

Antibacterial Efficacy of Aqueous and Ethanolic Extracts of *Hibiscus sabdariffa* and *Ocimum gratissimum* on some selected Gram-Negative Bacteria

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Abstract

This study is aimed at looking at the most effective between extracts of *Hibiscus sabdariffa* calyces and *Ocimum gratissimum* leaves on *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae*. Antibacterial efficacy of distilled water and ethanolic extracts of *Hibiscus sabdariffa* calyces and *Ocimum gratissimum* leaves on the above-named organisms were evaluated by agar well diffusion technique and broth dilution method. The antibacterial efficacy of these extracts was compared with the commercially available antibiotics. The dried residue obtained was reconstituted with distilled water to a concentration of 400mg/ml. Some of the organisms showed varying degree of resistances to the antibiotics while some were sensitive. The aqueous and ethanolic extracts of *H. sabdariffa* and the ethanolic extract of *O. gratissimum* inhibited growth of the organisms at concentrations of 100 -400 mg/ml (zones of inhibition ranged between 6 and 24 mm). The minimal inhibitory concentrations (MICs) of the extracts were between 24 and 93.75 mg/ml. This revealed that ethanolic extract of *O. gratissimum*, the aqueous and ethanolic extracts of *H. sabdariffa* have antimicrobial activity on all the organisms and probably could be used to control infections associated with them.

Keywords: *Hibiscus sabdariffa*, *Ocimum gratissimum*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*

Introduction

Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans (Nwachukwu *et al.*, 2010). Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Although medicinal plants produce slow recovery, the therapeutic use of medicinal plants is becoming popular because of their lesser side effects and low resistance in microorganisms (Seyyednejad and Motamedi, 2010).

Despite the advancement in, and increased availability of orthodox medicines, there has been a growing interest in the complementary and alternative medicines particularly by those who have apprehension concerning toxicity and safety of drugs (Cowan, 2005). Medicinal plants are gaining popularity over synthetic drugs due to rapid increase in the rate of infections and antibiotic resistance in microorganisms (Babu and Subashree, 2009).

Hibiscus sabdariffa is a medicinal plant in the Malvaceae family which originated from Egypt (Ali *et al.*, 2005). It is thought to be native to Asia or Tropical Africa (Mahadevan *et al.*, 2009). The antimicrobial properties of *Hibiscus* calyces have been studied and were demonstrated to be effective against a wide range of pathogens. Methanol extractions of the calyces have shown to have antimicrobial activity against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia mascences*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Pseudomonas* sp. (Olaleye, 2007). *Ocimum gratissimum* (Lamiaceae), is active against several species of bacteria, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Shigella*, *Salmonella* and *Proteus* (Janine de Aquino *et al.*, 2005). This study was carried out primarily to compare the effectiveness of aqueous and ethanolic extracts of the calyx of *H. sabdariffa* and leaves of *O. gratissimum* on selected bacteria which have developed multi-drug resistance to commercially available antibiotics.

Materials and Methods

Pure cultures of *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* obtained from the main laboratory of the Department of Microbiology, University of Ilorin were used in this study.

Collection of plant materials

Dried calyces of *H. sabdariffa* were obtained from Ago market in Ilorin, Kwara state, while leaves of *O. gratissimum* were obtained from the school garden of Warrah Community Secondary School, Warrah-Oja, Ilorin. The plant specimens were verified and their identities confirmed at the Department of Plant Biology, University of Ilorin, Ilorin.

Standardization of Test Organisms

Colonies of each organism was inoculated aseptically into test tubes containing 9 ml of sterile nutrient broth and incubated at 37°C for 24 hours. The organisms were standardised using the 0.5 McFarland standards (to give cell concentration approximately 1.5×10^8 per ml). The absorbance of each organism was measured at 625 nm using a spectrophotometer (Andrews, 2001).

