Qualitative phytochemical analysis of some selected medicinal plants occurring in local area of Faisalabad, Pakistan

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The Qualitative analysis is very essential to identify the phytochemical constituents present in medicinal plants. The medicinal value of plants is due to the presence of particular bioactive constituents. In present study qualitative analysis of seven medicinally important plants, namely Carica papaya (Papaya), Cichorium intybus (Chicory), Foeniculum vulgare (Fennel), Nicotiana tabacum (Tobacco), Rosa damascena (Red rose), Solanum nigrum (Makao) and Trachyspermum ammi (Ajwain) was done. Saponins, tannins, terpenoids, steroids, starch, total sugar, free reducing sugars, ascorbic acid, alkaloids, phenols, flavonoids and glycosides were analyzed qualitatively by following the standard protocols. Rosa damascena and Foeniculum vulgare contained all tested constituents. Terpenoids and starch were present in all plant species except Nicotiana tabacum. Saponins were present in all plants instead of Cichorium intybus.

Keywords: Qualitative analysis, bioactive constituents, standard protocols.

1. INTRODUCTION

Phytochemical screening is to isolate various constituents of the plants for assessing their biological activity or medicinal uses. The medicinal value of plants is due to the presence of particular chemical substances that have a definite physiological action on the living system (Aslam et al., 2009). Carica papaya (Family: Caricaceae) is a well-known, short lived, fast growing, woody, large herb. The plant is traditionally used for the treatment of gastric ulcers, dental caries, to expel intestinal worms, cardio-tonic, anti-inflammatory and to treat the hemorrhoids (Adeyeye and Olagunju, 2009).

Cichorium intybus Linn (Family: Asteraceae) is commonly known as Chicory used as hepatoprotective, hypoglycemic, anti-hyperlipidemic, anti-cancerous activity, anti-hepatotoxic and hypoglycemic agent. It can also be act as an antioxidant, anti-inflammatory and anti-bacterial agent (Nayeemunnisa, 2009). Foeniculum vulgare Mill (Family: Apioaceae) is an aromatic plant, which is used as a traditional medicine. Fruit has been found to possess diuretic, analgesic, antipyretic and antioxidant activity. Essential oil of fennel possesses antibacterial, antifungal, relaxant, estrigentic, analgesic and anti-inflammatory activities (Nickavar et al., 2009).

Nicotiana tabacum Linn (Family: Solanaceae), commonly known as tobacco, is a renowned plant used for its narcotic properties. Dried leaves, stalks and the whole herb of tobacco are widely used in the sub-continent for their antispasmodic, emetic, purgative, sedative, analgesic and insecticidal properties. It can also be utilized in the ethno-veterinary practice as an antiinflammatory, antirheumatic and anthelmintic agent (Iqbal et al., 2006).

Rosa damascena Mill (Family: Rosaceae) has been used as cardiotonic, mild laxative, anti-inflammatory, cough suppressant, antieptic, antidiabetic (Boskabady et al., 2011). Recent studies demonstrated the antioxidant, anti-HIV, anti-bacterial, anti-tussive, respiratory smooth muscle relaxant, analgesic and anti-inflammatory effects of Rosa damascena (Hajhashemi et al., 2010). Solanum nigrum (Family: Solanaceae) is a medicinal plant commonly known as Makao or black nightshade. S. nigrum has been extensively used traditionally to treat various ailments such as an antitumor, antioxidant, anti-inflammatory, diuretic, antipyretic
and as hepatoprotective agent (Zakaria et al., 2006). *Trachyspermum ammi* (Family: Apiaceae) a highly valued medicinally important seed spice. *T. ammi* has been shown to possess antimicrobial, hypolipidemic, hepatoprotective, antispasmodic, bronchodilating, antiplatelet aggregatory effects, antiinflammatory, antitussive, gastroprotective and anthelmintic activity (Gilani et al., 2005). The aim of present study was to investigate the bioactive constituents of above mentioned plants and correlate their bioactive constituents with their pharmacological activity.

