

Short Communications

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ANTIMICROBIAL ACTIVITY OF THE EXTRACTS OF SEEDS OF *TRIGONELLA FOENUM-GRAECUM*

Abstract.- *In vitro* antibacterial activity of hexane, chloroform and ethanol extracts of the seeds of *Trigonella foenum-graecum* were determined by using 5, 50 and 100mg/ml concentrations against three Gram +ve (*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*) and three Gram-ve (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella cholerae-suis*) bacteria. Hexane and ethanol extracts had prominent antibacterial activity against *P. aeruginosa* and *E. coli*, as compared with the chloroform extract. Two fractions of ethanol extract isolated by vacuum liquid chromatography showed similar antibacterial activity against *P. aeruginosa* and *E. coli*.

Key Words: *Trigonella foenum-graecum*; antimicrobial principles, flavonoid.

The herbal drugs used throughout the world have received greater attention in recent time because of its diversity of curing diseases, safety and well tolerated remedies when compared to the conventional medicines (Farnsworth *et al.*, *Vogel's Textbook of practical organic chemistry*. 4th Ed, p. 137. The English Language Book Society and Longman, U.K, 1994). World Health Organization estimated that nearly 80% of the people living in the developing countries selectively use the traditional medicines. Leguminosae (Fabaceae) is an important family of dicotyledonous plants. It includes about 700 genera and 17,000 species. Plants of this family is used in Indian folk medicine as antipyretic, anthelmintic, diuretics, croupy, in heart diseases, chronic cough and in enlargement of spleen and liver (Adebanjo *et al.*, *International symposium on medicinal plants*, University of Ife, Nigeria, pp. 152-158, 1983). The seeds are detersive, resolvent, concoctive nervine tonic, aphrodisiac and expectorant. It has tonic effects on stomach and intestines, and is carminative and laxative, uterine

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tonic, emmenagogue activities and relieves pain in the uterus and also in colic, flatulence, diarrhea, dysentery, dyspepsia and loss of appetite. Suppositories made from seeds of *Trigonella foenum-graecum* are useful application for uterine and vaginal inflammation (Usmanghani *et al.*, *Indusynic Medicine: Traditional medicines of herbal, animal and mineral origin in Pakistan*. Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan, pp. 432-433, 1997). Essential oils in seeds enhance the insulin sensitivity (Talpur *et al.*, *Diab. Obes. Metab.*, **7**: 193-199, 2005). Ethanolic extract is effective in regulating the high blood sugar and high blood lipids (Li *et al.*, *Faming Zhuanli Shengqing Gong Kai Shuomingshu*, **6**: 144, 2003).

Following constituents have already been reported from the seeds of *Trigonella foenum-graecum*: Malonic acid (Barkat *et al.*, *Monatsh. Chem.*, **95**: 1613-1622, 1964), diosgenin (Maeream *et al.*, *Phytochemistry*, **13**: 1513-1514, 1974) saponins and sapogenin (Varshney *et al.*, *Indian J. appl. Chem.*, **34**: 208-210, 1971), vitexin, isovitexin (Rehman *et al.*, *Rev. Pharmacol. Biol.*, **37**: 202-207, 2001), amino acids (Kolousek *et al.*, *J. Sci. Food Agron.*, **6**: 455-461, 1955; Kmath *et al.*, *J. scient. indust. Res.*, **15**: 121-123, 1956), ascorbic acid (Barkat *et al.*, *Monatsh. Chem.*, **95**: 1613-1622, 1964), flavonoid glycosides and flavonoid aglycons (Varshney *et al.*, *J. Indian chem. Soc.*, **43**: 564-567, 1966; *Planta Med.*, **26**: 26-32, 1974; Zombo *et al.*, *Anal. Chem. Symp. Ser.*, **10**: 511-517, 1982), saponins (Mitscher *et al.*, *J. nat. Prod.*, **50**: 1025-1039, 1987), α -ketoacids (Baggett *et al.*, *Electrophoresis*, **23**: 1642-1651, 2002), medicarpin (Homans and Fuchs, *J. Chromatog.*, **51**: 327, 1970).

Present investigation is preliminary screening of commonly occurring species of *Trigonella foenum-graecum* seeds for their antibacterial activity.

Materials and methods

Trigonella foenum-graecum L. (Fabaceae) seeds were purchased from the local market (Paper Mandi) Lahore and the voucher specimen was

deposited in the Herbarium of Govt. College University, Lahore.