Preparation of Plant Extracts

The dried calyces of *H. sabdariiffa* and leaves of *O. gratissimum* were rinsed thoroughly with cold water to remove dirt and then air dried at room temperature for a week. They were then grounded into fine powder using a blender. Fifty grams each of grounded powder was suspended in a conical flask and 200 ml of distilled water or 70% ethanol was added. It was placed on a rotary shaker for 72 hours and filtered with muslin cloth and Whatman No 1 filter paper. The filtrates obtained were evaporated using a rotary evaporator and further evaporated to complete dryness using a water bath. Four hundred mg of dried residues obtained were then reconstituted in distilled water for aqueous extract or in 70 %ethanol for ethanolic extract to a concentration of 400 mg/ml which serves as the stock (Cowan, 2005).

Sterility Test of Plant Extracts

This was done as described by Sule and Agbabiaka (2008) with slight modifications. Each extract was tested for the presence of microbial contaminants by dissolving 1 g of each extracted residue in 9 ml of distilled water and plating on a nutrient agar plate using the pour plate method and incubated at 37°C for 24 hours. Absence of growth on the plates indicated a sterile extract which was then used for the antibacterial sensitivity testing of the organisms.

Antibacterial Sensitivity Testing of the Extracts (Agar Well Diffusion Method)

From the stock concentration (400 mg/ml) of the extracts (aqueous and ethanolic), 300 mg/ml, 200 mg/ml and 100 mg/ml were prepared according to method of Andrews (2001). Solidified Mueller Hinton agar plates were inoculated with the test organisms using a sterile swab stick to streak each organism. Sterile cork borer of size 6 mm was used to bore wells aseptically on the plates that have been previously labelled. About 1 ml of the different concentrations of the extracts and control were introduced into labelled wells using a micropipette. The plates were allowed to stand for 1 hour for the extracts to diffuse into the media. The plates were incubated side-up aerobically at 37°C for 24 hours and the zones of inhibition were observed and measured using a ruler (Alo *et al.*, 2012).

Determination of Minimum Inhibitory Concentration (MIC)

Different concentrations of the extracts 95 mg/ml, 70 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml were prepared and 1 ml of each was pipetted into test tubes containing 9 ml of nutrient broth. About 0.1 ml of standardised inoculum of each organism was then added to the test tubes. Two control tubes were set up i.e. a tube containing the extract and nutrient broth and another containing the organisms and nutrient broth. The tubes were incubated aerobically for 16 hours. The lowest concentration that produced no turbidity (visible growth) when compared with the controls was taken as the MIC (Aneja, 2014).

Determination of the Minimum Bacteriostatic Concentration (MBC)

Samples from the tubes that showed no growth from the MIC were inoculated on sterile nutrient agar plates and incubated aerobically for 24 hours. The ability of the organisms to grow on the nutrient agar plates indicates the bacteriostatic effect of the extracts (Aneja, 2014).

Antibacterial Sensitivity testing of the organisms against commercially available antibiotics

Commercially sold antibiotics were also tested against the organisms using the disc diffusion method. The standardised organisms were inoculated on sterile Mueller Hinton agar using swab stick. The antibiotic disc which contained different types of antibiotics was then placed on the inoculated plate using sterile forceps. The plates were then incubated aerobically at 37°C for 24 hours. The sensitivity and resistance of organisms to the antibiotics were recorded. The antibiotics used and their different concentrations are: Augmentin 30µg; Ceftriaxone 30µg; Nitrofurantoin 200µg; Gentamicin 10µg; Cotrimoxazole 25µg; Ofloxacin 5µg; Amoxicillin 25µg; Ciprofloxacin 10µg; Tetracycline 30µg; Pefloxacin 5µg as indicated by the manufacturer.

Results

Five pathogenic bacteria; *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were subjected to antibiotic sensitivity testing using both the disc diffusion method and the agar well diffusion method.

