Leaf extract of *Smilax schomburgkiana* exhibit selective antimicrobial properties against pathogenic microorganisms

R.C. Jagessar[1,*], A. Mars[1], G. Gomes[2]

[1]*Department of Chemistry*, *University of Guyana*, *Faculty of Natural Sciences*, *South America*; [2]*Department of Biology*, *John's Campus*, *University of Guyana*, *Faculty of Natural Sciences*, *South America*

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Abstract

The antibacterial and antifungal activities of *Smilax schomburgkiana* were investigated against pathogenic microorganisms: *S. aureus* (gram+ve), *E. coli* (gram-ve) and *C. albicans* using the stokes disc diffusion, well diffusion, streak plate methods and a dilution method. The solvent type extracts were obtained by three extractions each with hexane, CH_2Cl_2, EtOAc and CH_3CH_2OH respectively. Solvents were removed in vacuo to yield viscous oils and paste which were made up to a concentration of 0.035 g in 10 ml of the respective solvents. These were tested in varying volumes of 0.2 – 0.6 ml/plate (i.e. concentrations of 0.03 – 0.18 mg/10 ml agar). Solvents were used as control whereas ampicillin and nystatin were used as references for bacteria and fungal species respectively. The solvents had no effect on the microorganisms whereas ampicillin and nystatin inhibited microbial growth. *Smilax schomburgkiana* showed selective antimicrobial inhibitory activity at 0.18 mg/10 ml plate of medium with activity most prominent with the CH_3CH_2OH and EtOAc extracts and negligible with the hexane. This study suggests that the CH_3CH_2OH and EtOAc extracts of *Smilax schomburgkiana* can be used as herbal medicines in the control of *E. coli* and *S. aureus* and *C. albicans* induced diseases, following clinical trials. [Life Science Journal. 2009; 6(1): 76 – 83] (ISSN: 1097 – 8135).

Keywords: antimicrobial; *Smilax schomburgkiana*; stokes disc diffusion; well diffusion; streak plate; dilution method; herbal medicines

1 Introduction

This paper focuses on the antimicrobiological (antibacterial and antifungal) properties of leaves of *Smilax schomburgkiana* also known as "Dorok Waropimpla" from the coastal plain of the Guyana flora and its possible use as an herbal cream/herbal medicine. Antimicrobial properties were investigated against *S. aureus* (*SA*) (gram+ve), *E. coli* (*EC*) (gram-ve) and *C. albicans* (*CA*) strains using the stokes disc diffusion sensitivity technique, well diffusion, streak plate and a dilution method. An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity), viruses (antiviral activity) or parasites (antiparasitic activity).

There is an urgent need to revolutionalsed research in herbal medicine and isolated drug discovery considering the presence of incurable diseases such as HIV AIDS and the threat of new emerging disease such as SARS, bird flu etc. Plants are a good source of herbal medicine and natural products/phytochemicals[1–26]. Guyana has a rich biodiversified flora whose crude extracts, both organic and aqueous can be investigated for their antimicrobial activity in addition to their role as global CO_2 sinks (in the context of global warming). Also, the specified plants parts of the same species, fractionated or screened for natural products whose antimicrobial activity can also be investigated and compared with the crude extracts. Following this, clinical trials of crude extracts or fractionated natural products can lead to the formulation of an herbal plant cream or herbal medicine. A few herbal medicine shops have now been established in Guyana and the "bush" medicine man is an important figure in Guyana's culture. Plants extracts and fractionated plant extracts have been used for

