Invitro Assessment of Anti Bacterial Effect of Extracts of *Ocimum Gratissimum* and *Carica Papaya* Leaves

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Abstract

Extracts of the leaves of Ocimum gratissimum and Carica papaya were assessed for antibacterial activity against Gram positive (Staphylococcus aureus) and Gram negative (Escherichia coli) bacteria. A 300grams of the fresh leave from each sample was washed, trimmed, sliced, blended and squeezed to extract the liquid. The undiluted extracts at different millilitres were pipetted into prepared Mac Conkey agar plates. Two discrete colonies from pure culture of each bacterium were inoculated into each of these plates and incubated at 37 ^{0}C for 24 hours, and the total plate count determined. Similarly, two discrete colonies of the bacterium were inoculated into a prepared Mac Conkey agar plate without the extract (control) and incubated at 37 ^{o}C for 24 hours. The result showed that the extracts inhibited bacterial growth as compared with the control. A 5 ml extract of Ocimum gratissimum reduced the visible colonies of S. aureus from 36 cfu/ml to 5 cfu/ml and E. coli from 65 cfu/ml to 14 cfu/ml. A 4.5 ml extract of Carica. papaya exhibited 100 % inhibition of S. aureus and reduced E. coli from 65 cfu/ml to 24 cfu/ml. As the concentration of the extract increases the microbial count decreases significantly (P<0.05). The highest antibacterial activity was achieved using the extract of Carica papaya leaf.

Keyword: Invitro assessment, anti bacteria, extract, *O. graticinum*, *Carica. papaya*, *Staphylococcus aureus* , *Escherichia coli*

Introduction

Herbs had been used by all cultures throughout history. Science has isolated the medicinal properties of large number of herbs and their healing components have been extracted and analyzed (Anthony, et. al; 2005). The search for newer sources of antibiotic is a global challenge preoccupying research institutions, pharmaceutical companies and academic institutions, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). The situation has further been complicated with the rapid development of multidrug resistance by the microorganisms to the antimicrobial agents available. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs (Pretorious and Watt, 2001). *Ocimum gratissmum* and *Carica papaya* are both perennial plants and they have antibacterial effects on pathogenic organisms, both Gram positives and Gram negatives including *Escherichia coli* and *Staphylococcus aureus*. *Ocimum gratissimum* is an aromatic medicinal plant grown in the tropic of Africa and Asia The plant has pleasant smell which gives it the common name scent leaf plant.

Ocimum gratissimum is rich in alkaloid, tannis, phytates, flavonoids, oligosaccharides and has tolerable cyanogenic content (Ijeh, et. al., 2004). According to Gill (1992), the plant contains terpenoids, eugenol, thymol, saponins and alkaloids. The plant has both economical uses and medicinal peoperties. The dried or fresh leaves are used as insect repellant, smell distinguisher and for colic pains (Adebolu and Oladimeji, 2005). The leaves of *O. gratissimum* are rubbed between the palms and sniffed as a treatment for blocked nostrils (Kokwaro, 1993). The essential oil of *O. gratissimum* has antimicrobial activities against pathogenic strains of Gram positive (*S. aureus, Bacillus spp*) and Gram negative (*E. coli, P. aeruginosae, S. typhi, K. pneumonia, P. mirabilis)* bacteria and pathogenic fungus *C. albicans* (Matasyoh, 2007).

Carica papaya is one of the major fruit crops cultivated in tropical and sub-tropical zones. The plant is a large herb or soft-wood tree measuring 1.8 to 6 meters. Its fruit is consumed world-wide as fresh fruit and as a vegetable or used as processed products. *C. papaya* contains many biologically active compounds including chymopapain and papain which are widely known as being useful for treatment of digestive disorders and disturbances of the gastrointestinal tract.

