# Selective Antimicrobial properties of Leaf extract of *Samanea Saman* against *Candida albicans, Staphylococcus aureus* and *Escherichia coli* using several microbial methods

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ABSTRACT: Antibacterial and antifungal activities of *Samanea Saman* 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 0.035g in 0.01L(10 mL) of the respective solvents. These were tested in varying volumes of 0.2-0.6ml/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. Saman Samanea showed selective antimicrobial inhibitory activity, with activity most prominent for the $CH_3CH_2OH$ and $CH_2Cl_2$ extracts and negligible with the hexane. Its the first time in our study that the $CH_2Cl_2$ extracts is found to be more potent antimicrobially than the EtOAc extract. This study suggests that the $CH_3CH_2OH$ and $CH_2Cl_2$ extracts of *Saman Samanea* can be used as herbal medicines in the control of *E.coli* and *S.aureus* and *C.albicans* induced diseases, following clinical trials.

**[**Raymond .C. Jagessar, Akini. .Mars, Subramanian Gomathinayagam. **Selective Antimicrobial properties of Leaf extract of Samanea Saman against Candida albicans, Staphylococcus aureus and Escherichia coli using several microbial techniques.** Journal of American Science 2011;7(3):108-119]. (ISSN: 1545-1003). http://www.americanscience.org.

**Key words**: Antimicrobial; *Samanea Saman; S.aureus; E.Coli*; *C. albicans*, Stokes Disc diffusion; Well diffusion; Streak plate; Dilution Method; Selective; herbal medicines.

## 1. Introduction:

This paper focuses on the antimicrobial (antibacterial and antifungal) properties of leaf extract of *Samanea Saman*, commonly called "rain tree or "monkey pod" 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 assay, Well diffusion, Streak plate and a dilution method.

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 extracts and fractionated plant extracts have been a good source of herbal medicine and natural products/ phytochemicals over the years (Kendal et. al, 1994 to Jagessar, R.C, 1998). Guyana has a rich biodiversified flora whose crude extracts, both organic and aqueous are currently been screened for their antimicrobial activity (Jagessar, R.C et. al, 1994 to Jagessar, R.C.et.al, 2008) in addition to their role as global $CO_2$ sinks (in the context of global warming) and national Low carbon development strategy (Jagdeo, B, 2009). Also, the specified plants parts 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 are established in Guyana and the "bush" medicine man is still an important figure in Guyana's culture. Plants are known to synthesize antimicrobial natural products whose structure usually correlates with biological activity. However, there are a large number of plants whose antimicrobial activity need urgent investigations. 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, alkannin (1), shikonin (2) and their derivatives (3-8) isolated from the extract of *Arnebia euchroma* have been found to be the active principles against methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *enterococci* (VRE). The derivatives (3)-(9)

showed stronger anti-MRSA activity (minimum inhibitory concentrations (MICs) that ranged from 1.56 to 3.13 ug/ml than alkannin or shikonin (MIC = 6.25 ug/ml). Anti-MRSA activity of derivatives was bacterial with minimum bactericidal concentration (MBC/MIC) < 2. Bactericidal activity against MRSA was achieved within 2h. Derivatives (3)-(9) were also active against vancomycin-resistant *Enterococcus faecium* (F935) and vancomycin resistant *Enterococcus faecalis* with MICs values similar to those with MRSA (Shen, C. C, 2002).

