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Comparative Anti-Nutrient Composition and Anti-Peroxidative Activities of Various Parts of Candle Bush (*Senna Alata* Linn) Plant

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Abstract: The study compared the antinutritive, antimicrobial and anti-peroxidative activities of the leaf, seed and root of the Candle bush (*Senna alata* (Linn)) plant *in vitro.* The plant parts were air-dried and analysed according to standard methods. The leaf was found to contain significantly higher concentration (P≤0.05) of oxalate (8.24±0.57 mg/g) than the seed (3.06±0.00 mg/g) or root (3.29±0.06 mg/g) while Tannin concentration was significantly higher in the seed (9.89±1.17 mg/g) than the leaf (7.42±0.00 mg/g) or root (0.16±0.00 mg/g). Phytate concentration was significantly higher in the root (3.29±0.00 mg/g) than the leaf (1.92±0.04 mg/g) or seed (0.3±0.04 mg/g).The root of the plant showed a significantly higher (P≤0.05) anti-peroxidative ability (35.82±0.00%) than the leaf (19.66±0.36%) or seed (31.84±0.70%). The aqueous and ethanolic extracts of the plant parts were screened against some bacteria isolates. The ethanolic extract of the leaf showed higher zones of inhibition than the aqueous extract on all organisms tested except on *E.coli* and *P. aeruginosa*. Futhermore, the ethanolic extract of the seed showed a higher inhibition on *Salmonella typhii*, *Staphylococus aureus* and *Clostridium* spp. while the aqueous extract of the seed showed higher inhibitory effect on *E.coli*, *P.aeruginosa* and *B.subtilis*. The ethanolic extract of the root showed a higher inhibitory activity against all tested organisms compared to the aqueous extract. Hence, the foregoing shows the plant as a rich source of antinutritive components with potential therapeutic and antimicrobial properties which could replace the conventional synthetic drugs, the cost of which is getting out of reach of large populations in the developing world.

Keywords: *Senna alata*; Anti-peroxidation; Anti-nutrients; Anti-microbial; Isolates.

1. Introduction

Apart from being sources of food, the use of plants by people for therapeutic purposes in all cultures dates back to antiquity. In the developing world, many people depend on wild, edible medicinal plants to meet their healthcare needs [1]. Among these populations, nutrition and healthcare are interconnected as many plants are consumed as food in order to benefit health [2, 3]. Therapeutic claims attributed to medicinal plants have been linked to their content of certain phytochemicals most of which possess among other qualities anti-oxidative [4], antimicrobial [5] and hypocholesterolemic [6] properties. In recent times, research interest is increasing on the nutraceutical value of wild, semi cultivated and uncommon plant species. One of the plants traditionally used in the developing world is the Candle bush tree (*Senna alata)* locally known as Asunwon oyinbo (Yoruba-Southern Nigeria). It is a pantropical ornamental shrub belonging to ceasal piniaceae. It is commonly known as ringworm plant and widely distributed from tropical America to India [7]. The large, leathery compound leaves are bilateral and fold together at night. The fruit is a pod, while the seeds are small and square in shape. It has been used frequently in herbal medicine to treat different kinds of pathogenic conditions, particularly as a laxative and in treatment of skin infections.

Previous workers have reported phytochemical compositions of the Senna plant leaf [8, 9] without reference to the root. The search for novel plants/plant parts with medicinal properties which could serve as substitutes to the conventional drugs which are often expensive in the developing world is imperative. This study aims at comparing the anti-nutrient, anti-microbial and anti-peroxidative compositions of the leaf, seed and root of the Candle bush plant with a view to exploiting their potentials to improve human health.

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2. Materials and Methods

2.1. Plant Collection, Identification and Preparation

Candle bush (*Senna alata*) plant was obtained from a residential Apartment around the Federal University Technology, Akure, Nigeria. The plant samples were taxonomically identified at the Plant science Department, Ekiti State University, Ado-Ekiti, Ekiti State Nigeria. The samples were air dried, finely powdered using a Saisho Electric blender and subjected to the following analysis.

