4.0 RESULTS AND DISCUSSION

Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases (Dash *et al.*, 2008). Diseases that remain most challenging in today's healthcare system tend to be complex involving multiple mechanisms, targets and drugs for effective disease management. In contrast to current combination therapies, however, plant based drugs contain a mixture of multiple components thereby saving considerable time and expense (Awoyinka *et al.*, 2007).

It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Currently, there is a growing interest towards natural antioxidants of herbal resources, medicinal plants and vegetables supporting that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Souri *et al.*, 2008). Phytochemicals (especially secondary metabolites) have been recognized as antioxidant sources in crude extracts and dietary foods (Tung *et al.*, 2008). Medicinal plants are also known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth. Higher plants have been shown to be a potential source for new antimicrobial agents (Varahalarao and Chandrashekar, 2010).

In the present investigation the leaf and stem samples of *Mukia maderaspatana* (L.) were evaluated qualitatively for phytochemical constituents namely alkaloids, anthroquinones, flavonoids, glycosides, phenols, reducing sugars, saponins, phytosteroids, tannins and terpenoids and some of these

constituents were determined quantitatively. The protein and carbohydrate contents were also estimated in the above samples. Various free radicals scavenging activity of the plant sample was evaluated. Enzymatic and nonenzymatic antioxidants were analysed in leaf and stem samples of the selected medicinal plant. The cytotoxic effect was identified in the ethanolic extracts of the both leaf and stem extracts. The antibacterial activity was also determined in the different extracts of leaf and stem samples of the experimental plant.

The findings of the present study are discussed under the following headings:

4.1 Yield of the plant samples

4.2 Qualitative screening of phytochemicals of the plant samples

4.3 Determination of phytochemicals in the plant samples

4.4 Nutritive value of the plant

4.5 Free radical scavenging activity of the plant samples

4.5.1 DPPH radical scavenging activity

4.5.2 ABTS radical scavenging activity

4.5.3 Hydrogen peroxide scavenging activity

4.5.4 Ferrous ion chelating activity

4.5.5 Inhibition of superoxide generation

4.5.6 Inhibition of nitric oxide generation

4.5.7 Inhibition of hydroxyl ion generation

4.5.8 Inhibition of *In vitro* lipid peroxidation

4.6 Antioxidant potential of the plant samples

4.6.1 Enzymatic antioxidants

4.6.2 Nonenzymatic antioxidants

4.7 Cytotoxic effect of the plant samples

4.8 Antibacterial activity of the plant samples

4.1 Yield of the plant samples

Leaf and stem sample of the selected medicinal plant was extracted with the solvents such as petroleum ether, ethanol, ethylacetate, and water by Soxhlet extraction and maceration method. The percentage yield of different extracts of the selected plant parts of *Mukia maderaspatana* (L.) is given in Table 1.

It is clear from the above table that the highest yield was recorded when extraction was performed with water and the lowest value was observed in the petroleum ether extract. Extraction of various phytochemicals revealed that petroleum ether and ethanol extracted the phytochemicals from leaf and stem samples to a greater extent by Soxhlet extraction, whereas maceration method produced higher yield with ethylacetate and water. The order of extraction with various solvents by two different methods was not found to be the same.

In Soxhlet extraction the order was ethanol > water > ethylacetate > petroleum ether for leaf sample and water > ethylacetate > ethanol > petroleum ether for stem sample. In the case of maceration method, the order was water > ethylacetate > ethanol > petroleum ether for leaf sample and ethylacetate > water > ethanol > petroleum ether for stem sample.

Chan *et al.*, (2008) documented that, the three different methods of extraction namely boiling in water, maceration and blending yielded different yield of the plant extract. Extraction yield is dependent on the solvent and the method of extraction. The extraction method must allow complete extraction of the compounds and it must avoid their chemical modification (Hayouni *et al.*, 2007).

TABLE 1

YIELD OF SOLVENT EXTRACTS FROM LEAF AND STEM SAMPLES OF *Mukia maderaspatana* (L.)

PLANT PARTS	PERCENTAGE YIELD							
	SOXHLET EXTRACTION				MACERATION EXTRACTION			
	PE	E	EA	W	PE	E	EA	W
LEAF	5.1	19.1	13.5	14.5	3.3	14.0	24.6	54.6
STEM	5.0	10.0	12.6	19.5	1.0	8.5	22.1	17.2

PE- Petroleum ether E- Ethanol
EA-Ethylacetate W-Water

4.2 Qualitative screening of phytochemicals of the plant samples

The qualitative analysis of the phytochemicals in the leaf and stem sample of *Mukia maderaspatana* (L.) is shown in Table 2.

The results showed the presence of phytochemical constituents such as alkaloids, anthroquinones, flavonoids, phenols, reducing sugars, saponins, phytosteroids and tannins. Glycosides and terpenoids were found to be absent in both leaf and stem.

Phytoconstituents are known to support bioactive activities in medicinal plants. Tannins are known to be useful in the treatment of inflammed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Akinpelu *et al.*, 2009). Flavonoids, phenols and saponins have been shown to exhibit their actions through effects on membrane permeability, antioxidative action and also to produce inhibitory effect on inflammation (Olayinka and Okoh, 2010). Alkaloids are haemolytically active and are also toxic to microorganisms. Alkaloids are widely used as therapeutic agents in the management of cancer (Soetan and Aiyelaagbe, 2009). Anthroquinones also possess antiparasitic,

bacteriostatic, antidepressant and antimicrobial properties (Peteros and Uy, 2010). Glycosides are known to inhibit tumor growth and also protect against gastrointestinal infections (Adeshina *et al.*, 2010). Terpenoids have been demonstrated to be active against bacteria, fungi, viruses and protozoa (Maiyo *et al.*, 2010).

Thus the presence of these secondary metabolites in the plant *Mukia maderaspatana (L.)* suggests some of its medicinal properties and further investigations can lead to the discovery of novel therapeutics.

TABLE 2
QUALITATIVE SCREENING OF PHYTOCHEMICALS IN LEAF AND STEM SAMPLES OF *Mukia maderaspatana* (L.)

PHYTOCHEMICALS	LEAF	STEM
ALKALOIDS		
Mayer's test	**+ve**	**+ve**
Wagner's test	**+ve**	**+ve**
ANTHROQUINONES	**+ve**	**+ve**
FLAVONOIDS	**+ve**	**+ve**
GLYCOSIDES	**-ve**	**-ve**
PHENOLS	**+ve**	**+ve**
REDUCING SUGAR	**+ve**	**+ve**
SAPONINS	**+ve**	**+ve**
PHYTOSTEROIDS	**+ve**	**+ve**
TANNINS	**+ve**	**+ve**
TERPENOIDS	**-ve**	**-ve**

+ve – detected; -ve – not detected

4.3 Determination of phytochemical content of the plant sample

Quantitative estimation of the phytochemicals present in the plant sample was carried out and the estimated phytochemicals of the plant were alkaloids, phenols, tannins, reducing sugars and chlorophyll. The content of the phytochemicals are given in Table 3.

TABLE 3

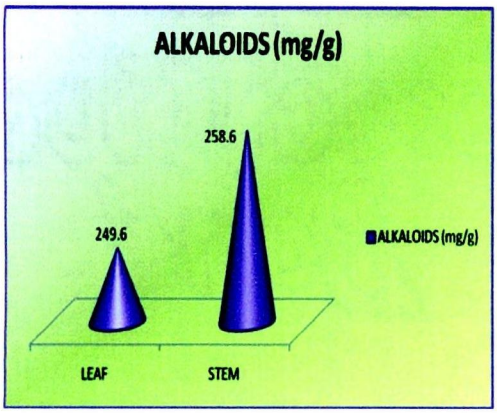
PHYTOCHEMICAL CONTENT IN LEAF AND STEM SAMPLES OF *Mukia maderaspatana* (L.)

