Study of Phytochemistry and Antioxidant Activity of *Hertia intermedia* (Boiss.) Flowers of Balochistan

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Abstract: *Hertia intermedia* (Boiss) O. Kuntze has been used as a traditional remedy for pain killer. The sample was extracted with methanol and used for the evaluation of phytochemical constituents and its antioxidant potential. The phytochemical compounds present in the sample were tannins, terpenoids, flavonoids, steroids, coumarins and carbohydrates. Total phenolic and total flavonoid contents investigated for the tested sample were 211.8 mg tannic acid equivalent/g and 162.7 mg quercetin equivalent/g extract respectively. The antioxidant assays applied for this study were DPPH free radical scavenging assay, Ferric-ion reducing antioxidant power assay, Reducing power assay and Phosphomolybdate assay. The results on the basis of antioxidant capacity methods and high contents of phenols and flavonoids of the flower extract suggest that the sample extract can be a valuable source of antioxidant.

Keywords: *Hertia intermedia*, methanolic extract, TFC, TPC, DPPH, FRAP, phosphomolybdenum,

INTRODUCTION

An absolute resource of remedies has been provided by the nature in the form of medicinal plants to be used for the health care and treatment and is the best companion of pharmacy. The plants globally constitute a single larger functional group and are most effective in action as herbal drugs without any side effects (Khan et al, 2011). According to an estimate, just from 95 plant species through the world are prescribed for 120 or so plant-based drugs. In rural areas of many developing countries the use of medicinal plants (or their parts) is well known as folk medicines (Onuekwusi *et al.*, 2014).

The studies of folk medicines should be more intensified, in order to encourage the use of herbal medicines and determination of their valuable potentials (Khan *et al.*, 2011). The two classes of chemicals present universally in all plants are primary and secondary metabolites. The primary metabolites consist of proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophylls etc., whereas secondary bioactive chemicals include alkaloids to terpenoids and acetogenins to different phenol (Khan *et al.*, 2011; Onuekwusi *et al.*, 2014). These phytochemicals differ in the qualitative and quantitative distribution from plant to plant and part to part of plants.

The phenolic compounds are found in high concentrations as compared to the alkaloids whereas alkaloids are present in high amounts in storage tissues (roots, fruits and seeds) of plants relative to the green leaves. The synthesis of aromatic compounds (mostly phenols or their oxygen-substituted derivatives) has been the limitless ability of plants. The isolation of 12,000 secondary metabolites (most of the natural products) has been reported so far that serve as plant defense mechanism against predation by microorganisms, insects and herbivores Khan *et al.*, 2011).

Biomolecules (protein, lipid, amino acids and DNA) can be oxidized by the accumulation of excess free radicals (FRs) leading to cellular damage and can induce various diseases (Basri and Setty, 2015). Oxidative stress is caused by an imbalance between reactive oxygen species (ROS) and antioxidants (AO) and it has been related to cancer, cardiovascular diseases(Kumar et al, 2013), ageing, atherosclerosis, arthritis, CNS

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damage, degenerative diseases (Shahid-Ud-Daula et al., 2015), Parkinson’s disease, cataracts and inflammatory diseases. Antioxidant serves as hydrogen donor or electron donor and help in reducing the oxidative stress. Phenols have antioxidant activity, which allow them to quench FRs (Basri and Setty, 2015).

Synthetic antioxidants (such as butylated hydroxyanisole [BHA], butylatedhydroxytoluene [BHT], etc.) have certain disadvantages and harmful side effects to human health. In recent years, the novel antioxidant substances derived from plant tissues are finding great interest for use in human diet and drug industry (Shahid-Ud-Daula et al., 2015).

The Gorgena of Mastung and Shabaan of Quetta city of Balochistan province has provided numerous herbal plants. Thus, there is a need to carry out the proper identification of the wild species, their antioxidant activity and to know their phytochemical constituents. This valuable information will be essential later for conservation purposes and advanced drug manufacturing in Pakistan. We carried out a research work on an indigenous plant namely Hertia intermedia of Composite (also called Asteraceae) family. This indigenous plant locally called Manguli has been used as a remedy of pain-killer (for headache, stomach problems and during menstrual cycle) in the hilly areas of Pakistan (Tareen et al., 2010).

