ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF
SABA FLORIDA (BENTH) EXTRACTS

Omale James \(^1\) Enemuor Simeon Chukwuemeka \(^2\) and Hussaini Elssie Eyenetu \(^1\)

1. Department of Biochemistry, Kogi State University Anyigba, Nigeria.
2. Department of Microbiology, Kogi State University, Anyigba, Nigeria.

Email: jamesomale123@yahoo.com, Tel: +23408068291727

ABSTRACT

In this study in vitro antibacterial and in vivo antioxidant activity of Saba florida parts (leaf, pulp and pericarp) extracts were investigated following standard methods. The pericarp and the pulp extracts had greater antibacterial activities compared to the leaf extract at 200 and 400mg/ml. All the extracts showed no antibacterial activity against Shigella dysentri at both 200 and 400mg/ml concentrations respectively. The minimum inhibitory concentration (MIC) of the extracts ranged from 200 to 400mg/ml. The minimum bactericidal concentration (MBC) of the extracts was 400mg/ml for two isolates. The extracts were bacteriostatic against the other isolates. The extracts at the dose of 200mg/kg body weight, administered orally once daily, demonstrated antioxidant activity. The antioxidant activity of the extracts increased in the following order: Pulp < leaf < pericarp. In conclusion, the present investigation indicated that Saba florida parts are potential sources of natural antioxidant and antimicrobial agents and therefore are potential drug candidates that require further study and development. These activities justified the ethno medical uses of the plant.

Key words: Saba florida, Antibacterial activity, Antioxidant activity, in vivo and in vitro

1. INTRODUCTION

Many plants contain a variety of useful compounds that have not yet been identified. Natural plant resources that contain antimicrobial and antioxidant effects have been extensively studied and utilized as additive agents in food \([1, 2]\). Therefore, the development and commercialization of novel functional compounds derived from these plants must be pursued to improve the functionality and safety of foods. The application of natural ingredients containing antioxidants and antibiotics may prove useful \([3]\). These antioxidants and antibiotics derived from natural resources are perceived by consumers as being better and safer than synthetics \([4]\).

In addition to providing additional taste and flavor to foods, certain spices have been used as remedies in traditional medicine for centuries \([5]\). Oxidative stress, the consequence of the imbalance between pro oxidants and antioxidants in an organism is considered to play a very important role in the pathogenesis of several degenerative diseases, such as diabetes, cancer, and cardiovascular diseases, including atherosclerosis.

Reactive oxygen species (ROS) including hydroxyl radicals, superoxide radicals and singlet oxygen, as well as reactive nitrogen species, are continuously generated in the cell, as a result of natural human metabolism and can be harmful as they can attack biological macromolecules, cause membrane and DNA damage and enzyme inactivation. The mechanism by which free radical interfere with cellular functions are not yet fully understood, but one of the most important process seems to be the formation of lipid hydro peroxides \([6]\).

Antioxidants can protect the human body from free radical and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation. Hence, a need
has appeared to identify alternative natural and safer sources of food antioxidants and the search for natural antioxidants especially of plants origin, has notably increased in recent years. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation and they act by inhibiting the initiation and propagation steps, leading to the termination of the reaction and delaying the oxidation process [7, 8]. At present, the most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluence (BHT), Propylgallate and tert-butylhydroquinone. However, BHA and BHT have been restricted by legislative rules due to doubts over their toxic and carcinogenic effects.

Therefore, there is a growing interest in natural and safer antioxidants for food applications and a growing trend in consumer preference towards natural antioxidants, all of which have given impetus to the attempts, to explore natural sources of antioxidants [9].

Recently, there is an increasing interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidants in plants are capable of terminating a free radical mediated oxidative reaction and would have beneficial activities in protecting the human body from such diseases [10, 11].

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics, carrying out research to better understand the genetic mechanisms of resistance, and continuing investigations aiming at the development of synthetic or natural new drugs. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

Saba florida belongs to the family Apocynaceae. The plant is very abundant in undisturbed forest, coastal areas and around great Lake regions of Africa from sea level to 12 50 M [12] but are in open areas. The plant is found in Ibaji and other parts of Kogi State, Nigeria. The fruit is edible and it makes a refreshing sour drink.

The stem yield latex that is an inferior rubber. Traditionally, barks decoctions are used to treat rheumatism. The leaves are used in Senegal to prepare sauces and condiments as salty appetizer. In cote d, I Voire, the latex is used as an adhesive for poison preparation for arrows as it hardens upon exposure. The inferior rubber, produced from the latex is sometimes used to adulterate genuine rubber [13].

