Qualitative and Quantitative Evaluation of Phytochemicals of *Cassia podocarpa*

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**Abstract:** The usefulness of *cassia podocarpa* as locally antifungal agent necessitated the qualitative and quantitative determination of the phytochemicals present. The screening of the leaf and flower showed the presence of flavonoid, tannin, saponin, alkaloid and glycoside. The quantitative analysis showed that the flavonoid in the leaf (6.73 mg/g) was more than that of the flower (5.83 mg/g). Also, the tannin in the leaf (10.11 mg/g) doubled that of the flower (5.24 mg/g). The saponin content in the leaf (27.36 mg/g) was higher than that of the flower (13.91 mg/g). The alkaloid content in the leaf (19.70 mg/g) was more than that of the flower (6.59 mg/g). However, the glycoside of leaf (7.10 mg/g) was just a little bit higher than that of flower (6.32 mg/g). From the quantitative evaluation of the leaf and flower of this plant, this has really confirmed the local use in the treatment of eczema in human body when the liquid is being extracted and the plant is a reservoir of many novel compounds which can be of immense use to the pharmaceutical world.

**Key words:** Cassia podocarpa • Qualitative • Quantitative • Reservoir

**INTRODUCTION**

The pharmacological usefulness of plants are known to us all because the variety of plants is a treasure house of potential drugs and in the recent years, researchers have beam their search light on plants that have medicinal activities. Medicinal plants contain some organic compounds that have pharmaceutical benefits to human being and these compounds include flavonoids, tannin, saponin, alkaloids [1] and host of others. A large number of phytochemicals that belong to different chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro [2].

Products from plants have been part of phytomedicines since and this can be obtained from barks, leaves, flowers, roots, fruits and seed [3]. Knowledge of the chemical contents of plants is desirable because such useful information will be valuable for the synthesis of novel compounds.

In this research work, both qualitative and quantitative phytochemical analysis were carried out on cassia podocarpa collected at the back of a house in Ajilosun area of Ado-Ekiti, Nigeria.

**MATERIALS AND METHODS**

**Phytochemical Screening**

**Test for Saponins:** Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

**Test for Glycosides**

**Keller-Kilani Test:** Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl₃. The mixture was then poured into another test tube containing 2 ml of concentrated H₂SO₄. A brown ring at the interphase indicated the presence of cardiac glycosides.

**Test for Alkaloids**

**Alkaline Reagent Test:** Crude extract was mixed with 2 ml of 1% HCl and heated gently. Mayer’s And Wagner’s reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

**Test for Flavonoids**

**Alkaline Reagent Test:** Crude extract was mixed with 2 ml
of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

**Test for Tannins:** Crude extract was mixed with 2ml of 2% solution of FeCl$_3$. A blue-green or black coloration indicated the presence of tannins.

**Quantitative Analysis**

**Tannin Determination:** 0.2g of finely ground sample was weighed into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2hours at 30°C. Each solution was then centrifuged and the supernatant store in ice. 0.2ml of each solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water. 0.5ml of Folinciocateau reagent was added to both sample and standard followed by 2.5ml of 20% Na$_2$CO$_3$ the solution were then vortexed and allow to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared [4].

**Saponin Determination:** The spectrophotometric method of [5] was used for Saponin determination. 2g of the finely ground sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol was added. Shaker was used to shake the mixture for 5hours to ensure uniform mixing. The mixture was filtered with No 1 Whatman filter paper to obtain a clean colourless solution. 1ml of the colourless solution was taken into 50ml volumetric flask using pipette, 2ml of 5% iron (iii) chloride (FeCl$_3$) solution was added and made up to the mark with distill water. It was allowed to stand for 30minutes for the colour to develop. The absorbance was read against the blank at 380nm.

**Alkaloid Determination:** 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4min. This was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is then alkaloid which was dried and weighed [6].

$$\%\text{alkaloid} = \frac{W_3-W_2}{W_1} \times 100$$

**Cardiac Glycoside Determination:** The procedure described by [7] was used 10ml the extract pipetted into a 250ml conical flask. 50ml chloroform was added and shaken on vortex mixer for 1hour. The mixture was filtered into 100ml conical flask. 10ml of pyridine and 2ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10min. 3ml of 20% NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin). A concentration which range from 0 – 50mg/ml were prepared from stock solution the absorbance was read at 510nm.

