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# Synergistic Antibacterial Activity of *Hibiscus sabdariffa*, Honey and Ciprofloxacin on Selected Uropathogens

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**ABSTRACT**

New treatment methods have been sought to alleviate resistance by uropathogens. The aqueous extract of *H. sabdariffa* calyx was subjected to gas chromatography-mass spectrophotometry, Fourier transform infrared assays and phytochemical screening following standard methods. I*n-vitro* interactive activity of the *H. sabdariffa*, honey and ciprofloxacin was conducted by Agar diffusion method. Among the various compounds revealed by GC-MS, the presence of 9-Octadecanoic acid (Z) methyl ester in the aqueous extract validates the antioxidant, anti-inflammatory, anticancer and antimicrobial properties of *H. sabdariffa* calyx. Fourier transform infrared spectrum of the extract and honey shows the functional groups present at different peaks. Phytochemicals revealed in *H. sabdariffa* calyx and honey include tannin, phenol, saponin, steroids, terpernoids, flavonoid and glycoside while, alkaloids and phlobatannin were absent. The antibacterial assay showed the efficacy of both plant extract and honey on clinical and typed bacterial strains. *H. sabdariffa* extract had its minimum inhibitory concentration at 12.5 mg/ml with highest (15.33±0.33) zone against *Pseudomonas aeruginosa* while honey was only effective at 100% with inhibition zones ranging from 9.54±0.88 – 14.6±0.33 on all tested organisms. The result of the interactive combination showed a synergistic reaction of aqueous extract of *H. sabdariffa* at higher concentration and lower concentrations of honey and ciprofloxacin giving the highest zone of inhibition (42.00±1.52 mm) on *Escherichia coli* ATCC 25922. This study has validated the folkloric use of *H. sabdariffa*, honey and ciprofloxacin to show an overall increase in the activity of their combination against uropathogens which has proved to safeguard the public health of persons with suspected urinary tract infections.

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## 1. Introduction

Uropathogenic *Escherichia coli* (UPEC) is usually the causal uropathogen for both uncomplicated and complicated urinary tract infection (UTIs). Other causal agents that are involved in uncomplicated UTIs are; *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* sp. (Flores-Mireles et al., 2015). UTIs result in major economic and public health problems and affect the quality of life of individuals (Kostakioti et al., 2012). Antibiotics are known to be used in treating and/or preventing bacterial infections (Leekha et al., 2011). Ciprofloxacin is recognized to be potent against both Gram-positive and Gram-negative bacteria. It is used in treating extensive kind of infections such as urinary tract infection that is caused by vulnerable bacteria (Kostakioti et al., 2012). In some cases of antibiotics resistance, microorganisms could produce enzymes that destroy the active drug or even change the permeability of the drugs (Willey et al., 2011). *Hibiscus subdariffa* (Linn.) is a shrub belonging to the family (Malvaceae) which is commonly found in Asia and tropical Africa. *H. sabdariffa* L. also known as Roselle, is one of the important and well-known **medicinal plants** in several countries and Nigeria (Qi et al., 2005). Roselle is considered as one of the most prominent folk medicinal plants, with many chemical constituents making it a potential panacea for health benefits and sustenance. The ethno-medicinal use of Roselle in stimulating cardiovascular health and averting hypertension, pyrexia and liver disorders, sedative, reduction in microbial proliferation, as well as a diuretic and digestive importance has been reported by (Al-Ansary et al., 2016; Al-Okbi et al., 2017). The red calyces contain anti-oxidants such as flavonoids, gossypetine, hibiscetine and sabdaretine which have been reported by (Qi et al., 2005). Honey primarily contains sugars and water. Sugars in honey are largely made up monosaccharides and oligosaccharide and known to give additional energy compared to synthetic sweeteners (Vallianou et al., 2014). Some components of honey are amino acids, antibiotic-rich inhibine, proteins and phenol antioxidants Wang and Li (2011). The use of honey in traditional medicine in treating a number of infections has been in existence since ancient time (Manndal and Mandal, 2011). Owing to the worrisome global resistance of uropathogens to contemporary antimicrobials, this factorial study design is focused on evaluating the antimicrobial synergistic efficacy *H. sabdariffa*, honey and antibiotics (ciprofloxacin) as an alternative in tackling bacterial resistance and reoccurrence of bacterial infection associated with uropathogens.

## 2. Materials and method

### 2.1. Collection and identification of plant

*Hibiscus sabdariffa* calyx purchased from Oba market Akure, was carefully transported in clean sample bags to the department of Crop, Soil and Pest Management of the Federal University of Technology Akure (FUTA) for analysis. The identity of the *Hibiscus sabdariffa* calyx was initially confirmed at the Crop, Soil and Pest Management department, FUTA before further studies.

