Objective: To investigate the presence of various phytochemicals from the flowers extracts of *Ixora coccinea* belonging to the Rubiaceae family.

Methods: Quantitative estimations were performed for tannin, phenol, flavonoids and terpenoids. *In vitro* antioxidant potential of the three extracts were performed using DPPH assay, Superoxide anion radical scavenging assay, LPO assay, hydrogen peroxide (H$_2$O$_2$), nitric oxide (NO) scavenging assays and MTT assay.

Results: Preliminary phytochemical analysis revealed the presence of tannin, phenol in Methanol extract, tannin, phenol, flavonoid and terpenoids were found to be present in ethyl acetate extract and none of phytochemicals was found to be present in high amount in petroleum ether extract. Quantitative determination revealed the presence of high amount of Tannin (68.74±4.26 mg/g extract) and total phenol (216.78±8.49 mg/g extract) in Methanolic extract flavonoids (41.46±1.17mg/g extract) and terpenoids (207.47±8.76 mg/g extract) in ethyl acetate extract where as in petroleum ether none of these phytochemicals were found to be present in high amounts. Ethyl acetate extract was found behave high total antioxidant capacity (0.63 ± 0.04 gm extract which contains equivalent of Vit E). The EA extract was found to be potent in scavenging free radicals like DPPH (12.86±1.4 µg/10µl), LPO (121.72±17.14µg/ml), NO (48.57±3.85µg/0.5ml ) and H$_2$O$_2$ (27.54±1.39). ME and EA were found to have equal potential in scavenging the other radicals like superoxide anion and hydroxyl radical. Cytotoxic potential was high in EA (42.28±1.63µg/µl).

Conclusion: These results reveal that EA extract of *Leccineas* has strong antioxidant potential compared with other extracts. Further study on the in vitro anticancer activity was extended to elucidate the molecular Mechanism of action of the EA extract.

Key words: *Ixora coccinea*, Ethyl acetate, Flavonoids, Terpenoids, Total antioxidants, Free radicals, Cytotoxicity

INTRODUCTION

Plants show enormous versatility in synthesizing complex materials, which have no immediate obvious growth or Metabolic functions. These complex materials are referred to as secondary Metabolites. Secondary Metabolites present in plants are biologically active and these bioactive compounds are believed to be effective in combating or preventing disease due to their antioxidant effect which is due to their capability to quench lipid peroxidation, prevent DNA oxidative damage and scavenge reactive oxygen species[8]. Oxidative modification of macromolecules like DNA, proteins, lipids and small cellular molecules by free radicals plays a major role in numerous diseases and age related degenerative conditions. Free radicals play an important role, in both health and disease, and have been implicated in manifold human disease processes. The antioxidant and antimicrobial properties of various plants have been reported by several studies. Abundant natural antioxidants have been reported in fruits and vegetables[2,3,4], oilseeds[5], herbs[6], tea[7]. Antioxidant defence systems can reduce side effects induced by ROS in living cells. Synthetic antioxidants, such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are widely used in the food and cosmetic industry. It has been reported that administration of high dose of BHT was found to be hepatotoxic and induce lung damage[8] and long term administration of BHT is capable to induce oxidative and metabolic alterations in heart similarly to some pathological disorders[9]. World health organization has recommended the evaluation of systemic toxicity for ornamental purpose were also used in traditional Indian medicine. Antimicrobial activity of *I.coccinea* leaves and flower extracts have been reported[10,11]. Anti-inflammatory and antimitic activities from leaf extracts have been reported. They have also been reported to have anti-inflammatory activity comparable to indomethacin[12]. Flowers were also reported to possess cytotoxic and antitumour activity in mice injected with Dalton’s lymphoma ascetic (DLA) cells[13]. Flowers extracts were reported to contain triterpenoid, ursolic acid[14]. The flowers afforded two new cycolartenol esters, lupeol fatty ester, lupeol, oleancolic acid and sitosterol. Flowers showed protective effects against cyclophosphamide and cisplatin induced systemic toxicity[15,16]. Wound healing properties of alcoholic extract of flower was also reported and it was shown to significantly increase the enzymatic profiles of Wistar rats[17].