The Hertia genus belongs to the Compositae family and Senecioneae tribe. The genus Hertia consists of twelve species distributed all over South- and North-Africa and South West Asia. Hertia intermedia (Boiss.) O. Kuntze is one of the species of the genus Hertia, which grows wildly in hilly regions of Balochistan and Khyber Pakhtunkhwa provinces of Pakistan. It is also distributed westward towards Iran. Hertia intermedia is also called as Othonnopsis intermedia and found in Balochistan mostly in Quetta, Koeie, Chaman, Kanozai-Moorga, and Wazir, and is also found in Kurram and the regions below Parachinar. These are small shrubs, grow up to 30-50 cm, with beautiful and attractive yellow flowers, and are used as a remedy of painkiller in the hilly areas of Pakistan (Akhgar et al., 2012; Malik et al., 2011; Yasmeen et al., 2009).

Based on above apprehensions, the present study is an effort to screen the methanolic extract of flowers of H. intermedia for phytochemicals and to assess the antioxidant and antibacterial activities.

MATERIALS AND METHODS

Reagents and Chemicals
The reagents and chemicals used were of analytical grade and were products of MERCK Darmstadt, Germany, Applichem Darmstadt, Germany, Scharlab S.L., Spain, BDH Chemicals Ltd, Poole, England, and SIGMA-ALDRICH St. Louis, USA unless otherwise stated.

Sample Collection and Extract Preparation
The flowers of the plant used for this study were collected from Gorgena and Shabaan, Balochistan. H. intermedia was taxonomically identified by Dr. Rasool Bakhsh Tareen, Taxonomist and Dean of Life Sciences, University of Balochistan, Quetta. The fresh flowers of H. intermedia were screened to get rid of the bad ones, washed, shade-dried at room temperature (22 ± 5) °C for 20 days and was extracted with methanol being soaked and stirred at room temperature (21 ± 5) °C for one month. The macerated sample was filtered prior to evaporation of methanol below 50°C under reduced pressure through rotary evaporator (IKA-WERKE GMBH & Co. KG Staufen, Germany). Subsequently, the greenish-black (25 g concentrated extract) was stored below 4°C until further analysis.

Qualitative Tests of Phytochemicals
The crude methanolic extract of H. intermedia flowers was subjected to different qualitative chemical tests for the screening and identification of secondary metabolites using standard procedures of Sofowara (1993), Trease and Evans (1989) and Harborne (1973) as illustrated in Table-1.

Quantitative Tests of Phytochemicals

Total Phenolic Content Estimation in the Extract
The total phenolic amount in the methanolic extract was estimated by using
Folin-Ciocalteau (FC) reagent based method as formerly described by Uddin et al. (2013) with minor modifications. Briefly, to 0.5 ml of extract

<table>
<thead>
<tr>
<th>Secondary Metabolites</th>
<th>Preliminary Test</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Braymer’s Test: 0.2 g extract + 5 ml H2O + 1-2 drops FeCl3 (0.1 %)</td>
<td>Green colored precipitates</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski Test: 0.2 g extract + 2 ml chloroform + 2-3 drops conc. H2SO4</td>
<td>Deep red coloration</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing Test: 0.2 g extract + 4 ml H2O</td>
<td>Froth formation</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>a) Shinoda Test: extract + 5-6 Mg fragments + 2-3 drops conc. HCl</td>
<td>Pink scarlet color</td>
</tr>
<tr>
<td></td>
<td>b) Alkaline Reagent Test: i. Picric acid test: 0.2 g extracts + 3 ml hexane + shake + filter + 5 ml (2%) HCl + heat + few drops picric acid</td>
<td>Yellow color precipitates</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>ii. Mayer’s and Wagner’s test: extract + 2 ml (1%) HCl + heat gently + Mayer’s &amp; Wagner’s reagent</td>
<td>Turbidity appears</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>iii. Hager’s test: 2 ml extract + few drops Hager’s reagent</td>
<td>Yellow precipitates</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>iv. Dragendorff’s test: 2 ml extract + 0.2 ml (1%) HCl + 1 ml Dragendorff’s reagent</td>
<td>Orange brown precipitates</td>
</tr>
<tr>
<td>Steroids</td>
<td>Libermann-Burchard: extract + 2 ml CH3COOH + cool + few drops H2SO4</td>
<td>Violet to blue steroidal ring at interface</td>
</tr>
<tr>
<td>Coumarins</td>
<td>2 ml extract + 3 ml (10%) NaOH</td>
<td>Yellow coloration</td>
</tr>
<tr>
<td>Emodins</td>
<td>2 ml extract + 2 ml NH4OH + 3 ml C6H6</td>
<td>Red coloration</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>extract + 1 % HCl + boil</td>
<td>Red color precipitates</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>extract + 6 ml (1%) HCl + filter + 5 ml C6H6 + filter + 2 ml (10%) NH3 solution + shake</td>
<td>Pink, violet or red color</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>Keller-Killiani Test: extract + 2 ml glacial acetic acid + few drops FeCl3 + 1 ml H2SO4</td>
<td>Brown ring at interface</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch Test: extract+ 5 ml H2O + few drops Ethanolic α-naphthol (20%) + 1 ml conc. H2SO4</td>
<td>Violet ring at interface</td>
</tr>
</tbody>
</table>