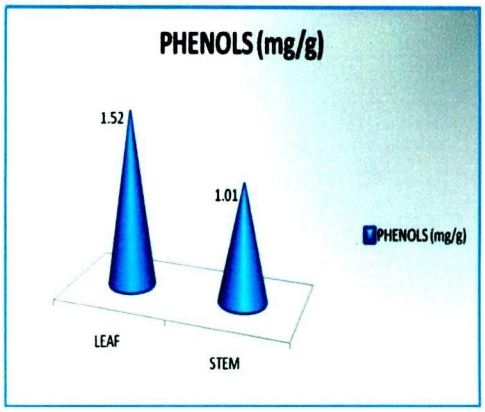
PLANT PARTS	PHYTOCHEMICALS (mg/g)				
	ALKALOIDS	PHENOLS	TANNINS	REDUCING SUGARS	CHLORO-PHYLL
LEAF	249.6±5.19	1.52±0.08	1.48±0.09	1.90±0.32	12.47±0.22
STEM	258.6±5.73	1.01±0.05	1.19±0.38	0.21±0.11	3.60±0.10
t-value	0.243^{ns}	7.755*	1.043^{ns}	6.994*	52.770*

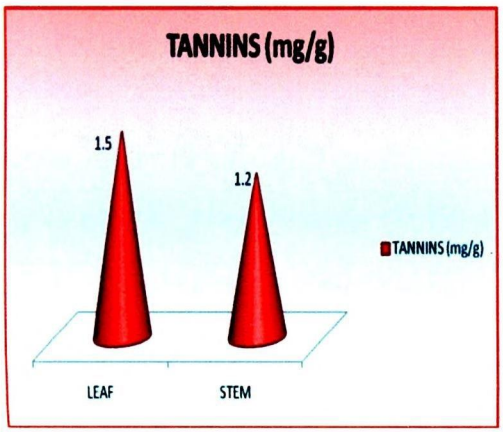
Values are mean ± S.D of triplicates
*- Significant at $p<0.05$ level
ns - not significant

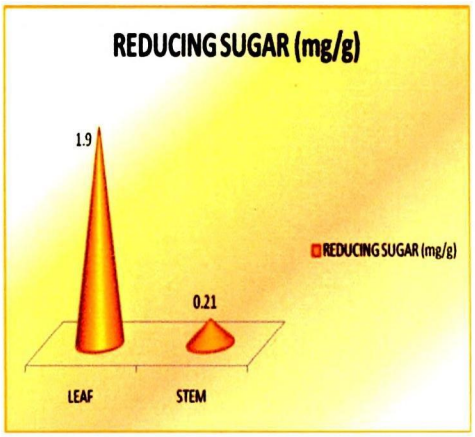
FIGURE 1

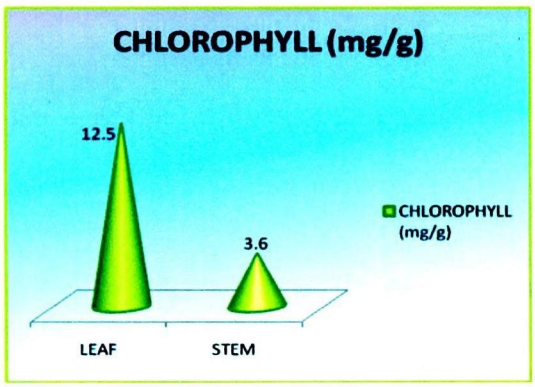
PHYTOCHEMICAL CONTENT OF *Mukia maderaspatana*(L.)











From Table 3 and Figure 1, it is evident that, the amount of phenols, tannins, reducing sugars and chlorophyll was found to be increased in the leaf sample and the amount of alkaloids was high in the stem sample. But the increase was statistically insignificant in the case of alkaloids and tannins.

It was documented that, the plants containing alkaloids and steroids are used traditionally for treatment of hypertension, dysentery and other intestinal disorders and also aids in wound healing (Krishnaiah *et al.*, 2009). Pharmacological investigations on the alkaloids have demonstrated interesting biological activities and *in vitro* studies show antioxidative and neuroprotective actions (Moura *et al.*, 2007). It is well known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma and flavor and also in providing health beneficial effects. They also counteract reactive oxygen species in order to prevent molecular damage (Sengul *et al.*, 2009). Paaver *et al.*, (2010) suggested that tannins and related polyphenols have been implicated to various pharmacotherapeutic effects. In particular, the tannin containing remedies are in use as antihelmintics, antioxidants, antimicrobials, antivirals and for the cancer treatment. Consumption of tannins as green tea and wines prevents different illnesses and inhibits viral reverse transcriptase (Gertrudes, 2006).

Lower content of reducing sugar in medicinal plants are encouraged in the diabetic patient's diet to control blood sugar (Kochhar *et al.*, 2006). It has been reported that chlorophyll, the green pigment of the plant is used as a health enhancing nutritional substance with many benefits. It defends against free radicals and toxins and also has expanded benefits like boosts immune system, resists bacteria in wounds, aids haemophilia conditions and antioxidant properties. (www.healthyyounaturally.com).

Thus the qualitative and quantitative determination of phytochemicals in *Mukia maderaspatana* (L.) revealed that the leaf and stem sample possess compounds with pharmacological properties which can be further investigated for the discovery of medicines.

4.4 Nutritive value of the plant sample

Table 4 and Figure 2, predict the nutritive value of the plant parts of the selected plant *Mukia maderaspatana* (L.). The carbohydrate content was found to be higher than the protein content in the leaf and stem samples of the plant. The leaf sample recorded significantly higher amount of carbohydrate and protein than the stem.

TABLE 4

NUTRITIVE VALUE OF LEAF AND STEM SAMPLES OF *Mukia maderaspatana* (L.)

PLANT PARTS	NUTRITENT CONTENT(mg/g)	
	TOTAL CARBOHYDRATES	TOTAL PROTEINS
LEAF	230.5±9.54	14.90±0.36
STEM	126.5±12.99	10.34±0.16
t-value	9.115*	16.274*

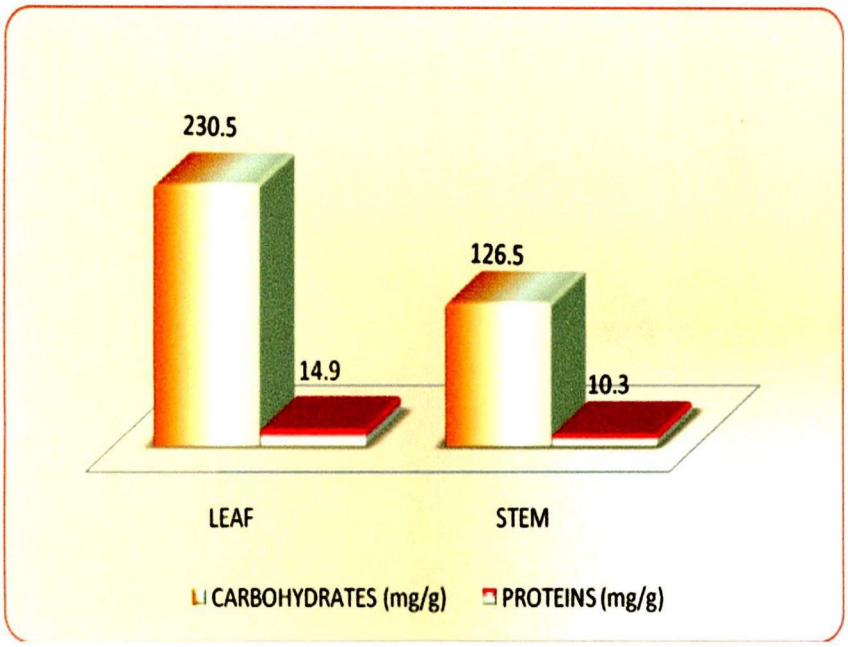
Values are mean ± S.D of triplicates
*- Significant at $p<0.05$ level

Similar to the findings of the experimental plant, the leaves of *Carthamus oxyacantha* and *Eruca sativa* contained high content of carbohydrates than proteins (Bukhsh *et al.*, 2007). Also stem part of *Khaya grandifoliola* contained high content of carbohydrates than the proteins (Ojokku *et al.*, 2010). Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological

functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes (Adnan *et al.*, 2010).

FIGURE 2

NUTRITIVE VALUE OF *Mukia maderaspatana* (L.)



4.5 Free radical scavenging effect of the plant sample

The extent of scavenging the free radicals namely DPPH, ABTS, Hydrogen peroxide and Ferrous ion by the plant sample at 100µg/ml concentration is shown in Table 5.

4.5.1 DPPH radical scavenging activity

In the DPPH test, the ability of the plant extracts to act as a donor of hydrogen atoms or electrons in the transformation of $DPPH^{\cdot}$ into its reduced form DPPH-H was measured spectrophotometrically (Salehi *et al.*, 2007).

From the Table 5 and Figure 3, DPPH test revealed that free radical scavenging effect of leaf extracts was higher than the stem extracts. The high level of scavenging effect was found to be in ethanolic extracts of leaf and stem samples. Followed by ethanol extract of leaf sample, water extract of leaves showed good scavenging effect. The lowest level of scavenging effect was found to be in the ethylacetate extract of leaves. In the case of stem, the ethylacetate extract exhibited good scavenging effect and the least scavenging effect was observed in petroleum ether extract.

Hayet *et al.*, (2008) reported that the ethylacetate, chloroform and methanol extracts of *Retama raetam* plant showed effective antioxidant activities with the DPPH. The aqueous, methanolic and ethanolic extracts obtained from *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus* promoted high inhibitory potency of DPPH radical (Picada *et al.*, 2009).

4.5.1 ABTS radical scavenging activity

The radical scavenging activity of the extracts was also determined by the ABTS radical decolourisation assay. The technique for the generation of ABTS radical involves the direct production of the blue/green $ABTS^{\cdot+}$ chromophore through the reaction between potassium persulfate and ABTS. The decrease in $ABTS^{\cdot+}$ radical in the presence of antioxidant species in the extracts was evaluated (Martini *et al.*, 2009).

