Open Science Journal of Clinical Medicine
2019; 7(2): 32-35
http://www.openscienceonline.com/journal/osjcm
ISSN: 2381-4748 (Print); ISSN: 2381-4756 (Online)

The Antimicrobial Activities of *Citrus Aurantifolia* Leave Extracts Against Some Bacteria

Orhue Philips Ogbeide[1, *], Momoh Abdul-Razaq McSionel[2]

[1]Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria

[2]Department of Medical Microbiology, Faculty of Clinical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria

Email address

orhue_philips@yahoo.com (O. P. Ogbeide)

[*]Corresponding author

To cite this article

Orhue Philips Ogbeide, Momoh Abdul-Razaq McSionel. The Antimicrobial Activities of *Citrus Aurantifolia* Leave Extracts Against Some Bacteria. *Open Science Journal of Clinical Medicine.* Vol. 7, No. 2, 2019, pp. 32-35.

Received: April 2, 2019; **Accepted**: May 19, 2019; **Published**: May 28, 2019

Abstract

Different solvents are known to present different extraction potency for plants and their active constituents and metabolites and for this reason, when used for antibiotic extraction, different inhibitory activities and potency should be expected. This study evaluates the antibacterial potential of different extracting solvents of *Citrus aurantifolia* leave against some infection causing microorganisms. In a bid to evaluate the aim of this study, 5 grams of dried blended leaves of *Citrus aurantifolia* was mixed with 95ml of six different extraction solvents (Hydrochloric acid, Propylene glycol, Hot water, Acetic acid, Acetone and Ethanol) for 24 hours. The constituted filter extracts were aseptically assessed and their minimum inhibitory concentration (MIC) on different miro-organism (*Pseudomonas aeruginosa, Escherichiia coli, Staphylococcus aureus* and *Klebsiella spp*) were determined. The results showed that hot water extraction of *Citrus aurantifolia* leave has no effect on the herein studied micro organisms. However, ethanol extract of *Citrus aurantifolia* leave was the most sensitive to the micro-organisms but *Klebsiella spp* was 100% resistant. Acetic acid, Propylene glycol and acetol extracts were sensitive to all the micro-organism at different MICs. Based on these findings, it is suggested that solvent extraction potency be investigated for plants known or acclaimed to have antibacterial activity.

Keywords

Antibacterial, Plant Extracts, Extracting Solvent, *Citrus Aurantifolia* Leave

1. Introduction

Human infections constitute a major problem, especially in tropical and subtropical developing countries. These infections are caused by various microorganisms which cause various diseases. Remedial supremacy of herbs has been noted and believed even by the earlier ancient civilization [1, 2] and nations like India, fulfils the requirement of medicinal sources mainly from plants to cure infectious diseases [3] and Africans practiced plant medicines for the treatment of many diseases and infections [4]. Medicinal plants had provided great impact in the field of curing disease and as a source of medicine for a wide variety of human ailment [5]. In fact, several up to date research and practical experience have shown that using medicinal plants is better that allopathic drugs by being safer besides having synergistic effect [6].

Concurrently, many herbs/spices and plant derived products have been used extensively in medicinal field from ancient era to present and had been proven to cure certain illness in replacement of chemical compounds or antibiotics particularly in many Asian, African and other countries. Referring to World Health Organization, 80% of world requirements on medicines for health needs attained from botanical preparations (plant extracts or their bioactive compounds) [2, 7-11].

Citrus aurantifolia (lime) is a small, densely and irregular branch tree with short, sharp spines and characterized by either sour or sweet greenish-yellow fruits. There are several species of citrus trees whose fruits are called limes; including

the key limes *(Citrus aurantifolia)*, Persian lime, Kaffir lime, and dessert lime. The plant is said to be rich in carbohydrate, sugar, soluble and insoluble fibre, sodium, vitamins, minerals, fatty acids, amino acids [12] and the fruits and leaves have been documented to possess medicinal properties.

In Nigeria, lime is used to suppress stomach ache and the juice as an excellent cough remedy when mixed with sugar, palm oil or honey. It also exhibits bioactive activities for colds, fevers, sore throats, bronchitis and asthma [13]. Especially by the Yorubas of Nigeria, the rind is burnt in homes as insecticides against mosquitoes. The meso-carp is used as a very good facial scrub to prevent pimples [14]. The traditional uses or phytochemical properties of C. aurantifolia from several literature described it as antibacterial [6, 15, 16], antifungal [17-19], anti-parasitic [20-23], and can be used for insecticide activity [24, 25]. In addition to antimicrobial activities, lime has several medicinal properties and potential health benefits which make it a good candidate as a natural antimicrobial. However, various solvents are known to possess varying extraction potencies and as such, may affect their potentials against microorganisms. It is therefore the aim of this study to evaluate the antibacterial potentials of different extracting solvents of *Citrus aurantifolia* leave against some infection causing microorganisms.

