Qualitative phytochemical screening and proximate analysis of Dialium guineense stem bark

Abstract: Phytochemical screening is a necessary step in the investigation of chemical constituents of plants. It leads to the isolation of novel compounds with promising bio-activities. Dialium guineense (Velvet Tamarind), is a tall, tropical, fruit-bearing tree. It belongs to the Leguminosae family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it grows in dense forests along the southern edge of the Sahel. The bark and leaves have been reported to possess medicinal properties and are used against several diseases. In the present study, qualitative phytochemical screening and proximate analysis were performed on the plant stem bark. The results obtained revealed the presence of saponins, tannins, alkaloids and other polyphenolics. Xanthoprotein, oxalate and acids were not detected. Proximate analysis also revealed the presence of moisture (fresh sample) (45.47 ± 1.49 %), ash (3.10 ± 0.10 %), fibre (2.05 ± 0.05 %), fat (11.75 ± 0.25 %), crude protein (5.69 ± 0.44 %) and nitrogen-free substances (NFS, 59.86 ± 0.09 %).

Keywords: Dialium guineense, Extract, Phytochemicals, Proximate analysis, Stem bark.

INTRODUCTION

Plants are natural source of a wide range of phytochemicals. Different bioactive phytoconstituents have been isolated and characterized since the middle of the 19th century. Many of which are used as active ingredients in pharmaceutical formulations and modern medicine. Plant-derived products such as those used in the treatment of coronary heart diseases and cancer have been reported to be rich in phenolic compounds (Scalbert, 1993; Hertog et al., 1995). These secondary plant metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Acharya and Shrivastava, 2008). Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agent with general as well as specific activity (Gowthami et al., 2012a).

Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases (Gowthami et al., 2012b; Manokaran et al., 2008).

Dialium guineense (Velvet Tamarind), is a tall, tropical, fruit-bearing tree. It belongs to the Leguminosae family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it grows in dense forests along the southern edge of the Sahel. The bark and leaves have been reported to possess medicinal properties and are used against several diseases. Each fruit typically has one hard, flat, round, brown seed, typically 7 - 8 mm across and 3 mm thick (Dalziel, 1973). The seed somewhat resembles a watermelon seed (Citrullus lanatus). Some have two seeds. The seeds are shiny, and coated with a thin layer of starch. The pulp is edible and may be eaten raw or soaked in water and consumed as a beverage (Dalziel, 1973). The bitter leaves are ingredients in a Ghanaian dish called domoda. Its wood is hard and heavy, and used for construction. The wood is also used for firewood and charcoal production (Dalziel, 1973).
Materials and Methods

Phytochemical Analysis

Qualitative phytochemical screening was performed using standard procedures (Sofowora, 1993; Harborne, 1998; Trease and Evans, 2002). Portion of the pulverized plant material (5 g) was boiled with 20 mL of distilled water gently on a water bath for 10 min. The mixture was allowed to cool and filtered. The resultant filtrate was used for the different tests.

Test for Tannins

A few drops of 0.1% ferric chloride was added to 3 mL of the filtrate and observed for brownish green or a blue-black colouration.

Test for Phlobatannins

Deposition of a red precipitate when 3 mL of the filtrate was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Saponins

A portion of the filtrate (10 mL) was diluted with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and then observed for the formation of an emulsion.

Test for Flavonoids

A given volume (5 mL) of 1% ammonia solution was added to a portion of the aqueous filtrate followed by addition of concentrated H₂SO₄. Appearance of yellow colour which disappeared on standing indicated the presence of flavonoids (Harborne, 1973; Sofowora, 1993).

Test for Steroids (Liebermann-Burchard’s Test)

To 2 mL of the filtrate was added few drops of chloroform, 3 – 4 drops of acetic anhydride and a drop of concentrated H₂SO₄. The colour changed from violet to blue or green in some cases, indicating the presence of steroids (Finar, 1986).

Test for Terpenoids (Salkowski Test)

Aqueous filtrate (5 mL) was mixed with 2 mL of chloroform, and 3 mL of concentrated H₂SO₄ was carefully added to form a layer. Reddish brown colouration at the interface was taken as a positive test for terpenoids.

Test for Cardiac Glycosides (Keller-Killiani Test)

Aqueous filtrate (5 mL) was mixed with 2 mL of glacial acetic acid containing a drop of ferric chloride solution. This was underlaid with 1 mL of concentrated sulphuric acid, and appearance of a brown ring at the interface was indicative of the presence of cardiac glycosides. Gradual formation of a violet ring below the brown ring, or a green ring in the acetic acid layer was also taken as a positive test.

