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Biochemical Studies of *Ocimum sanctum* and *Olax subscorpioidea* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NNW, EEB and SCJ designed the study. Authors FBJ and GAI collected all data and performed the statistical analysis. Authors FBJ, GAI and UMU did the literature search. Authors SCJ, NNW, EEB and GAI wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The leaves of *Ocimum sanctum* and *Olax subscorpioidea* are known in Nigerian traditional medical practices for the treatment of different ailments according to folklore. This study was undertaken to compare and appraise the phytochemical constituents from ethanol extracts, antimicrobial resistance, proximate and mineral analysis. Data obtained revealed that alkaloids, cardiac glycosides, steroids, tannins and terpenoids were detected in the *Ocimum sanctum* leaf samples whereas, flavanoids, phenol, steriods, saponins, tannins and terpenoids appeared in the *Olax subscorpioidea* ethanol extracts. Resistance to a selected range of infectious disease

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pathogens, using chloramphenicol (0.2 mg/mL) and fluconazole (0.5 mg/mL) as controls revealed that the leaf extract of *O. subscorpioidea* exhibited higher activity with zone of inhibition values of 18±0.50, 20±0.50, 15±2.00, 16±0.50, 18±0.50, 19±1.50 and 22±0.50 mm whereas that of *O. sanctum* was 15±1.0, 19±1.40, 15±1.20, 22±1.30, 15±1.10, 16±0.90, 16±1.10 mm. The proximate and mineral analysis of both leaves revealed that *Olax subscorpioidea* contain crude protein (10.15%), crude fiber (10.20%), moisture (5.70%), total ash (15.40%), total carbohydrate (58.55%), while that of *Ocimum sanctum* was crude protein (2.38%), crude fiber (11.45%), moisture (6.20%), total ash (10.42%), total carbohydrate (66.35%). The leaves indicated the presence of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), magnesium and copper at different concentrations. The results obtained suggested that the leaves of both studied plant possess anti-microbial activities with the major activity tailored to the phyto-constituents from the ethanol extracts.

Keywords: Phytochemical; Olax subscorpioidea; Ocimum sanctum; proximate analysis; mineral analysis.

1. INTRODUCTION

Infectious diseases remain as one of the most prevalent causes of human and animal mortality particularly in developing countries, owing to a number of factors in which the upsurge of drug resistant varieties which has diminished the potency of regular anti-microbial agents and the occurrence of opportunistic infections take precedence [1,2]. Apart from death, these diseases; bacterial, fungal, parasitic or viral in origin, greatly diminishes the reproductive capabilities of livestock as well as the overall effectiveness of humans thereby impacting negatively on commerce, economy and tourism in the affected locale [3]. The re-occurrence of opportunistic infections associated with upper respiratory and gastrointestinal tract infections, prolongs the prevalence of more lethal conditions such as encephalitis, gastroenteritis, tuberculosis and other acute respiratory complications [4].

Microbial infections commonly associated with upper respiratory and gastrointestinal tract infections include but are not limited to *Escherichia coli, Klebsciella sp, Pseudomonas sp, Staphylococcus sp, Aspergillus sp, Penicillum sp* and *Proteus sp*. These microorganisms give rise to the initial development of symptoms like diarrhoea, dysentery, pneumonia and thrombophlebitis [5]. Prolonged exposure and re-occurrence of these nosocomial infections creates a platform for invasion by more lethal pathogens [6]. Such phenomenon necessitates the search and investment for more effective drug alternatives against these microbial diseases.

Plants have been exploited for years towards the treatment of infectious diseases due to the assortment of bioactive compounds that serve as both valuable nutritional supplements and antimicrobial agents [7,8,9,10]. In Africa, traditional medicine system continues to dominate the health care practice whereby consumers reply on traditional medicinal plants as their primary source of health care and food [11]. This practice has gained considerable attention as the track record in terms of efficacy, cultural acceptability, safety concerns and side effects are generally better than that of synthetic drugs [12].

Studies show that there is approximately 250,000 or more plant species globally of which about 25,000 only have been screened for certain biological functionality [13].

