

Phytochemical screening and *in-vitro* activities of dichloromethane leaf extract of *Leptadenia hastata* (pers.) decne against pathogens

Abstract

Phytochemical screening and antibacterial activities of Dichloromethane leaves extract of *Leptadenia hastata* was investigated to evaluate the active component of the plant. Phytochemical analysis of the aerial parts showed the presence of bioactive secondary metabolites; alkaloids, steroids, terpenoids, saponins, flavonoids, Carbohydrate, Glycoside are present while tannins, and saponin were absent. The *in vitro* antimicrobial activity against four bacterial strains *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumonia*, was also assessed. The results; Alkaloids, steroids, terpenoids, saponins, flavonoids, Carbohydrate, Glycoside were the main compounds of the Dichloromethane extract from *Leptadenia hastata*. This extract has significant activity, they exhibited an antimicrobial property against bacterial strains. *Escherichia coli* and *Staphylococcus aureus* were the most sensitive strains with inhibitory concentration values of 1.23 ± 0.06 mm and 1.33 ± 0.06 mm at 1000ppm respectively. In conclusion; *Leptadenia hastata* Dichloromethane Leaves extract was found to be selectively antimicrobial as a result of the pharmacologically active components, which support its Ethno-botanical claims of traditional Medical practitioners in the treatment of various ailments.

Keywords: dichloromethane, phytochemical, *Leptadenia hastata*, metabolites, antibacterial

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Introduction

Natural products, especially those of vegetable origin, have always been an important source of therapeutic agents. About 25%–30% of drugs available for the treatment of diseases are derived from natural products.¹ Many studies carried out in the field of ethno pharmacology show that plants used in traditional medicine and which have been tested are often efficient plants in pharmacological models. Thereby, the medicinal plants and natural extracts have been considered as alternative therapy against various diseases.² *Leptadenia hastata*, a plant that is widely distributed throughout the world. This plant species is used in various applications especially for medicinal purposes. They are significant element of the world cultural heritage; they resort for treating health problems. This knowledge 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.³ It is a perennial plant of the family of Asclepiadaceae, the leaves are used as sausages among African community, it has flowers that are racemes, fruits with follicles. The size of the leaves is between 9–10cm, while the colour of the flower are creamy in nature.⁴

The plant *Leptadenia hastata* constituent different names depending on location and environment of collection. In north-east Nigeria where this plant was collected is called yadiya, it was also called yadiya in Niger. Some African countries like Chad call it Hagalhadjar, Ethiopian people call it Hayla among kusume ethnic.⁵ The plant *Leptadenia hastata* can resist drought and accommodate high pH and high exchangeable sodium and potassium within the environment.^{6,7} Villagers in their community grow this plant as a source of food and vegetable.⁸ Traditional medical practitioner treats their patient from hypertension with the leaves of the plant. They also use it for skin infection as well as controlling diarrhoea among children. Animals with placental retention at birth were administered

the plant to arrest the situation.^{9–11} The objective of this study was to perform the preliminary phytochemical screening and to determine the antimicrobial activities of Dichloromethane Leaves extract of *Leptadenia hastata*.

Materials and methods

Sample collection extraction

The stem-bark of *Leptadenia hastata* were collected in an open field non-cultivated area, washed with distilled water and allowed to dry in an open and aerated room for seven days.

Preparation of sample

Stem-bark of the plant were reduced into small pieces in a mortar and pestle and then powdered with laboratory blender. 150mg of the sample was dissolved in Dichloromethane three times and allowed to stand with an interval of 24hrs each, then filtrate was combined and evaporated. The crude extract was collected and stored until use.

Reconstitution of the extracts

2g of the extract was weighed and dissolved in 20ml of 50% Dimethyl sulfoxide (DMSO) to make a stock concentration of 100mg/ml from which the various concentrations used were calculated.

Selected bacteria

The selected bacteria were obtained from University Malaysia Sarawak, Virology Laboratory. Faculty of resource science; *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia* and *Escherichia coli*

Standard

The standard control (Tetracycline) for the test was obtained from

Oxoid, all other reagent and solvent were of standard laboratory and used based on the manufactures instructions.

Phytochemical analysis

Screening test for;

Sterols: To test for the presence of sterols in the dichloromethane extract, Salkowski and Liebermann-Burchard test was adopted. From the extract of *Leptadenia hastata*, the solvent chloroform and sulphuric acid (H_2SO_4), 2ml each were collected add together and shaken. Two layers of red and greenish-yellow indicated the presence of sterols, this shows that Salkowski test is positive. However, the Liebermann-Burchard test, the solvent used was chloroform, acetic anhydride and sulphuric acid. The extract (2ml) was collected and mixed with 2ml each of the solvent, gently added two drops of sulphuric acid from the side of the test tube. A red blue colour start to appear and finally turn to green thus indicated that sterol is present in the extract.

Terpenoids: This was determined by Salkowski test which gave a positive result thus confirms the presence of Terpenoids. The extract was also mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) is carefully added by through the side to form a layer. A reddish brown colouration of the interface was formed which confirm the presence of terpenoids.