### 2.2. Source and dilution of honey

The Nectar honey used in this study was obtained from Topfit xpress Nig. Ltd. Nigeria. The honey sample was aseptically filtered with sterile mesh to remove debris. The honey filtrate was divided into two portions. A portion was subjected to proximate analysis while the second portion was diluted with sterile distilled water in concentrations of 6.25, 12.5, 25.0 and 50.0 (v/v).

### 2.3. Test isolates

A total of four (4) clinical bacterial isolates were used in this experiment with their corresponding typed strains for zone of inhibition, minimum inhibitory concentration and antibacterial interactive effect. The clinical uropathogens were obtained from the stock culture of the Microbiology department of the

Federal University of Technology Akure, Ondo State while the corresponding typed isolates were obtained from the Federal Institute of Industrial Research Oshodi (FIRRO).

### 2.4. Preparation of Hibiscus sabdariffa for extraction

The plant calyxes were air dried at room temperature without exposing to the direct sunlight for two weeks, grinded into fine powder and kept in air tight containers. Exactly 100 g of the plant sample was measured and soaked in aqueous solvent for 72 hours; thereafter the menstrum was cooled and evaporated to obtain the dried extract.

#### *2.4.1. Structural elucidation of aqueous extract of Hibiscus sabdariffa*

The analysis of the sample was performed using GC-MS equipment on Varian 4000 GC-MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Runtime was 40 minutes. MS Varian 3800 mass spectrometer, coupled to a Varian 4000 gas chromatograph was used according to the manufacturer's protocol. An Agilent column, HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used. Experimental conditions of GC-MS system were as follows: carrier gas: nitrogen, injection temperature: 250 °C, split ratio 10:1, Film thickness: 0.25 µm Flow rate of mobile phase (carrier gas: $N_2$) was set at 1.0 ml/min. In the gas chromatography part, programed with oven temperature of 40 °C raised to 280 °C at 5 °C/min and injection volume was 1 µl. The instrument was set to an initial temperature of 110 $^{o}$C, and maintained at this temperature for 2 min (Colombini et al., 2010).

### 2.5. Phytochemical analysis of aqueous extract and honey

Phytochemical tests were carried out on the plant extract and honey using Standard laboratory techniques. Alkaloids, glycosides, (Salkowski test) and saponins (frothing test), terpernoids were identified with the method of Sofowora (1993). Chemical tests for the screening and identification of phytochemicals in the medicinal plant extract and honey under study was conducted according to Douye et al. (2013) and Paul et al. (2013).

#### *2.5.1. Fourier transform infrared spectrum assay*

Dried powder of the solvent extract of plant material and honey were used for FTIR analysis. Exactly10 mg of the samples was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs (Arockia et al., 2015).

### 2.6. Proximate composition of honey

Using the micro- Kjeldahl Procedure, crude protein, fat, carbohydrate, ash and moisture contents were determined for the honey sample according to the method outlined by AOAC (1990). Total carbohydrate content of the samples was calculated by difference method (subtracting the percent moisture, crude protein, crude fibre, crude fat, and ash from 100 %. This was carried out in other to ascertain the originality of the honey.

### 2.7. Sterilization and sterility proving of extract and honey

Aqueous extract of *H. sabdariffa* was sterilized using 0.22 µm Millipore membrane filter (Delson Pascal Nig. Ltd.). After sterilizing the extract, sterility proving test was determined on both aqueous extract of plant and honey according to Sule and Agbabiaka (2008). Two millimeters (2 ml) of the extract and honey were introduced into six (6) test tubes each containing 10 ml of Muller Hinton broth and incubated at 37 ˚C for 24 hours. The absence of turbidity signifies a well sterile sample.

#### *2.7.1. Determination of antibacterial activity of aqueous extract of H. sabdariffa and honey*

The antimicrobial assay was carried out according to the technique of Morales-Cabrera et al., (2013) with some modifications. One gram (1g) of the extract was measured into a sterile test tube of 10 ml and 30% dimethyl sulfuroxide (DMSO) which was added to dissolve it; this signifies 100 mg/ml concentration of the extract. Other concentrations were 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. A sterile cork borer with 6 mm diameter was used to bore 3 wells in plates containing the Muller Hinton agar medium which were initially seeded with 1 ml of the standardized inoculum of each clinical and typed strains. Each

of the well was filled with 100 µl of honey as well as the extract in different concentrations. The plates were left for 1 hour for diffusion to take place and then incubated at 37 ˚C for 18-24 hours. The zones of inhibition were recorded using a Vernier caliper and the average of the three replicates was recorded.

### 2.8. Antibiotic sensitivity testing

The test clinical isolates and typed strains were tested for their sensitivity against ciprofloxacin at 10µg/ml, 5µg/ml, 2.50 µg/ml, 1.25µg/ml and 0.625 µg/ml using similar methods for the determination of the antibacterial activity of *H. sabdariffa* and honey.

