QUALITATIVE PHYTOCHEMICAL ANALYSIS OF LEAVE AND STEM BARK OF
Zanthoxylum zanthoxyloides AND Zanthoxylum gilletti

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ABSTRACT

This study reveals result on qualitative phytochemical analysis, mineral analysis on powdered leaves and stem bark of Zanthoxylum zanthoxyloides and Zanthoxylum gilletti, micromorphology of the leaves of Zanthoxylum zanthoxyloides and Zanthoxylum gilletti. The phytochemicals analysis of the two species showed significant phytocompounds such as (saponnin, tannin,alkaloid, steroids, anthraquinone, cardiac glycosides and flavonoids) at varying strength of availability in the powdered leaves and stem bark. The leaves of Zanthoxylum zanthoxyloides were found to contains tannin, terpenoids, steroids, cardiac glycosides, phenol, phlobotannin and flavonoids while the stem bark contained all of the phytochemicals tested except alkaloid and anthraquinone. The leaves of Zanthoxylum gilletti were also found to contains saponnin, tannin,alkaloid, terpenoids, steroids, cardiac glycosides, phenol, phlobotannin and flavonoids while the stem bark contains all the phytochemicals tested except phenol and sterol. The presence of phytochemicals and minerals in the leaves and bark of Z. zanthoxyloides and Z. gilletti justify their popularity as a very potent plant that could be used in the treatment of various diseases.

Keywords: Phytochemical, Zanthoxyloides, phytocompounds, Antioxidants and Alkaloids.

INTRODUCTION

Phytochemistry is the art of resolving plants into chemically pure individual constituents. As a result of recent interest in the plant kingdom as a potential source of new drug strategies for the fraction of plant extracts based on biological activity rather than a particular class of compound developed [1].

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine [2].

Plants produce these chemicals to protect themselves, but recent research demonstrates that they can also protect humans against diseases. Phytochemicals help to repair damage or prevent the damage of these oxidants by acting as antioxidants. Phytochemicals may also inhibit the growth of cancer cells, improve immune function, block carcinogens, and help clear out toxins or
other damaging substances, among other actions [3].

Therefore, phytochemical studies may provide a useful tool for the authentication and discrimination between similar plant species.

Zanthoxylum species commonly called orin ata in Yoruba (Syn. Fagara species) of the Rutaceae family are widely used in many countries as food and in trado-medicinal practice due to their wide geographical distribution and medicinal properties. Peer reviewed journal articles and ethnobotanical records that reported the traditional knowledge, phytoconstituents, biological activities and toxicological profiles of Z. species with a focus on metabolic and neuronal health has been reviewed by [4].

Zanthoxylum species (rutaceae) namely Zanthoxylum gilletti and Zanthoxylum zanthoxyloides are well-known for their potential activities against constipation, complicated gastro-intestinal, malarial, rheumatism and skin infections [5].

**MATERIALS AND METHOD**

Fresh stem bark and leaves of Zanthoxylum zanthoxyloides was collected from a forest at Oni Gambari, Ido Local Government Area and Zanthoxylum gilletti was also collected from herbal garden in forestry research institutes of Nigeria, Jericho hills, Ibadan. Identification was carried out in the institute’s taxonomy department and the chemical analysis was carried out at the Forestry Research Institute of Nigeria Biomedicinal Research Centre Laboratory. The samples were cleaned and oven dried. The leaves were oven dried at 500c for a week and the bark at 1000c for a week. They were thereafter blended into fine powder and coarse powder separately and then kept in an air tight container for further analysis.

The finely powdered samples were screened for phytochemical constituents (alkaloids, flavonoids, saponins, tannins, steroids, anthraquinone, cardiac glycosides). 1g of powdered samples will be dissolved separately in various solvents in the order of increasing polarity of solvents. The collected 10% (v/v) extracts was used for phytochemical screening.

**Qualitative analysis for powdered sample** [6]

**Alkaloids**

1g of powdered sample was stirred in 10ml of (v/v) HCl on in water bath and then filtered. The filtrate was divided into 3 parts. Dragendorff’s, Wagner’s and Meyer’s reagent were added separately to each portion:

Formation of orange/orange-red precipitate/turbidity indicates the presence of alkaloids in dragendorff’s

A reddish-brown or yellow precipitate will be regarded as positive for the presence of alkaloid in Wagner’s
Formation of white or pale-yellow precipitate will indicate the presence of alkaloid in Meyer’s

**Flavonoids**

1g of sample was boiled in 10ml of ethanol and subject to the following test after filtering:

Aluminium solution: few drops of 1% aluminium solution was added to 5ml of the filtrate. The observation of a yellow coloration indicates the presence of flavonoids.

