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*Original Research Article*

# Production of Antiseptic Solutions Containing Extracts of *Momordica Charantia* L. and *Azadirachta Indica* A. Juss and Evaluation of its Antimicrobial Activity Against Microorganisms Associated with Folliculitis

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ABSTRACT

This study was designed to investigate the effectiveness of *Azadirachta indica* and *Momordica charantia* leaves and their formulated antiseptic solutions against selected organisms associated with folliculitis. Ethanol extracts of *A. indica* and *M. charantia* leaves were obtained and phytochemical analyses conducted using standard procedures. Antimicrobial tests on six microorganisms were conducted using standard methods. Three solutions each with varying percentage of extracts (1, 2.5 and 5%) were produced from the ethanol extracts of *A. indica* (FAi1, FAi2 and FAi3) and *M. charantia* (FMc1, FMc2 and FMc3) respectively through step-wise mixing. The antiseptic solutions produced were evaluated for their physicochemical properties and antimicrobial assay conducted on six selected microorganisms. The solutions were stored at 29 ± 4ºC for 40 days and evaluated using pH, viscosity and organoleptic properties. The formulations were Newtonian fluids with pH compatible with the skin. FMc1 significantly inhibited *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans* and *Trichophyton rubrum* (20.5 ± 0.7, 28.0 ± 0.0, 14.0 ± 0.0, 17.0 ± 1.4, 13.0 ± 1.4 and 15.0 ± 1.4 mm respectively) than others. FMc2, FAi1 and Gentamicin showed the highest inhibition against *Staphylococcus aureus* (22.00 ± 0.01) followed by FMc1 (20.5 ± 0.70), FAi3 (20.0 ± 0.01), FMc3 (19.0 ± 1.40), Dettol (18.0 ± 0.02) and FAi2 (17.0 ± 1.40). *Momordica charantia* formulated antiseptic solutions were more effective than those containing *Azadirachta indica.*

***Keywords***: Antimicrobial activity, *Momordica charantia*, *Azadirachta indica*, antiseptic solutions, folliculitis

## Introduction

*Momordica charantia* L. belongs to the family Curcurbitaceae and is commonly known as bitter gourd or bitter melon.[1] It is used in herbal medicine as an antidiabetic, abortifacient, anthelmintic, contraceptive, antimalarial and laxative.[2] *In vitro* investigations have demonstrated that *M. charantia* extracts can be used as a broad-spectrum antibacterial treatment against infections.[3]
*Azadirachta indica* A. Juss. commnly known as Neem tree and belongs to the family Meliaceae.[4] The leaves of neem possess antifungal, antibacterial, anti-inflammatory and antitumour activities.[5,6] In vitro investigations suggest that the leaves of neem contain secondary metabolites which are known to inhibit the growth of microorganisms.[7]

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Folliculitis is a common skin/scalp disorder in which the hair follicle becomes inflamed.[8] The most frequent type of folliculitis is superficial bacterial folliculitis, which is usually caused by the bacteria *Staphylococcus aureus*.[8] The majority of uncomplicated cases of folliculitis with few pustules would go away on their own after a few days. Topical antiseptics, on the other hand, may be used to treat more severe infections.[9] The antibacterial activity of the plants under study has been previously documented, however, the production of the plant extracts into antiseptic solutions have not been done. This study seeks therefore to undertake the formulation of the extracts into antiseptic solutions and also assess the activity of the solutions in microorganisms. It is expected that such effort will broaden the usefulness of the plant and the formulation will improve patient acceptance.

