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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF NEEM LEAVES AND LEMON GRASS ESSENTIAL OIL EXTRACTS

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ABSTRACT

*In this study, the antimicrobial activity and phytochemical constituents of neem leaves and lemon grass oil extracts were evaluated. Oil extracts of neem leaves and lemon grass were obtained by solvent extraction method using hexane and ethanol. Antimicrobial activity screening of plants' oil extracts were conducted using agar well diffusion method and the oil extracts were tested against three gram negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella specie*, *Escherichia coli*), one gram positive bacteria (*Staphylococcus aureus*) and two fungi (*Candida albicans*, *Rhizopus specie*). Phytochemical components of the ethanolic oil extracts were anthocyanin and betacyanin; quinones; terpenoids and acid for lemon grass. In addition to other phytochemicals present in lemon grass ethanolic oil extract, neem ethanolic oil extracts tested positive to flavonoids. Lemon grass oil extract shows high activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* which are representative of the three categories of microorganisms considered. Neem leaves oil extracts have relatively low activity against most of the selected microorganisms.*

Keywords: microbial resistance, lemon grass extract, neem leave extract, bioactive compounds, susceptibility mechanism.

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1. INTRODUCTION

There have been cases of microbial resistance to antibiotics, mostly, in some developing countries and better understanding of medicinal potentials of plants could give great scientific and medical insights towards strategic and effective approach to combat drug resistant microbes. Plants, unarguably serve as major source of food for most herbivores. It is however important to iterate that not only can plant provide the dietary requirements for living organisms, some plants are found useful in folk medicine for combating several health challenges and the medicinal values of a lot of plants still remains largely unexplored due to lack of in depth knowledge about the potentials of these plants. The ability of some plants to be used for medicinal purposes have been linked to the existence of certain chemically active substances in various parts of these plants and their extracts. These active substances are known as phytochemicals and are capable of producing specific physiological action on target organisms[1], [2].

Plants produce an extensive range of phytochemical components which are secondary metabolites [3]. These secondary metabolites work uniquely and are used directly or indirectly in the pharmaceutical industry. Phytochemicals have the ability to act as antioxidants by preventing cell damages which is usually caused by free radicals such as those associated with heart disease and cancer. Examples of phytochemicals with antioxidant activity are allyl sulphides, flavonoids, polyphenols and caternoids [4], [5]. Phytochemicals are also known to possess the ability to interfere with enzyme action. For instance, indoles stimulate enzymes that make estrogen less potent, and thus could lessen the risk of cancer of the breast [4]. The ability of Phytochemicals to interfere with DNA replication has been documented; in cases where harmful cells are present in a biological system, phytochemicals have prevented the replication of those cells [6], [7]. Phytochemicals have the ability to act like hormones. For example, isoflavones can imitate human estrogen and help decrease menopause symptoms and osteoporosis. Phytochemicals are known to possess the capability to destroy or impede the growth of bacteria. For instance, allicin is one of the phytochemicals known to possess anti-bacterial properties. Phytochemicals help in the prevention of the adhesion of pathogens to cell walls. For example, proanthocyanidins have anti-adhesion tendencies which help reduce the risk of urinary tract infections and enhance dental health. Due to the presence of diverse phytochemicals in essential oils and other plant extracts, there is high potential for extracts from different plants to have anti-microbial properties. The varied phytochemical constituents and unique functioning of each phytochemical, if properly investigated and harnessed could lead to discovery of novel substances and active compounds that are effective against seemingly drug resistant microorganisms while simultaneously alleviating several of the side effects that usually accompany synthetic antimicrobials

In this study, the antimicrobial activity of neem plant and lemon grass essential oil extracts is examined with the use of the following microorganisms; *Klebsiella specie*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Rhizopus specie*. The antimicrobial activities of neem crude oil extracted with hexane, neem crude oil extracted with ethanol, commercial neem plant essential oil and lemon grass crude oil extracted with ethanol are examined by the means of agar well diffusion method on the aforementioned microorganisms.

2. MATERIALS AND METHODS

2.1. Plant Material and Essential Oil Extraction

Neem leaves and Lemon grass were collected from the covenant university farm and covenant university campus respectively in Ota, Ogun State, South West of Nigeria. The plants collected were washed with distilled water before crushing for extraction. Extraction of the oil was carried out using method described by Ojewumi et al. [8]. With the aid of the Soxhlet apparatus, hexane and ethanol were used to extract oil from the neem leaves while extraction of oil from lemon grass was done using ethanol. The crushed leaves were placed in a muslin cloth, tied and put in the extraction chamber of the Soxhlet apparatus. The mixture of solvent and oil extracts collected at the boiling flask is distilled to separate the solvent from oil extracts. The oil extracts were stored in silica vials with Teflon caps at room temperature in cool cupboards until the antimicrobial screening and phytochemical analysis were carried out.

