The rationale of the present investigation was to assess the antioxidant potential of various fractions of *Justicia peploides* (Nees) T. Anders. To get various fractions, methanolic extract of the plant was dissolved in distilled water and partitioned with n-hexane, chloroform, ethyl acetate and n-butanol sequentially. Phytochemical screening showed presence of phenolics and flavonoids in more amounts in ethyl acetate and n-butanol fraction. The antioxidant potential of all these fractions and remaining aqueous fraction was evaluated by four methods: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, total antioxidant activity, Ferric reducing antioxidant power (FRAP) assay and ferric thiocyanate assay along with determination of their total phenolics. The results revealed that ethyl acetate soluble fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 82.20±1.02% inhibition of DPPH radical at a concentration of 60µg/mL. The IC₅₀ of this fraction was 14.567±1.80µg/mL, relative to butylated hydroxytoluene (BHT, a reference standard), having IC₅₀ of 12.33±0.87µg/mL. It also showed highest total antioxidant activity i.e. 0.92±0.06 (absorbance at 695 nm) as well as highest FRAP value (298.5±1.48 TunM/mL), highest total phenolic contents (89.9±1.98 GAE/mg) and highest value of inhibition of lipid peroxidation (55.04±1.46%) at concentration of 500 µg/mL as compared to the other studied fractions. n-butanol fraction also showed good results.

**Key words:** *Justicia peploides* (Nees) T. Anders, phytochemical screening, DPPH assay, total antioxidant activity, FRAP value, total phenolics, inhibition of lipid peroxidation.

**INTRODUCTION**

Herbal medicine involves the use of plants for medicinal purposes. Antioxidants have been used as important protective agents for human health. The crude extracts of various parts of plants contain antioxidants. Currently there is much interest in the protection of low density lipoprotein and important cells and organs, as well as food systems, against oxidative damage caused by superoxide, hydroxyl and peroxyl radicals. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts or isolated products from plant origin rather than looking for synthetic ones. It is an established fact that polyphenolic compounds, such as flavonoids, anthraquinones, anthocyanidins and xanthones, possess remarkable antioxidant activities which are present quite commonly in the plant family. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant potential.

Antioxidants are used to preserve foods by retarding discoloration, rancidity, or deterioration due to autooxidation. A large number of civilization-associated diseases such as autoimmune diseases, inflammation, cardiovascular-neurological diseases, cancer and aging are attributed to oxidative stress. An adequate intake of natural antioxidants could protect macromolecules against oxidative damage in cells. The antioxidant activity might be due to the presence of phenolic compounds such as flavonoids, phenolic acids and phenolic diterpine. Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by the disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack. Free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are associated with many pathological conditions such as atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. However, synthetic antioxidants have been reported to be carcinogenic. Hence, several attempts to replace synthetic antioxidants with natural antioxidants have been developed. Anti-oxidative substances obtained from natural sources, such as oil seed, grains, beans, vegetables, fruits, leaf waxes, bark, roots, spices, hulls and sea weeds, have been investigated. So, in this study, the antioxidant activity of a medicinal plant *Justicia peploides* has been investigated and reported.

*Justicia peploides* belongs to family Acanthaceae. Locally this plant is known as “Pamund”. Ethnobotanically this plant is used for fodder for cattle but its leaves are used in diuretic and in gastrointestinal complaints. The various plants of genus Justicia are considered to be the medicinal plants which have anticonvulsant activities, anti-cancer activity against P₃₈₈ lymphocytic leukaemia and also used traditionally in India and China to treat inflammatory disorders like rheumatoid arthritis. Some plants of this genus have anti-tumor effects, immunomodulatory effects, estrogenic and progestagenic effects which manage the menopausal symptoms and dysmenorrheal effect on hepatic metabolism. Phytochemical studies on genus Justicia led to the isolation of several compounds such as lignans, amide and a number of triterpenoidal glycosides. According to our knowledge, there has been no exploration of *Justicia peploides* as a natural antioxidant source. Therefore, the present study was aimed to investigate the phytoconstituents and antioxidant potential of different fractions of this plant.
MATERIALS AND METHODS

Plant Material
The plant Justicia peploides was collected from Azad Kashmir in August 2011, and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, Government College University, Lahore. A Voucher specimen (G.C.Herb.Bot.986) has been deposited in the herbarium of the Botany department of the same university.