Distilled hexane, chloroform and ethanol were

Table I.- Effect of different concentrations (5, 50 and 100 mg/ml) of hexane, chloroform and ethanol extracts on three Gram positive and three Gram negative bacteria.

Drug (Concentration)	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Micrococcus luteus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella cholerae-suis</i>
Hexane extract						
5mg/ml	-	-	-	6.83±4.54*	9.70±3.78	-
50mg/ml	-	-	-	7.98±3.21	10.32±3.13	-
100mg/ml	-	-	-	6.78±3.80	11.04±2.1	-
Chloroform extract						
5mg/ml	-	-	-	17.32±.28	-	-
50mg/ml	-	-	-	-	-	-
100mg/ml	-	-	-	-	-	-
Ethanol extract						
5mg/ml	-	-	-	6.81±4.54	9.71±3.78	-
50mg/ml	-	-	-	7.98±3.21	10.32±3.13	-
100mg/ml	-	-	-	6.78±3.62	10.53±1.49	-
VLC fraction (Fr-2)						
80 µ l/ml	-	-	-	12.31±1.44	-	-
Ampicillin (1mg/ml)	22.17±2.86	22.76±1.72	20.57±1.31	20.45±1.95	22.90±2.95	30.24±2.49
Streptomycin (1mg/ml)	30.76±1.51	34.37±1.89	21.48±0.98	27.20±2.44	29.30±2.24	27.10±0.74

*Inhibition zone diameter (m.m.)

Each value represents Mean±SE obtained from 6 replicates. All values of different concentrations of an extract were not significantly different from each other at P>0.05; -, no effect.

used successively for the extraction of active principles from the seeds which were washed with distilled water, dried under shade and pulverized with pestle and mortar using Soxhlet extraction method (Furnis *et al.*, *Vogel's Textbook of practical organic chemistry*. 4th Ed, pp. 137. The English Language Book Society and Longman, U.K, 1978). The extracts were concentrated in vacuum rotary evaporator (Tokyo, Rikakikai Co. Ltd.) and their percentage yields were calculated.

Concentrated hexane (28%), chloroform (6.11%), ethanol (12%) extracts and Vacuum Liquid Chromatography (VLC) isolated fractions (Fr-1 and Fr-2) of seeds were tested against pure cultures of three Gram positive bacteria, (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341), and three Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella cholerae-suis* ATCC 13312) collected from "Schazo

Laboratories, Lahore and Schering Pharmaceutical Industries Ltd. Lahore by using the hole plate diffusion method (Havvik and Johanssen, *J. Gen. Microbiol.*, **76**: 451, 1973).

All the chemicals used were of analytical grade.

Pure cultures of three Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341), and three Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella choleraesuis* ATCC 13312) collected from Schazo Laboratories, Lahore and Schering Pharmaceutical Industries Ltd. Lahore were used for testing antibacterial activity of hexane, chloroform and ethanol extracts of *Trigonella foenum-graecum* L.

The ethanol extracts of seeds of *Trigonella foenum-graecum* was further fractionated according to the vacuum liquid chromatography (Homans and

Fuchs, *J. Chromat.*, **51**: 327, 1970).

Means value and standard error were calculated. The data was presented as Mean \pm SEM Student's t-test was used for determining significant difference between the two means. Analysis of Variance (Pagano and Gauvreau, *Principles of biostatistics* 2nd Ed. pp. 38-41. Duxbury, California, U.S.A., 2000) with two way randomized complete block experimental designs was used to determine significance between different extracts. Further, the range of the significant differences between various observations of the experimental units were calculated by Duncan's Multiple Range Test which were evaluated with least significant difference at (P<0.05).

Results and discussion

Table I shows the effect of different concentration of hexane, chloroform and ethanol extracts on three gram positive and three gram negative bacteria. Hexane extract did not exhibit any activity against any of the microorganisms used except *P. aeruginosa* and *E. coli*. Chloroform extracts with the concentration 5mg/ml was found to be effective only against *P. aeruginosa*. Ethanolic extract showed antibacterial activity against *P. aeruginosa* and *E. coli*. None of the extracts showed antibacterial activity closer to ampicillin and streptomycin (1mg/ml), whereas chloroform extract displayed antibacterial response closer to ampicillin than that of streptomycin.

