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# Proximate Composition, Phytonutrient Analysis and Antimicrobial Activity of Ethanolic Extracts of the Young and Mature Leaves of *Greenwayodendron suaveolens*

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**ABSTRACTS:** The antimicrobial activity, proximate composition and phytonutrient contents of the ethanolic extracts of the young and mature leaves of *Greenwayodendron suaveolens* were analyzed using standard microbiological methods. The moisture contents of the young and mature leaves were 9.9% and 11.882% respectively. The ash contents of the young and mature leaves were 6.142% and 6.418% respectively. The crude fiber content of the young and mature leaves was 5.550% and 5.080% respectively. The crude fat content of the young and mature leaves was 14.750% and 18.870% respectively. The crude protein content of the young and mature leaves was 1.338% and 1.025% respectively. The carbohydrate content of the young and mature leaves was 72.220% and 68.607% respectively. The phytonutrient analysis carried out indicated that percentage alkaloid was 24.43 and 11.45 in the young and mature leaf extracts respectively. Phenolic contents were 64.00 µg/mL and 51.00 µg/mL in the young and mature leaf extracts respectively. Flavonoid content was 39.50 µg/mL and 13.75 µg/mL in the young and mature leaf extracts at respectively. Saponin content at 1,147 µg/mL and 1,205 µg/mL in the young and mature leaf extracts respectively. While tannin content was 65.00 mg/mL and 47.25 mg/mL in the young and mature leaf extracts at respectively. The ethanolic leaf extracts of *Greenwayodendron suaveolens* inhibited the growth of all the test organisms at the concentration of 25 mg/mL. However, the minimum inhibitory concentration (MIC) of the young and mature leaf extracts against *Bacillus subtilis* and *Trichodermaharzianum* was 6.25 mg/mL while the minimum inhibitory concentration (MIC) of the young leaf extract against *Acinobactercalcoaceticus* and *Candida albican* was also 6.25 mg/mL. The minimum inhibitory concentration (MIC) of the young and mature leaf extracts against *Aspergillus tamari* and *Aspergillus niger* was 12.5 mg/mL. The antimicrobial results indicated that the ethanolic leaf extracts of *Greenwayodendron suaveolens* are active against bacteria and fungi and therefore can be utilized to remedy infections caused by the test organisms.

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*Greenwayodendron suaveolens* is member of the Annonaceae family and species of plant in the genus *Greenwayodendron*. It is a deciduous tree circumscribed to Tropical Africa and is mostly found in Southern Nigeria, Western Uganda, Northern Tanzania, Southern Democratic Republic of Congo and Cabinda in Angola (Odaro and Sobotie, 2019). The mature tree is usually medium sized to large sized-up to 35–45 meters tall (Idu *et al.*, 2017). Some of its Nigerian native names include: ewai (Edo), eleku (Isekiri), Osharo (Urhobo), Agudugbu (Yoruba). (Odaro and Sobotie, 2019). The leaves of *Greenwayodendron suaveolens* slightly vary in shape but usually have a narrow elliptic shape with an elongated leaf tip and are mostly glabrous. Ajayi *et al.* (2013) reported that the leaf of *Greenwayodendron suaveolens* along with its root and bark is used in traditional medicine for the treatment of fever, rheumatic pains, oedema, swollen glands, headache, stomach ache, constipation, hernia, facilitation of child birth, fertility and as anthelmintic and aphrodisiac. The seed extract of *Greenwayodendron suaveolens* has antimicrobial activities on *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* (Idu *et al.*, 2017). This study is aimed at analyzing the antimicrobial activity, proximate composition and phytonutrient contents of young and mature leaves of *Greenwayodendron suaveolens*.

## MATERIALS AND METHODS

*Sample Collection and Preparation*: The young and mature leaves of *Greenwayodendron suaveolens* were collected from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. The young leaves were collected towards the tip of the branches while the mature leaves were collected towards the base of the branches. The sample was authenticated by Dr. E. O. Oshomoh

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(Associate Professor) of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City. The young and old leaves were weighed and air-dried separately in the Phytomedicine and Drug Discovery Laboratory in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City. They was pulverized and weighed with a standard weighing balance. 1 kg each of the dried samples was soaked in 1.5 L of ethanol and was left to stand for four (4) days. They were then filtered and the filtrate were decocted and transferred into air-tight containers and stored in the refrigerator.

*Proximate Analysis:* The moisture content, ash content, crude fiber, crude fat, crude protein and carbohydrate contents of the young and mature leaves of *Greenwayodendron suaveolens* was analyzed following the methods outlined by the Association of Official Agricultural Chemists (AOAC 1997).

*Phytochemical Analysis:* Quantitative analysis for alkaloids, phenolics, flavonoid, saponin and tannin was carried out on the young and mature leaves of *Greenwayodendron suaveolens* following the methods described by Ejikeme *et al.* (2014).

