International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 1 (1): 69-74, 2007
ISSN 1819-155X

Comparative Studies on the Antimicrobial Activity of Leaf Extract from *Ocimum basilicum* and Antagonistic Activity of Isolates from Refuse on Some Selected Pathogens

Oladunmoye M. Kolawole
Department of Microbiology, School of Sciences, Federal University of Technology, P.M.B. 704, Akure, Nigeria

Abstract: Refuse samples were screened for the presence of resident bacteria and fungi antagonistic to the growth of some pathogens. Nine bacteria and four fungi were isolated. The pathogens used are clinical isolates which include; *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus*. The assay was carried out using the agar well diffusion method and the plugging method for the bacteria and fungi isolates respectively. Seven bacteria were antagonistic to at least a pathogen and all the fungi isolates showed antagonistic activity against the five pathogens at varying degrees. A 60% ethanol extract from the leaf of *Ocimum basilicum* (Labiatae) was investigated for *in vitro* antimicrobial activities against the same set of pathogens. The results showed no antimicrobial activity at concentration of 50, 100 and 200 mg mL^{-1} while at 300 mg mL^{-1} there were activities against all the pathogens except *Staphylococcus aureus*. *Bacillus cereus* and *Salmonella typhi* were found to be the most susceptible to the extract. Compared to the plant extracts, none of the bacteria isolated from the refuse showed antagonistic activity against *Klebsiella pneumoniae*. *Actinomyces* isolate was the most active of the bacteria isolates and the activity is comparable to that of the leaf extract of *Ocimum basilicum*. *Penicillium* isolates has the strongest activity of the fungi isolates and it has a broader zone of inhibition and spectrum of activity than the leaf extract.

Key words: Refuse sites, antagonistic activity, pathogens, antimicrobial efficacy, *Ocimum basilicum*

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities (Edeoga *et al*., 2005). It is estimated that there are between 200,000 and 700,000 species of tropical flowering plants that have medicinal properties (Atata *et al*., 2003). Their actions include: anti bacterial, antifungal, antiviral, anti-helminthic, antiallergic, anticarcinogenic, analgesic and larvicidal. These medicinal value lie in some chemical substances they contain. The most important of the bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga *et al*., 2005). The development of some modern drugs could not have been possible without the chemical blue print from the bioactive constituent of these plants. Medically important strains of bacteria have been found to be widely inhibited by various medicinal plants (Akinyemi *et al*., 2005).

Biological control, as classically defined is the use of microorganisms for the control of disease causing agents (Sylvia *et al*., 1999). Antagonists are microbial agents that reduce microbial population in a particular environment. Antagonism can be due to the metabolic products of microorganisms. These products include lytic agents, enzymes, volatile compounds and toxic substances (Slyvia *et al*., 1999). It could be due to a particular microorganism gaining a measure of

advantage for the usage of the limited available resources such as organic and inorganic nutrients, growth factors, oxygen or space. The antagonistic microorganisms are known to occur over a wide environmental range, especially in the soil. The refuse dump is a complex environment where biodegradation is a continuous process.

Ocimum bacilicum, commonly known as basil is a member of the family Lamiaceae and the subfamily Nepetoideae. This plant as well as the oil from it has received lots of attention for its potential medicinal properties (Adigozel *et al*., 2005). It is used as food additive because of it flavoring properties. It is used in cosmetics, liqours and perfumes. It has also been used as a folk remedy to treat various ailments such as feverish illness, poor digestion, nausea, abdominal pain, gastroenteritis, migraine, insomnia, depression, gonorrhea, dysentery and chronic diarrhea (Adigozel *et al*., 2005).

Ocimum bacilicum is an annual plant and can be grown and found throughout the world (Davegarden, 2005).

The objectives of the present investigation is to isolate characterize and identify microorganisms from refuse sites and to determine their antagonistic activity on some selected pathogens. Comparative evaluation of antimicrobial efficacy of the leaf extracts from *Ocimum basilicum* against the pathogens as well as accessed the bioactive principles of pharmacological importance in the extract.

MATERIALS AND METHODS

Test Organisms

The pathogens used are clinical isolates from the Microbiology Laboratory of University College Hospital (UCH), Ibadan, Oyo State Nigeria. The bacteria are *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Staphylococcus aureus*.

Collection of Refuse Samples and Isolation of Microorganisms

The refuse samples were collected from three different locations around Akure Township. The microorganisms associated with the refuse was isolated, characterized and identified using standard methods.

Plant Source, Extraction and Fractionation

The plant sample was purchased from herb sellers at the Oja Oba market in Akure, Ondo State Nigeria. The taxonomic identification of the plant material was confirmed by Mr. Aduloju of the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Ondo State.