2. Materials and Methods

2.1. Processing of Plant Samples

Plant materials; *Citrus aurantifolia* leaves, were collected from Ekpoma, Edo State, Nigeria. The leaves were collected, washed in tap water, rinsed in sterile distilled water and dried for 5 days at 60^0 C in Laboratory of the Department of Microbiology, Ambrose Alli University, Ekpoma. The dried leaves were ground to powder with a clean kitchen blender and stored in airtight glass containers kept in laboratory cupboard, until required for preparation.

2.2. Preparation of Extracts

5grams of the ground leaves powder were weighed into 100ml sterile reagent bottle and 95ml of different extraction solvents (Hydrochloric acid, Propylene glycol, Hot water, Acetic acid, Acetone and Ethanol) were added and left to extract on a mechanical shaker overnight at room temperature. This was done using the ground leave materials for each of the extraction solvents.

The extract solutions were then filtered separately aseptically into another 100ml reagent bottle using a watt-man filter paper No. 1. All the filtrate were screened for purity by inoculation unto MacConkey agar and blood agar plates and incubated at 37^0C for 48 hours. Filtrates yielding growth of any organism were re-filtered and rescreened for purity until a sterile extract solution was obtained, following the methods outlined by Orhue [26].

2.3. Micro Organism Preparation/Growth

The test organisms; *Pseudomonas aeruginosa, Escherichiia coli, Staphylococcus aureus* and *Klebsiella spp,* were all human pathogenic organisms of clinical origin. They were obtained from the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma-Nigeria, where they were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmation.

2.4. Determination of Minimum Inhibitory Concentration (MIC)

Using a 50ml specific gravity bottle (SGB), the density of the extract solution was determined. In a similar manner, the density of the plain solvent was also determined. To determine the concentration of the extract, the density of the plain solvent was subtracted from that of the extract solution. This was done for each of the extraction solvents. This method hereafter will be termed "eureka method" of determination of extract concentration. With the known extract's concentration and the clinical isolates, MIC of the extract solutions were determined. The experiments were performed in triplicates for each of the extraction solvents and the average was calculated.

2.5. Data Analysis

Data were keyed into SPSS (version 16) and the average of each determined MIC was then presented in suitable table for simple descriptive comparison. The MICs of the different extraction solutions were compared.

3. Results and Discussion

Table 1 shows the result of the determination of extract concentrations from the different solvents. Based on the results, propylene glycol had the highest concentration of 0.16 and as such better extraction potency compared to the other solvents. This was followed by acetone and hot waters with concentrations of 0.14 and 0.01 respectively. On the other hand, ethanol, acetic acid and hydrochloric acid have the least extraction potencies with concentrations 0.04, 0.08 and 0.09 respectively. This finding indicates that there are differences in quality of extract from different solvents. In agreement with this finding, differences between the compositions of oils distilled in industry and in laboratory have been reported [27].

Table 2 shows the minimum inhibitory concentration (MIC in mcg/ml) of the leave extracts of *Citrus aurantifolia* by the different solvent. Hot water extraction of *Citrus aurantifolia* leave did not show any effect on the micro organisms. Hydrochloric acid extraction of *Citrus aurantifolia* leave was not effective on *Pseudomonas aeruginosa* and *Staphylococcus aureus* but was most effective on *Klebsiella spp* with MIC of 112mcg/ml and least on *Escherichiia coli* with MIC of 480mcg/ml.

Table 1. *Extracts concentrations from the different solvents.*

Agent	Weight of SGB	Weight of plain solvent	Density of plain solvent	Weight of SGB and extract	Density of Extract	Conc
Hydrochloric acid	24.04	25.40	1.01	27.61	1.10	0.09
Propylene glycol	24.04	26.38	1.01	29.40	1.17	0.16
Hot water	24.04	25.00	1.10	27.52	1.10	0.10
Acetic acid	24.04	25.66	1.03	27.87	1.11	0.08
Acetone	24.04	19.88	0.79	23.28	0.93	0.14
Ethanol	24.04	20.19	0.80	21.19	0.84	0.04

