Annual Research & Review in Biology
7(1): 54-60, 2015, Article no.ARRB.2015.105
ISSN: 2347-565X

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial Evaluation of Aqueous and Ethanol Extracts of *Ocimum gratissimum* and *Carica papaya*

E. Evwierhurhoma[1], M. C. Ugwu[2], C. O. Eze[1], C. Annie[1], F. O. Enwa[1], P. M. Eze[2*] and C. O. Esimone[2]

[1]Faculty of Pharmaceutical Sciences, Delta State University, Abraka, Nigeria.
[2]Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author COE designed and supervised the study. Authors EE, MCU, COE, CA and FOE carried out the laboratory analysis. Authors EE and PME managed the literature searches and writing of the manuscript. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/ARRB/2015/16481
Editor(s):
(1) George Perry, Faculty of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Anonymous, Romania.
(2) Anonymous, Taiwan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=973&id=32&aid=8775

Original Research Article

Received 3rd February 2015
Accepted 7th March 2015
Published 13th April 2015

ABSTRACT

Aims: This study was carried out to evaluate the antibacterial activity of aqueous and ethanol leaf extracts of *Ocimum gratissimum* and *Carica papaya* against selected clinical isolates of Gram-negative bacteria and to also determine the phytochemical constituents of the plants.
Methodology: Extraction of plant leaves was carried using the cold maceration method and preliminary antimicrobial screening of the extracts was done using the disc diffusion method. The minimum inhibitory concentrations (MICs) of the plant extracts against test organisms were determined using the broth dilution technique. The minimum bactericidal concentrations (MBCs) were also determined.
Results: Phytochemical analyses of leaf extracts of both plants revealed the presence of tannins, alkaloids, saponins and anthraquinones. Antibacterial activity was recorded at concentrations ranging from 12.5-50 µg/mL. At 50 µg/mL, the aqueous and ethanol extracts of *O. gratissimum* showed best activity against *E. coli* and *S. typhi* respectively. Also, the aqueous and ethanol extracts of *C. papaya* recorded good activity against *E. coli*. The minimum inhibitory concentrations (MICs) of the aqueous extract of *C. papaya* against test isolates ranged from 12.5-25 µg/mL and

**Corresponding author: E-mail: ezep2004@hotmail.com;*

that of the ethanol extract ranged from 25-50 µg/mL. *O. gratissimum* recorded MICs that ranged from 12.5-25 µg/mL for the aqueous extract, and 25 µg/mL for the ethanol extract. The minimum bactericidal concentrations (MBCs) of *C. papaya* ranged from 25-50 µg/mL for the aqueous extracts and 50 µg/mL for the ethanol extract; and that of *O. gratissimum* ranged from 12.5-25 µg/mL for the aqueous extract, and 25−50 µg/mL for the ethanol extracts.

Conclusion: This study shows that *C. papaya* and *O. gratissimum* leaves have antibacterial activity and supports the traditional use of these plants as medicines.

Keywords: Ocimum gratissimum; Carica papaya; antibacterial evaluation; MIC; MBC; phytochemical analyses.

1. INTRODUCTION

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to the synthetic drugs [1]. Many works have been done which aimed at knowing the different antimicrobial and phytochemical constituents of medical plants [2] and using them for the treatment of microbial infections (both topical and systematic applications) as possible alternative to chemically synthetic drugs to which many infectious micro-organisms have become resistant [3].

The medicinal value of plant lies on the presence of a host of chemical substances referred to as secondary metabolites which are of no apparent importance to the plant’s own life, but may possess important therapeutic properties which can and have been utilized on the treatment of human and animal diseases. The plants which produce and accumulate constituents having medicinal value are generally designated as medicinal plants [4]. These phytoconstituents may either be contained in the leaves, stem, bark, root, bud, corm, rhizome, flowers, or seeds. These constituents are termed ‘active principles’ and they are expected to be inimical to the growth of at least some disease-causing organisms such as *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, etc. [5,6,7].