**Total Flavonoid Content:** Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1ml of sample plant extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) [8].

**RESULTS**

**Statistical Analysis:** This was done using T-test (Package =R studio).

Data 1: FLAVONOID (Leaf)and FLAVONOID (Flower)  
\[ t - \text{test} = 11.9333, \text{degree of freedom} = 1, \text{probability -value} = 0.05322 \]

Alternative hypothesis: True Difference in Means is not equal to 0  
95 percent confidence interval: -0.05796536(leaf): 1.847965369 (flower)  
Sample Estimates:  
Mean (leaf): mean (flower): 6.730mg/g: 5.835 mg/g
Fig. 1: Showing graph of the mean values of phytochemicals of leaf

Fig. 2: Showing graph of the mean values of phytochemicals of flower

Fig. 3: Showing comparison of the mean of phytochemicals of leaf and flower

Table 1: Showing phytochemical screening of *cassia podocarpa*

<table>
<thead>
<tr>
<th>Part of the plant</th>
<th>Flavonoid</th>
<th>Alkaloid</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Cardiac Glycoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flower</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Data 2: 
TANIN (Leaf) and TANIN (Flower) 
t-test = 235.4743, degree of freedom = 1.145, probability value = 0.001266 
95 percent confidence interval: 4.682361 (leaf): 5.074509 (flower) 
Alternative Hypothesis: True Difference in means is not equal to 0
Sample Estimates: 
Mean (leaf): Mean (flower): 10.11843 mg/g: 5.24000 mg/g
Data 3: SAPONIN (Leaf) and SAPONIN (Flower)
t-test = 35.0566, degree of freedom = 2, probability -value = 0.0008132
Alternative Hypothesis: True Difference in Means is not equal to 0
95 percent confidence interval: 11.80225(leaf): 15.10502 (flower)
Sample Estimates:
Mean (leaf): Mean (flower): 27.36364mg/g: 13.91000mg/g

Data 4: ALKALOID (Leaf) and ALKALOID (Flower)
t-test= 36.0159, degree of freedom = 1.162, probability -value = 0.01028
Alternative Hypothesis: True Difference in means is not equal to 0
95 percent confidence interval:9.762957(leaf):16.457043 (flower)
sample estimates:
Mean (leaf): Mean (flower) 19.70mg/g: 6.59mg/g

Data 5 GLYCOSIDE(Leaf) and GLYCOSIDE (Flower)
t –test = 26.1119, degree of freedom = 1, probability -value = 0.02437
Alternative Hypothesis: True Difference in means is not equal to 0
95 percent confidence interval:0.4021699(leaf): 1.1645421
Sample Estimates:
Mean (leaf): Mean(flower) 7.103356 mg/g: 6.320000mg/g

DISCUSSION

Phytochemical analysis carried out on the cassia podocarpa ethanolic extract showed presence of constituents; flavonoid, tannin, saponin, alkaloid and glycoside which are known to exhibit medicinal and pharmacological activities. From the analysis, it showed that flavonoid content in the leaf (6.73mg/g) was more than that of flower (5.83mg/g). Also, the leaf had more tannin (10.12mg/g) than the flower(5.24mg/g). The saponin content of the leaf (27.36mg/g) was more than the flower (13.91mg/g).Alkaloid content of the leaf (19.70mg/g) was higher than that of flower (6.59mg/g). The Cardiac glycoside in the leaf (7.10mg/g) was higher than the flower content (6.32mg/g). However, the presence of these metabolites is an indication that the plant both the leaf and the flower is a reservoir of novel and lead compounds. Tanninbind to proline rich protein and interfere with protein synthesis. Saponin was higher in the leaf, it has been reported that saponins are known to produce inhibitory effect on inflammation [9]. Saponin has the ability to precipitate and coagulate the red blood cells. Alkaloids have also been associated with medicinal uses and one of their common biological properties is cytotoxicity. Many workers have reported the analgesic [10, 6], antispasmodic and antibacterial properties of alkaloid [11, 12].

CONCLUSION

The analysis results have shown that the plant cassia podocarpa has identified phytochemical compounds which may be the bioactive constituents. If the plant is subjected to very intense research, novel and lead bioactive compounds may be isolated, characterized using various techniques such as NMR, Mass -spectrophotometer, IR and novel compounds may be revealed.

REFERENCES