In Kogi Sate, the latex is used as trap for birds, rats and smaller rodents. The leaves are also used in the treatment of skin ulcer, children’s headache and catarrhs. The fruit is a special delicacy for the monkeys in the forest and humans are beginning to compete with monkeys for the fruit. It appears in the local markets during the fruiting season. It is called hunters lunch fruit [14].

The aim of this study therefore, was to evaluate the antibacterial and antioxidant activities of the parts of Saba florida (Berth) commonly used as food and medicine in our local communities.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of plant sample
The plant samples were collected from Igboigbo- Unale in Ibaji Local Government Area, eastern part of Kogi State, Nigeria, during the rainy season. Dirt was removed from the plants by rinsing in water. The leaves were air-dried for three weeks and pulverize using motorized blender. The fruit
pulp and pericarp were dried in oven at 40 °C and pulverized into powder.

2.2. Plant Identification
The plant was identified in the Botany unit of the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria as *Saba florida* (Benth) and a sample was deposited in the herbarium.

2.3. Preparation of Extracts
Cold extraction method was followed. Portions (30g) of the powdered samples were weighed into 500ml conical flasks and 200ml of pure methanol (99.9%) was added and left for 72 hours. The mixtures were filtered under vacuum pressure and the filtrates were concentrated using rotary evaporator (Wheaton, England). The semi-solid extracts obtained were further evaporated in an oven at 110 °C until constant weight was obtained for each extract.

2.4. Preparation of Stock Solutions
Each solid extract was constituted using 0.25% dimethyl sulphoxide (DMSO) to obtain a stock solution of 200 and 400mg/ml concentrations. These stock solutions obtained were filtered using millipore membrane filter (0.45um pore size). They were then stored in sterile capped bottles.

2.5. Test Organisms and Source
The test organisms used were obtained from the Bacteriology Department of the National Veterinary Research Institute, Vom, Plateau State, Nigeria. They include: *Escherichia coli*, *Shigella dysenteri Salmonella typhi*, *Yersinia enterocolitica* and *Pseudomonas aeruginosa*.

2.6. Preparation and Standardization of Inoculums
Colonies of each organism used were picked into 10mls of sterile peptone water (nutrient broth) and incubated at 37°C for 24 hours for bacterial proliferation. The turbidity produced was adjusted to match 0.5McFarland standard [15].

2.7. Test for Antibacterial Activity of the Plant Extracts
The agar well diffusion method was employed for testing antibacterial activity of the plant extracts [16]. Sterile nutrient agar plates were prepared. A sterile cork borer of 3mm was used to bore holes on the nutrient agar plates. The holes or wells were flooded with 3-4 hours old cultures of the test organisms. A portion (0.1ml) of the extracts was introduced into the wells appropriately. Gentamicin was used as a positive control or standard. The plates were incubated at 37°C for 24 hours. The antibacterial activity of the plant extracts were determined by measuring the diameter of the inhibition zones in millimeter (mm).

2.8. Determination of Minimum Inhibitory Concentration (MIC)
Broth dilution method as described by [15] was used. A portion (9ml) of nutrient broth was suspended in test tubes (ten for each of the extracts that produced inhibition zones). A portion (1ml) of the extract was added to the first tube and shaken thoroughly. 1ml was taken from the first test tube and introduced into the second test tube and mixed thoroughly. This was done for all the test tubes. 1ml was taken from the last test tube (10th) and discarded. The test tubes were incubated at 37°C for 24 hour. The test organism without extracts served as control. The test tube which did not show turbidity or growth after 24 hours of incubation was taken and recorded as the minimum inhibitory concentration (MIC). This indicates the minimum concentration at which the extracts were able to inhibit the growth of the test organisms.
2.9. Determination of Minimum Bactericidal Concentration (MBC)

A portion (1ml) sample from the test tubes used in the determination of MIC which did not show any visible growth after the 24 hour period of incubation were sub-cultured into nutrient agar plates and incubated for 24 hours to determine the minimum concentration of the extract required to kill the organisms. These concentrations were indicated by the failure of the test organism to grow on subsequent transfer to NA. The lowest concentration of extract indicating a bactericidal effect after 24 hours of aerobic incubation was regarded as the minimum bactericidal concentration (MBC).

