PRELIMINARY IN-VITRO POTENTIAL PHYTOCHEMICALS INVESTIGATION OF BARKS OF RAVENALA MADAGASCARIENSIS
SONNERAT


1Department of Pharmacy, Progati Medical Institute, Dhaka-1207, Bangladesh.
2Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh.
3Department of Pharmacy, University of Development Alternative, Dhaka-1209, Bangladesh.
4Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh.

ABSTRACT

Nature is the roots of medicines. Every plants contents lots of chemicals which have several biological activities. Every day we take lots of medicine with foods. These foods protect us from several diseases and sometimes cause diseases. Diseases are imbalance of chemicals compound. In present investigation, the bark methanolic extracts of R. madagascariensis were subjected to the Preliminary phytochemical screening. This investigation suggested that the presence of various potential phytochemicals such as Anthraquinone, Tannins, Flavonoids, Saponins, Steroids, Terpenoids, Triterpenoids. The presence of these compounds exhibit potential biological activities such as antioxidant, analgesic, cardioprotective, Lipid lowering activity, hepatoprotective, antidiabetics, anti diarrhoeal, anticancer, antiasthmatic activities, antimicrobial and so on. This is only preliminary studies to isolate potential compounds which may be use as a lead compound for several biological activities.

KEY WORDS: Ravenala madagascariensis; phytochemicals, Anthraquinone, Flavonoids, Steroids, Cardiac Glycoside.
INTRODUCTION

Greek word "Phyto" indicate plant and "phytochemicals" related with plant pigments. So, routes, barks, fruits and vegetables contain bright and deep colors like as yellow, orange, red, green, blue and purple—generally those indicates several phytochemicals are available in the plant. These phytochemicals acts as nutrients and plays vital role in our daily life. We may use fruits and vegetables, whole grains, soy and nuts by eating 5-9 servings. In resent investigation more than 900 different phytochemicals have been identified in plant foods and more will be discovered. These phytochemicals are an emerging area of nutrition and health (Aiyelaagbe and Osamudiamen, 2009) and (Egwaikhide et al., 2007). All phytochemicals are natural bioactive compounds found in plant foods those works with nutrients and dietary fiber to protect against disease. Recent research suggest, phytochemicals working together with nutrients found in fruits, vegetables and nuts, may help slow the aging process and reduce the risk of many diseases including stroke, high blood pressure, cancer, heart disease, cataracts, osteoporosis, and urinary tract infections. Maximum plants enable exhibit antioxidant, analgesic, cardioprotective, Lipid lowering activity, hepatoprotective, antidiabetics, antidiarrhoeal, antidiabetics, antidiarrhoeal, anticancer, antiasthmatic activities and so on by overlapping mechanisms of action in the body (Md. Reyad-ul-ferdous et al., 2014). Ravenala madagascariensis (commonly Travelers-Tree) is Native of North America, cultivated for in many tropical and subtropical regions. It is small spreading trees with bark do not droop; showy; typically multi-trunked; thorns belonging to the family Strelitziaceae. Tree 30-60 ft (9-18 m) tall. Simple Leaf type, oblong Leaf shape, entire Leaf margin, broad leaf evergreen leaf type, pinnate Leaf venation evergreen, more than 36 inches length, green Leaf color. Flowers are white/cream/gray. Fruit s are less than .5 inch, .5 to 1 inch length, dry or hard Fruit covering, brown In color, does not attract wildlife; not showy; fruit/leaves not a litter problem ("Botanical Journeys Plant Guides" 2008-2010).

MATERIALS & METHODS

Plant material

The Bark of R. madagascariensis was collected from Mirpur Botanical Garden, Dhaka, Bangladesh, in November 2011. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. 38302). The Barks were picked and washed with water to remove all unwanted plant materials and sand, air dried under light exposure (27°C-30°C for 7 days), pulverized in a mill and stored in an airtight container for further use. The air dried and powdered Bark and fruit (500 gm) of R.
*R. madagascariensis* was macerated in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extracts were concentrated with a rotary evaporator at low temperature (40-45 °C) and reduced pressure. The concentrated methanolic extract (ME) was partitioned by modified Kupchan method (Van Wagenen *et al.*, 1993) and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

**Preparation of extract**

The powdered plant material (200 g) was extracted thrice in distilled water (5.5 L; 27°C-30°C) on shaker (Stuart Scientific Orbital Shaker, UK) for 48 hours. The extracts were filtered using a Buchner funnel and Whatman No.1 filter paper. The aqueous extract filtrate obtained was quickly frozen at -40°C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA) to give a yield of 30 g of dry extract. The resulting extracts were reconstituted with distilled water to give desired concentrations used in this study.