*Corresponding author. Email: raymondjagessar@yahoo.com

their antimicrobial properties[1–11,16–20]. Besides used as an herbal cream, following clinical trials, crude plant extracts can be chromatographed, leading to the isolation and purification of new and known bioactive natural products/phytochemicals, whose medicinal activity can also be investigated. For example, two new isopimaranes, 19-methylmalonyloxy-ent-isopimara-8,9,15-diene (5) and 19-malonyloxy-ent-isopimara-8 (9), 15-diene (6) were isolated using bioassay-guided fractionation of the CH_2Cl_2-MeOH (1 : 1) extract of the aerial part of *Calceolaria pinifolia*[21]. All compounds were assayed against *SA*, methicillin resistant *SA* (MR*SA*), *Bacillus subtilis* (*BS*) and *EC*. 4-Epi-dehydroabietinol (2) and ent-isopimara-9 (11), 15-diene-19-ol (8) were found to be active against MRSA with MIC values of 8 µg/ml and 2 µg/ml, respectively. Mechanistic studies of (8) in *BS* suggested rapid and non specific inhibition of uptake and incorporation of radiolabelled precursors into DNA, RNA, and protein consistent with membrane damaging effects in bacteria. Compound (8) didn't afford protection against an acute infection with *SA* in mice (Figure 1).

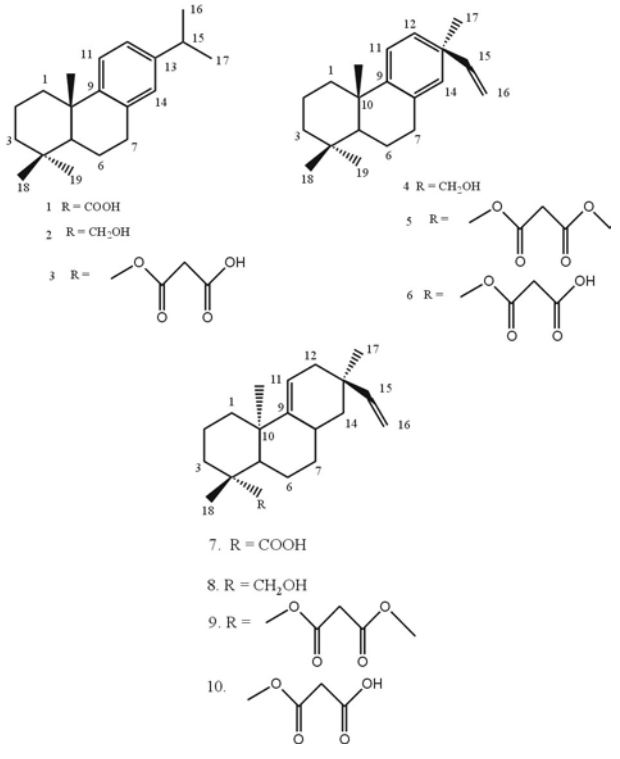

Figure 1. Structure of diterpenes (1) to (10).

Many synthetic drugs owe their discovery and potency as a result of a mimic of structures from natural products isolated from plants rather than to the creativity and imagination of contemporary organic chemists. For example, the drug taxol (a diterpenoid), first isolated from the bark of the yew tree *Taxus brevifolia* has yielded two approved drugs for breast and ovarian cancer[6].

In Guyana, there are many medicinal folklore practises but most are without scientific research. Its our scientific endeavour, to correlate antimicrobial activity of *Smilax schomburgkiana* with its folklore practices. In Guyana' s traditional medicine, a decoction of the root, and sometimes the woody stem may be mixed with kapadulla (*Doliocarpus* species), Sarsparilla (*Philodendron* species) and Devil-doer (*Strychnos* species) and used as an aphrodisiac or tonic. An infusion of the root is used to treat diseases of the urinary bladder and nervous conditions[29]. However, little is known of the antimicrobial properties of *Smilax schomburgkiana*[28]. As part of a project to investigate extracts and chromatographic fractions from plants of the Guyana's flora[16–20], for antimicrobial activity, we report here, the antimicrobial properties of *Smilax schomburgkiana*.

Smilax schomburgkiana is a high climber, often growing from a massive rootstock, stem twining, branched, terete, with a few spines and scattered blacklish tubercles, otherwise smooth and glabrous. Leaves alternate, ovate to lanceolate, acute or acuminate, rounded to cuneate at the base. Male and female flowers on separate plants in leafy racemose panicles of umbels from axils of the main stem. Its usually found in the forests of the Guianas and eastern Brazil[28]. The classification of the plant is given in Table 1.