### 2.9. Determination of the interaction between the plant extract, honey and ciprofloxacin

The antibacterial interactive activity was studied by combining with the standard antibiotics (ciprofloxacin) by standard method. The plant extract was combined at 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml while honey was combined at 100% and ciprofloxacin was combined at 0.625 µg/ml and 1.25µg/ml. Exactly 0.1 ml of each combination at different concentration was then introduced into the appropriately labeled Muller Hinton agar wells of 6 mm diameter. Plates from the combination and the control were incubated at 37 ˚C for 18-24 hours. The experiment was performed in triplicates while the inhibition zone diameter (IZD) was measured separately and the average of the three replicates were recorded.

### 2.10 Data analysis

Data are presented as mean ± standard error (SE). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using IBM SPSS version 20 software. For all the tests, the significance was determined at the level of $p \leq 0.05$.

## 3. Results

### 3.1. Structural composition of aqueous extract of *H. sabdariffa* calyx

Table 1 shows the biochemical compounds present in aqueous extract of *H. sabdariffa* calyx as revealed by gas chromatogram and mass spectroscopy analysis. *H. sabdariffa* extract showed eleven peaks which indicated the presence of eleven phyto-constituents. The compounds identified were; Methylcatechol, succinic Acid dimethyl ester, vanillic acid, protocatechuic acid, tyrosol, vanillin, 9-Octadecanoic acid (Z) methyl ester, dihydroeugenol, A-Terpinolene, hydroxytyrosol and gallic acid. The result revealed that gallic acid had the highest retention time of 31.18 min while, methycatechol had the least time of 5.06 min.

Table 2 shows the results for the qualitative and quantitative screening of plant and honey extracts respectively. Phytochemical screening of the plant extracts was carried out following air drying and the subsequent grounding of *H. sabdariffa* to powdered form. Phyto-compounds such as; saponins, terpenoid, flavonids, steroids, tannin, phenol and glycosides was determined, confirmed and quantified in mg/ml. The qualitative and quantitative test of honey was done following the order of the plant extract screening.

**Table 1:** Various compounds in aqueous extract of *H. sabdariffa* calyx using GC-MS method

*3.2. Phytochemical analysis of plant extracts and honey*

| S/N | Compound Detected | Formula | Mol. weight | Retention time (min) | % Area | Weight % | m/z |
|---|---|---|---|---|---|---|---|
| 1 | Methyl Catechol | $C_7H_8O_2$ | 124 | 5.06 | 2.23 | 7.17 | 53.81.109 |
| 2 | Succinic Acid Dimethyl ester | $C_6H_{10}O_4$ | 146 | 8.11 | 8.47 | 11.91 | 55.146 |
| 3 | Vanillic acid | $C_8H_8O_4$ | 168 | 10.04 | 4.93 | 6.46 | 70. 78. 126 |
| 4 | Protocatechuic acid | $C_7H_6O_4$ | 154 | 21.52 | 13.17 | 17.16 | 76.107.126 |
| 5 | Tyrosol | $C_8H_{10}O_2$ | 138 | 13.56 | 1.69 | 2.58 | 41.81.123.138 |
| 6 | Vanillin | $C_8H_8O_3$ | 152 | 14.43 | 2.22 | 3.61 | 109.122.151 |
| 7 | 9-Octadecanoic | $C_{18}H_{34}O$ | 282 | 16.88 | 2.23 | 3.22 | 53. 282 |
| 8 | Dihydroeugenol | $C_{10}H_{14}O_2$ | 166 | 17.24 | 1.70 | 2.90 | 31. 137 |
| 9 | A-Terpinolene | $C_{10}H_{16}$ | 136 | 12.00 | 10.00 | 13.34 | 41 .91 |
| 10 | Hydroxytyrosol | $C_8H_{10}O_3$ | 154 | 21.03 | 23.18 | 29.03 | 109.137 |
| 11 | Gallic acid | $C_7H_6O_5$ | 170 | 31.18 | 2.23 | 2.37 | 135.150 |

**Table 2:** Phyto-constituents of *H. sabdariffa* calyx and honey

| Phytoconstituents | Qualitative | | Quantitative (mg/ml) | |
|---|---|---|---|---|
| | Plant extract | Honey | Plant extract | Honey |
| Saponin | + | + | $76.16\pm0.16^{e}$ | $1.00\pm0.27^{b}$ |
| Tannin | + | + | $25.17\pm0.05^{e}$ | $0.42\pm0.01^{a}$ |
| Phenol | + | - | $0.34\pm0.14^{c}$ | $0.00\pm0.00^{a}$ |
| Steroid | + | + | $6.24\pm0.01^{e}$ | $0.24\pm0.21^{a}$ |
| Terpenoid | + | - | $0.82\pm0.01^{d}$ | $0.00\pm0.00^{a}$ |
| Flavonoid | + | + | $13.42\pm0.06^{e}$ | $1.07\pm0.05^{b}$ |
| Glycosides | + | + | $10.33\pm0.01^{e}$ | $6.98\pm0.03^{d}$ |
| Cardiac Glycoside | | | | |
| Keller Kiliani test | + | + | | |
| Salkwoski test | - | - | | |
| Lieberman test | + | + | | |

Key**:** + = positive, - = negative

Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$.