2.2.1. Determination of Oxalate content

Oxalate content of the samples was determined by soaking 1g of the sample in 75ml of 1.5N H_2SO_4 for one hour and then filtered through No 1 Whatman filter paper. 25ml of the filtrate was taken into a conical flask and this was titrated hot between 80^0C-90^0C against 0.1M $KMNO_4$ solution until a pink colour that persisted for 15 seconds was obtained [AOAC] [10].

2.2.2. Determination of Phytate Content

Phytate content of the sample was determined according to the method of Wheeler and Ferrel [11] 4g of the samples were soaked each in 100ml of 2% HCl for 3hours and filtered through No 1 Whatman filter paper. 50ml of the filtrate was placed inside a conical flask and 10ml of 0.3% ammonium thiocyanate solution was added to give it the proper acidity, this was titrated against 0.0056g per ml of standard iron (III) chloride solution that contained 0.00195g of iron per ml until a brownish yellow colouration which persisted for 5mins was obtained.

2.2.3. Determination of Tannin content

The spectrophotometric determination of tannin was done according to the method of Makkar, *et al.* [12] where 0.2g of the finely ground sample were weighed into each 250ml sample bottle. 10ml of 70% aqueous acetone was added and shaken for 10mins in ice water bath. The solution was centrifuged to obtain the supernatant. 0.5ml of the solution was pipetted into a test tube and 0.5ml of distilled water was added. Thereafter 0.5ml of Folin reagent (diluted 1:1) was added and 2.5ml of 20% Sodium carbonate was added. The tubes were shaken and incubated at room temperature for 40mins and the absorbance was read against the blank at 700nm.

2.2.4. Determination of Total Phenol Content

The total phenol content of the extracts was determined by the Singleton, *et al.* [13] method. 0.2ml of the extract was mixed with 2.5ml of 10% Folin Ciocalteau's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45^0C for 40mins and the absorbance was measured at 700nm in the spectrophotometer, Garlic acid was used as standard phenol.

2.3. Anti-peroxidative Ability Determination

Liver execised from anaesthesized rats was placed on ice, weighed and homogenized in cold Tris-HCl at pH 7.4 (1/10, w/v). The homogenate was centrifuged for 10mins at 4000g to yield a pellet (which was discarded) and a low-speed supernatant (SI).

An aliquot of 100µl of the SI fraction was added to a reaction mixture containing different extracts of the various plant parts (0-100 µl) with or without 50 µl freshly prepared sodium nitroprusside. The volume was made up to 300 µl with distilled water and incubated at 37^0C for 1hour.

2.3.1. Thiobabituric Acid Reactions

Production of thiobarbituric acid reactive species (TBARS) was determined as described by Ohkawa, *et al.* [14]. The colour reaction was developed by the addition of 200 µl of 8.1% sodium dodecyl sulphate (SDS) to SI, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% thiobarbituric acid (TBA). This mixture was incubated at 95^0C for 1hour. TBARS produced was measured at 532nm and the absorbance was compared with standard Malondialdehyde standard curve.

2.4. Determination of Antimicrobial Ability

Bacteria isolates viz: *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii, Staphylococcus aureus, Bacillus subtilis and Clostridium* spp used for the analyses were obtained from the Department of Medical Microbiology, University College Hospital (UCH), Ibadan, Nigeria were used for determination of the antimicrobial activity of the plant part extracts. All the pure cultures were suspended in nutrient broth and incubated at 37^0C for 24 hr. Mueller Hinton agar (MHA) and Nutrient agar were used to test for antibacterial activity.

2.4.1. Disc Diffusion Assay

The strains of microorganisms obtained were inoculated in conical flasks containing 100ml nutrient broth each. The flasks were incubated at 37^0C for 24h (seeded broth). Media was prepared using Mueller Hinton agar (MHA), poured on petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile 6mm discs previously impregnated with 20µl of test extract were introduced to the bored holes of the seeded agar plate.

The plates were incubated overnight at 37^0C. The antimicrobial activity was recorded by measuring the width (mm) of the clear inhibition zone around the discs using zone reader and Streptomycin sulphate as standard.