## 2. MATERIAL AND METHODS

### 2.1 Plant Material:
Leaves of *Carica papaya* and *Nicotiana tabacum*, roots of *Cichorium intybus*, flowers of *Rosa damascena*, fruit of *Solanum nigrum*, seeds of *Foeniculum vulgare* and *Trachyspermum ammi* were obtained from field and local market of Faisalabad, Pakistan. The plant materials was authenticated by botanist in the Department of Botany, University of Agriculture, Faisalabad (UAF), Pakistan. The plant materials was adulterant free plant material into coarse powder. The procedure of Triple maceration was adopted for the extraction purpose of coarse powdered material by soaking with 10% aqueous methanol in air tight amber glass bottles at 25 °C, with occasional shaking twice a day for one week (Hussain et al., 2014). After maceration, the soaked coarse powdered material was passed through muslin cloth (double layered), in order to remove vegetative debris and the obtained filtrate was subsequently filtered through a Whatman-1 filter paper. The filtrate was stored in amber glass air-tight container. The previously mentioned extraction procedure was subsequently repeated twice after each two days and filtrates of these three macerations were combined. Rotary evaporator (Rotavapor, BUCHI labotechnik AG, Model 9230, Switzerland) attached with a vacuum pump and a recirculation chiller was used for evaporation of the filtrate, under reduced pressure at 37 °C. The dark green crude extract was lyophilized to remove moisture contents. The dried extract was transferred to amber glass jar and stored at −4 °C in a refrigerator.

### 2.2 Preparation of Crude Extracts
Electrical grinder was used to crush the adulterant free plant material into coarse powder. The procedure of Triple maceration was adopted for the extraction purpose of coarse powdered material by soaking with 10% aqueous methanol in air tight amber glass bottles at 25 °C, with occasional shaking twice a day for one week (Hussain et al., 2014). After maceration, the soaked coarse powdered material was passed through muslin cloth (double layered), in order to remove vegetative debris and the obtained filtrate was subsequently filtered through a Whatman-1 filter paper. The filtrate was stored in amber glass air-tight container. The previously mentioned extraction procedure was subsequently repeated twice after each two days and filtrates of these three macerations were combined. Rotary evaporator (Rotavapor, BUCHI labotechnik AG, Model 9230, Switzerland) attached with a vacuum pump and a recirculation chiller was used for evaporation of the filtrate, under reduced pressure at 37 °C. The dark green crude extract was lyophilized to remove moisture contents. The dried extract was transferred to amber glass jar and stored at −4 °C in a refrigerator.

### 2.3 Phytochemical analysis:

#### 2.3.1 Test for Phenols
Test was performed by using the method of Sofowora, (1993). 2 ml of the aqueous extract was taken in a beaker. Then, 2 ml of ferric chloride solution was added. A deep bluish green solution indicated presence of phenols.

#### 2.3.2 Test for Carbohydrates
Test was performed by using the method of Sofowora, (1993). 3 ml of the aqueous extract was added to 2 ml of Molisch's reagent and the resulting mixture shaken. 2 ml of concentrated sulfuric acid was poured carefully down the side of the test tube. Formation of a red or dull violet color at the inter-phase of the two layers was indicative of positive test.

#### 2.3.3 Test for Terpenoids
Salkowski test was performed by using the method of Edeoga et al., (2005). 5 ml of aqueous extract was mixed in 2 ml of chloroform. Then 3 ml of concentrated sulfuric acid was added to form a layer. A reddish brown coloration of interface indicated presence of terpenoids.

#### 2.3.4 Test for Saponins
Test was performed by using the method of Edeoga et al., (2005). 2 g of the powdered sample boiled in 20 ml of distilled water in water bath and filtered the solution. Then 10 ml of the filtrate was mixed with 5 ml of distilled water and shake vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shakes vigorously which leads to formation of emulsion; indicated presence of saponins.

#### 2.3.5 Test for Flavonoids
Test was performed by using the method of Harborne, (2005). 1 g powdered sample was heated with 10 ml ethyl acetate over a steam bath (40–50°C) for 5 min. Filtrate was treated with 1 ml dilute ammonia. A yellow coloration demonstrated positive test for flavonoids.

#### 2.3.6 Test for Alkaloids
Test was performed by using the method of Harborne, (2005). Extracted 1 g powdered sample with 5 ml methanol and 5 ml of 2 N hydrochloric acid. Then filtrate was treated with Meyer's and Wagner's reagents. The samples were scored positive on the basis of turbidity.

