A Comparative Invitro Study on Antioxidant Properties of Selected Flowers

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ABSTRACT
Health is a central issue in today's life. Most illnesses are due to oxidative stress. Oxidation usually causes cytolyis. Plants such as Sesbania grandiflora, Spatodea campanulata (African tulip), Allamanda cathartica (golden trumpet) and Mirabilis jalapa (4 o'clock flower) are spreading in our environment. They are also used medicinally to treat various human illnesses. The antioxidant activity of the leaves of these plants was studied using aqueous extracts. The extract is subjected to phytochemical analysis. Sesbania grandiflora is known to contain alkaloids, saponins, carbohydrates, tannins, flavonoids and phenols. Protein, terpenoids, Spatodea campanulata is known to contain alkaloids, saponins, glycosides and flavonoids. Allamanda cathartica contains saponins, glycosides, tannins and flavonoids. Mirabilis jalapa contains alkaloids, saponins, glycosides, tannins, flavonoids, phenols and terpinoids. Two different in vitro methods, namely hydrogen peroxide capture activity, reducing power assay, were used to estimate antioxidant activity using a standard antioxidant (ascorbic acid). All four natural flower extracts showed antioxidant activity. However, Cathartica allamanda showed the highest activity. The results are roughly comparable to the standard drug ascorbic acid. Therefore, the flowers of these plants are useful as antioxidants.

KEY WORDS: Antioxidant, Free radicals, Phenols, Flavonoids, Oxidative stress, cancer

I. INTRODUCTION
ANTI OXIDANT ACTIVITY
Antioxidants are natural and synthetic substances that can prevent cell damage from free radicals, unstable molecules produced by the body in response to the environment and other stresses.

Free radicals are produced by humans during aerobic respiration, and free radicals produced by humans, plants and animals are inactivated by antioxidanats.[5] Free radicals can be generated by physiological or biochemical processes that can react with membrane lipids, enzymes, proteins, nucleic acids, and some molecules to damage cells. "Antioxidants act as free radical scavengers" and protect the body. Free radicals have a short half-life, are highly reactive, and damage macromolecules such as DNA, proteins, and lipids. Oxidative damage to DNA causes premature aging and wrinkles. Since the skin is the largest organ in the human body, it is important to protect it. Antioxidants reduce free radicals, thereby limiting the signs of aging, wrinkles, inflammation, scars, and sunburn damage.[8]. Naturally occurring antioxidants are rich in flavonoids, saponins, fresh fruits and vegetables, and whole grains are rich in antioxidant phytochemicals, alkaloids, tannins, phenols and ligans. Green leaves are the richest source of protein, vitamins and minerals. Plants are rich in free radical-removing antioxidants such as vitamins, phenolic acids, terpenoids, alkaloids, quinones, flavonoids, tannins, ligans, stilbens, amines, coumarins, betalains and other metabolites. Therefore, experts believe that antioxidants from foods are the healthiest[1]. Oxygen is essential for aerobic organisms, but can be toxic if taken in large amounts. Excessive intake of antioxidanats can be harmful. For example, vitamin E supplements may increase the risk of bleeding in people taking anticoagulants, and high-dose beta carotene supplements increase the risk of lung cancer in smokers. The most important antioxidants are beta-carotene, lycopene, and some vitamins such as vitamin A, vitamin C, and vitamin E. Decades of nutritional research suggest that eating large amounts of antioxidant-rich foods may
help protect against illness. Much research has been done on antioxidant supplements for these discoveries. [9] Rigorous studies of antioxidant supplements in many people have not found that high-dose antioxidant supplements prevent the disease. This section provides preliminary findings, in vitro findings, and possible explanations for the differences between the findings.[2]

II. MATERIALS AND METHODS

EXTRACT PREPARATION:

Plants can be thought of as biosynthetic laboratories where various types of organic compounds such as carbohydrates, proteins, lipids and flavonoids are synthesized. [14] It has been used as a bioactive ingredient since ancient times, such as glycosides, alkaloids, volatile oils, and tannins. However, the medicinal properties of herbs depend on the type of chemical component they contain and are called active ingredients. In this study, the active ingredients were extracted by ingesting medicinal herbal plants such as Cesbaniagrandiflora, Spatodeacampanulata (African tulip), Allamandacatarrica (golden trumpet), and Mirabilis jalapa (Oshiroibana).[11]

Solvent Purification: Distilled water obtained by distillation is used for water extraction of the powder drug substance.