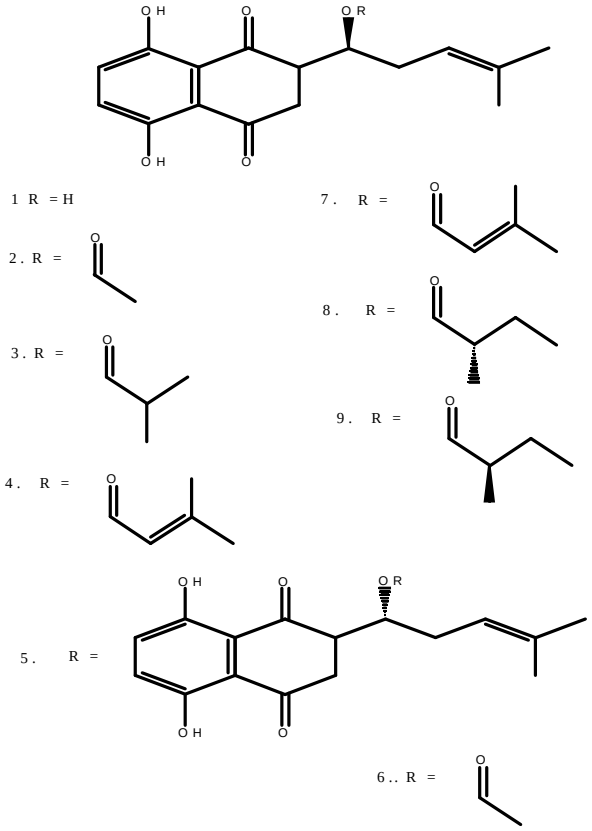


Fig. 1.0. Structure of Antibacterial Naphthazarins from *Arnebia euchrom*

Many synthetic drugs owe their discovery and potency as a result of a mimic of structures from natural products isolated from plants rather than totally to the creativity and imagination of contemporary organic chemists (Scalbert, A, 1991, Hadlington, 2006, Shen, C.C, 2002, Hepeng, 2008 to Macor, 2008). As part of an ongoing project to investigate extracts and chromatographic fractions from plants of the Guyana's flora (Jagessar, R. C. 2007 to Jagessar, R .C, 2008) for their antimicrobial activity, in correlation with folklore practices, we report here, the antimicrobial properties of *Samanea Saman* .

In Guyana's traditional medicine, there is no documentation of *Saman Samanea* as a medicinal plant, however, in neighbouring Venuzuela, the roots are used against stomach cancer and in the West Indies, the seeds are used for sore throat ((**http://www.tropilab.com/raintree.html,** En.wikipedia.org/wiki/*samanea_saman.*

*Samanea Saman*, the "rain tree", "monkey pod" also "cow tamarind", is a tall tropical canopied tree with a large symmetrical crown up to 240 ft broad with a beautiful shade (Scientific Classification of *Samanea Saman* (**http://www.tropilab.com/raintree.html,** En.wikipedia.org/wiki/*samanea_saman*). It is native to the mainland neotropics, from Mexico south to Peru, Brazil and Guyana but has been widely introduced to the Pacific islands, including Hawaii where it is categorized as an invasive species. The tree can reach a height of 25 meters and a diameter of 40 meters. Leaves are alternate bipinate, while the flowers are small pinkish green. It has flat oblong seedpods containing oblong brown seeds. Several flowering version are available e.g. with reddish pink and creamish golden colored flowers. Its usually found on the coastal land of Guyana. The classification of the plant is given in Table 1.0:

Table 1.0 Scientific Classification of *Samanea Saman* (**http://www.tropilab.com/raintree.html,** En.wikipedia.org/wiki/*samanea_saman.*

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| Kingdom | Plantae |
|---|---|
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Order | Fabales |
| Family | Fabaceae |
| Sub Family | *Mimosoideae* |
| Genus | *Samanea* |
| Species | S. *Saman* |

Pathogenic microorganism investigated were *Escherica coli (EC), Staphylococcus aureus* (*SA) and C. albicans (CA). Escherica. coli* can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia (http://en.wikipedia.org/wiki/Escheria_coli.). *Staphylococcus aureus*, the yellow type can cause furuncles (boils), carbuncles (a collection of furuncles)( http://en.wikipedia.org/wiki/Escheria_coli.). In infants, *Staphylococcus aureus* can cause a severe disease Staphylococcal scalded skin syndrome (SSSS). Staphylococcal *endocarditis* (infection of the heart valves) and pneumonia may be fatal. *Staphylococcus aureus* can cause food poisoning

(http://en.wikipedia.org/wiki/*Staphylococcus_aureus*). *Candida Albicans* is a diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans (http://en.wikipedia.org/wiki/Candida_albicans). It is responsible for the infectious disease, candidiasis, thrush etc (Chenn. P, Murray, J, 1997).

## 2. Materials and Methods

The leaves of the above plant was collected from the University of Guyana and the plant identified at the Biodiversity centre of the University of Guyana. A voucher specimen of the plant is deposited at the herbarium. The detached 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 of varying polarity.

Using selective solvent extraction, the ground leaves were first extracted thrice in hexane over a period of five days ( Jagessar, R.C, 2007 to Jagessar, R.C, 2008). .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.

Approximately 0.035g of dried crude extract of *Samanea Saman* 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 0.035g/0.01L. Pathogenic micro organisms: *Staphylococcus aureus, Escherichia coli and Candida albicans* were obtained from the Georgetown Public Hospital (GPH) microbiology laboratory and were stored in a refrigerator at the Berbice campus, Johns.

