

Full Length Research Paper

# Parasitocidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke

B. Ahmad<sup>1\*</sup>, N. Ali<sup>2, 4</sup>, S. Bashir<sup>2</sup>, M. I. Choudhary<sup>3</sup>, S. Azam and I. Khan<sup>1</sup>

<sup>1</sup>Centre for Biotechnology and Microbiology, University of Peshawar, N.W.F.P. Pakistan.

<sup>2</sup>Department of Pharmacy, University of Peshawar, N.W.F.P. Pakistan.

<sup>3</sup>International Centre for Chemical and Biological Sciences, H.E.J Research Institute of Chemistry, University of Karachi, Karachi. Pakistan.

<sup>4</sup>Department of Pharmacy, University of Malakand, Chakdara Dir, N.W.F.P. Pakistan.

Accepted 13 August, 2009

***Onosma griffithii* was screened for possible pharmacological activities. The crude methanolic extract (MeOH) and its fractions demonstrated parasitocidal activity ( $IC_{50}$  ( $\mu\text{g/ml} \pm \text{S.D}$ ) =  $31.03 \pm 0.23$ ) against *Leishmania major*. Based on the  $IC_{50}$  values, the potency of the standard drug (Pentamidine) and test fractions were of the order as: Pentamidine > crude extract > n-hexane fraction > ethyl acetate (ETOAc) fraction > chloroform fraction ( $\text{CHCl}_3$ ) fraction > n-butanol (BUOH) fraction > aqueous fraction. Similarly moderate antifungal activity was displayed by the crude methanolic extract against *Aspergillus flavus* and *Fusarium solani*. Against the *Staphylococcus aureus*, the aqueous fraction demonstrated moderate antibacterial activity.**

**Key words:** Parasitocidal, antileishmanial, antifungal, antibacterial and  $IC_{50}$  values.

## INTRODUCTION

The family Boraginaceae consists of about 100 genera and 2000 species distributed in temperate, especially in Mediterranean and tropical regions (Ali and Nasir, 1983). *Onosma* is an important genus of the family as extracts of some species of *Onosma*, except *Onosma hispidum*, have been used as antioxidant, antibacterial, antiviral and anti-inflammatory agents (Tosun et al., 2008). Traditionally, *Onosma* is used as stimulant in rheumatism, bladder pain, kidney irritation and palpitation of heart (Ahmed, 2004). Hispidone, a new flavanone has been isolated from *O. hispidum* that have cholinesterase inhibitory activity (Ahmad et al., 2003). *O. hispida* is also used as colorant (Shahina, 2005). Similarly, anticancer activity of *Onosma limitaneum* (Ahmad et al., 2005), antioxidant and antimicrobial activities of *Onosma argenatum* have been reported. It has been suggested that the roots of some plant of *Boraginaceae* contain naphthaquinone derivatives. Shikanins and alkanins do exist in the *Onosma* species (Ufuk et al., 2004).

Traditionally, *O. argenatum* is used in the treatment of

wound healing and burns with well documented anti-oxidant and antimicrobial activity (Sezik et al., 1997; Ozgen et al., 2003). This suggests that this genus is very potent from pharmacological activities point of view. Hence we carried out experiments to screen *Onosma griffithii* specie for possible pharmacological activities.

## MATERIALS AND METHODS

### Plant material

Aerial parts of the plant, *O. griffithii* (*Boraginaceae*), were collected from the nearby hills of Campus I of University of Malakand, in June-July 2005 and identified by Professor Dr. Jahandar Shah, Plant Taxonomist, University of Malakand, Chakdara Dir N.W.F.P Pakistan. A voucher specimen (OG-05) has been deposited in the herbarium of the University of Malakand.

### Extraction

The shade dried plant material was crushed into small pieces and finally pulverized into fine powder. The plant material (7 kg) was soaked in methanol with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (below

\*Corresponding author. E-mail: bashirdr2001@yahoo.com.

