

**ANALGESIC AND ANTI-MICROBIAL ACTIVITY OF *FAGONIA INDICA***

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**Summary**

The objective of the present study was to evaluate the anti-microbial and analgesic activity of the ethanol and aqueous extract of *Fagonia indica* leaves extracts. Anti-microbial study of ethanol extract of *Fagonia indica* leaves extracts (25, 50 and 100 mg/ml) were tested against gram negative and gram positive bacterial strains by observing zone of inhibition. The bacteria used in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus cereus* (ATCC 6633). Analgesic activity of various solvent extracts (200 and 400 mg/kg) of *Fagonia indica* was studied by tail flick method in rats. The results were analyzed statistically by regression method. The result was shown that the ethanol extract showed significant inhibitory effect against all bacterial strains but it showed maximum inhibitory effect against *Bacillus cereus* and minimum inhibitory effect against *Pseudomonas aeruginosa*. In the analgesic activity both extracts (ethanol and water) were shown significant ( $p < 0.05$ ) analgesic activity. So the extracts were shown significant anti-microbial and analgesic activity successfully.

**Keywords:** Analgesic, Antimicrobial, *E. Coli*, *Fagonia*

### Introduction

Human infections particularly those involving microorganisms i.e., bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases. Over the last centuries, intensive efforts have been made drugs (1-3).

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause (4). There is growing evidence that crude drug used in traditional medicine as remedies, mostly for arthritic diseases & for hyper pyrexia, possess analgesic & anti-inflammatory effects (5). The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficacy of traditional medicine (6). So world Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases (7).

The plant *Fagonia indica* belongs to the family Zygophyllaceae (8). *Fagonia indica* is a very common plant, widely distributed in India and Pakistan (9). The aqueous decoction of leaves and young twigs is a popular remedy for the treatment of skin lesions (boils and abscess) particularly amongst children. It is described as astringent and a cure for any disorder arising from poisoning. It is reputed in the indigenous system of medicine as a tonic, febrifuge and prophylactic against small pox (10).

*Fagonia indica* is found to have anti-cancer (11), anti-fungal (12) and anti-inflammatory (13) (alcoholic extract) activities.

The proposed pharmacological investigation has not been reported for the leaves of this plant. Therefore, our present aim was to investigate and evaluate the analgesic and anti-microbial activity of the *Fagonia indica*.

### Material and Methods

**Solvent and Chemicals:** Solvents viz. petroleum ether, benzene, chloroform, acetone, ethanol (95%), n-butanol and Di-methyl formamide (DMF) were used. For nutrient agar peptone, yeast and Di-potassium di-hydrogen phosphate were used. All the chemical and solvent were procured from local industry of india. All the chemical and drug used in the study were of analytical grade and commercially available.

**Plant Material:** The plant material (leaves) was collected from the Central Arid Zone Research Institute (CAZRI) and nearby areas of Jodhpur (Rajasthan), India.

The botanical identity of this plant was confirmed by the Dr. N.S. Shekhawat, Head of the Department of Botany, Jai Narayan Vyas University, Jodhpur, (Raj.), India. The specimen was deposited in the museum of the Department of Pharmacognosy, Jaipur National University, Jaipur-302025, (Raj.), India.

**Preparation of Extracts:** The plant material was dried and a coarse powder was prepared. It was soaked in ethanol and kept for 7 days with occasional shaking. After seven days it was filtered and the solvent was evaporated to get the concentrated extract. Likewise for aqueous extract the plant material was soaked in water instead of ethanol and the whole process was repeated. The ethanolic extract was syrupy in consistency and dark greenish in colour while the aqueous extract was syrupy in consistency and dark yellowish in colour.

**Preliminary Phytochemical Screening:** Preliminary phytochemical screening was carried out by using standard procedures described by Kokate (1986b) (14) and Harborne (1998) (15).

**Test Organisms:** Bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus cereus* (ATCC 6633).

#### ***Anti-microbial Activity by Cup Plate Method***

The antimicrobial activity of the extract was assessed by disc cup plate method (16-17). Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into Petri dishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* were spread over the media with the help of a sterile swab soaked in bacterium and is used for antibacterial study. Newly synthesized compounds (Test compounds) were dissolved in Di-methyl formamide (DMF) to produce three concentration 25, 50 and 100 µg /ml, which were used for the study. Ciprofloxacin (5 µg /ml) was used as the standard. Then cup was made with the help of sterile glass borer of size 6 mm and capacity 20 µl in solidified agar in such a way that there is no overlapping of zone of inhibition and different concentrations of each compound and standard drug were placed in each separate Petri dish. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism's inoculated Petri dishes were incubated at 37 °C for 24 hours. After the incubation period, the zone of inhibition produced by the samples and standard were measured.

