

Evaluation of the Antimicrobial Activity of *Strombosia grandifolia* Hook.f. ex Benth Extract Hand Sanitizer Formulation

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ABSTRACT

The hand is an easy agent for the spread and transmission of pathogens and community-acquired infections. This study aims at evaluating the antimicrobial activity of *Strombosia grandifolia* hand sanitizer compared with a commercially available alcohol-based hand sanitizer. Water, methanol, and ethyl acetate extract from the leaf of *Strombosia grandifolia* was used to formulate hand sanitizer. The antimicrobial activity of the hand sanitizers was carried out against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* and analyzed with one-way ANOVA. The sanitizers were smooth with a chilly feel on the skin. Aqueous and methanol extract sanitizer had a significantly better antimicrobial activity when compared with ethyl acetate extract with the rank order of susceptibility of the microorganisms as *Staphylococcus aureus* > *Bacillus subtilis* > *Pseudomonas aeruginosa* > *Escherichia coli* > *Candida albicans*. The incorporation of the extract synergized the antimicrobial activity of alcohol-based hand sanitizers.

Keywords: *Strombosia grandifolia*, antimicrobial activity, phytochemical screening, hand sanitizer.

INTRODUCTION

Hygiene is a condition and practice that helps to maintain health and prevent the spread of disease. Cleanliness is a necessity for the maintenance of well-being, so good hygiene and the usage of cleansers are requisite for healthy living. The hand is an easy agent for the spread and transmission of pathogens and community-acquired infections.¹ Proper hand hygiene can avert health care associated infections and reduce the spread of antimicrobial resistance.

Hence, hand hygiene is a term that refers to any action of hand cleansing which is an essential precautionary

method to prevent the transmission and spread of infections.² To achieve this, soap and water had been traditionally used to reduce microbial load.³ The use of soap and water is less effective in killing microorganisms and it is not very convenient and time-wasting in situations where health personnel will have to attend to so many clients,^{4,5} and in the absence of water, it is practically impossible to use soap and water to cleanse the hand.

Poor hand hygiene practices thrived in West Africa until the outbreak of the Ebola virus due to a lack of awareness, lack of knowledge of risk and non-availability of hand hygiene facilities.⁶ The Ebola virus disease outbreak in 2014 - 2016 was the genesis of the emphasis on the use of hand sanitizers in the West African sub-region as an infection control measure. This has made researchers to continue in the search for discovering and developing new hand sanitizers and improving on existing

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ones. Nwabueze et al.⁷ reported hand washing and use of hand sanitizers as measures to prevent Ebola virus disease. World health organization also advocated the use of hand sanitizers globally as one of the measures to prevent the spread of infectious diseases such as the recent coronavirus disease pandemic.⁸ Hand rubs have the advantage of good antimicrobial activity in a short time unlike the use of water and soap.

Strombosia grandifolia (*S. grandifolia*) Hook.f. ex Benth is a tree which grows up to 30m high and belongs to the family Olacaceae. It has simple and broad leaves. It is distributed mostly in lowland forests in tropical Africa. The plant is known in Yoruba, Nigeria as “Itako pupa” and it is used as an ethnomedicinal herb in the treatment of various infectious diseases such as skin infections, gonorrhoea, cough and cold possibly because of the presence of antimicrobial phytochemicals in the plant.⁹ Very little report on *S. grandifolia* is available in literature. Ekalu et al.⁹ reported antibacterial and antifungal activities of the stem bark of the plant. This study attempts incorporating various extracts of the leaf of *S. grandifolia* into an alcohol based hand sanitizer with the aim of improving its antimicrobial activities.

MATERIALS AND METHODS

Materials

The fresh leaves of *S. grandifolia* were collected from Sapoda, a village in Edo state, Nigeria in the month of June 2018. The plant was identified and authenticated by Mr A. S. Odewo of the Forestry Research Institute of Nigeria, Ibadan, Oyo state (Voucher Number: FHI 111932). The chemicals and reagent used were analytical grades purchased from BHD chemicals, Poole England

Plant Extraction

The leaves were washed and air dried for 7 days after which it was milled into powder with the aid of a mechanical blender. Two hundred gram (200g) of the powdered leaves was macerated in 500mL of water, methanol, and ethyl acetate for 72 hours at room

temperature with intermittent shaking. The mixture was filtered and the filtrate was concentrated using a rotary evaporator and dried.

