"Antibacterial and antifungal activity of leaf extracts of *Luffa operculata*, vs *Peltophorum Pterocarpum*, against *Candida albicans, Staphylococcus aureus and Escherichia coli*"

R. C. Jagessar*+, A. Mohamed®, G. Gomes+

*+Lecturer and Supervisor, Department of Chemistry, University of Guyana, Faculty of Natural Sciences, South America, raymondjagessar@yahoo.com

+Microbiologist, Department of Biology, John's Campus, University of Guyana, Faculty of Natural Sciences, South America

®Final Year Research student, Department of Biology

ABSTRACT: The antibacterial and antifungal activities of *Luffa operculata*, and *Peltophorum.pterocarpum* were investigated against *S. aureus* (gram+ve), *E. coli* (gram-ve) and *C. albicans* using the Stokes disc diffusion, the pour plate, well diffusion and streak plate method. These extracts were obtained by three extractions each with hexane, dichloromethane, ethyl acetate and ethanol. Solvents were removed in *vacuo* to yield viscous oils and paste which were made up to a concentration of 0.03g in 10 mL of the respective solvents. These were tested in varying volumes of 100-600 uL/plate (i.e. concentrations of 0.03-0.18 mg/10 mL agar). The 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. All three plants showed antimicrobial inhibitory activity at 0.18 mg/10mL plate of medium. Activity was also most prominent with the ethanol extracts and least or negligible with the hexane. This study suggests that the ethanol and ethyl acetate extracts of *Luffa operculata* and *Peltophorum.pterocarpum* can be used as herbal medicines in the control of *E. coli* and *S. aureus* induced medical diseases, following clinical trials. [Nature and Science. 2007;5(4):81-93].

Keywords: Antimicrobial, *S.aureus*, *E.Coli*, *C.albicans*, Stokes Disc diffusion, Pour plate, Well diffusion, Streak plate, herbal medicines.

Introduction

This paper investigates the microbiological properties of leaves of two plants from the coastal plane of the Guyana flora, in an attempt to evaluate their future use as possible herbal medicines. Plants studied are *Luffa operculata*, and *Peltophorum.pterocarpum*. Their antimicrobial properties were investigated against S.aureus (gram+ve), E.coli (gram-ve) and C.albicans using the Stokes disc diffusion sensitivity technique, Pour plate, Well diffusion and Streak plate.

Guyana has a rich diverse flora whose crude extracts, both organic and aqueous can be investigated for antimicrobial activity. Also, the specified plants parts of the same species be screened for natural products whose antimicrobial activity can also be correlated. Following this, clinical trials can lead to the formulation of an herbal plant cream. Plants extracts have been used for their antimicrobial properties[1-14]. In Guyana, there are many folk remedies but most are without scientific research. Thus, there exist an urgent need to correlate folklore herbal practices with scientific evidence. With an increasing emphasis on scientific research, Guyana stands well in this area. Besides used as an herbal cream, following clinical trials, plant extracts can be subjected to chromatographic separation, leading to the isolation and purification of new and un known and known bioactive natural products/phytochemicals whose medicinal activity can also be investigated [2,4,14].

Research in herbal medicine and isolated drug discovery need to be continued, considering the threat of new emerging disease such as SARS, bird flu, not to mention AIDS. Plants are a good source of herbal medicine and natural products/ phytochemicals[1-14]. Guyana stands well for the establishment of a phytopharm, a farm set aside for the sole purpose of cultivating plants rich in natural products/phytochemicals[14]. Phytopharm has been established in the UK since 1990 and is the first botanical development company. The company is developing treatments for Alzhemeimer's disease, appetite suppression and inflammation. One advantage of botanicals is that a company can start immediately to evaluate plant extracts for clinical efficacy in diseases if there is a history of its use. USA based Phytoceutica uses assay, informatics and clinical trials to discover and develop botanicals drugs[14]. Guyana stands

well for the establishment of phytopharms. This would require though scientific evidences to confirm folklore practices. One such evidence is an investigation of antimicrobial activity of selected plants using contemporary antimicrobial tests. Thus, the antimicrobial activity of *Luffa operculata*, and *Peltophorum.pterocarpum* are presented here.