The Table 5 and Figure 3, depict that in ABTS assay, the ethanolic extract of leaf and stem sample showed maximum scavenging effect and the water extract showed minimum scavenging effect. The ethylacetate and petroleum ether extracts of leaf and stem showed moderate scavenging activity. Of all the extracts, the leaf sample was found to be a better scavenger of ABTS radical than the stem sample.

TABLE 5

FREE RADICALS SCAVENGING ACTIVITY OF LEAF AND STEM SAMPLES OF *Mukia maderaspatana* (L.)

PLANT EXTRACTS	% SCAVENGING ACTIVITY							
	DPPH		ABTS		HYDROGEN PEROXIDE		FERROUS ION	
	L	S	L	S	L	S	L	S
Petroleum ether	54.6	44.3	51.2	45.3	38.6	44.9	31.3	24.8
Ethanol	81.5	77.9	83.0	72.5	44.2	46.8	57.7	48.0
Ethyl acetate	52.2	48.0	53.3	48.5	34.4	36.7	81.2	70.7
Water	59.3	47.0	41.9	25.6	39.0	45.5	63.2	66.2

Values are mean of triplicates; **L**- Leaf extract **S**-Stem extract

The ethylacetate fraction of the plant *Evax pygmaea* was the strongest radical scavenger whereas, the petroleum ether fraction of the same plant was found to be a weak radical scavenger (Boussaada *et al.*, 2008). The ethylacetate extracts of *Stevia rebaudiana* showed potential activity in the $ABTS^{+}$ decolorization assay (Ghanta *et al.*, 2007).

4.5.3 Hydrogen peroxide scavenging activity

Hydrogen peroxide is very important because of its ability to penetrate biological membranes. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical

in the cells. Thus, removal of hydrogen peroxide is very important for protection of food systems (Sunil and Dinesh, 2009).

It is evident from Table 5 and Figure 4 that, the hydrogen peroxide scavenging activity of different extracts of leaf sample was found to have lower scavenging activity than the stem samples of the plant. The ethanolic extract of leaf and stem samples showed higher scavenging activity whereas, the ethylacetate extract of leaf and stem samples showed least scavenging of hydrogen peroxide.

Deore *et al.*, (2009), documented that the ethanol extract of *Lagenaria siceraria* showed strong hydrogen peroxide scavenging activity. The ethylacetate extract of *Ficus racemosa* exhibited moderate activity in scavenging hydrogen peroxide (Surendra and Vivek, 2008).

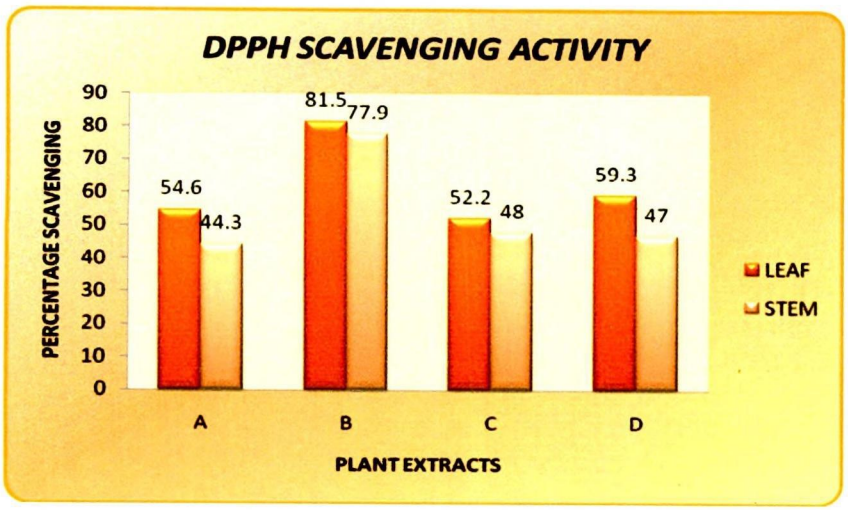
4.5.4 Ferrous ion chelating activity

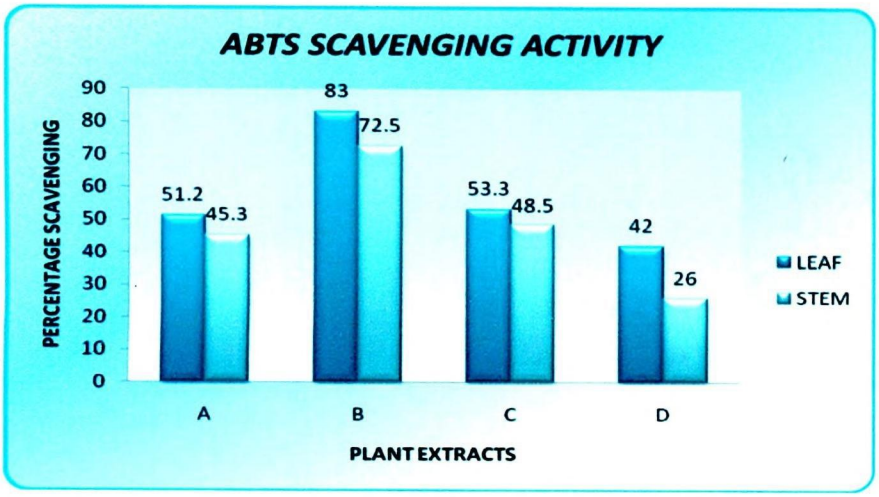
Iron, in nature can be found as either ferrous or ferric ion with the latter form of ferric ion predominating in foods. Ferrous ion (Fe^{2+}) chelation may render important antioxidative effects by retarding metal-catalyzed oxidation. Fe^{2+} ion is the most powerful prooxidant among the various species of metal ions. Minimizing ferrous (Fe^{2+}) ions may afford protection against oxidative damage by inhibiting production of ROS and lipid peroxidation (Gulcin *et al.*, 2010).

Investigation of ferrous ion chelating activity of the plant revealed that, the ethylacetate extracts of leaf and stem sample showed the highest chelating activity than the other extracts. Followed by ethylacetate extracts, water extracts of leaf and stem sample showed high activity in chelating ferrous ion. The ethanol and petroleum ether extract showed moderate and low ferrous ion chelating activity respectively. All the leaf extracts of different solvents showed higher chelating activity than the stem extracts, except the water extract, which showed higher activity in stem than the leaf sample.

FIGURE 3

DPPH AND ABTS RADICALS SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF *Mukia maderaspatana* (L.)



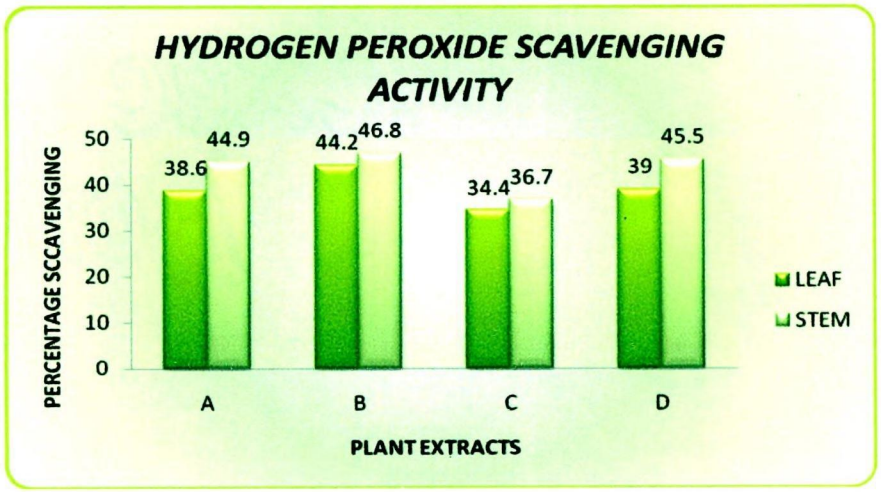


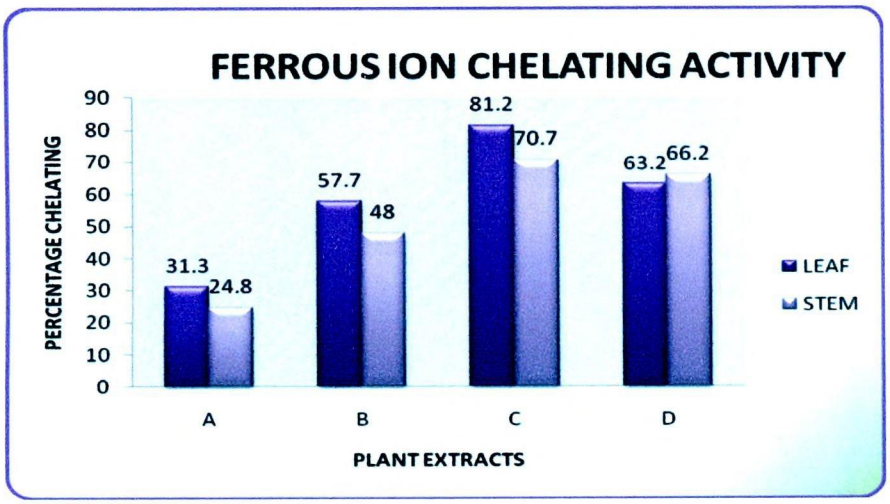
A- Petroleum ether **B- Ethanol**

C- Ethyl acetate **D- Water**

FIGURE 4

HYDROGEN PEROXIDE AND FERROUS ION CHELATING ACTIVITY OF DIFFERENT EXTRACTS OF *Mukia maderaspatana* (L.)





