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Investigating the Effect of African Basil (*Ocimum gratissimum)* LeafExtract on some Pathogenic Bacteria within Agricultural Environment in Southwestern Nigeria

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Abstract:
Background*: The advent of antibiotic-resistant strains of bacteria and reported side effects of synthetically manufactured drugs have further necessitated the search for plants and their extracts in the treatment of various infections and diseases in the area of health care delivery globally. Ethanolic and Aqueous (water) extracts of Ocimum gratissimum leaves known to be used in several traditional treatments of bacterial infections and diseases were evaluated for their antibacterial effects on three selected pathogenic bacteria namely: Shigella flexneri, Escherichia coli and Salmonella enteritidis.*
Materials and Methods*: Agar well diffusion techniques were used to test the in vitro activity of the plant extracts on Shigella flexneri, Escherichia coli and Salmonella enteritidis bacteria at different concentrations (25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml and 125mg/ml). The tests for the susceptibility of each of the bacteria at these different concentrations were done in triplicates. The average values of the diameter (mm) of the zones of growth inhibition in each of the test organisms at the different concentrations were recorded. 0.5mg/ml of Gentamycine was used as the positive control experiment on the test organisms while dimethyl sulphoxide (DMSO) was used as the negative control test. Phytochemical analysis of the extract was carried out following established methods.*
Results*: The extracts showed good but varying antimicrobial activities on the test bacteria. Salmonella enteritidis was susceptible to both Aqueous and ethanolic extracts at all concentrations. Escherichia coli were susceptible at concentrations of 100mg/ml and 125mg/ml in both extraction media. The growth of Shigella flexneri was not inhibited by aqueous extracts of the plant but was inhibited by the ethanolic extracts. The variations in actions of the extracts on the test bacteria could be a result of the varying presence, concentrations and interactions of the different phytochemicals in the extract as a result of the two different mediums of extractions used.*
Conclusion:*The results obtained from this research validate the known traditional use of Ocimum gratissimum in the treatment of bacterial infections and diseases and suggest that the plant extract has a great potential in the area of bacterial and infection disease management which can be further explored in drug manufacturing.*
Key Word*:African basil; Pathogenic bacteria; Ethanolic extract; Phytochemicals; Escherichia coli.*

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I. Introduction

Good health and productive agriculture are important in the economy of any nation, especially in the fight against poverty [1]. Empirical evidence abounds in the literature that health, as a form of human capital is one of the factors which affect crop productivity [2]. Health enhances work effectiveness and the productivity of an individual through an increase in physical and mental capacities. According to [3], there is a positive relationship between the health and productivity of skilled and unskilled labour. Good health as related to labour output or better production organization (since people with good health generally have better intellectual capacities), can enhance farmer's or household income and economic growth while poor health will result in

loss of work days or decrease worker capacity, decrease innovation ability and ability to explore diverse farming practices. Health influences total factor productivity growth directly through household income and wealth, and indirectly through labour productivity, savings and investments and demography, by reducing various forms of capital and technology adoption [4]. With lower mortality rates, the incentive to save increases and leads to higher total factor productivity growth. [5][6] [7][8] opined that health capital is affected by a number of preventable diseases: Malaria, musculoskeletal disorders, HIV/AIDS, farm injuries, yellow fever, typhoid fever, Schistosomiasis, Onchocerciasis, Diarrhea, respiratory diseases and skin disorders, among others which are mostly caused by pathogenic bacteria (*Shigella flexneri, Escherichia coli* and *Salmonella enteritidis,*). These diseases make farmers not to utilize fully all inputs at their disposal and dilapidate farmers' physical performance and equally impacts negatively on the farm profit levels. Some identified distinct and specific factors affecting health in developing regions include malaria, malnutrition, waterborne diseases and HIV/AIDS, all of which affect both morbidity and mortality and arguable morbidity in particular [3].

Traditional medicine is based on the use of herbs and plants that are abundant in nature and have a long history in the treatment of illnesses across the world [9]. A number of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific data (double-blind trials, for example) to confirm their efficacy [10]. The World Health Organization [11] reported that 80 percent of the world's population depends on the use of medicinal plants as therapies for different illnesses, involving the use of plant extracts or their active substance [9]. The reports on the roles of plants in the fight against infectious diseases and illness in different research works have led to an increase in the search for various alternatives to conventional medicine in healthcare delivery [12]. In recent times, governments of many countries have established regulatory councils for traditional medicines under their various health agencies for further development in the use of traditional medicine for health management [13].

