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Sensitivity of *Vernonia amygdalina* and *Ocimum gratissimum* on Some Selected Bacteria

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ABSTRACT

In this paper, sensitivity of water and ethanolic extracts of *Ocimum gratissimum* (scent leaf) and *Vernonia amygdalina* (bitter leaf) were carried out on *Escherichia coli*, *Staphylococcus aureus and Salmonella typhi and compared with* Tetracycline and Flagyl to ascertain their efficacy. Using Disc and Ditch methods, we determine the sensitivity of the plant extracts and the drugs. The diameter of the zone of the inhibition of plant extracts concentration of 100ml was between 15 to 20mm for the aqueous extracts,17 to 27mm for the ethanolic extracts and 16 to20mmfor the drugs. Water extracts of *Vernonia amygdalina and Ocimum gratissimum were not* effective on most of the test organisms, tetracycline inhibited the growth of *Escherichia coli and Staphylococcus aureus while* Flagyl only inhibited the growth of *staphylococcus aureus*, the drugs did not inhibit the growth of *Salmonella typhi* but ethanolic plants extracts inhibited its growth. The ethanolic extracts of *Ocimum Gratissimum and Vernonia amygdalina* were the most effective on majority of test organisms than the water extracts and the drugs used, the results of this study suggest the possibility of using the ethanolic extracts of these plants in treating disease caused by the test organisms.

KEYWORD: Sensitivity, *Ocimum gratissimum*, *Vernonia amygdalina*, Disc and Ditch Method

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1. INTRODUCTION

In response to World Health Organization directive culminating in several pre-scientific bases on the efficacy of many plants used in folk medicine to treat infections. The practice of complementary and alternative medicine is now on the increase in most developing countries. World Health Organization cited medicinal plants as one of the best source to obtain a variety of drugs; Hence, plants such as Neem (*Azadirachthaindica*), cotton leaf (*Gossypium spp*), scent leaf (Ocimum gratissimum), bitter leaf (*Vernonia amygdalina*), *Aframomummeleguets, Zingiber officinale, Myristicafragrans, Eucalypt (Eucalyptus citriodora) and schum (Phyllantusamarus*) are used in traditional medicine. Antimicrobial properties of many plants have been investigated extensively [(1)] for better understanding of the medicinal properties.

Medicinal plants provide enormous projections for new drug discoveries because of the matchless availability of chemical range. The practice of herbal medicine in Africa and Asia signifies a long antiquity of human interactions with the environment [(2)]. Medical uses of plants range from the administration of the roots, barks, stems, leaves and seed to the use of extracts and decoction from the plants [(3)]. Medicinal plants were used as excellent antimicrobial agents because it contains a variety of chemical constituent in nature. Recently, much attention has been directed towards extracts and biologically active compound from popular plant species [(4)]. Plants have ability to synthesize aromatic substances such as phenolic, (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites [(5)]. These substances serve as plant defense mechanism against predation by microbes, insects, herbivores [(6)]. Sensitivity is a measure of an antibacterial activity of a substance either to inhibit or damage the bacterial cells. Different type of antibiotics and chemotherapeutic agents are being used in the treatment of one form of diseases or the other. Most antibiotics are derived from microorganisms while the chemotherapeutic agents are from plants. Most countries in the West Africa, Nigeria inclusive are blessed with forests containing arrays of different herbs, shrubs and trees.

Vernonia amygdalina belongs to the family of compositate and is used widely as vegetable and medicinal plant in the treatment of typhoid fever by local people. It is a tropical shrub, 1-3m in height with petiole leaf of about 0.006m in diameter and elliptic in shape. The leaves are dark green coloured with characteristic odour and a bitter taste. *Ocimum gratissimum* is an herbaceous plant which belongs to the habitae family. It can be found in abundant in West Africa especially in Nigeria. In Nigeria, it is found in the savannah and coastal area. The different tribes in Nigeria use the leaf extract in the

treatment of diarrhea while the cold leaf infusion are used for the relief of stomach disorder and haemorrhoid.

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistance strains. The emergence of multiple drug resistance bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases [(7)]. As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many antibiotics as stated by [(8)]. The continuous spread of multidrug resistance pathogens has become a serious threat to public health and a major concern for infection control by practitioners worldwide. In addition to increasing cost of drug regimes this scenario has paved way for the re-emergence of high frequency of opportunity and chronic infection cases in developing countries. The slow pace of newer antibiotics development coupled with the availability of fewer antimicrobial actions centered on inhibition of ergosterol synthesis has provided the need to explore nature in search of phytotherapeutic agent work with novel targets and mode of actions.