## Materials and Methods

*Collection of plant materials*
*Azadirachta indica and Momordica charantia* leaves were collected in June, 2021 at Ilupeju, Liberty Academy, Ayegun road, Ibadan and Ilu Odo-Ona Elewe, Ibadan respectively. The plants were authenticated at the Department of Botany, University of Ibadan, Ibadan by Donatus O. Eseimokhuai, a Taxonomist. They were deposited at the Departmental Herbarium with the following numbers- *Momordica Charantia* L. – UIH-23085 and *Azadirachta Indica* A. Juss- UIH-23087

*Plant extraction*
The leaves of *Azadirachta indica and Momordica charantia* were collected and washed with distilled water then dried at room temperature. The air-dried samples were pulverised and macerated with absolute ethanol for 72 hours with occasional shaking. The liquid extracts were

decanted and filtered using Whatman filter paper No. 1; the filtrates were concentrated with rotary evaporator at 40ºC and dried to solid extract with a freeze dryer.

*Phytochemical screening for plant extracts*
About 500mg of ethanol leaf extracts of *Azadirachta indica* and *Momordica charantia* were dissolved and used for the detection of phytochemicals such as tannins, saponins, phenolics, flavonoids, alkaloids, anthocyanins, cardiac glycosides, terpenoids, anthraquinones, steroids, glycoside, quinones and coumarins using standard procedures.[10]

*Preparation of culture media*
The nutrient media was dissolved and autoclaved according to the manufacturer's instructions; 38 g of Muller Hinton Agar (Sigma Aldrich) was dissolved in 1 L distilled water and heated to boil; 20 ml each of the sterilized agar was dispensed into MacCartney Bottles, autoclaved at 121 ˚C, 15 psi (1 kg/cm$^3$) pressure for 15 minutes and then poured in sterile petri dishes and allowed to solidify.

*Antimicrobial sensitivity of the extracts*
The antimicrobial activity of the extracts in the cultured organisms was determined by agar well diffusion method as described by Arekemase *et al*,[11] The organisms used were clinical isolates obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan. A sterile cotton swab was inserted into the fungal/bacterial suspension (0.5 McFarland standard), the already prepared Muller-Hinton agar plate was then inoculated with the swab and the inoculums (test organism) was spread evenly on the agar medium, holes were bored on the medium using a sterile cork borer (8 mm). Each hole was filled with the appropriate plant extract concentration and standard drugs (Gentamicin, Ketoconazole) using sterile Pasteur pipette. The culture plates were incubated at 37 ˚C for 48 hours (for antibacterial test) and 72 hours (for antifungal test). Susceptibility of each test organism to the plant extract was examined and the zones of inhibition were measured with recorded in millimetres.

*Determination of Minimum Inhibitory Concentration (MIC)*
The Minimum inhibitory concentrations of the extracts were determined using agar diffusion method of Akinpelu and Kolawole.[12] Extract concentrations of 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml were prepared and 1 ml was introduced into 9 ml of Muller-Hinton agar in petri dishes. A sterile cotton swab was inserted into the fungal/bacterial suspension (0.5 McFarland standard), the Muller-Hinton agar and extract mixture was then inoculated with the swab and the inoculums (test organism) is spread evenly on the agar medium by swiping the swab left and right from the top to the bottom of the agar plates and incubated for 24 h at 35˚C.
The least concentration of the extract that did not permit any visible growth in the broth was taken as the MIC.

*Formulation of antiseptic solutions*
The ethanol extracts of each plant (0.5, 1.25 and 2.5 g) were dissolved in equal volume of ethanol (20 % $^w/_v$) and were continuously stirred till they dissolved completely. Phenol (2.5 % $^w/_v$) was dissolved in Sodium Lauryl Ether Sulfate (2.5 % $^w/_v$) and the ethanol and extract mixture was added to the phenol and Sodium Lauryl Ether Sulfate mixture and continually stirred. The mixture was made up to the required volume using distilled water and continually mixed to obtain pleasant products

*Physical evaluation of the antiseptic solutions*
The organoleptic properties (colour by visual look, odour by smelling) were recorded and each product was dispensed into plain tubes for different analyses.