Phytochemical Analysis

Qualitative phytochemical screening of the extracts was done by standard methods according to Trease & Evans.¹⁰

Test for Alkaloids

To 1g of the powdered leaf, 10mL of 10% HCl was added and heated on water bath for 10 min. The mixture was filtered and 1 mL of the filtrate was transferred into four test tubes. Few drops of Dragendorff’s reagent, Mayer’s reagent, Wagner’s reagent and 1% tannic reagent were added to test tube 1, 2, 3 and 4 respectively. Formation of an orange, light brown, reddish brown and golden yellow precipitates in test tube 1, 2, 3 and 4 respectively indicate the presence of alkaloids.

Test for Tannins

One gram (1g) of the powdered leaf was boiled for 5 min. in 20mL of water. It was filtered after cooling. One milliliter (1mL) of the filtrate was mixed with 5mL of water in a test tube and few drops of 0.1% ferric chloride solution were added. A greenish colour indicates the presence of tannins.

Test for Saponins (Frothing test)

One gram (1g) of the powdered leaf was boiled for 10 min. in 10mL of water. The mixture was filtered while hot and the filtrate was allowed to cool. Three milliliters (3mL) of the filtrate was mixed with 10ml of water and shaken vigorously for 2 min. The formation of froth on the upper surfaces of the liquid indicates the presence of saponin.

Test for Cardiac Glycosides

One gram (1g) of the powdered leaf was extracted with 10mL of 80% alcohol for 5 minutes on a water bath. The filtrate was diluted with equal volume of water. Two milliliters (2mL) of lead acetate was added and the mixture was filtered after standing for few minutes. Two milliliters (2mL) of concentrated H₂SO₄ was added along the side of the test tube to the filtrate. The formation of a light reddish brown colour at the interface with a green colour in the

acetic layer indicates the presence of cardiac glycosides.

Test for Anthraquinone

One gram (1g) of the powdered leaf was placed in a dry test tube and 10mL of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. The formation of a bright pink colour in the aqueous layer indicates the presence of free anthraquinones.

Test for flavonoids

One gram (1g) of the powdered leaf was boiled with 10mls of water for 5 min. and filtered while hot. The filtrate was allowed to cool. Two milliliter (2mL) of NaOH was added to 5mls of the cooled filtrate. A yellow colour indicates the presence of flavonoid.

Five milliliter (5mL) of dilute ammonia solution was added to 10mL of the cooled filtrate followed by the addition of 3mL of conc. H₂SO₄. Colour disappearance on standing indicates the presence of flavonoid.

Antimicrobial Evaluation of Plant Extracts

The screening of antimicrobial activity of the plant extracts was carried out against five test microorganisms (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* 27853, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6051 and *Candida albicans*

ATCC 10231) using the agar well diffusion technique according to the method described by Adeleye et al.¹¹ A standardized inoculum of each test microorganism was inoculated into sterile, cooled Mueller Hinton or Sabouraud dextrose agar, thoroughly mixed and transferred into a sterile Petri dish. After the agar had set, three holes were bored using a 6mm cork borer. The holes were filled with 0.5mL of 100mg/mL plant extract reconstituted with distilled water (DS), 0.5mL of 1% ciprofloxacin (positive control for bacterial) or 0.5mL of 1% fluconazole (positive control for fungal) and DS (negative control) respectively. The plates were allowed to stand for about one hour before incubation at 37°C for 24 hours for Mueller Hinton plate and at 25°C for 72 hours for Sabouraud dextrose agar. The zones of growth inhibition were then measured to the nearest millimetre.

Formulation of *S. grandifolia* Hand Sanitizer

The herbal hand sanitizer was prepared containing various concentrations of *S. grandifolia* extract as shown in Table 1. A modification of the method described by WHO, was adapted in the formulation of the herbal hand sanitizer.¹² The required quantities of the ingredients (Table 1) were accurately measured into a flask which was then shaken gently to mix the contents.