A- Petroleum ether **B- Ethanol**

C- Ethyl acetate **D- Water**

Aqueous extract of the plant *Dennettia tripetala* has higher chelating activity (Adedayo *et al.*, 2010). The ethylacetate extract of *Chionanthus virginicus* L. extract showed high activity in disruption of Fe^{2+}-ferrozine complex exhibiting high chelating activity (Gulcin *et al.*, 2007).

Thus, the above findings revealed that the leaf and stem samples of different extracts play an important in protecting against oxidative damage by sequestering the free radicals.

The effect of the plant samples in inhibiting superoxide, nitric oxide, hydroxyl ion generation and *in vitro* lipid peroxidation is shown in Table 6.

4.5.5 Inhibition of superoxide generation

Superoxide anion is a reduce form of molecular oxygen and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen (Ani *et al.*, 2006).

Table 6 and Figure 5 revealed that, the highest level of inhibitory effect of superoxide generation was observed in the ethanolic extracts of leaf and stem sample and the lowest inhibitory effect were produced by water extract of leaf and ethylacetate extract of stem sample. In all the solvent extracts, the leaf sample was found to have higher inhibitory effect. Except the aqueous extract, other extracts showed high inhibition of superoxide generation in stem sample.

Kaur and Arora, (2009) reported that, the extraction of *Cassia siamea and Cassia javanica* leaves with different solvents showed that ethylacetate extract caused maximum inhibition of superoxide generation and the water extract inhibited to a lesser extent. The plants namely *Melastoma malabathricum*, *Dicrunopteris linearis* showed high inhibition of superoxide generation in aqueous extracts of leaves (Zakaria, 2007).

4.5.6 Inhibition of nitric oxide generation

The plant/plant products may have the property to counteract the effect of nitric oxide formation and in turn may be considerable interest in preventing the ill effects of excessive nitric oxide generation in the human body. Further, the scavenging activity may also help to arrest the chain reactions initiated by excess generation of nitric oxide that are detrimental to human health (Mahmoudi *et al.*, 2009).

From Table 6 and Figure 5, it is evident that the ethylacetate extract of leaf and stem samples showed significantly the highest inhibition of nitric oxide generation followed by water extract. Least inhibitory effect was observed in the petroleum ether extracts of both the parts of the plant. Of all the leaf extracts, the ethylacetate, water and petroleum ether had higher inhibitory effect than stem and only the ethanolic extract of stem recorded higher inhibitory effect than leaf.

Hepsibha *et al.*, (2010) documented that, the leaf sample of *Azima tetracantha* Lam. showed greater scavenging effect of nitric oxide in ethylacetate extract than the petroleum ether extract. The ethanolic leaf extract of *Indigofera aspalathoides* effectively scavenged the nitric oxide radicals (Philips *et al.*, 2010). Also Meera *et al.*, (2009), reported that the ethanolic extract of *Ocimum basilicum* and *Trigonella foenum graecum* showed high inhibition of nitric oxide generation at the concentration 100µg/ml.

4.5.7 Inhibition of hydroxyl radical generation

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging the biomolecules of living cells (Zhang *et al.*, 2009).

From the Table 6 and Figure 6, it is predicted that the hydroxyl ion generation was highly inhibited in the ethanolic extracts of leaf and stem samples. Least inhibition was observed in petroleum ether extracts of leaf and stem samples. Inhibition of hydroxyl ion generation by various solvent extracts revealed high inhibitory effect in leaf samples and low inhibitory effect in stem samples.

Maximum inhibition of hydroxyl radical generation in ethylacetate extract of *Butea monosperma* Lam. was documented by Lavhale and Mishra, (2007). Basker *et al.*, (2007) reported that, the ethanolic leaf extract of *Annona squamosa*, *Annona muricata* and *Annona reticulate* showed moderate inhibition at 100µg/ml among various concentrations.

4.5.8 Inhibition of lipid peroxidation

Peroxidation is important in food deterioration and in the oxidative modification of biological molecules particularly lipids. Inhibition of lipid peroxidation by any external agent is often used to evaluate its antioxidant capacity. Kaviarasan *et al.*, 2007).

The findings of lipid peroxidation *in vitro* in the leaf and stem sample if the plant revealed that, high inhibitory effect in ethanol extracts and low inhibitory effect in petroleum ether extracts. Among all, the leaf extracts showed higher lipid peroxidation than the stem sample.

Deepa *et al.*, (2009), have indicated that the ethanolic extracts of *Commiphora caudata* and *Commiphora varpubescens* scavenged the free radicals in a dose dependent manner. The water extract of *Psoralea corylifolia* L. produced the least inhibition of lipid peroxidation among chloroform, hexane and alcoholic extracts of the plant (Kiran and Raveesha, 2010).

From the present findings, it is clear that the extracts of plant *Mukia maderaspatana (L.)* possess antioxidant properties and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

TABLE 6

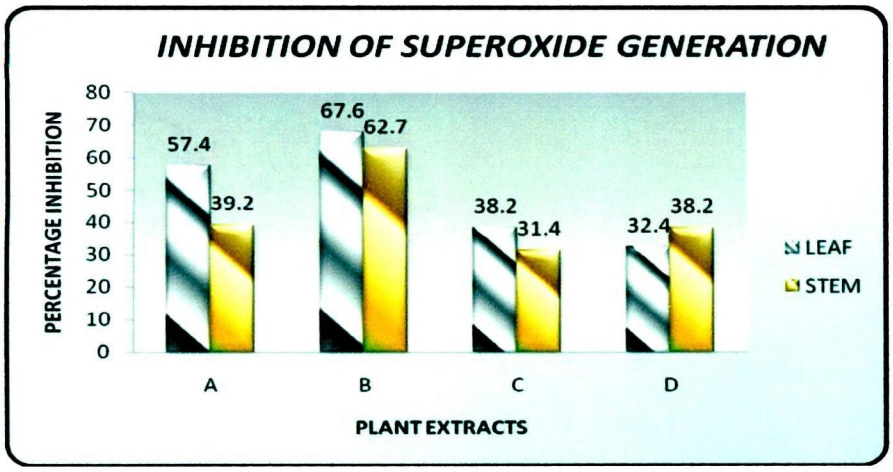
EFFECT OF LEAF AND STEM SAMPLES OF *Mukia maderaspatana* (L.) ON INHIBITION OF FREE RADICAL GENERATION

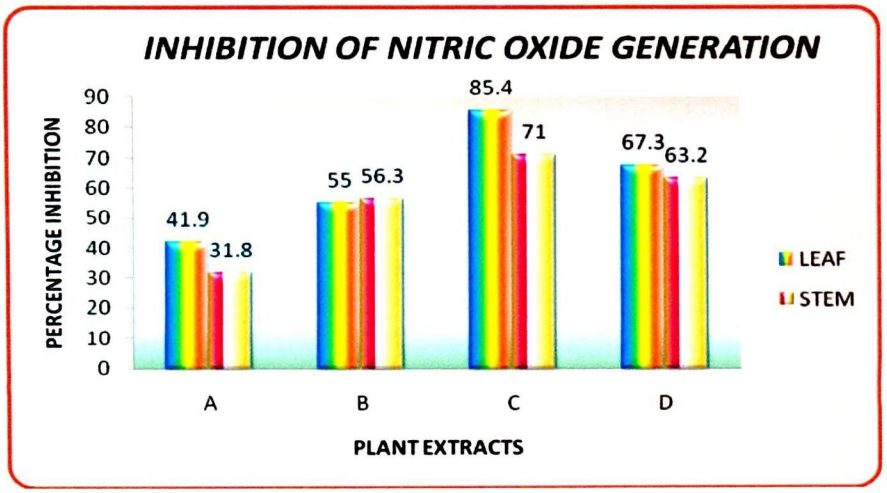
PLANT EXTRACTS	% INHIBITION							
	SUPEROXIDE GENERATION		NITRIC OXIDE GENERATION		HYDROXYL ION GENERATION		LIPID PEROXI-DATION	
	L	S	L	S	L	S	L	S
Petroleum ether	57.4	39.2	41.9	31.8	36.1	26.6	34.2	23.4
Ethanol	67.6	62.7	55.0	56.3	79.3	62.0	83.5	66.2
Ethyl acetate	38.2	31.4	85.4	71.0	73.3	64.1	77.8	63.1
Water	32.4	38.2	67.3	63.2	47.6	28.3	39.5	38.4