Among the VLC isolated fractions, only fraction-2 showed antibacterial activity against *P. aeruginosa*, which was almost closer to Ampicillin than that of Streptomycin used as standard antibiotic. This antibacterial activity may be due to flavonoid glycoside (hRf=46) and flavonoid aglycon (hRf=86). Such types of fractions were also isolated by Varshney *et al.* (*J. Indian chem. Soc.*, **43**: 564-567, 1966; *Planta Med.*, **26**: 26-32, 1974) and Zombo *et al.* (*Anal. Chem. Symp. Ser.*, **10**: 511-517, 1982). It is concluded that fraction-2 may be used pharmaceutically in topical preparation as well as in some other pharmaceutical dosage form which is similar to the work already reported by Usmanghani *et al.* (*Indusyunic Medicine: Traditional medicines of herbal, animal and mineral origin in Pakistan*. Department of Pharmacognosy,

Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan, pp. 432-433, 1997) against the pathogenic microorganism *P. aeruginosa*.

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RECORD OF GREAT GERBIL, *RHOMBOMYS OPIMUS*, FROM NAUKUNDI, BALOCHISTAN WITH COMMENTS ON EXTENSION OF ITS DISTRIBUTION RANGE IN PAKISTAN

Abstract.- The current record of *Rhombomys opimus* from west of Chagi extends the range of this species further west in Pakistan. It has a limited distribution along Pak-Afghan border in Chagi District of Balochistan.

Key words: Rodent, Muridae, *Rhombomys opimus*, great gerbil, giant day jird.

Great Gerbil is a large fossorial mammal inhabiting a variety of habitats in the desert regions. It belongs to the family Muridae, order Rodentia and resembles to various species of the Genus *Meriones*. The species, however, shows some differences from the latter in colouration and morphological characters.

This species was first recorded from Kara Kum in Russian Turkistan in 1920s. It was re-discovered from northeastern part of Iran after 100 years and again recorded in 1960s from the same locality (Lay *et al.*, *Comp. Biochem. Physiol.*, **40B**: 521-526, 1971). It is also distributed throughout northern Afghanistan in the steppe regions of north Hindukush, Herat, Maimana, Mazar-i-Sharif and Kunduz (Hassinger, *Fieldiana, Zoology*, **55**: 1-81, 1968.). It is also reported from Uzbekistan (Randall

and Rogovin, *Ethology*, **108**: 513-527, 2002) and from Gobi Desert (Kelt *et al.*, *J. Biogeog.*, **26**: 825-841, 1999).

In Pakistan, the first specimen of the Great Gerbil was collected from the northwest side of Nushki (Lay, *Fieldiana: Zoology*, **54**: 1-282, 1967). In 1986 the rodent was trapped from sandy areas bordering the orchards around Mastung (Mian, *J. Bombay Nat. Hist. Soc.*, **83**: 654-656, 1986). It has also been reported from Darzi Chach, Naushki (Roberts, *The mammals of Pakistan*, Oxford University Press, pp. 424, 1997). The present record of *Rhombomys opimus* is based on two specimens (PMNH-M-826), collected by senior author in 2005 from Rico Dik (29° 06' 05.7" N and 62° 07' 34.4' E), west of Naukundi near Koh-i-Dalil in district Chagi. Only one specimen was measured while the other was released. Distribution range of this species has been extended to some 450 km in west from its previously recorded site in Pakistan. Body measurements of the present specimens have been found different from the measurements given by Roberts (*The Mammals of Pakistan*, pp. 424, Oxford University Press, 1997).

Rhombomys opimus
(Fig. 1)

It is a female of total length, 205 mm; head and body, 96 mm; tail, 109 mm; hind foot, 28.5 mm; ear length, 10 mm; weight, 43.7 g.

Description

It is a large heavy rodent having black eyes with small white patch behind. Ears are small with hairs on inner and outer surface with white patch

behind. It has long and numerous vibrissae. Upper incisor have two longitudinal grooves. The body fur is soft and grayish-brown while belly fur is whitish in colour. The forelimb bears a vestigial thumb that differentiates it from all other species of Genus *Meriones*. The tail is uniformly coloured with tuft of dark brown hairs at the distal one-third portion of the tail.

Fig. 1. *Rhombomys opimus* Lichtenstein,



1823; Great Gerbil, or Giant Day Jird.

Comments

The current record of *Rhombomys opimus* from west of Chagi extends the range of this species further west in Pakistan. It has a limited distribution along Pak-Afghan border in Chagi District of Balochistan.

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