*Density determination*
A 2 ml syringe was first weighed, filled with 2 ml each of the produced antiseptic solutions and then reweighed. The density was calculated thus:

$$\text{Density (g/cm}^3) = (W_2\text{-}W_1)/V \qquad \text{Equation 1}$$

Where $W_1$ = weight of the syringe only
$W_2$ = weight of the syringe and antiseptic solution
V = Volume of the antiseptic solution

*Determination of pH*
The pH of the formulations was determined by a pH meter (Jenway, UK) at 25 ± 2˚C; the electrode was dipped into each formulation after calibration and readings were taken.

*Viscosity*
The viscosity of the formulations was determined with Brookfield viscometer (VT 181, Karls rule, Germany) at a temperature of 28 ± 2˚C using spindle number 3 at 5, 10, 20, 50 and 100 rpm respectively.[13]

*Antimicrobial Susceptibility of the formulated antiseptic solutions*
Two strains of Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*; two strains of Gram-negative bacteria: *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*; and two fungal isolates: *Trichophyton rubrum* and *Candida albicans* were obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, and used for the preliminary antimicrobial screening of the formulated antiseptic solutions.
The antimicrobial activities of the extracts in the cultured organisms were determined by agar well diffusion method[11]. A sterile cotton swab was inserted into the fungal/bacterial suspension (0.5 McFarland standard), the already prepared Muller-Hinton agar plate was then inoculated with the swab and the inoculums (test organism) is spread evenly on the agar medium, holes were bored on the medium using a sterile cork borer (8 mm). Each hole was filled with the appropriate plant extract concentration and standard antiseptic solution (Dettol®) using sterile Pasteur pipette. The solutions (1 ml) were diluted to 2 ml in a 1:1 ratio with ethanol and used for the test. The cultured plates were incubated at 37˚C for 24 hours (for antibacterial test) and 72 hours (for antifungal test). The susceptibility of each test organism to the antiseptic solutions was examined and the zones of inhibition were measured with a pair of divider and a ruler and recorded in millimetres.

*Stability studies of formulated antiseptic solutions*
The stability studies of the formulated antiseptic solutions for 40 days were determined by established methods;[14] using three parameters; pH, viscosity and the organoleptic properties of the solutions when stored at room temperature (29 ± 4˚C).

*Statistical analysis*
Data was presented as mean ± SD and statistical analysis was performed using Graphpad Prism 5.0 Software (GraphPad Software Inc., US) to compare the antimicrobial effects of the extract and formulation. The physicochemical properties of the formulations were also compared to that of the reference antiseptic solution. All tests were carried out using two-way analysis of variance. At 95% confidence interval, p values lower or equal to 0.05 were considered the limit of significance

## Results and Discussion

The results of the phytochemical composition of the extracts are presented in Table 1. The phytochemicals observed in this work indicates that both *Momordica charantia* and *Azadirachta indica* ethanol leaf extracts contained tannins, phenolics, flavonoids, alkaloids, anthocyanins, cardiac glycosides, terpenoids, steroids and coumarins; saponins were observed in *M. charantia* but absent in *A. indica*, quinones were observed in *A. indica* but absent in *M. charantia*.
The presence of tannins in *M. charantia* opposes the findings of Karmakar *et al*.,[15] who reported the absence of tannin in *M. charantia* ethanol leaf extract. According to chemical ecology, tannins show high variability in their structures with unique molecules which are unevenly distributed in the plant kingdom. Tannin composition also varies even within and among organs of the same plant, this could account for why they could be present or absent during screening (Salminen and Karonen.[16] The absence of saponins in *A. indica* opposes the findings of Ramadass and Subramanian,[17] however, lack of saponins agrees with the

findings of Muhammad *et al*.[18] The presence of cardiac glycoside and terpenoids in *M. charantia* opposes the findings of Adegbola *et al*.[19] The discrepancies observed can be traced to the different methods used by the various authors. Many of the phytochemicals observed in this study have been proved to be responsible for the antimicrobial activity.[20,21]