Extraction and Fractionation of Antioxidants
The shade-dried ground whole plant (0.5kg) was exhaustively extracted with methanol (5L) on the soxhlet apparatus. The extract was evaporated in rotary evaporator Laborta 4000-efficient Heidolph at 40°C under vacuum to yield the residue (148g), which was dissolved in distilled water (1L) and partitioned with n-hexane (1L × 5), chloroform (1L × 4), ethyl acetate (1L × 4) and n-butanol (1L × 4) respectively. These four organic fractions and remaining water fraction were concentrated separately on rotary evaporator (n-hexane at 38°C, chloroform at 40°C, ethyl acetate at 45°C, n-butanol 50°C and water at 60°C under vacuum) and the residues thus obtained were used to evaluate their in vitro antioxidant potential.

Chemicals and Standards
DPPH (1,1-Diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-Tripyridyl-s-triazine), Trolox, Folin Ciocalteu’s phenol reagent and BHT (butylated hydroxytoluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (n-hexane, chloroform, ethyl acetate, n-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride, ferrous chloride, hydrochloric acid, copper sulphate, aluminium chloride, lead acetate, acetic acid, lenoleic acid, tween 20 and ammonia from Merck (Pvt.) Ltd. (Germany).

Phytochemical Screening
Phytochemical screening of all the five crude extracts i.e. n-hexane soluble fraction, chloroform soluble fraction, ethyl acetate soluble fraction, n-butanol soluble fraction and remaining aqueous fractions was carried out to identify the phytochemical constituents (alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids, cardiac glycosides) using the standard procedures.28-30

Antioxidant Assays
Following antioxidant assays were performed on all the studied fractions.

DPPH Radical Scavenging Activity
The DPPH radical scavenging activities of each crude extract of plant were examined by comparison with that of known antioxidant, (BHT) using the reported method.31 Briefly, various amounts of the samples (1000μg/mL, 500μg/mL, 250μg/mL, 125μg/mL, 60μg/mL, 30 μg/mL, and 15μg/mL) were mixed with 3ml of methanolic solution of DPPH (0.1mM). The mixture was shaken vigorously and allowed to stand at room temperature for one an hour. Then absorbance was measured at 517nm against methanol as a blank in the spectrophotometer (CECIL Instruments CE 7200 Cambridge England). Lower absorbance of reaction mixture indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula:

\[
\text{Antiradical activity (\%) = } \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Each sample was assayed in triplicate and mean values were calculated.

Total Antioxidant Activity by Phosphomolybdenum Method
The total antioxidant activities of various fractions of plant were evaluated by phosphomolybdenum complex formation method.32 Briefly, 500μg/mL of each crude extract was mixed with 4mL of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in sample vials. The blank solution contained 4mL of reagent solution. The vials were capped and incubated in water bath at 95°C for 90 minutes. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695nm against blank. The antioxidant activity was expressed relative to that of BHT. All determinations were assayed in triplicate and mean values were calculated.

Ferric Reducing Antioxidant Power (FRAP) Assay
The FRAP assay was done according to Benzie and Strain33 with some modifications. The stock solutions included 300mM acetate buffer (pH 3.6), 10mM TPTZ solution in 40mM hydrochloric acid, and 20mM ferric chloride hexahydrate solution. The fresh working solution was prepared by mixing 25mL acetate buffer, 2.5mL TPTZ solution and 2.5mL ferric chloride hexahydrate solution and then warmed at 37°C before using. The solutions of plant samples and that of trolox were prepared in methanol (500μM/mL). 10μL of each of crude extract was taken in separate test tubes and 2990μL of FRAP solution was added in each to make total volume up to 3mL. The plant crude extracts were allowed to react with FRAP solution in the dark for 30 minutes. Absorbance of the coloured product [ferrous tripyridyltriazine complex] was checked at 593nm. The FRAP values were expressed as micromoles of trolox equivalents (TE) per mL of the sample solution using the standard curve constructed for different concentrations of trolox. Results were expressed in TE μM/mL.

Total Phenolic Content
Total phenolics of various fractions of plant were determined by the reported method.34 An aliquot of 0.1mL of each crude extract (0.5mg/mL) was combined with 2.8mL of 10% sodium carbonate and 0.1mL of 2N Folin-Ciocalteu reagent. After 40 minutes absorbance at 725nm was checked by UV-visible spectrophotometer. Total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. Results were expressed in GAE mg/g.