Test for Alkaloids

Mayer’s Test

To 1 mL of filtrate, few drops of Mayer’s reagent were added by the side of the test tube. Formation of white or creamy precipitate confirmed a positive result (Evans, 1997).

Wagner’s Test

To 1 mL of filtrate, few drops of Wagner’s reagent were added by the side of the test tube. Formation of reddish-brown precipitate confirmed the presence of alkaloids (Wagner, 1993).

Dragendorff’s Test

To 1 mL of filtrate, 2 mL of Dragendorff’s reagent was added and formation of prominent yellow precipitate was taken as a positive test (Waldi, 1965).

Test for Carbohydrates

Molisch Test

To 2 mL of the filtrate was added 2 drops of 10% alcoholic solution of naphthol followed by 2 mL of concentrated H₂SO₄, gently poured along the side of the test tube at an angle of 45°. Formation of a purple ring at the interface of the two liquid layers confirmed the presence of carbohydrates (Sofowora, 1993).

Fehling’s Test

Aqueous filtrate (1 mL) was boiled on water bath with 1 mL each of Fehling’s solutions A and B. Formation of a red precipitate was indicative of the presence of sugars (Ramakrishnan et al., 1994).

Barfoed’s Test

To 1 mL of filtrate, 1 mL of Barfoed’s reagent was added and heated on a boiling water bath for 2 min. Formation of a red precipitate confirmed the presence of sugars (Ramakrishnan et al., 1994).

Benedict’s Test

To 0.5 mL of filtrate, 0.5 mL of Benedict’s reagent was added. The mixture was heated on a boiling water bath for 2 min. Formation of a red precipitate confirmed the presence of sugars (Ramakrishnan et al., 1994).

Test for Proteins

Million’s Test

To 2 mL of filtrate, few drops of Million’s reagent were added. Formation of a white precipitate was indicative of the presence of proteins (Ruthmann, 1970).

Biuret Test

An aliquot of filtrate (2 mL) was mixed with drops of 2% copper sulphate (CuSO₄) solution. Then, 1 mL of ethanol (95%) was added, followed by excess KOH pellets. Appearance of pink colour in ethanol layer confirmed the presence of proteins (Gahan, 1984).
Test for Amino Acids (Ninhydrin Test)
    Two drops of ninhydrin solution (5 mg ninhydrin in 200 mL of acetone) were added to 2 mL of aqueous filtrate. A characteristic purple colour confirmed the presence of amino acids (Yasuma and Ichikawa, 1953).

Test for Coumarins
    Aqueous filtrate (1 mL) was mixed with 1 mL of 10 % NaOH. Formation of yellow colour confirmed the presence of coumarins.

Test for Quinones
    Concentrated sulphuric acid (1 mL) was added to 1 mL of filtrate. Formation of red colour was indicative of the presence of quinones.

Test for Acids
    Aqueous filtrate (0.5 mL) was mixed with sodium bicarbonate (NaHCO₃) solution. Formation of effervescence confirmed the presence of acids.

Test for Anthraquinones
    Few drops of 2 % HCl were added to 0.5 mL of filtrate, and the formation of red precipitate confirmed the presence of anthraquinones.

Test for Fixed Oils
    A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil (Kokate, 1999).

Test for Fat (Saponification Test)
    A few drops of 0.5 N alcoholic KOH solution and a drop of phenolphthalein were added to 2 mL of filtrate. The mixture was heated on water bath for 2 h. Formation of soap or partial neutralization of alkali confirmed the presence of fixed oils and fats.

Test for Resins
    Aqueous filtrate (5 mL) was mixed with 25 mL of absolute ethanol with constant stirring. Formation of white or cloudy precipitate was indicative of the presence of resins (Whistler and BeMiller, 1993).

Test for Oxalates
    To 3 mL portion of filtrate was added few drops of glacial acetic acid. Appearance of greenish black colouration was taken as positive test for oxalates.

Test for Xanthoprotein
    To 2 mL of the filtrate was added few drops of concentrated nitric acid and 2 – 3 mL of ammonia. Appearance of a red precipitate confirmed the presence of xanthoprotein.

RESULTS
Outcome of Phytochemical Evaluation of Aqueous and Ethanol Extracts and Proximate Analysis of Dialium guineense Stem Bark
    The Results of phytochemical analyses revealed the presence of saponins, tannins, alkaloids and other polyphenolics. Xanthoprotein, oxalate and acids were not detected (Table 1). Results of proximate analysis showed that the stem bark contained more Nitrogen-Free Substances (NFS) and low fibre content (Table 2).