(1 mg/ml extract concentration). FC phenol reagent (0.5 ml, ten times diluted) was added and allowed to stand for 5 min at room temperature. Subsequently, a 7 % (w/v) Na2CO3 solution (5 ml) was added in the mixture and after keeping in dark for 90 min, the absorbance of developed color was measured at 750 nm. Tannic acid (50-250 mg/L) solutions were used as standard for extrapolation of calibration curve (fig-1)by using the linear equation y=0.0372x+0.1266 (R²=0.9985) and the TPC was expressed as tannic acid equivalents (TAE mg/g of compound extracted).

**Total Flavonoid Content Estimation in the Extract**

Aluminum chloride colorimetric assay was used to measure total flavonoids described as per (Kaur and Mondal, 2014) using quercetin as positive control for the generation of calibration curve (Onuekwusi et al., 2014). Precisely, an aliquot of 1 ml of sample extract was mixed with 4 ml of distilled water and 0.3 ml of sodium nitrite (5%) in a 10 ml volumetric flask. To this 0.3 ml of AlCl3 (10%) was added after incubation of 5 min at room temperature. The reaction mixture was further allowed to stand for 6 min and then 2 ml of sodium hydroxide (1M solution) was added and immediately the total volume was made up to 10 ml with distilled water. Spectrophotometrically the absorbance of the mixture was measured at 510 nm.
Based on the standard curve (fig-2) using the following equation: 
\[ y = 0.0504x - 0.042 \]
\[ R^2 = 0.9992 \]
was used to estimate the total flavonoids and results were expressed as milligrams of quercetin equivalent (QE) per gram of compound extracted.

**Antioxidant Assays**

**Scavenging Ability of Stable DPPH Radical**

The antioxidant property was appraised by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect for both test extract and standard based on the previously documented method (Mohan et al., 2014). In this assay, the natural antioxidant, ascorbic acid was used as reference for comparison. Exactly, a volume of 0.5 ml of freshly prepared DPPH solution (0.004%) was mixed with 1.5 ml of each concentration (0.05-0.8 mg/L) of test extract. Prior to incubation for 25 minutes in dark, the reaction mixture was shaken vigorously. The reaction solution’s color reduction caused by DPPH radicals was measured spectrophotometrically at 517 nm. Control was prepared by adding DPPH (0.5 ml) to 1.5 ml ethanol in place of flower extract. The radical scavenging effect (RSE) was calculated by using following equation and expressed in RSE % values:

\[ \text{RSE} \% = \left( 1 - \frac{A_{TE}}{A_{DS}} \right) \times 100 \]

Where \( A_{TE} \) is the absorbance of test extract and standard and \( A_{DS} \) is the absorbance of DPPH solutions in the absence of sample extract. A greater anti-oxidant activity was indicated by the lower absorbance values.