1.MATERIALS AND METHODS

1.1.Chemicals and instruments

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, PMS, NBT, NADH, sulphanilamide, sodium nitroprusside, naphthyl ethyldiamine, deoxyribose, hydrogen peroxide, ferric chloride, trichloro acetic acid, thio barbitric acid, potassium dihydrogen phosphate and ferrous sulphate ascorbic acid were purchased from SRL chemicals India Ltd. DMEM, FBS, Penicillin and streptomycin were obtained from GIBCO, USA. All other chemicals and solvents used were of analytical grade. The absorbance measurements were recorded using the UV-Visible Thermo multiskan spectrophotometer.

1.2.HeLa cell lines and culture Medium

HeLa cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Cells were grown in DMEM Medium with 10% FBS, 100 U/ ml penicillin and 100 μg/ml streptomycin and Non essential amino acids and sodium pyruvate. The cells were grown in a humidified atmosphere in a CO$_2$ incubator at 37°C with 5% carbon dioxide.
1.3. Plant material and extraction

*Ixora coccinea* were collected from local herbal drug vendor and were authenticated by Dr. Amarjot, Department of Botany, Presidency College, Chennai, India. Flowers were washed thoroughly with water to remove the earthy matters and freed from debris. Raw flowers were shade dried, powered (80% coarse; 20% fine) and subjected to successive (petroleum ether, ethyl acetate and methanol) extraction by hot continuous percolation Method using Soxhlet’s apparatus. Extracts were concentrated under vacuum, in rotary evaporator, dried and stored in vacuum desiccators till use.

1.4. Phytochemical screening

Qualitative determination of phytochemical constituents of the three extract were carried out by standard protocols.[18,19,20]

1.5. Determination of tannin content

Tannin content was determined by the Method described by[21,22]. To 0.5ml of extracts (mg/ml concentration), 0.5ml of Folin’s phenol (1:2 ratio) and 1% 5ml sodium carbonate was added and incubated for 5 mins at room temperature. 0.5ml of folin’s phenol and sodium carbonate alone served as blank. Intensity of blue coloration of the reaction mixture was read at 640nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

1.6. Determination of total phenol content

The amount of total phenolics in all the extracts was determined by employing Folin’s Ciocalteau reagent[23]. Reaction of 0.5ml of each sample was mixed with 2.5ml of Folin’s phenol and 2ml of sodium carbonate and incubated for 30mins at room temperature. 0.5ml of folin’s phenol and sodium carbonate alone served as blank. Intensity of blue coloration of the reaction mixture was read at 765nm. 2.5ml of Folin’s phenol, 2ml of sodium carbonate and incubated for 30mins at room temperature. Intensity of color developed was read at 517nm. The antioxidant activity was calculated as inhibition (% of DPPH radical formation).

1.7. Determination of total flavonoid content

Aluminium chloride colorimetric[24] method was used for flavonoids determination. 0.5ml of the extracts was mixed with 0.1ml of 10% aluminium chloride, 0.1ml of 1M sodium acetate and the volume was made upto 5.3ml with distilled water and incubated at room temperature for 30min. The absorbance of the reaction mixture was measured at 402nm. Quercetin was used as standard.

1.8. Determination of Triterpenoids

Terpenoid was estimated by the method of Ing–Luen et al., 2009[25]. 0.2 ml of extract prepared in ethanol (mg/ml concentration) was evaporated by keeping it in boiling water bath and to the residue 0.3ml of Vanillin / glacial acetic acid (W/V), 1ml of perchloric acid was added and incubated at 60°C for 45min. Tubes were cooled in ice and to the mixture 5ml of glacial acetic acid was added and the color intensity was absorbed at 548nm.

1.9.Reducing power

Reducing capacity of the vitamin c was evaluated by standard procedures[26]. Briefly, 0.5 ml of extract was added to 0.1ml of DTC reagent (0.4g of thiourea, 0.05g of copper sulphate and 3g of DNPH) and incubated at 37°C for 3 hrs. To the mixture 0.75ml of 85% hydrochloric acid was added and incubated at room temperature for 30mins. Intensity of color developed was read at 520 nm using a spectrophotometer.