Table 1: Composition of herbal hand sanitizer formulations

MATERIALS	NO	A1	A2	A3	A4	A5	M1	M2	M3	M4	M5	E1	E2	E3	E4	E5
Isopropyl alcohol (% ^{v/v})	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
<i>S. grandifolia</i> extracts (% ^{w/v})	-	0.5	1.0	3.0	5.0	10	0.5	1.0	3.0	5.0	10	0.5	1.0	3.0	5.0	10
Glycerol (% ^{v/v})	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Distilled water	26	25.5	25	23	21	16	25.5	25	23	21	16	25.5	25	23	21	16

NO- Hand sanitizer without plant extract, A1- 0.5% *S. grandifolia* aqueous extract, A2- 1.0% *S. grandifolia* aqueous extract, A3- 3.0% *S. grandifolia* aqueous extract, A4- 5.0% *S. grandifolia* aqueous extract, A5- 10% *S. grandifolia* aqueous extract, M1- 0.5% *S. grandifolia* methanol extract, M2- 1.0% *S. grandifolia* methanol extract, M3- 3.0% *S. grandifolia* methanol extract, M4- 5.0% *S. grandifolia* methanol extract, M5- 10% *S. grandifolia* methanol extract, E1- 0.5% *S. grandifolia* ethyl acetate extract, E2- 1.0% *S. grandifolia* ethyl acetate extract, E3- 3.0% *S. grandifolia* ethyl acetate extract, E4- 5.0% *S. grandifolia* ethyl acetate extract, E5- 10% *S. grandifolia* ethyl acetate extract.

Physical characterization of the Herbal Hand Sanitizer

Physical evaluation of the hand sanitizer was done manually by observing colour, odour, texture and feel on the skin.^{13,14} The pH of the formulations was determined with a pH meter.

Antimicrobial Evaluation of Formulated Hand Sanitizers

The method used for the screening of antimicrobial activity of the plant extracts against the five test microorganisms was utilized for the formulated hand sanitizers.

Statistical analysis

Microsoft Excel 2010 and GraphPad Prism 5 were used to analyze data obtained. Data were presented as mean \pm standard deviation (SD). One-way analysis of variance and Tukey's Post Hoc test were used to check significant differences in mean. Parameters with p-value of < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical test

The result of the phytochemical screening of the crude

extracts is presented in Table 2. The powdered leaves contain moderate concentrations of alkaloids, saponins, flavonoids and tannins. Aqueous extract contains moderate concentrations of alkaloids with low concentration of saponins, flavonoids and tannins. Methanol extract contains moderate concentrations of alkaloids, saponins and tannins with low concentration of flavonoids, while ethyl acetate extract contains low concentrations of alkaloids, saponins and flavonoids.

Plants possess some secondary metabolites that protect them from attack by microorganisms and insects such as alkaloids, flavonoids, tannins, phenols, saponins, and other aromatic compounds.¹⁵ The phytochemical screening (Table 2) indicated that the plant extracts generally contain alkaloids, saponins, flavonoids and tannins. Plants synthesize flavonoids in response to microbial invasion.¹⁶ saponin acts by causing leakage of proteins and enzymes from the invading microorganism,¹⁷ while tannins act by interfering with protein synthesis in the invading microorganism.¹⁸ Since the different extracts of *S. grandifolia* leaves possess these secondary metabolites, there is the possibility of the plant being bioactive as an antimicrobial agent.