Alkaloids: Mayer's reagent: Mercuric chloride (1.36g) dissolved in water (60ml) and Potassium iodide (5g) in distilled water (20ml) were combined and adjusted 100ml distilled water. This was used to test for the presence of Alkaloid in the dichloromethane extract. The extract was warmed with 2% sulphuric acid for two minutes and added Mayer's solution in a drop a creamy-white was observed indicate alkaloid present in the sample. Wagner's reagent: Iodine (1.27g) and Potassium iodine (2g) in 5ml of distilled water, adjusted in 100ml of distilled water was used to test as well for the presence of Alkaloid in the extract. The extract was warmed with 2% sulphuric acid for about two minutes. The mixture was filtered and few drops of the prepared reagent was added, a reddish-brown was observed confirm the presence of alkaloid in the extract.

Carbohydrates: Carbohydrate test was determined using Molisch and Fehling reagent. The extract was treated with Molisch reagent a purple to violet colour ring appeared at the junction in the test tube. To confirm and the qualify the presence of Carbohydrate and Molisch test, the extract was again treated with Fehling solution mixed with few drops of the extract and boiled. A colour of brick red precipitate indicate the presence of carbohydrate.

Flavonoids: To test for flavonoid; The extract in small amount was heated with 10ml of ethyl acetate for 3minutes in a boiling water and filtered, the filtered is kept for the test. Ammonium and Aluminium chloride were used to confirm the presence of the flavonoid in the extract. One mil of Ammonia (1%) with the filtered was shaken a separate layer of yellow colouration was observed which indicated the presence of flavonoid. To confirm this test, the filtered was then shaken with one mil of aluminium chloride a light yellow colour was observed. The solution was further added dilute NaOH and HCl the yellow colour disappeared thus confirm the presence of flavonoid.

Tannins: To test for Tannin in the extract. The extract was subjected to ferric chloride and Lead sub-acetate test. 5ml of methanol (45%) mixed with small amount of the extract from dichloromethane, added drop wise was the ferric chloride and a transient greenish to black colour which was supposed to be the colour for presence of Tannin was absent during the test. However, to ascertain the absence of tannin

the extract filtrate (1ml) was also mixed by adding three drops of Lead sub-acetate. This creamy gelatinous precipitation was absent thus confirming tannin is not present in the extract.

Phenols: The presence of phenol in the extract was confirmed to be present by testing using Ellagic acid test. The extract mixture turn muddy with Niger brown precipitation was observed.

Glycosides: The Keller-Kiliani Test and Concentrate H_2SO_4 Test was used to confirmed the presence of Glycosides in the dichloromethane extract. A Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green, indicating the presence of glycosides. In the extract. The confirmation was done when 5ml of the extract and 2ml of the Glacial acetic acid are mixed and drop wise added $FeCl_3$ (5%) with concentrated sulphuric acid added a brown ring appears in the mixture thus indicating that the extract contain glycoside.

Saponin: The extract obtained was obtained subjected by drop to a blood placed on glass slide there was no presence of haemolysis observed. To confirm the absence of the compound in the extract foam test was conducted by subjecting the extract to 20ml of distilled water and mixed thoroughly, the mixture did not show any sign of foam thus indicate the absence of saponin.

Preparation of test samples for antibacterial studies

The crude extract of *Leptadenia hastata* was used in the antibacterial studies to ascertain the activity of the extract. The plant extract was tested using agar disc method as reported by Isaac et al.,¹² 5mg of the extract was collect and dissolved homogeneity in 5ml of methanol (1000 μ g/mL stock) as well as lower concentration of 25, 50, 100, 250, 500 and 1000ppm were prepared as the test concentration.

Preparation of agar plates

Nutrient agar was prepared according to manufacturer's instruction with 14 g of dried agar dissolved in 500 mL distilled water. The agar solution was heated until boiling followed by sterilization in autoclave at 121 °C. The agar solution was then poured into a sterile petri plate and allowed to cool down. The culture plate was divided into eight sections. The six sections were for each test samples namely the 25ppm, 50ppm, 100ppm, 250ppm, 500pp and 1000ppm samples, tetracycline 30 μ g (positive control) and methanol (negative control). The plate was sealed using parafilm and keep chilled at 4 °C upon bacteria inoculation.¹²

Preparation of bacteria broth

The pathogen to be used for this study are *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumonia* were obtained from the stock culture provided by Virology Laboratory, Faculty of Resource Science, Universiti Malaysia Sarawak. The nutrient broth was prepared according to manufacturer's instruction, with 2.6 g of the dried broth dissolved in 200 mL distilled water followed by sterilization in autoclave at 121 °C. The pathogen was sub-cultured in a 10 mL of broth, each in universal glass vail bottle for 16 hours inside an incubator equipped with shaker at 37 °C.¹³ The optical density of the bacterial broth was measured using UV spectrophotometer and compared with the nutrient broth standard. The optical density was measured at 575nm. the pathogen was used at density between 0.6 to 0.9.¹⁴