Table 1. Scientific classification of *Smilax schomburgkiana*

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Liliales
Family	Smilacaceae
Genus	*Smilax*
Species	*Schomburgkiana*

Pathogenic microorganism investigated were *EC*, *SA* and *CA*. *EC* can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia[30]. *SA* can cause furuncles (boils), carbuncles (a collection of furuncles)[31]. In infants, *SA* can cause a severe disease Staphylococcal scalded skin syndrome (SSSS). Staphylococcal endocarditis (infection of the heart valves) and pneumonia may be fatal. *CA* is a

diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans[32].

2 Materials and Methods

2.1 Collection of plant materials

The leaves of the above plant was collected from the University of Guyana. The deteched plant leaves were subjected to aerial drying for three weeks, removed and placed in separate conical flasks. They were then extracted with the required solvents.

2.2 Extraction

Using selective solvent extraction, the leaves were first extracted thrice in hexane over a period of five days[12–20]. Water was removed from the solvent extract by stirring over anhydrous Na_2SO_4 and extract was filtered. Solvents were removed in vacuo using a rotor vapor. The extracts was placed in sample vials and allow to evaporate. Further drying was done in a dessicator to remove residual solvents. Extracts were stored in capped vials and were weighed. The above procedure was repeated with the same leaves but with different solvents of increasing polarity: CH_2Cl_2, EtOAc, and then CH_3CH_2OH. At the end of drying process, plant extract was either viscous oils, solid or paste.

2.3 Antimicrobial activity tests

2.3.1 Making up extract solution. Approximately 0.035 g of dried crude extract of *Smilax schomburgkiana* was weighed and transferred to a 10 ml volumetric flask. The respective solvent was then added to make up the 10 ml solution, i.e 0.035 g/0.01 L.

2.3.2 Microorganisms. Pathogenic microorganisms *SA*, *EC* and *CA* were obtained from the Georgetown Public Hospital (GPH) microbiology laboratory and were stored in a refrigerator at the Food and Drug Microbiology Laboratory, Kingston.

2.3.3 Agar preparation. Two types of agar were used, nutrient agar to make up the medium for bacteria and Potato Dextrose Agar (PDA)[34,35] to make up the medium for fungi.

PDA. The potato was peeled and 100 g was measured, finely chopped and boiled to a mash in distilled water. The dextrose was measured (12.5 g) and placed in a 1 L measuring cylinder. Agar was measured (12.5 g) and added to the measuring cylinder (with the dextrose). The potato mash was stirred and strained into the cylinder. Hot distilled water was added to make up 500 ml. The contents was continuously poured and stirred until consistency was achieved. The content was then poured into a conical flask, plugged with cotton wool, over which aluminium foil was tightly wrapped. The flask was then autoclaved at 121 ºC for 24 hours. The pH range was between 6.5 – 7.0.

Nutrient agar. Nutrient agar was purchased from the International Pharmacy Association in Guyana. 14 g of nutrient agar was suspended in 500 ml of distilled water in a 1 L flask, stirred, boiled to dissolve and then autoclaved for 15 minutes at 121 ºC. The pH range was between 7.0 – 8.0. The plates were poured in a sterile environment and allowed to cool for 2 hours. Under aseptic conditions, microorganisms were streaked onto separate plates and the discs were applied with a forceps. They were labeled and placed in an incubator at 37 ºC for 24 hours and 48 hours for bacteria and fungi respectively.

2.3.4 References and control. The references were antibiotic in nature, ampicillin and nyastatin. Ampicillin was choosen as the reference for all bacterial species used, *EC* and *SA*. Nyastatin was used as the reference for the fungus, *CA*. The control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1 : 1 portion[34,35].

2.3.5 Aseptic conditions. The aseptic chamber which consists of a wooden box (1 m × 1 m × 0.5 m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from a lamp).

2.3.6 Mother plates. These were made by culturing *CA* on PDA. A sterilized 6 mm cork borer was used to cut agar discs in the plate.