**Ferric-ion Reducing Antioxidant Power (FRAP) Activity**

The ferric-ion reducing ability of plasma FRAP method of Benzie and Strain (1996) with minor modifications (Lahouar et al., 2014) was used to determine the total antioxidant potential of sample extract as a measure of anti-oxidant power. In-brief, the freshly prepared FRAP reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tri[2-pyridyl] s-triazine in 40 mMHC1) plus 20 mM FeCl\(_3\).7H\(_2\)O in a ratio of 10:1:1 (v/v/v) and warmed to 37°C prior to use. A volume of 0.1 ml of sample infusion at various concentrations (0.05-0.8 mg/L) and 0.3 ml of de-ionized water were added to 3 ml of working FRAP reagent and the reaction mixture was incubated at 37°C in the dark for 30 min. The increase in absorbance was monitored at 593 nm due to the reduction of colorless Fe (III)-TPTZ complex to a blue colored Fe (II)-TPTZ complex in the presence of antioxidants. The calibration curve was constructed using various concentrations of FeSO\(_4\).7H\(_2\)O. The reaction signal given by a Fe\(^{2+}\) solutions was referenced to calculate the reducing ability of the test extract. The resulting FRAP values were expressed as mmol of Fe\(^{2+}\)/g of dried matter.

**Reducing Power Antioxidant Assay**

The ferric-reducing power of sample extract and positive control (ascorbic acid) were assessed by using the method described by Thakralet al. (2010). Different concentrations of the crude extract were mixed with a volume of 0.75 ml phosphate buffer (pH 6.6, 0.2 M) plus 1 % potassium ferricyanide (0.75 ml), followed by
keeping the mixture for 20 min in a water bath at 50°C. An equal volume of 10% TCA (trichloroacetic acid) was added then to stop the reaction and centrifuged for 10 min at 3000 r/min to collect the supernatant of the solution. To 1.5 ml distilled water and 0.1 ml freshly prepared FeCl₃ solution (0.1%), 1.5 ml of supernatant was mixed. The absorption maxima was recorded at 700 nm; increase in absorbance indicated stronger reducing power.

Phosphomolybdenum (PM) Assay

The total antioxidant ability method is based on the formation of a green phosphomolybdenum [Mo (V)] complex due to the reduction of Mo (VI) by the sample analyte at acidic pH. The spectrophotometric method of Prieto et al. (1999) was used for the quantitative measurement of total antioxidant capacity. At various concentrations, the sample solution (0.1 ml) was taken and combined with 1 ml of molybdate reagent solution (0.6 M H₂SO₄, 28 mM sodium phosphate and 4 mM ammonium molybdate mixture). Subsequently, the tubes were capped and incubated for 90 min in water bath at 95°C. The reaction mixtures were normalized to room temperature. After normalizing, the absorbance of the mixture was recorded at 695 nm against blank. For blank appropriate volume of solvent used for sample was combined with 1 ml of reagent solution and incubated under same conditions. The antioxidant activity was expressed as mg of equivalent of ascorbic acid, for unknown composition of sample.

RESULTS AND DISCUSSION

Phytochemical characteristics

The phytochemical screening of medicinal plants revealed the presence of secondary metabolites which are known to exhibit curative as well as physiologicaal properties (Yadav & Agarwala, 2011). In table-2 the phytochemical characteristics of H. intermedia (Boiss.) flowers are summarized. From the results, it can be seen that, tannins, terpenoids, flavonoids, steroids, coumarins and carbohydrates were detected in tested sample, whereas, saponins, alkaloids, emodins, phlobatannins, anthraquinones and cardiac glycosides were absent. The absence of these phytocompounds in the flower extract suggesting thereby the absence of therapeutic properties associated with them.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Preliminary test</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Braymer’s</td>
<td>(+)ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski</td>
<td>(+)ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing</td>
<td>(-)ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda</td>
<td>(+)ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Picric acid</td>
<td>(-)ve</td>
</tr>
<tr>
<td></td>
<td>Mayer’s and Wagner’s</td>
<td>(-)ve</td>
</tr>
<tr>
<td></td>
<td>Hager’s</td>
<td>(-)ve</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s</td>
<td>(-)ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>Libermann-Burchard</td>
<td>(+)ve</td>
</tr>
<tr>
<td>Coumarins</td>
<td></td>
<td>(+)ve</td>
</tr>
<tr>
<td>Emodins</td>
<td></td>
<td>(-)ve</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>Precipitate</td>
<td>(-)ve</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Borntrager’s</td>
<td>(-)ve</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Keller-Killiani</td>
<td>(-)ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s</td>
<td>(+)ve</td>
</tr>
</tbody>
</table>