Many studies have been carried out to evaluate the antimicrobial properties of diverse plants. This study, however seeks to evaluate the antibacterial activity of the ethanol and aqueous leaf extracts of *C. papaya* and *O. gratissimum* against isolates of some clinical strains of Gram-negative organisms.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *O. gratissimum* and *C. papaya* used in this study were collected during the month of November, 2012, from Abraka, Delta state-Nigeria. They were identified in by a taxonomist in the Department of Botany, Delta State University Abraka-Nigeria.

2.2 Culture Media

Culture media used were Nutrient agar, Nutrient broth, and Mueller Hinton Agar (Titan Biotech Ltd, India).

2.3 Test Organisms

Clinical isolates of *Salmonella typhi*, *Escherichia coli*, *Proteus pyogenes* and *Pseudomonas aeruginosa* used in the work were obtained from Delta State University Teaching Hospital, Oghara, Delta State-Nigeria.

2.4 Extraction

The leaves of both plants were washed with distilled water and air dried after at room temperature. After drying, the leaves were grounded into fine powder with a mechanical grinder and macerated in distilled water and 95% ethanol respectively for 48 hours. After maceration, the aqueous and ethanol solutions of the plants were filtered through No. 1 Whatman filter paper and the filtrates were evaporated to dryness over a steam bath. The dried extracts recovered were placed in sterilized screw-capped bottles and stored at refrigeration temperature.

2.5 Phytochemical Analysis of the Extracts

A small portion of the extracts was used for phytochemical testing according to the methods described by Trease et al. [8] and Harbourne [9]. The phytochemicals that were screened for include; alkaloids, saponins, tannins, glycosides and anthraquinones.

2.6 Preparation of Antibiotic Discs and Primary Screening of Extracts for Antibacterial Activity

The disc diffusion technique was used to determine the antibacterial activity of the extracts. Sterile whatman No. 1 paper was punched into 5 mm diameter disc sizes. The discs were placed in a glass Petri plate and oven-sterilize at 170°C for 1 hour. Different dilutions of the plant extracts were prepared in sterile bottles and the sterile discs were allowed to soak in the extracts for 2 h for proper absorption, after which they were removed and allowed to dry. Twenty (20) mL of molten Mueller-Hinton (MH) agar were poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates. The crude extract discs were then gently placed on the plates and incubated immediately for 24 h at 37°C. The inhibition zones diameters (IZDs) were measured and recorded. This procedure was conducted in quadruplicate and the mean IZDs calculated and recorded.

2.7 Determination of Minimum Inhibitory Concentrations (MICs)

The MICs of the plant extracts against test organisms were determined using the broth dilution technique described by Baron and Finegold [10]. Serial dilutions of the extracts were prepared in peptone water, and challenged with small volumes (0.1 mL) of overnight broth culture of the test organisms. These were incubated at 37ºC for 24 hrs. The smallest concentration of the plant extract that inhibits the growth of the test organism was taken as the MIC.

2.8 Determination of Minimum Bactericidal Concentrations (MBCs)

The MBC is the lowest concentration of the antibacterial agent that kills at least 99.9% of the test organism. Those that showed no visible growth from the MIC test tubes were subcultured into nutrient agar plates and incubated at 37ºC for 24 hrs. The lowest concentration of the extracts that yielded no growth was noted as the MBC [11].

3. RESULTS AND DISCUSSION

3.1 Results

Results of the phytochemical screening of the two plants are shown in Table 1. Also, IZDs produced by the extracts of the plants against the test organisms in the antimicrobial assay are shown in Figs. 1-4. The MICs and MBCs produced by the extracts against the test organisms are presented in Tables 2 and 3 respectively.

Phytochemical screening of the aqueous and ethanol leaf extracts of both plants reveals the presence of alkaloids, saponins, tannins, glycosides, and anthraquinones.