Table 2: Phytochemical Screening of *S. grandifolia* leaves

Secondary metabolites	Powdered leaf	Distilled water	Methanol	Ethyl acetate
Alkaloids	++	++	++	+
Cardiac Glycosides	-	-	-	-
Antraquinone	-	-	-	-
Saponins	++	+	++	+
Flavonoids	++	+	+	+
Tannins	++	+	++	-

- = Not detected; + = Low concentration; ++ = Moderate concentration;

Antimicrobial Activity of Plant Extracts

The result of the antimicrobial activity of the plant extract (100mg/mL) is presented in Table 3. All the extracts showed a significant difference in antibacterial and antifungal activity against all the test microorganisms

with methanol extract having the highest antimicrobial activity. The rank order of antimicrobial activity was methanol > distilled water > ethyl acetate. The high antimicrobial activity of methanol extract as revealed in Table 3 could be due to the presence of high concentrations

of saponin and tannin than the other two extracts. Plants with extracts rich in tannin have been reported to show high antimicrobial activities mainly by hydrophobic interactions and hydrogen bonds leading to inhibition of bacteria metabolism.¹⁹ Methanol is known to be a good solvent for extraction, extracting various chemical groups from plant materials in large quantities as reported by Adeleye et al.¹¹, Murugan et al,¹⁹ Alayo et al.²⁰

The aqueous and methanol extracts had strong antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The rank order of susceptibility of the organisms to aqueous and

methanol extracts was *Staphylococcus aureus* > *Bacillus subtilis* > *Pseudomonas aeruginosa* > *Escherichia coli*. Ethyl acetate extract had the lowest antibacterial activity against all the test microorganisms. Only methanol extract showed a significantly comparable antibacterial activity to that of the positive control, ciprofloxacin on *Bacillus subtilis* and *Pseudomonas aeruginosa* (Table 3) probably due to the reason earlier mentioned. The rank order of antifungal activity of the plant extracts was methanol > distilled water > ethyl acetate which is similar to the antibacterial activity. The positive antifungal control (fluconazole) had better activity than all the plant extracts.

Table 3: Antimicrobial activity of extracts of *S. grandifolia*

Test organisms	Zones of inhibition (mm)				
	A	M	E	B	F
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
<i>Staphylococcus aureus</i>	12±0.01	16±0.39	04±0.10	20±0.04	-
<i>Escherichia coli</i>	08±0.11	11±0.24	06±0.08	16±0.01	-
<i>Bacillus subtilis</i>	10±0.04	16±0.08	07±0.24	18±0.06	-
<i>Pseudomonas aeruginosa</i>	12±0.08	14±0.11	08±0.02	16±0.10	-
<i>Candida albicans</i>	07±0.14	10±0.30	05±0.11	-	17±0.21

A = Aqueous extract, M = Methanol extract, E = Ethyl acetate extract, B = 1% Ciprofloxacin for bacterial positive control, F = 1% Fluconazole for fungi positive control,

Physical characteristics of the hand sanitizer formulations and pH

The physical properties of the hand sanitizer formulations and the pH of the formulations is presented in Table 4. The formulations exhibited different shades of green or brown coloration and they are all smooth in texture with a chilly feel on the skin. The pH of the formulations ranged from 6.0 to 7.5.

The ideal pH of the human skin is usually slightly acidic within the range of 4 – 6.5.^{21,22} Formulations for application on the skin should be within this range to prevent skin irritation. As shown in Table 4, only four formulations (A4, M3, M4 and M5) had a pH value within the ideal range. The pH of other formulations can be made to be within the ideal range with the use of suitable buffer.

Table 4: Physical properties and pH of formulations

Formulation code	Colour	Texture	Feel on skin	pH
NO	Colour less	Smooth	Chilly	7.4
A1	Light Brown	Smooth	Chilly	7.1

Formulation code	Colour	Texture	Feel on skin	pH
A2	Light Brown	Smooth	Chilly	7.0
A3	Light Brown	Smooth	Chilly	7.2
A4	Light Brown	Smooth	Chilly	6.4
A5	Light Brown	Smooth	Chilly	7.0
M1	Light Brown	Smooth	Chilly	7.5
M2	Light Brown	Smooth	Chilly	7.2
M3	Brown	Smooth	Chilly	6.5
M4	Brown	Smooth	Chilly	6.3
M5	Brown	Smooth	Chilly	6.0
E1	Light Green	Smooth	Chilly	7.4
E2	Light Green	Smooth	Chilly	7.5
E3	Light Green	Smooth	Chilly	7.3
E4	Green	Smooth	Chilly	7.1
E5	Green	Smooth	Chilly	7.1
C	Light Blue	Smooth	Chilly	6.4

NO = formulation with no extract, A1-A5 = *S. grandifolia* aqueous extracts, M1-M5 = *S. grandifolia* methanol extracts, E1-E5 = *S. grandifolia* ethyl acetate extracts, C = commercially available hand sanitizer containing 45% Ethyl alcohol.

