhibition was exhibited by methanolic extract against *Escherichia coli* followed by all other strains. All bacteria used were found to be sensitive to methanol and chloroform extracts. *Klebsiella pneumoniae, Shigella flexneri* and *Staphylococcus aureus* were

resistant to petroleum ether extracts. No zone was seen in aqueous extract against *Klebsiella pneumoniae* and *Staphylococcus aureus*.

According to Ogbonnia *et al.* (2008) the aqueous ethanol extract of the bark of *Treculia Africana Decne* was effective *invitro* in inhibiting the growth of gastrointestinal bacterial pathogens. Pavithra *et al.* 2010 have shown that chloroform and methanol extracts of *Denolix elata* exhibited significant antibacterial activity against gram positive and gram negative strains with minimum bactericidal concentration (MBC) ranging from 1.5 to 100 mg/ml.

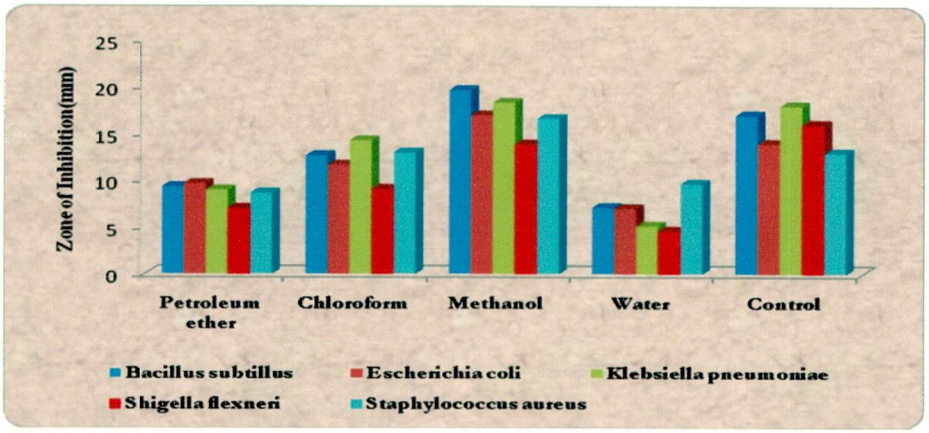
Among the various solvents used for extraction of leaf, flower and bark, the prominent activity was conferred by the extracts of methanol with the highest zone of inhibition against the tested bacterial isolates, whereas chloroform was able to reduce zone to moderate extent. There was only a meek inhibition produced by the extract of petroleum ether for all the plant samples. But aqueous extracts of *Denolix elata* tested against organism elicit only trace inhibition. However in general, the extracts obtained from methanol was able to tremendously inhibit the growth of bacterial isolates tested.

The antibacterial activity of the different parts of *Denolix elata* revealed that flowers were more effective in inhibiting the bacterial growth than leaf and bark. Thus the study reveals the potential use of the plant for developing new antibacterial compounds against bacterial isolates.