3.2 Discussion

The results of this study show that the ethanol and aqueous extracts of *C. papaya* and *O. gratissimum* possess antimicrobial activities against the test bacteria. The organisms showed greater sensitivities to aqueous extracts of *O. gratissimum* and *C. papaya* compared to their respective ethanol extracts. This is similar to the findings of Ijeh et al. [12] who reported on the antibacterial activity of the aqueous extracts of *O. gratissimum* against some Gram-negative bacteria; and Anibujuwon and Udeze [13] who reported on the antibacterial activity of the aqueous *C. papaya* leaf extract against both Gram positive and Gram negative bacterial.

Figs. 1 to 4 show the IZDs produced by the extracts of the two plants. Antibacterial activity was recorded at concentrations ranging from 12.5-50 µg/mL. At 50 µg/mL, the aqueous and ethanol extracts of *O. gratissimum* recorded best activity against *E. coli* and *S. typhi* respectively. Also, the aqueous and ethanol extracts of *C. papaya* showed good activity against *E. coli*. *E. coli* is an organism that has been incriminated in food poisoning incidence which is often associated with gastroenteritis [14]. The aqueous extracts of *O. gratissimum* and *C. papaya* demonstrated increased activity against *E. coli*. This finding supports the ethnomedicinal use of both plants in the management of stomach aches and gastroenteritis in the Nigeria. It can also be observed that the extracts of the two plants recorded considerable efficacy in their antibacterial activity in relation to the positive control, ciprofloxacin (10 µg). This can be seen with *P. aeruginosa* being sensitive to the plants but not to the control drug.

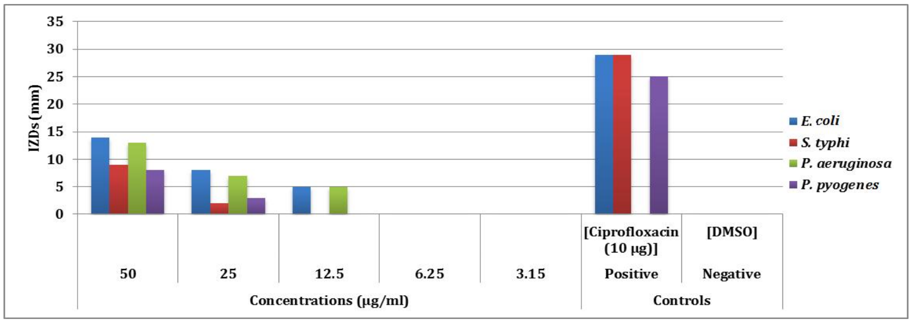

Fig. 1. Inhibition Zone Diameters (IZDs) (mm) produced by aqueous leaf extracts of *O. gratissimum*

The antimicrobial activity of the aqueous leaf extract of O. gratissimum was observed at concentrations of 12.5-50 µg/mL with IZDs that ranged from 2-14 mm. At 25-50 µg/mL, the extract recorded antibacterial activity against E. coli, S. typhi, P. aeruginosa, and P. pyogenes. At 12.5 µg/mL antibacterial activity was recorded only against E. coli and P. aeruginosa. The positive control [ciprofloxacin (10 µg)], recorded no antibacterial activity against P. aeruginosa, but had activity against E. coli, S. typhi, and P. pyogenes with IZDs of 29, 29, and 25 mm respectively

