Estimation of Total Flavonoids and Tannins in the Stem Bark and Leaves of *Anogeisus leiocarpus* Plant

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**Article history:** Received 18 May 2013, Received in revised form 18 June 2013, Accepted 23 June 2013 Published 9 July 2013.

**Abstract:** The powdered leaves and stem bark of *Anogeisus leiocarpus* were subjected to cold maceration using 100% and 80% methanol as solvents. The percent of crude recovery are 9.2% and 13.3% for 100% stem bark and leaves extract, and 7.8% and 17.1% for 80% stem bark and leaves extract, respectively. The phytochemical screening and total amount of flavonoids and tannins in both the stem bark and leaves of the plant were estimated using standard methods. The results of the total amount of flavonoids and tannins were 12.5% and 13.0% for stem bark and leaves, and 0.94% and 1.03% for stem bark and leaves, respectively. The results also reveal that the amount of flavonoids and tannins were higher in leaves than in stem bark. These secondary metabolites may be responsible for the medicinal uses of the plant.

**Keywords:** *Anogeisus leiocarpus*; stem; bark; leaves; flavonoids; tannins.

1. **Introduction**

Many of the plants materials used in traditional medicine are readily available to rural dwellers and these have made traditional medicine relatively cheaper than modern medicines. Over sixty percent of Nigerian rural population depends on traditional medicine for their health care need (Apulu et al., 1994). Medicinal properties of plants are normally dependent on the presence of certain...
phytochemical compounds, such as tannins, flavonoids, alkaloids and saponins which are bioactive base responsible for the antimicrobial property (Apulu et al., 1994). Many of these indigenous plants are used as spices and food plants. They are also sometimes added to food means for pregnant and nursing mothers for medical purpose (Okwu, 2004).

Okwu and Okeke (2003) reported that many of these plants possess bioactive compounds that exhibit physiological activity against microorganisms. These plants are used in the treatment of many diseases such as rheumatism, diarrhoea, dysentery, cough, asthma, malaria, cold and others (Burkill, 1995; Bartam, 1998). Many plant materials are useful herbs, from which important drugs could be prepared or agents which may serve as starting materials for the partial synthesis of some useful drugs. The usefulness of these plant materials medicinally is due to the presence of bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds.

Flavonoids are free radical scavenger, super antioxidant and potent water soluble which prevent oxidative cell damage and have strong anti cancer activity (Kim et al., 1994). Flavonoids apart from their antioxidant protection effects, inhibit the initiation, promotion and progression of tumour (Kim et al., 1994; Okwu, 2004). Anogeisus leiocarpus is an abundant plant in Nigeria. This study emphasizes on only two phytochemicals which are known to play important medicinal role in human body. These are tannins and flavonoids. Plants generally have active components in them which serve as bioactive. Flavonoids are known to possess antioxidant activity while tannins have anti-diarrhoea activities. Therefore there is a need to know the total amount of tannins and flavonoids in the plant which correspond to its antifungal and antioxidant activity.

Anogeisus leiocarpus belong to the family "Cambretaceae", is a tall ever green native to savanna of tropical Africa (Steentoft, 1998). It is the sole West African species of the genus Anogeisus, a genus otherwise distributed form tropical central and east Africa through tropical South East Asia (Steentoft, 1998). Anogeisus leiocarpus germinates in the new soils produced by seasonal wetlands and grows at the edges of the rain forest, although not in the rain forest in the savanna, and along river banks forming gallery forests. Anogeisus leiocarpus is one of the plants in which small branches with leaves are crushed to make one of the yellow dyes (Arbonnier, 2004). Anogeisus leiocarpus inner bark is used as a human and livestock anthelmintic for treating worms and for treatment of a couple of protozoan diseases in animals, nagana an animal trypanosomiasis, and babesiosis (Bizinama, 1994). The inner bark used as a chewing stick in Nigeria and extracts of the bark show antibacterial properties (Mann et al., 2008).

The scope covers the phytochemical screening of plant extract and estimation of total flavonoids and tannins in the leaves and stem bark of Anogeisus leiocarpus plant materials, and also quantitative estimation of percentage of crude yield of extract of the plant sample.
This research is aimed at estimation of total tannins and flavonoids in the leaves and stem bark of *Anogeisus leiocarpus* and the specific objectives of the research are: (1) carry out quantitative estimation of percentage of crude yield of extract of the plant sample, (2) qualitative estimation (phytochemical screening) of tannins and flavonoids in the stem bark and leaves of *Anogeisus leiocarpus*, (3) quantitative estimation of total flavonoids and tannins in the leaves and stem bark of plant.