The luffas species are tropical and subtropical annual vines comprising the genus Luffa[15]. Luffa belongs to the family called Cucurbitaceae. *L. acutangula* (Angled luffa, Ridged Luffa), L. *aegyptiaca* (Smooth luffa, Egyptian luffa), *L. operculata* (Sponge cucumber), are some species. It is commonly called "nenwa". The fruit of at least two species, *L. acutangula* and L. *aegyptiaca*, is grown to be harvested before maturity and eaten as a vegetable, sometimes called jhingey or nenwa.. The fruit of L. aegyptiaca may also be allowed to mature and used as a bath or kitchen sponge after being processed to remove everything but the network of xylems. In this research, *luffa operculata* was studied.

Peltophorum pterocarpum is a deciduous tree growing up to 15–25 m (rarely up to 50 m) tall, with a trunk diameter of up to 1 m[16]. The leaves are bipinnate, 30-60 cm long, with 16-20 pinnae, each pinna with 20-40 oval leaflets 8-25 mm long and 4-10 mm broad. The flowers are yellow, 2.5-4 cm diameter, produced in large compound racemes up to 20 cm long. The fruit is a pod 5-10 cm long and 2.5 cm broad, red at first, ripening black, and containing one to four seeds. Trees begin to flower after about four years. The bark of plant is used for dysentery, tooth powder, eye lotion, embrocation for pains and sores. The bark also gives a dye of a yellow colour.

Fig. 1.0 *Peltophorum pterocarpum*

The above two plants extracts were tested against *E.Coli*, *S.aureus* and *C.albicans*. *Escherica*. *coli* can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia[17]. Staphylococcus aureus can cause furuncles (boils), carbuncles (a collection of furuncles)[18]. 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. *Candida Albicans* is a diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans[19-20].

Procedure:

2.1: **Collection of Plant materials**: The leaves of the above two mentioned plants were collected off the coastal plain of Guyana. The detached plant leaves were subjected to aerial drying for two weeks and crushed into very small pieces and placed in separate conical flasks. This increased the surface area for extraction.

Extraction: The leaves were first extracted in hexane thrice over a period of five days[4-11].Water was removed from the accumulated extract by stirring over anhydrous Na_2SO_4 and extract filtered.Solvents were removed in vacuo using rotary evaporation.The extracts were placed in vials and then in a dessicator. Extracts were stored in capped vials and were weighed. The above procedure was repeated with the same leaves but with different solvents of differing polarity such as dichloromethane, ethyl acetate, and then ethanol.

2.3. Antimicrobial activity tests

2.3.1. **Making up extract solution**

0.03 g of each dry crude extract was weighed and placed in a 10mL volumetric flask. The respective solvent was then added to make up the 10 mL solution.

2.3.2. Microorganisms:

Micro organisms were obtained from the Georgetown Public Hospital (GPH) microbiology laboratory. These were *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. They were stored in a refrigerator at the Food and Drug microbiology lab.

2.3.3. **Potato dextrose agar (PDA)** [21]

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 1L 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 ^{0}C for 24 hrs.

2.3. 4. Reference and Control:

The references were antibiotic in nature. Ampicillin and Nyastatin. Ampicillin was choosen as the reference for all bacterial species used: *E.Coli* and *S.aureus*. Nyastatin 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 [21].

2.3.5. Aseptic conditions:

The aseptic chamber consists of a wooden box (1m x 1m x 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 before by culturing *C. albicans* on PDA. A sterilized 6 mm cork borer was used to cut agar discs in the plate.

2.3. 7. Nutrient Agar:

500 ml of nutrient agar was made by placing 14g of the powdered mixture in a 1L flask, stirred, boiled and then autoclaved for 15 minutes at 121^{0}C. The plates were poured in a sterile environment and allowed to cool for 2 hours. Under aseptic conditions, the 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.

2.3.8. **Colonies Counting**: Colonies were estimated with the assistance of a colony counter. The number was estimated for 1 cm^2 and then calculated for the entire plate. The plate radius was determined.

2.3.9. 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.

2.3.10. Disc diffusion: Stokes Disc diffusion sensitivity technique [21].

Using Stokes Disc diffusion sensitivity testing technique[21] , 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 10mg of antibiotic/disc. 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 0.2mL 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.