(+): Present; (-): Absent
Secondary metabolites such as terpenoids have been reported to possess various activities including antibacterial, antimalarial, antiviral, anti-inflammatory and inhibition of cholesterol synthesized in animals. Steroids are responsible for wide variety of effects such as cardiotonic activities (Wadood et al, 2013), antibacterial properties (Yadav & Agarwala, 2011) and a natural alternative to treat age- and disease-related muscle loss (Shahid-Ud-Daula et al, 2015). Dietary flavonoids have been recommended to oppose the coronary heart-disease (Wadood et al, 2013) and exhibit a vast range of biological activities including antiallergic, antithrombotic and vasodilatory activities (Shahid-Ud-Daula et al, 2015). Studies have revealed that tannins possess amazing stringent properties including the healing of wounds and inflamed mucous membranes. Carbohydrates and coumarins are important dietary supplements, contained in medicinal plants are known to have valuable action on immune system. The antimicrobial and anti-inflammatory properties of coumarins can be recommended to be helpful for hyper-proliferative skin diseases (Yadav et al, 2014). Hence, the identification of significant reservoir of bioactive compounds via preliminary phytochemical screening tests may lead to future medicine bank.

**Total Phenolics and Total Flavonoids Content**

Phenolic compounds (act as primary antioxidants) are able to protect human body from free radicals because their hydroxyl groups possess scavenging property. These phytochemicals are derived from phenylalanine and tyrosine (Saeed et al, 2012). They play an important role in curing major diseases such as diabetes, osteoporosis, neuro-degenerative and cardiovascular diseases (Shahid-Ud-Daula et al, 2015) and their oxidative products confer inhibitory action on various enzyme systems (Aparadh et al, 2012). Folin-Ciocalteu reagent was used to determine the total amount of phenols using tannic acid as standard. A mixture of phosphotungstic acid and phosphomolybdic acid forming the reagent oxidizes the phenols and hence, reduced to a mixture of blue oxides of tungsten and molybdenum. At 750 nm, a maximum absorption is recorded by the blue coloration. It is proportional to the total amount of phenolic compounds originally present (Narayanaswamy and Balakrishnan, 2011). The amount of TPC in *H. intermedia* flowers was found to be 211.8 mg TAE/g of dried matter reported in fig-3.

Flavonoids are the naturally occurring polyphenolic compounds including flavones, flavonols, flavanols, chalcones, flavanones, iso-flavonoids, neo-flavonoids, bi-flavonoids, flavanones and anthocyanins (Shahid-Ud-Daula et al, 2015). Studies on derivatives of flavonoids have revealed a wide range of biological effects, which also inhibit enzymes (such as aldose reductase and xanthine oxidase) (Mohan et al, 2014) and hinder the diffusion of free radicals, thereby restricting the peroxidation reaction (Shahid-Ud-Daula et al., 2015).

The total amount of flavonoid content for methanolic extract was determined by the aluminium chloride colorimetric assay using quercetin as reference. Stable complexes of aluminium chloride are formed with the C-4 keto groups and either the C-3/C-5 hydroxyl group of flavones and flavonols and it also forms liable complexes with ortho-dihydroxide groups in A or B rings of flavonoids. Fig-3 demonstrates the total flavonoid content of sample. It was found that TFC of the methanolic extract of *Hertia intermedia* flowers was 162.7 mg QE/g of dried extract. The high contents of phenols and flavonoids in the extracts are related to the antioxidant activity by neutralizing the free radicals (Mohan et al., 2014).
Antioxidant Assay

Antioxidant activity methods can be categorized as single-electron transfer (ST) and hydrogen-atom transfer (HT) based methods. HT methods are mainly kinetic based and includes a competitive reaction scheme among antioxidant and substrate for free radicals thermally generated whereas ST methods measure the reduction of an oxidant which decolorize when reduced (Phatak & Hendre, 2014).

Free-radical scavenging assay

The free radicals contain one or more unpaired electrons which make them highly unstable. They extract electrons from other molecules in order to gain stability. Therefore, radical scavenging activities are essential in biological systems due to the adverse role of free radicals in a wide range of pathological manifestations. The free radicals generated in biological system are neutralized by antioxidants which protect us from several diseases.

The widely accepted method for determining antioxidant ability of plant extracts is the assay based on the DPPH free radical. It is the stable, organic nitrogen centered radical which produces purple color in ethanol (Mohan et al., 2014). In this assay the hydrogen donor is an antioxidant which makes this method simple and extremely sensitive. The disappearance of DPPH radical in sample extract is monitored with UV/Vis spectrophotometer.

