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Haemagglutination and Antibacterial activities of *Strobilanthes urticifolia* Wall. ex Kuntze

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Abstract

According to literature review there is little or no such reference is available on proposed study. Moreover, the indigenous knowledge on medicinal properties of this plant is not documented so in the current studies, antibacterial activity of crude methanolic extract and its fractions of *Strobilanthes urticifolia* Wall. ex Kuntze was assessed against seven pathogenic bacterial strains using agar well diffusion method and minimum inhibitory concentration (MIC) was noticed after recording the zone of inhibitions. Crude methanolic extract and its fractions were also investigated for haemagglutination activity. Moderate, weak and no haemagglutination activity was observed against human red blood cells (RBCs) of blood groups by crude methanolic extract and fractions at various dilutions. Of the seven strains studied the widest zone of inhibition (75%) was showed by n-hexane fraction against *B. cereus* while crude methanolic extract, $CHCl_3$, EtOAc and aqueous fractions were also noticed as equally active against all pathogens. The result got from the study of antibacterial activity clearly suggest that the crude methanolic extract and its fractions of *S. urticifolia* Wall. ex Kuntze possess potent antibacterial activity and consequently can leads to antibiotics production.

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Introduction

According to record of archaeologists, the therapeutic use of plants certainly goes back to the Paleolithic Age (Stone Age) (Sumner, J. (2000). They contain therapeutic compounds that can be used to treat chronic as well as infectious diseases (Duraipandiyan *et al.*,2006). Infectious diseases are one of the major problems of the world and almost 57 million people die because of these diseases worldwide every year (Fauci *et al.*, 2005).

S. *urticifolia* Wall. ex Kuntze commonly known as Blue Nettle belonging to family Acanthaceae and genus *Strobilanthes,* is an erect shrub up to 1.2 m, mainly distributed in Afghanistan, Pakistan, India and Nepal at altitude of2000-3500m. *Strobilanthes* species are used as food plants by the larvae of some Lepidoptera species and locally it is used as fodder for animals. Due to less and no research reference, this plant could be a source of biologically active components against microorganisms.

Natural products have been an essential part of the ancient traditional medicine (TM) systems, e.g. Chinese, Ayurvedic and Egyptian (Sarker *et al.*, 2007). It has been shown that worldwide ~25% of the medications are derived from natural sources (Mukherjee *et al.*,2006). About 3.4 billion people in the developing world depend on plant-based traditional medicines. According to the World Health Organization (WHO), a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests (Abo *et al.*, 1991; Liu, 2004; Nweze *et al*, 2004).

The medicinal value of plants is related to their phytochemical component content and secondary metabolites, including: phenolic compounds, flavonoids, alkaloids, tannins, terpenoids, saponins and other stress gene response products (Mohammedi and Atik , 2011; Ghasemzadeh *et al.*, 2011). Phytochemicals have been isolated and characterized from fruits, vegetables, spices, beverages as well as many other sources (Doughari and Obidah,2008; Doughari *et al.*, 2009). Therefore, the search on new drugs must be persistent and natural products from plants, microorganisms, fungi and animals can be the source of innovative and powerful curative agents for newer, safer and affordable medicines (Lindequist *et al.*, 2005). Clinical trials are carefully planned to safeguard the health of the participants as well as answer specific research questions by evaluating for both immediate and long-term side effects and their outcomes are measured before the drug is widely applied to patients (Sasidharan *et al.*, 2011).

The present study aimed to check the pharmacological features of *Strobilanthes urticifolia* Wall. ex Kuntze like antibacterial and haemagglutination activities. Antibacterial activity was tested against different bacterial strains of Escherichia coli, Pseudomonas aeruginosa, *Staphylococcus aureus,* Salmonella typhi, *Micrococcus luteus*, *Streptococcus pneumoniae* and *Bacillus cereus.*

Materials and methods

Plant material

The plant *Strobilanthes urticifolia* Wall. ex Kuntze (whole) was collected of the year 2016 from Swat, the Northern region of Pakistan. The plant was identified in Department of Botany, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

Extraction

The plant material was kept in shade for drying. The shade dried plant materials were cut into small pieces and grinded to fine powder by using electric grinder.

The powdered material (12 kg) was soaked in analytical grade methanol for 15 days, twice, at room temperature, with occasional shaking. Each time the material was filtered and the filtrate was concentrated, at 40°C, under vacuum, by rotary evaporator till a greenish crude extract was obtained.

Fractionation

The crude methanolic extract (880 g) was suspended in distilled water (500 ml) and partitioned with n-hexane (3 × 500 ml), $CHCl_3$ (3 × 500 ml), EtOAc (3 × 500 ml) to yield n-hexane (250 g), $CHCl_3$ (220 g), EtOAc (85 g) and aqueous (325 g) fractions. About 50 g of crude methanolic extract was reserved for pharmacological/biological screenings.

Antibacterial activity

Percent zone of inhibition

The crude methanolic extract and various fractions of *Strobilanthes urticifolia* Wall.ex Kuntze were screened for possible antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, *Staphylococcus aureus*, Salmonella typhi, *Micrococcus luteus, Streptococcus pneumoniae* and Bacillus cereus employing agar well diffusion method (Bashir *et al*., 2009). On the sterile nutrient agar plates, 18 h old culture from nutrient broth was transferred and spread on it to make bacterial lawn. Wells were made in the plates using a sterile 6 mm borer. The test samples were prepared by dissolving 3 mg of the extract in 1 ml of DMSO, serving as stock solution. From each stock solution 100 µl was introduced in the respective well and incubated for 24 h at 37°C. Amoxicillin was used as positive and DMSO as negative control. Percentage zone of inhibition was measured in mm in comparison with positive control.