### 3.3. Fourier transform infrared spectrum of *H. sabdariffa* extracts and honey

Figure 1 shows the presence of the following functional groups obtained from aqueous fraction of *H. sabdariffa*. The result revealed that the aqueous fraction had twenty-five peaks with different functional groups. From this result it was discovered that cold water had the highest peak of 3379.29 $cm^{-1}$ with O-H stretching vibration functional group when compared with the lowest peak of 443.63 $cm^{-1}$ with C-Br stretching functional group. The Fourier transform infrared spectrum of honey in figure 2 showed the presence of seventeen peaks with diverse functional groups such as free O-H stretching, $CH_2$ asymmetry, and $CH_2$ symmetry stretching among others.

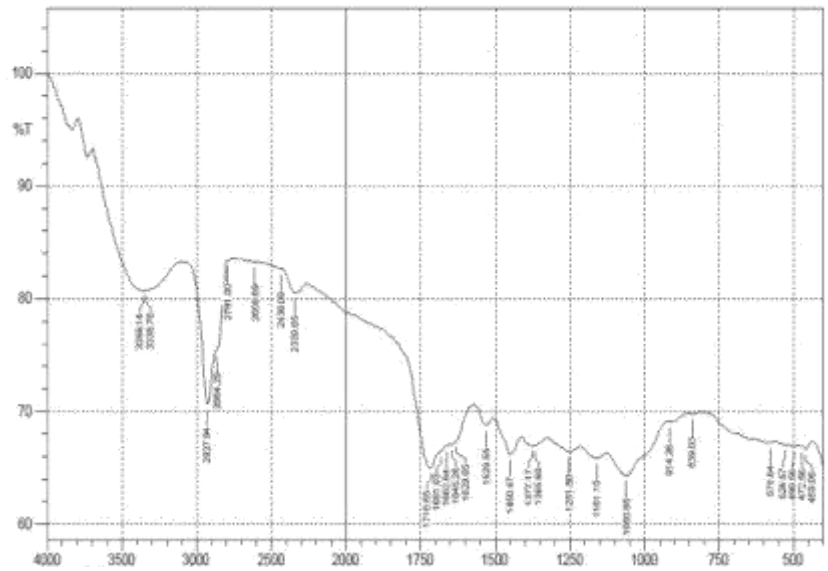


**Fig. 1**. Fourier transform infrared spectrum of cold-water fraction of *H. sabdariffa* calyx

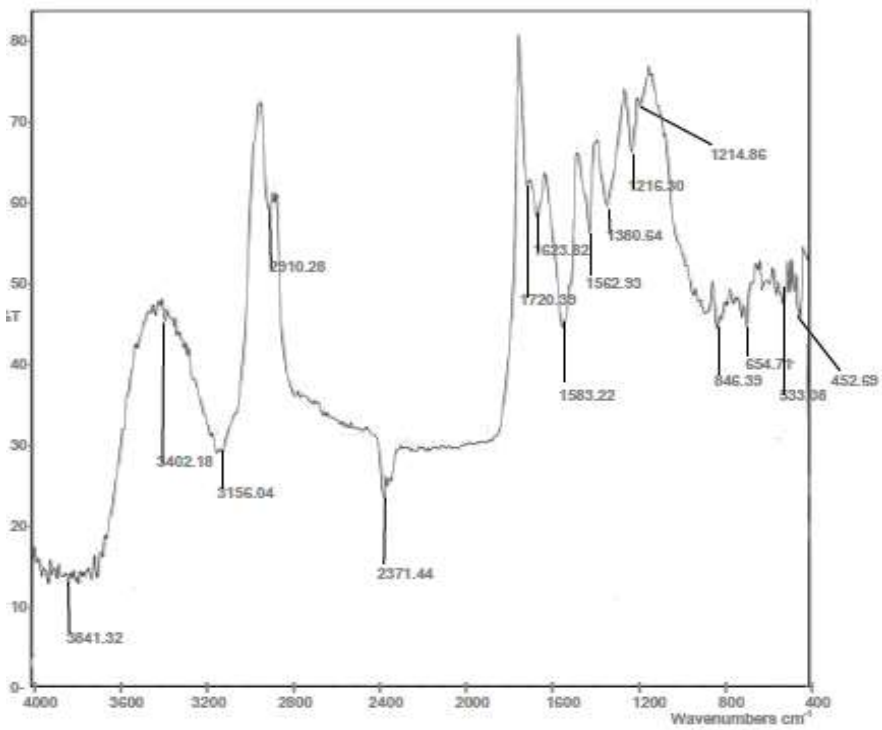


**Fig. 2.** Fourier transform infrared spectrum of pure honey

### 3.4. Proximate composition of honey sample

Table 3 shows the result for the proximate composition of the procured honey sample. The result revealed that the honey is free from adulterations. The percentage of the moisture content of the honey was 9.45%, the ash content was 1.12%. The protein content of the honey was 10.30% while the carbohydrate content was 77.95%.

**Table 3:** Proximate composition of honey

| S/N | Parameter | Quantity |
|---|---|---|
| 1. | Ash content | 1.18% |
| 2. | Moisture content | 9.45% |
| 3. | Crude fat content | 1.12% |
| 4. | Crude fiber content | 0.00% |
| 5. | Protein content | 10.30% |
| 6. | Carbohydrate content | 77.95% |
| 7. | Energy value | 1541.525 (KJ/g) |

### 3.5. Antibacterial activity of *H. sabdariffa* extract and honey against test bacteria

Table 4 shows the susceptibility profile of four uropathogens and four typed strains to aqueos extract of *H. sabdariffa* calyx and honey. All the test bacteria were susceptible to the antimicrobial agents at wavering degrees producing zones of inhibition (ZOI), with 100 mg/ml concentration as the most effective. For the clinical isolates, 100mg/ml of *H. sabdariffa* had the highest zone of inhibition of 26.67±0.67 against *S. aureus* and the least ZOI against *P. aerugonosa.