1.10. Total antioxidant capacity

Total antioxidant capacity was assayed[27] by adding 0.1ml of extract to 1 ml of vitamin E reagent (0.6M sulphuric acid, 0.028M sodium dihydrogen phosphate and 4mM ammonium molybdate). Then, mixture was incubated at 60°C for 30mins. Color developed was measured at 695nm using a spectrophotometer.

1.11. Antioxidant assay with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

DPPH is a free radical, when dissolved in ethanol has a violet color which when reacts with reducing agent, the solution loses its color that depends upon the number of electrons taken up. Hence, the loss of color indicates radical scavenging activity of test material and was performed according to Koleva[28]. DPPH was measured by adding 190µl of DPPH (3mg of was dissolved in 50ml ethanol) to 10µl of different concentrations (1.5-1000µg) of the extracts and incubated at room temperature for 30 min. Absorbance was read at 517 nm. The antioxidant activity was calculated as inhibition (% of DPPH radical formation).

% DPPH = ([Control - Asample] /Asample) X 100

1.12. Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity was assayed using the method of Kakkar[29] by taking 100µl of different concentrations of extracts (1.5-1000µg), 250µl of pyrophosphate buffer (0.025M, pH8.3), 25µl of PMS and 75µl of NBT. Reaction was started by addition of 7µl of NADH, 250µl of acetic acid and 2ml of butanol. After incubation of the reaction mixture at 37°C for 10 sec the tubes were centrifuged at 3500rpm for 10min. 2ml of n-butanol alone served as blank. The colour intensity was read at 560nm. Percentage inhibition was calculated by comparing the results of control and test samples.

% SOD = ([Control - Asample] /Asample) X 100

1.13. Determination of inhibition of Lipid peroxidation

Lipid peroxidation was evaluated by measuring the TBARS content according to the TBA test described by Okawa[30] with slight modifications. To different concentration of the extracts (1.5-1000µg), 1ml of 1% homogenate, 0.1ml of ferrous sulphate, 0.1ml of ascorbic acid, 0.1ml of potassium dihydrogen phosphate and 2.7ml of water was added and incubated at 37°C for 1hr. Then, 1ml of TCA and 1ml of 0.375% of TBA was added and the tubes were boiled for 30mins. After cooling, the solution was centrifuged at 3000rpm for 10mins. The intensity of color is the measure of MDA concentration. Absorbance at 532nm was determined using UV/VIS spectrophotometer against the blank.

% LPO = ([Control - Asample] /Asample) X 100

1.14. Nitrate / Nitrite radical scavenging activity

Aqueous sodium nitroprusside at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite, which can be estimated by use of Greiss reagent[31]. Nitrate/Nitrite was (NO/NO) was assayed by taking 0.5ml of extracts of various concentration (1.5-1000µg) followed by addition of 1.25ml of phosphate buffer, 1.25ml of sodium nitroprusside and incubated at room temperature for 2 and half hours. To the mixture, 1.25ml of Griess reagent (1g of sulphanilamide dissolved in small volume of water, 2 ml of orthophosphoric acid and 100mg of naphthyl ethyldiamine were added. Volume was made upto 100ml with distilled water and mixed well) was added. Intensity of color development was read 546 nm.

% NO = ([Control - Asample] /Asample) X 100

1.15. Hydroxyl (•OH) radical scavenging activity

Among oxygen-centered radicals, the hydroxyl radical (•OH) is the most reactive and can damage numerous biomolecules. Hydroxyl radical scavenging activity was assayed according to the method of Halliwell[32], by adding 1ml of extracts of various concentration (1.5-1000µg) to 360µl of deoxyribose and 100µl of EDTA, 10µl of ferric chloride, 100µl of hydrogen peroxide and 330µl of phosphate buffer (50mM, pH7.4). The mixture was preincubated for 30mins at 37°C. Then, 0.1ml of ascorbic acid was added and left undisturbed for 10mins. The reaction was arrested by adding 1ml of 10% TCA and 1ml of 0.5% TBA was added and the tubes were boiled for 30mins, cooled and then centrifuged at 3500rpm and the colour intensity was read at 532nm.