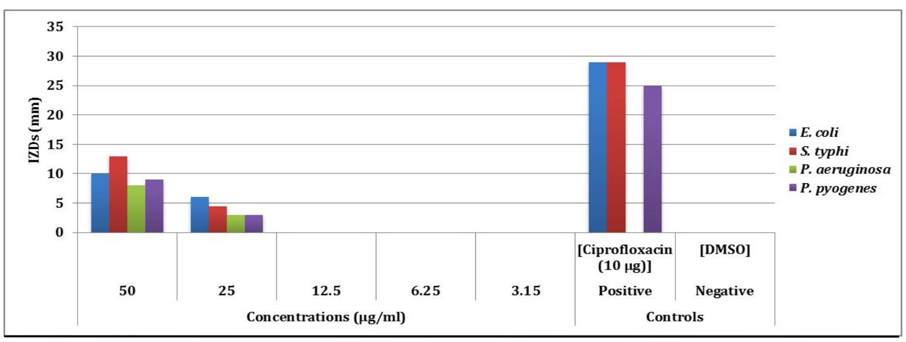

Fig. 2. Inhibition Zone Diameters (IZDs) (mm) produced by ethanol leaf extract of *O. gratissimum*

The antimicrobial activity of the ethanol leaf extract of O. gratissimum was observed at concentrations of 25-50 µg/mL with IZDs that ranged from 3-13 mm. At these concentrations, the extract recorded antibacterial activity against E. coli, S. typhi, P. aeruginosa, and P. pyogenes. The positive control [ciprofloxacin (10 µg)], recorded no antibacterial activity against P. aeruginosa, but had activity against E. coli, S. typhi, and P. pyogenes with IZDs of 29, 29, and 25 mm respectively

Table 1. Result of phytochemical analysis of extracts of *C. papaya* and *O. gratissimum*

Tests	*C. papaya*		*O. gratissimum*	
	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Glycosides	+	+	+	+
Anthraquinones	+	+	+	+

+: Present, -: Absent

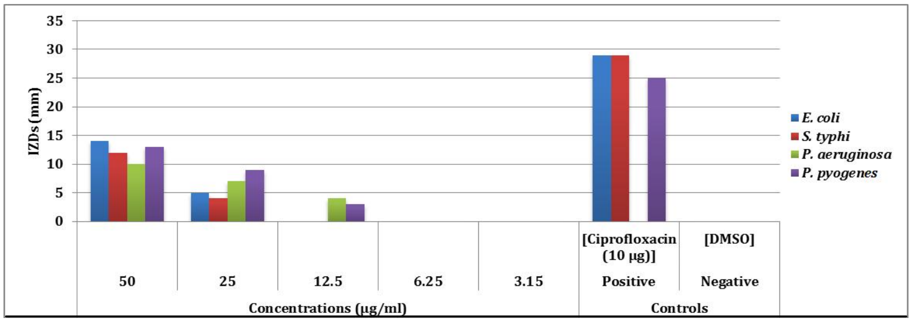

Fig. 3. Inhibition Zone Diameters (IZDs) (mm) produced by aqueous leaf extract of *C. papaya*

The antimicrobial activity of the aqueous leaf extract of C. papaya was observed at concentrations of 12.5-50 µg/mL with IZDs that ranged from 3-14 mm. At 25-50 µg/mL, the extract recorded antibacterial activity against E. coli, S. typhi, P. aeruginosa, and P. pyogenes. At 12.5 µg/mL antibacterial activity was recorded only against P. aeruginosa and P. pyogenes. The positive control [ciprofloxacin (1µg)], recorded no antibacterial activity against P. aeruginosa, but had activity against E. coli, S. typhi, and P. pyogenes with IZDs of 29, 29, and 25 mm respectively

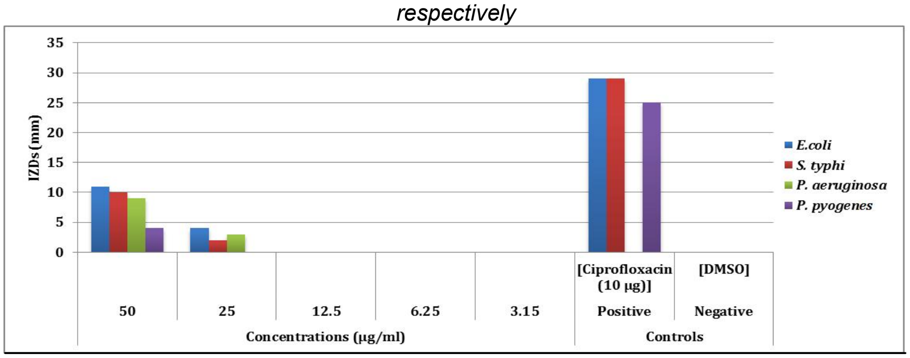

Fig. 4. Inhibition Zone Diameters (IZDs) (mm) produced by ethanol leaf extracts of *C. papaya*

