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Full Length Research Paper

Studies on *Buddleja asiatica* antibacterial, antifungal, antispasmodic and Ca^{++} antagonist activities

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Crude extract of *Buddleja asiatica* Lour and its fractions, chloroform (F1), ethyl acetate (F2) and n-butanol (F3) were evaluated for antibacterial, antifungal, antispasmodic and Ca^{++} antagonist activities. The antibacterial activity was performed against 11 types of bacteria. The crude extract and fractions F2 and F3 exhibited significant activity, while F1 showed low activity in killing the *Shigella flexenari*, *Sternostoma boydi* and *Escherichia coli.* In the rest bacteria, the crude extract and all the fractions (F1 to F3) revealed minimum to nil inhibitory effect. The fungicidal activity of the crude extract and all the fractions (F1 to F3) was also performed against six different fungi. The crude extract and fractions F1 and F3 displayed significant activity, while fraction F2 showed moderate activity against *Fusarium solani*. In the case of *Microsporum canis*, the crude extract and fraction F3 showed high activity but in the other four fungi, the inhibition area exhibited optimum to nil activity in crude extract and all the fractions (F1 to F3). In isolated rabbit jejunum preparations, *B. asiatica* crude extract caused concentration-dependent (0.03 to 1.0 mg/ml) relaxation of spontaneous and high K^+ (80 mM)-induced contractions. The results indicate the antibacterial, antifungal, antispasmodic and Ca^{++} antagonist potential of *B. asiatica* Lour.

Key words: *Buddleja asiatica*, antibacterial, antifungal, antispasmodic, Ca^{++} channel blocker.

INTRODUCTION

The genus *Buddleja* belonging to the family Scrophulariaceae, comprises approximately 100 species. In Pakistan, it is represented by four species (Abdullah, 1974). This plant has been used medicinally in different regions in the past and present. It has been used for skin complaints (Pande et al., 2007) and as an abortifacient (Nepal, 1970). A paste of its roots is used as a tonic when mixed with rice water (Gage, 1904). This plant is also used as a medicine for skin disease, abortion and as a cure for loss of weight (Guerrero, 1921). Roots and leaves of this plant are employed to treat head tumor (Hartwell, 1970). A fusion of roots is used in the treatment of malaria (Reis and Von, 1973). The leaves of *Buddleja asiatica* have a hypotensive effect on cats and dogs, probably due to α-adrenoceptor blocking activity (Singh et al., 1980). It is also reported that essential oil of the leaves has *in-vitro* antifungal activities (Garg and Dengre, 1992). The flowers are used in the treatment of cystitis and cold (Lee et al., 2008) and to treat edema (Zheng and Xing, 2009). The extracts of *B. asiatica* also showed strong cyclo-oxygenase inhibitory activities in elicited rat peritoneal leukocytes (Liao et al., 1999). The ethno-pharmacological and chemo-taxonomic importance of the genus *Buddleja* prompted us to carry out investigation on *B. asiatica* since no work has been carried out on the antimicrobial activities of the plants and different extracts/fractions. Also, in this investigation, the evidence that *B. asiatica* exhibited spasmolytic effect, which is mediated through Ca^{++} antagonist mechanism was shown.

MATERIALS AND METHODS

The whole plant of *B. asiatica* was collected from Banda Piran, Siran valley, 2900 meters above the sea level, in the of District Mansehra in October, 2007. It was identified by Professor Dr.

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Manzoor Ahmad, Plant Taxonomist, Department of Botany, Government Degree College, Abbotabad, Pakistan. A voucher specimen (Accession no. B-0015) has been deposited in the herbarium.

Preparation of crude extract and fractions

The whole plant of *Buddleja asiatica was* shade-dried, ground to powder (21 kg) and extracted with methanol (65 L) at room temperature for seven days. The extraction was repeated thrice. The combined methanolic extract was evaporated to obtain a greenish gummy residue (522 g) which was divided into *n*-hexane (63 g), chloroform (101 g), ethyl acetate (59 g), *n*-butanol (48 g) and water (70 g) soluble fractions.