Table 2. *MIC (mcg/ml) of the leave extracts of Citrus aurantifolia by different solvents.*

Micro organism	HCL	Acetic acid	PPG	Acetol	Hot water	Ethanol
Pseudomonas aeruginosa	0	800	600	400	0	400
Escherichiia coli	480	400	800	800	0	200
Staphylococcus aureus	0	200	400	350	0	100
Klebsiella spp	112	100	100	175	0	0

Also, ethanol extraction of *Citrus aurantifolia* leave was not effective on *Klebsiella spp* but was most effective on *Staphylococcus aureus,* intermediate on *Escherichiia coli* and least effective on *Pseudomonas aeruginosa.* Acetic acid, Propylene glycol and acetol extractions of *Citrus aurantifolia* leave were sensitive to all the micro-organism at different MICs. Comparatively, acetic acid and Propylene glycol were the most effective for *Klebsiella spp* while acetic acid was most effective for *Staphylococcus aureus* and *Escherichiia coli* but acetol was most effective for *Pseudomonas aeruginosa* among the three extracts. Although *Klebsiella spp* was resistant to ethanol extraction of *Citrus aurantifolia* leave, the ethanol extract was the most sensitive to the other micro-organisms. These findings indicate that antimicrobial activity of the different solvents may be due to compositions which the solvent may have been able to extract. In agreement with this assertion, Costa et al. [28] has mentioned that the antimicrobial activity of essential oils is strictly connected to their chemical composition.

4. Conclusion

Based on the findings of this study, solvent extraction potency need to be investigated for plants acclaimed to have antibacterial activity. Also, the dissimilarities in findings from researchers researching on the same plant may be due to the variations in the solvent used for extraction. Thus, there is the need to give an account of the solvents used for extraction and the procedures for extraction for reproducibility purposes.

Author's Contributions

Dr Orhue participated in the conception and design of the study. While both authors Dr Orhue and Dr Momoh contributed in experimental work, data collection and prepared the first draft of the manuscript.

Acknowledgements

The authors express their gratitude to all authors of literature cited and consulted and sincerely appreciate the Department of Microbiology, of the Ambrose Alli University for making the equipments available for the study.

Competing Interests

The authors declare that they have no competing interests.

Funding

There was no funding in the conduct of this study.

References

[1] Onyeagba, R., Ugbogu, O., Okeke, C. and Iroakasi, O. 2005. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). African Journal of Biotechnology 3 (10): 552-554.

[2] Nkambule, T. P. 2008. Antimicrobial Properties of Selected Asian Herbs. Master Thesis. University of Florida.

[3] Amrita, V., Sonal, D. and Shalini, R. 2009. Antibacterial Effect of Herbs and Spices Extract on *Escherichia coli.* Electronic Journal of Biology 5 (2): 40-44.

[4] Aboaba, O., Smith, S. and Olude, F. 2006. Antibacterial effect of edible plant extract on *Escherichia coli* O157: H7. Pakistan Journal of Nutrition 5 (4): 325-327.

[5] Iqbal Ahmad, Arina Z. Beg. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology. 2001; 74; 113-123.

[6] Pathan R, Papi R, Parveen P, Tananki G, Soujanya P. *In vitro* antimicrobial activity of *Citrus aurantifolia* and its phytochemical screening. Asian Pac J Trop Dis. 2012; 2: 328–31.

[7] Indu, M., Hatha, A., Abirosh, C., Harsha, U. and Vivekanandan, G. 2006. Antimicrobial activity of some of the south-Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila.* Brazilian Journal of Microbiology 37 (2): 153-158.

[8] Mohanta, T., Patra, J., Rath, S., Pal, D. and Thatoi, H. 2007. Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium.* Scientific Research and Essay 2 (11): 486-490.

[9] Hema, R., Kumaravel, S. and Elanchezhiyan, N. 2009. Antimicrobial Activity of Some of the South-Indian Spices and Herbals against Food Pathogens. Global Journal of Pharmacology 3 (1): 38-40.

[10] Tagoe, D., Baidoo, S., Dadzie, I., Kangah, V. and Nyarko, H. 2010. A comparison of the antimicrobial (antifungal) properties of garlic, ginger and lime on *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum* using organic and water base extraction methods. The Internet Journal of Tropical Medicine 7 (1): DOI: 10.5580/1099.

[11] Yan, S. and Asmah, R. 2010. Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. International Food Research Journal 17: 417-423.