African basil (*Ocimum gratissimum L.*) is an herbaceous shrub notably found in tropical countries including Nigeria, where it is commonly called Clove basil, Sweet basil, tea bush, Scent leaf, or fever plant popularly referred to as 'Effirin' in Yoruba, 'Nchanwu' in Igbo and 'Dai doya' in Hausa is noted for its aroma and used as spices for food or soup preparation in Nigeria [14]. It is widely known for its medicinal use in the treatment of various infections ranging from bacteria to fungal infections [15][16][17]. The plant (Figure 1) is widely cultivated on open fields and backyards farms in the southwestern part of Nigeria. Many species of the genus *Ocimum* namely: *Ocimumamericanum, Ocimumbasilicum, Ocimumcanum, Ocimumgratissimum, Ocimum sanctum* and *Ocimum suave* have been reputed for various medicinal uses [17]. *Ocimum gratissimum* is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips. Leaves opposite; petiole 2-4.5 cm long, slender, pubescent; blade elliptical to ovate, 1.5-16 cm x 1-8.5 cm, membranaceous, sometimes glandular punctate, base cuneate, entire, margin elsewhere coarsely crenate-serrate, apex acute, puberulent or pubescent. In its native area, *O. gratissimum* occurs from sea level up to 1500 m altitude in coastal scrub, along lake shores, in savanna vegetation, in the sub-montane forest, and on disturbed land [18].

Figure 1: African basil (*Ocimum gratissimum L.*)

[19] reviewed the Comprehensive biological activities of *O. gratissimum* and it is associated with antibacterial, antifungal, hypoglycemic, antipyretic, anti-nociceptive, antioxidant, anti-inflammatory, anthelmintic, chemo preventive, anti-carcinogenic, free radical scavenging, radio protective, antidermatophytic activities, and numerous others pharmacological use [20]. According to studies conducted by [9] and [14], it was

revealed that phytochemical contents of *Ocimumgratissimum* include saponin, alkaloids, glycosides, phenols, flavonoids, steroids, terpenoid and tannin. These phytochemicals when consumed are said to have antimicrobial properties that can prevent and treat diseases in humans [17]. Antimicrobial properties of the plant have been seen in the study conducted by [21], which revealed that extracts from *Ocimum gratissimum* were active against diarrhea-causing bacteria species of *Staphylococcus aureus* and *Salmonella sp*. The use of most medicinal plants is now heavily promoted as a result of recent cases of pathogens developing resistance to conventional antibiotics. This is now a global threat to humanity as a result of the inability to control the spread of some initially treatable diseases with synthetic antimicrobial drugs formerly used in some cases [22][14]. Another challenge in the healthcare sector on the administration of synthetic antibacterial drugs is the side effects of the drugs such as tendonitis, seizure and Steven-Johnson Syndrome [11]. In view of this, the aim of this study is to investigate the effect of aqueous and ethanolic extract of *Ocimum gratissimum* leaves extract on three selected pathogenic bacteria species; *Shigella flexneri, Escherichia coli* and *Salmonella enteritidis,* as they affect the health of farmer within the farming environment.

II. Material And Methods

Study Area

Ocimum gratissimum leaves used in this research were harvested from backyard farms in Olodo, Ajagbe and Kugba areas of Odeda local government area in the central part of Ogun state, southwestern part of Nigeria. The area is largely agrarian, has over 20 semi-urban areas and 860 villages and hamlets, the inhabitants are predominantly Egbas having homesteads and farmlands in the area (23). The entire Odeda council area has an extensive landmass mostly grassland with an area of 99,615Km^2 (24).

Identification and Preparation of Plant Material

The plant was identified by Koko Olusegun an Agricultural research officer in the Planning Research and Statistics department of the Ogun State Ministry of Agriculture, Nigeria. After the leaves were harvested, they were de-stalked, rinsed with distilled water to remove dirt, air dried for two weeks at room temperature and blended into powder using an electric blender [14].