In recent years, phytochemical compounds within plants are being screened to help access which active compounds or synergy of compounds produce their antimicrobial activities. This makes such bioactive compounds effective drug candidates. Furthermore, this also confirms the belief that local plants are the platform for traditional African medicine [14]. A variety of illnesses especially malaria as well as upper respiratory and urinary tract infections has been treated via traditional practice using indigenous plants [15]. The assortment of unique bioactive compounds including alkaloids, flavonoids, terpenoids, etc which elicit a series of immuno-stimulatory events within the body have been implicated in the ethno-medicinal properties of such indigenous plants [16]. Research has indicated that alkaloids possess antimicrobial, anticancer, cytotoxic and antimalarial properties while flavonoids are extremely antibacterial activity thereby making them highly effective towards the treatment of certain allergies, cancer, inflammations and viral infections [17].

The high demand for products of plant resources is due to their versatile applications [18].

The leaves of *O. sanctum* (holy basil) has been reportedly used to treat diarrhoea, pneumonia and other upper respiratory tract infections [19]. Ethno-botanical studies have also shown that its oil is active against several species of bacteria including *S. aureus*, *Listeria monocytogenes, E. coli, Shigella sp, Salmonella sp*, *Proteus sp* and fungi like *Trichophyton rubrum, Penicillium islandi cum* and *Candida albicans* [20].

Different parts of *O. subscorpioidea* is used across Nigeria for different conditions like for treating arthritis, constipation, cough, dermatosis, fever, headaches, jaundice, malaria, rheumatism, syphilis, ulcer, etc [21]. However, there are claims by traditional healers in some parts of Nigeria that treatment of a myriad of infections using extracts of either *O. sanctum* or *O. subscorpioidea.*

The present study was undertaken to compile data on the phyto and physicochemical composition of the leaves of *O. sanctum* (Fig. 1) *and O. subscorpioidea* (Fig. 2) including the antimicrobial activity and nutritional content, thereby justifying their use in the treatment of certain upper respiratory or gastrointestinal infections as possible new antibacterial drug candidates against infections.

Fig. 1. *Ocimum sanctum*

Fig. 2. *Olax subscorpioidea*

2. MATERIALS AND METHODS

Leaves of *Ocimum sanctum* and *Olax subscorpioidea* were collected in fresh condition at Sheda and Abakiliki regions of the FCT and Ebonyi state, Nigeria. The plants were identified, air-dried and kept in airtight containers until required for further laboratory analysis. Crispy plant leaf sample (100 g) was placed and soaked with ethanol in a Soxhlet apparatus for 6-8 hours. The crude extracts were later concentrated using rotary evaporator. Phytochemical screening was then performed using standard procedures [22].

2.1 Mineral Analysis

The metal analysis was determined using an Atomic Absorption spectrometer (iCE 3000, Thermo fisher). Five grams of oven dried samples were weighed into a crucible and transferred to a furnace at 600℃ and left to ash for 3 hours. The furnace was cooled to about 120℃ and then placed in a desiccator for an hour to cool before weighing. This process was repeated until a constant weight was obtained. The ashed samples (0.5 g) were weighed and transferred into the digestion tube. 5 ml each of distilled water, concentrated HNO_3 and perchloric acid were added and the content mixed. The tubes were placed into the digestion block inside a fume cupboard and the temperature was set at 150℃ for 90 minutes. The temperature was then adjusted to 230℃ and incubated for another 30 minutes to obtain white fumes. The temperature was then reduced to 150℃, followed by the addition of 1 ml of hydrochloric acid to the tubes within a few minutes. Water was added to the tube to make up to the mark, mixed and filtered.

Elemental analysis of the solution obtained was then performed using an atomic absorption spectrophotometer (AAS) at an appropriate wavelength, temperature and lamp-current for the different elements. The following elements were determined, calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), potassium (K), sodium (Na), lead (Pb) and Nickel (Ni).

2.2 Proximate Analysis

The Moisture, protein, fat, ash, crude fibre and carbohydrate content of the dried leaves was determined. For moisture content, 5 g of dried leaf samples was weighed and dried in an oven at 105℃ to a constant weight. The percentage weight loss was determined. Fat content was determined by extracting 5 g of dried leaf sample with hexane or petroleum ether in a Soxhlet apparatus for 8 hours. The ash content was estimated by incinerating 5 g of dried sample in a muffle furnace (Carbolite-RHF 1600) at 550℃ for 4 hours, and then the percentage ash content was determined. The micro-Kjedahl method was employed for estimation of crude protein by determining total nitrogen and converting to crude protein by multiplying with 6.25 and carbohydrate was determined by difference.