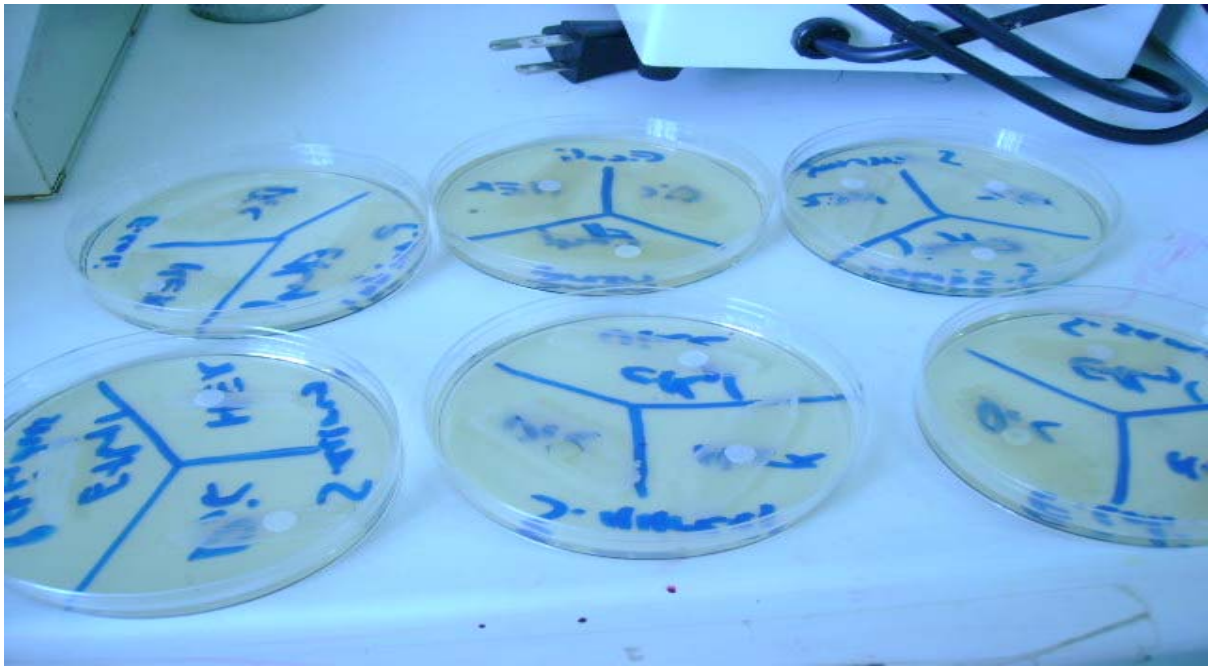


Fig. 2.0. Discs applied to the plates already streaked with bacteria and the fungus, Stokes disc diffusion method.

2.3.11. Pour Plate Method [21].

After the nutrient agar was placed in the autoclave at 120ºC for one and half hour, it was taken out and left to semi cool in a sterilized environment. 0.1mL of each solvent type extract and control were measured and placed

in separate sterile glass plates plates (100 mm diameter). 10 mL of nutrient agar was then poured into the 100 mm plate, with an even depth of 4 mm on a level surface shaken and allowed to cool. A sterile glass rod was used to uniformly stir the mixture into the nutrient agar which was left to solidify in the glass plate. The microorganisms were then streaked onto the plates and placed in an incubator at 37 ^{0}C for 24 and 48 hours for bacteria and fungi species respectively.

The inoculated plates were incubated in an inverted position (lid on bottom) to prevent collection of condensation on the agar surface. Unless the surface is dry, it will be difficult to obtain discrete surface colonies. The plates were examined for the appearance of individual colonies growing throughout the agar medium. The number of colonies were counted so as to determine how effective the plant extract were against bacterial and fungi.

2.3.12. Diffusion plate (well diffusion):

The fungus (Candida albicans) was mixed with the warm, melted, autoclaved PDA and poured into 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 h. When cooled, a well was made at the centre of the plate. The well was made by using a 6mm cork borer that was sterilized with alcohol and flame. The extracts were applied to different wells in volumes of 100-600uL using a micro liter syringe. The four solvents (hexane, dichloromethane, ethyl acetate and ethanol) were used as control whereas nystatin was used as the reference.The plates were labelled, covered, inverted and placed in a fume hood (no incubator was available) for 48h.

2.3.13. Streak plate for bacteria:

Nutrient agar was prepared as described above and 10mL was poured into plates. The plates were treated with the extracts and reference compound ampicillin in varying volumes of 100-600 uL.The plates were allowed to cool and then the bacteria were streaked onto the surface.These plates were left for 24 hours. The plates with inhibition were used in further experiments.

2.3.14. 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.They were examined under the UV/Vis lamp and the specks were circled with a pencil. The plates were then held in the iodine jar for a few seconds, shaken and taken out.The plate was further examined under UV lamp and any new specks were marked. The specks 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 determined.