The DPPH radical becomes a stable diamagnetic molecule as it captures an electron or absorbs a hydrogen atom. The antioxidants reducing the DPPH radical results in discoloration from deep purple to pale yellow. The lower the absorbance of reaction mixture, the greater is the discoloration of DPPH ethanol solution that shows the notable free radical scavenging ability (Morales and Paredes, 2014). The RSE (%) values are illustrated in fig-4. The results of this study suggest the increase in concentration of extract and standard notably increases the DPPH radical scavenging effect and therefore, are said to be highly dependent on extract concentration (Patel et al., 2010). The hydrogen donating ability of phenols and flavonoids might be the reason of the anti-radicalizing property of plant extract (Narayanaswamy & Balakrishnan, 2011).

FRAP activity

The FRAP method was used to assess the antioxidant property as it is simple, rapid and reliable method to perform. In addition, the reaction is reproducible and linearly related to molar concentration of the antioxidants present (Uddin et al., 2013). Although FRAP assay was found to react slowly with some antioxidants (such as glutathione) (Rabeta and NurFaraniza, 2013), but can still be used to determine antioxidant capacity in vast range of biological samples to pure compounds (Katalinic et al., 2006).

Redox reactions can be explained as the inactivation of oxidants by reductants, in which one oxidant (reaction species) is reduced at the cost of the oxidation of another antioxidant (reductant). In the reaction medium the antioxidant capacity of any substance is measured as reducing ability by the FRAP assay (Lahouar et al., 2014). Ferric reducing antioxidant power of examined methanolic extract of *H. intermedia* was 1.3 mMFeII/g extract (Table-3).
Table 3. Quantitative values of PM and FRAP assay

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Antioxidant parameters</th>
<th>Line of regression</th>
<th>Quantitative amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phosphomolybdenum assay</td>
<td>$y = 0.0333x + 0.1205$</td>
<td>100.21 mg Ascorbic acid/g extract</td>
</tr>
<tr>
<td>2.</td>
<td>Ferric-reducing antioxidant potential</td>
<td>$y = 0.0649x + 0.1483$</td>
<td>1.3 mM Fe$^{2+}$/g extract</td>
</tr>
</tbody>
</table>

*Ascorbic acid equivalent

Measurement of reducing power

The reducing power (RP) of *H. intermedia* methanolic extract was collated with the standard ascorbic acid (Fig-5). The RP was found to be correlated with increasing absorbance at 700 nm with increasing concentration. In this assay, the reduction of Fe$^{III}$-ferricyanide complex (yellow) to Fe$^{II}$ (Prussian-blue) form would result by electron donation by the sample extract (reducers) and this is reflected in present study. The putative reductants have been attributed to reduce the oxidized intermediates of peroxides decomposition and act as primary or secondary antioxidant substances (Mohan et al, 2014).

![Graph showing the relationship between absorbance and concentration](image)

Fig-5. The reducing power of methanolic extract of *H. intermedia* flowers

Phosphomolybdic Assay

The total antioxidant potential (TAP) of extract has been evaluated by the routinely used phosphomolybdic assay (Prieto et al, 1999) and was measured using the linear regression equation of the standard curve (Table-3). The resulting total antioxidant ability of the methanolic extract was 100.21 mg ascorbic acid equivalent/g dried weight, shown in Fig-3. Increased TAP is proportional to increased absorbance of reaction mixture (Morales & Paredes, 2014). The reduction rate of Mo (VI) to Mo (V) is evaluated by phosphomolybdate scavenging assay, thereby providing direct estimation of antioxidant reducing capacity. It is used to quantitatively assess the reduction reaction degree among antioxidant, oxidant and molybdenum ligand, by formation of green complex without the involvement of free metal ions (Phatak and Hendre, 2014).

CONCLUSION

The results obtained from different antioxidant potential assays, support the view that methanolic extract of *H. intermedia* flowers has shown a significant source of potent antioxidants. This may find efficient role as a therapeutic agent in some of the diseases. Besides this, it can also be considered as herbal source in pharmacy and can be utilized in food industry. However, further detailed studies on screening of biological activities is required to establish the scientific basis.

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REFERENCES