2.10. Animal Grouping and Extract Administration

Thirty two (32) albino rats of average weight of 86.4g were divided into eight groups of four rats; with group 1 and 2 serving as controls.

- Group 1 animals were administered a single dose of water (0.1ml) for five days and received liquid paraffin on day 2 and 3.
- Group 2 animals were administered 0.2ml carbon tetrachloride (CCl4) solution (CCl4: paraffin, 1:1).
- Group 3 received 200mg/kg b.w of Saba florida leaf extract and 0.2ml of CCl4 after 30 minutes of extract administration.
- Group 4 received 200mg/kg b.w of the leaf extract alone.
- Group 5 received 200mg/kg b.w of the pulp extract and 0.2ml of CCl4 after 30 minutes of extract administration.
- Group 6 animals received the pulp extract of 200mg/kg b.w alone.
- Group 7 animals were administered 200mg/kg b.w of percarp extract and 0.2ml of CCl4 after 30 minutes of extract administration.
- Group 8 animals were given 200mg/kg b.w of pericarp extract alone.

All extracts were administered orally for five days. CCl4 was administered intraperitoneally (I.P). On the sixth day, the animals were subjected to mild chloroform anesthesia and dissected. The whole blood was collected via cardiac puncture and stored in EDTA bottle.

2.11. Glutathione Peroxidase Activity Assay

The glutathione peroxidase activity was determined using the UV method as described by [17]. A mixture containing 0.05ml of diluted EDTA whole blood and 0.2ml of reagent was added 0.10ml of cumene. The reagent blank was prepared as above except that the diluted EDTA whole blood was omitted and 0.05ml of distilled water added instead. The initial absorbance of sample and reagent blank was read after one minute and a subsequent absorbance was read after one and two minutes at 340nm.

Lutathione peroxidase concentration was then calculated from the formula.

\[ \text{U/L of Hemolysate} = 84 \times \frac{12 \times \text{change in absorbance at 340 nm}}{\text{minute}} \]

2.12. Statistical Analysis

All data were expressed as mean ± SEM and Graph pad Instat (Data set 1.15D) was applied.

3. RESULTS AND DISCUSSION

As presented in table 1, the pericarp and the pulp extracts exhibited greater antibacterial activity as compared to the leaf extract, judging from the zones of inhibition produced against the isolates. The leaf extract had the least antibacterial activity against the test organisms. Inhibitory zone was produced only for salmonella typhi at 400 mg/ml. The pericarp extract showed the
greatest antibacterial activity against the isolates except *Shigella dysentri*.
All the extracts demonstrated no antibacterial activity against *Shigella
dysentri* at the different concentrations tested.

The result of the minimum inhibitory concentration determination is as presented in table 2. The pericarp extract inhibited the growth of *Pseudomonas aeruginosa* at 200 and 400 mg/ml whereas other isolates growth were inhibited at 400mg/ml. The leaf extract inhibited the growth of *Salmonella typhi* at a minimum concentration of 400 mg/ml. Similarly, pulp extract exhibited antibacterial activity with MIC at 400 mg/ml for the isolates except *Yersinia enterocolitica* and *Shigella dysentri* where growth was not inhibited at all. The fruit pericarp extract was more effective against the isolates with MIC of 200mg/ml against *Pseudomonas aeruginosa*.

The MBC of the extracts are as present in table 3. At 400mg/ml, the pericarp extract demonstrated bactericidal activity against *Pseudomonas aeruginosa* and *Escherichia coli*, similarly the pulp extract exhibited a killing effect against *Pseudomonas aeruginosa*. The leaf extract demonstrated no killing effect at all.

Table 3 represents the results of the effects of the crude extracts of *S. florida* on the activity of an antioxidant enzyme – glutathione peroxidase. In this present study, the pericarp and the leaf extract demonstrated antioxidant activity *in vivo* whereas the pulp exhibited weak antioxidant activity. This can be seen from the effect of the extracts on glutathione peroxidase activity when compared with the control groups (1 and 2) (Table 3).

The antioxidant activity of the extracts increased in the following order:-
Pulp -> Leaf - > Pericarp.

The fruit pericarp possessed the highest antioxidant effect, the mechanism by which the extract increased the activity of the antioxidant enzyme is not clear. It could probably induce the activity of the enzyme *in vivo* or contain the enzyme. *In vitro* experiment demonstrated also that the fruit pericarp possessed the highest antioxidant activity [18]. Thus, *S. florida* extracts are potential drug candidates for mopping up free radicals and could be used in preventing free radical mediated diseases.