* For the typed bacterial isolates, a high ZOI of 21.00±0.58 was observed against *E. coli* ATCC 25922 which the least ZOI of 15.33±0.33 was observed against *S. aureus* NCTC 6571. 100% concentration of honey showed appreciable inhibition pattern against clinical bacterial isolates (*S. aureus*), and two typed bacterial isolates (*S. aureus* NCTC 6571 and *E. feacalis* ATCC 13883) at ZOI of 14.00±0.58, 14.6±0.33 and 14.00±0.58 respectively indicating that honey was most effective at 100% concentration.

Table 5 shows the zones of inhibition diameters of plant extract at (6.25 mg/ml, 12.5 mg/ml and 25 mg/ml) when combined with honey at 100%. 25mg/ml of combined aqueous extract of *H. sabdariffa* with honey showed high ZOI against *E. feacalis* and *P. aerugonosa* (clinical bacterial isolates) at 19.67±0.88 and 18.67±0.88 respectively, while high ZOI was observed against all typed bacterial isolates at 23.33±0.88, 16.00±0.58, 18.67±0.88 and 18.67±0.88 respectively. The table shows that out of the twenty-four (24) different combination, eleven (45.83%) showed synergistic effect, two (8.33%) showed additive effect, five (20.83%) showed an antagonistic effect, two (8.33%) showed an indifferent effect and four (16.66%) showed no activity. The Chi square test was used in calculating the statistical significance ($p< 0.05$). The result revealed that the combination showed synergistic effect on all the test isolates at 25 mg/ml and an additive effect on *S. aureus.*

### 3.6 Antibacterial interaction of H. sabdariffa, honey and ciprofloxacin on test isolates

Table 6 shows the results of the interactions of the combination of aqueous extract of H. sabdariffa calyx, honey and ciprofloxacin on both Gram-positive and Gram-negative bacteria. Plant extract was combined at 12.5 mg/ml and 6.25 mg/ml, honey was combined at 100% while ciprofloxacin was combined at 0.625µg/ml and 1.25µg/ml. From the result it could be deduced that these three combinations were most effective on all clinical isolates and typed strains. There was synergistic effect on all test isolates except but, an additive effect on *S. aureus* at 1:1:1 proportion.

**Table 4:** Zones of inhibition produced by different concentrations of aqueous extract of *H. sabdariffa* calyx and honey (mm) on clinical and typed bacterial isolates