2.3.7 Disc diffusion: stokes disc diffusion sensitivity technique[34,35]. Using stokes disc diffusion sensitivity testing technique[24], an inoculum containing bacterial or yeast cells was applied onto nutrient agar plates. On each plate, a reference antibiotic was also applied. The reference antibiotic disc contained 200 mg antibiotic/ml. The discs were made by cutting discs (5 – 6 mm) from a filter paper with a perforator, placing 5 of these discs in a vial and adding 0.2 ml of each extract solution. These were left to dry. Discs were also made for the controls: ampicillin for the bacteria and nystatin for the fungus. Each disc was impregnated with the anticipated antimicrobial plant extract at appropriate concentration of 200 mg/ml using a microlitre syringe. This was then placed on a plate of sensitivity testing nutrient agar which was then incubated with the test organism: bacteria/fungi. Incubation was done at 37 ºC for 24 hours and 48 hours for the bacteria and *CA* species respectively. The antimicrobial compound diffuses from the disc into the medium. Following overnight incubation, the culture

was examined for areas of no growth around the disc (zone of inhibition). The radius of the inhibition zone was measured from the edge of the disc to the edge of the zone. The end point of inhibition is where growth starts. Larger the inhibition zone diameter, greater is the antimicrobial activities. It is anticipated through the antimicrobial activity of plant extract, no area of growth will be induced around the disc. Bacteria or fungal strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc. Discs applied to the plates already streaked with bacteria and the fungus.

2.3.8 Well diffusion (diffusion plate) method. A fungus (*CA*) was inoculated into test tube containing three ml of distilled water (medium), using a flamed loop. Drops of fungus/water culture was mixed with the warm, melted, autoclaved PDA and poured into separate plates under aseptic conditions. The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left for 2 hours. When cooled, a well was made at the centre of the plate. The well was made by using a 6-mm cork borer or puncher that was sterilized with alcohol and flame. Plant extracts dissolve in solvent at final concentration of 0.035 g/0.01 L was pipette into the different wells in a sterilized environment at different volumes (0.2, 0.4, 0.6 ml) in separate plates, using a micro liter syringe. The four solvents (hexane, CH_2Cl_2, EtOAc and CH_3CH_2OH) at different volumes were used as control whereas nystatin dissolved in CH_2Cl_2 at same concentration with plant extract (0.035 g/0.01 L) at different volumes (0.2, 0.4, 0.6 ml) was used as the reference. The plates were labelled, covered, inverted and placed in a fume hood (no incubator was available) for 48 hours.

2.3.9 Streak plate method. Nutrient agar was prepared as described above and 10 ml was poured into plates. Plant extracts dissolved in solvent at a final concentration of 0.035 g/0.01 L were pipette into three sterilized plates under asceptic conditions at different volumes (0.2, 0.4, 0.6 ml), using a micropipette. The plates were allowed to cool and then the bacteria were streaked onto the surface of the solidified agar/plant extract medium. A flame loop was used to inoculate the bacteria from their cultures. These plates were left for 24 hours in a dessicator. The plates with inhibition were used in further experiments. A reference experiment was setup using an antibiotic (ampicillin capsule) at the same concentration as plant extracts (0.035 g/0.01 L) at different volumes (0.2, 0.4, 0.6 ml). Controls were also setup using solvents: hexane, CH_2Cl_2 and EtOAc and CH_3CH_2OH at the different volumes.

2.3.10 Luria-Bertani (LB) broth[24]. LB broth is a rich medium used to culture bacteria such as *EC* and *SA*. To make it, tryptone (10 g), yeast extract (5 g) and sodium chloride (10 g) were measured and placed in a 1 L cylinder. Distilled water was added to make up the 1 L solution and the mixture was poured and re-poured until the contents were dissolved. The pH of the solution was adjusted to 7.4 using sodium hydroxide. 3 ml each of LB broth was placed in 56 test tubes. The tubes were plugged with cotton wool foil and wrapped over each top. The tubes were placed into a beaker and autoclaved at 121 ºC for 2 hours. These tubes were used in the dilutions experiments.