% OH = ([Control - Asample] /Asample) X 100

1.16. H$_2$O$_2$ Radical scavenging activity

Hydrogen peroxide radical scavenging activity was assayed by the method of Sinha[33] with slight modification. Reaction was started by taking 100µl of different concentrations (1.5-1000µg) of extracts, 500µl of buffer and 400µl of 2mM hydrogen peroxide. The reaction mixture was incubated at 37°C for 10 sec the tubes were centrifuged at 3500rpm for 10min. 2ml of n-butanol alone served as blank. The intensity of color is the measure of absorbance at 532nm. Percentage inhibition was calculated by comparing the results of control and test samples.

% H$_2$O$_2$ = ([Control - Asample] /Asample) X 100
room temperature for 5 min. Then, to the reaction mixture 2 ml of dichromate acetic acid reagent (5% Potassium dichromate and Glacial Acetic Acid in 1:3 v/v) and the decrease in color intensity was measured at 570 nm. 2 ml of dichromate acetic acid reagent alone served as blank whereas the reaction mixture without extracts served as control.

1.17. Cytotoxicity assessment

Cell growth inhibition was determined by the 3-(4, 5-diMeOthylthiazol-2-yl)-2,5 diphenyl-tetrazolium bromide (MTT) quantitative assay(33) capable of detecting viable cells. Cells were plated in 96 well microtiter (ELISA) plates at an initial density of 7000 cells/well.

After incubation for 24 h at 37°C, cells were treated with different concentrations (ranging from 100µg-1µg) of various extracts of IC and incubated for 18 h. MTT solution was added to each well and further incubated for 4 h at 37°C, optical density was read with an ELISA reader at 550 nm with 670 nm as the reference range.

1.18. Statistical Analysis

Triplicate of each sample were used for statistical analysis. Data were expressed as Mean±SD. The significance between the results was assessed using the Student’s t-test and significance was accepted for p-values <0.01 and 0.05.

2. RESULTS

2.1. Preliminary phytochemical screening

The results of phytochemical screening of the investigated extracts were showed in Table 1. The presence of various active constituents like saponins, tannins, steroids, alkaloids, terpenoids and cardiac glycosides were positive in ME and EAE extracts whereas PEE showed positive to flavonones and terpenoids alone.

### Table 1: Preliminary qualitative phytochemical analysis of I. coccinea flower extracts

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>PEE (gm extract)</th>
<th>EAE (gm extract)</th>
<th>ME (gm extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flavones</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Anthroquinones</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Phenol</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>Tannins</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5.</td>
<td>Sugars</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Alkaloid</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Quinones</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>8.</td>
<td>Terpenoids</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Saponins</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+: Present in low range; ++: Present in moderate range; +++: Present in high range; -: Absent

2.2. Quantitative determination of phytochemicals

Content of tannin, phenol, flavonoids and terpenoids were recorded in Table 2. ME was found to contain the highest amount of tannin (68.74±4.26 mg/g extract) and phenol (216.78±4.99 mg/g extract) and EAE with phenol (157.18±4.12 mg/g extract), flavonoid (41.46±1.17 mg/g extract) and terpenoids (207.47±8.76) whereas PEE found to contain high tannin (82.70±2.51 µg/100µl) and 0.05.

### Table 2: Comparison of yield of extracts and tannin, phenol, flavonoid and terpenoid content of flower extracts of I. coccinea.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Yield (mg/W)</th>
<th>Tannin (mg/g extract)</th>
<th>Total Phenol (mg/g extract)</th>
<th>Flavonoid (mg/g extract)</th>
<th>Terpenoid (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEE</td>
<td>0.720</td>
<td>20.99±1.27</td>
<td>9.91±1.34</td>
<td>10.00±0.65</td>
<td>93.66±4.86</td>
</tr>
<tr>
<td>EAE</td>
<td>2.300</td>
<td>36.68±2.82</td>
<td>15.18±1.12</td>
<td>41.46±1.17</td>
<td>207.47±8.76</td>
</tr>
<tr>
<td>ME</td>
<td>26.980</td>
<td>68.74±4.26</td>
<td>216.78±4.99</td>
<td>12.04±0.91</td>
<td>36.39±1.30</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. ** & * P value of 0.01 & 0.05 respectively, compared to PEE.

2.3. Reducing power and total antioxidant capacity

Reducing power of all the extracts were similar and found to be in the order of PEE< EAE< ME as shown in Table 3. Total antioxidant capacity was found to high in EAE (0.63 ± 0.04 gm extract which contains equivalent of Vit E) when compared to its counter parts.