2. Materials and Methods

2.1. Sample Collection and Preparation

The stem bark and the leaves of *Anogeisus leiocarpus* plant sample were collected from Abubakar Tarawa Balewa University (ATBU), Bauchi, Bauchi State. The samples were transferred into paper bags and taken directly to the laboratory for analysis. The samples (leaves and stem bark) were dried under shade and ground into fine powder to pass through 20 µ sieve. Powdered stem bark and leaves of *Anogeisus leiocarpus* were subjected to cold solvent extraction, using 100% and 80% methanol. About 100 g of dried powdered plant tissues (stem bark and leaves): each was soaked in 100% and 80% methanol for four days with occasional vigorous shaking on daily basis. The mixture was later filtered using Whatman filter paper No. 42 and the filtrate was then evaporated over a water bath.

2.2. Phytochemical Screening

Phytochemical screening is a qualitative method of determining the presence of plant chemicals in plant extracts by the use of physical and chemical tests. These processes were used to identify the presence of flavonoids and tannins in *Anogeisus leiocarpus* powdered extracts.

2.3. Test for Flavonoids

The presence of flavonoids in the sample extracts of the plant was determined using the Harbone and William (1992) and Sofowora (1993) methods. Three tests were carried out. Ten mL of ethyl acetate was added to 0.2 g of the powdered extract in a 250 mL beaker and heated in a water bath for 5 min. The mixture was cooled, filtered and the filtrate used for the test.

2.4. Ammonia Test

About 4 mL of the extract was shaken with 1 mL of dilute ammonia solution. The layers were allowed to separate and the yellow colour was formed in the ammoniacal layer. This indicated the
presence of flavonoids.

2.5. Ammonium Chloride Solution Test

The 1 mL of 1% ammonium chloride solution was added to 4 mL of the filtrate and shaken. A yellow colouration was formed which indicated the presence of flavonoids.

2.6. Tetraoxosulphate VI Acid Test

The 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract on addition of concentrated H₂SO₄, and a yellow colouration was observed in each extract which indicated the presence of flavonoids. The yellow colouration disappeared on standing.

2.7. Test for Tannins

The test for the presence of tannins was carried out using standard method by Harbone and William (2000). One gram of the powdered extract sample was boiled with 50 mL of water, filtered and the filtrate was used to carry out ferric chloride test. Few drops of ferric chloride solution were added to 3 mL of the filtrate in a test-tube. A blue black colouration was observed, which indicated the presence of tannins was formed.

2.8. Quantitative Estimation of Phytochemicals

Quantitative estimation of phytochemical is a chemical test carried out to estimate the total amount of flavonoids and tannins in *Anogeisus leiocarpus* plant sample.

2.9. Flavonoid Total Estimation

Flavonoids total estimation on a plant sample was carried out using Boham and Kocipai (1994) method. The 10 g of the plant material was extracted repeatedly with 100 mL of 80% aqueous methanol each for 3 days at room temperature. The whole solution was later filtered through Whatman filter paper No. 42 (125 mm). The filtrate was then transferred into a crucible and evaporated to dryness in a water bath and weighed to a constant weight. The weight obtained gave the estimation of flavonoids in the plant samples (*Anogeisus leiocarpus*).

2.10. Tannins Total Estimation

Tannins total estimation was done by using Okeke and Elekwa (2003) method. The 0.5 g of the plant sample was weighed into a test-tube and shaken with 10 mL of 2M HCl for 5 min. The whole
contents were then transferred into a volumetric flask made up to 50 mL and then filtered using filters paper. The 5 mL of filtrate was measured and introduced into a sample bottle, and 3 mL of 0.1M FeCl\textsubscript{3} in 0.1 N HCl and 3 mL of 0.008 M of potassium ferrocyanide [K\textsubscript{3}Fe(CN)\textsubscript{6}] were added. The absorbance was then read out at 720 nm wavelength within 10 min using UV/visible spectrophotometry. Then 10, 20, 30, 40 and 50 ppm were prepared from standard stock solution of tannic acid (1000 ppm). The absorbance of each serial dilution of stock solution of 1000 ppm of tannins was read at 720 nm, and the resultant absorbances were plotted against the serial dilution of tannin. The absorbance of the plant sample (Anogeisus leiocarpus) was then extrapolated from the graph to get the average gradient (ppm of the sample) and the percentage of the total amount of tannins was determined using the formula.

\[
\text{Percentage of total tannins} = \frac{\text{Absorbance of sample} \times \text{Av. grad.} \times \text{Dilutn factor}}{100}
\]

3. Results and Discussion

The results of phytochemical analysis and percentage of crude yield extract, the qualitative analysis of the phytochemicals and the quantitative estimation of total tannins and flavonoids in the leaves and the stem bark of Anogeisus leiocarpus determined were summarized in Tables 1-4.