**Table 1.** Antileishmanial activities of crude methanolic extract and fractions of *Onosma griffithii* against the susceptible strains of *Leishmania major* promastigotes (Pakistani isolates). IC<sub>50</sub> values in µg/ml ± S.D obtained from three separate experiments are shown.

Extract / Fraction / standard*	Control		IC <sub>50</sub> values (µg/ml ± S.D)	Mean relative index (IC <sub>50</sub> Sample / IC <sub>50</sub> Standard)
	Negative Control (% Survivors)	Positive control* (% Inhibition)		
Pentamidine*	100%	100%	2.5 ± 0.09	1
Crude methanolic extract	100%	100%	31.03 ± 0.23	12.41
n-Hexane	100%	100%	31.36 ± 0.34	12.54
Chloroform	100%	100%	42.60 ± 0.465	16.24
Ethyl acetate	100%	100%	38.39 ± 0.37	15.32
n-Butanol	100%	100%	41.87 ± 0.39	16.748
Aqueous	100%	100%	60.35 ± 0.87	24.14

40°C) using rotary evaporator. A blackish crude extract (150 g) was obtained.

### Fractionation

The crude methanolic extract (150 g) was suspended in distilled water (400 ml) and sequentially partitioned with n-hexane (3 x 350 ml), chloroform (3 x 350 mL), ethyl acetate (3 x 350 ml) and n-butanol (3 x 350 ml) to yield n-hexane (30 g), chloroform (20 g), ethyl acetate (2g), n-butanol (2 g) and aqueous (65 g) fractions, respectively. About 25-gram crude methanolic was reserved for other pharmacological screenings.

### Statistical analysis

All the experiments were performed in triplicate. Standard deviations and IC<sub>50</sub> values of compounds possessing antileishmanial activity were calculated by Software Ezfit 5.03 Perella Scientific. P values of 0.05 or less was considered significant using student's "t" test.

### Parasiticidal activity

The antileishmanial activity was performed according to the procedure of Rehman et al. (1991) described as follows:

### Parasite culture

*Leishmania major* parasite's Promastigotes were grown in bulk, early in modified NNN biphasic medium, using normal physiological saline. Then the Promastigotes were cultured with RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS). The parasites, at log phase, were centrifuged at 2000 rpm for 10 min and washed three times with saline at same speed and time. Finally the parasites were diluted with fresh culture medium to give a final density of 10<sup>6</sup> cells/ml.

In a 96-well micro titer plate, 180 µl of medium was added in different wells. 20 µl of the experimental compound was added in medium and serially diluted so that the minimum concentration of compound was one 1 µg/ml. 100 µl of parasite culture (final density of 10<sup>6</sup> cells/ml) was added in all wells. 2 rows were left one for negative and other for positive control. Negative controls received the medium while the positive control received Pentamidine as standard antileishmanial compound. The plate was incubated at 21

- 22°C for 72 h. The culture was examined microscopically for cell viability by counting the number of motile cells on an improved Neubauer counting chamber and IC<sub>50</sub> values of compounds possessing antileishmanial activity were calculated by Software Ezfit 5.03 Perella Scientific. IC<sub>50</sub> values of different fractions against the test pathogen are mentioned in Table 1.