2.3.15. **Results**:

Mass of dried leaves used for Luffa and P. pterocarpum were 4.38 g and 39.4 g respectively. The physical state of the dried extract are shown in Table 1.0.

Table 1.0 Shows physical properties of the dry extracts

Solvent	Plant	Dried extract
Hexane	*Luffa operculata* *Peltophorum.pterocarpum*	Opaque, oily. Hard, green, gummy.
Dichloromethane	*Luffa operculata* *Peltophorum.pterocarpum*	Rust brown, oily. Black, soft, gummy.
Ethyl acetate	*Luffa operculata* *Peltophorum.pterocarpum*	Rust brown, oily. Soft, black, gummy.
Ethanol	*Luffa operculata* *Peltophorum.pterocarpum*.	White flaky. Black, gummy.

These extracts were in the concentration of 0.03 g in 10 ml of solvent. This works out to 0.0003 mg/uL and 0.02 mg/uL of crude extract respectively. The ampicillin and nystatin controls were in concentration of 250 mg in 10 ml.

Disc diffusion:

Table 2.0. Antimicrobial activity of Plant extracts 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**	**Reference compound (mm^2)**	**Control Experiment**
			Luffa operculata		No zone of inhibition
< 5	**<5**	**<5**	Hexane extract	**27**	No zone of inhibition
<5	**<5**	**<5**	Dichloromethane extract	**27**	No zone of inhibition
10	**6**	**5**	EtOAc extract	**28**	No zone of inhibition
22	**18**	**21**	Ethanol extract	**31**	No zone of inhibition
			Peltophorum.pterocarpum		
< 5	**< 5**	**< 5**	Hexane extract	**27**	No zone of inhibition
< 5	**< 5**	**< 5**	Dichloromethane extract	**29**	No zone of inhibition
22	**23**	**22**	EtOAc extract	**28**	No zone of inhibition
24	**27**	**25**	Ethanol extract	**30**	No zone of inhibition

Pour Plate:

Table 3.0 showing the number of visible colonies when viewed under a colony counter

Bacterium	**Extract**	**Volume**	**# of colonies**
E.coli	*Peltophorum.pterocarpum* with ethanol	600 uL	1
S. aureus	*Luffa operculata* with ethyl acetate	600 uL	0
E. coli	*Luffa operculata* with ethanol	600 uL	$18/cm^2$x $0.3(63.6cm^2)$= 381.
S.aureus	*Peltophorum.pterocarpum* with EtOAc	600 uL	
Controls:	solvents	600 uL	Excess growth observed.
S.aureus *E.coli*	Nutrient agar only Nutrient agar only	-------- ---------	$26/cm^2$x $63.6cm^2$=1653. $20/cm^2$x $63.6cm^2$=1272.
Ampicillin	Nutrient agar only	600 uL	0

These colonies were estimated with the assistance of a colony counter. The number was estimated for 1cm^2 and then calculated for the entire plate. The plate radius was 45mm, therefore the area was 63.6 cm^2.

Table 4.0. Results of the well diffusion for plant extracts against C.albicans

Zones of inhibition (mm^2)	**Extract**	**Volume**	**Observations**
0	*Luffa operculata* with hexane	600 uL	No zones of inhibition visible.
0	*Luffa operculata* with ethyl acetate	100-400 uL	No zones of inhibition visible.
25x30	*Luffa operculata* with ethyl acetate	500uL	Zones of inhibition visible.
30x40	*Luffa operculata* with ethyl acetate	600uL	Zones of inhibition visible.
0	*Peltophorum.pterocarpum* with hexane	100-600 uL	No zones of inhibition visible.
30x40	*Peltophorum.pterocarpum* with dichloromethane	600 uL	Zones of inhibition visible.
0	*Peltophorum.pterocarpum* with dichloromethane	100-400 uL	No zones of inhibition visible.
5x10	*Peltophorum.pterocarpum* with ethyl acetate	100 uL	Zones of inhibition visible.
20x20	*Peltophorum.pterocarpum* with ethyl acetate	200 uL	Zones of inhibition visible.
22x28	*Peltophorum.pterocarpum* with ethyl acetate	300 uL	Zones of inhibition visible.
30x40	*Peltophorum.pterocarpum* with ethyl acetate	400 uL	Zones of inhibition visible.
35x48	*Peltophorum.pterocarpum* with ethyl acetate	500 uL	Zones of inhibition visible.
40x70	*Peltophorum.pterocarpum* with ethyl acetate	600 uL	Zones of inhibition visible.
75x45	*Luffa operculata* with ethanol	600 uL	Complete zones of inhibition.
75x45	*Peltophorum.pterocarpum* with ethanol	600 uL	Complete zones of inhibition.
20x30 30x50 50x70	Nystatin	200 uL 400 uL 600 uL	Zones of inhibition
Controls	Diffusion well with four solvents		Scattered colonies
Reference	Nyastatin	600 uL	Complete zones of inhibition