Fungal and bacterial strains were collected from the Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan. Acetylcholine (ACh), dimethyl sulfoxide (DMSO), sabouraud, glucose agar and imipenem were purchased from Sigma Chemicals Co., St. Lous, USA. The solvents: *n*-butanol, ethyl acetate, *n*-hexane, chloroform and methanol were from Merck, Germany. Chemicals used for making physiological salt solutions: potassium chloride, calcium chloride, glucose, magnesium chloride, sodium bicarbonate, sodium dihydorgen phosphate and sodium chloride were obtained from BDH Laboratories, England. All chemicals were of analytical grade A.

Rabbits (1.5 to 2 kg) of either sex or local breed were used in this study. They were housed at the Animal House of the Aga Khan University, maintained at 23 to 25°C and given a standard diet and tap water. Rabbits had free access to water, but food was withdrawn 24 h prior to the experiment and killed by a blow on the back of the head. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and were approved by the Ethical Committee of the Aga Khan University.

Antibacterial assay

The antibacterial activity was checked by the agar–well diffusion method (Kavanagh, 1963). In this method, one loop full of 24 h old culture containing approximately 10^4 to 10^6 CFU was spread on the surface of Mueller-Hinton agar plates. Wells were dug in the medium with the help of sterile metallic cork borer. Stock solutions of the test samples (crude extract and fractions F1 to F3) with the concentration of 1 mg/ml were prepared in DMSO and 100 µl dilutions were added in their respective wells. The antibacterial activity of crude extract and fractions (F1 to F3) were compared with the standard drug imipenem which served as the positive control.

Antifungal activity

The antifungal activity was determined by the agar well diffusion method (Kavanagh, 1963). In this method, amphotericin and miconazole were used as the standard drugs. The crude extract and fractions (F1 to F3) were dissolved in DMSO (50 mg/ 5ml). Sterile sabouraud's dextrose agar medium (5 ml) was placed in a test tube and inoculated with the sample solutions (400 µg/ml) and kept in slanting position at room temperature overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 29°C and growth inhibition was observed.

Antispasmodic effect

The spasmolytic activity of the plant material was studied by using isolated rabbit jejunum as described previously (Gilani et al., 1999). Respective segments of 2 cm length were suspended in 10 ml of Tyrode's solution and bubbled with carbogen at 37°C. The composition of Tyrode's solution in mM was: KCl, 2.68, NaCl, 136.9, $MgCl_2$, 1.05, $NaHCO_3$, 11.90, NaH_2PO_4, 0.42, $CaCl_2$, 1.8 and glucose, 5.55. A resting tension of 1 g was applied to each tissue and kept constant throughout the experiment. Intestinal responses were recorded isotonically using a Bioscience transducer and Harvard oscillograph. Each tissue was allowed to equilibrate for at least 30 min before addition of any drug, then stabilized with sub-maximal concentration of ACh (0.3 µM) and bath fluid was subsequently replaced with normal Tyrode's solution before starting the experiment. Under these experimental conditions, jejunum exhibited spontaneous rhythmic contractions, which allowed the testing of the relaxant (spasmolytic) effect directly, without the use of any agonist.

Determination of Ca^{++} antagonist action

To assess whether the antispasmodic effect of the plant materials is mediated through calcium channel blockade, high K^+ (80 mM) was used to depolarize the preparations as described by Farre et al. (1991). Addition of high K^+ to the tissue bath produced a sustained contraction. Relaxation of intestinal preparations by the plant material, precontracted with K^+, was expressed as percent of the control response mediated by K^+.

Statistical analysis

Data expressed were mean ± standard error of mean (SEM, n = number of experiments).