Values are mean of triplicates
L- Leaf extract **S**-Stem extract

FIGURE 5

EFFECT OF DIFFERENT EXTRACTS OF *Mukia maderaspatana* (L.) ON INHIBITION OF SUPEROXIDE AND NITRIC OXIDE GENERATION



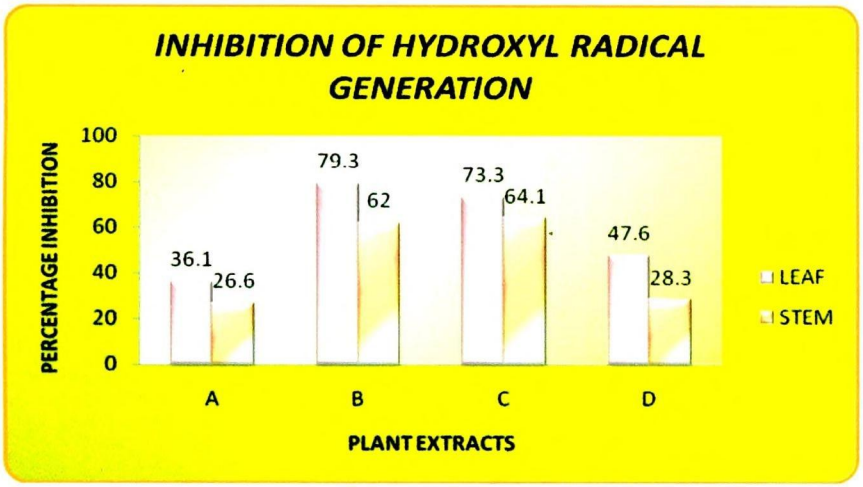


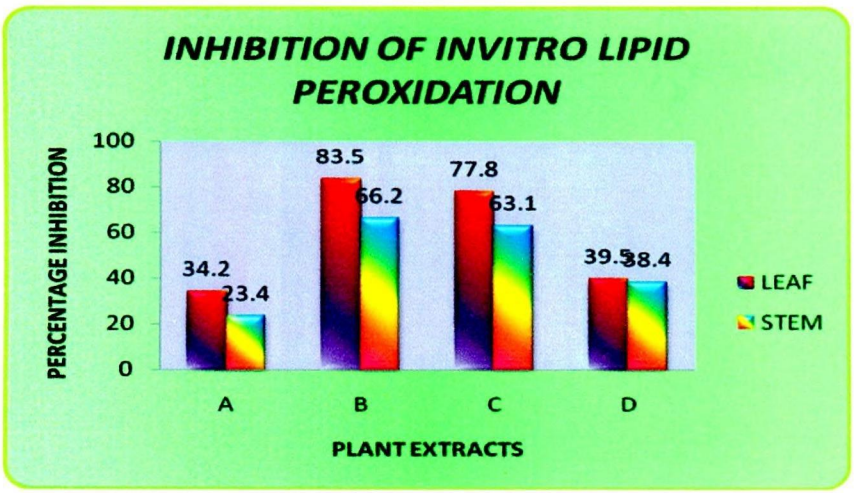
A- Petroleum ether **B- Ethanol**

C- Ethyl acetate **D- Water**

FIGURE 6

EFFECT OF DIFFERENT EXTRACTS OF *Mukia maderaspatana* (L.) ON INHIBITION OF HYDROXYL ION GENERATION AND *INVITRO* LIPID PEROXIDATION





A- Petroleum ether B- Ethanol

C- Ethyl acetate D- Water

4.6 Antioxidant potential of the plant sample

The levels of enzymatic and nonenzymatic antioxidants assessed in two different parts of the plant are collectively represented in the following Tables.

4.6.1 Enzymatic antioxidants

The levels of various antioxidative enzymes were determined and presented in Table 7.

Table 7 and Figure 7 revealed the activity of superoxide dismutases, catalase, peroxidase, glutathione peroxidase, glutathione-s-transferase and polyphenol oxidase in the plant samples. From the Table it is evident that, the activity of the enzyme catalase, peroxidase, glutathione peroxidase and polyphenol oxidase was higher in leaf sample than the stem sample but the difference was statistically significant in the case of catalase and glutathione peroxidase. The stem sample recorded significantly higher level of superoxide dismutases. Though the activity of glutathione-s-transferase was greater in stem sample, it was statistically insignificant.

Bharali *et al.,* (2003), reported increased levels of antioxidants like glutathione peroxidase, superoxide dismutases and catalase in *Moringa oleifera.* High peroxidase and catalase activity was inferred in the leaves of *Ficus deltoidea* (Hakiman and Maziah, 2009). Increased activity of polyphenol oxidase and peroxidase was documented by Arun *et al.*, (2010) in different genotypic varieties of *Pennisetum glaucum* L., a food crop.

The three primary scavenging enzymes involved in detoxifying the free radicals are superoxide dismutases, catalase and glutathione peroxidase. Superoxide dismutases dismutase the highly reactive superoxide anions to the less reactive species hydrogen peroxide. Catalase efficiently reacts with hydrogen peroxide to form water and molecular oxygen. Glutathione peroxidase catalyses the

reduction of hydroperoxides against the oxidative damage (Srikumar *et al.*, 2006). Glutathione peroxidase is also involved in the reduction of fatty acid hydroperoxides generated during the production of prostaglandin, leukotrienes and related compounds (Reddy *et al.* 2007). The presence of catalase is of great importance as its scavenging of hydrogen peroxide protects the organelles against the damaging effects (Salvi *et al.*, 2007). Polyphenol oxidase is widely distributed in higher plants; it plays a role in plant resistance against diseases and insect herbivory (Yamasaki *et al.*, 2008).

Glutathione-s-transferase is a protective antioxidant; it plays an important role in detoxification and elimination of xenobiotics and also plays a major role in protecting the cells form free radicals (Otitoju and Onwurah, 2007). Anticancer drugs have electrophilic centers and can easily form the adduct with glutathione in the presence of glutathione-s-transferase and will be excreated from the body. This would lower the efficiency of the chemotherapeutic agent (Ata *et al.*, 2007). Thus the insignificant level of glutathione-s-transferase in the plant selected for the present study will not have any inverse reactions in the case of cancer treatment.

Thus the results obtained from the assessment of various enzymatic antioxidants revealed *Mukia maderaspatana* (L.) as the good source of antioxidative enzymes.

TABLE 7

LEVELS OF ENZYMATIC ANTIOXIDANTS IN LEAF AND STEM SAMPLES OF *Mukia maderaspatana* (L).

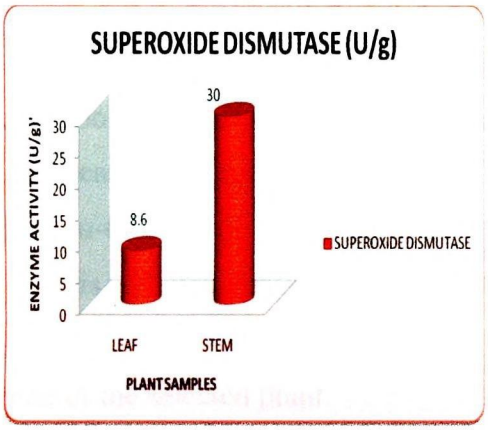
PLANT PARTS	ENZYMATIC ANTIOXIDANTS (U/g)					
	SOD[1]	CAT[2]	POX[3]	GPx[4]	GST[5]	PPO[6]
LEAF	8.6 ± 1.308	237.3 ± 33.020	9.2 ± 0.489	65.4 ± 0.141	1.3 ± 0.360	0.03 ± 0.004
STEM	30.0 ± 0.326	167.2 ± 3.959	8.5 ± 0.410	44.3 ± 0.640	2.6 ± 1.368	0.02 ± 0.009
t-value	22.509*	2.979*	1.635ns	44.980*	1.254ns	1.1569ns

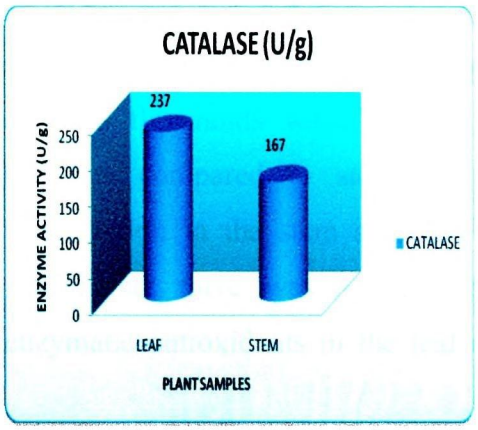
Values are mean + S.D of triplicates
*- Significant at $p<0.05$ level
ns - not significant