Papain and chymopapain are important proteolytic enzymes found in the milky white latex of *carica papaya*. Papaya is used in tropical folk medicine. According to Reed (1976), papaya latex is very much useful for curing dyspepsia and is externally applied to burns and scalds. Papaya milk latex shows anti-bacterial properties, inhibits fungal growth, especially that of *Candida albicans* (Giordani and Siepai, 1991), and thus would be useful in the treatment of skin eczema caused by this fungus. The Dutch and Malays use leaves and young fruit extracts to eradicate intestinal worms and to treat boils (Burkill, 1966) while young shoots and male flowers are consumed as a vegetable dish in the Malay Peninsula. In Mauritius, the smoke from dried papaya leaves relieves asthma attacks. In Australia it is believed in some quarters that several cancer diseases can improve after drinking papaya leaf extract. Despite the fact that the leaf decoction is administered as a purgative for horses in Ghana and in the Ivory Coast, it is a treatment for genito-urinary ailments. The dried leaf infusion is taken for stomach troubles in Ghana and it is used as a purgative (Burkill, 1966).

Escherichia coli is a gram negative rod that is naturally found in the intestinal tract, in the soil and water (Cheesbrough, 2006). It causes urinary tract infections, infections of the wounds sepsis, bacteraemia, meningitis and diarrhoeal diseases. Morphologically, *E. coli* is usually motile but inactive strains are non-motile and a minority of the strains is capsulate (Cheesbrough, 2006). E. coli grows in optimum temperature of 36-37^{0}C with most strains growing over the range of 18-44 ^{0}C. On MacConkey agar, *E. coli* ferment lactose producing smooth pink colonies. It is indole positive, citrate negative and reduce nitrate to nitrite.

Pathogenesis of *E. coli*

Pathogenic strains of *E. coli* are responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis). The diseases caused by a particular strain of *E. coli* depend on distribution and expression of an array of virulence determinants, including adhesins, invasins, toxins, and abilities to withstand host defenses. (Todar, 2008).Neonatal meningitis is produced by a serotype of *E. coli* that contain capsular antigen called K1. The colonization of the new born's intestines with these stems, that are present in the mother's vagina, lead to bacteriemia which leads to meningitis.

Most of the strains of E. coli are harmless but some, such as serotype O157:H7 can cause serious food poisoning in humans, and are occasionally responsible for product recalls (Vogt, 2005). The serotype O157:H7 (O refers to somatic antigen; H refers to flagellar antigen) is uniquely responsible for causing HUS (hemolytic uremic syndrome). The virulent strains of *E. coli* typically cause a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world (Nataro, 1998). More virulent strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immune compromised (Hudault, *et. al*; 2001; Nataro, 1998).

The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K (Bentley, 1982) and by preventing the establishment of pathogenic bacteria within the intestine (Hudault, *et. al*; 2001; Reid, *et. al*; 2001). Non-pathogenic *Escherichia coli* strains Nissile 1917 also known as Mutaflor is used as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases (Grozdanov et. al., 2004) including inflammatory bowel disease (Kamada et. al., 2005).

Staphylococcus aureus is a Gram positive organism that occurs characteristically in group. They are non-motile and non-capsulate. *S. aureus* can be found in the anterior nares of man and can be isolated from the sputum, infected food and vomit. *S. aureus* is the causative organism of boils, styes, septicaemia and toxic shock syndrome and it produces toxins and extracellular enzymes such as staphylokinase and enterotoxins that contribute to its invasiveness and pathogenicity. On MacConkey agar, *S. aureus* produced smaller colonies that measured 0.1-0.5mm at 35-37 ^{0}C. It is coagulase positive, catalase positive and ferment lactose. Most strains are resistant to methicillin and penicillins and are difficult to be treated (Brayshaw, 1999).

Pathogenesis of *Staphylococcus aureus*

Staphylococcus aureus is a potential pathogen of humans, which causes a wide range of suppurative (pus forming) infections, as well as food poisoning and toxic shock syndrome. It causes superficial skin lesions such as boils, styes, pustules and infections of the wounds. It also cause infections such as pneumonia, mastitis, phlebitis, meningitis, urinary tract infections and subconscious infections, such as osteomyelitis and endocarditis (Cheesbrough, 2006)

Herbal preparations are known to have an important role in disease control due to their antioxidant and antimicrobiological activities (Prasad and Padhyoy, 1993).