RESULTS AND DISCUSSION

Antibacterial activity of crude extract and various fractions (F1 to F3) of *B. asiatica* L. was performed against 11 human pathogens including *Escerichia coli, Bacillus subtilis, Staphylococcus aureus, Shigella flexneri*, *Sternostoma boydi, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Klebsiella pneumoniae, Pseudomonas maltophilia* and *Mycobacterium leprae.* The results are shown in Table 1. The crude extract displayed high to low activity in killing the *S. boydi* (56%), *E. coli* (44%), *S. flexneri* (36%), *S. aureus* (25%), *V. cholera*, (25%), *B. subtilis* (17%), *M. leprae* (13%) and *P. aeruginosa* (10%) but no activity was seen against *S. typhi, K. pneumoniae* and *P. maltophilia*. F1 was most effective against *S. flexenari* (23 %) and *S. boydi* (16 %), while less activity was seen in the case of inhibiting the growth of *P. aerigenosa* (12 %) and *E. coli*. F1 was totally ineffective against the rest of the bacteria. F2 exhibited significant activity against *S. boydi* (67%) and *E. coli* (44%), while low activity was seen in the killing of *S. flexneri* (29%), *P. aeruginosa* (21%), *S. aureus* (18%), *B. subtilis* (10%) and *V. cholera* (7%), but no activity was observed in the rest of the bacteria. The fraction F3 revealed moderate to low activity in killing the *S. flexneri*(58%), *S. boydi* (32%), *V. cholera* (29%), *E. coli* (27%), *P. aeruginosa* (17%) and *S. aureus* (11%), and

Table 1. Antibacterial activity of crude extracts and various fractions of *Buddleja asiatica.*

Bacteria	Z.I of standard drug (imipenem)	Crude extract		F1		F2		F3	
		Z.I	Inh	Z.I	Inh	Z.I	Inh	Z.I	Z.I
E. coli	34	15	44	5	9	15	44	9	27
B. subitiltis	30	5	17	-----	-----	3	10	-----	-----
S. aurous	28	7	25	2	7	5	18	3	11
S. boydi	25	14	56	4	16	16	67	8	32
S. flexenari	31	11	36	7	23	9	29	18	58
P. aerigenosa	30	3	10	4	12	2	21	5	17
S. typhi	29	-----	-----	-----	-----	-----	-----	-----	-----
V. cholera	24	6	25	-----	-----	3	7	7	29
K. pneumoniae	25	-----	-----	-----	-----	-----	-----	-----	-----
P. maltophilia	28	-----	-----	-----	-----	-----	-----	-----	-----
M. leprae	31	4	13	3	10	-----	-----	2	7

Z.I = Zone of Inhibition in mm; Inh = Inhibition in %. The plates were inculcated at a concentration (mg/ml) of DMSO.

Table 2. Antifungal activity of the crude extracts and various fractions of *B. asiatica*.

Fungi	Control L.G	Crude extract		F1		F2		F3		Standard drug	
		L.G	Inh	L.G	Inh	L.G	Inh	L.G	Inh	Name	MIC (µg/ml)
A. flavus	100	25	71	60	40	80	20	65	35	Amphotericin-B	30
F. solani	100	70	66	35	65	54	46	41	59	Miconazole	105
C. albicans	100	100	-----	100	-----	100	-----	100	-----	Miconazole	20
T. longifusus	100	50	59	44	46	65	35	55	4	Miconazole	88
M .canis	100	40	60	80	20	75	25	35	65	Miconazole	94
C. glaberata	100	100	-----	100	-----	100	-----	100	-----	Miconazole	103

L.G= Linear growth in mm; Inh = Inhibition in %. The plates were inculcated at a concentration (mg/ml) of DMSO.

Table 3. Concentration-dependent inhibitory effect of the crude extract of *B. asiatica* on spontaneous contractions of isolated rabbit jejunum preparations.

Concentration (mg/ml)	Inhibition (%)
0.03	5.7 ± 4.8
0.1	11.7 ± 9.3
0.3	38 ± 8.5
1.0	100

Values shown are mean ± SEM (n = 3).

did not show any activity against the others. These results were compared with standard drug (imipenem) which was more effective by showing maximum inhibition zones (Table 1).