The plant material was air dried and ground into uniform powder using a milling machine. It was extracted with 60% ethanol and the extract concentrated in vacuo using rotary evaporator. The extract was dissolved in 0.1 M Tris-HCL buffer (pH 7.0, 5 mL) and applied to a column (5×85 cm) of Sephacryl S-300 HR, pre-equilibrated and developed with the same buffer. Fractions corresponding to the peak were pooled together concentrated and freeze dried. The powder was dissolved in water and applied to a Sephadex G-25, column (1.5×50 cm), then eluted with water and fractions were collected. The eluate obtained was concentrated and lyophilized.

Antimicrobial and Antagonistic Activity of the Extract and Isolates

Antimicrobial activity was measured using the agar well diffusion method (Schillinger and Lucke, 1989).

Phytochemical Screening

The extract was screened for bioactive molecules using standard methods described by Sofowora (1993).

RESULTS AND DISCUSSION

A total of nine bacterial isolates and four fungi were obtained based on their morphological and biochemical properties from the refuse sites (Table 1 and 2). This suggests that the refuse site is populated by myriad if microorganisms' that were responsible for the biodegradation.

The result of the antagonistic activity of the bacterial isolates (Table 3) indicated that out of the nine organisms, seven were active against the test organisms. *Pseudomonas* specie and *Clostridium* specie did not show any antimicrobial activity against any of the test organisms. It might be that the type and the concentration of the metabolite(s) produced could not be enough to inhibit the growth of the test organisms. *Actinomyces* isolate was the most active with activity against all the test organisms except *Klebsiella pneumoniae*, with the largest zone of inhibition against *Escherichia coli* and the least against *Salmonella typhi*. This study is in conformity with Krasil (1997) who stated that there are very large quantities of actinomycete-antagonists with clearly expressed antimicrobial properties. The resistance of *Klebsiella pneumoniae* may be due to the presence of a resistance plasmid that codes for resistant gene in the cell. *Shigella* isolate was the next with a wide spectrum of activity; it showed activity against three of the five test organisms. The reason for the difference in sensitivity between gram-positive and gram-negative bacteria might be ascribed to the difference in morphological constitutions with respect to variation in the complexity of the cell wall and membrane (Mariam *et al*., 2005).

Listeria, Proteus and *Enterobacter* isolates inhibited the same set of two test organisms at varying degree, *Escherichia coli* and *Staphylococcus aureus. Acinetobacter* and *Bacillus* species inhibited a test organism each. None of the nine bacteria isolates showed activity against *Klebsiella pneumoniae*.

The antimicrobial activity of fungi is presented in Table 4. Fungi were found to be more potent in inhibiting the growth of the test organisms than the bacterial. *Penicillum* isolate was able to show activity against *Klebsiella pneumoniae,* in contrast to the result obtained with the bacteria isolates. Metabolites from fungi had been shown to have broad spectrum antimicrobial activity (Krasil, 1997). Antagonism by these bacteria and fungi isolates may be due to various specific and non specific metabolic products formed by the isolates. These non-specific substances include organic acids, alcohols, peroxides and other compounds. The specific substances are the antibiotics and bacteriocins. Similar findings have been reported by Krasil (1997). The results obtained with the fungi isolates compared favourably with those of the commercial antibiotics like ofloxacin, chloramphenicol, cefuroxine, ampicillin, gentamycin and tetracycline (Table 6).

The results of the antimicrobial bioassay of the leaf extract of this plant are shown in Table 5. Similar findings have been reported (Adigozel *et al*., 2005; Calzada *et al*., 2005). The extract was not active at low concentrations of 50, 100 and 200 mg mL^{-1}. The minimum inhibitory concentration was

Table 1: Morphological and biochemical characteristics of bacterial isolates from refuse

Isolates	Morphology	Gram reaction	Spore stain	Catalase	Coagulase	Glu	Dex	Gal	Lac	Ara	AP	GP		Identity microorganism
1	Greenish on MA spread, raised	-ve short rods	-ve	+ve	-ve	✓	✓	✓	✓	✓	+ve	-ve	Early fermenter	*Pseudomonas* sp.
2	Sharp pink, round and raised	-ve tiny rods	-ve	+ve	-ve	X	✓	✓	✓	X	+ve	-ve	Late fermenter	*Acinetobacter* sp.
3	Light pink, spread and flat	+ve short rods	-ve	+ve	-ve	✓	✓	✓	✓	✓	+ve	+ve	Early fermenter	*Listeria monocytogenes*
4	Orange, entire, raised	+ve tiny rods	-ve	-ve	-ve	✓	✓	✓	✓	✓	+ve	-ve	Early fermenter	*Actinomyces* sp.
5	Cream, entire, flat	+ve long rods	+ve	-ve	-ve	✓	✓	✓	✓	✓	+ve	+ve	Early fermenter	*Clostridium* sp.
6	Cream, entire, flat	+ve rods	+ve	+ve	-ve	✓	✓	✓	✓	✓	+ve	+ve	Early fermenter	*Bacillus* sp.
7	Whitish, spread and flat	-ve long rods	+ve	-ve	-ve	✓	✓	✓	✓	✓	+ve	-ve	Early fermenter	*Shigella* sp.
8	Cream, spread, flat	-ve long rods	+ve	-ve	-ve	✓	✓	✓	✓	✓	+ve	-ve	Early fermenter	*Proteus mirabilis*
9	Cream, entire, raised	-ve long rods	+ve	-ve	-ve	✓	✓	✓	✓	✓	+ve	+ve	Early fermenter	*Enterobacter aerogenes*