Streak plate:

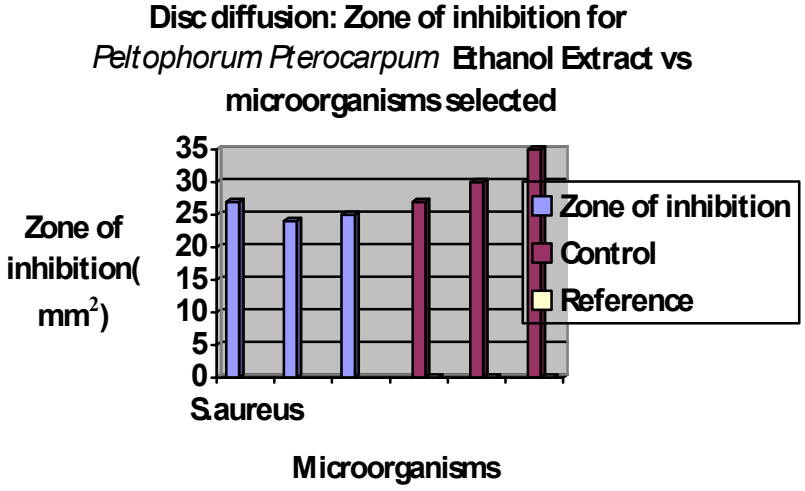
Table 5.0. showing the results of the streak plate method

Bacterium	Solvent Extract	Volume	Observations
S.aureus	*Luffa operculata* with ethyl acetate	600uL	Limited growth
	Peltophorum.pterocarpum with ethanol	600uL	Limited growth
E.coli	*Peltophorum.pterocarpum* with ethyl acetate	200-600uL	Limited growth
	Luffa operculata with ethanol	200-600uL	Limited growth
	Peltophorum.pterocarpum with ethanol	600uL	Limited growth
Controls	The four solvents: hexane,dichloromethane, ethyl acetate and ethanol	600uL	Growth of microorganism
Reference (Ampicillin)		200-600uL	Inhibition

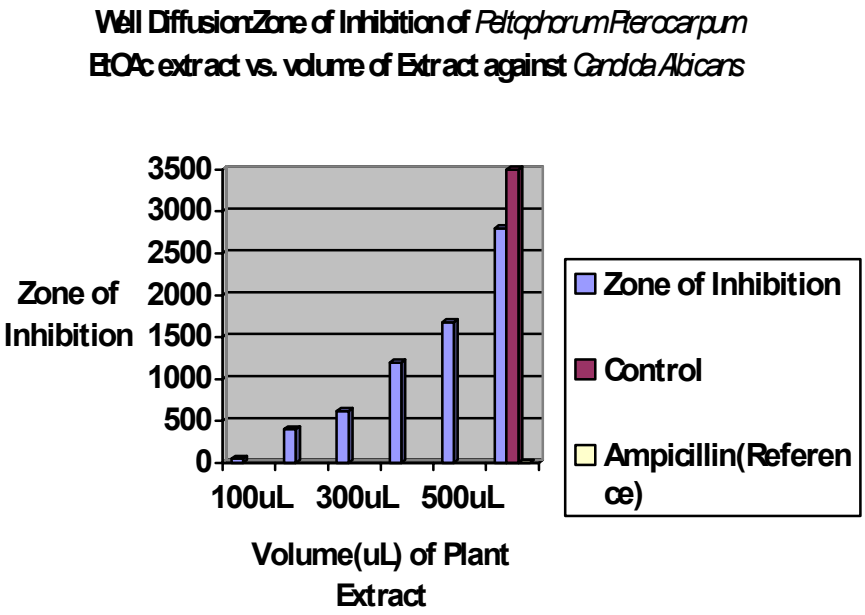
Table 6.0. showing the results of the TLC for all the extracts