*Experimental Microorganisms: Staphylococcus aereus, Candida albicans, Mucormucedo* and *Penicilium chrysogenum* were obtained from the Pharmaceutical Microbiobiology Laboratory, Faculty of Pharmacy; *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* were obtained from Microbiology Laboratory, Faculty of Life Sciences; *Providencia rettgeri*, *Arthrobacter globiformis*, *Acinetobactercal coaceticus*, were obtained from Science Laboratory Technology Laboratory, Faculty of Life Sciences; *Aspergillus tamari*, *Aspergillus niger*, *Trichoderma harzianum* and *Escherichia coli* were collected from the Plant Biology and Biotechnology Laboratory, Faculty of Life Sciences, all in the University of Benin, Benin City. Each of these microorganisms was already identified by the various laboratory technologists/scientist before collection. The microorganisms were stored in the refrigerator.

*Preparation of Extract Concentrations:* 0.5 g each of both young and mature leaf extracts was weighed into different sterile beakers containing 10 ml of ethanol which were taken as the stock concentrations. Further concentrations were made from the stock concentration by double serial dilutions; for each extract, 5 ml of the stock concentration was transferred into a test tube containing 5 ml of ethanol to get 25 mg/ml of the extracts. 5 ml was transferred from the 25 mg/ml concentrations into test tubes containing 5 ml of ethanol to obtain 12.5 mg/ml of the samples. 5 ml was transferred from the 12.5 mg/ml concentrations into test tubes containing 5 ml of ethanol to obtain 6.25 mg/ml of the samples. Finally, 5 ml was transferred from the 6.25 mg/ml concentrations into test tubes containing 5 ml of ethanol to obtain 3.125 mg/ml of the samples.

*Antimicrobial Plating:* Antimicrobial plating was carried out as described by Elek and Hilson (1954). The Inhibition Zone Diameter (IZD) was measured after incubation using a transparent meter rule.

## RESULTS AND DISCUSION

*Proximate Composition:* The young leaves are slightly higher in crude fiber, crude protein and carbohydrate while the mature leaves are higher in moisture content, ash content and crude fat (Table 1).

Table 1: Proximate composition of the young and mature leaves of *Greenwayodendron suaveolens.*

| Parameters | Young Leaves | Mature Leaves |
|---|---|---|
| Moisture Content (%) | 09.98±0.290 | 11.88±0.003 |
| Ash Content (%) | 06.14±0.001 | 06.42±0.002 |
| Crude Fiber (%) | 05.55±0.012 | 05.08±0.012 |
| Crude Fat (%) | 14.75±0.012 | 18.87±0.012 |
| Crude Protein (%) | 01.34±0.001 | 1.023±0.001 |
| Carbohydrate (%) | 72.27±0.024 | 68.61±0.060 |

*Values are represented as mean ± S.E.M. where n = 3.*

The mature leaves have higher moisture content (11.882±0.003%) than the young leaf leaves (9.980±0.001%). Since high moisture content is favorable for microbial attack and is also an index of spoilage, the mature leaves tend to spoil faster than the young leaves. The ash content of the young leaves was slightly lower which implies that minerals establish as the leaf mature and so the mature leaves will provide more minerals for animals when used as fodder (Bamishaiye, 2011). The carbohydrate content is very high in both young (72.27±0.024) and mature (68.61±0.06) leaves though there is a slight decrease in the mature leaves. This can be related to the findings of Bamishaiye *et al.* (2011) who analyzed the proximate composition of the leaves of *Moringa oleifera* and reported that the carbohydrate content showed a slight decrease as the leaves mature. There was no significant difference in the crude fiber content of the young (5.55±0.012) and mature (5.08±00.012) leaves of *Greenwayodendron suaveolens* though the young leaves have a slightly higher fiber content. *Greenwayodendron suaveolens* has higher crude fiber content than *Cnidoscolus chayamansa* (0.92%), *Solanium nodiflorum* (0.78%), *Seneciobiafrae* (0.92%) and lower crude fiber content compare with *Acalyphahispada* (10.25%,), *Acalyphara cemosa* (7.20%) and *Acalypha marginata* (11.50%) as reported by Bamishaiye *et al.* (2011). The young leaves are higher in crude protein while the mature leaves are higher in crude fat.

*Phytonutrient Contents:* The quantitative analysis of phytonutrient contents of the young and mature leaves of *Greenwayodendron suaveolens* show that they contain alkaloid, phenolics, flavonoid, saponins and tannin (Table 2).

