

Determining the antibacterial effect of *Leptadenia hastata* extracts against *Klebsiella Pneumonia* and *Staphylococcus aureus*

Abstract

Introduction: As the worldwide mortality rate is high due to the pathogens and especially because of the bacteria associated dysenteriae, antibacterial effect is well known from centuries these plant *Leptadenia hastata* can be used to annihilate *klebsiella pneumonia* and *staphylococcus aureus*.

Objective: The study aims to determining the antibacterial effect of *Leptadenia hastata* Stem-bark solvent extracts of hexane, dichloromethane, ethyl acetate, chloroform, and methanol against *Klebsiella Pneumonia* and *Staphylococcus aureus*.

Methods: The crude extracts were tested by disc method on nutrient agar medium; the bacterial was sub-cultured in a 10mL of broth, each in universal glass bottle for 16 hours inside an incubator equipped with shaker at 37°C. After 16 hours' incubation, turbidity (optical density/OD) of the bacterial broth was measured by using UV mini spectrophotometer (model 1240 of Shimadzu brand), comparable to that of nutrient broth standard tube for further use. Measurement was performed at wavelength 575nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6 to 0.9. Nutrient broth was used to adjust the turbidity until the desired value was obtained.

Result: The results of antibacterial sensitivity assay of the six concentration of the different solvent extract of *Leptadenia hastata* are presented in Table 2 and Table 3. The antibacterial activities were observed in various ways with zone of inhibition diameters ranging from 0.70±0.00mm to 1.27±0.06mm for *staphylococcus aureus* and *klebsiella pneumonia* respectively among the six-concentration selected (25, 50, 100, 250, 500, and 1000 ppm).

Conclusion: It is investigated in present studies that *Leptadenia hastata* parts extracts can be utilized against the management of antibacterial diseases particularly *Klebsiella Pneumonia* and *Staphylococcus aureus*.

Keywords: *Klebsiella Pneumonia*, *Staphylococcus aureus*, *Leptadenia hastata*

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Introduction

Plant metabolites are good agent of African cultural heritage; the products are used for disease and ailment among the villagers. This information is passed down from generation to the next generation with or without little written information was available on the active, safety and effectiveness of this medicine.¹ Infectious diseases caused by bacteria have become of great concern to the health workers all over the world, the mortality rate as a result of this pathogen is higher especially the dysenteriae bacteria's even with the effort played in scientific research.² The effect was found to be enormous in developing countries as a result of lack of modern medicine and the resistance of the pathogen to some of the agents.³ The interest of the medical world and the researchers in natural products towards pathogens was so significant because of the growing rate of resistance pathogen against some of the antibiotics. Plants are custodians of millions number of bioactive molecules, thus making a rich source of different types of agents for diseases and ailments. This plant source especially the higher plants play a great role to the human health stability.⁴ Plants because of their diverse range of phytochemicals has become a greater option for a better agent for medicines.⁵ The world health organization reported that 80% and above of world population depends on the plant medicine for their primary health needs⁶ this brought about self-medication all over the world because it's cheap and affordable.⁷ Among the various medicinal plants *Leptadenia hastata* (Pers.) Decne is a climber, the plant is perennial and belongs to the family

of Asclepiaceae, it has been used by man since the beginning of our existence and have passed the test of time.⁸ The plant is one among the oldest remedies of mankind.⁹ It is found to be an important plant in ethnobotanical studies. The plant is found grown in the tropical region and used as vegetable by man and animals as well,¹⁰ it is a drought resistant plant and can tolerate high pH,¹¹ Local medical practitioner uses the plant extracts for skin disease, hypertension, catarrh, ulcer and diabetics.¹² while breeder use the leaves and stems for against parasite and placental retention (Table 1).¹³⁻¹⁵

Table 1 Traditional uses stem-bark of *Leptadenia hastata*

Plant (parts)	Traditional usage
Stem-bark	Wound healing ^{14,17,18}
	α -glucosidase inhibitory properties ¹⁹
	Diabetes mellitus management ²⁰
	Skin diseases ¹⁴