Solvents	**Plants**	**No of spots visible by UV**	**R_f value= d moved by sample / d moved by solvent**
Hexane/ dichloromethane, 50:50, v/v)	*Luffa operculata*	3	0.23 0.73 1.0
Hexane/ dichloromethane, 50:50, v/v)	*Peltophorum.pterocarpum*	3	0.33 0.67 0.94
Dichloromethane/hexane, 90: 10, v/v)	*Luffa operculata*	1	0.03
Dichloromethane/hexane, 90: 10, v/v)	*Peltophorum.pterocarpum*	5	0.08 0.17 0.22 0.40 0.97
Ethylacetate/dichloromethane, 90: 10, v/v)	*Luffa operculata*	1	0.5
Ethylacetate/dichloromethane, 90: 10, v/v)	*Peltophorum.pterocarpum*	3	0.18 0.53 0.92
Ethanol/hexane, 90: 10, v/v)	*Luffa operculata*	1	0.07
Ethanol/hexane, 90: 10, v/v)	*Peltophorum.pterocarpum*	6	0.09 0.17 0.38 0.57 0.64 0.95

Graphs: Bar graphs are shown in Fig. 3.0 (a) and (b) whereas the corresponding line graphs for (b) and (c) are shown in Fig. 4.0 (a) and (b).

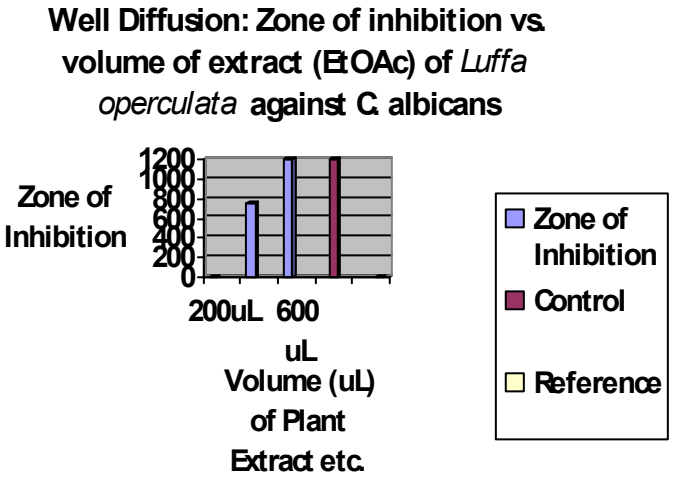


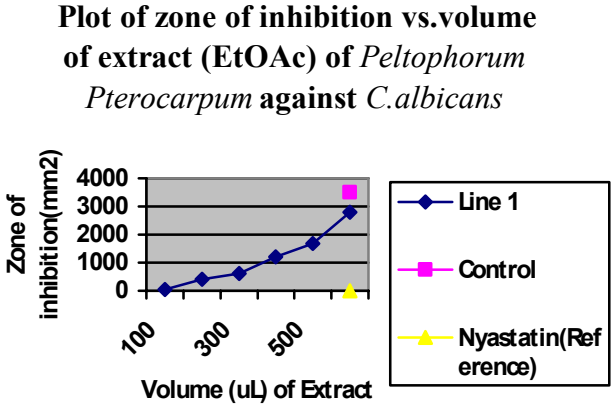
(a)



(b)

Fig. 3.0. (a) and (b)





(a)

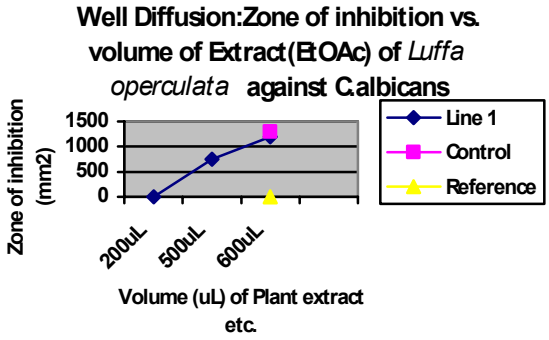


Fig. 4.0. (a) and (b).

2.3.17. Discussion:

All four methods: Stokes disc diffusion sensitivity techniques, pour plating, Well diffusion and Streak plate were successful in determining the two plants antimicrobial activities. In general antimicrobial activity follow the sequence: Ethanol extract > EtOAc extract > dichloromethane extract > hexane extract. Stokes disc diffusion indicates that the hexane and dichloromethane of *Luffa operculata* and *Peltophorum Pterocarpum* had neglible antimicrobial activity. Only a maximum zone of inhibition of 10mm^2 was observed against E.coli.However, in constrats, *Peltophorum Pterocarpum* EtOAc extract was effected against all microorganisms. For example, a maximum zone of inhibition of 23 mm^2 was noted against S.aureus. In all cases, all two plants ethanol extracts were effected against all three microorganisms studied. Significant zone of inhibition were observed. This range from 18 mm^2 to 27 mm^2. The former was obtained for Luffa operculata against *S.aureus* whereas the latter was obtained for *Peltophorum*. *Petrocarpum* against *S.aureus*.