2.3.11 Dilution method. This method was used to test the plant extracts for antimicrobial activities against bacteria by investigating whether there was turbity or not. Turbidity represents microbial growth, while no turbidity represents inhibition of microbes. One set of tubes containing LB was inoculated with *SA* and the second set was inoculated with *EC* using a loop, flame and alcohol. Under aseptic conditions, the plant extracts (dissolved in solvent at concentration 0.035 g/0.01 L) and showed inhibition in the streak plate were added to the one set of test tubes containing *EC* and the other set, *SA* with LB broth in differing volumes (0.2, 0.4, 0.6 ml). Two sets of four tubes each were treated with the four solvents (hexane, CH_2Cl_2 and EtOAc and CH_3CH_2OH). One set was inoculated with *SA* and the other with *EC*. Cotton wool was used to plug test tubes. The tubes were observed after 24 hours.

2.3.12 Retention factor. R_f = (Distance moved by sample)/(Distance moved by solvent front).

In general, the most polar compound has the lowest R_f value.

2.3.13 Thin layer chromatography (TLC). A baseline was drawn on the TLC plate. A spot of the plant extract was placed on the baseline with use of the pipette and allowed to dry. The plate was placed in the developing jar with the solvent. When taken out of the jar, the solvent front was drawn. The plates were then held in the iodine jar for a few seconds, shaken and taken out. They were examined under the UV/Vis lamp and the spots were circled with a pencil. The plate was further examined under UV lamp and any new spots were marked. The spots were labeled and their distances from the baseline were measured. The distance between the baseline and the solvent front was measured. The R_f values were calculated.

3 Results

Mass of dried leaves used for *Smilax schomburgkiana* species was 8.55 g respectively. These extracts were in the concentration of 0.035 g in 10 ml of solvent except for *Smilax schomburgkiana* with CH_3CH_2OH which was 0.5 g in 25 ml. This works out to 0.0003 mg/µl and 0.02 mg/µl of crude extract respectively. Ampicillin and nystatin controls were in concentration of 200 mg/ml.

3.1 Disc diffusion (Table 2)

Table 2. Antimicrobial activity of plant extract as shown by the inhibition zone diameter

Plant extracts	Reference compound (Ampicillin) (mm^2)	Area of inhibition (mm^2)			Control
		EC	*SA*	*CA*	
Hexane	28	< 5	< 5	< 5	No zone of inhibition
CH_2Cl_2	30	< 5	< 5	< 5	
EtOAc	33	23	18	20	
CH_3CH_2OH	35	24	22	23	

3.2 Well diffusion (Table 3)

3.3 Streak plate (Table 4)

Results of Table 4 were obtained from streak plate method for the bacteria's *EC* and *SA* against different volumes of dissolved plant extracts at a final concentration of 0.035 g/0.01 L and controls.

3.4 Results of dilution method (Table 5)

Table 5 showed the degree of turbidity of dissolved *Smilax schomburgkiana* extracts at concentration of 0.035 g/0.01 L at different volumes against *EC* and *SA* microbe.

3.5 TLC analysis (Table 6)

4 Discussion

All four methods, stokes disc diffusion sensitivity techniques, well diffusion, streak plate and dilution method were successful in determining *Smilax schomburgkiana* antimicrobial activities. Several trends are noted and these will be discussed first. Antimicrobial activity follow the sequence: CH_3CH_2OH extract > EtOAc extract > CH_2Cl_2 extract > hexane extract. For example, with disc diffusion method, zone of inhibition of 24 mm^2, 22 mm^2 and 23 mm^2 were obtained for the CH_3CH_2OH extract against *EC*, *SA* and *CA* in constrast to 23 mm^2, 18 mm^2 and 20 mm^2 for the EtOAc extract. For the hexane extract, zone of inhibition of < 5 mm^2 were obtained against all microbes. In a comparative method, the well diffusion, zone of inhibition of 87 mm^2 was obtained for the CH_3CH_2OH extract when the well

Table 3. Results of the well diffusion for plant extracts *Smilax schomburgkiana* against *CA*