All experiments were done in triplicate and results were expressed as the averages on dry weight basis.

2.3 Preparation of Inoculum

The bacterial isolates were collected from Medical Microbiology Department of Specialist Teaching Hospital, University of Abuja, F.C.T., Nigeria on a slant Nutrient agar. The isolates were restored on Nutrient broth and confirmed using standard biochemical tests according to the Bergey's manual of Bacteriology [23].

2.4 Biological Screening of Extracts

The crude extract was screened for antimicrobial activity using agar well diffusion technique with little modification. Clinical isolates of *E. coli, S. aerus, P. aeruginosa, K. pneumonia and S. thypi* were tested to determine the antibacterial activity of the ethanolic extract, using agar well diffusion method. The media Mueller hinton agar (Sigma Aldrich) was prepared based on the manufacture's instruction. The agar plates were sterilized at 37℃. The sterilized Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile cotton bud and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The wells were made in each culture plates using a sterile cork borer. Chloramphenicol (0.20 mg/mL) and ampicillin (0.25 mg/mL) were used as controls. Approximately, 0.1 mL of the crude extract concentrations from (1000, 500, 250 mg/mL) respectively was dispensed in each well and incubated for 24 hours at 37℃. The plates were examined for the presence of bacterial and fungal inhibition zones around each well. The zones of inhibition were measured using a ruler and the results were reported in millimeters (mm).

3. RESULTS

3.1 Phytochemical Screening

Table 1 represents the phytochemical composition of ethanol extracts from the leaves of *O. sanctum* and *O. subscorpioidea*. The qualitative tests performed on the extracts revealed the presence of similar secondary plant metabolites with the exception of alkaloids and cardiac glycosides which were detected only in the *O. sanctum* fractions just as flavanoids and phenols were only recorded in the *O. subscorpioidea* leaf extract. The data revealed that both plants contained saponins, terpenoids and tannins.

Table 1. Phytochemical screening of plant extracts

Compounds	Ethanol	
	Ocimum sanctum	***Olax subscorpioidea***
Alkaloids	+	-
Carbohydrate	+	+
Cardiac glycosides	+	-
Flavonoids	-	+
Glycoside	-	-
Phenol	-	+
Saponins	-	-
Steroids	+	+
Tannins	+	+
Terpenoids	+	+

Key: (+) – Present (-) – Absent

The presence of alkaloids and cardiac glycosides in *O. sanctum* leaf extracts suggests that the plant possesses antimicrobial potential, particularly against intestinal infections although they may have a diminished spectrum of application [24]. The presence of phenols and flavonoids in the leaf extract of *O. subscorpioidea* suggests that the plant possesses a higher antimicrobial potential than *O. sanctum* as a number of antibiotics with strong antifungal activity are phenolic in nature [25].

3.2 Proximate Analysis

Data from this study (Table 2) reveal that the leaves of *O. subscorpioidea* (10.15%) are richer in protein than that of *O. sanctum* (2.38%). Leaves of the former may possess necessary building blocks that underscore the development of hormones towards the regulation of different body functions [26].

The crude fiber values; 11.45% and 10.20%, as well as the high carbohydrate content values of

66.35% and 58.55% for *O. sanctum* and *O. subscorpioidea* leaves indicates that both plants maybe highly digestible. The fat and ash content values of 3.2% and 10.42% was obtained for *O. sanctum* and *O. subscorpioidea* 4.2% and 15.4% respectively.

Table 2. Proximate composition data of leave samples

Components	*Ocimum sanctum* (% w/w)	*Olax subscorpioidea* (% w/w)
Crude Protein	2.38	10.15
Crude Fiber	11.45	10.20
Moisture	6.20	5.70
Ash	10.42	15.40
Carbohydrate	66.35	58.55
Fat	3.20	4.20

The moisture content of 6.20% was obtained for *O. sanctum* while a value of 5.7% was obtained for *O. subscorpioidea* leaves.