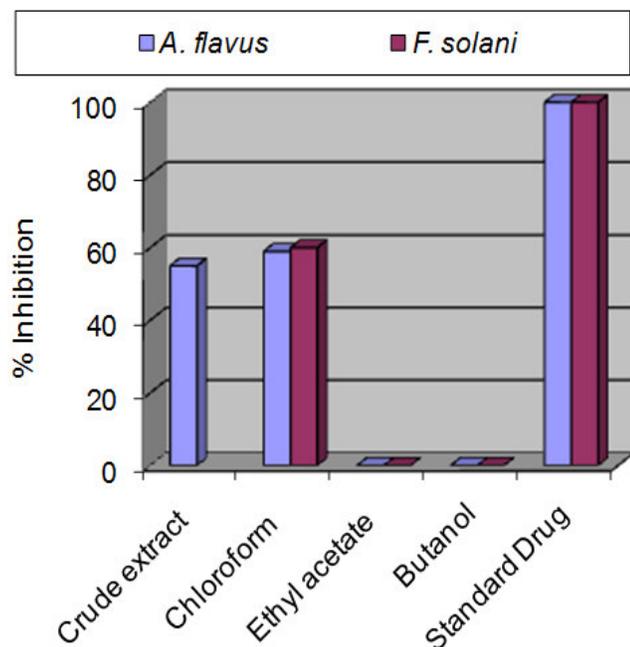
### Antifungal activity

Similarly antifungal activity was evaluated by agar tube dilution method as per our reported procedure (Bashir et al., 2007). Describing the procedure, the test samples in the concentration of 24 mg/ml were dissolved in the sterile (autoclaved) dimethyl sulfoxide (DMSO, Merck), which served as stock solution. Sabouraud Dextrose Agar (SDA, Sigma-Aldrich, Germany) was prepared by mixing 32.5 g sabouraud, 4% glucose agar and 4.0 g of agar-agar in 500 ml distilled water and the mixture was mixed thoroughly with magnetic stirrer. Then 4 ml amount was dispensed into screw cap tubes, which were autoclaved at 120°C for 15 min and then cooled to 15°C. The non-solidified SDA media was mixed with stock solution (66.6 µl) giving the final concentration of 400 µg of the extract per ml of SDA. Tubes were then allowed to solidify in the slanted position at room temperature. Each tube was inoculated with a piece (4 mm diameter) of inoculums removed from a seven days old culture of fungi for non-mycelial growth; an agar surface streak of *Aspergillus flavus* and *Fusarium solani* was employed. Other media supplemented with dimethyl sulfoxide (DMSO) and standard antifungal drugs served as negative and positive control respectively (Figure 1). Inhibition of fungal growth was observed after 7 days of incubation at 28 ± 1°C. Humidity (40 - 50%) was controlled by placing an open pan of water in the incubator.

### Antibacterial activity

The crude extract along with fractions was screened against various human pathogens including *Escherichia coli*, *Bacillus subtilis*, *Shigella flexneri*, *Staphylococcus aureus* and *Salmonella typhi* as per our reported procedure (Bashir et al., 2007). In this method, 10 ml aliquots of nutrients broth (Sigma-Aldrich, Germany) was inoculated with the test organism and incubated at 37°C for 24 h.

Using a sterile pipette, 0.6 ml of the broth culture of the test organism was added to 60 ml of molten agar, which had been cooled to 45°C, mixed well and poured into a sterile petri dish (for the 9 cm petri dish, 0.2 ml of the culture was added to 20 ml of agar). Duplicate plates of each organism were prepared. The agar



**Figure 1.** Antifungal activities of crude extract and fractions of *Onosma griffithii* against *Aspergillus flavus* and *Fusarium solani* using Amphotericin B (20 µg/ml) and Miconazole (98.4 µg/ml) as standards respectively.

was allowed to set and harden and the required numbers of wells were dug in the medium with the help of a sterile metallic cork borer ensuring proper distribution of the wells in the periphery and one in the center. Agar plugs were removed. Stock solutions of the test samples in the concentration of 1 mg/ml were prepared in the sterile dimethyl sulfoxide (DMSO) and 100 and 200 µl of each dilution was added in their respective wells. The control well received only 100 and 200 µl of DMSO. *Imipenem* was used as standard drug. The plates were left at room temperature for 2 h to allow diffusion of the samples then incubated face upwards at 37°C for 24 h. The diameter of the zones of inhibition was measured (mm).

## RESULTS AND DISCUSSION

### Parasiticidal activity

Leishmaniasis particularly cutaneous leishmaniasis is a parasitic disease with diverse clinical manifestations that depends on both infecting species of *Leishmania* and the immune response of the host (WHO, 1984). The results of the crude methanolic extract and its fractions are summarized in Table 1.