The ethanolic and aqueous extracts of *H. sabdariffa* and *O. gratissimum* were used in this study to determine their antimicrobial effect on *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. All the organisms were resistant to the aqueous extracts of *O. gratissimum* but showed different zones of inhibition to different concentrations of the ethanolic extracts of the two plants and the aqueous extract of *H. sabdariffa* (Figures 1-5).

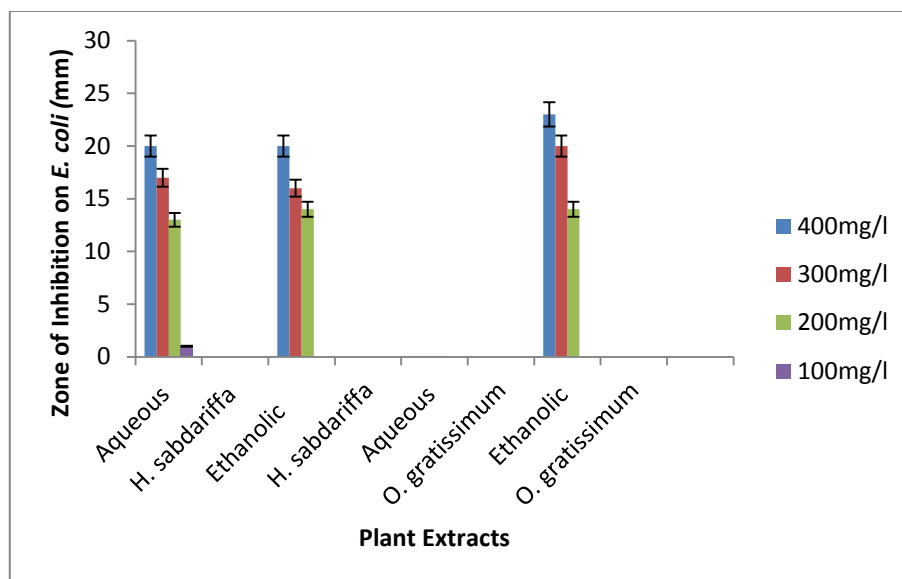


Figure 1: Antibacterial activities of aqueous and ethanolic extracts of *H. sabdariffa* and *O. gratissimum* against *E. coli*

Figure 1 shows the zones of inhibition of all the extracts against *E. coli* at concentrations between 100–400 mg/ml. The ethanolic extract of *O. gratissimum* at 400mg/ml had the highest zone of inhibition of 23 mm and the lowest diameter of 9 mm was at the 100mg/ml of aqueous *H. sabdariffa*.

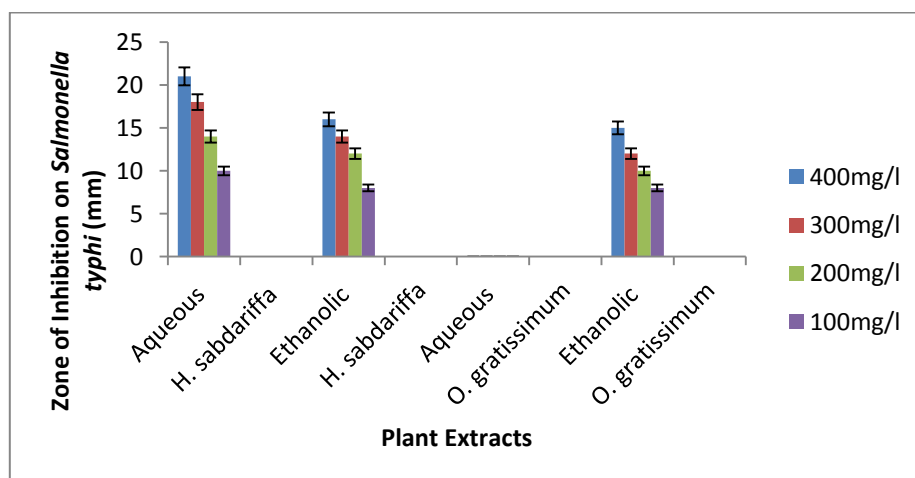


Figure 2: Antibacterial activities of aqueous and ethanolic extracts of *H. sabdariffa* and *O. gratissimum* against *Salmonella typhi*

Figure 2 shows the zones of inhibition of all the extracts against *S. typhi* at concentrations between 100mg/ml – 400mg/ml. The aqueous extract of *H. sabdariffa* at 400mg/ml had the highest zone of inhibition of 21 mm and the lowest diameter of 8 mm was at the 100mg/ml ethanolic extracts of *H. sabdariffa* and *O. gratissimum*.