The antimicrobial activity of the ethanol leaf extract of C. papaya was observed at concentrations of 12.5-50 µg/mL with IZDs that ranged from 2-11 mm. At 50 µg/mL, the extract recorded antibacterial activity against E. coli, S. typhi, P. aeruginosa, and P. pyogenes. At 25 µg/mL antibacterial activity was recorded only against E. coli, S. typhi and P. aeruginosa. The positive control [ciprofloxacin (10 µg)], recorded no antibacterial activity against P. aeruginosa, but had activity against E. coli, S. typhi, and P. pyogenes with IZDs of 29, 29, and 25 mm respectively

Table 2. Minimum inhibitory concentrations (MICs) of extracts of *C. papaya* and *O. gratissimum*

Test organisms	MIC (µg/mL)			
	***C. papaya* (aqueous)**	***C. papaya* (ethanol)**	***O. gratissimum* (aqueous)**	***O. gratissimum* (ethanol)**
E. coli	25	25	12.5	25
P. aeruginosa	12.5	25	12.5	25
S. typhi	25	25	25	25
P. pyogenes	12.5	50	25	25

Table 3. Minimum bactericidal concentrations (MBCs) of extracts of *C. papaya* and *O. gratissimum*

Test organisms	MBC (µg/mL)			
	***C. papaya* (aqueous)**	***C. papaya* (ethanol)**	***O. gratissimum* (aqueous)**	***O. gratissimum* (ethanol)**
E. coli	50	50	12.5	50
P. aeruginosa	25	50	25	50
S. typhi	50	50	25	25
P. pyogenes	25	50	25	50

The MICs and MBCs of the two plants extracts are shown in Tables 2 and 3 respectively. The MICs of aqueous extract of *C. papaya* ranged from 12.5-25 µg/mL and that of the ethanol extract ranged from 25-50 µg/mL. *O. gratissimum* recorded MICs that ranged from 12.5-25 µg/mL for the aqueous extract, and 25 µg/mL for the ethanol extract. The MBC of *C. papaya* ranged from 25−50 µg/mL for the aqueous extracts and 50 µg/mL for the ethanol extract. That of *O. gratissimum* ranged from 12.5−25 µg/mL for the aqueous extract and 25−50 µg/mL for the ethanol extracts. This study relates considerably with the work of Ijeh et al. [12] who reported the MICs of ethanol and aqueous extracts of *O. gratissimum* against test organisms that ranged from 6.25-25 µg/mL.

These results reveal that the aqueous extracts of the two plants showed a higher activity compared to their ethanol extracts. This is quite contrary to the findings of Alo et al. [15] who indicated ethanol to be a better solvent than water for the extraction of the active ingredient in these plants. However, the results of this research show that both the aqueous and ethanol extracts of these plants have potential for use as an antimicrobial agent.

The results of the phytochemical analyses of the extracts of both plants are shown in Table 1. The extracts revealed the presence of tannins, alkaloids, saponins and anthraquinones. These phytocompounds are known to be biologically active and are believed to be responsible for the observed antibacterial effects. Although the mechanism of action of the *C. papaya* and *O. gratissimum* plant extracts used for this study is not understood, it has been proposed that its action against the bacteria may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the bioactive components of the extract [16].