Antimicrobial Activity of herbal hand sanitizers

The antimicrobial activity of the leaves extract of *S. grandifolia* formulated into hand sanitizer was determined by the diameter (measured in mm) of the zone of inhibition and presented in Fig. 1, 2 and 3. The antimicrobial activities are concentration-dependent. Statistical analysis of the formulations containing 0.5% extracts of different solvents [A1, M1 & E1] showed no significant difference in activity against all the test microorganisms except *Bacillus subtilis* [P = 0.0270] with methanol extract. At 1% extract concentration [A2, M2 & E2] there was no significant difference in activity against *Staphylococcus aureus* and *Candida albicans* but there was a significant difference in activity against *Escherichia coli* [P = 0.0025] (the exact difference was between formulation A2 and E2; M2 and E2) with A2 and M2 having higher activity, *Pseudomonas aeruginosa* [P = 0.0080] (the exact difference was between formulation M2 and E2) with M2 having higher activity and *Bacillus subtilis* [P = 0.0025] with the exact difference between formulation A2 and M2;

M2 and E2 with A2 and M2 having higher activity. At 3% extract concentration [A3, M3 and E3] there was significant difference in activity against all test microorganisms, *Staphylococcus aureus* [P = 0.0025] (the exact difference was between formulation A3 and M3; M3 and E3) with M3 having the highest activity, *Escherichia coli* [P = 0.0020] (the exact difference was between formulation A3 and M3; M3 and E3) with M3 having the highest activity, *Pseudomonas aeruginosa* [P = 0.0270] (the exact difference was between formulation M3 and E3) with M3 having higher activity, *Bacillus subtilis* [P = 0.0270] (the exact difference was between formulation M3 and E3) with M3 having higher activity and *Candida albicans* [P = 0.0066] with the exact difference between formulation A3 and M3; A3 and E3 with A3 having the highest activity. At 5% extract concentration [A4, M4 and E4] there was no significant difference in activity against *Pseudomonas aeruginosa* and *Candida albicans* but there was significant difference in activity against *Staphylococcus aureus* [P = 0.0025] (the exact difference

was between formulation A4 and E4; M4 and E4) with A4 and M4 having the higher activity, *Escherichia coli* [P = 0.0066] (the exact difference was between formulation A4 and M4; A4 and E4) with M4 having the highest activity and *Bacillus subtilis* [P = 0.0270] with the exact difference between formulation M4 and E4 with M4 having the highest activity. At 10% extract concentration [A5, M5 and E5] there was no significant difference in activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* but there was significant difference in activity against *Escherichia coli* [P = 0.0027] (the exact difference was between formulation M5 and E5) with M5 having the higher activity and *Candida albicans* [P = 0.0080] with the exact difference between formulation A5 and E5 with A5 having the higher activity. There was significant difference in the activity of all the formulations against the test microorganisms when compared with the commercially available hand sanitizer.

Statistical analysis of the results of the antimicrobial activity of herbal hand sanitizers as shown in shown Fig. 1, 2 and 3 revealed that aqueous and methanol extract of *S. grandifolia* hand sanitizer formulation had a significantly better antimicrobial activity when compared with ethyl acetate extract. However, methanol extract had a slightly

better activity when compared with the aqueous extract although generally not statistically significant except the formulations containing 1% concentration against *Bacillus subtilis*, 3% concentration against *Staphylococcus aureus* and *Escherichia coli*, and 5% concentration on *Escherichia coli*. The reason for these variations in antimicrobial activities could be correlated to the concentration of the phytochemicals present in the extracts (Table 2). As highlighted previously, the presence of phytochemicals such as tannins, saponins and flavonoids could be responsible for these activities.^{11,20,23} The extent of these activities may be due to the concentrations of these phytochemicals.¹¹ In this study, the probable mechanism of action of the plant extract as a result of the phytochemicals could be disruption of bacterial cell membrane as reported by Gonelimali et al.²⁵ The antimicrobial activity of the formulations were significantly higher against all the test microorganisms when compared with the commercially available hand sanitizer due to synergism as a result of the incorporation of the leaf extract of *S. grandifolia*. This synergistic effect may prevent microorganisms from developing alcohol-tolerance as highlighted by Golin et al.²⁶