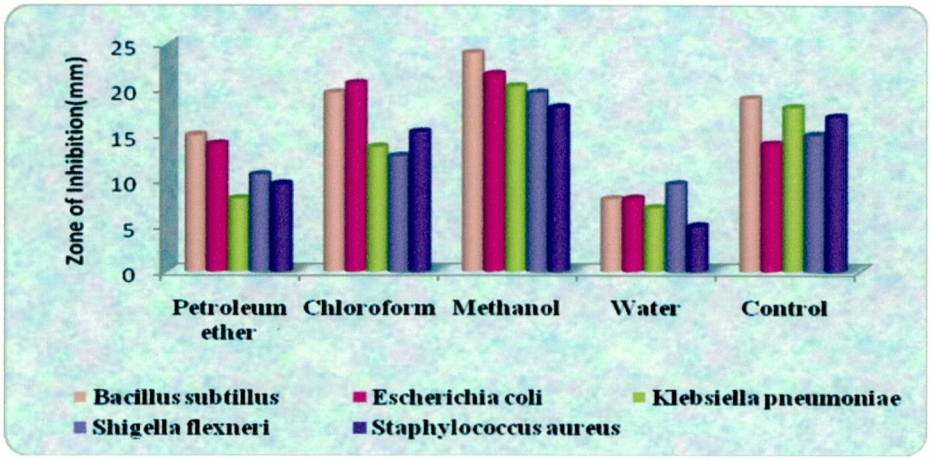
FIGURE VIII

ANTIBACTERIAL ACTIVITY OF *Denolix elata*

LEAF



FLOWER



BARK

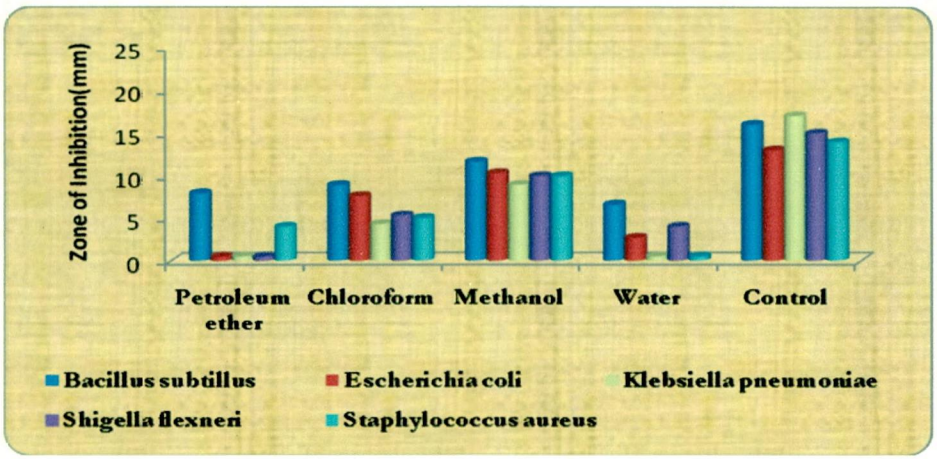
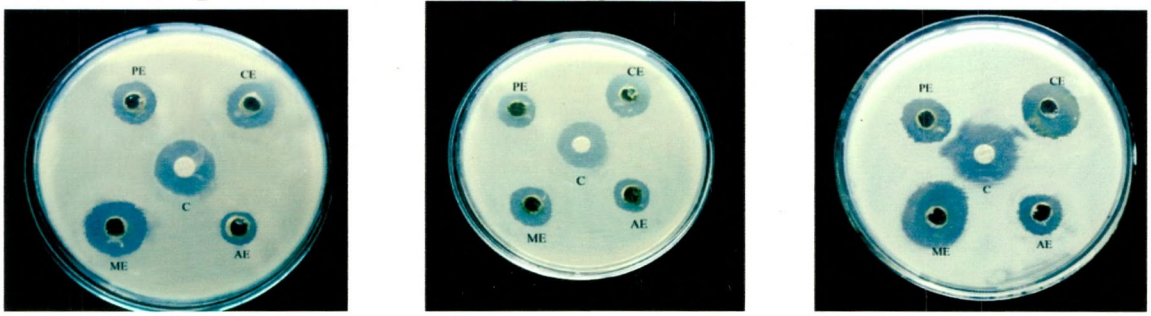


PLATE NO.II

ANTIBACTERIAL ACTIVITY OF LEAF, FLOWER AND BARK OF *Denolix elata* AGAINST DIFFERENT BACTERIAL ISOLATES

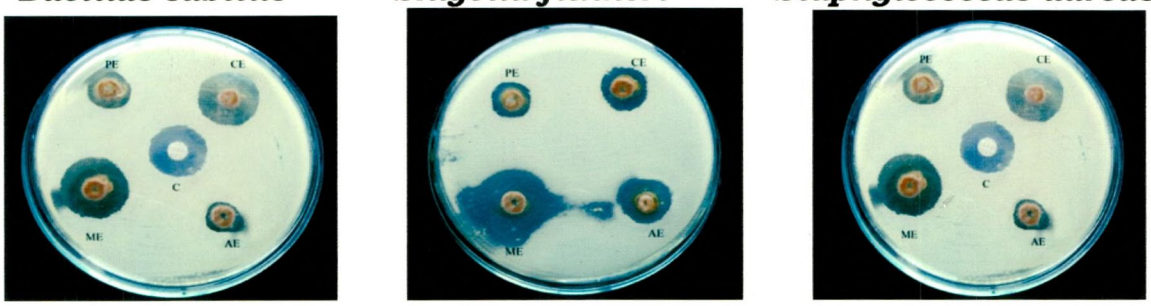
Antibacterial activity of *Denolix elata* leaf against bacterial isolates

Klebsiella pneumoniae ***Shigella flexneri*** ***Bacillus subtilis***



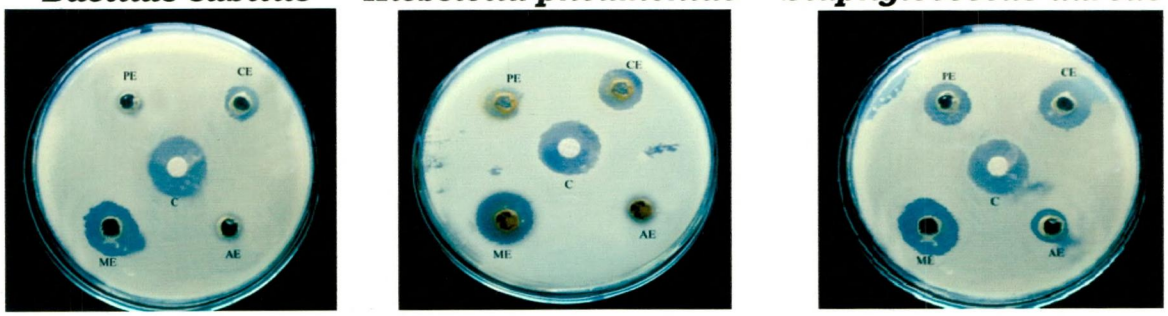
Antibacterial activity of *Denolix elata* flower against bacterial isolates

Bacillus subtilis ***Shigella flexneri*** ***Staphylococcus aureus***



Antibacterial activity of *Denolix elata* bark against bacterial isolates

Bacillus subtilis ***Klebsiella pneumoniae*** ***Staphylococcus aureus***



PE-Petroleum ether extracts **CE- Chloroform extracts**

ME-Methanol extracts **AE - Aqueous extracts**

C-Chloromphenicol disc