#### 2.3.7 Test for Glycosides
Kellar – Kiliani test was performed by using the method of Parekh and Chanda, (2007). 2 ml of
filtrate was added with 1ml of glacial acetic acid. Then 1ml of ferric chloride was added with 1ml concentrated sulfuric acid. Green-blue coloration of solution indicated the glycoside presence

2.3.8 Test for Tannins
Test was performed by using the method of Kumar et al., (2007). Alcoholic ferric chloride solution (10%) was added in 2-3ml of methanolic extract (1:1). The development of dark blue color of solution indicated the presence of tannins

2.3.9 Test for Ascorbic Acid
Test was performed by using the method of Ganesan and Bhatt, (2008). 2ml of 2% w/v solution, add 2ml of water, 0.1g of sodium bicarbonate and about 20mg of Ferrous sulfate was shaken and allowed to stand. A deep violet color produced which was disappeared by adding 5ml of 1M sulfuric acid

2.3.10 Test for Steroids
Identification of steroids was done by adopting the method described by Edeoga et al., (2005). To 1 ml of extract, 2 ml acetic anhydride and 2 ml concentrated sulfuric acid was added, color change from blue to dark green indicated the presence of steroids

2.3.11 Test for Free Reducing Sugars
Test performed by using the method of Sofowora, (1993). Fehling solution used as reagent and appearance of a red precipitates of cuprous oxide indicated presence of free reducing sugars

2.3.12 Test for Starch
Test performed by using the method of Ganesan and Bhatt, (2008). By using Iodine as reagent appearance of dark blue color which disappeared on heating and reappears on cooling indicated presence of starch in sample

3. RESULTS
The pharmacological effects of these all plants are due to the presence of bioactive chemical constituents. R. damascena and F. vulgar contained all tested constituents as shown in Table. Terpenoids and starch were present in all plant species except N. tabaccum. Saponins were present in all plants instead of C. intybus. Steroids were present only in C. intybus, F. vulgar and R. damascena. Free reducing sugar was absent in N. tabaccum and C. papaya. Glycosides were absent in T. ammi. C. papaya contained all constituents except steroids and free reducing sugars.

Following table shows the results of qualitative analysis of different medicinal plants:

<table>
<thead>
<tr>
<th>Class of compounds</th>
<th>C. papaya</th>
<th>C. intybus</th>
<th>F. vulgar</th>
<th>N. tabaccum</th>
<th>R. damascena</th>
<th>S. nigrum</th>
<th>T. ammi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Free reducing sugar</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>++</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>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>++</td>
<td>++</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>
<td>+</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</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>
<td>+</td>
<td>+</td>
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<tr>
<td>Terpenoids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total sugar</td>
<td>+</td>
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<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Where; + Positive, ++ Strong positive, ± Trace, - Negative.
4. DISCUSSION

The presence of ascorbic acid in plant species has shown high total antioxidant properties of plants, glycosides are characterized by their actions on contractile forces of cardiac muscle and saponins show anti-fungal, antibacterial, anti-protozoal and lipid lowering effects (Aslam et al., 2009). Saponins present in all plant species shows that they can be used as lipid lowering agent as well as has anthelmintic and antibacterial activity. Due to presence of saponins these all may be used as cytotoxic and as expectorant through the stimulation of a reflex of the upper digestive tract (Ayoola and Adeyeye, 2010).

Tannins act as astringent, antioxidants, free radical scavengers, promote healing of wounds and effective in peptic ulcers while presence of reducing sugars in these plants has a reductive properties (Rajurkar and Gaikwad, 2012). Due to presence of terpenoids these might be act as cardio protective and antioxidant (Kusmic et al., 2004). Steroids are frequently used signaling molecules biologically and decrease fluidity of membranes (Sadava et al., 2011).

Phenolic compounds widely distributed in all plants have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic. Due to presence of phenolic compounds these might play role in the prevention of several chronic diseases such as cardiovascular disease, cancer, diabetes, bacterial and parasitic infections (Canini et al., 2007). Flavonoids can also inhibit the activity of many enzymes such as xanthine oxidase, peroxidase and nitric oxide synthase, which are supposed to be involved in free radical generation, thereby resulting in decreased oxidative damage of macromolecules (Cazarolli et al., 2008).

5. Conclusion

In conclusion, the overall results of study suggest that all plants contain one or other pharmacologically active constituent in them. It is mandatory to conduct the chemical characterization to isolate and evaluate active phyto-constituents in order to develop the therapeutics that has a promising role in the treatment of dysfunction diseases.

Conflict of Interests

Authors declared no competitive interests for the presented work.

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