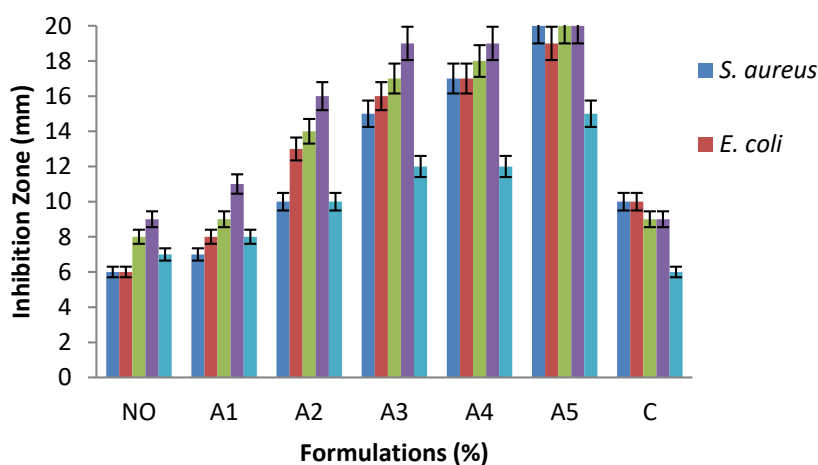


Figure 1: Zone of inhibition (mm) of aqueous extract hand sanitizer formulations

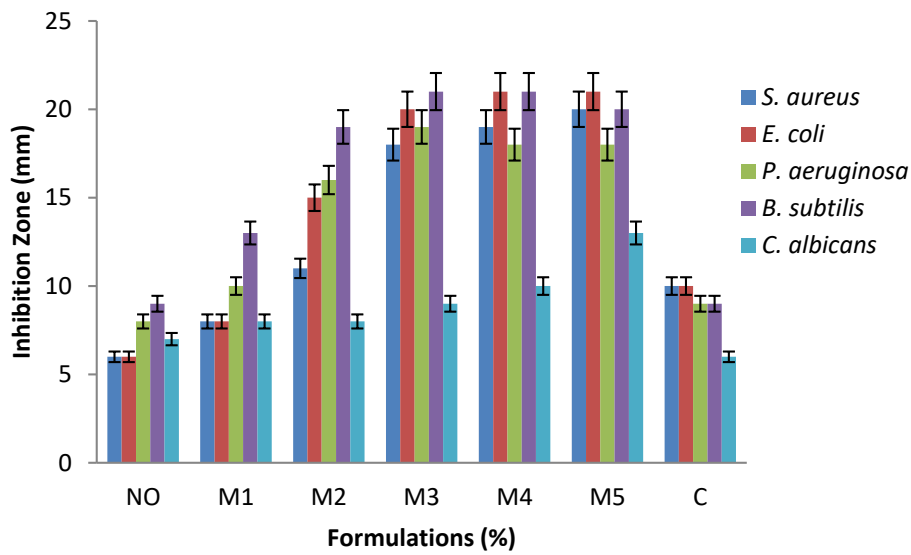


Figure 2: Zone of inhibition (mm) of methanol hand sanitizer formulations

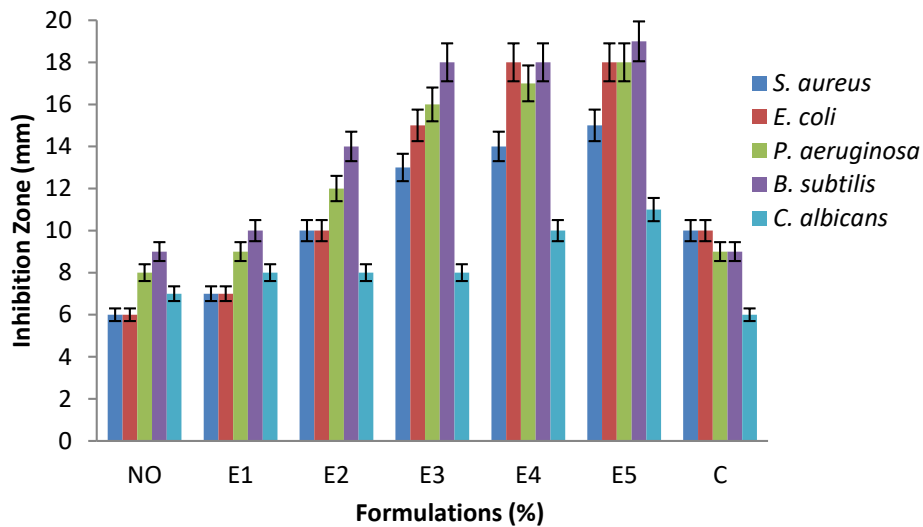


Figure 3: Zone of inhibition (mm) of ethyl acetate extract hand sanitizer formulations

CONCLUSION

This study justified the antimicrobial activity of *S. grandifolia* as reported in literature based on its ethnomedicinal use in the treatment of infectious diseases. The plant would likely be safe when included in hand

sanitizers since presently there is no reported adverse consequences with its use in ethnomedicine. It was observed that the antimicrobial activity of all the extract was concentration dependent up to a point when activities became almost static, at this point rate of activity declined.

The highest antimicrobial activity was obtained from the formulations containing methanol extract which follows similar trend with the antimicrobial activity of the leaf extract only. At all concentration of the extract, the *S. grandifolia* hand sanitizer formulations were more effective than the alcohol based sanitizer formulation. All formulations except those containing 0.5% extract are more effective than the commercially available sanitizer.

