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Phytochemical Screening, Acute Toxicity and Antibacterial Activity of Methanolic Stem Bark Extract of *Hibiscus Rosa-Sinensis*

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Abstract

This study was conducted to determine the phytochemical constituents, acute toxicity and antibacterial activity of methanolic stem bark extract of. *Hibiscus rosa-sinensis.* The stem bark was collected, air dried, grounded and extracted using maceration method with methanol. The methanol extract of the stem bark was used for phytochemical screening, acute toxicity studies and anti-bacterial activity. The phytochemical screening of this extract revealed the presence of alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, cardiac glycosides, carbohydrates except anthraquinones which was not present. The acute toxicity studies of the extract indicated that: *Hibiscus rosa-sinensis* had $LD_{50\ value\ of}$ above 5000 mg/kg. The result of antibacterial activity showed that the stem bark extracts was found to have a significant effect ($P<0.05$) against the growth of *Staphylococcus aureus and Escherichia coli.* The Methanolic stem bark extract had MIC of 50.0 mg/ml and 200 mg/ml and MBC of 100 mg/ml and >200 mg/ml, against *S. aureus* and *E. coli* respectively. The result obtained is an indication that the stem bark of the plant can be used for treatment of bacterial ailment as claimed by traditional practitioners in Giambi Local Government Area of Kaduna State, Nigeria. Further research such as isolation of the active component should be carried out to validate the use of this plant in the traditional medicine as antibacterial agents.

Keywords: Acute toxicity, Antibacterial activities, Hibiscus rosa-sinensis, Methanolic extract, Phytochemical screening.

Introduction

Traditional medicine is the most ancient art of medical practice on which many of rural population of developing countries relied for their health care, due to economic accessibility and the belief that natural products are more acceptable to the body and as such have fewer side effects than synthetic drugs [1].

A medicinal plant is one in which one or more of its organs comprises of materials that can be used for treatment purposes or that are used for reasoning of beneficial medicines [12].

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Hibiscus rosa-sinensis: Are tropical Hibiscus species and the flowering plant of Hibiscus family of Malakai, the plant species was named by Carolos Linnaeus in 1753. The Latin term "*rosa-sinensis"* literally means 'rose of china'. This plant is bushy and evergreen shrubs that is growing 2.55m range tall and then 1.5-3m range wide, it has a shiny leaf and a solitary, in the summer and the autumn. The back is pale grey to whitish. The stem is upright and cylindrical in branches. The Leaf has oval-shape, it has margin and acute tip and is serrated. The plant is cultivated in China for a very long time, it was originated from South China.

Hirose-sinensis leaves were used for medication of diarrhoea, dysentery and to encourage draining of the abscesses and analgesic in folk medicine of Japan and Mexico [9]. The plant flowers were used for diabetes, epilepsy and leprosy [11].

Many natural products isolated from plants serve as "template" or lead molecules for the design and generation of completely recent drugs. The prospect of plants as source of recent medicine is not yet available. Out of all the different species of the plants in the world, very few have been examined phytochemically and fraction screened for pharmacological [1].

The use of plants to cure several kinds of human diseases has a long history. Various parts of plants such as leaf, stem bark, root, etc. are being used to prevent, allay symptoms or revert abnormalities back to normal. Since the practice of "herbal remedies" does not adhere strictly to facts using scientific approaches, orthodox medicine sees "herbal medicines" as an alternative medicine. However, most of the pharmaceutical products currently dispensed by physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis and quinine. Modern medicine today utilizes active compounds isolated from higher plants, and about 80% of these active ingredients indicate a positive correlation between their modern therapeutic use and the traditional uses [6].

MATERIALS AND METHODS

Collection and Identification

The collection of *Hibiscus rosa-sinensis* was carried out at Giambi Local Government Area (Lat: 10^0 48′ 21.71 ″N, long: 7^0 42′ 51.95 ″E) of Kaduna State. It was identified and documented by a taxonomist in the herbarium section of the Department of Biological Sciences Kaduna State University. Voucher specimen number of 9713 was assigned to it and the specimen was deposited at the Herbarium unit for future reference.