Minimum inhibitory concentration (MIC)

The MIC was considered as the lowest concentration of the crude extract that inhibited the visible growth of bacteria (Mukherjee, PK. (2002). After recording the zone of inhibitions, experiments were performed to determine the MIC50, using slightly modified procedure of (Banso, A. (2009). This was performed by taking 4 ml of nutrient broth in sterile test tubes inoculated with 18 - 24 h old culture of the test organisms. 50, 100, 150, 200, 250, 300 and 350 µl from the stock solution (8 mg/ml) of the samples were poured into the test tubes containing test pathogens. After 24 h of incubation at 37°C, results were recorded based on the percent clarity of the test tubes. After 24 h of incubation at 37°C, results were recorded based on the percent clarity of the test tubes.

Haemagglutination activity

Haemagglutination activity of the crude extract and fractions was tested against human erythrocyte blood groups ABO (Naqvi *et al*., 1992). Stock solution of the test sample was prepared at concentration of 1 mg/ml and each solution was serially diluted. Fresh blood was collected from healthy persons, centrifuged and the erythrocytes were separated. 2% erythrocyte suspension was prepared in phosphate buffer (pH 7.4) of all blood groups. 1 ml of the test sample dilution was taken with 1 ml of 2% erythrocyte and incubated at 25°C. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of haemagglutination was determined from the extent of deposition.

Results and discussion

Haemagglutination activity

Certain plant contains lectins. Lectins have become a well-established means for understanding varied aspects of cancer and metastasis in the past few years. Lectins can be used for cell adhesion and localization, tumor cell recognition (surface markers), signal transduction across membranes, augmentation of host immune defense, mitogenic stimulation, cytotoxicity and apoptosis (Mody *et al*., 1995). Crude methanolic extract and fractions of *Strobilanthes urticifolia* Wall. ex Kuntz with different dilutions were screened for possible haemagglutination activity against human erythrocytes of all blood groups (ABO). The results showed that weak haemagglutination were detected against B+ve, O+ve and B-ve blood groups at different dilutions and fractions while no sign of haemagglutination were noticed against the remaining blood groups. Results are summarized in Table 1.

Table 1. Haemagglutination activity of the crude methanolic extract and various fractions of *Strobilanthes urticifolia* Wall. ex Kuntz atvarious dilutions.

Blood group	Crude methanolic extract				n-hexane				CHCl3				EtOAc				Aqueous			
	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8
A +	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B +	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
AB +	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O +	+	+	-		-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-
A –	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B –	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
AB -	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O –	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-, No activity; +, low activity; ++, moderate activity; +++, strong activity.

Table 2. Antibacterial results of the crude methanolic extract and various fractions of *Strobilanthes urticifolia* Wall. ex Kuntz.

Bacterial Species	Zone of inhibition of standard (amoxicillin) 10µg/disc	Cr. Met. Ext.		*n*-hexane		Chloroform		Ethyl Acetate		Aqueous	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
S. aureus	27	13	48	14	51.85	10	37	16	59.25	10	37
E. coli	21	12	57.14	13	61.90	11	52.38	13	61.90	11	52.38
S. typhi	25	14	56	10	40	16	64	10	40	13	52
P. aeruginosa	23	9	39.13	12	52.17	13	56.52	11	47.82	9	39.13
S. pneumoniae	26	15	57.69	11	42.30	14	53.84	15	57.69	12	46.15
Micrococcus luteus	24	13	54.16	14	58.33	12	50	12	50	10	41.66
Bacillus cereus	20	10	50	15	75	13	65	13	65	14	70

Antibacterial activity

Crude methanolic extract along with various fractions of *S. urticifolia* were screened for antibacterial activity. The results are summarized in Table 2. From the results, it is discovered that the widest zone of inhibition (75%) was presented by n-hexane fraction against *B. cereus*. This fraction proposed substantial biological activity against all other bacterial strains.

Nevertheless Cr. Met. Ext, CHCl3, EtOAc and aqueous fractions were noticed as equally active against all pathogens and were considered as significant because of broad zone of inhibitions. The similar pattern of bacterial inhibition of the crude methanolic extract and all other fractions was proposed by MIC50 values (mg/ml) which are summarized in Table 3.The lower the MIC50 values, the higher will be its potency and vice versa. The EtOAc fraction showed lower MIC50 value i.e. it inhibited 50% growth at 1.2 mg/ml concentration against *M. luteus* while CHCl3 fraction also showed good MIC50 value (inhibited 50% growth at concentration of 1.6 mg/ml) against *S. typhi, S. pneumoniae* and *B. cereus*.

Table 3. MIC50 values of Crude methanolic extract and various fraction of *Strobilanthes urticifolia* Wall. ex kuntz.

Bacterial species	MIC_{50} Values (mg/ml)				
	Crd. MeOH Ext	*n*-hexane	$CHCl_3$	EtOAc	Aqueous
S. aureus	2.0	2.0	2.0	1.6	2.4
E. coli	2.0	2.4	2.4	2.4	2.0
S. typhi	2.0	2.0	1.6	2.0	1.6
P. aeruginosa	2.4	2.0	2.0	1.6	2.4
S. pneumoniae	2.0	2.0	1.6	2.0	2.0
M. luteus	2.0	2.4	2.0	1.2	2.4
B. cereus	2.0	2.0	1.6	2.0	2.0

Conclusion

Results of the present study clearly showed that *S. urticifolia* Wall. ex Kuntz extracts contain potent antimicrobial property. Furthermore, the extracts of the plant also contain lectins and could be a useful source of phytolectins. The research should be extended for the isolation of active compounds so that, further investigations on this plant are recommended to exploit its hidden medicinal importance.

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