In this study, anti-bacteria effect of direct herbal preparations will be determined using extracts from *Ocimum gratissimum* and *Carica papaya* leaves on E. coli and S. aureus. The objective of this study is to obtain the extract of *Ocimum gratissimum* and *Carica papaya* and evaluate the antimicrobial potential of these extracts on *Staphylococcus aureus* and *Escherichia coli* as test organisms.

Materials and Method

Sources of materials

The plant materials, *Ocimum gratissimum* and *Carica papaya* were bought Ochuba farm in Oba; and were identified and authenticated at the Department of Botany, Nnamdi Azikiwe Unversity, Awka as *Ocimum gratissimum* and *Carica papaya* leaves. The media used was purchased from Gepet Global resource Ltd; No. E291 bridge head market Onitsha Anambra State and the bacterial used were collected form Chizoba hospital and maternity and laboratory services; No. 55 Mgbaemena street, Awada Obosi Anambra state-Nigeria.

Sample preparation

The leaves of *Ocimum gratissimum* and *Carica papaya* were separated from the stalk after collection and washed with tap water and rinsed with sterile laboratory distilled water. The leaves were allowed to drop the excess water and were shredded, weighed using a weighing balance and were blended using sterile electric blender. The liquid was extracted by pressing and the extract was later separated aseptically using filtration technique to obtain the clear extract.

Biochemical confirmation of *Escherichia coli*

Escherichia coli was confirmed using biochemical methods described by Cheesbrough (2006). The organism was first identified using Gram staining technique and it was negative. The test organism was inoculated in bijou bottle containing 3ml of sterile peptone water (a general purpose growth medium that can be used as a base for carbohydrate fermentation studies and it contains high level of tryptone making it suitable for use in Indole test) for 48hrs at a temperature of 35 – 37 ^{0}C. The organism was tested using Indole biochemical test and it showed positive with the presence of red surface layer within ten minutes.

Biochemical confirmation of Staphylococcus *aureus*

The method described by Cheesbrough (2006) was used for the confirmatory test of *Staphylococcus aureus*. The organism was tested using Gram staining technique and was identified as gram positive organism. Then, catalase test was used to differentiate bacteria from non-catalase producing bacteria such as streptococci. It showed catalase positive by producing active bubbles. The organism was later confirmed to be pathogenic strains of staphylococcus by carrying out coagulase test and the organism showed positive with the presence of clumping within ten minutes.

Determination of bacterial plate count

Pour plate count was carried out using the method described by Cheesbrough, (2006). A 5.2g of MacConkey agar was measured carefully using a weighing balance for every 100mls. The agar was transferred into a conical flask, dissolved with small quantity of distilled water and was made up to 100 mls. The mixture was boiled, sterilized at 121 ^{0}C for 15 minutes, allowed to cool to about 45 ^{0}C and was poured into sterile petri dishes (20 mls for each petri dish). 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4.0 ml, 4.5 ml, 5.0 ml of the leaf extract was differently pipetted into different petri dishes containing media numbering 1 to 10 (in duplicate). No leave extract was added to the media No. 11 and it was used as control plate. Two colonies of the test organism were introduced into the media and to the control plate. The culture plates were incubated at the temperature of 35 – 37 ^{0}C for 18-24 hours. The bacterial plate count was determined by counting the visible colonies of the test organism.

Statistical analysis of data

Statistical analysis of data was carried out using SPSS version 17.0. Data generated were subjected to one-way Analysis of Variance (ANOVA) and the averages were compared at the 5% level of probability. Significant difference between means was separated using least significant difference (LSD) test

Results and Discussion

Table 1 and figure 1 exhibit the effect of *O. gratissimum* leaf extracts on *S. aureus* and *E. coli*. From the table, increase in concentration reduces the number of visible *S. aureu* colonies from 65 cfu/ml to 5 cfu/ml and for

E. coli from 36 cfu/ml to 5 cfu/ml. This showed that the leaf extracts of the plants were sensitive to these bacteria used as the test organisms.