Plate inoculation

Inoculation of the bacteria was carried out in a biohazard cabinet,

about one milliliter of the ready bacterial broth were transferred into mini centrifuge tubes. The bacteria were applied and streaked over the whole agar performed in all four direction of the agar plate and allow for 4-8min before introducing the plant extract. A volume of 10 μ L of the test samples of concentration 10, 25, 50, 100, 250, 500 and 1000 μ g/mL, negative and positive control were each pipetted onto the discs and placed onto the agar plate by using sterile forceps and gently pressed to ensure contact. The cultured disc was left for 10min to allow diffusion of the test agents. This procedure was repeated three times. The cultured plate was then transferred to incubator regulated at 37°C for 24hrs. After which the inhibition rate was measured in mm and recorded.

Statistical analysis

The statistical analysis values were expressed in Mean \pm SD for three determinations using the software-SPSS one-way ANOVA.

Table 1 Biological activities of dichloromethane leaves extract of *Leptadenia hastata*

Values are Mean \pm 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

Bacteria	Control	25ppm	50ppm	100ppm	250ppm	500ppm	1000ppm
Salmonella typhi	2.05 \pm 0.06	0.55 \pm 0.07b	0.63 \pm 0.15b	0.67 \pm 0.15b	0.70 \pm 0.20b	0.77 \pm 0.12b	0.93 \pm 0.06b
Escherichia coli	2.02 \pm 0.01	0.47 \pm 0.06	0.50 \pm 0.00	0.70 \pm 0.10	0.93 \pm 0.06	1.03 \pm 0.06	1.23 \pm 0.06
Staphylococcus aureus	2.05 \pm 0.01	0.60 \pm 0.00	0.90 \pm 0.10	1.00 \pm 0.10	1.10 \pm 0.10	1.23 \pm 0.06	1.33 \pm 0.06
Klebsiella pneumonia	2.05 \pm 0.02	0.57 \pm 0.06	0.70 \pm 0.00	0.77 \pm 0.06	0.97 \pm 0.06	1.00 \pm 0.10	1.13 \pm 0.15

Table 2 Phytochemical screening of dichloromethane leaves extract of *Leptadenia hastata*

Phytochemicals	Alkaloids	Steroids	Terpenoids	Flavonoids	Carbohydrate	Tannins	Saponins	Glycoside	Phenol
Present	+	+			++	-	-		+
Strongly Present	++			++				++	
Absent									

Discussion

The antibacterial value of various concentration of Dichloromethane crude extract of the plant *Leptadenia hastata* was tested against the selected pathogenic bacterial (Table 1) which showed varied level of inhibition. All the concentration was found active against gram positive as well as in Gram negative bacteria. The highest activity was shown at concentration 500ppm and 1000ppm (1.03 \pm 0.06mm, 1.23 \pm 0.06mm and 1.23 \pm 0.06mm, 1.33 \pm 0.06mm) against Escherichia coli and staphylococcus aureus. Significant activity against Escherichia coli and staphylococcus aureus were also shown by this extract throughout the concentrations 25ppm-1000ppm when compared with the other pathogens as well as the control. The Dichloromethane extract contains a greater proportion of the phytoconstituents. Different bioactive components were obtained from the extract during the evaluated for their phytoconstituents. Alkaloids, Steroids, Terpenoids, Flavonoids, Carbohydrate, Tannins, Saponins, Glycoside and Phenol. However, alkaloids, flavonoid, carbohydrate and Glycosides are the major constituents present in the fraction of Dichloromethane followed by steroid, terpenoid and Phenol. The presence of this phytochemical has given the plant parts its potential activity against the pathogen and other activities as claimed by the traditional healers.

Conclusion

The Dichloromethane extract of *Leptadenia hastata* exhibited

Results and discussion

Results

The treatment of *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* with 25ppm, 50ppm, 100ppm, 250ppm, 500ppm, and 1000ppm showed an increase in the inhibition rate with increase in concentration. The level of selected pathogen inhibition rate at 500 and 1000ppm recorded highly significant increase ($P<0.05$) in the bacterial culture of all the pathogen as shown in Table 1. The obtained test report from the phytochemical result are presented in Table 2. This preliminary phytochemical screening revealed the presence or absence of these secondary metabolites. The examination of this table showed the presence Alkaloids, Steroids, Terpenoids, Flavonoids, Carbohydrate, Glycoside and absence of Tannins, Saponins, in the extracts with varying intensities.

an antibacterial effect with a rate of inhibition within the range of 0.47 \pm 0.06mm to 1.33 \pm 0.06mm against all the pathogen used. This showed that the plant is a good agent. This was made possible because of the presence of the identified phytochemical in the plant extract such as Alkaloids, Steroids, Terpenoids, Flavonoids, Carbohydrate, Tannins, Saponins, Glycoside. The extract displayed antimicrobial property against bacterial strains this could be attributed to the content of bioactive compounds.

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Conflict of interest

The authors declare that there is no conflict of interest.

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