Material and methods

Sample preparation

Plant extract from different solvent were prepared from Hexane, dichloromethane, ethyl acetate, chloroform and methanol, the test for the microorganism was prepared as reported by Umaru et al.¹⁶

Three (3mg) of the extract from the various extract were dissolved separately in three (3mL) of methanol to give a stock solution of 1000mL. 25ppm, 50ppm, 100ppm, 250ppm, 500ppm, and 1000ppm were also prepared respectively.

Agar preparation

The nutrient agar was prepared by taking 14g of the powdered agar and dissolved in 500ml distilled water and heat until boiling. The preparation is put in the autoclave adjusted at 121°C as reported by Pundir & Jain.²¹ The agar after an interval of few minutes was poured in the petri plate to solidify. The petri plate was sectioned into eight each for the extracts, the negative and the positive control after which the plate was sealed using parafilm and kept at 4°C before bacterial inoculation.

Broth Preparation

The selected pathogen for this test were *Staphylococcus aureus* and *Klebsiella pneumonia*. This was obtained from Virology Laboratory, Faculty of Resource science University Malaysia Sarawak. The broth, 2.6g of the powdered broth dissolved in 200mL of distilled water and sterilized in the autoclave at 121°C. The bacterial was then subculture in a 10mL broth in a universal bottle (Vail) for 16hrs on a shaker in an incubator at 37°C.²² The optical density of the bacterial broth was ascertained using spectrophotometer at wave length 575nm. The bacterial broth will be ready for use if the optical density is within the range of 0.6-0.9.²³

Inoculation procedure

The inoculation of the selected pathogen was carried out as reported by Pundir & Jain²¹ with little modifications. The bacterial broth was then transferred into mini centrifuge about 1mL, a sterile cotton swap was used to collect the pathogen in the mini centrifuge by

dipping and streaked over the whole plate in the entire circumference and left for 6-10minutes before applying the crude extracts. Ten (10µL) each of the crude extract and the controls were pupated on to the prepared disc and then placed firmly using a sterile forceps. The controls are methanol and 30µg tetracycline. The inoculated plate which are in triplicate was left for 10minutes at room temperature and then incubated at 37°C for 24hrs. After 24hrs, the inhibition growth zone was measured in millimetre and recorded in triplicate for onward statistical calculation.

Results

The growth rate of inhibition of the crude extract from the solvents as shown in Table 2 and Table 3 indicated a significant result. From these tables it was observed that the zone of inhibition ranges from 0.47±0.15mm to 1.23±0.12mm and the degree of inhibition indicated increase in the activity of extract with increase in concentration. The highest growth inhibition rate was observed at 1000ppm in Table 2 with 1.27±0.06mm for hexane extract, followed by 1.20±0.10mm for chloroform extract, 1.17±0.06mm for dichloromethane, 1.10±0.10mm for ethyl acetate and the least was observed from methanol with 0.97±0.06mm. This indicates that hexane and chloroform extracts are more potent compared to the other extract concentrations. However, Table 3 showed same pattern of inhibition for *Klebsiella Pneumonia* within the range of 0.70±0.00mm at 25ppm, to 1.27±0.06ppm all the test concentration with hexane extract at 1000ppm, having the highest growth inhibition when compared to the other concentrations. Thus, the extract from chloroform and hexane showed more potent as an agent for *Klebsiella Pneumonia* and *Staphylococcus aureus* when compared to the control.

Statistical analysis: Data on inhibition of antibacterial growth of different pathogen were subjected to One-way (ANOVA).