Two types of agar were used, nutrient agar to make up the medium for bacteria and PDA (Potato Dextrose Agar) (Murray, 1995 and Chenn, 1997) to make up the medium for fungi. The potato *was* peeled and 100g was measured, finely chopped and boiled to a mash in distilled water.The dextrose was measured (12.5g) and placed in a 1L measuring cylinder. Agar was measured (12.5g) 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 to 500mL.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 $^0$C for 24hrs. The pH range was between 6.5-7.0.

Nutrient agar was purchased from the International Pharmacy Association in Guyana. 14g of nutrient agar was suspended in 500ml of distilled water in a 1L flask, stirred, boiled to dissolve and then autoclaved for 15 minutes at 121$^0$C. 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, micro organisms were streaked onto separate plates and the discs were applied with a forceps.They were labeled and placed in an incubator at 37 $^0$C for 24 and 48 hours for bacteria and fungi respectively.

*Luria –Bertani* broth (LB broth) **(** Murray, 1995 and Chenn, 1997) is a rich medium used to culture bacteria such as *E.Coli and S.aureus.* To make it, tryptone (10g), yeast extract (5g) and sodium chloride (10g) were measured and placed in a 1L cylinder. Distilled water was added to make up the 1L 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. 3mL 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 $^0$C for 2h. These tubes were used in the dilutions experiments.

The references were antibiotic in nature. *Ampicillin* and *Nystatin*. *Ampicillin* was choosen as the reference for all bacterial species used: *E.Coli* and *S.aureus. Nystatin* was used as the reference for the fungus, *Candida. albicans.* The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion (Murray, 1995 and Chenn, 1997)

The aseptic chamber which consists of a wooden box (1m x 1m x 0.5m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from a lamp). These were made by culturing *C.albicans* on PDA. A sterilized 6 mm cork borer was used to cut agar discs in the plate. Stokes Disc diffusion sensitivity technique, Well Diffusion (Diffusion plate) method, Streak Plate Method and Dilution method.

Using Stokes Disc diffusion sensitivity testing technique (Murray, 1995 and Chenn, 1997), 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 200mg antibiotic/ml. The

discs were made by cutting discs (5-6mm) from a filter paper with a perforator, placing 5 of these discs in a vial and adding 0.2mL 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 37ºC for 24 hr and 48 hr for the bacteria and *Candida albicans* 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.

For the Well Diffusion (Diffusion plate) method, A fungus (*Candida albicans*) 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 2h. 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 0.035g/0.01L was pipette into the different wells in a sterilized environment at different volumes (0.2-0.4-0.6ml) 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 (0.035g/0.01L) at different volumes(0.2-0.4-0.6ml) was used as the reference. The plates were labelled, covered, inverted and placed in a fume hood (no incubator was available) for 48h

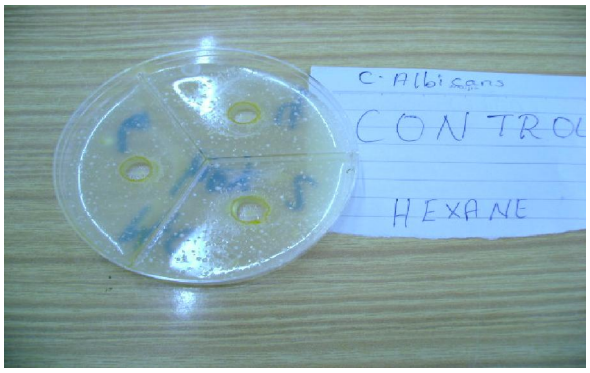



Fig. 3.0. Well diffusion for *C.albicans*, Control Experiment.

Nutrient agar was prepared as described above and 10 mL was poured into plates. Plant extracts dissolved in solvent at a final concentration of 0.035g/0.01L 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 (0.035g/0.01L) at different volumes (0.2-0.4-0.6ml). Controls were also setup using solvents: hexane, $CH_2Cl_2$ and EtOAc and $CH_3CH_2OH$ at the different volumes.