The Well diffusion method was also used against *C. albicans*. The Well diffusion indicate that the ethyl acetate extract of *Luffa operculata* at volume of 100-400 uL respectively induce no zones of inhibition i.e microbial. Instead scattered colonies were observed.Also, both Luffa operculata and P. pterocarpum dichlormethane extract showed zero zone of inhibition at 100 uL. However, P. pterocarpum dichloromethane extract at 600uL showed zero zone of inhibition. However, Luffa operculata EtOAc extract at a volume of 500uL and 600 uL induce zone of inhibition of 750 mm^2 and 1200 mm^2 respectively. *Peltophorum*. *pterocarpum* ethylacetate extract at a volume of 500 and 600 uL induced zone of inhibition of respectively. Furthermore, as the volume of extract increased from 100uL to 600uL, zones of inhibition increased from 50 mm^2 to 280 mm^2 respectively. The ethanol extract of Luffa operculata and P.Pterocarpum showed complete zones of inhibition.Interestingly, the zone of inhibition was observed to be of the same magnitude: 75 x 45 mm^2. P.pterocarpum hexane extracts at 100-600 ul showed zero zone of inhibition. However, *Peltophorum*. *pterocarpum* dichloromethane extract against C. albicans showed zone of inhibition of 1200 mm^2 at 600 uL. The reference antibiotic: Nystatin showed zone of inhibition of 600 mm^2 to 3500 mm^2 as the volume of the extract increase from 200uL to 600 uL respectively. For the control experiment, the well with the four solvents induced scatterred colonies i.e negative inhibition

For the pour plate method, *Peltophorum*.*Pterocarpum* ethanolic extract at a volume of 600 uL was antimicrobial i.e zero colonies survived. In constrast, the ethanolic extract of Luffa operculata species at 600 uL against *E. coli* was microbial, inducing the growth of 381 colonies. Both ethyl acetate extract of *Luffa operculata* and *Peltophorum*. *Pterocarpum* at a volume of 600 uL showed 100% inhibition against *S. aureus*. Control experiments for pour plate indicate that the solvents induce growth of bacteria: S.aureus and E. coli, Table 2.0. However, the reference compound ampicillin completely inhibit the growth of microorganisms.

For the Streak plate method, both plant extracts were used against bacterial species: S.aureus and E.Coli. The EtOAc and ethanol extract of *Peltophorum*.*Pterocarpum* and *Luffa operculata* at a volume of 600 uL showed limited growth against S.aureus. and E. Coli. The control experiment indicate that all four solvents showed growth of the two bacterial species whereas the reference compound Ampicillin showed inhibition at 200-600 uL.

Fig. 3.0. (a) represents a disc diffusion plot of the zone of inhibition vs. volume (uL) of *Peltophorum*.*Pterocarpum* ethanol extract against all three microorganisms (b) represents a plot of the zone of inhibition vs. volume (uL) of *Peltophorum*.*Pterocarpum* EtOAc extract for well diffusion against *Candida albicans* whereas Fig. 4.0 (a) and (b) represent plots of the corresponding line graphs. As is evident, as the volume of plant extract increased so too is the zone of inhibition for the well diffusion method.

TLC analyses in various solvent system for each solvent type extract revealed the presence of spots that range from one to a maximum of six. Each spot is presumbably 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 5.0. For example for the dichloromethane extract, P. pterocarpum has five spots with R_f values of 0.08, 0.17, 0.22, 0.40 and 0.97 in dichloromethane/hexane, 90: 10, v/v respectively. The largest number of spots of six was seen for the ethanol extract of P. Pterocarpum. These having R_f value of 0.09, 0.17, 0.38, 0.57, 0.64 and 0.95 respectively. The solvent system for elution been ethanol: hexane, 95:5)

Conclusions:

It is clearly seen that these two plants have antimicrobial properties.However, antimicrobial activity is solvent dependent with the ethanol extract, the most potent and hexane the least. In general the order of antimicrobial activity follow the sequence: Ethanol extract > EtOAc extract > dichlormethane extract > hexane extract. However, the other solvent extracts such as EtOAc were in some cases also very effective. Thus, the ethanol extract and in the case of *Peltophorum*.*Pterocarpum*, the EtOAc extract can be used as the active constituent of an antimicrobial cream. Future work such as isolation and purification of bioactive constituents should target the ethanol and ethylacetate extract of these plants.

