

Full length Research Paper

Comparison of antimicrobial potential of *Piper umbellatum*, *Piper guineense*, *Ocimum gratissimum* and *Newbouldia laevis* extracts

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The antimicrobial potential of seeds of *Piper umbellatum* Linn. and *Piper guineense* Schum and Thonn. and leaves of *Ocimum gratissimum* Lin. and *Newbouldia laevis* have been reviewed. The results revealed that all the plant extracts showed antimicrobial activity to various extents against three organisms tested; *Coliform bacilli*, *Staphylococcus aureus* and *Salmonella typhi*. The ethanol extract of *O. gratissimum* exhibited the greatest antimicrobial activity against the microorganisms, followed by *P. umbellatum* extract while *N. laevis* leaf extract showed the least activity. The comparative susceptibility of each microorganism to different extracts showed that *P. umbellatum* exhibited the greatest activity against *Coliform bacilli* with inhibition zone diameter of 27mm at 1.0mg/ml concentration while *O. gratissimum* showed the greatest activity against *Salmonella spp.* with inhibition zone diameter of 28 mm at the same concentration. *P. guineense* showed the greatest activity against *S. aureus* with inhibition zone diameter of 35 mm at 1.0mg/ml concentration. Thus, a mixture of these plants could provide a herbal formula for the treatment of diseases caused by these microorganisms.

Keywords: Antimicrobial, microorganisms, plants.

INTRODUCTION

In Nigeria and other developing nations of the world, especially in Africa, many low income earners and residents of remote villages rely almost exclusively on traditional medicine for the treatment of several diseases, including; malaria, convulsion, epilepsy, infertility, dysentery, etc. Extracts from barks, leaves, roots or seeds of plants (in local gin) are used in the preparation of syrups or other medical formulations. Medicinal plants often used in such preparations include *P. guineense* seeds (Uzizza, in Igbo language) usually given to nursing mothers, for the normalization of the womb after delivery and leaves of *N. laevis* ("Tree of life" or "Fertility plant", also called "Ogirishi", in Igbo language) often administered as fertility formula in cases of infertility.

The antimicrobial potential of plant extracts have been investigated by several workers (Anesini and Perez, 1993; Jussi-Pekka *et al.*, 2000; Adesokan *et al.*, 2007; Mahesh and Satish, 2008; Kotzekidou *et al.*, 2008

Bakkali *et al.*, 2008; Alinnor and Ejele, 2009). Anesini and Perez (1993) tested 122 known plant species often used for local treatment of several diseases and found that twelve plant species inhibited growth of *staphylococcus aureus*, ten were effective against *Escherichia coli* while four inhibited *Aspergillus niger*. Jussi-Pekka *et al.*, (2000) carried out the antimicrobial screening of 13 phenolic substances and 29 plant extracts against selected microbes and found that some of the phenolic compounds (such as flavone, quercetin and naringenin) were active and inhibited the growth of the microorganisms. Similarly, some plant extracts also inhibited the growth of different microorganisms and showed strong inhibition effects against gram-positive *Staphylococcus aureus*.

Adesokan *et al.* (2007) studied the aqueous extract of the stem bark of *Enantia chlorantha* and found that it possessed broad-spectrum antibacterial activities; with gram-positive bacteria showing more susceptibility to the extract at all concentrations. The authors concluded that the broad-spectrum antibacterial activity of the plant

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extract was possibly due to the presence of alkaloids. Mahesh and Satish (2008) screened local medicinal plants for antibacterial and antifungal activities. The results showed that some extracts possessed antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, etc, in addition to antifungal and anti-inflammatory properties. The authors concluded that the antibacterial and antifungal activities varied with species of plants and plant materials used.