Plant Extraction Process

The extraction was performed by adopting the [13] method, by adding 100g of Ocimum gratissimum leaf powder in 900ml of ethanol for the ethanol extract and another 100g of the leaf powder for the water extract. The two different extracts of water and ethanol to be used for the study were then poured into two separate sterile flasks and swirled to ensure effective mixing and stoppered to avoid loss of volatile liquid content at ambient temperature (28±20C). The two mixtures were extracted by agitation on a rotary shaker for 48hours, decanted, filtrate was then poured into stainless trays and extracts were allowed to evaporate to dryness under microbiological hood set at appropriate conditions using rotary evaporator. The concentrated extract obtained was then scooped into a container, covered and labeled accordingly, then stored in the refrigerator to be later reconstituted.

Reconstitution and Antimicrobial Screening of Plant Extract

the plant extract was reconstituted in a sterile distilled water for the water extraction and dimethyl sulfur Oxide (DMSO) for ethanol extract to give dilutions of the two extracts to give 25-125mg/ml. the extracts were screened for antibacterial activity using the agar well diffusion method by [9].

Test Isolates

Pure isolates of some bacteria from the family of Enterobacteriaceae; *Salmonella enteritidis, Shigella flexneri* and *Escherichia coli* were used in this research work. They were checked for purity and confirmed by Gram staining and sub culturing them on selective media; *Salmonella Shigella* Agar MacConkey Agar. Colony characteristics and morphology of the cells were observed for confirmation. The bacteria selected can cause gastroenteritis in humans, affecting the gut leading to inflammation of the stomach and intestine, causing vomiting, severe abdominal cramps and diarrhea [23]. This can occur in a case of poor personal and environmental hygiene or when in close contact with infected animals or consuming food or water contaminated with the bacteria or toxic substances produced by the bacteria[24].

Determination of the Antimicrobial Activity of the Plant Extract

The method employed in testing the antimicrobial actions of the plant extracts on the selected bacteria was "Agar well diffusion techniques.' 24 hours old cultures were transferred into a nutrient broth and incubated at 370C for 5 hours and standardized to Macfarland standard. The three test organisms from the broth were streaked on 5 different Mueller Hinton agar plates in triplicates under sterilized conditions and labeled to avoid

mix-up. The three sets of 5 plates each makes a total of 15 plates with each set representing the three different geographical locations where the plants were harvested. Wells of 6mm in diameter were drilled on the surface of the inoculated agar medium using a sterile cork borer no.1. The plates were further labeled with a marker indicating the concentration of the plant extract filled in each well (25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml, 125mg/ml,). 0.5mg/ml of Gentamycine a broad spectrum antibiotics was used as a positive control while DMSO was used as a negative control. The experiment was performed in triplicate. The zones of inhibition for the 5 different concentrations of the extract on each of the three bacteria were recorded in millimeters (mm) as the average of the three triplicates for each concentration and the minimum Inhibitory concentration in mg/ml was also determined.

Statistical analysis

Range and mean values of data collected in this work were analyzed using Statistical package for Social Science (SPSS) software version 25 and presented in tables and bar charts.

III. Result

Phytochemical analysis of Ocimum gratissimum of ethanolic and water (Aqueous) extract showed the presence of alkaloids, saponin, tannin, Terpernoid, flavonoid and steroid as presented in Table 1. The test for antimicrobial activity of ethanolic extract and aqueous extracts showed that *Salmonella enteritidis* was susceptible to the extract at all concentrations tested. Average zones of growth inhibition for the aqueous extract ranged between 13-18mm and 7-11mm for ethanolic extract as presented in table 2. This could be due to the presence of the phytochemicals or interactions between some bioactive compounds present in the extract against the test organism. Salmonella enteritidis from the result was the most susceptible amongst the test organism for both medium of extraction of the plant but highly susceptible to the aqueous extract at concentrations of 100mg/ml and 125mg/ml. The result obtained was similar to the work of [21].

Escherichia coli was less susceptible to the extracts of both aqueous and ethanol. Zones of inhibition were only recorded at higher concentrations of 100mg/ml and 125mg/ml as presented in Table 2. This could be due to low concentrations of active substances that could inhibit the activities of the organisms at lower concentrations. The result at this concentration agrees with the work of [25]that showed that the Volatile oil of the leaves of *Ocimum gratissimum* was active against *Escherichia coli*.