2.2. Bacterial Strains

The clinical isolates of *Klebsiella specie*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Rhizopus specie* were obtained at the Department of Biological Sciences Covenant University, Ota, Ogun State for the tests against neem leaves and lemon grass oil extracts.

2.3. Antimicrobial Screening

The agar well diffusion method as described by Adebayo et al. [9] was used in stating the antimicrobial activity of neem leaves and lemon grass oil extracts. For the method employed in determining the antimicrobial activity of the oil extracts, 0.1 ml of the broth culture of each isolate was inoculated in 18 ml Mueller Hinton agar plates in sterile petri-dishes in which 4 mm holes were bored to insert 1000 µl of essential oil or oil extract (as the case may be). Gentamicin was used as control in another plate, which was setup with 1000 µl of 70% methanol. The plates were allowed to stand for 1hr at ambient temperature to permit adequate diffusion of the oil after which they were incubated at 37 °C for 24 hr. until there was an obvious show of waning in the potency of the plant oil extract to impede the growth of the test isolates. The experiment was carried out in duplicates. The zone of inhibition was measured in millimeters and average values were calculated and recorded.

2.4. Minimum Inhibitory Concentration (MIC) Analysis

By adopting the approach presented by Adebayo et al. [9] the minimum inhibitory concentration was determined after the essential oil displayed sensitivity against the growth of the isolates, the zone of inhibition of the essential oil was above 20 mm. A 38 g/500 ml medium of Mueller Hinton Agar solution was used. It was regulated and dispersed into McCartney bottles and sterilized in an autoclave at 121 °C for 15 min. the agar solution was cooled to 45 °C and each graded solution was poured into petri-dishes and let to solidify for 1 hr. Extract concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.562 and 0.781 mg/ml were prepared by serial dilution. The plates were then divided into sections and labelled appropriately. The 5 mm diameter paper discs were placed into each labelled section of the plate aseptically with the aid of sterilized forceps. 0.1 ml of each isolate was injected into the labelled paper discs on the agar plates appropriately with the aid of an automatic micropipette. The plates were incubated for 24 hr at 37 °C after which they were observed to determine the growth or death of the test organism. The lowest concentration inhibiting growth was considered to be the minimum inhibitory concentration.

2.5. Minimum Bactericidal Concentration (MBC) Analysis

Determining the minimum bactericidal concentration is done using sterilized Mueller Hinton Agar. The paper discs used during the minimum inhibitory concentration tests were reactivated using a mixture of 0.5% egg lecithin and 3% Tween 80 solution Adebayo et al. [9]. The isolates were uniformly streaked on labelled quadrants using a wire loop after the reactivated isolates were sub – cultured into the quadrants of the sterilized Mueller Hinton Agar plates. The isolates were incubated at 37 °C for 24 hr after which growth was observed and recorded, the minimum bactericidal concentration is the quadrant with the lowest concentration of the essential oil without growth.

2.6. Phytochemical Analysis

The following methods were used for qualitative phytochemical analysis of oil extracts from neem leaves and lemon grass according to Guevara [10] and Harborne [11].

Test for Carbohydrates: 1 ml of Molisch's reagent was added to 2 ml of the oil extract after which a few drops of concentrated sulphuric acid was added. A purple colouration depicts the presence of carbohydrates.

Test for Tannins: 2 ml of 5% ferric chloride was added to 1 ml of oil extract. A greenish black colouration depicts that tannins are present.

Test for Saponins: 2 ml of distilled water was added to 2 ml of oil extract and shaken for 15 minutes. Foam formation indicates that Saponins are present.

Test for Flavonoids: 5 ml of dilute NH_3 solution was added to 1 ml of oil extract prior to the addition of concentrated sulphuric acid. A yellow colouration depicts that flavonoids are present.

Test for Anthocyanins and Betacyanins: 1 ml of 2N NaOH was added to 2 ml of oil extract and then heated for 5 minutes at 100°C. A yellow colouration depicts their presence.

Test for Alkaloids: 2 ml of concentrated HCl was added to 2 ml of oil extract before a few drops of Mayer's reagent were added. A greenish colouration depicts that alkaloids are present.