The antimicrobial activity of the extracts as shown by the zones of inhibition and minimum inhibitory concentration are shown in Tables 2 and 3 respectively. These results showed that both *M. charantia* and *A. indica* ethanol leaf extracts are active against all the bacteria tested, with the exception of *A. indica* which was not active against *Escherichia coli*. This agrees with the findings of Rutanga *et al*,[22] but opposes that of Gajendrasinh *et al*.[23] Only *Momordica charantia* was active against *Candida albicans* at the concentration of 100 mg/ml, both plants were not active against *Trichophyton rubrum*. However, *Momordica charantia* ethanol leaf extracts showed highest antimicrobial activity which can be credited to the presence of saponins in it, which was absent in *Azadirachta indica* ethanol leaf extract. In comparing the two plants, *Momordica charantia* showed higher zones of inhibition against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*, and *Candida albicans* while *Azadirachta indica* showed higher zones of inhibition against *Bacillus subtilis.*

The results of the minimum inhibitory concentration (MIC) of the extracts showed that *Mormodica charantia* utilised 0.781 mg/ml against *Klebsiella pneumonia, Escherichia coli, Trichophyton rubrum* and *Candida albican* while the concentration against *Staphylococcus aureus* and *Bacillus subtilis* is 0.391 mg/ml showing higher activity. For *Azadirachta indica*, the MIC was 6.25 mg/ml against *Klebsiella pneumonia, Escherichia coli and Trichophyton rubrum,* 3.125 mg/ml against *Staphylococcus aureus* and 0.391 mg/ml *against Bacillus subtilis* and *Candida albicans.* Generally, the ranking of MIC was *Mormodica charantia* < *Azadirachta indica.* The composition of the antiseptic solutions are presented in Table 4. All the formulations elicited a pleasant visual outlook with dark green colour but which deepens in intensity as the extract concentration increased. The odour of the formulations can be related to that of Dettol$^{R}$ which could be due to the presence of phenol. The evaluated physicochemical properties of the antiseptic solutions are shown in Table 5.

**Table 1:** Phytochemical analysis of *Momordica charantia* and *Azadirachta indica* ethanol leaf extracts

| **Phytochemicals** | ***Momordica charantia*** | ***Azadirachta indica*** |
|---|---|---|
| Saponins | + | - |
| Tannins | + | + |
| Flavonoids | + | + |
| Cardiac glycosides | + | + |
| Terpenoids | + | + |
| Steroids | + | + |
| Alkaloids | + | + |
| Phenolics | + | + |
| Quinones | - | + |
| Coumarine | + | + |
| Anthocyanin | + | + |

Key: Present + ; Absent -

**Table 2:** Zones of inhibition by *Momordica charantia* and *Azadirachta indica* ethanol leaf extracts on selected bacteria and fungi

| **Extract** | **Microorganism** | **Extract concentrations (mg/ml)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **25** | **50** | **100** | **Gent.** | **Ethanol** | **Ketoconazole** |
| | | **Zone of inhibition (mm)** | | | | | |
| *Momordica* | *Staphylococcus aureus* | 12 | 12 | 14 | 20 | 0 | NA |
| *charantia* | *Bacillus subtilis* | 12 | 12 | 14 | 20 | 0 | NA |
| | *Klebsiella pneumonia* | 12 | 16 | 18 | 10 | 0 | NA |
| | *Escherichia coli* | 10 | 10 | 12 | 18 | 0 | NA |
| | *Trichophyton rubrum* | 0 | 0 | 0 | NA | 0 | 22 |
| | *Candida albicans* | - | - | 14 | NA | 0 | 20 |
| *Azadirachta* | *Staphylococcus aureus* | 0 | 10 | 12 | 20 | 0 | NA |
| *indica* | *Bacillus subtilis* | 10 | 14 | 20 | 20 | 0 | NA |
| | *Klebsiella pneumonia* | 0 | 0 | 10 | 10 | 0 | NA |
| | *Escherichia coli* | 0 | 0 | 0 | 18 | 0 | NA |
| | *Trichophyton rubrum* | - | - | - | NA | 0 | 22 |
| | *Candida albicans* | - | - | - | NA | 0 | 20 |