Table 2 and figure 2 show the effect of *C. papaya* leaf extracts on *S. aureus* and *E. coli*. A 5 ml extract of both leaves completely inhibited the growth of *S. aureu* but reduced the number of visible colonies of *E. coli* from 65 cfu/ml to13 cfu/ml. As the volume of the added extract increases, the number of visible colonies decreases. At the concentration of 4.5mls, there is total inhibition. Total inhibition was observed at the concentration of 4.5 mls of *C papaya* extract on *S. aureus* (gram positive) but 4.5 ml of the extract reduced the colonies of *E coli* from 65 cfu/ml to 26 cfu/ml.

This indicates that *O. gratissimum* and *C. papaya* extracts have the potential of a broad spectrum of activity against both gram-positive and gram-negative bacteria. But one could observe the variation in number of visible colonies between the two groups of bacteria. In addition, it was observed that the antibacterial activity of the plant materials were higher against the gram-positive organism as compared to the gram-negative. This phenomenon has been observed elsewhere (Matasyoh, 2007) and one reason for this may be the fact that gram negative bacteria are more resistant to the action of antimicrobial compounds compared to their gram positive counterparts as a result of the more complex cell wall of the former (Aulton, 2002).

This resistance may also be due to the lipid content of the membranes of the different groups of the micro organisms and the differences in the rate of permeability of active phytochemicals of *O. gratissimum* and *C. papaya*. There have been a greater number of studies showing antimicrobial activity of *O. gratissimum* and *C. papaya* against bacteria, fungi, virus and human intestinal protozoan parasites. Matasyoh (2007) observed the antimicrobial activity of the essential oil of *O. gratissimum* against pathogenic strains of Gram positive (*Bacillus spp*), Gram negative (*E. coli, P. aeruginosae, S. typhi, K. pneumonia, P. mirabilis*) and pathogenic fungus (*C. albicans*). Papaya milk latex shows anti-bacterial properties and it inhibits fungal growth, especially that of *Candida albicans* (Giordani and Siepai, 1991).

Since many of the pathogenic bacteria are resistant to antibiotics used for therapy these days, natural products of higher plants may offer a new source of antibacterial agents and from this result it is clear that the anti bacterial potential of *O. gratissimum* and *C. papaya* are promising.

Conclusion

From this study it is clear that, *O. gratissimum* and *C. papaya* satisfy all of the criteria for antibacterial agents, being cheap and safe. Further investigation upon the principles of the antimicrobial activity of juices from *Ocimum* and *Carica* species could be furthered. From the foregoing study, it can be concluded that both extracts demonstrated antibacterial activities against both *S. aureus* and *E. coli*. This indicates that these plants extracts could be used in the management of ailment caused by these bacteria.. Further formulation studies should be conducted with extracts of different part of these plants and also be tried on food products as a novel preservative.

Table 1: Effect of *Ocimum gratissimum* leaf extract on *Staphylococcus aureus* and *Escherichia coli.*

	***Ocimum gratisimum* extracts (ml) / number of colonies (cfu/ml)**										
Test organisms	Control	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
E. coli	65.0^{z} ± 7.0	40.0^{a} ±1.6	38.0^{a} ± 1.0	33.0^{ab} ± 2.5	32.0^{b} ± 2.0	30.0^{c} ±0.0	27.0^{d} ±1.6	24.0^{e} ±1.0	19.0^{f} ±1.6	18.0^{f} ±2.5	14.0^{g} ± 3.5
S. aureus	36.0^{w} ± 2.6	15.0^{a} ±0.7	13.0^{b} ±1.6	10.0^{b} ±0.0	9.0^{b} ±1.0	8.0^{ab} ±1.0	8.0^{ab} ±0.7	6.0^{c} ±1.0	6.0^{c} ±0.7	5.0^{d} ±1.0	5.0^{d} ±0.7

Values are means of duplicate determinations ± SD. Values in the same row bearing different superscript differ significantly ($P < 0.05$).