Preparation of Materials

The collected samples of *Hibiscus rosa-sinensis* were washed in running water and air dried under shade and then pulverized by using pestle and mortar [7]. The powder of stem bark materials of the plant was kept in clean air tight containers and they were subsequently macerated with methanol.

Extraction of Materials

The whole powdered of stem bark material (120 g) of the plant was macerated with methanol for three days and the extract was filtered. The filtrate of the stem bark material was evaporated to dry on the water

bath and the yield of the stem bark was calculated in percentage. The extract of the plant was used for acute toxicity and biological studies. This method was adopted by [3].

Phytochemical Screening

Phytochemical analysis of the stem bark extract was carried out with slight modifications by [13]. By this analysis, the presence or absence of several phytochemicals compounds investigated in the stem bark extract ware alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids.

Acute Toxicity Studies

The method described by Lorke, (1983) was adopted and used for the methanolic stem bark extract. A pilot study was carried out with methanolic extract of the plant on albino rats, by using nine rats of three groups (three rats per group). Animal in each group was administered with 10, 100 and 1000 mg respectively. From the result of the pilot studies, suitable dose intervals were chosen and used to conduct the final toxicity study. Four rats of four groups were used for final toxicity of the methanolic extract of stem bark of *Hirose-sinensis*.

Anti-Bacterial Activities

Two strains of Bacterial namely: *E. coli* and *S. aureus* were obtained from the Department of Microbiology, Ahmadu Bello University Zaria. The two bacteria strains were isolated, cultured and used for the research. A modified method described by [10] and [2] were adopted. Standard inoculant of the bacterial isolates was streaked on sterilized Mueller Hinton agar plates with the aid of a sterile swab stick. Five wells were punched in each inoculated agar plate with a sterile corn borer (8 mm). The wells were properly label according to the concentrations of different extract prepared, which were 200, 100, 50, 25, and 12.5 mg/ml (serial dilution). Each well was filled up with 0.2 ml of the methanolic stem bark extract.

The inoculated plates with the different methanol stem bark extract e.g. Agar well diffusion method was placed on the bench to stay for about one hour, to enable the extract to diffuse on the agar. The plates were then incubated at 37℃ for 24 hours. Plates were observed for any evidence of inhibition at the end of incubation period, which appear as a clear zone of growth inhibition that was completely around the wells. The diameter of zone of inhibition for each concentration of the stem methanolic extract were measured using a transparent ruler calibrated in millimetre and the results obtained were recorded [10].

Data Analysis

Analysis of Variance (ANOVA) was used to evaluate significant differences among the concentrations of the extract against the isolates and control. Least significant difference (LSD) was also used to separate the means where significant difference occurs among the concentration of the plant extract and control P-Value 0.05 was considered to be significant.

RESULTS

Phytochemical Screening

The bioactive ingredients such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids were detected in the plant parts tested, except anthraquinone which was not detected.

Table 1: Phytochemical constituents of crude methanolic extract of stem bark of Hibiscus *rosa-sinensis*

Test	Constituent	Observation	Inference
Shinoda Test	Alkaloids	Formation of reddish precipitate	+
Shinoda Test	Flavonoids	Formation of intense yellow colour, which became colourless on addition of dilute hydrochloric acid	+
Froth's	Saponins	Persistent frothing on worming	+
Lieberman Burchard's	Steroids	Formation of dark pink or red colour precipitate	+
Braymer's	Tannins	Formation of blue or greenish colour Solution	+
Liebermann-Burchard's	Terpenoids	Appearance of a reddish-brown colouration at the interface precipitate	+

Keller-Killian's	Cardiac glycosides	Deep red coloration which fades too brownish yellow	+
Fehling's	Carbohydrates	Formation of reddish-brown precipitate	+
Borntrager's	Anthraquinones	Absence of bright pink colour in the aqueous layers	-

Key: (+) = present, (-) = absent.