Ferric thiocyanate (FTC) Assay
The antioxidant activities of various fractions of plant on inhibition of linoleic acid peroxidation were assayed by thiocyanate method.35 Each crude extract (0.1ml, 0.5mg/mL) was mixed with 2.5mL of linoleic acid emulsion (0.02M, pH 7.0) and 2.0mL of phosphate buffer (0.02M, pH 7.0). The linoleic emulsion was prepared by mixing 0.28g of linoleic acid, 0.28g of Tween-20 as emulsifier and 50.0mL of phosphate buffer. The reaction mixture was incubated for 5 days at 40°C. The mixture without extract was used as control. The mixture 0.1mL was taken and mixed with 5.0mL of 75% ethanol, 0.1mL of 30% ammonium thiocyanate and 0.1mL of 20mM ferrous chloride in 3.5% hydrochloric acid and allowed to stand at room temperature. Precisely 3 minutes after addition of ferrous chloride to the reaction mixture, absorbance was recorded at 500nm. The antioxidant activity was expressed as follows:

\[
\text{Inhibition of lipid peroxidation (\%) = } \left( \frac{1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) }{100} \right)
\]
The antioxidant activity of BHT as reference standard was assayed for comparison.

**Statistical Analysis**

All the measurements were done in triplicate and statistical analysis was performed by Statistical software. All the data were expressed as ± S.E.M. Statistical analysis were determined using one way analysis of variance (ANOVA) followed by post-hoc Tukey’s test.

Table 1: Phytochemical constituents of various fractions of Justicia peploides

<table>
<thead>
<tr>
<th>Test</th>
<th>n-Hexane soluble fraction</th>
<th>Chloroform soluble fraction</th>
<th>Ethyl acetate soluble fraction</th>
<th>n-Butanol soluble fraction</th>
<th>Remaining Aqueous fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sugars</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(“+” represents presence and “-” represents absence).

Table 2: 1, 1-Diphenyl-2-picryl hydrazyl radical (DPPH) radical scavenging activity of the various fractions of Justicia peploides

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>Concentration in assay (μg/ml)</th>
<th>% scavenging of DPPH* S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Hexane soluble fraction</td>
<td>1000</td>
<td>85.57±1.23*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>60.90±0.98*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>42.44±1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>33.01±0.72</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform soluble fraction</td>
<td>250</td>
<td>77.18±0.39*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>59.12±1.24*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>46.27±1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>32.2±0.89</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate soluble fraction</td>
<td>60</td>
<td>82.20±1.02*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>62.30±1.11*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>49.07±0.99</td>
</tr>
<tr>
<td>4</td>
<td>n-Butanol soluble fraction</td>
<td>60</td>
<td>80.75±0.79*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>62.66±1.21*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>40.12±0.45</td>
</tr>
<tr>
<td>5</td>
<td>Remaining Aqueous fraction</td>
<td>500</td>
<td>79.9±0.63*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>58.78±1.60*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>44.63±0.58</td>
</tr>
<tr>
<td>6</td>
<td>BHT</td>
<td>60</td>
<td>91.35±0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>75.46±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>42.57±0.05</td>
</tr>
</tbody>
</table>

*a) All results are presented as mean ± standard mean error of three assays. *p < 0.05 when compared with negative control i.e. blank/solvent (p<0.05 is taken as significant)