According to Kone et al. [17] some medicinal plants might indeed be potential sources of new antibacterial agents. This present study also demonstrates that herbal medicines can be as effective as modern medicines to combat some pathogenic microorganisms.

4. CONCLUSION

From this study, it can be concluded that *C. papaya* and *O. gratissimum* leaves have antibacterial activity and supports the traditional use of these plants as medicines. The study has also shown that the observed antibacterial effects of the extracts of *C. papaya* and *O. gratissimum* leaves against the test bacteria appear reasonable and could serve as a promising alternative to the synthetic antibiotics, since several bacterial species have developed resistance to most existing synthetic antimicrobials. It is therefore recommended that further experiments using different solvents-extracts against a broader spectrum of micro-organisms, other pharmacological evaluations, toxicological studies and possible isolation of the therapeutic compounds from these plants be carried out.

COMPETITING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Latha S, Kanaabiran K. Antimicrobial activity and phytochemicals of *Solanum trinobactum* Linn. Afr J Biotech. 2006; 5(23).
2. Adamn HM, Abayeh OJ, Agho MO, Abdullahi DL, Ubah A, Dukku HU. Antimicrobial activities of medicinal plants. Journal of Ethnopharmacology. 2005; 99(1):1-4.
3. Anyamene CO, Ezeakdila JO. Antimicrobial activities of medicinal plants. Journal of Applied Science. 2010; 13(1):8940-8948.
4. Tavs AA. A practical approach to evaluation of phytomedicine.1st edition published by Ambik press,Benin. 2012, pp 267−278.
5. Effraim KD, Jacks TW, Sodipo OA. Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of rabbits. African Journal of Biomedical Research. 2003;6:21-25.
6. Okigbo RN, Mweka EC. Evaluation of the toxicity of antimicrobials on some organs of rats. Afr J. Trad. 2006;5(3):224-228.
7. Fumilayo VD, Okaniran OO, Kanife UC. Studies on the effect of antimicrobials on some organs of rabbit. International Journal of Biology. 2010;2(2):126-131.
8. Trease GE, Evans WC. Textbook of pharmacognosy. 12th ediction (Balliere, Tindall, London; 1983.
9. Harbourne JB. Phytochem. Methods- A guide to modern technique of plant analysis chapman and Hall. 1973;271.

10. Baron JE, Finegold SM. Method for testing antimicrobial effectiveness: Bailey Scotts Diagnostics Microbiology. Mosby, Missouri. 8th ed. 1990;171−194
11. Geo F, Brooks JSB, Stephen AM. Janetic, Meinick and Addbergs medical microbiology, 22nd ed. 2001;607.
12. Ijeh II, Omodamiro OD, Nwanna IJ. Antimicrobial effect of *Ocimum gratissimum* and *Xylopia aethiopica*. African J Biotech. 2005;4(9):953-956.
13. Anibujuwon II, Udeze AO. Antimicrobial activity of *Carica papaya* on some pathogenic organism of clinical origin from Southern Nigeria. Ethnobotanical Leaflets. 2009;13:850-64.
14. Entani E, Agari M, Trujihata S, Tsukamoto T, Ohta M. Antimicrobial action of vinegar against food borne pathogenic bacteria including *E. coli*. 0157-717. J Food Prot. 1998;61(8):953-989.
15. Alo MN, Anyim C, Igwe JC, Uchenna DS. Antibacterial activity of water, ethanol and methanol extract of *O. gratissimum*, *V. amygdalina* and *A. meluegueta*. Advances in Applied Science Research. 2012; 3(2):844-848
16. Bais HP, Walker TS, Schweizer HP, Vivanco JM. Plant Phytochemistry. Biochem. 2002;40:983-995.
17. Kone WM, Atindehou KK, Terreaux C, Hosettman K, Traore D, Dosso M. Traditional medicine in North cote d ivoire; Screening of 50 medicinal plants for antibacterial activity. J Ethnopharmacol. 2004;93:43-49.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=973&id=32&aid=8775