**Animals:** Adult Albino rats of either sex weighing 150–200 g bred in the animal house in the School of Pharmaceutical Sciences, Jaipur National University, Jaipur were housed in

a controlled room with a 12 h light-dark cycle, at room temperature of  $22\pm 02$  °C, humidity 30-60%, and kept on standard pellet diet (altromin pellets) and water *ad libitum*. Animal maintenance and handling were in accordance to internationally accepted standard guidelines for use of laboratory animals. Animals kept under fasting for overnight, but allowed for free access of water before commencement of experiments. The experiment were conducted according to the guidelines and ethical norms, approved by Ministry of Social Justice and Empowerment, Government of India and the study was got approved from the Institutional Animal Ethical Committee (IAEC). The no. is 010/2009/CPCSEA/JNU (Approval no. 1054/ac/07/CPCSEA) of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

**Acute Toxicity Studies:** The acute toxicity test ( $LD_{50}$ ) of extracts were determined according to the OECD guidelines no. 420 (Organization for Economic Corporation and Development). Adult albino rats (180-200 g) of either sex were used. Starting dose of  $2000\text{ mg kg}^{-1}$  (P.O) of each extract was given to three groups (n=6) each. The treated animals were monitored for 14 days for mortality and general behavior. No death was observed till the end of study. The extracts were safe up to the dose of  $2000\text{ mg kg}^{-1}$  and from results suitable dose was chosen for each activity in each extract for further experimentation (18).

#### ***Analgesic Activity by Tail Flick Method***

The albino rats were divided into six groups consisting of six animals each group. Group I received 0.2 ml of 2% CMC suspension orally as a control group, group II received  $200\text{ mg/kg}$ ; P.O. of ethanolic extract of *Fagonia indica*, group III received  $400\text{ mg/kg}$ ; P.O. of ethanolic extract of *Fagonia indica*, group IV received  $200\text{mg/kg}$ ; P.O. of aqueous extract of *Fagonia indica*, group V received  $400\text{mg/kg}$ ; P.O. of aqueous extract of *Fagonia indica* and group VI received pethidine as a standard drug. A cut of period 10 sec. was observed to prevent damage to the tail. The reaction time was recorded using tail flick analgesiometer at 0, 15, 30, 45, 60 and 120 min time interval after drug administration (19). The temperature was maintained at  $50\text{-}55^{\circ}\text{C}$  and data are represented in table.

#### ***Statistical Analysis***

Result are expressed as mean $\pm$ SEM, statistical significance was calculated by applying one way ANOVA.  $P<0.05$  was considered as significant.

#### **Results**

The result of phytochemical analysis of ethanolic and aqueous extracts of *Fagonia indica* were shown that flavonoids, terpenoids, saponins, glycosides and amino acids were present in both the extracts but alkaloids, tannins, steroids, fat, gum and proteins were absent (Table 1). In the present study the phytochemical occurring in the various solvent extracts of plant leaves (ethanolic and aqueous) were analyzed qualitatively by phytochemical screening. These phytochemicals present in this study may be responsible for the antibacterial and analgesic activity of plant leaves extracts.