**Results and Discussion**

The phytochemical screening was done on all the studied fractions and results have been shown in table 1. It was observed from the results that chloroform soluble fraction, ethyl acetate soluble fraction and n-butanol soluble fraction contain phenolics and flavonoids, as well as alkaloids however these constituents were absent in n-hexane soluble fraction. Cardiac glycosides were present only in ethyl acetate fraction. Terpenoids were present in all fractions but in more amount in ethyl acetate fraction. Tannins and saponins were found in ethyl acetate fraction and n-butanol soluble fractions. Sugars were detected in all fractions except in n-hexane and chloroform soluble fraction. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron.
or hydrogen radical to become a stable diamagnetic molecule\textsuperscript{36}. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compound is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow\textsuperscript{37}. DPPH is a preformed stable radical used to measure radical scavenging activity of antioxidant samples. The method is based on the reaction of DPPH radical that is characterized as a stable free radical with deep violet colour and any substance that can donate hydrogen atom to DPPH thus reduces it to become stable diamagnetic molecule. DPPH radical decolourizes in the presence of antioxidants. It contains an odd electron which is responsible for visible deep purple colour\textsuperscript{38}. Reduction of DPPH radical was observed by the decrease in absorbance at 517nm whereas colour changes from purple to yellow. This assay was performed on all the studied crude fraction of the plant and the values of percent scavenging of DPPH radical have been shown in table II. It was observed that activity was increased by increasing the concentration of the samples. The various concentrations of ethyl acetate soluble fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 82.20±1.02 % inhibition of DPPH radical at a concentration of 60µg/mL. The various concentrations of the fractions which showed percent inhibition greater than 50% were found to be significant (p < 0.05). IC\textsubscript{50} value can be defined as the concentration of substrate that causes 50% inhibition of DPPH radical and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds\textsuperscript{38}. IC\textsubscript{50} values of all the fractions were also calculated and results have been shown in table III. The lower the IC\textsubscript{50} value, higher will be scavenging power. Ethyl acetate soluble fraction exhibited lowest IC\textsubscript{50} value i.e.14.56±1.80µg/mL as compared to other fractions. n-butanol soluble fraction also showed good IC\textsubscript{50} value (22.00±1.80µg/mL). Chloroform soluble fraction showed moderate IC\textsubscript{50} value (108.67±1.04µg/mL) while n-hexane soluble fraction and remaining aqueous fraction showed poor values relative to butylated hydroxytoluene (BHT), a reference standard, having IC\textsubscript{50} of 12.5 ± 0.87µg/mL. The IC\textsubscript{50} values of chloroform soluble fraction, ethyl acetate soluble fraction and n-butanol soluble fraction were found to be significant (p < 0.05) while that of n-hexane soluble fraction and remaining aqueous fraction were found to be non-significant (p > 0.05) when compared with BHT, a reference standard.

The total antioxidant activity was measured by phosphomolybdenum complex formation method. In this assay, the reduction of Mo(VI) to Mo(V) took place by various fractions of plant which was detected at 695nm by spectrophotometer due to the formation of green phosphate Mo (V) compounds\textsuperscript{39}. The assay was successfully used to quantify vitamin E in seeds\textsuperscript{40} and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant polyphenols. A higher absorbance indicates a higher antioxidative activity. It was revealed from the results (table III) that ethyl acetate fraction showed highest total antioxidant activity i.e. 0.92±0.06 as compared to other fractions. Chloroform and n-butanol soluble fraction also exhibited good activity i.e. 0.61±0.03 and 0.74±0.05 respectively. The n-hexane soluble fraction and remaining aqueous fraction exhibited very less activity (0.27±0.02 and 0.38±0.04 respectively). The results were compared with BHT, a reference standard having total antioxidant activity 0.96±0.06. The total antioxidant activity shown by chloroform soluble fraction, ethyl acetate soluble fraction and n-butanol soluble fraction was found to be significant (p < 0.05) while that of n-hexane and aqueous fraction was found to be non-significant (p > 0.05) when compared with BHT. FRAP assay is used for the determination of the reducing power of various samples which is shown by the colour change of the test solution from yellow to blue and green in proportionate to the reducing power of various samples. In a redox- linked colorimetric method antioxidants are used as reductants in FRAP assay and in stoichiometric excess it provides an easy reduced oxidant system. Ferric form in a ferric tripyridyltriazine complex changes to ferrous form showing intense blue colour. This change was observed by measuring the absorption at 593nm. In the reaction mixture the absorption change was linked directly with the total reducing power of electron donating antioxidants which reduced the ferric form (ferric cyanide complex) to the ferrous form\textsuperscript{41}. From the results (table III) it was revealed that among all the fractions the ethyl acetate soluble fraction showed highest FRAP value (298.54±1.48 TE\textsubscript{nM/mL}). n-butanol soluble fraction also showed good FRAP value i.e. 226.93±1.66 TE\textsubscript{μM/mL}, while chloroform fraction showed moderate value i.e. 116.02±1.95 TE\textsubscript{μM/mL}. n-hexane soluble fraction and remaining aqueous fraction showed very less FRAP values i.e. 23.90±1.45 TE\textsubscript{μM/mL}, 72.64±1.21 TE\textsubscript{μM/mL} respectively. High FRAP values obtained for polar fractions may be ascribed partially to the presence of phenolic and flavonoid contents. The FRAP values of chloroform soluble fraction, ethyl acetate soluble fraction, and n-butanol soluble fraction were found to be significant (p < 0.05) while that of n-hexane soluble fraction and remaining aqueous fraction were found to be non-significant (p > 0.05) when compared with blank.