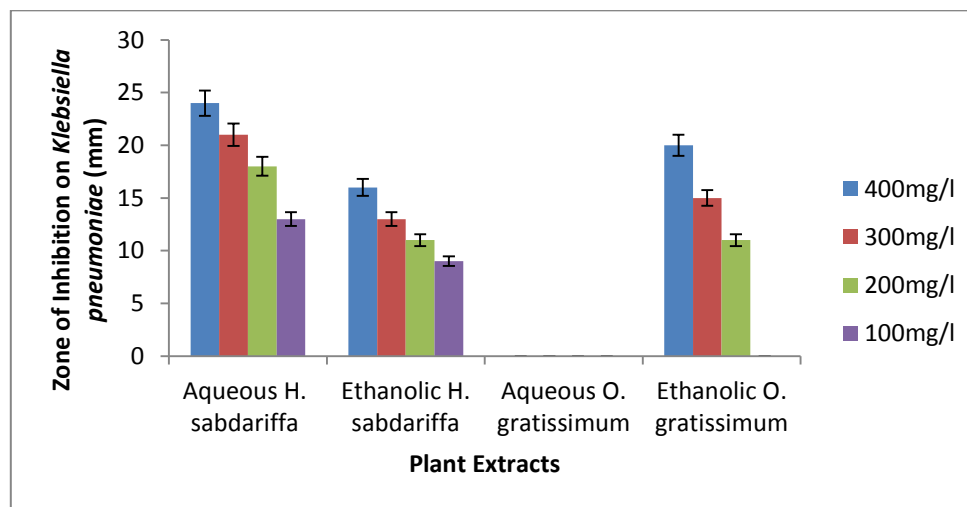


Figure 3: Antibacterial activities of aqueous and ethanolic extracts of *H. sabdariffa* and *O. gratissimum* against *Klebsiella pneumoniae*

Figure 3 shows the zones of inhibition of all the extracts on *K. pneumoniae* at concentrations between 100mg/ml - 400mg/ml. The aqueous *H. sabdariffa* at 400mg/ml had the highest zone of inhibition of 24mm and the lowest diameter is at the 100mg/ml concentration of ethanolic *H. Sabdariffa* while the organism was not inhibited at 100mg/ml concentration of ethanolic *O. gratissimum*.