### Table 3: Reducing capacity and total antioxidant content of extracts of I. coccinea.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Reducing capacity (gm extract which contains equivalent of Vit C)</th>
<th>Total antioxidant capacity (gm extract which contains equivalent of Vit E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEE</td>
<td>0.09 ± 0.01</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>EAE</td>
<td>0.11 ± 0.01</td>
<td>0.63 ± 0.04**</td>
</tr>
<tr>
<td>ME</td>
<td>0.12 ± 0.02</td>
<td>0.38 ± 0.01**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, ** & * P value of 0.01 & 0.05 respectively, compared to PEE.

2.4. Antioxidant assay with 1, 1-diphenyl-2-pircylyldrazyl (DPPH) radical DPPH is a stable molecule and considered to be a lipophilic radical model. A chain reaction in lipophilic radicals was initiated by lipid auto-oxidation. The radical scavenging activity of PEE, EAE and ME was determined from the reduction in absorbance at 517 nm resulting from the scavenging of stable DPPH free radicals. The positive DPPH test suggested that the samples were free radical scavengers.

### Table 4: Free radical scavenging potential of I. coccinea flower extracts.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Free radicals</th>
<th>IC₅₀ value</th>
<th>PEE</th>
<th>EAE</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DPPH</td>
<td>82.70±2.51</td>
<td>12.86±1.14</td>
<td>37.92±2.76</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Superoxide anion</td>
<td>317.27±2.9</td>
<td>56.31±2.3</td>
<td>38.2±2.51</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Lipid Peroxide</td>
<td>308.08±4.29</td>
<td>121.72±7.17</td>
<td>169.31±7.62</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Hydroxyl radical</td>
<td>136.35±4.81</td>
<td>50.25±4.29</td>
<td>46.35±3.17</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Hydrogen Peroxide</td>
<td>81.27±4.92</td>
<td>27.54±1.39</td>
<td>46.21±2.76</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.

2.5. Determination of inhibition of Lipid peroxidation DNA base modification by ROS and RNS induce lipid peroxidation end-products (malondialdehyde, 4-hydroxynonenal, etc.) to form cyclic adducts while lipid peroxides (LOOH) in the presence of heme iron, as in hemoglobin, can also induce strand breakage and form abasic sites[34]. Hence it is necessary to evaluate the lipid peroxidation scavenging potential of herbs before proceeding with further studies. The results in Table 4 showed the lipid peroxide scavenging potential of the three extracts. EAE extract showed strong radical scavenging activity with IC₅₀ value of 121.72±7.17µg/ml. Followed by ME showing strong scavenging potential with 169.31±7.62 µg/ml and PEE with 308.08±4.29 respectively.
2.7 Nitrate / Nitrite radical scavenging activity
The presence of nitrite, a stable oxidised product of NO, was determined
by Griess reagent. Nitric oxide (NO), which is derived from the oxidation
of L-arginine through three isoforms of nitric oxide synthase (NOS), is
recognised as a mediator and regulator in pathological reactions, especially
in acute inflammatory responses\(^{35}\). Table 4 illustrates a significant decrease
in the concentration of nitric oxide radical due to the scavenging ability of
IC extracts. All of the extracts had NO-scavenging activity, with all exhibiting
a two-stage NO-scavenging dose–response curve. The results showed that
EAE had the highest (48.57±3.85) scavenging potential when compared to
the other extracts. The IC\(_{50}\) values of ME and PEE were 103.09±9.93 and
257.90±10.88 µg/0.5ml respectively.

2.8 Hydroxy (·HO) radical scavenging activity
Antioxidant capacity was assessed via inhibition of deoxyribose degradation.
Once hydroxyl radicals are formed, they can degrade deoxyribose into
fragments that on heating with thiobarbituric acid generate a pink chromogen.
When a scavenger molecule is added to the reaction mixture, it competes
with the deoxyribose for hydroxyl radicals and can inhibit deoxyribose
degradation depending on its concentration. Hence it is important to assess
the hydroxyl radical scavenging ability of the herbal samples before further
investigations on the molecular mechanism. Table 3 represents the hydroxyl
radical scavenging activity of IC extracts. The IC\(_{50}\) values were in the order
of ME<EAE<PEE and the values are 46.35±3.17, 50.25±4.29 and
136.35±4.81.