It has been acknowledged that phenolics and flavonoids show significant antioxidant action on human health and fitness. These compounds act through scavenging or chelating process\textsuperscript{40, 41}. The high potential of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they possess\textsuperscript{42}. Most commonly encountered flavonoids are flavonols, quercetin, flavanols and anthocyanins. Table III shows the phenolic concentration in the different fractions, expressed as milligrams of gallic acid equivalents (GAEs) per gram of fraction. Among the studied fractions, the ethyl acetate soluble fraction showed the highest amount of total phenolic compounds i.e. 89.9±1.98 GAE mg/g). n-butanol soluble fraction also showed good value of total phenolic contents (78.43±1.03 GAE mg/g). Chloroform fraction exhibited moderate amount of total phenolic contents i.e. 53.44±1.04 GAE mg/g respectively). The total phenolic contents of n-hexane soluble fraction and aqueous fraction were found to be 24.61±1.45 and 28.08±0.39 GAE mg/g respectively. The results for total phenolic contents of chloroform soluble fraction, ethyl acetate soluble fraction and n-butanol soluble fraction were found to be significant (p < 0.05) while that of n-hexane soluble fraction and remaining aqueous fraction were found to be non-significant (p > 0.05) when compared with blank.
ion was formed upon reaction of peroxide with ferrous chloride. The ferrous ion will then unite with ammonium thiocyanate producing ferric thiocyanate, a red-coloured substance\textsuperscript{43}. The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities\textsuperscript{44}. Low absorbance values measured via the FTC method indicate high antioxidant activity\textsuperscript{45}. The % inhibition of lipid peroxidation was measured for all the studied samples and results were shown in table III. It was observed that highest percentage of inhibition of lipid peroxidation was exhibited by ethyl acetate soluble fraction (55.04±1.46%). n-butanol soluble fraction also showed good value of percentage of inhibition of lipid peroxidation (43.91±0.95%). n-hexane, chloroform fraction and aqueous fractions exhibited percent inhibition of lipid peroxidation 9.47±0.68%, 21.06±1.53% and 19.28±0.95% respectively which were not found good. The results were compared with butylated hydroxytoluene (BHT), a reference standard which showed 62.73 ± 0.96% inhibition of lipid peroxidation. The results for percent inhibition of lipid peroxidation of ethyl acetate soluble fraction and n-butanol soluble fraction were found to be significant (p < 0.05) while that of n-hexane soluble fraction, chloroform soluble fraction and aqueous fraction were found to be non-significant (p > 0.05) when compared with BHT.

CONCLUSION

It was observed from the results that ethyl acetate soluble fraction and n-butanol soluble fraction contain more phenolics and flavonoids. Because of presence of such compounds these fractions showed good antioxidant activity. Chloroform soluble fraction showed moderate activity due to fewer amounts of such compounds while n-hexane soluble fraction and remaining aqueous fraction showed no activity due to absence of all these compounds. Ethyl acetate soluble fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 82.20±1.02% inhibition of DPPH radical at a concentration of 60\(\mu\)g/mL. The IC50 of this fraction was 14.567±1.80\(\mu\)g/mL, relative to BHT, having IC50 of 12.33±0.87 \(\mu\)g/mL. It also showed highest total antioxidant activity (0.92±0.06) as well as highest FRAP value (298.54±1.48 TEu M/mL), highest total phenolic contents (89.9±1.98 GAE mg/g) and highest value of inhibition of lipid peroxidation (55.04±1.46% at concentration of 500\(\mu\)g/mL) as compared to the other studied fractions. n-butanol fraction also showed good results. So it was concluded from the present study that ethyl acetate soluble fraction and n-butanol soluble fractions of this plant are rich in strong antioxidants, so these fractions are potentially valuable sources of natural antioxidants and bioactive materials and further phytochemical investigations may bring new natural antioxidants into the food industry that might provide good protection against the oxidative damage which occurs both in the body and our daily foods.

REFERENCES

30. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Ohowe K, Ezenina EC, Atangbayila TO. Phytochemical screening and antioxidant activities of some selected medicinal Plants used for malaria

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