| Test organism | Plant extract | | | | | Honey | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 100 mg/ml | 50 mg/ml | 25 mg/ml | 12.5 mg/ml | 6.25 mg/ml | 100% | 50% | 25% | 12.5% | 6.25 % |
| *S. aureus* | 26.67±0.67$^{f}$ | 21.00±0.58$^{bc}$ | 15.67±0.33$^{c}$ | 13.67±0.33$^{bc}$ | 5.10±0.03$^{b}$ | 14.00±0.58$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| *E. coli* | 24.33±0.88$^{de}$ | 18.67±0.88$^{d}$ | 15.67±0.33$^{c}$ | 14.00±0.58$^{b}$ | 10.67±0.33$^{b}$ | 10.00±0.33$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| *E. feacalis* | 21.00±0.00$^{d}$ | 20.33±0.33$^{d}$ | 15.67±0.33$^{d}$ | 12.00±3.51$^{b}$ | 7.00±6.00$^{b}$ | 11.33±0.88$^{ab}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| *P. aeruginosa* | 20.00±0.58$^{d}$ | 18.00±0.58$^{cd}$ | 17.00±1.15$^{c}$ | 15.33±0.33$^{d}$ | 9.67±0.88$^{b}$ | 9.54±0.88$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| *S. aureus* NCTC 6571 | 15.33±0.33$^{d}$ | 13.00±0.58$^{c}$ | 10.67±0.33$^{b}$ | 5.00±0.58$^{a}$ | 0.00±0.00$^{a}$ | 14.6±0.33$^{d}$ | 5.10±0.03$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| *E. coli* ATCC 25922 | 21.00±0.58$^{e}$ | 16.00±0.58$^{d}$ | 15.33±0.33$^{d}$ | 13.00±0.58$^{c}$ | 5.00±0.58$^{a}$ | 11.33±0.88$^{ab}$ | 5.00±0.58$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| *E. feacalis* ATCC 13883 | 20.33±0.88$^{d}$ | 16.00±0.58$^{c}$ | 13.00±0.58$^{b}$ | 10.67±1.76$^{a}$ | 0.00±0.00$^{a}$ | 14.00±0.58$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| *Pseudomonas aeruginosa* ATCC 10145 | 18.67±0.88$^{de}$ | 17.67±0.33$^{cde}$ | 14.33±0.33$^{b}$ | 12.33±0.33$^{c}$ | 5.00±0.58$^{a}$ | 10.33±0.33$^{b}$ | 5.10±0.$^{03b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a row are not significantly different at p≤0.05.

**Tables 6:** Synergistic antibacterial combination of aqueous extract of *H. sabdariffa* calyx, honey and ciprofloxacin

| Test isolates | E + Ho + Cip 1:1:1 | Interpretation | E + Ho + Cip 2:1:1 | Interpretation | E + Ho + Cip 1:1:2 | Interpretation |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 19.00±0.58$^{a}$ | Additive | 30.00±0.58$^{c}$ | Synergism | 25.00±0.58$^{b}$ | Synergism |
| *Escherichia coli* | 31.00±0.58$^{b}$ | Synergism | 34.00±0.58$^{c}$ | Synergism | 26.00±0.58$^{a}$ | Synergism |
| *Enterococcus feacalis* | 28.33±0.88$^{a}$ | Synergism | 32.33±1.20$^{b}$ | Synergism | 30.00±1.15$^{a}$ | Synergism |
| *Pseudomonas aeruginosa* | 26.33±0.88$^{a}$ | Synergism | 36.00±0.58$^{c}$ | Synergism | 30.67±0.88$^{b}$ | Synergism |
| *S. aureus* NCTC 6571 | 24.00±0.58$^{a}$ | Synergism | 31.67±0.88$^{b}$ | Synergism | 23.00±0.58$^{a}$ | Synergism |
| *E. coli* ATCC 25922 | 36.33±0.67$^{b}$ | Synergism | 42.00±1.52$^{c}$ | Synergism | 26.00±0.58$^{a}$ | Synergism |
| *Enterococcus feacalis* ATCC 13883 | 30.33±0.88$^{a}$ | Synergism | 34.67±1.20$^{b}$ | Synergism | 30.33±0.91$^{b}$ | Synergism |
| *P. aeruginosa* ATCC 10145 | 26.33±0.67$^{a}$ | Synergism | 37.00±0.58$^{c}$ | Synergism | 31.66±0.88$^{b}$ | Synergism |

Key**:** E = aqueous extract of *H. sabdariffa* (1= 6.25 mg/ml, 2 = 12.5 mg/ml), Ho = Honey (1= 100%), Cip = Ciprofloxacin (1 = 0.625 µg/ml, 2 = 1.25 µg/ml). Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a row are not significantly different at p≤0.05.