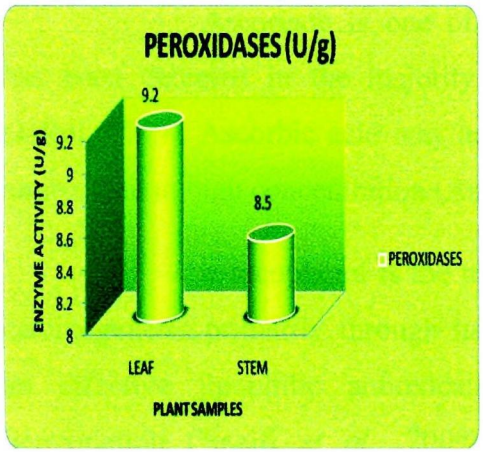
Enzymes	Units
1. Superoxide dismutases (SOD)	Amount that causes 50% reduction in the extent of NBT oxidation
2. Catalase (CAT)	Amount of enzyme required to decrease the optical density by 0.05 units
3. Peroxidase (POX)	1µmole of pyrogallol oxidized / min
4. Glutathione peroxidase (GPx)	µmoles of GSH consumed/ min/g sample
5. Glutathione-S-transferase (GST)	µmoles of CDNB-GSH conjugate/min/g sample
6. Polyphenol oxidase (PPO)	Amount of enzyme that transforms 1µmole of dihydrophenol to 1 mole of quinines/min

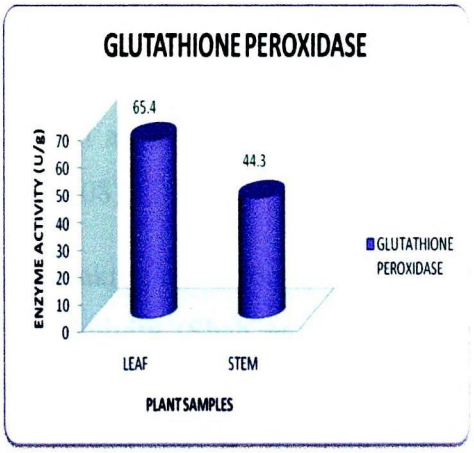
FIGURE 7

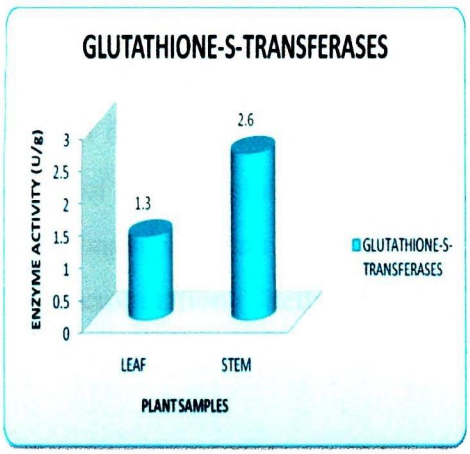
LEVELS OF ENZYMZTIC ANTIOXIDANTS IN *Mukia maderaspatana* (L).

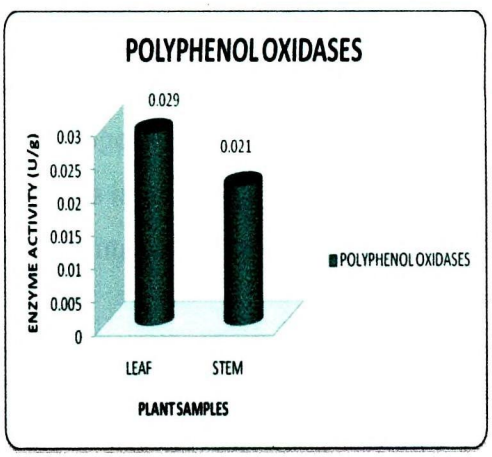












4.6.2 Nonenzymatic antioxidants

Table 8 and Figure 8 indicate the content of various nonenzymatic antioxidants namely ascorbic acid, α-tocopherol, carotenoids, reduced glutathione, polyphenols and flavonoids of the plant samples.

It was observed from Table that, the content of ascorbic acid, carotenoids, reduced glutathione, polyphenols and flavonoids was found to be significantly increased in the leaf sample when compared to stem sample. Only α-tocopherol was present at higher concentration in the stem sample. The analysis of nonenzymatic antioxidants revealed that there was a statistically significant difference in the content of nonenzymatic antioxidants in the leaf and stem of the selected plant.

Ascorbate is one of the most extensively studied antioxidant and has been detected in the majority of plant cell types, organelles and apoplast (Jaleel, 2009). Ascorbic acid may act as an oxidant at low concentration and as an antioxidant at high concentration (Andrabi *et al.*, 2008).

α-tocopherol is the major chain breaking antioxidant, which inhibits carcinogenesis primarily through its antioxidant activity (Shah *et al.*, 2009). It is an effective lipophilic antioxidant which protects lipid membranes against peroxidation (Nazifi *et al.*, 2009). Reduced glutathione is an important free radical scavenger in the mammalian nervous system and an endogenous anticonvulsant (Xu and Stringer, 2008). High levels of reduced glutathione protects the cell from oxidative stress and hence from damage (Chaturvedi, 2008). Increased levels of reduced glutathione with other antioxidants in alcoholic and aqueous extract of *Ocimum sanctum* significantly decreased percentage of wound contraction and lipid peroxidation (Shetty *et al.*, 2008).

TABLE 8

LEVELS OF NONENZYMATIC ANTIOXIDANTS IN LEAF AND STEM SAMPLES OF *Mukia maderaspatana (*L*).*

PLANT PARTS	NONENZYMATIC ANTIOXIDANTS (mg/g)					
	Ascorbic acid	α - tocopherol	Carote-noid	Reduced glutathione	Poly-phenols	Flavo-noids
LEAF	0.812 ± 0.023	0.008 ± 0.001	0.193 ± 0.009	0.237 ± 0.040	0.965 ± 0.032	5.141 ± 0.444
STEM	0.603 ± 0.124	0.013± 0.001	0.094 ± 0.001	0.123 ± 0.026	0.596 ± 0.032	2.558 ± 0.215
t-value	6.525*	4.558*	16.265*	3.396*	11.395*	7.399*

Values are mean ± S.D of triplicates
*- Significant at $p<0.05$ level

Carotenoids protect cells and tissues from free radicals and singlet oxygen with antioxidant activity (Golkar *et al.*, 2009). Experimental studies suggest that a high consumption of vegetables, fruits and medicinal plants rich in carotenoids protects the body against certain kinds of diseases and disorders resulting from free radical activity (Gajewski *et al.*, 2010).

High content of polyphenols and flavonoids was observed in the plant extracts namely *Mellilotus officinalis, Adiantum capillus-veneris* and *Urtica dioica* (Pourmorad *et al.*, 2006). High concentration of polyphenols and flavonoids in alcoholic extract of *Agrimonia pilosa* was reported by He *et al.*, (2009). Perera *et al.*, (2010) suggested that, polyphenols with the inhibition of lipid

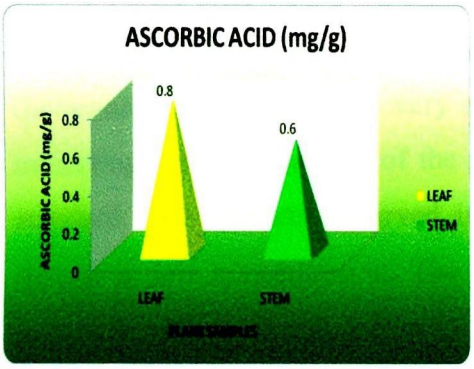
peroxidation, there is evidence about their ability to scavenge radicals such as hydroxyl, superoxide and peroxyl, which are important in cellular prooxidant states. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Raja *et al.*, 2006).

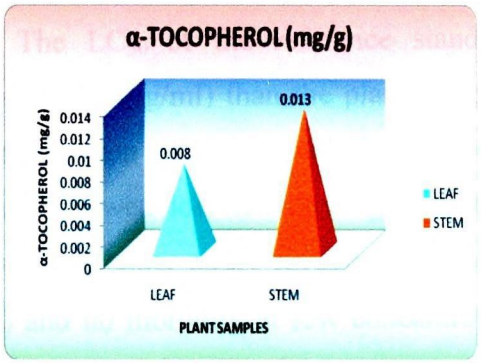
The results obtained from the evaluation of various nonenzymatic antioxidants revealed *Mukia maderaspatana* (L.*)* is a good reservoir of all nutritional content which evidences for antioxidant activity.