3. Results

Table-1. Anti-nutrient Composition (mg/g) of Candle bush (*Senna alata*) Leaf, Seed and Root

Antinutrient	Leaf	Seed	Root
Oxalate	8.24 ± 0.57^a	3.06 ± 0.00^b	3.29 ± 0.06^c
Phytate	1.92 ± 0.04^a	0.3 ± 0.04^b	3.29 ± 0.00^c
Tannin	7.42 ± 0.00^a	9.89 ± 1.17^b	0.16 ± 0.00^c
Phenol	1.21 ± 0.01^a	3.38 ± 0.01^b	3.47 ± 0.04^c

Results were expressed as means ± Standard deviation with superscripts indicating significant differences.

Table-2. Anti-peroxidative ability (%) of Candle bush on rat liver homogenate

Plant Parts	Thiobarbituric acid reactive species (TBARS)
Leaf	19.66 ± 0.36^a
Seed	31.84 ± 0.70^b
Root	35.82 ± 0.00^c

Results were expressed as means ± SD superscript indicating significant differences.

Table-3. Zones of growth inhibition (mm) showing Antimicrobial Activity of the Aqueous and Ethanol Extract of the Leaf, Seed, and Root of (*Senna alata).*

Organism	Leaf	Seed	Root	Leaf	Seed	Root	Standard
	Ethanol Extract			Aqueous Exract			(Streptomycin sulphate)
E.coli	15.90^a	13.20^b	20.19^c	23.85^a	14.57^b	14.57^b	35.27^d
Salm typhii	19.06^a	19.83^a	18.66^b	14.78^a	14.78^a	11.08^b	33.30^c
Staph aureus	17.17^a	13.53^b	17.00^a	10.62^a	13.06^b	11.26^c	34.06^c
Clostridium spp	16.78^a	15.59^b	15.60^b	16.37^a	14.24^b	3.25^c	33.55^c
P.aeruginosa	13.99^a	10.53^b	17.50^c	14.90^a	14.17^a	12.36^b	32.81^d
B.subtilis	15.05^a	15.14^a	19.02^b	11.50^a	17.18^b	10.00^c	42.89^c

Salm typhii Salmollena typhii, Staph aureus Staphylococcus aureus $P \leq 0.05$ Superscript ^{a,b,c,d} indicate significant differences

4. Discussion

Table 1 shows the result obtained for the anti-nutrient composition of the various parts of Candle bush (*Senna alata*) plant parts. Anti-nutritional factors are natural or synthetic compounds that interfere with the absorption of nutrients. Depending on their concentration, antinutrients can influence the functional and nutritional properties of food. They affect the availability of nutrients required by the body and interfere with metabolic processes so that growth and development of the body is negatively affected.

The leaf extract showed a significantly higher ($P \leq 0.05$) oxalate concentration (8.24±0.57mg/g) than the seed (3.06±0.00mg/g) or root (3.29±0.06mg/g). These values were higher than (0.08±0.06mg/g) obtained by Abdulwaliyu, *et al.* [15] for the methanolic extract of *S.alata* leaf. The oxalate composition of the seed (3.06±0.00mg/g) was significantly higher than 0.06±0.62mg/g in earlier reports [16] for the methanol extract of *S.alata* seed. The root bark oxalate concentration (3.29±0.06mg/g) was also higher than 0.10±0.62mg/g reported by Abubakar, *et al.* [17] for the methanol root bark extract of *S.alata*. Oxalate causes oxidation, leading to inflammation and damage of tissues including the digestive tract. It binds to calcium, interfering with its absorption and in the long run leading to the formation of calcium oxalate which is found in kidney stones.

The phytate concentration (3.29±0.00mg/g) of the Candle bush plant root was significantly higher ($P \leq 0.05$) than the leaf (1.92±0.04mg/g) or seed (0.3±0.04mg/g) concentrations. This concentration was higher than (0.13±0.31mg/g) reported by Abubakar *et al.*, [15] for the methanolic extract of *S.alata* seed. The seed had a lower phytate content compared to that of Baobab seed (0.73±0.9mg/g) [18]. Phytates have the ability to potentially lower blood glucose, cholesterol and triglycerols and reduce the risk of cancer development through the absorption of divalent and multivalent minerals which cancerous cells require for growth [19].