The lower the IC<sub>50</sub> value of a drug against a particular pathogen, the more will be its potency and vice versa. Based on the IC<sub>50</sub> values (Table 1), the crude methanolic extract and its fractions showed statistically significant ( $P < 0.05$ ) antileishmanial activities against the *L. major*, Pakistani isolates. In comparison with the Pentamidine as a standard drug used, the crude methanolic extract and

its various fractions demonstrated the order of potency as: Pentamidine > crude extract > n-hexane fraction > ethyl acetate > chloroform fraction > n-butanol > aqueous fraction. Cutaneous leishmaniasis being a challenge and threat to Pakistani society as its incidence increases day by day. There were 350 patients in 1995 - 1997, 1210 patients in 1996-2001 and 1640 patients reported in 2008 in Pakistan (Bhutto et al., 2008). Therapy results of already existing topical preparation of paromomycin and gentamicin combination are not so much satisfactory because of 50% relapse and resistance (Grogil et al., 1999; El-On et al., 1991). Therefore, search for new antileishmanial drugs is necessary.

Very few medicinal plants have been reported for their antileishmanial activities (Kvist et al., 2006), hence the plant specie could be a potential target for activity guided isolation of bioactive compounds as the crude methanolic extract and its fractions are very potent because of their low IC<sub>50</sub> values.

### Antifungal activity

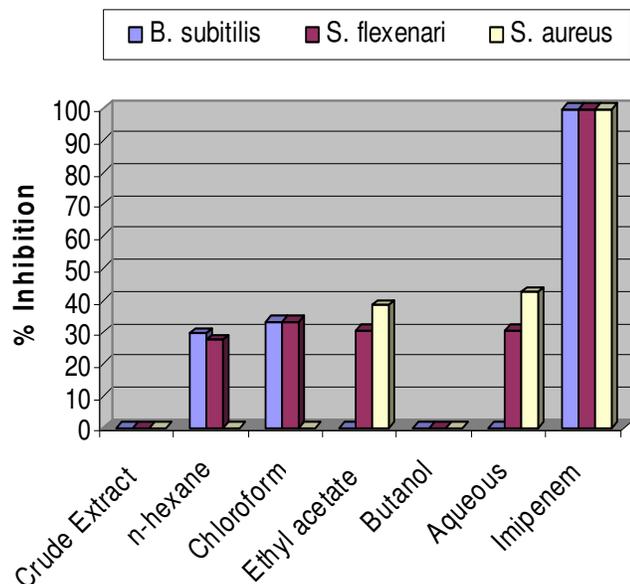
The crude methanolic extract of *O. griffithii* displayed moderate antifungal activity against *Aspergillus flavus* (55%) and *Fusarium solani* (40%). Whereas, the chloroform fraction showed good antifungal activity against *A. flavus* (59%) and *Fusarium solani* (60%). While rest of the fractions showed no antifungal activity against the stated fungal strains (Figure 1). This confirms the folkloric uses of the species of the *onosma* genus in the treatment of wounds (Sezik, et. al, 1997).

### Antibacterial activity

Crude methanolic extract and all fractions of *O. griffithii* were screened for antibacterial activity. The results are summarized in Figure 2. It is evident that against *S. flexenari* the fractionated extracts showed antibacterial activity of the order as CHCl<sub>3</sub> Fraction (33.3%) > EtOAc Fraction = Aqueous Fraction (30.55%) > n-hexane Fraction (27.77%). Against *B. subtilis* n-hexane Fraction and CHCl<sub>3</sub> Fraction showed 29.72 and 33.42 % antibacterial activity respectively. Against the *S. aureus*, the aqueous fraction demonstrated moderate antibacterial activity (42.3%) and the EtOAc fraction showed 38.46% antibacterial activity. The rest of the fractions showed no activity against the tested pathogens.

### Conclusion

*O. griffithii* has potent antileishmanial and moderate antifungal and antibacterial activities that strongly encourage the activity guided isolation of biologically active compounds.