Recently,[(9)] showed that the antibacterial activity of *Vernonia amygdalina* and *Ocimum gratissimum* leaves extract on selected food borne pathogens, result to high zones of inhibition at low concentration proved the plants to be medically useful.[(10)], investigates the antibacterial activity of the extract of leaves of *Ocimum gratissimum* on *Listeria monocytogenes*. Their findings yielded great significance in health delivery system, since it could be used as an alternative treatment to orthodox antibiotics in the treatment of diseases caused by the bacterial isolates. [(11)] documented antibacterial activity of these plants on selected gram positive and negative bacterial isolates. Extracts of *Zingiber officinale, Myristicafragrans, Ocimum gratissimum,* thyme, sage, rosemary, yarrow and guava showed antibacterial activity against antibiotic resistant bacteria such as *P.aeroginosa, K.pneumonia, proteussp, Shigellasp* as reported by [(12)].[(13)] found ethanolic extracts and essential oil of *Zingiber officinale* and *Myristicafragrans* to be effective against the *Enterobactericae*. In view of increasing uses of *Vernonia amygdalina* and *Ocimum gratissimum* traditionally coupled with the popular prescription and administration as decoction of leaves, we investigate their pharmacological properties and antibacterial activities to ascertain their possible curative abilities for the acclaimed diseases.

2. MATERIALS AND METHOD

Collection and Preparation of samples

Fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina* weighing about 30gram were obtained around Lagos State Polytechnic gate at Ikorodu, Lagos State between 08-10 hours at an ambient temperature of 32^0C. *Ocimum Gratissimum* and *Vernonia Amygdalina* leaves were thoroughly rinsed using tap water and distilled water respectively then air dried for a week. Two solvents; distilled water and ethanol were used for the extraction. The aqueous extract was prepared by weighing out 20g of leaves of *Ocimum gratissimum* and *Vernonia amygdalina* each; were soaked in 100ml of distilled water in a conical flask and stirred vigorously. The combination was allowed to settle for 72hours. The ethanol extract were also prepared by soaking the leaves (20g) in 70% ethanol and allowed to settle for 72hours, the extracts were then filtered using Whatman's No. 1 filter paper and poured into amber bottle each and kept in the refrigerator at 4^oC.

The following phytochemical analysis; alkaloids, saponins, tannins, flavonoids, steroids and terpenoids were carried out on the extracts to determine the qualitative analysis of the phytochemical constituent as specified by [14] and [15]. Clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Salmonellatyphi* obtained from Microbiology Laboratory of Lagos University Teaching Hospital and Nigerian institute of medical research Yaba Lagos were used in this study to ascertain the sensitivity of the organisms to the leaf extracts. The later microorganisms were inoculated on each nutrient agar and spread uniformly using a sterile glass spreader. The antimicrobial tests of the plant extracts were afterward carried out on the test organisms using disc and ditch methods.

Ditch method

Cavities of 5mm diameter were made on the three Nutrient agars using a sterile cork borer. The agar chunks were removed with sterilized forceps. In each, plant extract of distilled water and ethanol were introduced in the cavities in quantity of 1ml of distilled water and ethanol respectively. It was allowed for 1hour on the bench for diffusion to occur before the growth of organisms commence and incubated at 37^oc for 24hours.The average readings from the cavities were taken and presented.

Disc method

Each of the discs, which are approximately 3mm in diameter, was cut from WhatmanNo. 1 filter paper. The discs were put into a Petri dish and then sterilized in the oven at 160^{o}c for 2hours.The discs were then impregnated with the extract by soaking it in the extract for 24hours. Each of the disc contained approximately 100ml of the aqueous and ethanolic extract of *Ocimum gratissimum* and *Vernonia amygdalina*. With the aid of sterilized forceps, each disc was recovered from the extract, held for a few seconds for the ethanol to evaporate before being applied aseptically unto the agar surface in a plate, which had initially being inoculate with a pure culture of the test organism.