**Table 3:** MIC of the leaf extracts against the test organisms.

| **Microorganisms** | **Extracts** | |
|---|---|---|
| | ***Mormodica charantia*** | ***Azadirachta indica*** |
| | **Minimum inhibitory concentration (mg/ml)** | |
| *Staphylococcus aureus* | 0.391 | 3.125 |
| *Bacillus subtilis* | 0.391 | 0.391 |
| *Klebsiella pneumoniae* | 0.781 | 6.250 |
| *Escherichia coli* | 0.781 | 6.250 |
| *Trichophyton rubrum* | 0.781 | 6.250 |
| *Candida albicans* | 0.781 | 0.391 |

The density of the formulated antiseptic solutions as shown in Table 5 increased as the concentration of the extracts increased *M. charantia* antiseptic solution formulation (5 %$^{w/}_{v}$) had the highest density, while the *M. charantia* antiseptic solution formulation (1 %$^{w/}_{v}$) had the least density (1.04 ± 0.03 and 1.02 ± 0.03 g/cm$^3$ respectively). Also, *A. indica* antiseptic solution formulation (5% $^{w/}_{v}$) had the highest density, while the *A. indica* antiseptic solution formulation (1% $^{w/}_{v}$) had the least density (1.06 ± 0.02 and 1.03 ± 0.03 g/cm$^3$ respectively). Analyses of the results showed that the differences were not significant ($p>0.05$). This is understandable because, irrespective of the extract concentration, dissolution has been achieved and density should not be significantly affected. The densities were generally higher than 1.0 which is the density of water. This is also satisfactory because solutes are within the system hence the density will be higher than that of water. Furthermore,

there were no significant differences ($p>0.05$) in the densities of all the formulations and that of the reference samples. For all drugs manufactured for use topically, the pH is a vital parameter to consider. In addition, the potential impact of pH on solubility is critical in determining the stability of the active ingredient in the product[20]. The solubility of acid and basic medications is pH-dependent and pH generally catalyses aqueous processes. There have been studies that have measured the rate of degradation at various pH levels while keeping the temperature, ionic strength, and solvent concentration constant. The pH of the formulations was generally acidic and ranged between 4.43 ± 0.03 to 5.97 ± 0.01, In addition, the pH reduced with increase in extract concentration. In comparison with Dettol antiseptic solution, with a pH of 9.61; the formulated solutions had pH compatible with the skin surface. The pH range of the skin is 4.0-6.0 with an average of 5.5, this indicates that the pH of the formulated solutions will not disturb the normal flora of the scalp nor cause irritation due to pH differences[24]. The pH of the reference antiseptic solution was significantly higher ($p<0.05$) compared to that of the formulated ones. However, the pH of the formulated solutions was within the range acceptable for use in the scalp and skin.

The viscosity of the formulations was generally low and the use of different shear rates (data not shown) did not significantly influence the values. In addition, increase in extract concentration did not have significant effect on the viscosity of the formulations. The viscosity of the formulations is therefore regarded as constant irrespective of shear rates or concentration of solutes. These indicate that they have a Newtonian flow property. Generally, all the formulations are miscible with water and this might have been influenced by the nature of the solvents used in the dissolution of the extracts. This attribute is considered satisfactory with respect to rheology. Solutions demonstrate Newtonian rheological profile like water and ethanol. The advantage for the patient is easy application and there would be no need for vigorous rubbing. In addition, the reference solution had significantly higher ($p<0.05$) viscosity compared to the formulated solutions although, it remained Newtonian hence was satisfactory.