This Dilution 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 (*Louria Bertinieia*) was inoculated with *Staphylococcus aureus* and the second set was inoculated with *Escherichia coli* using a loop, flame and alcohol. Under aseptic conditions, the plant extracts (dissolved in solvent at concentration 0.035g/0.01L) and showed inhibition in the streak plate were added to the one set of test tubes containing *E. Coli* and the other set, *S.aureus* with LB broth(medium) in differing volumes (0.2-0.4-0.6ml). Two sets of four tubes each were treated with the four solvents (hexane, $CH_2Cl_2$, EtOAc and $CH_3CH_2OH$). One set was inoculated with *S.aureus* and the other with *E.coli*. Cotton wool was used to plug test tubes. The tubes were observed after 24 hrs.

Retention Factor, $R_f$ was calculated using the formula,

$$R_f = \frac{\text{Distance moved by sample}}{\text{}}$$

Distance moved by solvent front.

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

For 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 *Samanea Saman species was* 8.55g respectively. These extracts were in the concentration of 0.035g in 10 ml (0.0003mg/uL) of solvent except for *Samanea Saman* with $CH_3CH_2OH$ which was 0.5g in 25ml (0.02mg/uL). Reference compounds were: Ampicillin and nystatin in a concentration of 200mg/ml.

Table 2.0. Using the Disc diffusion method above, the Antimicrobial activity of Plant extract as shown by the inhibition zone diameter**.**

| **Area of inhibition. ($mm^2$) using** *E.Coli* | **Area of inhibition. ($mm^2$) using** *S.aureus* | **Area of inhibition. ($mm^2$) using** *Candida albicans* | **Plant Extracts** *Samanea Saman species* | **Reference compound (Ampicillin) ($mm^2$)** | **Control Experiment** |
|---|---|---|---|---|---|
| **< 5** | **< 5** | **< 5** | Hexane extract | **27** | No zone of inhibition |
| **20** | **27** | **26** | $CH_2Cl_2$ extract | **28** | No zone of inhibition |
| **<5** | **< 5** | **<5** | EtOAc extract | **30** | No zone of inhibition |
| **22** | **29** | **28** | $CH_3CH_2OH$ extract | **33** | No zone of inhibition |

Table 3.0. Results of Well diffusion for plant extracts *Samanea Saman* against *C.albicans.*

| **Plant Extract dissolved in solvent at concentration: 0.035g/0.01L** | **Volume of Extract (mL)** | **Presence of zone of Inhibition** | **Diameter of Zone of Inhibition ($mm^2$)** |
|---|---|---|---|
| *Samanea Saman* with Hexane | 0.2 | No zones of inhibition visible, scattered colonies. | **<5** |
| | 0.4 | **“ “** | **<5** |
| | 0.6 | **“ “** | **<5** |
| *Samanea Saman* with $CH_2Cl_2$ | 0.2 | Zones of inhibition visible. | **45** |
| | 0.4 | **“ “** | **55** |
| | 0.6 | **“ “** | **74** |
| *Samanea Saman* with EtOAc | 0.2 | No Zones of inhibition visible. | **<5** |
| | 0.4 | **“ “** | **10** |
| | 0.6 | | **< 5** |
| *Samanea Saman* with $CH_3CH_2OH$ | 0.2 | | **50** |
| | 0.4 | | **61** |
| | 0.6 | | **65** |
| **Reference(Nystatin)** | 0.2 | Zone of Inhibition | **76** |

| | | | |
|---|---|---|---|
| | 0.4 | “ “ | **76** |
| | 0.6 | “ “ | **76** |
| **Controls** Hexane, $CH_2Cl_2$, EtOAc, $CH_3CH_2OH$ | 0.2 | No zone of inhibition | **< 5** |
| | 0.4 | No zone of inhibition | **< 5** |
| | 0.6 | No zone of inhibition | **< 5** |

Table 4.0. Results obtained from Streak plate method for the bacteria's *Escheria coli(EC)* and *Staphylococcus aureus(SA)* against different volumes of dissolved plant extracts at a final concentration of 0.035g/0.01 L and controls. Inhibition or no growth of microbes were represented by a positive sign (+), while the negative sign (-) represents no inhibition or growth of microbes**.**