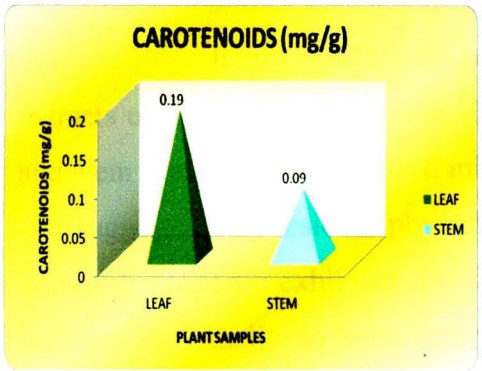
Thus the findings of enzymatic and nonenzymatic antioxidants revealed that, the selected plant for the study *Mukia maderaspatana (*L.*)* will be a promising source of all antioxidants which evidences anticancer and antioxidant activity.

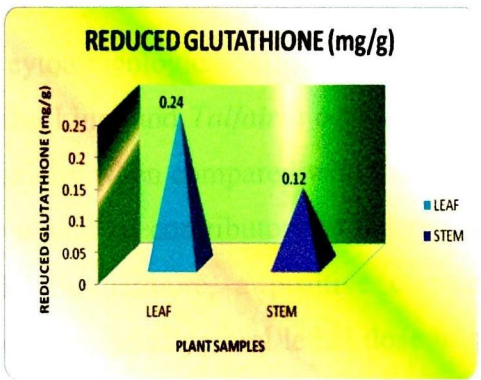
FIGURE 8

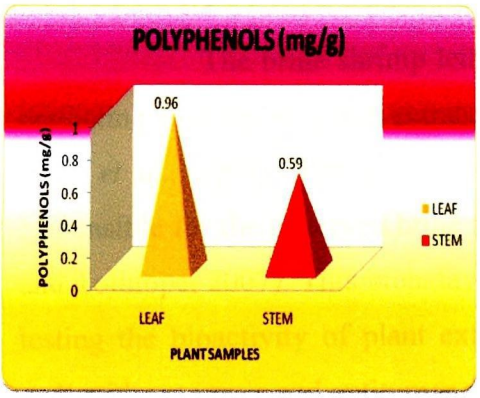
LEVELS OF NONENZYMATIC ANTIOXIDANTS IN *Mukia maderaspatana* (L).

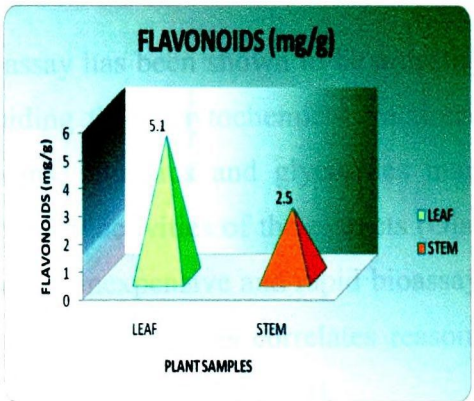












4.7 Cytotoxic effect of the plant sample

Effect of ethanolic extracts of leaf and stem sample of *Mukia maderaspatana (L.)* on the percentage lethality of brine shrimp (*Artemia salina)* and the LC_{50} values of the samples are shown in Table 9 and Figure 9.

The LC_{50} values of ethanolic extract of leaf and stem were 588.8µg/ml and 631.0µg/ml respectively. The LC_{50} of the reference standard (potassium dichromate) was very much lower (100.5µg/ml) than the plant samples indicating the least toxicity of the plant extracts when compared with the reference standard. The extracts observed in this study showed low lethality against brine shrimp at low concentration. The evaluation of lethality assay revealed maximum mortality at high concentration (1000µg/ml) and no mortality at low concentration (100µg/ml) in both leaf and stem samples.

James, (2009) documented cytotoxicological effect of methonolic extracts of *Gongronema latifolia, Besella alba* Linn. and *Talfairia occidentalis* leaf and stem samples which showed mild cytotoxicity when compared with the standard and suggested that resident phytochemicals would be contributory to the relatively nontoxic property exhibited by the plants. Furthermore, a positive correlation between the lethality to brine shrimp and the corresponding oral lethal dose in mice of medicinal plants has been demonstrated (Bastos *et al.*, 2009).

The brine shrimp lethality bioassay has been shown to be a useful for predicting toxicity of plant extracts and guiding their phytochemical fractionation (Ayo *et al.*, 2007). The presence of saponins, alkaloids and glycosides may be responsible for the observed brine shrimp lethality activities of the extracts (Olaleye and Tolulope, 2007). This bioassay represents an inexpensive and rapid bioassay for testing the bioactivity of plant extracts which in most cases correlates reasonably well with cytotoxic and antitumor properties (James and Jacob, 2010).

TABLE 9

EFFECT OF ETHANOLIC EXTRACTS OF LEAF AND STEM SAMPLES *Mukia maderaspatana (L.)* ON BRINE SHRIMP LETHALITY

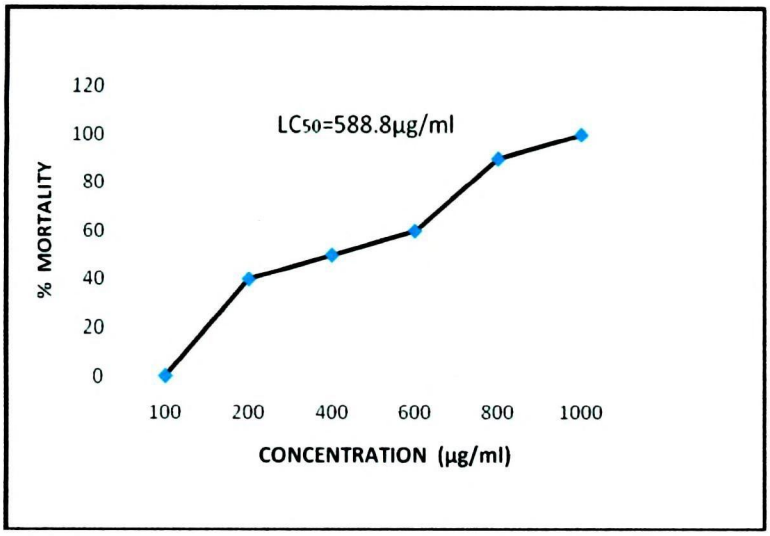
SAMPLE	CONCENTRATION (µg/ml)	% LETHALITY	LC_{50} (µg/ml)
LEAF	1000 800 600 400 200 100	100 90 60 50 40 0	588.8
STEM	1000 800 600 400 200 100	80 60 50 20 0 0	631.0
POTASSIUM DICHROMATE*	150 100 50	70 50 30	100.5

* - Reference standard

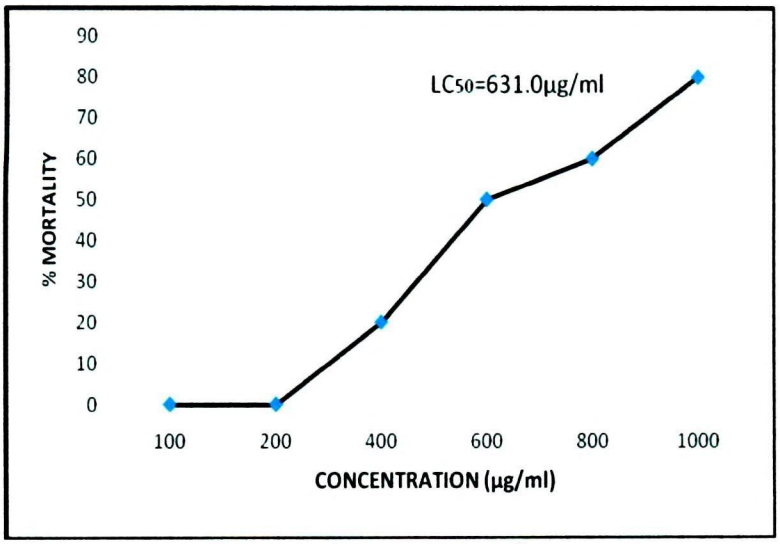
FIGURE 9

EFFECT OF ETHANOLIC EXTRACTS OF *Mukia maderaspatana (L.)* ON BRINE SHRIMP LETHALITY

LEAF



STEM



Therefore, the positive response obtained in this leathality assay suggests that the leaf and stem extracts of *Mukia maderaspatana (*L.) will contain antitumor, antibacterial or pesticidal compounds. However further study is necessary to find out the active principles responsible for these activities.

4.8 Antibacterial activity of the plant sample

The antibacterial activity in the different solvent extracts of leaf and stem sample of *Mukia maderaspatana (*L.) at a concentration of 100µg/ml was determined by agar well diffusion assay. The bacterial strains used to screen the antibacterial activity of the plant extracts were *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Shigella dysentriae.*

The antibacterial activity of the plant samples against five bacterial species is summarized in Table 10 and shown in Plates II & III. All extracts of the leaves of *Mukia maderaspatana (*L.*)* showed varying degrees of antibacterial activity against all microorganisms tested. These variations could be due to the nature and level of antibacterial agents present in the plant, their mode of action and the typical differences in the microbial cell walls between the strains. The ethanol extract exhibited a higher degree (14mm) and broad spectrum antibacterial activity than the petroleum ether extract in leaf sample.