Kotzekidou *et al.* (2008) studied the efficacy of commercially available plant extracts and oils used in confectionery products as antimicrobials using the disc diffusion method. The microorganisms studied included *Escherichia coli*, *Salmonella Enteritidis*, *Salmonella Typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. The authors observed inhibition zone diameters >20 mm and found that *E. coli* strains were the most susceptible microorganisms inhibited by 18 extracts, followed by *S. Typhimurium* and *S. aureus* which were inhibited by 17 extracts. Lemon flavour, lemongrass essences, pineapple and strawberry flavour inhibited the foodborne pathogens at the lowest concentration. Kotzekidou *et al.* (2008) also tested the plant extracts and essential oils with potent antimicrobial activities in chocolate at 7° and 20°C and found that the highest inhibition was performed by lemon flavour applied on chocolate inoculated with *E. coli* culture. The authors concluded that plant extracts tested on chocolate showed an enhanced inhibitory effect indicating that their application may provide protection in case of storage at 20°C or higher temperatures.

Alinnor and Ejele (2009) studied the antimicrobial properties of crude extracts of *Gongronema lafolium* using ethanol, toluene and distilled water as solvents and observed that ethanol extract inhibited growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Coliform bacilli*. In this paper, we report on the phytochemistry and antimicrobial potential of crude extracts of seeds of *P. umbellatum* and *P. guinense* and leaves of *O. gratissimum* and *N. laevis* in an attempt to relate the phytochemistry and antimicrobial potential of the medicinal plants.

MATERIALS AND METHODS

Sample Collection and Extraction

The seeds of *P. umbellatum* and *P. guinense* as well as leaves of *O. gratissimum* and *N. laevis* were obtained from the open market in Owerri, Imo State of Nigeria, and authenticated as such in Department of Plant Science and Technology, Federal University of Technology, Owerri. The samples were sun-dried and ground to semi

powder. 30 g of each sample was extracted separately with 300 ml of ethanol for 12 h in a soxhlet extractor equipped with a reflux condenser. The ethanol extracts were allowed to evaporate at room temperature to give gel-like solids, which were dissolved in ethanol/water mixture (4:1) and filtered. The filtrates were used without further purification for preliminary phytochemical screening and antimicrobial experiments.

Antimicrobial Tests

The experiments were carried out in Microbiology Department, Federal Medical Centre, Owerri, Imo State of Nigeria. Test microorganisms used were *Coliform bacilli*, *Salmonella typhi* and *Staphylococcus aureus* and the method used was Agar disc diffusion method. An inoculating loop was touched to three isolated colonies of the test bacteria on an agar plate and used to inoculate a tube of culture broth, which was incubated at 35 –37°C until it became slightly turbid and was diluted to match the turbidity standard. Then a sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of the agar plate. After the agar surface has dried for 5 minutes, the appropriate extract test disks were placed with a multiple applicator device. The agar plate was incubated at 35-37°C for 16-18 hours, after which the diameters of inhibition zones (areas showing little or no microbial growth) were measured to the nearest mm (Garred and O-Graddy, 1983).

Determination of Minimum Inhibitory Concentration (MIC)

The determination of MIC was carried out to obtain an idea of the antibacterial activities of the plant extracts, since agar disc diffusion assay is a quantitative method based on the method of European Society of Clinical Microbiology and Infectious Diseases (2000) for evaluation of antimicrobial potential. Standard solutions of extracts were prepared: 1.0mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml and 0.0625mg/ml, in agar nutrient and distributed into sterile test tubes. One milliliter of each extract dilution was separately added into the agar plates and poured into Petri-plates. The test microorganism was spotted onto the surface of the solidified extract-agar mixture and the plates were inoculated, starting from the lowest concentration to the highest concentration. After inoculation the plates were allowed to dry for 30 min and incubated at 37°C for 18h, after which the samples were examined for microbial growth. The lowest concentration of the extract which showed little or no visible growth of the microorganism

Table 1. Preliminary antimicrobial screening

Test microorganism	<i>Piper umbellatum</i> (seed)	<i>Piper guineense</i> (seed)	<i>Ocimum gratissimum</i> (leaf)	<i>Newbouldia laevis</i> (leaf)
<i>Coliform bacilli</i>	++	+	++	+
<i>Salmonella typhi</i>	++	+	++	++
<i>Staphylococcus aureus</i>	+	+++	++	++