| Plant extract dissolved in solvent | Volume of dissolved plant extract used in (ml) at concentration 0.035g/0.01L | Inhibition or no growth of microbe, *Escherichia coli* | Inhibition or no growth of microbe *Staphylococus aureus* |
|---|---|---|---|
| *Samanea Saman* dissolved in hexane | **0.2** | - | - |
| | **0.4** | - | - |
| | **0.6** | - | - |
| *Samanea Saman* dissolved in $CH_2Cl_2$ | **0.2** | + | - |
| | **0.4** | + | - |
| | **0.6** | + | - |
| *Samanea Saman* dissolved in Et(OAc) | **0.2** | - | - |
| | **0.4** | - | - |
| | **0.6** | - | - |
| *Samanea Saman* dissolved in $CH_3CH_2OH$ | **0.2** | + | + |
| | **0.4** | + | + |
| | **0.6** | + | + |
| Reference (Ampicillin with same concentration as dissolved plant extracts(0.035g/0.01L). | **0.2** | + | + |
| | **0.4** | + | + |
| | **0.6** | + | + |
| Hexane | **0.2** | - | - |
| | **0.4** | - | - |
| | **0.6** | - | - |
| $CH_2Cl_2$ | **0.2** | - | - |
| | **0.4** | - | - |
| | **0.6** | - | - |
| EtOAc | **0.2** | - | - |
| | **0.4** | - | - |
| | **0.6** | - | - |
| $CH_3CH_2OH$ | **0.2** | - | - |
| | **0.4** | - | - |
| | **0.6** | - | - |

For the Dilution Method , Results were recorded in terms of turbidity.

$T_0$ = No Turbidity = Inhibition
$T_1$ = Slightly Turbid = Moderately Inhibited
$T_2$ = Moderately Turbid = Lightly Inhibited
$T_3$ = Very Turbid = No Inhibition

Table 5.0 shows the degree of turbidity of dissolved *Samanea Saman* extracts at concentration of 0.035g/0.01L at different volumes against *Escheria coli* (EC) microbe.

| Plant extract dissolved in solvents at concentration of 0.035g/0.01L | Volume of dissolved plant extract (ml) 0.2 ml | Volume of dissolved plant extract (ml) 0.4ml | Volume of dissolved plant extract (ml) 0.6 ml |
|---|---|---|---|
| *Samanea Saman* with hexane | **$T_3$** | **$T_3$** | **$T_3$** |
| *Samanea Saman* with $CH_2Cl_2$ | **$T_3$** | **$T_0$** | **$T_0$** |
| *Samanea Saman* with EtOAc | **$T_3$** | **$T_3$** | **$T_3$** |
| *Samanea Saman* with $CH_3CH_2OH$ | **$T_0$** | **$T_0$** | **$T_0$** |

Table 6.0. Table showing the degree of turbidity of dissolved plant extract at concentration of 0.035g/0.1 L at different volumes against *Staphylococcus aureus (SA)* microbe.

| Plant extract dissolved in solvents at concentration of 0.035g/0.01L | Volume of dissolved plant extract (ml) 0.2 ml | Volume of dissolved plant extract (ml) 0.4ml | Volume of dissolved plant extract (ml) 0.6 ml |
|---|---|---|---|
| *Samanea Saman* with hexane | **$T_3$** | **$T_3$** | **$T_3$** |
| *Samanea Saman* with $CH_2Cl_2$ | **$T_0$** | **$T_0$** | **$T_0$** |
| *Samanea Saman* with EtOAc | **$T_3$** | **$T_3$** | **$T_3$** |
| *Samanea Saman* with $CH_3CH_2OH$ | **$T_0$** | **$T_0$** | **$T_0$** |

Table 7.0. Antimicrobial activity of different controls used in the dilution method.

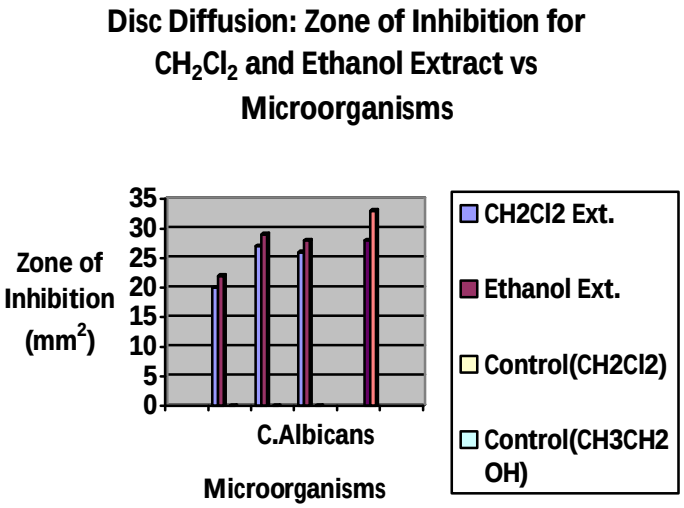
| | Volume of dissolved plant extract (ml) 0.2 ml | Volume of dissolved plant extract (ml) 0.4ml | Volume of dissolved plant extract (ml) 0.6 ml |
|---|---|---|---|
| Reference (Ampicillin at same concentration as dissolved plant extract 0.035g/0.01L | $T_0$ | $T_0$ | $T_0$ |
| **Control** | | | |
| Hexane | $T_3$ | $T_3$ | $T_3$ |
| $CH_2Cl_2$ | $T_3$ | $T_3$ | $T_3$ |
| EtOAc | $T_3$ | $T_3$ | $T_3$ |
| $CH_3CH_2OH$ | $T_3$ | $T_3$ | $T_3$ |