Glu = Glucose, Dex = Dextrose, Gal = Galactose, Lac = Lactose, Ara-Arabinose, AP = Acid production, GP = Gas production

Table 2: Morphological characterization of fungi

Morphology on plate	Morphology under microscope	Identity of isolates
Fluffy with green colour in dry biseptal chains	An upright conidophore that terminates in a clavate swelling and bearing phialides at the apex of radiating from the entire surface, conidia are 1-celled and globose.	*Aspergillus flavus*
Fluffy with dark colour that covers the plate	An upright conidophore that terminates in a clavate swelling and bearing phialides at the apex.	*Aspergillus niger*
Fluffy with dirty brown colour that covers the plate.	An upright conidophore that terminates in a clavate swelling and bearing phialides at the apex.	*Aspergillus repens*
Deep green mycelin that cover the plate	Septate mycelium bearing single conidiophores which are branched near the apex ending in phialides that carried the conidia.	*Penicillium* sp.

Table 3: Antagonistic activity of bacterial isolates from refuse against test organisms

	Zones of inhibition (mm) test organisms								
	Isolate								
Test organisms	1	2	3	4	5	6	7	8	9
Bacillus cereus	NI	NI	NI	6.0	NI	NI	9.0	NI	NI
Escherichia coli	NI	NI	8.5	7.0	NI	4.0	7.0	5.0	9.0
Klebsiella pneumoniae	NI	NI	NI	NI	NI	NI	NI	NI	NI
Salmonella typhic	NI	NI	NI	3.5	NI	NI	NI	NI	NI
Staphylococcus aureus	NI	5.5	4.0	6.5	NI	NI	9.0	6.0	6.5

NI = No inhibition

Table 4: Antagonistic activity of fungi isolates from refuse against test organisms

	Zones of inhibition (mm)			
Test organisms	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Bacillus cereus	11.0	3.0	11.0	4.0
Escherichia coli	2.0	3.0	11.0	3.0
Klebsiella pneumoniae	3.0	2.0	12.0	2.0
Salmonella typhic	3.0	4.0	12.0	2.0
Staphylococcus aureus	4.0	4.0	13.0	4.0

Table 5: Minimum Inhibitory Concentration (MIC) of leaf extract of *ocimum basilicum* against test organisms

	Zones of inhibition (mm)			
	50	100	200	300
Test organisms	mg mL^{-1}			
Bacillus cereus	NI	NI	NI	6.5
Escherichia coli	NI	NI	Ni	6.0
Klebsiella pneumoniae	NI	NI	NI	4.0
Salmonella typhi	NI	NI	NI	6.5
Staphylococcus aureus	NI	NI	NI	NI

NI = No inhibition

Table 6: Antimicrobial activity of antibiotics against test organisms (positive control)

	Zones of inhibition (mm)									
Test organisms	OF	C	CF	AM	GN	N	CIP	TE	NB	AX
Escherichia coli	17	7	NI	NI	4.5	2	17	7	12	2
Klebsiella pneumoniae	7	NI	NI	NI	NI	NI	5	NI	NI	NI
Salmonella typhi	16	10	NI	4	4	2	18	8	12	5
	OF	E	CIP	CD	GN	CX	CO	AP	FX	AU
Bacillus cereus	14	23.5	23.5	4	NI	NI	NI	NI	NI	NI
Staphylococcus aureus	29	42	45	9	NI	NI	NI	NI	NI	NI

NI = No inhibition; OF = Ofloxacin; C = Chloramphenicol; E = Erythromycin, CIP = Ciprofloxacin; CD = Clindamycin; CF = Cefuroxine; AM = Ampicilin, GN = Gentamycin; N = Nitrofurantion; CX = Cephaplexin; CO = Cotrimoxazole; TE = Tetracycline; NB = Norfloxacin; AX = Amoxycillin; AP = Ampicillin/Cloxacillin; FX = Floxapen; AU = Augumentin