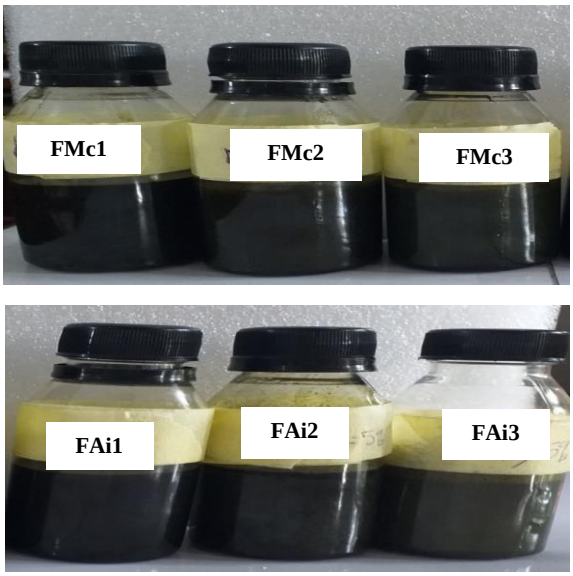


**Figure 1:** Macroscopic view of *Momordica charantia* and *Azadirachta indica* formulated antiseptic solutions
FMc1 = M. charantia antiseptic solution formulation (1 % $^{w/}_{v}$), FMc2 = M. charantia antiseptic solution formulation (2.5 % $^{w/}_{v}$), FMc3 = M. charantia antiseptic solution formulation (5 % $^{w/}_{v}$), FAi1 = A. indica antiseptic solution formulation (1 % $^{w/}_{v}$), FAi2 = A. indica antiseptic solution formulation (2.5 % $^{w/}_{v}$), FAi3 = A. indica antiseptic solution formulation (5 % $^{w/}_{v}$)

**Table 4:** Composition of the formulated antiseptic solutions

| COMPONENTS (%$^{w}/_{w}$) | SOLUTIONS | | | | | |
|---|---|---|---|---|---|---|
| | FMc1 | FMc2 | FMc3 | FAi1 | FAi2 | FAi3 |
| Extract | 1.0 | 2.5 | 5.0 | 1.0 | 2.5 | 5.0 |
| Phenol | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Sodium Lauryl Ether Sulfate | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Ethanol | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Water to | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

FMc1 = Momordica charantia antiseptic solution formulation (1%$^{w/}_{v}$), FMc2 = Momordica charantia antiseptic solution formulation (2.5%$^{w/}_{v}$), FMc3 = Momordica charantia antiseptic solution formulation (5%$^{w/}_{v}$), FAi1 = Azadirachta indica antiseptic solution formulation (1%$^{w/}_{v}$), FAi2 = Azadirachta indica antiseptic solution formulation (2.5%$^{w/}_{v}$), FAi3 = Azadirachta indica antiseptic solution formulation (5%$^{w/}_{w}$).

**Table 5:** Physicochemical properties of *Momordica charantia* and *Azadirachta indica* formulated antiseptic solutions (Mean ± SD; n = 2).

| Solutions | Density (g/cm$^3$) | pH | Viscosity (cp) | Colour |
|---|---|---|---|---|
| FAi1 | 1.03 ± 0.03 | 5.34 ± 0.09 | 8.50 ± 0.70 | Dark green |
| FAi2 | 1.05 ± 0.02 | 4.58 ± 0.03 | 4.50 ± 6.36 | Dark green |
| FAi3 | 1.06 ± 0.02 | 4.43 ± 0.03 | 9.50 ± 0.71 | Dark green |
| FMc1 | 1.02 ± 0.03 | 5.97 ± 0.01 | 9.50 ± 0.71 | Dark green |
| FMc2 | 1.03 ± 0.04 | 5.95 ± 0.02 | 10.50 ± 0.71 | Dark green |
| FMc3 | 1.04 ± 0.03 | 5.92 ± 0.03 | 8.50 ± 0.70 | Dark green |
| Dettol | 1.07 ± 0.00 | 9.61 ± 0.00 | 24.00 ± 0.00 | Amber gold |