+++ = Strongly Positive, ++ = Positive, + = weakly positive; - = very weak

Table 2. Minimal Inhibitory Concentration of *P. umbellatum* seed extract

Concentration	1.0mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
<i>Coliform bacilli</i>	27mm	24mm	20mm	10mm	7.5mm
<i>Salmonella typhi</i>	21mm	15mm	8mm	2.0mm	-
<i>Staphylococcus aureus</i>	15mm	10mm	4.5mm	-	-

Table 3. Minimal Inhibitory Concentration of *P. guineense* seed extract

Concentration	1.0mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
<i>Coliform bacilli</i>	7mm	2.5mm	-	-	-
<i>Salmonella typhi</i>	5mm	1.5mm	-	-	-
<i>Staphylococcus aureus</i>	35mm	24mm	15mm	9mm	4.5mm

Table 4. Minimal Inhibitory Concentration of *O. gratissimum* leaf extract

Concentration	1.0mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
<i>Coliform bacilli</i>	21mm	14mm	9mm	—	—
<i>Salmonella typhi</i>	28mm	22mm	16mm	12mm	8.5mm
<i>Staphylococcus aureus</i>	25mm	18mm	12mm	7.5mm	-

was taken as the MIC of the extract (Garred and O-Graddy, 1983).

Phytochemical Analysis of Extracts

The ethanol/water filtrates of different extracts were used for preliminary phytochemical screening. The following phytochemicals were tested for using standard methods: alkaloids, aldehydes / ketones, amino acids, carboxylic acid, cardio-active glycosides, esters, flavonoids, phenols, saponins, tannins and steroid / triterpenes. The results are presented (Table 7).

RESULTS AND DISCUSSION

Table 1 presents the results of preliminary tests for antimicrobial activities of the plant extracts. The results revealed that all the plant extracts showed antimicrobial activity against the three organisms tested to various extents. The extract of *O. gratissimum* appeared to exhibit the greatest antimicrobial activity against the microorganisms, followed by the extract of *P. umbellatum* while the *N. laevis* extract appeared to show the least activity against the organisms.

Tables 2 to 5 showed the MIC for the various plant extracts with the inhibition zone diameters ranging from

Table 5. Minimal Inhibitory Concentration of *N. laevis* leaf extract

Concentration	1.0mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
<i>Coliform bacilli</i>	12mm	8mm	–	–	–
<i>Salmonella typhi</i>	25mm	18mm	12mm	8.5mm	–
<i>Staphylococcus aureus</i>	28mm	20mm	15mm	10.5mm	7mm

Table 6. Susceptibility of Extracts against Microorganisms [Conc = 1.0mg/ml]

Plant Extract	<i>Coliform bacilli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
	1.0mg/ml	1.0mg/ml	1.0mg/ml
<i>P. umbellatum</i>	27mm	21mm	15mm
<i>P. guineense</i>	7mm	5mm	35mm
<i>O. gratissimum</i>	21mm	28mm	25mm
<i>N. laevis</i>	12mm	25mm	28mm

7–35mm for different extracts at varying concentrations. It could be seen from the Tables that *P. umbellatum* seed extract showed greatest activity against *Coliform typhi* with MIC of 0.0625mg/ml and the least activity against *S. aureus* with MIC of 0.25mg/ml.

For the *P. guineense* seed extract, the greatest activity was against *S. aureus* with MIC of 0.0625mg/ml and the least activity was against *S. typhi* with MIC of 0.50mg/ml. The greatest activity of *O. gratissimum* leaf extract was recorded against *S. typhi* with MIC of 0.0625mg/ml while its least activity was against *C. bacilli* with MIC of 0.25mg/ml. *N. laevis* leaf extract showed its greatest activity against *S. aureus* with MIC of 0.065mg/ml and least activity against *C. bacilli* with MIC of 0.50mg/ml. Thus, a mixture of these plants could provide herbal formulation that would be very effective against these and other microorganisms