Extract preparation: The newly collected botanical material was obtained from the Botanical Gardens of Sir C.R. Reddy Pharmaceutical Science College, Eluru A.P. Collect, wash thoroughly with tap water, dry in the shade at room temperature (32 °C), powder and use for solvent extraction. Plant samples were sequentially extracted with distilled water using a Soxhlet extractor. Prior to each extraction, the material was dried in a hot air oven at 40 °C. The solvent was evaporated using a rotary vacuum evaporator at 50 °C. Extract recovery was expressed as a percentage of the dry matter of the plant sample. The lyophilized extract thus obtained was dissolved in an aqueous solvent at a concentration of 1 mg/ml and used to evaluate antioxidant capacity by various chemical assays.[18]

Qualitative phytochemical analysis.

Alkaloid test: a. Dragendorff's test: Add 1 ml of Dragendorff's reagent (potassium iodide bismuth solution) to 1 ml of extract. An orange-red precipitate indicates the presence of alkaloids.

Meyer test: Add 1 ml of Meyer reagent (mercury
iodide potassium solution) to 1 ml of extract. A whitish yellow or off-white precipitate indicates the presence of alkaloids.

**Hager's test:** Add 3 ml of Hager's reagent (saturated aqueous solution of picric acid) to 1 ml of extract. A yellow precipitate indicates the presence of alkaloids. That is, Wagner test: Add 2 ml of Wagner reagent (iodine in potassium iodide) to 1 ml of extract. The formation of a reddish brown precipitate indicates the presence of alkaloids.

**Saponin test:** Take a small amount of alcoholic extract and aqueous extract separately, add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes. The absence of a layer of foam indicates the absence of saponins.

**Glycoside test:** A. Legal test: Dissolve the extract in pyridine and make it alkaline with sodium nitroprussidesolution. The absence of pink to red formation indicates the absence of glycosides.

**Baljet test:** Add 1 ml of sodium picricate solution to 1 ml of test extract. Yellow to orange indicates the presence of glycosides.

**Keller-Killiani test:** Extract 1 g of powdered drug with 10 ml of 70% alcohol for 2 minutes, filter, add 10 ml of water and 0.5 ml of strong lead acetate solution to the filtrate, filter and shake the filtrate. Increase. With 5 ml of chloroform. The chloroform layer was separated in a porcelain dish and gently evaporated to remove the solvent. Dissolve the cooled residue in 3 mL of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2 ml of concentrated sulfuric acid. Reddish brown layer. Formed at the junction of two liquids, the upper layer slowly turns turquoise and darkens when standing.

**Borntreger test:** Add a few ml of diluted sulfuric acid to 1 ml of extract. The filtrate is boiled, filtered and extracted with chloroform. Treat the chloroform layer with 1 mL of ammonia. The red formation of the ammonia layer indicates the presence of anthraquinone glycosides.

**Carbohydrate test:**

**Molisch's test:** Add 1 ml of naphthol solution to 2 ml of extract and add concentrated sulfuric acid from the side of the test tube. The purple or purplish red color at the junction of the two liquids indicates its presence. Fehling's test: Add equal volumes of Fehling's solutions A and B to 1 ml of extract and heat to form a red brick-colored precipitate, indicating the presence of sugar. Benedict test: Add 1 ml of extract to 5 ml of Benedict reagent, boil for 2 minutes and allow to cool. The formation of red precipitates indicates the presence of sugar.

**Tannin test:** a. Take a small amount of the test solution and mix it with the basic lead acetate solution. The formation of white precipitates indicates the presence of tannins. When ferric chloride solution is added to 1 ml of the extract, the formation of a dark blue or greenish black product indicates the presence of tannins. The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins. To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic compounds.

**Test for Flavonoids:**

The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light; formation of fluorescence indicates the presence of flavonoids. Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of flavonoids.

**Shinoda's Test:** The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence of flavonols. The extract of the extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones. The extract is treated with concentrated H2SO4, formation of yellow or orange color indicates flavones. The alcoholic and aqueous extract is treated with 10% sodium chloride; formation of yellow color indicates the presence of coumarins.

**Test for Steroids:**

Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform. 3ml of acetanhydride, 3ml of glacial acetic acid warmed, cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

**Salkowski test:** Dissolve the extract in chloroform and add equal volume of conc. H2SO4. Formation of bluish red to cherry color in chloroform layer
and green fluorescence in the acid layer represents the steroidal components in the tested extract.

**Test for Proteins:**

**Biuret test:** Add 1 ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1 ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

**Ninhydrin test:** Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides or amino acids.

**Xanthoproteic test:** To 1 ml of the extract, add 1 ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of aromatic amino acids.