Acute Toxicity of Crude Methanolic Extract of Stem Bark of *H. rosa-sinensis*

The acute toxicity study of the methanolic extract of *H. rosa-sinensis* revealed that it has LD_{50} value of above 5000 mg/kg.

Table 2: Acute toxicity of crude methanolic extract of stem bark of Hibiscus *rosa-sinensis* in Albino rats per oral

Phase of toxicity test	**Dose (mg/kg)**	**Observation**
First Phase	10	0/3
	100	0/3
	1000	0/3
Second Phase	1200	0/3
	1600	0/3
	2900	0/3
	5000	0/3

LD_{50} value = > 5000 mg/kg.

Key: 0/3 = All the Albino rats survive in both first and second phase.

Antibacterial Activity

The antibacterial activity of crude extracts of stem bark of H. *rosa-sinensis* against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) are presented in Table 3. Crude methanolic extract of stem bark of *H. rosa-sinensis* showed no antibacterial activity at concentration of 12.5 mg/ml, 25 mg/ml, 50mg/ml, 100 mg/ml and 200 mg/ml against the two tested bacterial strains.

However, Ciprofloxacin treated (control) group was used against the two bacterial strains tested where it has zone of inhibition of 35.0 mm and 33.0 mm against *S. aureus* and *E. coli* respectively. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against *Staphylococcus aureus* were presented below. The MIC and MBC of the extracts against *S. aureus* had shown that *H. rosa-sinensis* had MIC of 50 mg/ml and MBC of 100 mg/ml, While the (MIC) and (MBC) against *E. coli*, showed, that *H. rosa-sinensis* had MIC of 200 mg/ml and MBC above 200 mg/ml.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Stem Bark Extract of *H. rosa-sinensis* Against *S. aureus* And *E. coli*

	S. aureus	*E. coli*
MIC	50.00 mg/ml	200 mg/ml
MBC	100 mg/ml	>200 mg/ml

Table 4: One way ANOVA of the data on the anti-bacterial activities treated with *H. rosa-sinensis* stem bark extracts against *Staphylococcus aureus.*

Source of variance		**Mean sum of square**
	DF	*H. rosa-sinensis*
Between group	5	389.251*
Within group	6	0.029
Total	11	

*Significant at $p < 0.05$

Table *5:* One way ANOVA of the data on the anti-bacterial activities treated with *H. rosa-sinensis* stem bark extract against *Escherichia coli.*

Source of variance	**Mean sum of square**

	DF	*H. rosa-sinensis*
Between group	5	314.594*
Within *group*	6	0.367
Total	11	

* Significant at $p < 0.05$

DISCUSSION

The local heritage with global importance is medicinal plants. Crude drugs contain natural drug of plants, animal or minerals origin which have not undergone any treatment other than collection and drying or are drugs that has not been advanced or improved by any physical or chemical treatment. Traditional drugs are produced from plants and pharmaceutical preparation for man and other animals. According to a survey shows that the United Nations Commission for Trade and Development (UNCTAD), more than 33 % of modern drugs and medicinal products are derived from plants. The chemical substances present in plants are regarded as chemical constituents. Not all the chemical compounds produced by plants have the same activities but the so called active constituents frequently used as crude drugs are alkaloid, glycosides, flavonoids coumarins, tannins, anthraquinone, steroidal saponins and volatile oil [4].

The most extensive areas of natural product of research today are the extraction of bioactive agents from plants. A reason for screening plants for bioactive agent is to use the extracted compound for pharmaceutical purposes [8].

The bioactive ingredients such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids were detected in *H. rosa-sinensis* plant tested. Except anthraquinone which was not present.