Table 8.0. TLC analyses for all the extracts of *Samanea Saman.*

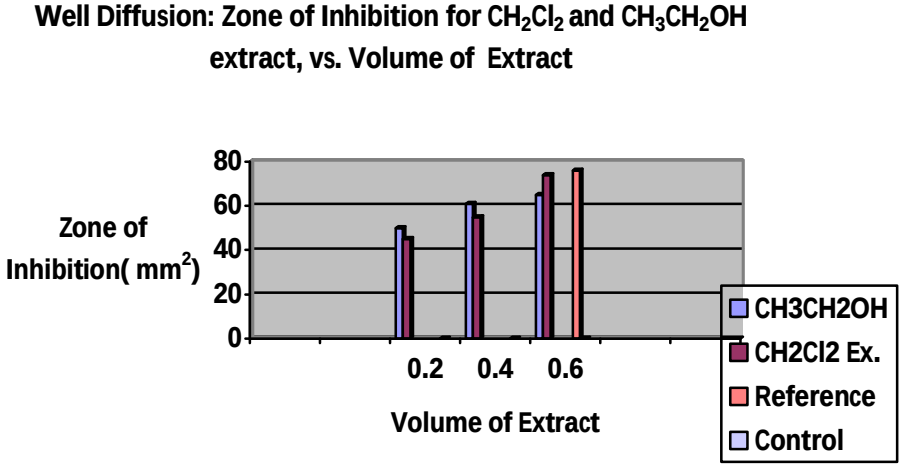
| Eluent (90:10, v/v) | Plants Extracts | Number of spots visible by UV | $R_f$ value |
|---|---|---|---|
| $CH_2Cl_2$/Hexane | Hexane Extract | 2 | 0.043<br>0.109 |
| $CH_2Cl_2$/Hexane | $CH_2Cl_2$ Extract | 2 | 0.051<br>0.974 |
| EtOAc/Hexane | EtOAc Extract | 3 | 0.032<br>0.081<br>0.35 |
| Ethanol/Hexane, | $CH_3CH_2OH$ Extract | 3 | 0.081<br>0.251<br>0.512 |

Bar graphs are shown in Fig. 4.0 (a) and 4.0 (b)

(a)




(b)



4. .**Discussion**:

All four methods: Stokes disc diffusion techniques,Well diffusion Streak plate and a dilution method were successful in determining crude hexane, $CH_2Cl_2$ and $CH_3CH_2OH$ *extracts of Samanea Saman* for antimicrobial activities. Several trends are noted and these will be discussed first. Each solvent type extracts was added in increasing volume (0.2-0.4-0.6) ml to the microbial medium. Unlike previous publications (Jagessar, R.C, 2007 to Jagessar, R.C, 2008) antimicrobial potency follow the sequence: $CH_3CH_2OH$ extract > $CH_2Cl_2$ extract> EtOAc extract > hexane extract, suggesting that *Samanea Saman* antimicrobial compounds are localized in the $CH_3CH_2OH$ and $CH_2Cl_2$ extract. For example, with disc diffusion method, zone of inhibition of 22 $mm^2$, 29 $mm^2$ and 28 $mm^2$ were obtained for the $CH_3CH_2OH$ extract against *E.Coli*, *S.aureus* and *C.albicans* in contrast to 20 $mm^2$, 27 $mm^2$ and 26 $mm^2$ for the $CH_2Cl_2$ extract. For the hexane and EtOAC extract, zone of inhibition of < 5 $mm^2$ were obtained against all microbes. In a comparative Well diffusion method, zone of inhibition of 65 $mm^2$ was obtained for the $CH_3CH_2OH$ extract when the well was filled with 0.6 ml of extract compared with a zone of inhibition of 74 $mm^2$ for $CH_2Cl_2$ extract when the volume of the well was 0.6 ml, suggesting greater potency. This is the first example of a plant, we have studied whose $CH_2Cl_2$ extract is more potent that the EtOAc extract against microbes. All of our plants reported to date displayed the common antimicrobial potency trend: $CH_3CH_2OH$ extract > EtOAc extract > $CH_2Cl_2$ extract > hexane extract (Jagessar, R.C, 2007 to Jagessar, R.C, 2008).. 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 (*Luria-Bertani)* broth with bacterial microbes. The reference antibiotic ampicillin for bacteria and Nystatin for fungi induced positive results as anticipated, Figure 1.0 and Figure 2.0. For example, for the disc diffusion method, ampicillin induced zone of inhibition of 33 $mm^2$ for the $CH_3CH_2OH$ extract whereas Nystatin induced zone of inhibition of 76 $mm^2$ at a volume of 0.6 ml for the Well diffusion method. These results suggest that *Samanea Saman* antimicrobial properties are due to the plant active constituent rather than to a solvent effect.