To the 5ml of the filtrate was added 2 drops of ferric chloride. The observation of dusty green color indicates presence of flavonoids.

To 5ml of the filtrate was added a small quantity of dilute NaOH and then drops of conc. HCl was allowed to run down the side of the tube. Reddish color formation indicated the presence of flavonoids.

**Test 2**

1g of the powdered sample was treated with 10ml of ethyl acetate over a steam bath for 30min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration signified the presence of alkaloids

**Saponins**

1g of powdered sample was boiled with 10ml of distilled water for 10mins. The sample was filtered while hot and allowed to cool. The resulting filtrate was subjected to:

Frothing test: 2.5ml of the filtrate was diluted to 10ml with distilled water and shaken vigorously for 20mis. Formation of persistent frothing indicated the presence of saponins.

**Tannins**

1g of the powdered sample was boiled in 10ml of distilled water, filtered hot and then cooled. The filtrate was adjusted to 10ml with distilled water, few drops of ferric chloride was added to 1ml of the filtrate. The formation of blue, dark brown and blue-black; green or green-black coloration or precipitate indicates the presence of tannins

**Test for Anthraquinones**

A) Combined Anthraquinone

1g of the powdered sample was boiled with 50ml of 10% HCl for 5 min and filtered while hot. The cooled filtrate was proportioned against equal volume of chloroform while avoiding vigorous shaking. A clean pipette or syringe was then used to transfer the chloroform layer to a clean tube taking care not to include the aqueous layer. An equal volume of 10% ammonia was added to the chloroform extract. Formation of pink, red or violet color indicates the presence of Anthraquinone.

B) Free Anthraquinone

0.5g of powdered sample was shaken with 5ml of chloroform for 10 minutes and filtered. 5mls of 10% ammonia solution was added to the filtrate and the mixture was shake. Formation of pink,
red or violet color will signify the presence of free anthraquinone.

**Test for Cardiac glycosides**

1g of the powdered sample was extracted with 10ml of 80% ethanol for 5 minutes on a water bath and filtered. The filtrate was diluted with an equal volume of distilled water. A few drops of lead acetate solution was added, shaken and filtered after standing for few minutes. The filtrate was then extracted with aliquot of chloroform and divided into two portions in an evaporating dish and evaporated to dryness on a steam bath.

One portion was dissolved in 2mls of glacial acetic acid containing one drop of FeCl$_3$ solution in a clean test tube. 2ml of conc. H$_2$SO$_4$ was then poured down the side of the tube so as to form a layer below the acetic acid. The formation of a purple or reddish-brown ring at the interface and a green color in the acetic layer was taken for a positive result.

**Test for Steroids**

1g of the powdered sample was dissolved in 10ml of chloroform on a steam bath and filtered. The filtrate was allowed to cool while ten drops of acetic anhydride and two drops of conc. H$_2$SO$_4$ was then carefully added down the side of the test tube. The formation of bluish green, green, or blue coloration will confirm the presence of steroids.

**RESULT AND DISCUSSION**

**Phytochemical estimation**

The table below presents the results obtained for the qualitative phytochemical analysis of leaves and bark of *Zanthoxylum zanthoxyloides* and *Zanthoxylum gilletti*. All the medicinal plants had the presence of some secondary metabolites in varying quantities.

<table>
<thead>
<tr>
<th>S/n</th>
<th>Phytochemical</th>
<th>Test</th>
<th>Z.zl</th>
<th>Z.zb</th>
<th>Z.gl</th>
<th>Z.gb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponin</td>
<td>Frothing test</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannin</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Alkaloids</td>
<td>Dragendorff test</td>
<td>D. -</td>
<td>D. -</td>
<td>D. -</td>
<td>D.  +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meyer test</td>
<td>M. -</td>
<td>M. -</td>
<td>M. +</td>
<td>M. +</td>
</tr>
</tbody>
</table>

Table 1: Phytochemical qualitative analysis of leaves and bark of *Zanthoxylum zanthoxyloides* and *Zanthoxylum gilletti*. 
<table>
<thead>
<tr>
<th></th>
<th>Wagner test</th>
<th>W. -</th>
<th>W. -</th>
<th>W. +</th>
<th>W. -</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Steroids</td>
<td>Liebermann-Burchard’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Anthraquinone</td>
<td>Borntrager test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Cardiac Glycoside</td>
<td>Keller kiliani test</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Key: + low, ++ moderate, +++ high, - absent