[12] Ibukun, A., Tayo, A., Toyin, A, Tolu, O. (2007). Evaluation of the Antimicrobial properties of Different parts of Citrusaurantifolia (Lime Fruit) as used Locally. Afri J Tradt Complement Altern Med, 4 (2): 185-190.

[13] Khan, P. R., Gali, P. R., Pathan, P., Gowthan, T., Pasupuleti, S. (2012). In Vitro antimicrobial activity of Citrus aurantifoliaand its phytochemical screening. Live Sciences feed, 1 (2): 13 – 16.

[14] Onyeagba, R. A, Ugbogu, O. C, Okeke, C. U. Iroakasi, O. (2004). Studies on the antimicrobial effects of garlic (Alliumsativum Linn), ginger (Zingiber officinale Roscoe) and Lime (Citrus aurantifolia Linn). Afri J Biotechnol, 3 (10): 552–554.

[15] Nwankwo IU, Osaro-Matthew RC, Ekpe IN. Synergistic antibacterial potentials of *Citrus aurantifolia* (Lime) and honey against some bacteria isolated from sputum of patients attending Federal Medical Center, Umuahia. Int J Curr Microbiol Appl Sci. 2015; 4: 534–44.

[16] Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Odugbemi T. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr J Tradit Complement Altern Med. 2006; 4: 185–90.

[17] Balamurugan S. *In vitro* antifungal activity of *Citrus aurantifolia* Linn plant extracts against phytopathogenic fungi *Macrophomina phaseolina*. Int Lett Nat Sci. 2014; 13: 70–4.

[18] Dongmo PJ, Tatsadjieu L, Sonwa ET, Kuate J, Amvam Zollo P, Menut C. Essential oils of *Citrus aurantifolia* from Cameroon and their antifungal activity against *Phaeoramularia angolensis*. Afr J Agric Res. 2009; 4: 354–8.

[19] Tagoe D, Baidoo S, Dadzie I, Kangah V, Nyarko H. A comparison of the antimicrobial (antifungal) properties of garlic, ginger and lime on *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum* using organic and water base extraction methods. Int J Trop Med. 2009; 7: 1–8.

[20] Domínguez-Vigil I, Camacho-Corona M, Heredia-Rojas J, Vargas-Villarreal J, Rodríguez-De la Fuente A, Heredia-Rodríguez O, et al. Anti-giardia activity of hexane extract of *Citrus aurantifolia* (Christim) Swingle and some of its constituents. Afr J Tradit Complement Altern Med. 2015; 12: 55–9.

[21] Enejoh S, Suleiman M, Ajanusi J, Ambali S. *In vitro* anthelmintic efficacy of extracts of *Citrus aurantifolia* (Christm) Swingle fruit peels against *Heligmosomoides bakeri* ova and larvae. Int J Curr Pharm Res. 2015; 7: 92–6.

[22] Enejoh S, Shuaibu K, Suleiman M, Ajanusi J. Evaluation of anthelmintic efficacy of extracts of *Citrus aurantifolia* fruit juice in mice experimentally infected with *Heligmosomoides bakeri*. Int J Biol Res. 2014; 4: 241–6.

[23] Abdelqader A, Qarallah B, Al-Ramamneh D, Das G. Anthelmintic effects of *Citrus* peels ethanolic extracts against *Ascaridia galli*. Vet Parasitol. 2012; 188: 78–84.

[24] Soonwera M. Efficacy of essential oils from *Citrus* plants against mosquito vectors *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) J Agric Technol. 2015; 11: 669–81.

[25] Effiom O, Avoaja D, Ohaeri C. Mosquito repellent activity of phytochemical extracts from fruit peels of *Citrus* fruit species. Glob J Sci Front Res Interdiscip. 2012; 12: 5–8.

[26] Orhue, P. O. (2004). Antibiogram types of urinary tract infection bacteria isolates and the susceptibility to some indigenous plant extract. Ph.D Thesis, Ambrose Alli University, Ekpoma, Nigeria.

[27] Dugo G., Cotroneo A., Bonaccorsi I. and Trozzi A., Composition of the volatile fraction of Citrus peel oils. In Citrus oils – Composition, advanced analytical techniques, contaminants, and biological activity. Edits. G. Dugoand L. Mondello, pp. 1–161, CRC Press, Boca Raton, FL (2011).

[28] Costa R, Bisignano C, Filocamo A, Grasso E, Occhiuto F and Spadaro F. (2014) Antimicrobial activity and chemical composition of Citrus aurantifolia (Christm.) Swingle essential oil from Italian organic crops, Journal of Essential Oil Research, 26: 6, 400-408.