Table 2: Effect of *Carica papaya* leaf extracts on *Staphylococcus aureus* and *Escherichia coli*

	***Carica papaya* extracts (ml)/numbers of colonies(cfu/ml)**										
Test organisms	Control	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.
E. coli	65.0^{z} ± 7.0	37.0^{a} ±1.0	36.0^{a} ±0.7	34.0^{ab} ±0.7	33.0^{bc} ±0.7	$30.0a^{bc}$ ±0.0	29.0^{d} ±1.6	28.0^{d} ±0.0	26.0^{e} ±1.0	24.0^{f} ±0.7	13.0^{g} ±1.0
S. aureus	36.0^{w} ± 2.6	7.0^{a} ±0.7	4.0^{b} ±0.7	3.0^{b} ±0.0	3.0^{b} ±0.7	2.0^{bc} ±0.0	2.0^{bc} ±0.7	1.0^{cd} ±0.0	1.0^{cd} ±0.7	0.0^{d} ±0.0	0.0^{d} ±0.0

Values are means of duplicate determinations ± SD. Values in the same row bearing different superscript differs significantly ($P < 0.05$).

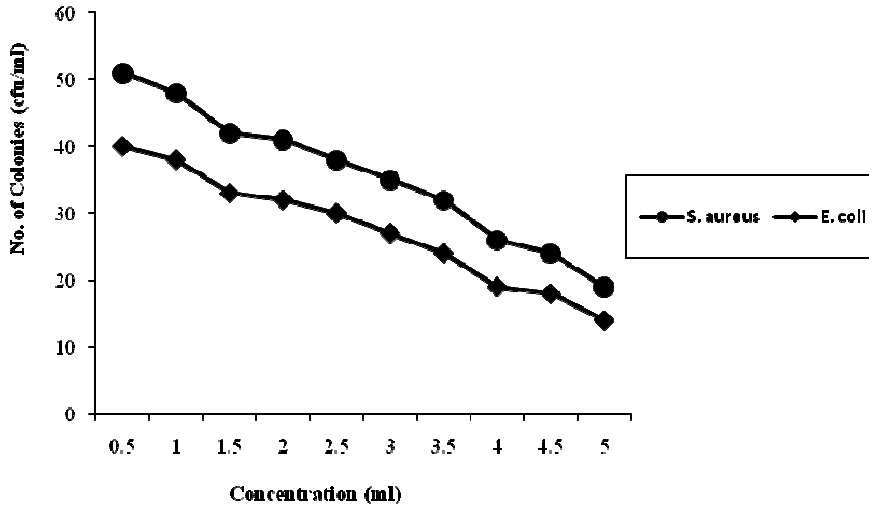

Fig. 1: Graph of effect of *Ocimum gratissimum* leaf extracts on *Staphylococcus aureus* and *Escherichia coli.*

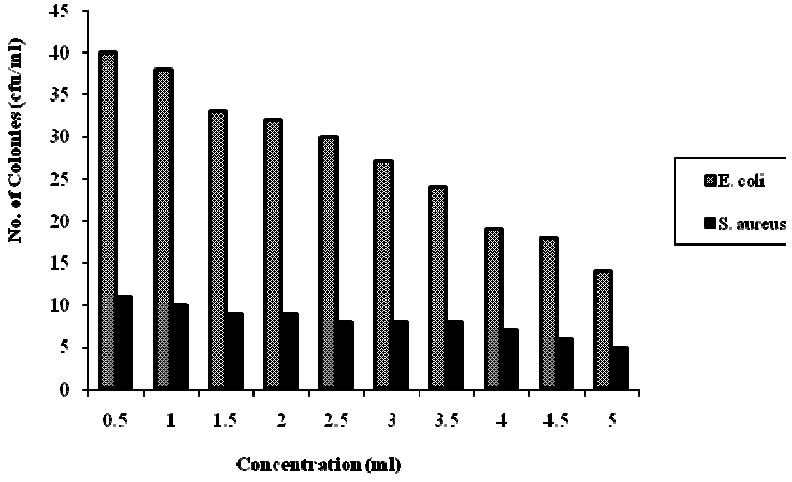

Fig**.** 2: Effect of *Ocimum gratissimum* leaf extracts on *Staphylococcus aureus* and *Escherichia coli.*