**Qualitative screening of Phytochemicals**

One gram of the methanol extract of *R. madagascariensis* was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents (Harborne, 1998; Egwaikhide *et al.*, 2007 and Md. Reyad-ul-ferdous *et al.*, 2014). A small portion of the dry extract was used for the phytochemical tests for compounds which include Anthraquinone, tannins, flavonoids, alkaloids, saponins, steroids, Triterpenoids and Cardiac Glycoside in accordance with the methods of with little modifications. We identified several compound in table-1.

**Anthraquinone (Borntrger’s Test)**

0.5 g of the extract was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 min. The extract was filtered then the filtrate was shaken with equal volume of 10% ammonia solution. A red or pink violet color in the ammonical layer indicates presence of anthraquinone.

**Tannins**

Small quantity of extract was mixed with distilled water and heated on H2O bath. Then filtered and Ferric chloride was added to the filtrate. A dark green color indicates presence of
tannins. 5ml of extract and a few drops of 1% lead acetate were added. Yellow precipitate was formed which indicates the presence of tannins.

Flavonoids
0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution was format that turns colorless indicates the presence of flavonoids.

Saponins
0.2 g of plant extracts were taken and 5 mL of distilled water was added and then boiled. Frothing persistence indicates presence of saponins.

Steroids (Liebermann-Burchard Reaction)
1ml plant material in 10 mL chloroform filtered. 200 milliliter of acetic anhydride was added to 2 mL filtrate with 2 mL H$_2$SO$_4$. The color changes from violet to green or blue in some samples indicating the presence of steroids. 1 ml of the extracts was dissolved in 10ml of chloroform and equal volume of concentrated H$_2$SO$_4$ was added by sides of the test tube. The upper layer turns red and H$_2$SO$_4$ layer showed yellow with green fluorescence which indicates the presence of steroids.

Phlobatanins
0.5 g of plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution and red precipitate shows which indicate the presence of phlobatanins.

Terpenoids (Salkowski Method)
0.5 g of extract added in 2 mL of chloroform filtered. Then Concentrated H$_2$SO$_4$ carefully added to form a layer. Reddish brown color of the interface was formed to show positive results for the presence of terpenoids.

Triterpenoids
10 mg of the extract was dissolved in 1 ml of chloroform; 1ml of acetic anhydride was added following the addition of 2 ml of conc. H$_2$SO$_4$. Reddish violet color was formatting which indicates the presence of triterpenoids.

Cardiac Glycoside
0.5g of each was treated with 2 mL of glacial acetic acid containing a drop of FeCl$_3$ solution. This was underlayered with 1 mL of conc. H$_2$SO$_4$. Brown ring obtained at the interface indicated the presence of de-oxy sugar characteristics of cardenolides.
Table-1: Phytochemicals screening of barks of Traveler’s-Tree (*R. madagascariensis*)

<table>
<thead>
<tr>
<th>Name of the Test</th>
<th>Color</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthraquinone (Borntrger’s Test)</td>
<td>pink violet or red color</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>dark green color</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>yellow solution that turns colorless</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>frothing persistence</td>
<td>+</td>
</tr>
<tr>
<td>Steroids (Libermann-Burchard Reaction)</td>
<td>The upper layer turns red and H₂SO₄ layer showed yellow with green fluorescence</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatanins</td>
<td>red precipitate</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids (Salkowski Method)</td>
<td>formation of reddish violet colour</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>H₂SO₄, formation of reddish violet colour</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycoside</td>
<td>brown ring obtained</td>
<td>+</td>
</tr>
</tbody>
</table>

(+)= present and (-)= absence

RESULTS & DISCUSSION

The result obtained in the present investigation phytochemicals screening of the methanol extract of barks of *R. madagascariensis* revealed that the crude extract contained flavonoids, saponins, steroids, tannins and triterpenoids, terpinoids, cardiac glycosides and Anthraquinones (Table-1). *R. madagascariensis* barks can also have various medicinal values such as anti-inflammatory, membrane stabilizing and thrombolytic activity. The presence of flavonoids exhibit significance cardioprotective, anticancer, Anti-microbial activity may demonstrate gram-positive, gram-negative as well as fungi also. Most of the microorganisms are resistance to several drugs. We isolate pure lead compound from this plant for future drug development. Other potential phytochemicals also present in this plant, which exhibit several biological activities. Each though, this is only a preliminary study of the occurrence of certain properties of *R. madagascariensis* barks an in-depth study will provide a good concerted base of all the phytochemicals functions mention above.

CONCLUSION

In the present study, we have found that most of the biologically active phytochemicals were present in the methanolic extract of *R. madagascariensis* barks. This is only a preliminary study and to make final comment the extract should thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.
ACKNOWLEDGMENT
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CONFLICT OF INTEREST
All authors were declared no conflict of interest.

REFERENCE