Table 2 Effect of Stem Extract of *Leptadenia hastata* on *Staphylococcus aureus*

Extract	Control	25ppm	50ppm	100ppm	250ppm	500ppm	1000ppm
Hexane	2.06±0.01	0.70±0.00	0.93±0.12	1.00±0.20	1.17±0.06	1.16±0.12	1.27±0.06
Dichloromethane	2.05±0.12	0.40±0.00	0.60±0.00	0.63±0.21	0.97±0.06	1.03±0.06	1.17±0.06
Ethyl acetate	2.05±0.02	0.63±0.15	0.63±0.06	0.80±0.17	0.93±0.12	1.00±0.10	1.10±0.10
Chloroform	2.04±0.01	0.67±0.06	0.90±0.10	0.73±0.15	1.00±0.00	1.07±0.06	1.20±0.10
Methanol	2.07±0.01	0.40±0.00	0.53±0.15	0.70±0.10	0.80±0.10	0.87±0.06	0.97±0.06

Values are Mean±SD for three determinations

^aSignificantly (p<0.05) higher compared to different extract at the same concentration

^bSignificantly (p<0.05) lower compared to the control

Table 3 Effect of Stem Extract of *Leptadenia hastata* on *Klebsiella Pneumonia*

Extract	Control	25ppm	50ppm	100ppm	250ppm	500ppm	1000ppm
Hexane	2.06±0.02	0.73±0.12	0.87±0.31	1.10±0.10	1.13±0.12	1.1±0.12	1.13±0.21
Dichloromethane	2.06±0.11	0.53±0.12	0.6±0.15	0.87±0.12	0.93±0.23	1.00±0.20	1.03±0.15
Ethyl acetate	2.06±0.04	0.90±0.10	0.80±0.00	0.93±0.12	0.87±0.12	1.07±0.12	1.17±0.06
Chloroform	2.06±0.06	0.77±0.12	0.85±0.07	0.80±0.00	0.93±0.12	0.93±0.12	1.13±0.12
Methanol	2.06±0.05	0.87±0.06	0.80±0.10	0.93±0.12	1.00±0.00	1.10±0.10	0.70±0.62

Values are Mean±SD for three determinations

^aSignificantly (p<0.05) higher compared to different extract at the same concentration

^bSignificantly (p<0.05) lower compared to the control

Discussion

The effect of the *Leptadenia hastata* extract from various solvents after 24 hours, the Petri disc was observed for growth inhibition rate of five different extract, four of the extract significantly showed antimicrobial activity. The hexane, dichloromethane, ethyl acetate, chloroform showed progressive inhibition of the two bacterial with increase in concentration. But the methanol extract of the stem bark inhibition rate was less compared to the other solvent extracts. The extract indicated a significant rate of growth inhibition for *Staphylococcus aureus* as well as *Klebsiella Pneumonia* having a maximum zone inhibition (1.27 ± 0.06 mm and 1.13 ± 0.21 mm) at 1000 ppm respectively for hexane extracts, followed by dichloromethane for *Staphylococcus aureus* and ethyl acetate for *Klebsiella Pneumonia* Table 3: However methanol crude extract from both tables didn't showed significant activity against any of the two bacterial when compared to the rest of the extract as well as to the control. This indicates that active compounds present in the four solvent extract (hexane, dichloromethane, ethyl acetate, and chloroform) has more potential than the methanol extracts thus, the extracts general inhibited growth of gram positive bacteria *Staphylococcus aureus* and *Klebsiella Pneumonia*. Since four different extracts showed antimicrobial activity, it can be predicted that variety of antimicrobial compounds are present in this plant.

Conclusion

From history it was reported that plant extract has been a great agent to cartel the menace of disease and ailment. Since then different crude extracts were tested by great scientist to unveil the phytochemical composition of the plant bioactive composition. This present solvent extract from hexane, dichloromethane, ethyl acetate, chloroform and methanol, from this present research with its bacterial activity against this selected compound could not be possible if not for the presence of some metabolites responsible for the growth inhibition, thus this plant should be exploited to ascertain this bioactive towards developing a bacterial agent as an alternative to for the drug resistance microorganism.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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