*S. florida* extracts demonstrated antibacterial activity against the tested isolates. This is in consonance with the findings of [19, 20] using different plant extracts. The inhibitory activity of the plant extracts could be due to the presence of some bioactive compounds such as alkaloids, flavonoids, tannins, saponins [21]. Tannins are secondary metabolites known for their antimicrobial activities. Alkaloids found in higher plants are also known to exhibit marked physiological activities. [22]. In general, the pericarp extract produced the highest antibacterial activity and the leaf extract had the least activity against the tested isolates.

All the extracts showed no activity against *Shigella dysentri* at the tested concentrations. Thus, at a higher concentration of the extracts, antibacterial activity may be produced against this organism. The strong antibacterial activity of the pericarp and pulp extracts on *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* supports the ethnomedicinal uses of the plant for the treatment of diseases such as typhoid fever, dysentery, and diarrhea and also in the management of wound infections.
Table 1:
Antibacterial activity of the extracts of *S. florida* parts

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Pericarp (mg/ml)</th>
<th>Leaf (mg/ml)</th>
<th>Pulp (mg/ml)</th>
<th>Gentamicin (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>15</td>
<td>17</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>9</td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>7</td>
<td>12</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td><em>S. dysentri</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- No zone of inhibition observed

Table 2:
Minimum Inhibitory Concentration (MIC) of the crude extracts of *S. florida* on the isolates

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Pericarp (mg/ml)</th>
<th>Leaf (mg/ml)</th>
<th>Pulp (mg/ml)</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>200</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>+</td>
<td>-</td>
<td>400</td>
<td>-</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>+</td>
<td>-</td>
<td>400</td>
<td>+</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td><em>S. dysentri</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- No growth observed, + Growth observed, * No MIC recorded

Table 3:
Minimum Bactericidal Concentration (MBC) of the crude methanol extracts of *S. florida*

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Pericarp (mg/ml)</th>
<th>Leaf (mg/ml)</th>
<th>Pulp (mg/ml)</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>400</td>
<td>NIL</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td></td>
</tr>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>400</td>
<td>NIL</td>
<td>NIL</td>
<td></td>
</tr>
</tbody>
</table>

NIL = No killing effect observed

Table 4:
Effect of *S. florida* extracts on Glutathione Peroxidase activity in albino rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group of rats</th>
<th>Glutathione peroxidase activity (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>290.14±0.001</td>
</tr>
<tr>
<td>2.</td>
<td>CCl₄</td>
<td>351.80±0.011</td>
</tr>
<tr>
<td>3.</td>
<td>Leaf + CCl₄</td>
<td>353.28±0.002</td>
</tr>
<tr>
<td>4.</td>
<td>Leaf</td>
<td>360.34±0.021</td>
</tr>
<tr>
<td>5.</td>
<td>Pulp + CCl₄</td>
<td>337.21±0.002</td>
</tr>
<tr>
<td>6.</td>
<td>Pulp</td>
<td>348.57±0.010</td>
</tr>
<tr>
<td>7.</td>
<td>Pericarp + CCl₄</td>
<td>375.75±0.020</td>
</tr>
<tr>
<td>8.</td>
<td>Pericarp</td>
<td>378.99±0.012</td>
</tr>
</tbody>
</table>

Values in the results are expressed as Mean ± S.E.M, (n=4)
4. CONCLUSION
This study points to the possible antimicrobial and antioxidant potentials of *S. florida* parts. The observed antimicrobial effects of *S. florida* extracts on the bacterial isolates used, though *in vitro* appear to be interesting and promising. This implies that the plant extracts may indeed be effective in the management of diseases caused by these organisms, supporting its ethno medicinal uses; thus the plant may be presented as potential source of novel antimicrobial drugs. There is need for further investigation of this plant in order to identify and isolate its active antibacterial and antioxidant principle(s). The results of this investigation will also need to be validated utilizing *in vivo* models as far as antibacterial activity is concerned.

5. ACKNOWLEDGEMENTS
The authors are grateful to Mr Gabriel Ichife for providing the plant sample. The assistance given by Miss Magdalene Ukpata of National Veterinary Research Institute, Vom, Plateau State, Nigeria, where the test organisms were obtained is hereby acknowledged. The technical assistance given by Mr Friday T. Emmanuuel is gratefully acknowledged.

6. REFERENCES