Test for Quinones: 1 ml of concentrated H_2SO_4 was added to 1 ml of oil extract. A red colouration depicts that quinones are present.

Test for Cardiac Glycosides: 2 ml of glacial acetic acid and a few drops of 5% ferric chloride was added to 0.5 ml of oil extract before 1 ml of concentrated sulphuric acid was added to the mixture. A brown ring formation at the interface depicts that cardiac glycosides are presence.

Test for Terpenoids: 2 ml of chloroform and concentrated H_2SO_4 was added to 0.5 ml of oil extract. A red brown colouration at the interface depicts that terpenoids are present.

Test for Phenols: 2 ml of distilled water and a few drops of 10% ferric chloride was added to 1 ml of oil extract. A green colouration depicts that phenols are present.

Test for Acids: Sodium bicarbonate solution was added to 1 ml of oil extract. Formation of effervescence depicts the presence of acids.

Test for Glycosides: 3 ml of chloroform and 10% NH_3 solution was added to 2 ml of oil extract. A pink colouration depicts that glycosides are present.

3. RESULTS

3.1. Antimicrobial Screening

Table 1 Antimicrobial analysis result, zone of inhibition (mm)

Organisms	Control-Gentamicin	Neem oil extracted with hexane	Neem oil extracted with ethanol	Commercial Neem oil	Lemon grass oil extracted with ethanol
-Klebsiella specie	20	5	15	7	18
-Escherichia coli	10	4	4	10	25
+Staphylococcus aureus	20	10	4	4	30
-Pseudomonas aeruginosa	15	6	4	12	4
Candida albicans	-	24	30	4	21
Rhizopus specie	-	11	12	10	12

3.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration Analysis

Table 2 MIC and MBC analysis results

Organism	Lemon grass oil		Neem oil extracted with ethanol via		Neem oil extracted with hexane	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	3.125	1.562	-	-	-	-
Escherichia coli	3.125	1.562	-	-	-	-
Candida albicans	1.562	0.781	12.5	6.25	25	12.5

3.3. Phytochemical Analysis

Table 3 phytochemical analysis result

NAME OF PHYTOCHEMICAL	NEEM OIL	LEMON GRASS OIL
Alkaloid test	-	-
Carbohydrate test	-	-
Saponins test	-	-
Flavonoid	+	-
Anthocyanin and Betacyanin test	+	+
Quinones	+	+
Glycosides test	-	-
Cardiac glycosides test	-	-
Terpenoids test	+	+
Phenols	-	-
Acids	+	+
Tannins	-	-

Key: Detected: + Not Detected: -

4. DISCUSSION

The results in table 1 reveal that neem plant essential oil has low antimicrobial activity when compared to that of lemon grass. The antimicrobial activity of the essential oils varies with each organism tested against and most of the samples of oil investigated have high activity against *Candida albicans*. *Candida albicans* which is a fungus have the largest zone of inhibition at 30 mm for neem oil extracted with ethanol and the lowest zone of inhibition at 4 mm for commercial neem oil. Ethanolic lemon grass oil extract shows high activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The mentioned organisms are gram negative bacteria, gram positive bacteria and fungus respectively. This indicates ethanolic lemon grass oil extract effectiveness against a larger range of organisms than neem plant oil extract.

Neem oil extracted with both solvents has low sensitivity to all bacteria tested against but has high sensitivity to *Candida albicans* which is a fungus. *Candida albicans* have been considered as prototypical organisms for studying fungal pathogens and it is part of the natural human microflora that ordinarily exists harmlessly in gastrointestinal tract, genitourinary tracts and the oral cavity [12]. However, despite its benign nature under normal circumstances, in immunocompromised individuals, recalcitrant infections ranging from mild and severe cutaneous infections, oral candidiasis, vaginal candidiasis and life – threatening systemic infections can be suffered [12], [13]. Based on the literature information on *Candida albicans* and by drawing inference from the results in Table 1, the activity of neem leaf extract against *Candida albicans* can be explored in mouthwash formulae to maintain oral health, and in the formulation of other products in which action against fungi is required for the benefit of skin and human system.