**Table 5**: Antibacterial effect of different concentrations of aqueous extract of *H. sabdariffa* calyx and honey at 100% (mm)

| Test isolates | Extract concentrations | Honey 100% (mm) | Extract + Honey (mm) | % change IZD | Interpretation |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | A | 14.33±0.33$^{b}$ | 10.67±1.76$^{a}$ | -28.57 | Antagonism |
| | B | | 11.33±0.88$^{ab}$ | 0 | Indifference |
| | C | | 15.33±0.33$^{d}$ | 7.14 | Additive |
| *Escherichia coli* | A | 10.67±1.76$^{a}$ | - | - | - |
| | B | | 10.67±1.76$^{a}$ | 0 | Indifference |
| | C | | 13.67±0.33$^{bc}$ | 30.0 | Synergism |
| *Enterococcus feacalis* | A | 11.33±0.88$^{ab}$ | 10.67±0.33$^{b}$ | -9.09 | Antagonism |
| | B | | 13.67±0.33$^{bc}$ | 18.18 | Synergism |
| | C | | 19.67±0.88$^{de}$ | 72.72 | Synergism |
| *Pseudomonas aeruginosa* | A | 9.67±0.88$^{b}$ | 10.67±1.33$^{ab}$ | 5.26 | Additive |
| | B | | 15.33±0.33$^{d}$ | 57.89 | Synergism |
| | C | | 18.67±0.88$^{de}$ | 89.47 | Synergism |
| *Staph. aureus* NCTC 6571 | A | 14.00±0.58$^{b}$ | 13.67±1.33$^{bc}$ | -7.14 | Antagonism |
| | B | | 16.00±0.58$^{c}$ | 14.29 | Synergism |
| | C | | 23.33±0.88$^{de}$ | 64.29 | Synergism |
| *E. coli* ATCC 25922 | A | 11.33±0.88$^{ab}$ | - | - | - |
| | B | | 10.67±1.33$^{ab}$ | -9.09 | Antagonism |
| | C | | 16.00±0.58$^{c}$ | 45.45 | Synergism |
| *E. feacalis* ATCC 13883 | A | 14.00±0.58$^{b}$ | - | - | - |
| | B | | 11.33±0.88$^{ab}$ | -21.42 | Antagonism |
| | C | | 18.67±0.88$^{de}$ | 28.57 | Synergism |
| *Ps. aeruginosa* ATCC 10145 | A | 10.67±1.76$^{a}$ | - | - | - |
| | B | | 15.33±0.33$^{d}$ | 50.0 | Synergism |
| | C | | 18.67±0.88$^{de}$ | 80.0 | Synergism |

Keys**:** A = 6.25 mg/ml, B = 12.5 mg/ml, C = 25 mg/ml, IZD = inhibition zone diameter.
Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$.

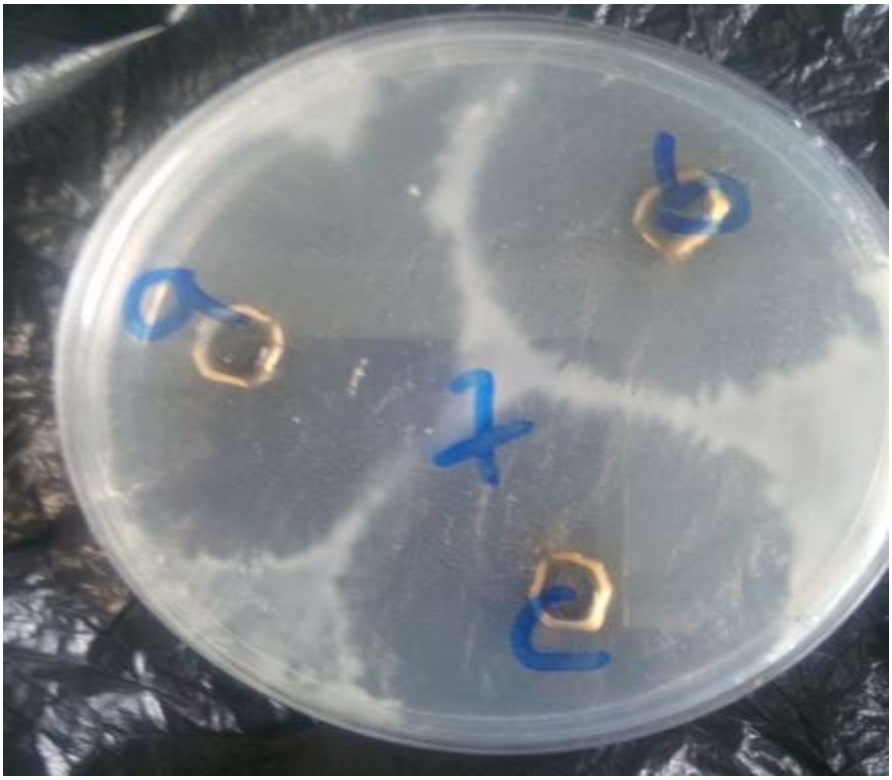


**Plate 1**: Synergistic activity of *H. sabdariffa*, honey and ciprofloxacin against *E. coli* ATCC 25922 in triplicates are represented as a, b, and c respectively