The comparative analyses of susceptibility of each microorganism to different extracts were presented in Tables 6. The susceptibility of *Coliform bacilli* to different extracts showed that *P. umbellatum* exhibited the greatest inhibitory zone of 27mm while *P. guineense* showed the lowest activity against this microorganism with a zone of inhibition of 7mm at 1.0mg/ml concentration respectively. The susceptibility of *Salmonella typhi* to the extracts showed that *O. gratissimum* exhibited the greatest inhibitory zone of 28mm while *P. guineense* showed the lowest activity against the microorganism with zone of inhibition of 5mm at 1.0 mg/ml concentration respectively.

The susceptibility of *S. aureus* to the extracts showed that all the extracts exhibited inhibitory zones equal to or greater than 25mm except *P. umbellatum* with inhibitory zone of 15mm at 1.0 mg/ml concentration. *P. guineense* showed the greatest activity against this microorganism

with an inhibitory zone of 35mm at 1.0 mg/ml concentration.

The inhibition of growth and activity of microorganisms is one of the major purposes for the use of chemical preservatives in the food industry because these are able to inhibit microbial growth by interfering with cell membranes, enzyme activity or genetic mechanisms of the microorganisms. Chemical preservatives may also be used as coatings to keep out microorganisms, prevent loss of water and hinder undesirable microbial, enzymatic and chemical reactions (Fulton, 1981; Branan and Davidson, 1983). Many antibiotics have also been used as preservatives for raw foods, especially protein foods like meat, fish, poultry, etc and inhibited protein synthesis in microbial cells; but microorganisms soon became resistant to these antibiotics and new strains of these organisms developed (WHO Report 1974; Levy, 1998) hence the use of medicinal plant extracts became necessary and several reports have been published concerning the use of medicinal plants for treatment of diseases (Ouattara *et al.*, 2006; Huang *et al.*, 2006; Adesokan *et al.*, 2007; Odugbemi *et al.*, 2007; Hlaing *et al.*, 2008; Mahesh and Satish, 2008; Alinnor and Ejele, 2009; Uhegbu *et al.*, 2009);

The bactericidal effects of plant extracts have been reported and several attempts have made to destroy bacteria by the application of these extracts (Jussi-Pekka *et al.*, 2000; Smith-Palmer *et al.*, 2001; Okwu, 2005; Kotzekidou *et al.*, 2008; Ejele, 2010; Ugbo *et al.*, 2010). When compared with chemical preservatives, plant extracts were found more effective and performed better than chemical additives in terms of antimicrobial activities because they exhibited greater bactericidal effects and inhibited the action of several microorganisms (Smith-Palmer *et al.*, 2001; Kotzekidou *et al.*, 2008). In

Table 7. Phytochemical Screening of crude plant extracts

Phytochemicals	<i>Piper umbellatum</i> (seed)	<i>Piper guineense</i> (seed)	<i>Ocimum gratissimum</i> (leaf)	<i>New bouldia laevis</i> (leaf)
Tannins	+++	+++	+++	+++
Saponins	+++	+++	+++	+++
FLavonoids	+	+	+	+
Steroid / triterpenes	++	++	++	++
Cardio active glycosides	++	++	++	++
Phenols	+++	+++	+++	+++
Carboxylic acids	++	++	+	+
Esters	+	+	+	+
Aldehydes / Ketones	-	-	-	-
Alkaloids	-	-	++	++
Amino acids	++	++	++	++

+++ = Strongly Positive; ++ = Positive; + = weakly positive; - = not detected

addition, plants extracts have an advantage over chemical preservatives because they promote human health and several plant extracts are effective against various human pathogens including *Candida albicans* and *Staphylococcus aureus* (Jussi-Pekka *et al.*, 2000; Okwu, 2005; Ugbogu *et al.*, 2010). According to Jussi-Pekka *et al* (2000), plant extracts such as *Lythrum salicaria* (purple loosestrife) and *Solanum tuberosum* (potato) were the most active against *Candida albicans* and *Staphylococcus aureus* respectively.