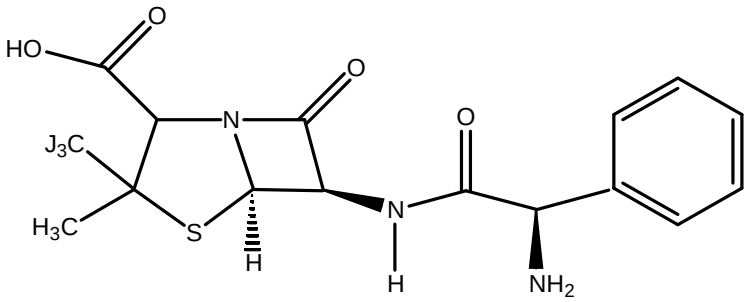



Figure. 1.0 Ampicillin

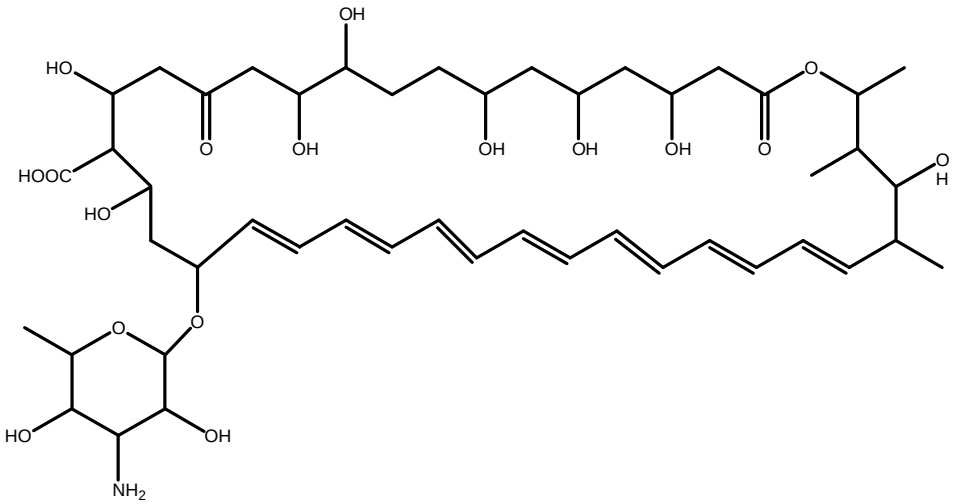



Figure. 2.0. Nystatin

Each method display interesting results and these can be further discussed. Stokes Disc diffusion indicates that the plant extract was more selective potent against *S.aureus* as compared against *E.coli* and *C.albicans*. For example, for the $CH_2Cl_2$ extract, zone of inhibition of 26 $mm^2$ and 27 $mm^2$ were obtained for *C.albicans and S.aureus* respectively. Of the two bacteria, *S.aureus*. showed a larger zone of inhibition as compared with *E.Coli*, inspite of the fact that *E.coli* has an outer membrane that acts as a selective barrier against antibiotics and toxins. Contrary, gram positive *S. aureus* lack the outer membrane but possess a much thicker cell wall of peptidoglycan layer which is not an effective permeability barrier. However, for the Streak plate method, *E.Coli* showed inhibition whereas *S. aureus* showed no inhibition for the $CH_2Cl_2$ extract. Comparatively, for the EtOAc extract, no inhibition zone was observed. For the $CH_3CH_2OH$ extract extract, inhibition was observed against both *E.Coli* and *S.aureus*.