The ethanol and ethylacetate extracts of leaf showed increased *invitro* antibacterial activity against all 5 bacterial species tested. Petroleum ether extract of leaf showed no inhibition against *Klebsiella pneumoniae* and *Shigella dysentriae* whereas, the water extract showed no inhibition against only *Shigella dysentriae* Stem samples showed lesser antibacterial activity in all the solvent extracts against all the bacterial species tested when compared to leaf samples. Among the various stem extracts aqueous extract exerted greater inhibition against all the microorganisms except *Shigella dysentria.* But no zone of inhibition was

observed against *Bacillus subtilis* and *Shigella dysentriae* by petroleum ether extract of stem sample.

TABLE 10

ANTIBACTERIAL ACITIVITY OF LEAF AND STEM EXTRACTS OF *Mukia maderaspatana (L.)*

MICROORGANISMS	DIAMETER ZONE OF INHIBITION (mm)								
	C	LEAF				STEM			
		PE	E	EA	W	PE	E	EA	W
Staphylococcus aureus	15	3	14	10	8	3	5	10	12
Bacillus subtilis	15	9	15	16	10	NZ	12	10	15
Escherichia coli	10	2	9	5	4	2	7	4	13
Klebsiella pneumoniae	10	NZ	2	5	4	8	10	12	15
Shigella dysentriae	9	NZ	9	4	NZ	NZ	5	9	3

C - Chloramphenicol
PE Petroleum ether extract
EA – Ethylacetate extract
NZ - No Zone
E - Ethanol
W- Water

Mathabe *et al.*, (2006) documented that, the ethanol, methanol and water extract from two plants namely, *Punica granatum* and *Ozoroa insignis* showed bactericidal effect against *Staphylococcus aureus, Escherichia coli and Shigella sp.* The antibacterial activity of ethanolic stem extract of Plumeria acutifolia against *Bacillus subtilis, Klebsiella sp., Staphylococcus aureus* and *Psuedomonas aeruginosa* has also been indicated by Rasool *et al.*, (2008).

The phytochemicals like alkaloids, flavonoids, phenols, tannins and terpenoids could be potential alternatives to the traditional chemical control of

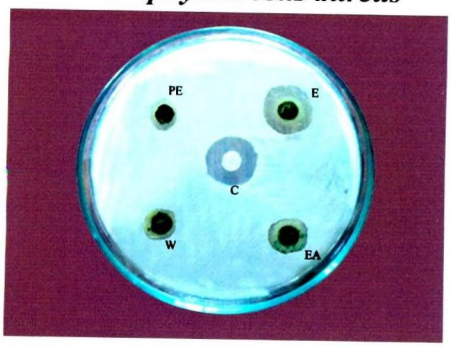
clinical pathogen and phytopathogeic bacteria (Abdul and Hopper, 2010). It was documented that the occurrence of phytochemicals namely glycosides, flavonoids, which either individual or in combination in *Landolphia oqrrience* exert antibacterial activity (Rani *et al.*, 2009). Polyphenols have been shown to exercise their antibacterial action through membrane perturbations (Aiyegoro and Okoh, 2009).

Thus, the extracts of leaf and stem of *Mukia maderaspatana* (L.) indicate potentiality in treatment of infectious diseases caused by tested strains and the plant could be a source of new potent antibiotic agents that would serve as selective agents for the maintenance of animal and human health. However, further efforts are still needed to isolate the biological active compounds from the extracts studied in order to know specific active principles responsible for the antibacterial activity of *Mukia maderaspatana* (L.).

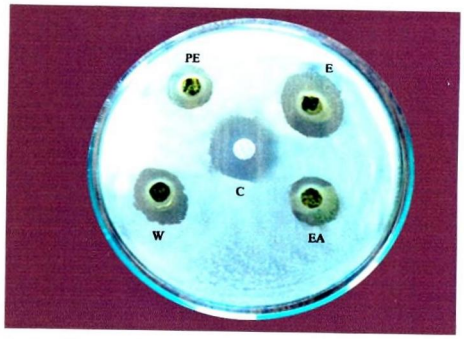
PLATE II

ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *Mukia maderaspatana(L.)*

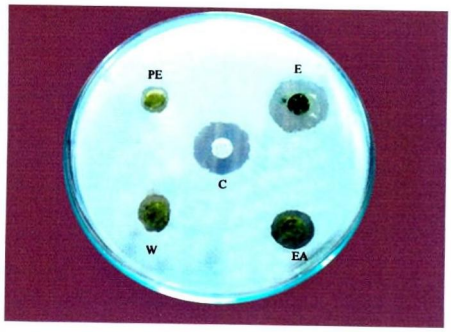
Staphylococcus aureus



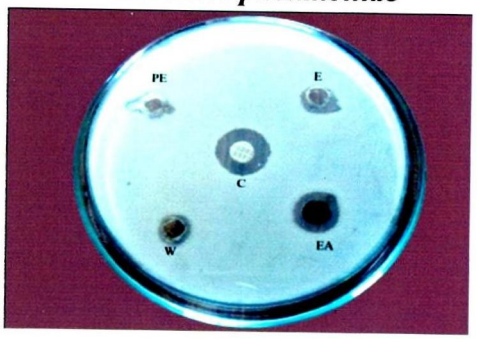
Bacillus subtilis



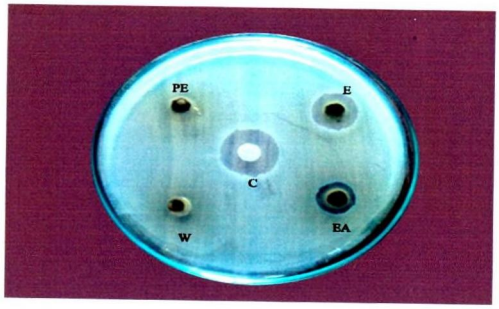
Escherichia coli



Klebsiella pneumoniae



Shigella dysentriae



PE- Petroleum ether **E- Ethanol**

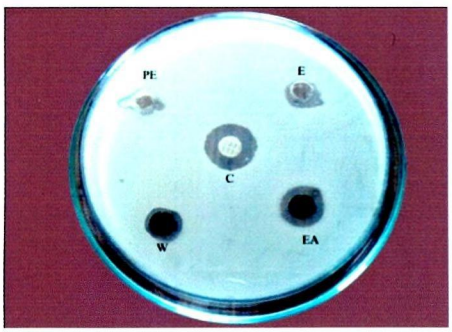
EA- Ethylacetate **W- Water**

C- Chloramphenicol

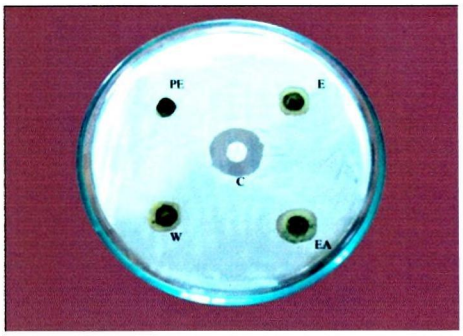
PLATE III

ANTIBACTERIAL ACTIVITY OF STEM EXTRACTS OF *Mukia maderaspatana(L.)*

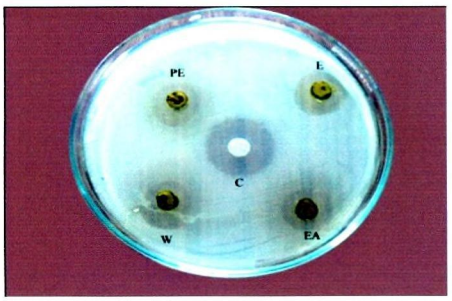
Staphylococcus aureus



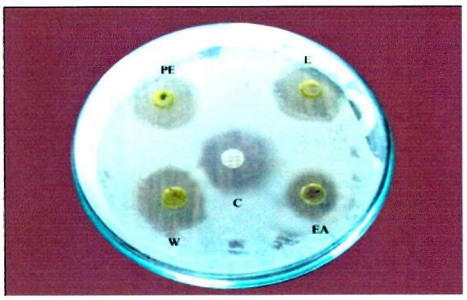
Bacillus subtilis



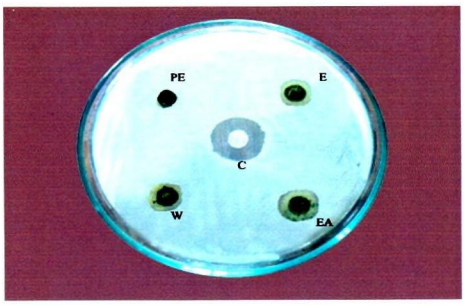
Escherichia coli



Klebsiella pneumoniae



Shigella dysentriae



PE- Petroleum ether **E- Ethanol**

EA- Ethylacetate **W- Water**

C- Chloramphenicol