3.3 Mineral Composition

Table 3 presents the result of the mineral element composition of the leaves of *O. sanctum* and *O. subscorpioidea* in mg/g. The shows the presence of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and copper (Cu) at different concentrations in both plants. The concentrations of these elements are well within the acceptable limits for minerals that are useful for human and animal health.

Table 3. Mineral composition of leaves

Minerals	*Ocimum sanctum* (mg/g)	*Olax subscorpioidea* (mg/g)
Calcium	41.2041	1.4586
Copper	0.4014	0.0245
Manganese	-	0.2665
Magnesium	4.7941	1.2549
Sodium	0.1346	0.1346
Zinc	0.804	0.0815
Iron	2.8343	-
Potassium	-	1.2441

3.4 Antimicrobial Screening

The mean zones of inhibition of different plant extracts tested against 4 bacterial and 3 fungal species are summarized in Table 4. In the bacteria screening, the ethanol extract of *O. sanctum* leaves were found more active against *K. pnuenomia* with 18-22±0.55 mm zone of inhibition across the different concentration of the extract used. The highest zone of inhibition recorded for the fungal screen of the same plant extract was between 15-16±0.90 mm against *P. aeruginosa.* The least zone of inhibition of 12-15±0.70 mm was observed for the same plant extract against *Pseudomonas sp*.

Data from Table 4 suggests that with the exception of *K. pneumonia*, the ethanol leaf extracts of *O. subscorpioidea* proffer greater resistance to disease causing agents for most respiratory tract infections than that of *O. sanctum*. Furthermore, *O. subscorpioidea* leaf extracts possess better anti-fungal activities than its counterpart in this study.

4. DISCUSSION

The use of plant derived drugs for the treatment of different diseases has long been exploited traditionally for decades by herbalists with a good knowledge of local flora. In the treatment of diseases or infections, medicinal plants are considered as the best alternative to synthetic drugs. Consumed either as a preventive measure or as a viable treatment solution, phyto-drugs are not prone to certain side effects commonly associated with the use of synthetic drugs. The presence of bioactive compounds within plants are being studied to help elucidate which of them individually or collectively are responsible for their antimicrobial activities thereby confirming the belief that local plants are the platform for traditional African medicine [14].

In Nigeria, native plants are used either in whole or in combination with other plants as herbal medicine to develop new cures to disease or physical ailment. Like in other developing countries, native Nigerian plants are consumed as ‘plants for medicine and plants for food’ [27]. The therapeutically and dietary needs that specific native plant species meet has been underscored in many developing countries [28].

Disease causing pathogens like *P. aeruginosa* is a major opportunistic infection that is commonly associated with respiratory diseases, urinary tract infections, etc [29]. Another cause of life-threatening diseases arising from infections is triggered by *S. aureus* which has been identified as a major gastrointestinal and urinary tract pathogen that is gaining a lot of attention owing to its raising prevalence among community acquired infections [30]. Entero-toxigenic strains of *E. coli* have also been identified among the array of causative agents responsible for acute diarrhoea and other urinary tract infections globally [31].

Table 4. Antimicrobial activity of *Ocimum sanctum* leaf and *Olax subscorpioidea leaf extracts*

Test organism	***Ocimum sanctum* (mm)**			***Olax subscorpioidea* (mm)**				
	125 (mg/ml)	**250 (mg/ml)**	**500 (mg/ml)**	**125 (mg/ml)**	**250 (mg/ml)**	**500 (mg/ml)**	**Chloramphenicol (0.2 mg/ml)**	**Fluconazole (0.5 mg/ml)**
Escherichia coli	13±1.40	14±1.50	15±1.00	16±1.00	17±1.50	18±0.50	22±1.20	-
Staphylococcus aureus	12±1.20	14±1.20	19±1.40	15±0.50	16±1.00	20±0.50	21±1.10	-
Psuedomonas sp	12±1.10	13±0.70	15±1.20	13±0.50	14±1.50	15±2.00	22±0.30	-
Klebsciella pneumonia	18±1.60	20±0.90	22±1.30	14±0.50	15±1.00	16±0.50	25±1.10	-
Proteus mirabilis	13±0.70	14±0.80	15±1.10	16±1.00	17±1.50	18±0.50	23±1.10	-
Aspergillus niger	14±1.00	15±1.30	16±0.90	16±1.00	18±0.50	19±1.50	-	18±1.10
Penicullum sp	15±1.30	15±1.40	16±1.10	15±1.00	21±1.00	22±0.50	-	21±1.10

Analysis of the data obtained from Table 1 suggests that the leaves of both studied plants contain certain ethanol soluble compounds whereby the presence of Alkaloids and Cardiac glycosides was detected in the *O. sanctum* fractions alone. Similarly, the presence of Flavonoids and Phenols were detected in only the *O. subscorpioidea* leaf extracts. The disparities in phytochemical content is responsible for the observed antimicrobial effectiveness exhibited in this study [32].