The fungicidal activity of the crude extract and various fractions (F1 to F3) of *B. asiatica* was evaluated against six fungi including *Aspergillus flavus, Fusarium solani, Candida Ibicans, Trichophyton longifusus, Microsporum canis and Candida glaberata*. The results (Table 2) indicated that the crude extract and fractions F1 and F3 displayed significant activity, while fraction F2 showed moderate activity in killing *F. solani*. In the case of *A. flavus,* and *T. longifusus,* the area of inhibition were almost the same and exhibited very high activity in crude extract, while in fractions F1 to F3, it showed optimum activity. It was further observed that the crude extract and fraction F3 showed high activity and fractions F1 and F2 revealed weak activity against *M. canis.* The crude extract and fractions F1 to F3 remained totally ineffective in killing *C. albicans* and *C. glabrata.*

B. asiatica crude extract inhibited spontaneous contractions at concentrations of 0.03, 0.1, 0.3 and 1.0 mg/ml by 5.7 ± 4.8, 11.7 ± 9.3, 38 ± 8.5 and 100% (mean ± SEM, n = 3), respectively (Table 3), showing an antispasmodic effect. The contraction of smooth muscle preparations including rabbit jejunum was dependent upon an increase in the cytoplasmic free Ca^{++}, which activates the contractile elements (Karaki et al., 1997; Grasa et al., 2004). The increase in intracellular Ca^{++} was due to either influx via voltage dependant L-type Ca^{++} channels

Table 4. Concentration-dependent relaxant effect of the crude extract of *B. asiatica* on K^+-induced contractions of isolated rabbit jejunum preparations.

Concentration (mg/ml)	Relaxation (%)
0.1	6.7 ± 4.4
0.3	32.7 ± 9.8
1.0	100

Values shown are mean ± SEM, n = 3.

Table 5. High effective fractions amongst F1, F2 and F3 against various pathogens.

Pathogen	Common disease	Active fraction
Bacteria		
E. coli (Gram-negative)	Gastroenteritis , UTI, Meningitis in infants, septicaemia	F2
S. boydi (Gram-negative)	Dysentery, Diarrhea	F3
S. flexneri (Gram-negative)	Diarrhea	F3
Fungi		
F. solani	Onychomycosis, nails infection, cornea, plant diseases	F1
A. flavus	Cause allergy, liver carcinoma, brain infections, plant diseases	F1
T. longifusus	UTI infections, skin diseases	F1

(VDCs) or release from intracellular stores in the sarcoplasmic reticulum. Periodic depolarization regulates the spontaneous movements of intestine and at the height of depolarization, the action potential appears as a rapid influx of Ca^{++} via VDCs (Brading, 1981). The inhibitory effect of the plant extract on spontaneous movements of jejunum may be due to interference either with the Ca^{++} release or with the Ca^{++} influx through VDCs. In our earlier studies, we observed that the spasmolytic effect of the medicinal plants was usually mediated through Ca^{++} channel blockade (Gilani et al., 2007).

To see whether the spasmolytic effect of this plant is also mediated via the same mechanism, the *B. asiatica* was tested on high K^+ (80 mM) induced contraction, which was relaxed by the plant extract at 0.1, 0.3 and 1.0 mg/ml by 6.7 ± 4.4, 32.7 ± 9.8 and 100% (mean ± SEM, n = 3), respectively (Table 4). At high concentration (> 30 mM), K^+ is known to cause smooth muscle contractions through opening of VDCs, thus allowing the influx of extra cellular Ca^{++} and causes a contractile effect (Bolton, 1979), and a substance which causes inhibition of high K^+-induced contraction is considered as an inhibitor of Ca^{++} influx (Fleckenstein, 1977, Godfraind et al., 1986).