The extract of *H. rosa-sinensis had* an LD_{50} value of above 5000 mg/kg obtained for the crude methanolic extract of stem bark of H. *rosa-sinensis.* Extracts with high LD_{50} value when used as drugs may likely possess low toxic effect. This finding was supported by (Lorke,1983) which indicated that less value obtained in the acute toxicity study is an indication that the crude methanolic extract is not save and when used as drugs may have high toxic effect and the higher LD_{50} value obtained is an indication of the plant extract when used as drugs to have low toxic effect.

The antibacterial activity of the crude methanolic extract of stem bark of *H. rosa-sinensis* revealed no antibacterial activity at concentration of 12.5mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml, against the two tested bacterial strains.

CONCLUSION

The present studies had established that the methanolic extracts of stem bark of *H. rosa-sinensis* plant species had significant ($p<0.05$).

The phytochemical analysis of the extracts revealed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and the terpenoids. except anthraquinones which was absent.

The acute toxicity study of H. *rosa-sinensis* extract helped to provide the safety dose to be used. It also Extracts with high LD_{50} value when used as drugs may likely possess low toxic effect.

The Methanolic extract of the plants against *staphylococcus aureus* revealed that *H. rosa-sinensis* had MIC and MBC of 50.0 and 100. While the methanolic extract of the plants against *Escherichia coli* showed that, *H. rosa-sinensis* had MIC and MBC of 200 and >200.

References

[1] Aidil, P., Rosa, V., Blame, F., Tomas, A. and Salvador, C. (2001). Paraguayan Plants used in Traditional Medicine. *Journal of Ethnopharmacology*.16: 93-98.

[2] Akter, A., Neela, F.A., Khan, M.S., Islam, M. S. and Alarm, M. (2010). Screening of Ethanol, Petroleum ether and Chloroform Extracts of medicinal plants *Laws onia interims L.* and *Mimosa pudic L*. for antibacterial activity. *Journal of Ethnomedicine* 73 (3),388-392.

[3] Anas, K., Durga, J., Ashutosh, M., Dinesh, C., Indranil, K.Y. and Jain, D. A. (2010). *International Journal of Chemical Technology Research*. 2:744-751.

[4] Antony, G., Rinku, D., Sumana, S., Roshana, M., Sanghamitra, M. and Shanik, B. (2010). Herbs and herbal constituents active against snake bite. *Indian*

[5] *Journal of Experimental Biology.* 48:865 – 878.

[6] Collins and Minta (2000). *Medieval Herbals: The Illustrative Traditions. University of Toronto Press. p. 32. ISBN 978-0-8020-8313-5.*

[7] Kumara, D., Khan, M.E., Punnai, A.M. and Hassan, M. (2009). "Phytochemical and Antimicrobial Screening of *Ficus* against Human/Animal pathogens". *Pacific Journal of Science and Technology*, 10 9(1):382-386.

[8] Mors, W.B., Nascimento, M.C., Pereira, B. and Pereira, N.A., (2000). Plant natural product active against snake bite, the molecular approach. *Journal of Phytochemistry.* 55:627-642.

[9] Nath, P. and Yadav, A.K. (2015). Acute and sub-acute oral toxicity of the methanolic extract from leaves of *Hibiscus rosa-sinensis in mice. Journal of assessment Interculture Ethnopharmacology*. 4(1), 70-73.

[10] Nweze, E. I., Okafor, J. K. and Njoku, O. (2004). Antimicrobial activities of Methanolic extracts of *Trema Guinness Schumm and* Marinda *lucida* Benth used in Nigeria Herbal Medical Practice. *Journal of Biological Research and Biotechnology* 2(1), 36–30.

[11] Peka war, S.S., Kalamkari, T.M. and Jadhav, A.C. (2013). *Hibiscus rosa-sinensis:* A review on ornamental plant. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2(6),4719-4727.

[12] Sophomore, A (1982). Medicinal plant and Traditional Medicinal in Africa Spectrum Book Limited.

[13] Trease, G. and Evans, W. (1989), Pharmacognosy London: Bailliere (11th ed., pp. 45-50).