Tannin distribution shows that the seed had a significantly higher ($P \leq 0.05$) composition (9.89±1.17mg/g) than the leaf (7.42±0.00mg/g) and root (0.16±0.00mg/g). The seed of Sesame plant was reported to have higher tannin concentration in the seed than the leaf [20] The leaf was observed to have the highest concentration of oxalate (Table 1) this could explain why the aqueous extract of the leaf showed higher antimicrobial activities against microbial strains (Table 3). Tannins decrease the efficiency of the body to convert absorbed nutrients to new body substances. Certain cancers such as oesophageal cancer have been reported to be related to the consumption of tannin-rich foods [21].

The *S.alata* root contains significantly higher (P≤0.05) phenol concentration (3.47±0.04 mg/g) than the leaf (1.21±0.01 mg/g). Low concentrations of phenol in water have been injected into nerve tissues to lessen pains associated with certain nerve disorder. Phenol acts as an antiseptic when applied to the skin in small amounts, this may explain the use of *Senna alata* plant in the treatment of ringworm, a skin infection.

Table 2 shows the anti-peroxidative ability of the *Senna alata* plant parts. The leaf extract showed a significantly higher anti-peroxidative ability than the root or seed extract. Thiobabituricacid reducing substance (TBARS) are formed as a by-product of lipid peroxidation (degradation products of fats) and are detected by the TBARS assay. The leaf extract produced the least concentration of TBARS (19.66%) of the three plant parts analyzed. This concentration was significantly (P≤0.05) lower that formed on treatment with extracts from the seed (31.84%) or root (35.82%). This shows that the leaf extract was able to control lipid peroxidation in the rat liver than other extracts. Hence, this suggests the leaf extract as the best part to be used in the treatment or suppression of the effects of lipid peroxidation. This is consistent with the reports of Butterfield and Lauderback [22] for the inhibition of Fe^{2+} induced lipid peroxidation in rat brain *in-vitro* by Tigernuts extracts

The high inhibitory effect of leaf extract on Fe^{2+}-induced lipid peroxidation on liver homogenates may be due to its high iron chelating ability. *Senna alata* leaf may possess a good iron chelating property which must have prevented the oxidation of iron (II) to iron (III), thereby preventing the generation of hydroxyl radical and inhibiting oxidative assaults on the tissue in the process. This finding is similar to results obtained for the leaf extract of *Tetracarpidium conophorum* which caused a significant and dose-dependent reduction in lipid peroxidation levels in rat reproductive organs [23].

The anti-microbial susceptibility test showed that the aqueous and ethanolic extracts demonstrated significant activity against bacteria isolates (Tables 3). The ethanolic root extract showed significantly higher (P≤ 0.05) growth inhibitory effect on *E.coli*, *B.subtilis* and *P.aeruginosa* than the leaf and seed extracts. The ethanolic leaf extract had a significantly higher inhibitory effect on *S.aureus* and *Clostridium spp* while the ethanolic seed extract showed a significantly higher (P≤ 0.05) growth inhibition on *S.typhii* than the leaf and root extracts. The high antimicrobial activity of the ethanolic leaf extract against *S.aureus* is similar to previous reports [24]. The aqueous extract of the leaf showed a higher inhibition on *E.coli* and *Clostridium spp* than the seed and root extracts while the aqueous seed extract revealed higher growth inhibition on *S.aureus* and *B.subtilis* *S. aureus* is known to cause skin infections like boils, carbuncles and rashes of the beard [25] hence, the extract could be effective in the treatment of skin diseases. Similar inhibitory effects were demonstrated on *S.typhii* by the aqueous leaf and seed extracts. The aqueous root extract was observed to show a significantly low inhibitory effect on all isolates. The ethanolic root and aqueous leaf extracts inhibited microbial growth effectively while the most susceptible isolates to both extracts were *S.typhii*, *E.coli* and *Clostridium spp*. The leaf extract was observed to have the highest concentration of oxalate (Table 1) This explains higher antimicrobial activities of the leaf extract.

5. Conclusion

The foregoing shows that ethanolic extracts of *S.alata* Plant exhibited greater antimicrobial activity than the aqueous extract. Organic solvents hare been found to be more suitable for extraction of phytochemicals [26].This study therefore suggests the *Senna alata* plant as a rich source of phytochemicals with therapeutic and antimicrobial properties, which if properly domesticated and harnessed will serve as an important potential source of bioactive agents for pharmaceutical use.

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