Conclusions

The results of this study indicate that ethyl acetate (F2) and *n*-butanol (F3) fractions of *B. asiatica* are strong inhibitor of Gram negative bacteria such as *S. boydi, S. flexneri* and *E. coli*. Amongst all, the chloroform (F1) fraction possessed outstanding antifungal activity against *A. flavus*, *F. solani* and *T. longifusus* (Table 5). The crude extracts also exhibited antispasmodic and Ca^{++} antagonist effects. Thus, this study showed that the medicinal plant *B. asiatica* contains antibacterial, antifungal, antispasmodic and Ca^{++} antagonist constituents. The active fractions will be further investigated through bioassay guided isolation to obtain the crude drugs and active compounds.

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REFERENCES

Abdullah P (1974). Flora of West Pakistan. University of Karachi, Karachi, pp. 1-4.

Bolton TB (1979). Mechanism of action of transmitters and other substances on smooth muscles. Physiol. Rev. 59: 606-718.

Brading A (1981). How do drugs initiate contraction in smooth muscles. Trends Pharmacol. Sci. 2: 261-265.

Farre A, Colombo M, Fort M, Gutierrez B (1991). Differential effects of various Ca^{2+} antagonists. Gen. Pharmacol. 22: 177-181.

Fleckenstein A (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Ann. Rev. Pharmacol. Toxicol. 17: 149-166.

Gage AT (1904). The vegetation of the district of Minbu in upper Burma. Records of the Botanical Survey of India 3: 141-143.
Garg SC, Dengre SL (1992). Composition of the essential oil from the leaves of *Buddleja asiatica* Lour. Flavour Fragr. J. 7: 125-127.
Gilani A, Shaheen F, Zaman M, Janbaz K, Shah B, Akhtar M (1999). Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica* Pods. Phytother Res. 13: 665-669.
Gilani AH, Bashir S, Khan A (2007). Pharmacological basis for the use of *Borago officinalis* in gastrointestinal, respiratory and cardiovascular disorders. J. Ethnopharmacol. 114: 393-399.
Godfraind T, Miller R, Wibo M (1986). Calcium antagonism and calcium entry blockade. Pharmacol. Rev. 38: 321-416.
Grasa L, Rebollar E, Arruebo MP, Plaza MA, Murillo MD (2004). The role of Ca^{+2} in the contractility of rabbit small intestine *in-vitro*, J. Physiol. Pharmacol. 55: 639-650.
Guerrero LA (1921). Medicinal uses of Philippine plants. Philippines Bureau Forest. Bull. 22: 149-246.
Hartwell J (1970). Plants used against cancer. Lloydia, 33: 87-94.
Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano KI, Harada KI, Miyamoto S, Nakazawa H, Won KJ, Sato K (1997). Calcium movements, distribution and functions in smooth muscle. Pharmacol. Rev. 49: 157-230.
Kavanagh F (1963). Analytical Microbiology. Natl. Acad. Press, London, pp. 125-141.
Lee S, Xiao C, Pei S (2008). Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, SW China. J. Ethnopharmacol. 117: 362-377.
Liao Y, Houghton P, Hoults J (1999). Novel and known constituents from Buddleja species and their activity against leukocyte eicosanoid generation. J. Nat. Prod. 62: 1241-1245.
National Research Council (1996). Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, pp. 12-35.
Nepal HM (1970). Medicinal Plants of Nepal. Ministry of Forests, Kath-Mandu, pp. 105-106.
Pande P, Tiwari L, Pande H (2007). Ethnoveterinary plants of Uttaranchal-A review. Ind. J. Trad. Knowl. 6: 444-458.
Reis AS, Von S (1973). Drugs and Foods from Little-known Plants. Harvard University Press, Cambridge, pp. 221-223.
Singh S, Mathur A, Sinha J, Singh N, Kohli R (1980). A Study on the Mechanism of the Hypotensive Activity of Buddleja asiatica (Newarpati). Pharm. Biol. 18: 83-87.
Zheng X, Xing F (2009). Ethnobotanical study on medicinal plants around Mt. Yinggeling, Hainan Island, China. J. Ethnopharmacol. 124: 197-210.