The Well diffusion method was used to investigate *C. albicans's* antimicrobial activity. A larger zone of inhibition was observed compared with the Stokes Disc diffusion method. This difference may be ascribed to the higher sensitivity of the Well diffusion as compared with the Disc diffusion method. For example, for the $CH_3CH_2OH$ extract, a zone of inhibition of 65 $mm^2$ was observed at a volume of 0.6 ml. Compared with the Well Diffusion method, the ethanol extract induce zone of inhibition of 28 $mm^2$ against *Candida albicans* for the Disc diffusion method.

The Streak plate method indicated selective solvent and microbial inhibition at increasing volume of 0.2-0.6 ml. Results are tabulated as positive or negative inhibition. Hexane and EtOAc extract showed negative inhibition against *E.Coli* and *S.aureus*. However, for the $CH_2Cl_2$ extract at a volume of 0.2 to 0.6 ml, positive inhibition was observed for *E.Coli* whereas a negative inhibition was observed for *S.aureus*. Ethanolic extract induce positive inhibition against both *E.Coli* and *S.aureus* from 0.2 to 0.6 ml. The reference ampicillin showed positive inhibition.

The Dilution method was used to test plant extracts for antimicrobial activity against bacteria: *E.Coli* and *S.aureus*. 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 ($T_0$) indicates inhibition. LB (*Louria Bertinieia*) broth was used as a rich medium to foster or stimulate the growth of the bacteria. *E.Coli and S.aureus* microbes induced no inhibition (very turbid mixture, $T_3$) for the hexane and EtOAc extract. Complete inhibition was observed for the $CH_2Cl_2$ extract at a volume of 0.4-0.6ml for *E.Coli* and *S.aureus*. $CH_3CH_2OH$ extract at a volume 0.2 to 0.6 ml induce complete inhibition, $T_o$. The reference compound, ampicillin and solvent control at a volume range from 0.4-0.6 showed inhibition and non inhibition respectively. It is interesting to note that the zone of inhibition induced by the antibiotics ampicillin and nystatin was greater than each solvent type extract.

Figure 4.0 (a) represent for the Disc diffusion method, using the $CH_3CH_2OH$ and $CH_2Cl_2$ extract, a comparative bar graph plot of the zone of inhibition ($mm^2$) vs. type of microorganism. Figure. 4.0 (b) represent for the Well Diffusion method, a bar graph plot of the zone of inhibition for $CH_2Cl_2$ and $CH_3CH_2OH$ extract vs volume of extract. For the disc diffusion technique, a larger zone of inhibition was observed for the $CH_3CH_2OH$ extract as compared with the $CH_2Cl_2$ extract. The largest zone of inhibition was observed for *S.aureus* in both cases. For the Well Diffusion method, as the volume of plant extract increased in the well so too is the zone of inhibition. However, the largest zone of inhibition was seen for the reference antibiotic, ampicillin (light blue). In contrasts, zero inhibition was seen for the solvent control (red).

TLC analyses in various solvent system for each solvent type extract revealed the presence of spots that range from one to two, Table 8.0. Each spot has a specific $R_f$ value. The larger the $R_f$ value, the lower the polarity of natural product/phytochemicals For example for *Samanea Saman* ethanol extract using the solvent system, Ethanol/hexane (90:10, v/v), three spots of $R_f$ values 0.081, 0.251 and 0.512 were seen.

In conclusion, *Samanea Saman* has antimicrobial properties which are localized primarily in the $CH_3CH_2OH$ and $CH_2Cl_2$ 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 > $CH_2Cl_2$ extract > EtOAc extract > hexane extract. In our study, it's the first plant in which the $CH_2Cl_2$ extract is found to be more potent than the EtOAc extract against microbes. Thus, the $CH_3CH_2OH$ and $CH_2Cl_2$ extract of *Samanea Saman* 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 $CH_2Cl_2$ extract of *Samanea Saman*.

**Acknowledgements**:

This research was carried out by a final year research student A.Mars under my constant supervision and also that of Dr. S. Gomathinayagam, microbiologist at John's campus, University of Guyana in the county of Berbice. We thank the University of Guyana, Faculty of Natural Sciences, Chemistry Department for the provision of laboratory space for the extraction process and equipment to carry out this

research. Special thanks extended to the Georgetown Public hospital (GPH) for the provision of microorganisms essential for research. This research was supported via a grant to Dr. R. C. Jagessar from the Research and Publication Agency of the University of Guyana, South America.

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7/11/2010