FMc1 = M. charantia antiseptic solution formulation (1%$^{w/}_{v}$), , FMc2 = M. charantia antiseptic solution formulation (2.5 % $^{w/}_{v}$), FMc3 = M. charantia antiseptic solution formulation (5 % $^{w/}_{v}$), FAi1 = A. indica antiseptic solution formulation (1 % $^{w/}_{v}$), FAi2 = A. indica antiseptic solution formulation (2.5 % $^{w/}_{v}$), FAi3 = A. indica antiseptic solution formulation (5 % $^{w/}_{v}$)

**Table 6:** Antimicrobial inhibition by *Momordica charantia* and *Azadirachta indica* antiseptic solution formulation (Mean ± SD; n = 2)

| Formulations | Extract | *S. aureus* | *B. subtilis* | *P. aeruginosa* | *K. pneumonia* | *C. albicans* | *T. rubrum* |
|---|---|---|---|---|---|---|---|
| | | Zone of inhibition (mm) | | | | | |
| FMc1 | *M. Charantia* | 20.5 ± 0.7 | 28.0 ± 0.0 | 14.0 ± 0.0 | 17.0 ± 1.4 | 13.0 ± 1.4 | 15.0 ± 1.4 |
| FMc2 | *M. Charantia* | 22.0 ± 0.0 | 23.0 ± 1.4 | 14.0 ± 0.0 | 13.0 ± 1.4 | 15.0 ± 1.4 | 14.0 ± 0.0 |
| FMc3 | *M. Charantia* | 19.0 ± 1.4 | 16.0 ± 0.0 | 14.0 ± 0.0 | 11.0 ± 1.4 | 15.0 ± 1.4 | 16.0 ± 0.0 |
| FAi1 | *A. indica* | 22.0 ± 0.0 | 25.0 ± 1.4 | 14.0 ± 0.0 | 14.0 ± 0.0 | 19.0 ± 1.4 | 14.0 ± 0.0 |
| FAi2 | *A. indica* | 17.0 ± 1.4 | 19.0 ± 1.4 | 13.0 ± 1.4 | 17.0 ± 1.4 | 14.0 ± 0.0 | 11.0 ± 1.4 |
| FAi3 | *A. indica* | 20.0 ± 0.0 | 21.0 ± 1.4 | 19.0 ± 1.4 | 12.0 ± 0.0 | 17.0 ± 1.4 | 13.0 ± 1.4 |
| Ethanol | | 0 | 0 | 0 | 0 | 0 | 0 |
| Dettol | | 18.0 ± 0.0 | 10.0 ± 7.1 | 10.0 ± 0.0 | 10.0 ± 0.0 | 16.0 ± 0.0 | 0 |

*A 1:1 dilution of the formulations and of Dettol was prepared and used for the test.

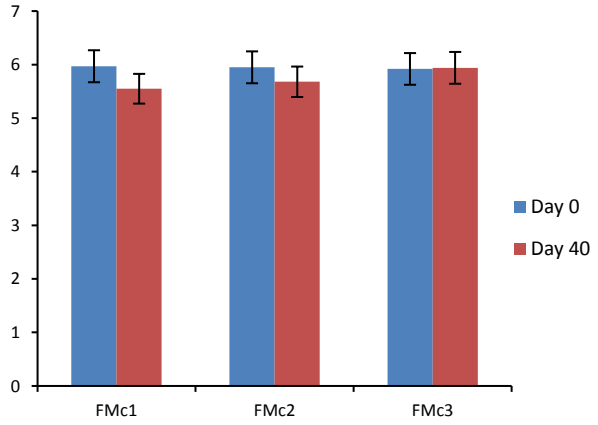


**Figure 2:** Mean pH of *Momordica charantia* stored for 40 days
FMc1 = M. charantia antiseptic solution formulation (1%$^{w/v}$), FMc2 = M. charantia antiseptic solution formulation (2.5 % $^{w/v}$), FMc3 = M. charantia antiseptic solution formulation (5 % $^{w/v}$)