2.9 Hydrogen peroxide radical scavenging activity
In the present study inhibition was found to be dose dependent in all the
cases and the H\(_2\)O\(_2\) radical scavenging activity of IC extracts was
found to be high in EAE with IC\(_{50}\) 27.54±1.39, followed by ME and PEE
with 46.21±2.76 and 81.27±4.92 respectively as represented in Table 4. 
H\(_2\)O\(_2\) directly or indirectly plays crucial role in malignant transformation,
but can also sensitize cancer cells to H\(_2\)O\(_2\)-induced cell death\(^{36}\). Cellular
production of superoxide anion and H\(_2\)O\(_2\) favours the formation of other
reactive oxygen and nitrogen species such as hydroxyl radical (OH\(^-\)) and
peroxynitrite (ONOO\(^-\)) and over production of these species causes oxidative
stress and plays an important role in carcinogenesis\(^{37}\).

2.10 Cytotoxicity assessment
In the present study, the effect of IC extracts on the extent of survival of
HeLa cells were recorded using the MTT reduction assay. According to the
basic principle of MTT, the number of living cells is proportional to the
absorbance of reduced MTT formazan. A dose dependent increase in activity
was observed in EAE when compared to the PEE and ME. After the 24hr
exposure of the EAE the amount of reduced formazan is increased in case of
EAE whereas the formation of formazan has found be decreased in the
counter parts. Figure 1 represents the IC\(_{50}\) value determined from the graph.
EAE showed potent cytotoxic effects with IC\(_{50}\) value of 42.28±1.63µg/µl
followed by ME with 73.50±3.22. Whereas PEE found to be less potent
with IC\(_{50}\) value of 119.47±4.01µg/µl in comparison with other extracts.

DISCUSSION
Medicinal plants constitute a common alternative for several ailments in
many countries around world\(^{38}\) and more than 3000 plants were reported
to have anti-cancer properties. The main strategies for the selection of plant
species in cancer drug discovery include random screening with antioxidant,
cytotoxic, anti proliferative activities. Anticancer agents with a combination
of antioxidant, cytotoxic and apoptotic activities might provide better healing
efficacy in comparison to those with only cytotoxic property, as they are
not only delaying the complex process of carcinogenesis by inhibiting cancer
cells growth, but also prevent chronic complication by inducing cancer
cell death without injuring neighboring cells and reduce side effect by
countering chemotherapy/carcinogenesis generated free radicals\(^{39}\).
Phytochemicals are well established to exert anticancer activities, partially
based on their ability to quench reactive oxygen species and thereby
protecting critical cellular targets (i.e. DNA, proteins, lipids) from oxidative
stress.
insult\textsuperscript{[40]}. Several epidemiological studies have found an inverse association between the intake of diets rich in polyphenols (such as fruits, vegetables, and grains) and the risk of age-related diseases in humans\textsuperscript{[41]}. This association is often attributed to the powerful antioxidant activities of flavonoids and other polyphenols, as established in vitro, to scavenge a wide range of reactive oxygen, nitrogen, and chlorine species\textsuperscript{[42]}. Herbs have been claimed for their therapeutic intervention for decades. In the emerging trends of drug development, plants and plant products attain much prominence. In the current scenario plant based drugs have extensive application in clinical trials. Free radicals are identified as one of the main culprits in neurodegenerative disorders like Parkinson’s disease, life threatening diseases like cancer and coronary heart diseases.

Antioxidants are proposed as therapeutic options to combat the free radical generation and maintenance. It is evident from current research that EAE and ME possessed effective antioxidant activity. These findings suggest possible therapeutic activity of EAE and ME in management and control of diseases. In addition, EAE exhibited potent cytotoxic activity on human cancer cell line, HeLa. These findings suggest EAE as a salutary choice in treatment of cancer. Although the antioxidant and cytotoxic activity of EAE and ME have been well studied\textsuperscript{in vitro}, the suggestion of EAE and ME as a therapeutic choice requires more attention and remains as an option of research interest in drug discovery which presently consists of multiple sophisticated test that yield a novel innovative drug at the end.

Declaration of conflict of interest

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article

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