## 4. Discussion

Gas chromatography- mass spectroscopy carried out on the aqueous extract of *H. sabdariffa* calyx was able to identify the phytochemical compounds present based on the Peak area (%), molecular weight, retention factor and molecular formula. The result from this analysis revealed eleven (11) known compounds. The presence of these major compounds in the aqueous extract of *H. sabdariffa* calyx is in support with some scientific evidences that this plant can be used as potential medicinal products as opined by Bothon et al. (2016) who have revealed 9-Octadecanoic acid (Z) methyl ester, as an essential compound for basal metabolism in humans. Phytochemical analysis revealed the presence of tannin, phenol, saponin, steroids, terpernoids, flavonoid and glycoside in *H. sabdariffa* calyx and honey while, alkaloids and phlobatannin were absent. Large number of biologically active molecules has been identified from *H. sabdariffa* calyx as maintained by Okereke et al. (2015) and Jasmeet-Kaur et al. (2017). *H. sabdariffa* calyx extract also contains steroids which are in conformity with the findings of Okwu et al. (2001) who stated that steroids are of pharmaceutical importance because many of them form sex hormones. Akinoso and Suleiman (2011) added that honey possess high level of phenolic and flavonoid compounds that might have contributed to its antimicrobial properties while, the absence of phlobatannin and alkaloids may be in line with the findings of Markon et al. (2007) and Adeyemo et al. (2017), while screening for the phytochemicals in plant.

The result derived from this FTIR analysis is in corroboration with the findings of Muruganatham et al. (2009) that analyzed *Eclipta alba* and *Eclipta prostrate* plants and reported that there is strong absorption band for the plant owing to the N-H stretching and also reported the presence of functional groups such as amines, polysaccharides, carboxylic acid, carbohydrates and nitrates. The curve of the FTIR result of the honey sample shows the chemical composition and the band region from 4000-460 $cm^{-1}$. The honey sample is composed of bands of carbohydrate, water, organic acids and carboxylic acids. The presences of these essential groups could have been responsible for the antibacterial activity of both aqueous plant extract and honey (Anguebes et al., 2016; Rajiv et al., 2017).

Proximate analysis of pure honey was done to confirm the originality of the honey. The result showed the quality in the ash content, moisture content, crude fat, carbohydrate content, protein content and the energy value of the honey. The result of this study revealed the moisture content of honey to be 9.45% which is in

accordance with the findings of Ambaye and Mekonen (2016) who reported that, honey whose moisture content is lesser than 17.1% is usually safe from bacterial growth and additionally has great antibacterial properties.

Conventional drug antibacterial activity on both clinical and typed strains revealed the susceptibility patterns of different bacterial organisms. The pattern of inhibition of the microbial growth of both clinical and typed strains showed the varying abilities of each organism to resist the antibacterial activity of the conventional drug. However, these disparities could be due to variations in the structure and components of the microbial cell wall, because these attributes are the ultimate target of any antibacterial agent in accordance with Oladunmoye (2006). The antibacterial result revealed the plant aqueous extract to be effective at lower concentration as lower inhibitory concentration of any antibacterial agent indicates its potentials as a safer and better drug as detailed by Navarro-Garcia et al. (2006).

The result of the interactive effects of the aqueous extract of *H. sabdariffa* calyx and pure honey after combination in different proportions revealed increase in the antibacterial activities against the clinical and typed strains. While some have additive potentials, others showed antagonistic upshot which correlates to the findings of Oladunmoye (2006). However, there was none of the test organisms that were resistant to combinations. This work shows higher susceptibility rates of some of the organisms to higher concentration of the extract when combined with honey. This in turn, is also higher than when these natural products are applied singly. The result from the synergistic combination in this study revealed a promising future of a very effective combination of natural products and conventional drug in treating urinary tract infections. The findings from these three combinations showed a wider zone of inhibition when compared to the initial two combinations as none of the uropathogens were resistant to these three combinations as supported by Pankey et al. (2005).

The findings in this study has answered some of the questions and doubts emanating from the effectiveness of ciprofloxacin and natural products as combination therapy in treating mild to severe urinary tract infections. The synergistic result of this study is in conjunction with Haidan et al. (2016) who stated that, one of the advantages of traditional medicine's therapeutics is the "synergism" which indicates that multiple components in natural products play a synergistic role which is greater than that of individual drug.

## 5. Conclusion

The synergistic study of *H. sabdariffa*, honey and ciprofloxacin were investigated and found to be very effective. Ciprofloxacin was found to boost the synergistic effect in the combination. The antibacterial activity shown by the combination of *H. sabdariffa* calyx and honey suggests in great deal the future potentials and prospects of these three combinations to be of unique traditional medicinal use and ethno-medicinal product resource due to the synergistic properties demonstrated to contribute to safeguarding the public health of persons with suspected urinary tract infections. Further studies should be done on the immuno-modulatory and toxicological effect of the combinations.

## Conflict of interest

All authors declare none

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