The presence of tannins in both plants is indicative of their use in the treatment or prevention of diarrhea. The presence of cardiac glycosides in *O. santum* alone validates reports that this plant has also been used as a stimulant for cardiovascular applications [33], thereby corroborating its use according to folklore in the management of hypertension. In addition to this, the presence of Alkaloids and Steroids in this plant suggests it possesses sufficient activity against disease causing pathogens like *S. aureus* and *S. thypi* which are responsible for diarrhoea and stomach upsets [5]. However, the presence of Phenols in the leaf extract of *O. subscorpioidea* alongside phyto-compounds like Steriods, Tannins and Terpenoids suggests that this plant would possesses slightly better antimicrobial properties as Phenols generally exhibit antiseptic properties which has resulted in the synthesis and production of a number of therapeutic agents including griseofulvin, natamycin and nystatin [25]. Phenolics, which also comprises of Flavonoids and Tannins are one of the most abundant phyto-constituents in plants, as a result are not only an essential part of our diet but has also been associated with good mental performance through the consumption of items including berries, chocolate, red wine and tea [34].

Overall, data from this study (Table 4) shows that with the exception of *K. pneumonia*, the ethanol leaf extract of *O. subscorpioidea* exhibits a stronger antimicrobial activity than *O. sanctum*. The selected organisms used in this study proved to be of great impact medically as these organisms are all associated with community infections, ranging from sub-mucosa obsesses, broncho-pneumonia, diarrhea, etc [35,36].

Members of the *Olacaceae* family are generally considered to be rich in minerals, proteins and vitamins. Data obtained from this study revealed that the crude protein, ash and fat content of *O. subscorpioidea* leaves is higher than that of *O. sanctum*.

The higher moisture content in the *O. sanctum* leaves may account for the lower antimicrobial activity whereby it encourages microbial growth. Overall, the values obtained for the tested macronutrients (Table 2) revealed that although *O. sanctum* has a higher percentage of crude fiber (11.45%) than *O. subscorpioidea* (10.2%), both plants are highly digestibility. The low fat content of both plants; 3.2% and 4.2%, respectively, suggests that they are both good as sources of animal feed coupled with the protein content which favours the consumption of *O. subscorpioidea* due to its higher content (10.15%). Since these substances are essential for growth, metabolism and other body functions, the leaves of *O. subscorpioidea* are thus a better food source for human consumption. The higher ash content (15.4%) indicates that the leaves should possess a high mineral composition. Table 3 revealed concentrations of Calcium (1.4586 mg/g), Magnesium (1.2549 mg/g), Zinc and Iron are much higher in dried leaf samples of *O. sanctum* than that of *O. subscorpioidea*. This means that the plant in this study is useful towards bone formation, energy, normal functioning of metabolic and metallo-enzymes [37,38]. The presence of zinc and other trace minerals detected further boosts the plants' application as an anti-microbial amongst many functions. Variations observed in the observed mineral and nutritional content could be attributed to the diversity in genetic material, geography and soil fertility.

5. CONCLUSION

This study serves as an indication to the varied possible applications of indigenous medicinal plants towards the treatment of different infections. Also, it validates traditional knowledge and adds to the growing literature on herbal sources of emerging nutraceuticals. The information on the therapeutic effect of the both plants in the treatment of upper respiratory and gastrointestinal infections seems to be sparsely known throughout Nigerian cultures. The findings in this study have hence provided scientific support for the ethnomedical anti–microbial activity of *O. sanctum* and *O. subscorpioidea* extracts. Moreover, the phyto-chemistry of the extracts of *Olax subscorpioidea* contains more phenolics than that of *O. sanctum* thereby validating the stronger anti-microbial activity of the two.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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