Shigella flexneri was not susceptible to aqueous extract at all concentrations this could be due to the absence of bioactive compounds in the extract as a result of the medium of extraction. This was similar to the work of [14]. Ethanolic extract on *Shigella flexneri* showed growth inhibitions at all concentrations with the Minimum Inhibitory Concentration to be 25mg/ml as presented in figure 2, with an average zone of inhibition of 9mm. The inhibitory action of the ethanolic extract could be as a result of the medium of extraction making available bioactive substances in the extract that can affect the activities of the test organisms. The average zone of inhibition observed for the positive control test using 0.5mg/ml of Gentamycine showed zones of inhibition in the entire test organism, with the highest average zone of inhibition in *Salmonella enteritidis* with 26mm in diameter and the lowest was 8mm recorded for *Escherichia coli* as presented in Table 3. The growth of the three test organisms were not inhibited by the presence of DMSO similar to the findings of [14].

Table 1: Result for Phytochemical Analysis of *Ocimum gratissimum* Extract.

Chemical test	Ethanolic extract	Aqueous extract
Alkaloid	+	+
Saponin	+	-
Tannin	+	+
Terpernoid	+	+
Flavonoid	+	+
Steroid	+	+

+ Present, - Absent

Table 2: Average Zone of Growth Inhibition (mm) for the Test Organisms at Different Concentrations

Bacteria	Concentration extract (mg/ml)	Aqueous extract (mm)	Ethanolic Extract (mm)
Salmonella enteritidis			
	25	13	7
	50	14	8
	75	15	9
	100	17	10
	125	18	11
Escherichia coli			
	25	-	-
	50	-	-
	75	-	-
	100	7	7
	125	8	8
Shigella flexneri			
	25	-	9
	50	-	11
	75	-	12
	100	-	13
	125	-	14

– Negative, 1.0-3.0 resistant, 4.0-11 susceptible, < 11 highly susceptible

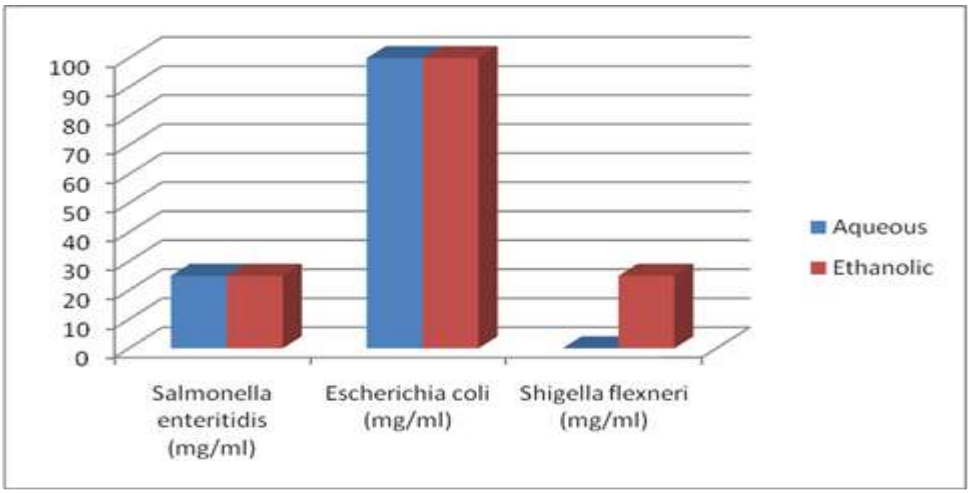

Figure 2: minimum Inhibitory Concentration (mg/ml) for the Extracts

Table 3: Zones of Inhibition (mm) in the Control Experiment

Control/Concentration	*Escherichia coli* (mm)	*Shigella flexneri*(mm)	*Salmonella enteritidis*(mm)
Gentamycine (0.5mg/ml)	8	13	26
DMSO	-	-	-

– Negative

IV. Conclusion

The result from this work shows that Ocimum gratissimum have antimicrobial properties either extracted using ethanol or extraction with water. This implies that the plant is a potential source for drug production for the treatment of diseases/infections caused by the test organism. This also supports traditional application of the plant in the treatment of infections that can be caused by the test organisms as well. This result can throw more insight as to how best this plant can be used in the treatment of the diseases caused by the test organisms, further toxicological studies, pharmacological evaluation, mode of action of the bioactive substances in the plants and in-vivo analysis is recommended for further studies.

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