Table 1: Phytochemicals Present in Aqueous and Ethanol Extracts of Dialium guineense Stem Bark

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous</th>
<th>Ethanol</th>
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<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xanthoprotein</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxalates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fat</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = detected; - = not detected
DISCUSSION

Plants produce phytochemicals as part of their normal metabolic activities which they use for defence against predators (Muller, 1998). Phytochemicals are bioactive non-nutrient plant compounds present in fruits, vegetables, grains and other plant foods, whose ingestion has been linked to reductions in the risk of major chronic diseases. The different compounds included in this group are classified according to common structural features as carotenoids, phenolics, alkaloids and nitrogen containing and organosulfur compounds. Phenolics, flavonoids and phytosterols are of particular interest because of their potential effects as antioxidants, anti-inflammation, immunomodulatory, cardioprotective and anticancerogenic compounds. In the present study, qualitative phytochemical screening and proximate analysis were performed on the *D. guineense* stem bark. Findings suggest that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity (Mathai, 2000). The physiological properties of relatively few phytochemicals are well understood (Mathai, 2000). Phytochemicals have been promoted for the prevention and treatment of diabetes mellitus, high blood pressure, and muscular degeneration (Mathai, 2000). Results of this study have shown that the stem bark of *D. guineense* is rich in important phytochemicals. Phytochemical screening showed that *D. guineense* stem bark contains carbohydrates, protein, amino acids, fat, coumarins, fixed oil, tannins, saponins, flavonoids, alkaloids, glycosides, steroids, phlobatannins, and terpenoids. Phlobatannins, steroids, terpenoids, and fat were absent in the aqueous extract, but present in the ethanol extract. Studies have shown that environmental factors and time of collections affect the type and quantity of secondary metabolites in a particular part of a plant. The medicinal value of *Dialium guineense* may be correlated to the presence of various bioactive chemical constituents (Raaman, 2006). The specific activity of this plant may be attributed to the presence of quinones, terpenoids, steroids and phenolic compounds which need further investigation. Saponins are known to reduce blood cholesterol by preventing its reabsorption (Osagie and Eka, 1998) and may also be a potent inhibitor of hydroxyl methylglutaryl CoA (HMG-CoA) reductase: an enzyme that catalyzes the conversion of HMG-CoA to mevalonate an early and rate limiting step in cholesterol biosynthesis. Medicinal agents containing tannins have been shown to possess anti-diabetic properties (Iwu, 1983). Saponins, flavonoids, quercetin and ferulic acid synergistically reduce blood glucose level via the correction of defective insulin secretion and peripheral insulin resistance (Mahesh, 2004). The presence of alkaloids in *D. guineense* stem bark could make it effective against cardiovascular diseases (Tan and Reinhold-Hurek, 2003). Alkaloids are known to possess pharmacological activities such as antihypertensive, antiarrhythmic and anticancer effects. A number of alkaloids are used as drugs and the best known is quinine used as an antimalarial (Cordell, 1983). Steroids and cardiac glycosides are presently used for the treatment of cardiac failure. These agents increase the force of contraction in a failing heart by increasing the interaction of actin and myosin filament of cardiac sarcomere, thereby increasing calcium concentration in the vicinity of the contractile protein during systole (Prohp and Onoagbe, 2012).

CONCLUSION

The stem bark of *D. guineense* is a reservoir of potentially useful chemical compounds which may serve as drugs and provide newer leads and clues for modern drug design.

REFERENCES


Table 2: Proximate Composition of Pulverized Stem Bark of *Dialium guineense*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (Fresh sample)</td>
<td>45.47 ± 1.49</td>
</tr>
<tr>
<td>Moisture (Dried sample)</td>
<td>17.55 ± 0.05</td>
</tr>
<tr>
<td>Ash</td>
<td>3.10 ± 0.10</td>
</tr>
<tr>
<td>Fibre</td>
<td>2.05 ± 0.05</td>
</tr>
<tr>
<td>Fat</td>
<td>11.75 ± 0.25</td>
</tr>
<tr>
<td>Crude protein</td>
<td>5.69 ± 0.44</td>
</tr>
<tr>
<td>Nitrogen-Free Substances</td>
<td>59.86 ± 0.09</td>
</tr>
</tbody>
</table>

Data are percentage proximate composition and are expressed as mean ± SEM (n = 3), on “Dry Weight (DW)” basis.