Volume of extract (ml)	Presence of zone of inhibition/Diameter of zone of inhibition (mm^2)					
	Hexane	CH_2Cl_2	EtOAc	CH_3CH_2OH	Reference (Nystatin)	Controls
0.2	–	+/79	–	+/80	+/79	–
0.4	–	+/79	+/79	+/85	+/79	–
0.6	–	+/79	+/79	+/87	+/79	–

Table 4. Results of streak plate method for the bacteria's *EC* and *SA* against different volumes of dissolved plant extracts

Bacteria	Volume of dissolved plant extract used in ml at concentration 0.035 g/0.01 L	Inhibition or no growth of microbe (Plant extract dissolved in solvent/ Ampicillin with same concentration as dissolved plant extracts)				Reference (Ampicillin with same concentration as dissolved plant extracts)
		Hexane	CH_2Cl_2	EtOAc	CH_3CH_2OH	
EC	0.2	–/–	–/–	+/–	+/–	+
	0.4	–/–	+/–	+/–	+/–	+
	0.6	–/–	+/–	+/–	+/–	+
SA	0.2	–/–	–/–	–/–	–/–	+
	0.4	–/–	–/–	–/–	+/–	+
	0.6	–/–	–/–	–/–	+/–	+

Inhibition or no growth of microbes were represented by a positive sign (+), while the negative sign (–) represents no inhibition or growth of microbes.

Table 5. Results of the degree of turbidity of dissolved *Smilax schomburgkiana* extracts at different volumes against *EC* and *SA*

Bacteria	Volume of dissolved plant extract (ml)	Turdity (Plant extract dissolved in solvent/Ampicillin with same concentration as dissolved plant extracts)				Reference (Ampicillin with same concentration as dissolved plant extracts)
		Hexane	CH_2Cl_2	EtOAc	CH_3CH_2OH	
EC	0.2	T_3/T_3	T_3/T_3	T_2/T_3	T_0/T_3	T_0
	0.4	T_3/T_3	T_3/T_3	T_1/T_3	T_0/T_3	T_0
	0.6	T_3/T_3	T_3/T_3	T_0/T_3	T_0/T_3	T_0
SA	0.2	T_3/T_3	T_3/T_3	T_2/T_3	T_0/T_3	T_0
	0.4	T_3/T_3	T_3/T_3	T_0/T_3	T_0/T_3	T_0
	0.6	T_3/T_3	T_3/T_3	T_0/T_3	T_0/T_3	T_0

T_0 = No turbidity = Inhibition; T_1 = Lightly turbid = Moderately inhibited; T_2 = Moderately turbid = Lightly inhibited; T_3 = Very turbid = No inhibition.

Table 6. TLC analyses for all the extracts of *Smilax schomburgkiana*

Solvents	Plants extracts	No. of spots visible	R_f value
Hexane	Hexane	3	0.043
			0.173
			0.826
CH_2Cl_2	CH_2Cl_2	9	0.051
			0.077
			0.154
			0.205
			0.282
			0.436
			0.641
			0.897
			0.974
EtOAc/CH_2Cl_2 (90 : 10, v/v)	CH_3CH_2OH	8	0.048
			0.079
			0.158
			0.349
			0.381
			0.444
			0.492
			0.984

was filled with 0.6 ml of extract. For the EtOAc extract, zone of inhibition of 79 mm^2 was obtained when the volume of the well was 0.6 ml. Again, these results strongly suggest that *Smilax schomburgkiana* antimicrobial active constituents are localized in the EtOAc and CH_3CH_2OH extracts. For all methods used, the control experiments which necessitate the use of pure distilled solvent alone, rather than pure plant extract induced negative result i.e no zone of inhibition or in the case of the dilution method, turbidity in test tubes containing LB broth with bacterial microbes. The reference antibiotic ampicillin for bacteria and nyastatin for fungi induced positive results. For example, for the disc diffusion method, ampicillin induced zone of inhibition of 35 mm^2 for the CH_3CH_2OH extract whereas nystatin induced zone of inhibition of 79 mm^2 at a volume of 0.6 ml for the well diffusion method. These results suggest that *Smilax schomburgkiana* antimicrobial properties are due to the plant active constituent rather than to a solvent effect. Each solvent extracts was added in increasing volume (0.2, 0.4, 0.6 ml) to the microbial medium.