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In conclusion, the incorporation of *S. grandifolia* extract synergized the antimicrobial activity of alcohol only based hand sanitizers which could be used to reduce the spread of infectious pathogens in health care settings.

Competing Interest

Authors have declared no competing interest.

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تقييم النشاط المضاد للميكروبات لـ *Strombosia grandifolia* Hook.f. خلاصة بنث السابقة تركيبة مطهر اليد

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ملخص.

اليد عامل سهل لانتشار وانتقال مسببات الأمراض والالتهابات المكتسبة من المجتمع. تهدف هذه الدراسة إلى تقييم النشاط المضاد للميكروبات لمعقم اليدين *Strombosia grandifolia* مقارنة بمطهر الأيدي الكحولي المتاح تجاريًا. تم استخدام الماء والميثانول ومستخلص أسيتات الإيثيل من ورقة سترومبوزيا غرانديفوليا لتكوين معقم لليدين. تم إجراء النشاط المضاد للميكروبات لمطهرات اليدين ضد المكورات العنقودية الذهبية، الزائفة الزنجارية، الإشريكية القولونية، العسوية الرقيقة والمبيضات البيضاء وتم تحليلها باستخدام ANOVA أحادي الاتجاه. كانت المطهرات ناعمة مع شعور بالبرودة على الجلد. كان للمطهر المائي وخلاصة الميثانول نشاط مضاد للميكروبات أفضل بشكل ملحوظ عند مقارنته بمستخلص أسيتات الإيثيل بترتيب حساسية للكائنات الدقيقة مثل *Staphylococcus aureus* > *Candida albicans* > *Escherichia coli* > *Pseudomonas aeruginosa* > *Bacillus subtilis* أدى دمج المستخلص إلى تضافر النشاط المضاد للميكروبات لمطهرات اليد التي تحتوي على الكحول.

الكلمات الدالة: سترومبوزيا غرانديفوليا، نشاط مضاد للميكروبات، فحص كيميائي نباتي، معقم لليدين.

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