**Millon’s test:** 1 ml of test solution is made acidify with sulphuric acid and add Millon’s reagent and boil this.

**Noller’s test:** Dissolve two or three granules of tin metal in 2 ml thionyl chloride solution. Then add 1 ml of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids.

**Test for Gums:**

Add about 10 ml of aqueous extract slowly to 25 ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrate minutes, and measure the absorbance at 240 nm after 20 minutes for a phosphate buffer blank containing no H2O2. The absorbance of hydrogen peroxide at 240 nm was measured after 10 minutes.

$\% \text{ inhibition of H}_2\text{O}_2 = \frac{(\text{AC} – \text{AS})}{\text{AC}} \times 100$

where

$\text{AC} = \text{control absorbance}$

$\text{AS} = \text{absorbance of the sample tested.}$

**FIG-3**

**Reducing power assay**

Required concentration of sample + 2.5 ml Phosphate buffer 6.6 + 2.5 ml Potassium ferric cyanide (1%) Next, incubate in water at 50 °C for 10 minutes. Add 2.5 ml of trichloroacetic acid (10%). To the above solution. The mixture was centrifuged at 800 rpm for 10 minutes at 10 °C.

Take 2.5 ml supernatant + 2.5 ml distilled water + 0.5 ml ferric chloride (0.1%). Measure the absorbance at 700 nm for the blank.

$\% \text{ reduction} = \frac{(\text{AC-AS})}{\text{AC}} \times 100$

where

$\text{AC} = \text{control absorbance}$

$\text{AS} = \text{absorbance of the sample tested.}$
III. RESULTS AND DISCUSSION

TABLE -1 Qualitative phytochemical analysis of aqueous extract of the powdered medicinal crude drugs

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Sesabinagrandiflora</th>
<th>Mirabilis jalapa</th>
<th>Allamandacathartic a</th>
<th>Spathodeacampanulata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
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</table>

TABLE-2 Hydrogen peroxide scavenging activity

<table>
<thead>
<tr>
<th>S.no</th>
<th>conc µg/ml</th>
<th>Sesbania Grandiflora Abs %Rd</th>
<th>Cathartica Allamanda Abs %Rd</th>
<th>Spathodeacampanulata Abs %Rd</th>
<th>Mirabilis Jalapa Abs %Rd</th>
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<tr>
<td>1</td>
<td>100</td>
<td>0.057 43%</td>
<td>0.059 41%</td>
<td>0.056 44%</td>
<td>0.059 41%</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>0.040 60%</td>
<td>0.049 51%</td>
<td>0.051 49%</td>
<td>0.051 49%</td>
</tr>
<tr>
<td>S.no</td>
<td>Conc. µg/ml</td>
<td>( \text{Abs} )</td>
<td>( % \text{Rd} )</td>
<td>( \text{Abs} )</td>
<td>( % \text{Rd} )</td>
</tr>
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<td>----------------</td>
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<tr>
<td>1</td>
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<td>53</td>
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<td>51</td>
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</tbody>
</table>

**FIG-5**

**TABLE 3: Reducing Power Assay**

- **Sesbania Grandiflora**
- **Cathartica Alba Manda**
- **Spathodea Campanulata**
- **Mirabilis Jalapa**
IV. DISCUSSION
The results obtained indicate that the selected flower extract has antioxidant activity comparable to ascorbic acid. The presence of strong antioxidant capacity can be attributed to the regulation of free radical production, thereby protecting cellular components from their harmful effects. Flower extracts contain high concentrations of phenolic and other flavonoid compounds that have been shown to have strong free radical trapping capacity and provide associated antioxidant capacity. Dark flower extract is rich in phenolic compounds, flavones and flavanols and has high antioxidant capacity.

V. CONCLUSION
The results obtained in our study show that extracts of various flowers (Cesbaniagrandiflora, catarrticaallamanda, spatodeacamanulate, mirabilis jalapa) have antioxidant properties. The antioxidant activity of various flower extracts is almost comparable to standard ascorbic acid in the order Catharticaallamanda>Spathodeacampanulate>Sesbaniagrandiflora>Mirabilis jalapa. Hydrogen peroxide suppression rate Next, the iron ion reduction rate is in the order of Sesbaniagrandiflora>Spathodeacampanulate>Mirabilis jalapa>Catharticaallamanda. This may be due to high levels of phenol and flavonoids in the flower extract. Finally, we conclude that due to its antioxidant properties, flower extracts can be used to protect us from cell damage caused by oxidative stress. However, further research is needed on its therapeutic effect in humans and its stability when used in the formulation.

REFERENCES