**Table 2:** Phytonutrient analysis of the young and mature leaves of *Greenwayodendron suaveolens*.

| Parameter | Young Leaves | Mature leave |
|---|---|---|
| Total Alkaloids (%) | 24.41±0.34 | 11.66±0.14 |
| Total Phenolics (µg/mL) | 63.97±0.49 | 51.57±0.35 |
| Total Flavonoid (µg/mL) | 38.50±0.51 | 14.15±0.33 |
| Total Saponin (µg/mL) | 1150.67±4.7 | 1221.0±9.24 |
| Total Tannin (µg/mL) | 65.77±0.41 | 47.58±0.22 |

Values are represented as mean ± S.E.M. where n = 3.

However, phytonutrient analysis carried out by Odaro and Sobotie (2019) reported that saponins, phenols and flavonoids are present in the aqueous leaf extract of *Greenwayodendronsuaveolens*, but alkaloid was not detected contrary to the result of phytonutrient analysis in Table 2. This could owe to the fact that the content and level of phytochemicals and macronutrients in plants can be affected by the various factors as like varieties, climatic conditions, cultural practices, and level of maturity at harvest and storage conditions (Jannatul *et al.*, 2020). Odaro and Sobotie (2019) made use of mature leaves and this can be related to the much lower alkaloid content (11.45±0.04%) as shown in Table 2. The young leaf extract of *Greenwayodendron suaveolens* contains all the phytonutrients in higher proportion than the mature leaf extract except saponin which is higher in mature leaf extracts (Table 2). This result is supported by the result of analysis conducted on the different developmental stages of *Clausena lansium* leaf by Chang *et al.* (2018). The total and free phenolic content of leaf buds, young leaves, mature leaves and old leaves of *Clausena lansium* were analyzed and it showed a significant decreased as the leaves got older.

*Antimicrobial Analysis:* The results of antimicrobial analysis show that the test bacteria and fungi were all susceptible to the ethanolic extracts of *Greenwayodendron suaveolens* at the concentration of 25 mg/mL (Table 3).

**Table 3:** The antimicrobial activity of the ethanolic extracts of young and mature leaves of *Greenwayodendron suaveolens* against bacteria isolates in comparison with ciprofloxacin (positive control) and water (negative control).

| Organisms | 25 mg/mL (A) | 25 mg/mL (B) | 12.5 mg/mL (A) | 12.5 mg/mL (B) | 6.25 mg/mL (A) | 6.25 mg/mL (B) | 3.125 mg/mL (A) | 3.125 mg/mL (B) | Cipro-floxacin | Water |
|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | 9.7±1.76 | 10.7±1.20 | 6.3±0.88 | 4.3±0.88 | 4.0±0.5 | 1.7±0.67 | 2.0±0.5 | 0.3±0.3 | 9.7±0.33 | 0.0±0.0 |
| *P aeruginosa* | 8.3±0.88 | 8.7±o.33 | 5.7±0.88 | 5.0±1.00 | 3.3±0.8 | 5.0±0.58 | 0.7±0.3 | 1.0±0.5 | 14.7±0.33 | 0.0±0.0 |
| *B. subtilis* | 9.3±0.88 | 11.7±0.67 | 5.7±0.33 | 6.7±1.20 | 3.3±0.3 | 2.0±0.58 | 0.0±0.0 | 0.0±0.0 | 7.0±0.58 | 0.0±0.0 |
| *E.faecalis* | 6.0±1.53 | 6.7±1.33 | 5.7±0.88 | 7.0±0.58 | 4.7±0.8 | 4.3±1.45 | 2.0±0.0 | 1.7±0.3 | 3.7±0.33 | 0.0±0.0 |
| *M. luteus* | 10.3±1.33 | 13.3±0.67 | 6.7±1.20 | 6.3±2.19 | 4.0±1.1 | 4.3±1.20 | 0.7±0.3 | 1.7±0.3 | 5.7±0.33 | 0.0±0.0 |
| *K. pneumonia* | 13.3±0.33 | 11.3±2.73 | 11.3±0.33 | 7.3±1.45 | 5.7±0.8 | 5.7±0.88 | 1.0±0.5 | 4.0±0.5 | 10.0±0.58 | 0.0±0.0 |
| *A. calcoaceticus* | 5.3±0.88 | 9.3±0.88 | 3.0±0.58 | 7.0±1.16 | 2.7±0.3 | 4.3±0.88 | 0.0±0.0 | 3.0±1.0 | 0.7±0.33 | 0.0±0.0 |
| *A. globiformis* | 5.0±0.58 | 7.0±0.58 | 4.7±0.88 | 6.0±0.58 | 2.3±0.8 | 4.3±0.67 | 0.3±0.3 | 2.7±0.8 | 7.3±0.88 | 0.0±0.0 |
| *P. rettgeri* | 9.7±1.20 | 16.0±1.16 | 6.7±0.88 | 14.0±0.58 | 5.0±0.5 | 12.3±0.33 | 1.3±0.3 | 6.3±0.8 | 4.0±0.58 | 0.0±0.0 |
| *S.aureus* | 9.0±0.58 | 16.7±1.20 | 5.0±0.58 | 9.7±1.45 | 3.3±0.8 | 4.7±0.67 | 1.7±0.6 | 2.0±0.5 | 13.0±0.58 | 0.0±0.0 |