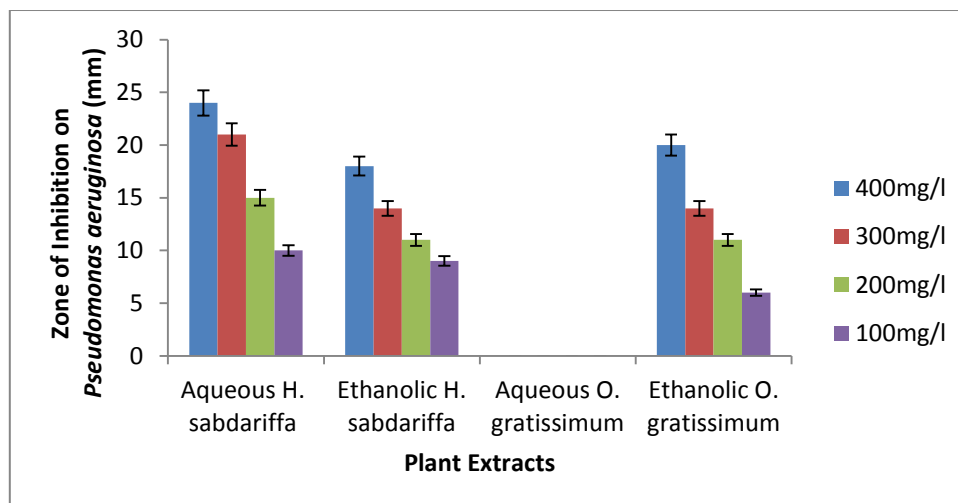


Figure 4: Antibacterial activities of aqueous and ethanolic extracts of *H. sabdariffa* and *O. gratissimum* against *Pseudomonas aeruginosa*

Figure 4 shows the zones of inhibition of all the extracts on *P. aeruginosa* at concentrations between 100mg/ml - 400mg/ml. The aqueous *H. sabdariffa* at 400mg/ml had the highest zone of inhibition of 24mm and the lowest diameter of 6mm is at the 100mg/ml concentration of ethanolic *O. gratissimum*.

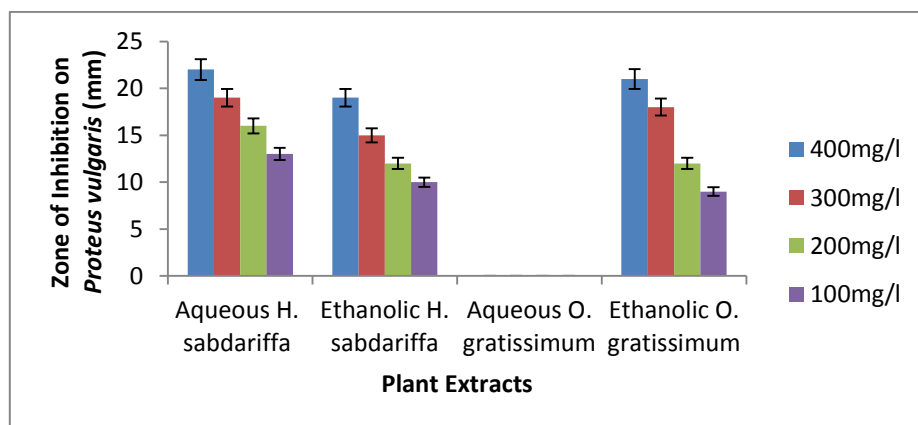


Figure 5: Antibacterial activities of aqueous and ethanolic extracts of *H. sabdariffa* and *O. gratissimum* against *Proteus vulgaris*

Figure 5 shows the zones of inhibition of all the extracts on *P. vulgaris* at concentrations between 100mg/ml - 400mg/ml. The aqueous *H. sabdariffa* at

400mg/ml had the highest zone of inhibition of 22mm and the lowest diameter of 9mm is at the 100mg/ml concentration of ethanolic *O. gratissimum*.

The MICs for all the organisms were between the range of 24mg/ml and 93.75mg/ml for the extracts that were effective against the organisms (Table 1).

The plant extracts were found to have bacteriostatic effects on all the tested organisms as they were all able to grow back on the recovery media. *E. coli* and *P. vulgaris* were sensitive to ofloxacin while *K. pneumonia*, *S. typhi* and *P. aeruginosa* showed no zone of inhibition. However, all the organisms showed resistance to one or more of the antibiotics. No single antibiotic was effective against all the test organisms (Table 2).

Table 1: Minimum Inhibitory Concentration (mg/l) values on test organisms

Organisms	Aq. <i>H. sabdariffa</i>	Ethan. <i>H. sabdariffa</i>	Ethan. <i>O. gratissimum</i>
<i>E. coli</i>	55	70	24
<i>S. typhi</i>	52	69.25	49.25
<i>K. pneumoniae</i>	93.75	92.25	0
<i>P. aeruginosa</i>	91.25	93.5	71.25
<i>P. vulgaris</i>	49.25	71.25	49.25

P. aeruginosa was resistant to gentamicin, cotrimoxazole and amoxicillin. *E. coli* was also resistant to all the antibiotics except ofloxacin that was able to inhibit its growth. *P. vulgaris* was resistant to augmentin, ceftriaxone and nitrofurantoin. *K. pneumoniae* was only sensitive to gentamicin while *S. typhi* was not inhibited by any of the antibiotics (Table 2).