Table 1. The percentage yields and nature of the extracts

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Plant sample</th>
<th>Description of extract</th>
<th>Weight of extract (g)</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Stem bark (s)</td>
<td>Brown, Coarse powder</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>100%</td>
<td>Leave (l)</td>
<td>Dark green, Coarse powder</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Methanol</td>
<td>Stem bark (s)</td>
<td>Brown, Coarse crystalline</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>80%</td>
<td>Leave (l)</td>
<td>Brownish green, Coarse powder extract</td>
<td>17.1</td>
<td>17.1</td>
</tr>
</tbody>
</table>

Table 1 showed the nature and percentage yield of 100% and 80% methanol extracts of the leaves and stem bark of Anogeisus leiocarpus plant. It was observed that the colour of the stem bark extracts were brown for both 100% and 80% methanol, and dark green, brownish green for 100% and 80% leaves respectively. The texture of the extracts of the plant samples (stem bark and leaves) were all coarse powdered extract in both 100% and 80% methanol. The percentage yields of the extracts were found to be high in the leaves of 80% and 100% methanol, and the yields ranged from 7.8% to 17.1% which were obtained from Anogeisus leiocarpus plant. Generally, the percentage of the extracts
obtained from *Anogeissus leiocarpus* plant from Abubakar Tafawa Balewa University (ATBU) Bauchi using methanol of various percentages for cold extraction shows that high amount of crude extracts were obtained in the leaves than in the stem with 80% methanol as solvent. Methanol is a good solvent of extraction for the plant due to its polar nature that gives wide spectrum (separation/extraction) of chemical constituent in the plant; as such solvents of various percentages are needed for extraction of a particular active ingredient in the plant materials.

**Table 2.** Qualitative analysis of the phytochemicals in the leaves and stem bark of *Anogeissus leiocarpus* plant extract sample

<table>
<thead>
<tr>
<th>Constituents (Phytochemicals)</th>
<th>Test</th>
<th>Plant Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stem (S)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S 100%</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl₃ (Reduction)</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Ammonium Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Aluminium Chloride Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Cone. H₂SO₄ Test</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: + = Presence of phytochemical, - = Absence of phytochemical.

**Table 3.** Quantitative estimation of percentage of total flavonoids in the leaves and stem bark of *Anogeissus leiocarpus* plant powdered sample

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Total amount (g/mL)</th>
<th>Percentage of total amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem Bark</td>
<td>2.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Leaves</td>
<td>2.6</td>
<td>13.0</td>
</tr>
</tbody>
</table>

**Table 4.** Quantitative estimation of percentage of total amount of tannins in both stem bark and leaves of *Anogeissus leiocarpus* plant material

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Absorbance of sample (nm)</th>
<th>Average gradient (ppm)</th>
<th>Percentage of total tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem bark</td>
<td>0.032</td>
<td>25.0</td>
<td>0.94</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.034</td>
<td>26.0</td>
<td>1.03</td>
</tr>
</tbody>
</table>
The extracts from the plant contain secondary metabolites which are the major constituents of the phytochemicals (Adamu et al., 2001). The presence of flavonoids in the leaves and the stem bark of Anogeisus leiocarpus plant indicated their medicinal value. Flavonoids are antioxidant and free radical scavenger which prevent oxidative cell damage, and have strong anticancer activity and protect the cell against all stages of carcinogenesis. Flavonoids in intestinal tract lower the risk of heart diseases (Okwu, 2004). Anogeisus leiocarpus has been used in treatment of anthelmintic in herbal medicine (Morton, 1987). The stem bark and the leaves of Anogeisus leiocarpus were found to have flavonoids and tannins. These phytochemicals (flavonoids and tannins) are bioactive bases responsible for antimicrobial property (Ebana et al., 1993).

The presence of tannins was detected in the Anogeisus leiocarpus plant, which is high in the leaves. Tannins have been found to possess astringent properties, and hasten the healing of wounds and inflamed mucus membrane (Hakkinen, 1999). The Anogeisus leiocarpus extract of the bark showed antibacterial properties (Bizinnama, 1994).

4. Conclusions

The investigation has revealed that the Anogeisus leiocarpus plant has certain medicinal value in the leaves and the stem bark due to their phytochemical content (flavonoids and tannins) which can be utilized in the treatment of many diseases and also be exploited for use in pharmaceutical industries, food preparation, and also in areas like hide and skin, cosmetics and raw materials for other products.

References


Garden Keid, p. 522.