The observed antimicrobial activities exerted by the extracts of different plants are due to the phytochemicals contained in them (Alinnor and Ejele, 2009; Adesokan *et al*, 2007). The results of phytochemical screening presented in Table 7 showed the presence of tannins, saponins, phenols, glycosides, amino acids and flavonoids in the extracts to various extents. However, aldehydes and ketones were probably absent while the presence of esters was doubtful. On the other hand, carboxylic acids were found in the seed extracts but not in the leaves while alkaloids were present in the leaf extracts but not in the seed showing that the phytochemicals varied with parts of the plant used; the same was also true of the antimicrobial activities of the plants. A similar conclusion had earlier been made by Mahesh and Satish (2008). In other words the variations observed in the antimicrobial properties of the various extracts may be due to the phytochemicals present in the different parts of the plants.

Since the middle ages, plant extracts and oils have been used widely for bactericidal, fungicidal, virucidal, antiparasitical, insecticidal, medicinal and cosmetic purposes. Even now they are often employed in sanitary, pharmaceutical, cosmetic, agricultural and food industries (Bakkali *et al*, 2008). Although the mechanism of action

of plant oils is not known with certainty, *in vitro* physicochemical assays characterize most of them as antioxidants, which possess ability to limit oxidation. The antioxidant properties of essential oils are well known and have been documented (Jussi-Pekka *et al*, 2000; Smith-Palmer *et al*, 2001; Cimanga *et al*, 2002; Bakkali *et al*, 2008; Kotzekidou *et al*, 2008) and plant phenols are currently of growing interest because they promote human health.

The plant extracts contained different phytochemicals but we cannot say (for now) which of these compounds were responsible for the observed antimicrobial properties. However, we may suspect and speculate that polyphenols (flavonoids and tannins) and/or simple phenols, which were found present in all the plant extracts may be responsible for the observed effects because these had earlier been shown to possess bactericidal, fungicidal, virucidal, antiparasitical, insecticidal, medicinal and antioxidant properties (Jussi-Pekka *et al*, 2000; Smith-Palmer *et al*, 2001; Cimanga *et al*, 2002; Bakkali *et al*, 2008; Kotzekidou *et al*, 2008).

CONCLUSION

The antimicrobial potential of the seeds of *P. umbellatum* and *P. guineense* as well as leaves of *O. gratissimum* and *N. laevis* have been studied. The results revealed that all the plant extracts showed antimicrobial activity to various extents against the three organisms tested; *Coliform bacili*, *Salmonella typhi* and *Staphylococcus aureus*. The ethanol extract of *O. gratissimum* exhibited the greatest antimicrobial activity against the microorganisms, followed by *P. umbellatum* extract while *N. laevis* leaf extract showed the least activity.

The comparative susceptibility of each microorganism to different extracts showed that *P. umbellatum* exhibited the greatest activity against *Coliform bacilli*. with inhibition zone diameter of 27mm at 1.0mg/ml concentration whereas *O. gratissimum* showed the greatest activity against *Salmonella typhi* with inhibition zone diameter of 28 mm at the same concentration. However, *P. guineense* showed the greatest activity against *Staphylococcus aureus* with inhibition zone diameter of 35mm at 1.0mg/ml concentration.

The observed antimicrobial activities exerted by the different plant extract were interpreted in terms of the presence of various phytochemicals contained in them. The results of phytochemical screening of different plant extracts showed the presence of tannins, saponins, phenols, glycosides, amino acids and flavonoids to various extents. Although we cannot say which phytochemicals were responsible for the antimicrobial activities, however, we suspect and speculate that polyphenols (flavonoids and tannins) and/or simple phenols, which were found present in all the plant extracts could be responsible for these effects because these had earlier been shown to possess bactericidal, fungicidal, virucidal, antiparasitical, insecticidal, medicinal and antioxidant properties. Therefore a mixture of these plants could provide the herbal formula for treatment of diseases caused by these microorganisms.

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