In comparing the effects of the oils against the microorganisms, each sample of oil gives a unique reaction to the microorganisms, lemon grass oil extract shows high activity towards *Staphylococcus aureus* which is gram positive bacteria more than any other organisms tested against. Commercial neem plant essential oil and neem leaf oil extracted with ethanol show more activity against gram negative bacteria than against gram positive bacteria. Neem leaf oil extracted with hexane shows higher activity against gram positive bacteria than against gram negative bacteria. In general, most of the samples of oils show a good level of activity against gram negative bacteria which indicates that gram negative bacteria are slightly more susceptible to the oils than gram positive bacteria. Understanding the mechanism of susceptibility of microbes to antimicrobial agents or techniques should be of scientific interest as it could lead to breaking new grounds in the field of medicine and healthcare. In a research conducted by Mai – Prochnow et al. [14], the team discovered that gram positive and gram negative bacteria exhibited varied sensitivity to cold atmospheric – pressure (CAP), which is the technique of antimicrobial activity being investigated by the researchers. These researchers reported correlation between the efficacy of the adopted method and bacterial cell wall thickness. Also, they observed that when planktonic cultures of bacteria were subjected to CAP, gram positive bacteria were less susceptible. However, when the bacteria were grown in co – culture, gram negative species of bacteria was more resistant to the investigated antimicrobial method. In addition to the submission that mechanism of susceptibility could be investigated for deeper understanding and cutting edge discovery, the results presented in Table 1 also indicate that, apart from the plant source, the methods used in oil extraction and where applicable, the processing the oils undergo after extraction also contribute to the antimicrobial activity that the plant extracts will possess. For oils proven to have high sensitivity (values > 20mm) to the isolates tested against, Table 2 shows the minimum concentrations of ethanolic lemon grass oil extract, ethanolic neem oil extract and hexane extracted neem oil required to inhibit the growth of microorganisms.

The phytochemicals detected in the oil samples are presented in Table 3. The results obtained show that neem oil contains flavonoid; anthocyanin and betacyanin; quinones; terpenoids; and acids. Whereas lemon grass oil also contains anthocyanin and betacyanin; quinones; terpenoids; and acids, flavonoid was not detected in it. One of the factors that may have affected the phytochemicals detected in the extracts include the level of solubility of the phytochemical. The presence of these phytochemicals in the extracts means that both neem oil and lemon grass oil can both be used in herbal treatment. The neem oil may serve culinary purposes and possess anti – inflammatory properties because of the presence of flavonoids in it [15]. Also, the presence of appreciable amount of flavonoid have been linked with good free radical scavenging activity of *N. latifolia* ethanolic extract which is an attribute of excellent antioxidants. This imply that the neem oil extract in this study may possess more unique antioxidant property when compare with the lemon grass oil extract [16].

Anti – oxidant and anti – inflammatory activities of betacyanin have also been documented [17]. The presence of terpenoids in both oils make it possible for them useful for dietary purposes such as in the management of metabolic disorders which are induced by obesity [18] and also justifies the use of neem leave extract for the treatment of diabetes in traditional medicine. Some of the identified roles of quinones in medicine include anti – cancer, anti – aging and arteriosclerosis agents [19]. The synergistic activities of these phytochemicals may partly be responsible a level of anti – bactericidal and anti – fungal properties exhibited by the extract samples

5. CONCLUSION

As antimicrobial resistance to synthetic drugs and medication continues to be a dreadful and worrisome challenge in the medical field, unraveling and exploring the untapped nutritional and medicinal wealth of leaves, roots and herbs remains a potent choice in incapacitating recalcitrant infection/disease causing microorganisms. In this study, antimicrobial activity and phytochemical constituents of neem leaves and lemon grass essential oil extracts were evaluated and the results revealed that ethanolic neem oil extracts had highest activity against *Candida albicans* which is a fungus. Based on the selected microorganisms which include gram positive bacteria, gram negative bacteria and fungi, ethanolic lemon grass extracts had more appreciable activity against wider range of microbes. Ethanolic lemon grass oil and ethanolic neem leaves oil had different activity towards the microorganisms; ethanol and hexane extracted neem leaves oils had different antimicrobial behavior and also, the susceptibility of the microbes to the commercial neem oil and the laboratory solvent extracted neem oil was different. These indicate that several factors affect susceptibility of microbes to antimicrobial agents.

Five phytochemicals were detected in the neem leave oil extract while four phytochemicals were detected in the lemon grass oil extract. The synergistic actions of the phytochemical are very like to be responsible for the various anti – bactericidal and anti – fungal properties exhibited by the oils. It is suggested that more research efforts should be geared towards understanding the mechanisms of antimicrobial susceptibility of the microorganism as this will improve the chances of coming up with novel products which can effectively combat seemingly drug resistance microbes.

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