Zzl: *Zanthoxylum zanthoxyloides* leaf

Zzb: *Zanthoxylum zanthoxyloides* bark

Zgl: *Zanthoxylum gilleti* leaf

Zgb: *Zanthoxylum gilleti* bark

**Discussion**

Saponins are glycosides of both triterpenes and sterols generally possessing five sugar units and gluconic unit as a component. The occurrence of saponins has been reported in over seventy families of higher plants. They are used to reduce body cholesterol by preventing its reabsorption. They also find applications in foaming fire extinguishers, emulsifiers and insecticides [7]. The data shows that saponin is moderately present in the bark of *Zanthoxylum zanthoxyloides* and the leaves of *Zanthoxylum gilletti*. It is low in the bark of *Zanthoxylum gilletti* but however absent in the leaves of *Zanthoxylum zanthoxyloides*. This is due to the slimy nature of the leaves powder and its swelling capability upon addition making extract unobtainable. This is in contrast to the report by [8] which shows the extraction of saponins from the leaf of *Z. zanthoxyloides* using methanol yielded 8.2% w/w of the dried powdered extract. This variation occurred as a result of methods employed.

Tannins are polyphenolic compounds naturally found in leaves, bark, stem of almost all plants. Their presence in nature has prompted their historical use in many different ways [9]. Tannin-containing herbs are used to tighten up tissues (varicose veins), dry up excessive watery secretions (diarrhea), protect damaged tissue (skin), helps to stop bleeding (heavy menstrual flow) and to keep infection in check. They also
act to inhibit enzymes such as 5-lipoxygenase & hyaluronidase, lending to their action as anti-inflammatories, antimicrobials & keratolytics [10]. Tannin content is found to be low in leaves of *Zanthoxylum zanthoxyloides* and bark of *Zanthoxylum gilletti* but it is moderately present in the leaves of *Zanthoxylum gilletti* and bark of *Zanthoxylum zanthoxyloides*.

Alkaloids are well known nitrogen-containing natural bioactive compounds. They are one of the most prominent secondary metabolites in the genus Zanthoxylum. From this study, three types of tests were carried out to evaluate the presence of alkaloids in the samples under study which were; dragendoff test (D), Meyer’s test and Wagner test. The three test showed that alkaloid is absent in the leaves and bark of *Zanthoxylum zanthoxyloides*, Meter and Wagner showed that alkaloid is present in the leaves of *Zanthoxylum gilleti* and Dragendoff test and Meyer test show the presence of alkaloid in the bark of *Zanthoxylum gilleti*.

Steroids were found to be absent in the bark of *Zanthoxylum gilletti* but present in the rest of the samples under study although at a relatively low level.

Anthraquinones impart color to plants and have been widely utilized as natural dyes. In addition, they are also used as laxatives and possess antifungal and antiviral activities [11]. Anthraquinones were found to be absent in all the four samples under study except at a relatively low level in the bark of *Zanthoxylum gilletti*.

Cardiac glycosides are frequently used for heart rate control in patients with atrial fibrillation. Additionally, glycosides can be considered in heart failure patients with resistant symptoms despite optimal medial therapy with beta-blocker, ACE inhibitor/ARB/ARNI, and MRA [12]. According to the table presenting the results, cardiac glycosides are the most abundant secondary metabolite in *Z. zanthoxyloides* leaves. It is also moderately present in the bark of *Z. zanthoxyloides* and at a low level in the leaves and bark of *Z. gilletti*.

Flavonoids generally serve as flavouring ingredients in plants. Besides their role as flavouring agents they are also expressed in plants in response to microbial infection suggesting their antimicrobial activity. Flavonoids have also been implicated as antioxidants both in physiological and diseased states [6]. Flavonoid was found to be present in all the samples under study at a moderate level except in the bark of *Z. gilletti* where it was found to be low.

**CONCLUSION**

The presence of phytochemicals and minerals in the leaves and bark of *Z. zanthoxyloides* and *Z. gilletti* justify their popularity as a very potent plant that could be used in the treatment of various diseases. This study showed that medicinal attributes of leaves and bark of *Z. zanthoxyloides* and *Z. gilletti* is evident. Their folkloric benefits could be associated with the
presence of secondary metabolites and minerals in them.

REFERENCES

1. Evans C.W., Trease and Evance, Pharmacognosy, 15th ed. 2002; 3