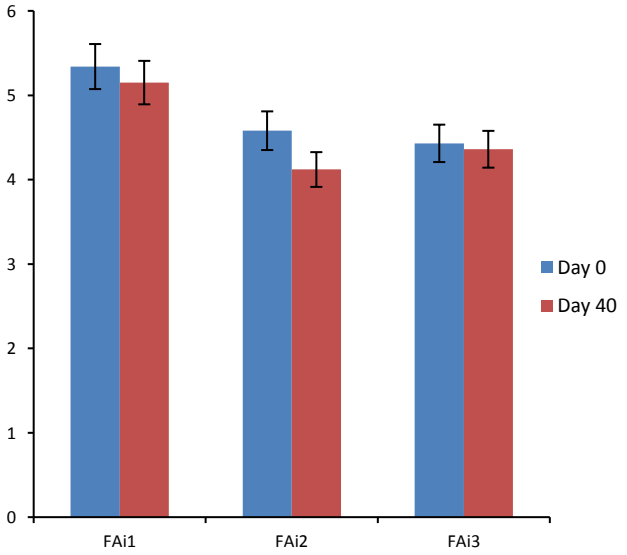


**Figure 3:** Mean pH of *Azadirachta indica* stored for 40 days
FAi1 = A. indica antiseptic solution formulation (1 % $^{w/v}$), FAi2 = A. indica antiseptic solution formulation (2.5 % $^{w/v}$), FAi3 = A. indica antiseptic solution formulation (5 % $^{w/v}$)

The formulation showed higher zones of inhibitions when compared with the reference drugs. Dettol$^{R}$ was observed to have lower zones of inhibition and also, it was not active against *Trichophyton rubrum*. However, all the formulations were active against the test organisms with the *M. charantia* antiseptic solution formulation (1%$^{w/v}$) (FMc1), having the optimum activity. Surprisingly, the formulations were active against *Trichophyton rubrum* and *Candida albicans* as against the results obtained with extracts alone, this shows that formulation ingredients did not hinder the activity of the extracts. The formulation had significantly higher ($p<0.05$) activity than the extracts, the fluidity of the formulations would also have aided in the diffusion of the through the agar medium, thus, helping in the release of the active constituents present in the plants. The antimicrobial activity of the formulations were observed to be concentration- dependent and it varied against the tested pathogens (Table 6)

There were insignificant changes in the organoleptic properties of *M. charantia* and *A. indica* antiseptic solution formulations stored under room temperature (29 ± 4ºC) for 40 days. Degrading pharmaceutical products usually present with bad odour, colour change, turbidity for fluids that are supposed to be free-flowing. Since the formulations did not show any of these deleterious effects, this indicates that the formulations are stable with respect to visual appearance (Figure 1). The observed stability is considered as compatibility of the extracts with other ingredients used in the products. The stability of the products with respect to pH has been presented in Figures 2 and 3. The results showed that the differences observed in the pH for day zero and day 40 were not significant ($p>0.05$). In addition, all formulations remained within the acceptable range of pH for topical pharmaceutical products.

## Conclusion

Both *M. charantia* and *A. indica* leaves had useful phytochemical constituents and this suggests that they could be applicable in herbal drug production and also serve as a more economical substitute for conventional drugs since they are readily available.

The formulated antiseptic solutions demonstrated satisfactory physicochemical properties both at production time and after storage. The stored products were stable and could be commercially developed as a herbal antiseptic solution. In addition, both *M. charantia* and *A. indica* antiseptic solution formulations showed appreciable antimicrobial activity against the test organisms, hence, they can be applied in the treatment of folliculitis.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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