Acknowledgements:

This research was carried out by a final year research student A.Mohamed under my constant supervision and also that of Dr. G. Gomes, 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 also extend to Food and drugs, Kingston, Georgetown for partial use of the microbiology laboratory there at the initial microbial stage of this project. Also, 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.

Correspondence to:
Dr. R. C. Jagessar, BSc, PhD, PDF
Lecturer and Supervisor,
Department of Chemistry,
University of Guyana, Faculty of Natural Sciences,
South America
raymondjagessar@yahoo.com

References:

1. Kandil, O; Redwan, N.M; Hassan, A.B., Amer, A.M.M., El-Banna, H.A., 1994. Extracts and fractions of Thymus capitatus exhibit antimicrobial activities. *Journal of Ethnopharmacology* 44, 19-24.
2. Barre, J.T, B.F. Bowden, J.C.Coll, J.Jesus, V.E. Fuente, G.C.Janairo, C.Y. Ragasa. 1997. "A bioactive triterpene from Lantana camara, *Phytochemistry* 45: 321-324.
3. Batista, O., A. Duarte, J.Nascimento and M.F.Simones. 1994. Structure and antimicrobial activity of diterpenes from the roots of Plectranthus her *J. Nat. Products*. 57: 858-861.
4. Rojas, A., L. Hernandez, R. Pereda-Miranda, R.Mata. 1992. "Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants". *Journal of. Ethnopharmacology*. 35: 275-283.
5. Silva, O., A. Duarte, J.Cabrita, M.Pimentel, A. Diniz and E. Gomes. 1996. "Antimicrobial activity of Guinea-Bissau traditional remedies". *Journal of.Ethnopharmacology*. 50: 55-59
6. "The isolation and structural elucidation of natural products from a marine organism and a plant species, a research project in collaboration with the University of the West Indies, St. Augustine campus", R.C.Jagessar*, University of Guyana library, 2004.
7. Research abstracts "Extractions and isolation of natural products from *Momordica Charantia*", R.C.Jagessar*, A.Mohamed, 15th Annual conference of the Caribbean Academy of Sciences, Guadeloupe, 2006.
8. Research abstracts "The making of perfumes from the essential oils of local flowers", R.C.Jagessar*, W.Narine, 14 th annual conference of the Caribbean Academy of Sciences, Mount Hope, Trinidad, 2004.
9. Research abstracts "Phytochemical screening of the stems, twigs, roots and bark of *Conacarpus erectus* L", R.C.Jagessar*, M. F. Cox , 14 annual conference of the Caribbean Academy of Sciences, Mount Hope, Trinidad, 2004.
10. Research abstracts "Antimicrobial activities of selected plants", R.C.Jagessar*, N.Mohamed, 1st International conference on the status of Biological Sciences in the Caribbean and Latin America Societies, Buddy's International hotel, Providence, Guyana, 2007

11. Research abstracts "Antimicrobial activities of selected tropical plants", R.C.Jagessar*, N.Mohamed, 1st International conference on the status of Biological Sciences in the Caribbean and Latin America Societies, Buddy's International hotel, Providence, Guyana, 2007.
12. Paper presentation "Antimicrobial activities of selected tropical plants", R.C.Jagessar*, N.Mohamed, 1st International conference on the status of Biological Sciences in the Caribbean and Latin America Societies, Buddy's International hotel, Providence, Guyana, 2007.
13. Research Proposal for funding from the Royal Society of Chemistry, England and the University of Guyana,"Extraction, Isolation and structural elucidation of Natural products from the Guyana flora", R.C.Jagessar*, 2006 and 2007.
14. Chemistry in Britain, December, 2003; pg 27-29.
15. http://en.wikipedia.org/wiki/Luffa
16. http://en.wikipedia.org/wiki/*Peltophorum_pterocarpum*
17. http://en.wikipedia.org/wiki/Escheria_coli.
18. http://en.wikipedia.org/wiki/*Staphylococcus_aureus*
19. http://en.wikipedia.org/wiki/*Candida_albicans*
20. http://hcd2.bupa.co.uk/fact_sheets/html/fungal_skin_infections.html
21. Murray,P.R.,Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolke, R.H., 1995. Manual of Clinical Microbiology, 6th ed. Mosby Year Book, London.