*Values are represented as mean ± S.E.M. where n = 3; A = young leaf; B = mature leaf.*

**Table 3:** The antimicrobial activity of the ethanolic extracts of young and mature leaves of *Greenwayodendron suaveolens* against fungi isolates in comparison with ciprofloxacin (positive control) and water (negative control).

| Organisms | 25 mg/mL (A) | 25 mg/mL (A) | 12.5 mg/mL (B) | 12.5 mg/mL (B) | 6.25 mg/mL (A) | 6.25 mg/mL (A) | 3.125 mg/mL (B) | 3.125 mg/mL | Metro-nidazole | Water |
|---|---|---|---|---|---|---|---|---|---|---|
| *P.chrysogenum* | 10.3±0.88 | 9.7±0.88 | 5.7±0.88 | 6.0±1.73 | 1.7±0.33 | 4.7±1.20 | 0.7±0.33 | 2.7±0.33 | 11.0±1.00 | 0.0±0.00 |
| *M. mucedo* | 12.3±0.88 | 14.3±2.40 | 6.7±0.88 | 14.0±2.31 | 5.7±0.33 | 8.0±0.58 | 3.0±0.00 | 4.3±0.88 | 12.3±0.88 | 0.0±0.00 |
| *A. niger* | 3.7±0.88 | 3.3±0.88 | 1.7±1.20 | 0.7±0.33 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 6.7±0.88 | 0.0±0.00 |
| *A. tamari* | 4.7±0.67 | 5.3±1.20 | 1.7±0.88 | 2.0±0.58 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 10.7±0.88 | 0.0±0.00 |
| *T. harzianum* | 4.3±0.88 | 6.3±1.20 | 1.3±0.67 | 4.7±0.88 | 0.3±0.33 | 3.7±0.88 | 0.0±0.00 | 0.0±0.00 | 10.3±0.88 | 0.0±0.00 |
| *C. albicans* | 2.0±0.58 | 3.0±0.56 | 1.0±0.00 | 2.0±0.58 | 0.0±0.00 | 0.7±0.33 | 0.0±0.00 | 0.0±0.00 | 9.0±1.53 | 0.0±0.00 |

*Values are represented as mean ± S.E.M. where n = 3; A = young leaf; B = mature leaf.*

Idu *et al.* (2017) also reported that the aqueous and chloroform seed extract of *Greenwayodendron suaveolens* were remarkably active against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. At the concentration of 3.125 mg/mL, the extracts were not able to inhibit the growth of most of the bacteria (Table 3). At the concentration of 6.25 mg/mL and below, the extracts were not able to inhibit the growth of most of the fungi (Table 4). This trend could be attributed to the presence of phytochemicals such as alkaloids, saponins, tannin, flavonoids and phenolics (Idu *et al.*, 2017). Aruoma (2003) reported that mixtures of such chemicals have been known to exhibit a broad spectrum of biological effects and pharmacological properties. The bacteria are more susceptible to the mature leaf extract than the young leaf extract. The young and mature leaves of *Greenwayodendron suaveolens* were active against *Escherichia coli* and are therefore conceivable that these extracts can be used in medicine to treat cases of diarrhea caused by *Escherichia coli* (Ellis *et al.*, 2020). *Klebsiella pneumoniae* showed one of the highest susceptibility to these extracts. This can indicate the possibility of treating infections such as pneumonia, blood stream

infections, wounds and surgical site infection which are caused by *Klebsiella pneumonia* with extracts of young and mature leaves of *Greenwayodendron suaveolens*.

Idu *et al.* (2017) revealed that the seed extracts of *Greenwayodendron suaveolens* were active against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. However, *Aspergillus niger* and *Candidaalbicans* were not susceptible to the aqueous extract of *Greenwayodendron suaveolens* seeds. This shows some conformation with the relatively low susceptibility of these two organisms to the leaf extracts of *Greenwayodendron suaveolens* (Table 4).

*Conclusion:* The results of this work indicate that the young and mature leaf extracts of *Greenwayodendron suaveolens* possess phytochemicals which are effective against the test organisms used. This shows that these phytochemicals can be utilized in treating infections caused by these test organisms. The young leaves of *Greenwayodendron suaveolens* are relatively richer in nutrients while the mature leaves prove to have better antimicrobial properties than the young leaves.

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