Table 2: Antibiotic susceptibility of the test organisms to commercially available antibiotics

Antibiotics	Organisms				
	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>P. vulgaris</i>
Augmentin	R	R	R	I	R
Ceftriaxone	R	R	R	I	R
Nitrofurantoin	R	R	R	I	R
Gentamicin	R	R	I	R	I
Cotrimoxazole	R	R	R	R	S
Ofloxacin	S	R	R	R	S
Amoxicillin	R	R	R	R	S
Ciprofloxacin	R	R	R	I	S
Pefloxacin	R	R	R	I	I

KEY: S- Sensitive (Zone of Inhibition between 6–11 mm); I- Intermediate (Zone of Inhibition between 3-6 mm); R - Resistant

Discussion

None of the tested organisms was inhibited by the aqueous extract of *O. gratissimum*. This is in line with the findings of Ladipo *et al.* (2010) and Alo *et al.* (2012) who found out that aqueous extract of *O. gratissimum* had no inhibitory effect on *E. coli*, *Salmonella* spp., *Klebsiella* spp. and *Shigella* spp. This may be due to insolubility of the active compounds in water or the presence of inhibitors to the antimicrobial components. However, when Junaid *et al.* (2006) studied the antimicrobial efficacy of cold and hot water, hexane and methanolic extracts of fresh and dried leaf of *O. gratissimum* against *S. typhimurium*, *E. coli*, *Yersinia enterocolitica*, *Bacillus cereus* and *Aeromonas hydrophila* they found that the cold water extract of fresh leaf was most potent. This could be due to denaturing of the active antimicrobial constituents of the leaf during drying. Hence the local populace has always employed the method of the cold water extraction of the fresh leaf in

tackling gastrointestinal diseases. Both the aqueous and ethanolic extracts of *H. sabdariffa* were inhibitory at all concentrations to all the test organisms.

The development of resistance by these organisms could be as a result of antibiotics proliferation and careless use of the drugs as suggested by Todar (2011).

Inhibition of all the organisms by the ethanolic extract at different concentrations in this study is similar to what was obtained by Ladipo *et al.* (2010) and Alo *et al.* (2012). *E. coli* showed the highest zone of inhibition i.e. 23 mm at 400mg/ml (Figure 1) and *P. aeruginosa* with the lowest zone of inhibition of 6 mm at 100mg/ml (Figure 4). The effectiveness of the ethanolic extract could be as a result of the solubility of the active components of the plant in ethanol. This is in line with the reports of Obi and Onuoha (2000), who reported that alcohol (ethanol), is the best solvent for the extraction of the active principles of medical importance in most plants. This may be due to the non-polar nature of the active principles.

The MIC range of the two extracts on all test organisms was between 24mg/ml and 93.75g/ml. The results obtained from this study correlates with previous findings that demonstrated the antimicrobial properties of aqueous and ethanolic extracts of *H. sabdariffa* to be effective against a wide range of pathogens (Navarro *et al.*, 2006; Olaleye, 2007 and Chao and Yin, 2009).

Ethanolic extract of *O. gratissimum*, the aqueous and ethanolic extracts of *H. sabdariffa* have antimicrobial activity on all the organisms and probably could be used to control infections associated with them. These plants are readily available in our local environment, they are relatively cheap and biodegradable, thus not likely to pose any negative side effect to the user and the environment.

Conclusion

There were observed antimicrobial effects of calyx of *H. sabdariffa* and leaf of *O. gratissimum* on *E. coli*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *P. vulgaris*. This implies that extracts from these plants possess antimicrobial activities and hold great promise as antimicrobial agents against the tested organisms.

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