Table 7: Qualitative analysis of the phytochemicals in the leaf extract of *Ocimum basilium*

Chemical constituents	
Alkaloids	-ve
Anthraquinones	+ve
Cardiac glycosides	+ve
Flavonoids	+ve
Phylobatannins	-ve
Saponins	+ve
Tannins	+ve
Terpenoids	+ve

+ve: Presence of constituent, -ve: Absence of constituent

fond to be at 300 mg m L^{-1} and the highest antimicrobial activity was observed against *Bacillus cereus* and *Salmonella typhi* with both having zones of inhibition of 6.5 mm. The antimicrobial action could have been due to inhibition of protein synthesis or other means apart from the inhibition of cell wall synthesis. The antimicrobial activity of the leaf extract against *Escherichia coli* at a concentration of 300 mg m L^{-1} was found to be almost comparable to chloramphenicol, gentamycin, nitrofurantion and tetracycline.

The phytochemical screening of the leaf extract of *Ocimum basilicum* (Table 7) showed that the leaves were rich in anthraquinones, cardiac glycosides, flavonoids, saponins and tannins. These bioactive molecules were known to show medicinal activity as well as exhibiting physiological and antimicrobial activities (Vlictinck and Pieters, 2005).

Comparative evaluation of the antimicrobial activity of the leaf extract and the isolates (Table 3-5), revealed that the leaf extract had a broader spectrum of activity than most of the bacteria isolates. The fungi isolates are more active than the leaf extracts. These observations may be attributed to the nature of the biological active components present in the plant (anthraquinones, flavonoids, saponins, tannins and terpenoids). It has been documented that tannins, saponins and alkaloids are plant metabolites well known for antimicrobial activity (Akinyemi *et al*., 2005). Also, antibiotics produced from bacteria are commonly known to be narrow in spectrum of activity while the antibiotics from fungi are broad in spectrum of activity (Krasil, 1997).

CONCLUSION AND RECOMMENDATION

The results of this study may suggest that *Ocimum basilicum* extracts possesses compounds with antimicrobial properties against the pathogenic bacteria, therefore offer a scientific basis for the traditional use of the leaf to cure infectious diseases. Also, it suggests that microbial isolates from refuse dumps can produce metabolites with antimicrobial activities against the some pathogens. It was also evident that the leaf extract of *Ocimum basilicum* is comparable to the fungi isolate, with regards to their spectrum of activity against the pathogens. The leaf extracts and the fungi isolates have a broader spectrum of activity when compared with the bacteria isolates. Therefore the leaf extract may be a better source for drug development.

In vivo study is necessary and should seek to determine toxicity of the active constituents, their side effects, pharmacokinetics properties and diffusion in different body sites. Also, effort should be geared up towards purification and characterization of the metabolites.

REFERENCES

Adigozel, A., M. Gulloce, M. Senqul, H. Oqutco, F. Sahin and I. Karaman, 2005. Antimicrobial effects of *Ocimum basilicum* (Labiatae) Extract. Turkish J. Biotechnol., 29: 155-160.

Akinyemi, K.O., O. Oladapo, C.E. Okwara, C.C. Ibe and K.A. Fasure, 2005. Screening of crude extracts of six medicinal plants used in South West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine, 5: 32-38.

Atata, R.F., A. Sani and S.M. Ajewole, 2003. Effects of stem Bark Extracts of *Enantia chloranta* on some clinical isolates. Biokemistri, 15: 84-92.

Calzada, F., A.D. Alanis, J.A. Cervantes, J. Jorres and G.M. Ceballos, 2005. Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J. Ethnopharmacol., 100: 153-157.

Davegarden, 2005. Plants and flowers. http://davegarden.com/pf/go/304/index.html.

Edeoga, H.O., O.E. Okwu and B.O. Mbaeble, 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4: 685-688.

Krasil, N.A., 1997. Soil Microorganisms and Higher Plants. 1st Edn., Academy of Science Moscow, pp: 364.

Mariam, T.G., H. Tadey, E. Mohammed and K. Asres, 2005. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J. Ethnopharmacol., 100: 168-175.

Schillinger, U. and F. Lucke, 1989. Antibacterial activity of *Lactobacillus sake* isolate from meat. Applied Environ. Microbiol., 55: 1901-1906.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books Limited, Ibadan, Nigeria, pp: 298.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel and D.A. Zuberer, 1999. Principles and Applications of Soil Microbiology. 1st Edn., Prentice Hall Inc., Upper Saddle River, New Jersey, pp: 312.

Vlietinck, A.J. and L. Pieters, 2005. Bio-guided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds? J. Ethnopharmacol., 100: 57-60.