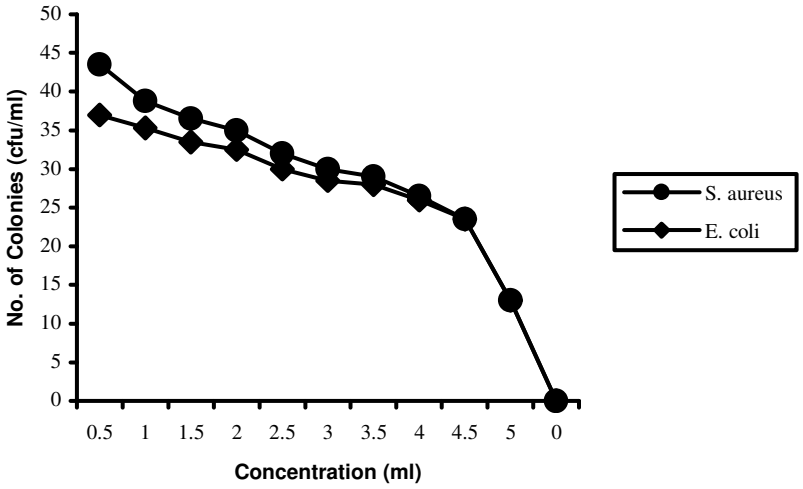

Fig. 3: Effect of *Carica papaya* leaf extracts on *Staphylococcus aureus* and *Escherichia coli.*

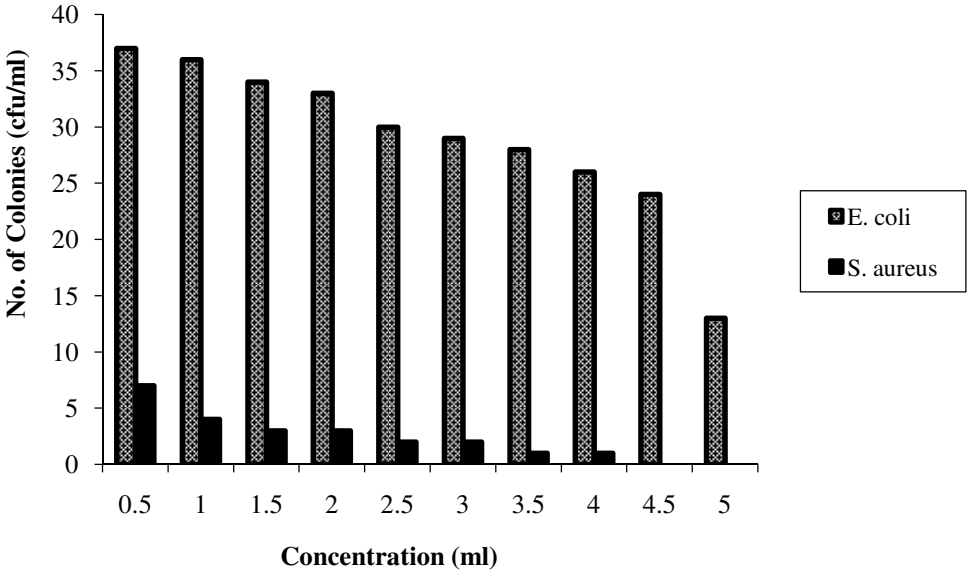

Fig. 4: Effect of *Carica papaya* leaf extracts on *Staphylococcus aureus* and *Escherichia coli.*

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