Each method display interesting results and these can be further discussed. Stokes disc diffusion indicates that the plant extract induced a larger zone of inhibition against *EC* as compared against *SA*. For example, for the EtOAc extract, zone of inhibition of 23 mm^2 and 18 mm^2 were obtained for *EC* and *SA* respectively. A similar trend was noted for the streak plate method. *EC* showed inhibition whereas *SA* showed negligible inhibition (< 5 mm^2) for the CH_2Cl_2 and EtOAc extract. However, for the CH_3CH_2OH extract extract, inhibition was observed against both *EC* and *SA*.

To investigate *CA* antimicrobial activity, the well diffusion method was used. A larger zone of inhibition was observed compared with the stokes disc diffusion method. For example, for the CH_3CH_2OH extract, a zone of inhibition of 79 mm^2 was observed at a volume of 0.6 ml. Compared with the well diffusion method, the ethanol extract at a well volume of 0.6 ml induce zone of inhibition of 87 mm^2. This difference may be ascribed to the higher sensitivity of the well diffusion method as compared with the disc diffusion method.

The streak plate method indicated selective solvent and microbial inhibition at increasing volume of from 0.2 ml to 0.6 ml. Hexane extract showed negative inhibition

against *EC* and *SA*. However, for the CH_2Cl_2 extract at a volume of 0.4 ml and EtOAc extract from a volume of 0.2 to 0.6 ml positive inhibition was observed for *EC* whereas a negative inhibition was observed for *SA*. Ethanolic extract induce inhibition against both *EC* and *SA* from 0.2 ml to 0.6 ml.

The dilution method was used to test plant extracts for antimicrobial activity against bacteria, *EC* and *SA*. The plates with inhibition from the streak plate method were used in these experiments. Results were recorded in terms of turbidity. In general, no turbidity indicates inhibition. LB broth was used as a rich medium to foster or stimulate the growth of the bacteria. *EC* and *SA* microbe induced no inhibition (very turbid mixture, T_3) for the hexane and CH_2Cl_2 extract. Complete inhibition was observed for the EtOAc extract at a volume of 0.6 ml and 0.4 ml to 0.6 ml for *EC* and *SA* respectively. CH_3CH_2OH extract at a volume 0.2 ml to 0.6 ml induce complete inhibition. The reference compound ampicillin and solvent control showed inhibition and non inhibition respectively.

TLC analysis in various solvent system for each solvent type extract revealed the presence of spots that range from three to eight (Table 6). Each spot is probably due to a pure natural product or phytochemical. Each also has a specific R_f value. The larger the R_f value, the lower the polarity of natural product/phytochemicals. The number of spots and R_f value for each spot is recorded in Table 6. For example for *Smilax schomburgkiana* EtOAc, extract using the solvent system, EtOAc/CH_2Cl_2 (90 : 10, v/v), eight spots at R_f values of 0.048, 0.079, 0.158, 0.349, 0.381, 0.444, 0.492 and 0.984 were seen.

5 Conclusion

It is clearly seen that *Smilax schomburgkiana* has antimicrobial properties which are localized primarily in the CH_3CH_2OH and EtOAc extract. However, antimicrobial activity is selective and solvent dependent with the CH_3CH_2OH extract, the most potent and hexane the least. In general, the order of antimicrobial activity follow the sequence: CH_3CH_2OH extract > EtOAc extract > CH_2Cl_2 extract > hexane extract. Thus, the CH_3CH_2OH and EtOAc extract of *Smilax schomburgkiana* can be used as the active constituent of an antimicrobial cream. Future work such as isolation and purification of bioactive constituents should target the CH_3CH_2OH and EtOAc extract of *Smilax schomburgkiana*.

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