**Figure 2.** Antibacterial activity of fractions of *Onosma griffithii* extracts

## ACKNOWLEDGMENT

The author wants to thank the Sponsor Higher Education Commission, Islamabad for Research Grant No: 1-23/HEC/RandD- 2007/CASE/34-Local.

## REFERENCES

- Ahmad I, Anis I, Malik A, Nawaz SA, Choudhary MI (2003). Cholinesterase Inhibitory Constituents from *Onosma hispida* Chem. Pharm. Bull. 51(4): 412-414.
- Ahmad VU, Kousar F, Khan A, Zubair M, Iqbal S, Tareen RB (2005). A New Ketone and a known anticancer triterpenoid from the leaves of *Onosma limitaneum*, Helv. Chim. Acta. 88(2): 309-311.
- Ali SI, Nasir YJ (1983). *Onosma griffithii* Vatke (Boraginaceae) (Eds.) In: Flora of Pakistan vol. 191, Shamim Printing Press Karachi p.103.
- Bashir A, Shah SMH, Bashir S, Nisar M, Chaudry MI (2007). Antibacterial and antifungal activities of *Andrachne cordifolia* muell. J. Enzyme Inhib. Med. Chem. 22(6): 726-729.

- Bhutto AM, Soomro FR, Katakura K (2008). Leishmaniasis in Sindh, Pakistan: Outbreak and review of literature. J. Pak. Assoc. Dermatol. 18: 212-219.
- El-On J, Sulitzeanu A, Shnur LF (1991). Leishmania major: resistance of promastigotes to paromomycin, and susceptibility of amastigotes to paromomycin- methylbenzethonium chloride ointment. Ann. Trop. Med. Parasitol. 85(3): 323-328.
- Grogl M, Schuster BG, Ellis WY, Berman JD (1999). Successful topical treatment of murine cutaneous leishmaniasis with a combination of paromomycin (Aminosidine) and gentamicin. J. Parasitol. 85(2): 354-359.
- Kvist LP, Christensen SB, Rasmussen HB, Mejia K, Gonzalez A (2006). Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. J. Ethnopharmacol. 106(3): 390-402.
- Dr. Ahmed T (2004). Medicinal Herbs and products, page no xxi and xxii; a printed version of *herbal medicines* from online drug information system: www.druginfosys.com.
- Rehman AU, Choudhary MI, Thomsen WJ (1991). In: (eds.) Manual of Bioassay Techniques for Natural Product Research, Harward Academic Press, Amsterdam, p. 17.
- Sezik E, Silada EY, Tabata M, Honda G, Takaishi Y, Fujita T, Tanaka T, Takeda Y (1997). Traditional Medicine in Turkey VIII. Folk Medicine in East Anatolia; Erzurum, Erzincan, Agri, Kars, Igdir Provinces Ekrem. Econ. Bot. 51: 195-212.
- Shahina N (2005). Structure and functionality of the pigments isolated from *Onosma hispidum* (ratanjot) *Terminalia catappa* (jangli badam) and other tropical plant. Ph. D thesis, University of Karachi, Karachi.
- Ozgen U, Houghton PJ, Ogundipe Y, Coskun M (2003). Antioxidant and antimicrobial activities of *Onosma argenatum* and *Rubia peregrina*. Fitoterapia, 74(7-8): 682-685
- Tosun A, Akkol EK, Bahadır O, Yeşilada E (2008). Evaluation of anti-inflammatory and antinociceptive activities of some *Onosma L.* species growing in Turkey. J. Ethnopharmacol. 120(3): 378-831.
- Ufuk O, Maksut CK, Cavit K, Hasan SK (2004). Naphthoquinones from the Roots of *Onosma argenatum* Hub. Mor. (Boraginaceae). Turk. J. Chem. 28: 451-454
- World Health Organization (1984). The leishmaniasis. Report of a WHO expert committee. Tech. Rep. Series WHO No 701, Geneva.