3. RESULTS AND DISCUSSION

Table 1: Phytochemical constituent of Ocimum gratissimum Leaf (Scent Leaf)

Active ingredients	**Inference**
Alkaloids	+
Saponins	-
Tannins	+
Flavonoids	+
Steroids	+
Terpenoids	+

Table 2: Phytochemical constituent of Vernonia amygdalina Leaf (bitter leaf)

Active ingredients	**Inference**
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Steroids	-
Cardiac Glycosides	-
Terpenoids	+

Key: (+) means present, (-) means absent

Disc method

Table 3: Diameter of zone of inhibition (mm) of the water extracts (100ml) on the test organisms

Test organisms	Scent leaf	Bitter leaf	Scent leaf + Bitter leaf	Tetracycline	Flagyl
E.coli.	Nil	15mm	20mm	20mm	Nil
S.aureus	Nil	Nil	Nil	20mm	20mm
S.Typia	5mm	Nil	Nil	Nil	Nil

Table 4: Diameter of zone of inhibition (mm) of the ethanolic extracts (100ml) on the test organisms

Test organisms	Scent leaf	Bitter leaf	Scent leaf + Bitter leaf
E.coli	18mm	23mm	Nil
S.aureus	Nil	20mm	20mm
S.Typhi	20mm	20mm	Nil

Ditch method

Table 5: Diameter of zone of inhibition (mm) of the water extracts (100ml) on the test organisms

Test organisms	Scent leaf	Bitter leaf	Scent leaf + Bitter leaf	Tetracycline	Flagyl
E.coli	Nil	Nil	20mm	16mm	Nil
S.aureus	13mm	Nil	Nil	17mm	11mm
S.Typhi	Nil	Nil	Nil	Nil	Nil

Table 6: Diameter of zone of inhibition (mm) of the ethanolic extracts (100ml) on the test organisms

Test organisms	Scent leaf	Bitter leaf	Scent leaf + Bitter leaf
E.coli	17mm	12mm	Nil
S.aureus	19mm	5mm	22mm
S.Typhi	24mm	27mm	Nil

After the plants were extracted using ethanol and water, the ethanolic extract color was dark green while the water extracts was brown, the phytochemical screening of the plant extract of Ocimum gratissimum revealed the presence of Alkaloid, Tannins, Flavonoids**,** Steroids, Terpenoids; Vernonia amygdalina reveals Alkaloids, Saponins, Tannins, Flavonoids**,** Terpenoids. In table 3 (Disc method),

aqueous extract of Ocimum gratissimum has no inhibition on E. coli and S. aureus but S. typhi with low inhibition. On bitter leaf only E.coli has diameter of 15mm; other bacteria show no inhibition as well combination of the two leaves except E.coli with 20mm. however, in table 4 with ethanolic extract of scent leaf, all the bacteria except S. aureus grows; E. coli and S. typhi.have zone of inhibitions of 18mm and 20mm respectively. Bitter leaf ethanolic extract was very sensitive to all bacteria with 23mm, 20mm and 20mm respectively. The combination of the two ethanolic extract shows an inhibition on only S.aureus.

Using ditch method in table 5 with aqueous extract of scent leaf, only S. aureus showed inhibition of 13mm; but no inhibition of all the three organisms with bitter leaf aqueous extract. The combination of the two aqueous extract of the leaves only has inhibition on E.coli with 20mm. ethanolic extract of shown in table 6 for scent leaf showed inhibition on all the three selected organisms with 17mm, 19mm and 24mm respectively. While bitter leaf extract also show inhibition zone of 12mm, 5mm and 27mm where as scent leaf and bitter leaf extract together only inhibition S. aureus with 22mm. Tetracycline was only sensitive to E. coli and S.aureus with zone of inhibition between 16-20mm while Flagyl was sensitive to only S.aureus.

In the overall result, disc and ditch method were both effective for the sensitivity test on the selected organism, but the disc method seem to be more effective due its higher inhibitory zone. Also, it appeared that ethanolic extract of the leaves are more effective than aqueous extract. Nevertheless the two leaves namely scent leaf and bitter leaf are effective against the three organisms is just depend on the method employed and solvent used for extraction of leaves.

4. CONCLUSION AND RECOMMENDATION

Although, the condition at which the leaves would work at in vivo might be different from those used in vitro which could also affect the result obtained; but it may likely have some adverse effect on administration. This may be due to impurities, synergistic effect of the active ingredient or compound made up with. Also from the fact that dosage is not even specific and effective compound be seasonal; hence the effectiveness to inhibit the organism might not be rely on [(5)].In conclusion, the concentration used could be increased to get better inhibitory activities on the selected organisms, increase the range of organisms used and know the minimum inhibitory concentration of the extracts; this will help in the commercial manufacturing of these extracts that contain antibiotic properties.

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