**Table 1.** Preliminary phytochemical screening of extracts of *Fagonia indica*

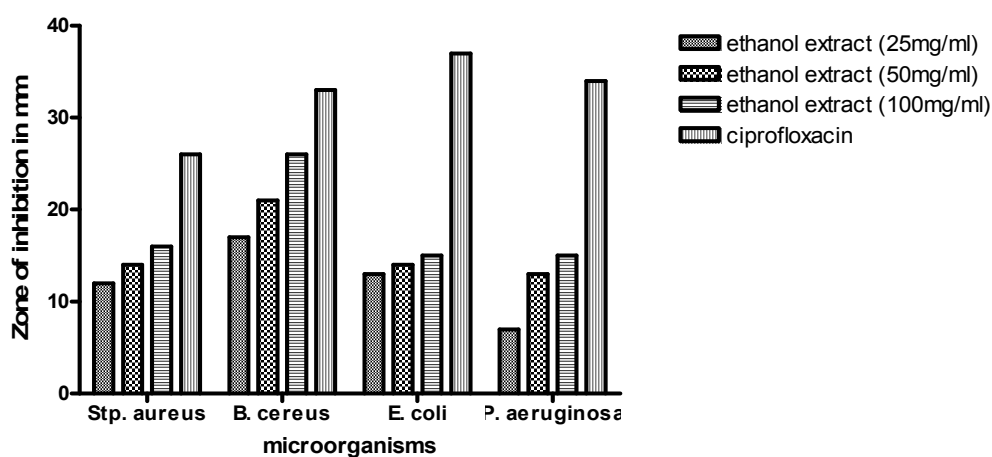
Tests	Ethanol Extract	Aqueous Extract
Alkaloid	+	+
Terpenoids	+	+
Flavanoids	+	+
Phenolics compound & Tannins	-	-
Amino Acids	+	+
Steroids	-	-
Glycosides	+	+
Fat & oils	-	-
Saponins	+	+

+ Denotes presence of respective class of compounds

The result of antibacterial activity of ethanol extract was shown inhibitory effect against all gram negative and gram positive bacteria's. But it showed maximum inhibitory effect against *Bacillus cereus* and minimum inhibitory effect against *Pseudomonas aeruginosa* (Table 2) (fig. 1). Because every medicine have his own spectrum against micro-organisms so *that plant also* have his own spectrum against bacteria's so we can say that it is more effective against *Bacillus cereus* and less effective against *Pseudomonas aeruginosa*.

**Table 2.** Anti-microbial activity of ethanolic extract of leaves of *Fagonia indica* against gram positive and gram negative bacteria.

Bacterial Strains	Diameter of zone of inhibition in mm of <i>Fagonia indica</i>			
	Ethanol extract of leaves			Standard
	25 mg/ml	50 mg/ml	100 mg/ml	Ciprofloxacin 5 µg/ml
Gram Positive Bacteria				
<i>Staphylococcus aureus</i> (ATCC 29213)	12	14	16	26
<i>Bacillus cereus</i> (ATCC 6633)	17	21	26	33
Gram Negative Bacteria				
<i>Escherichia coli</i> (ATCC 25922)	13	14	15	37
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	07	13	15	34

Anti-microbial activity of ethanolic extract of *Fagonia indica* at various concentrations

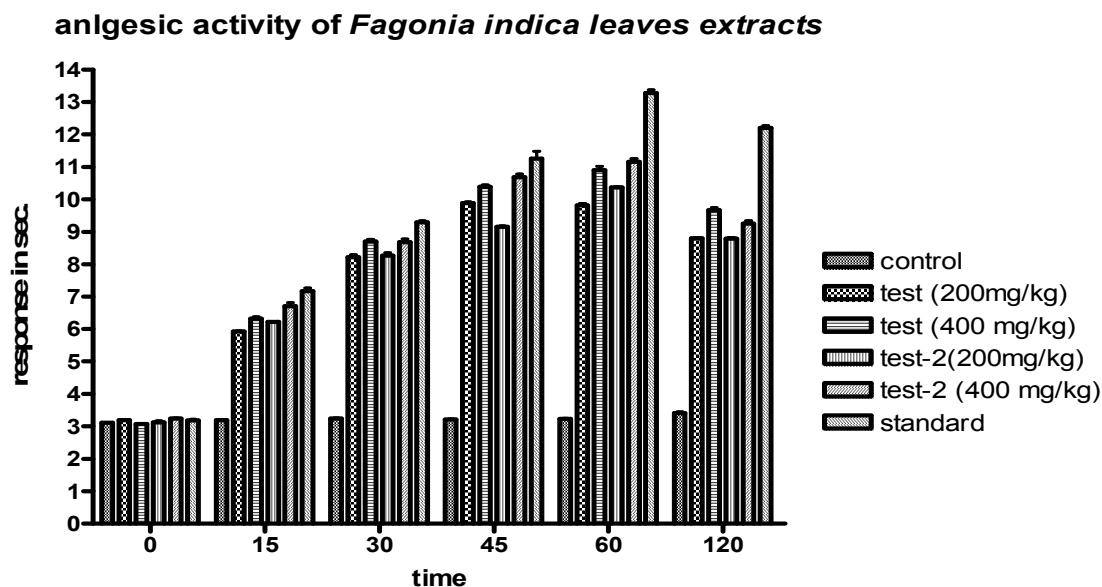
**Fig.1** Anti-microbial activity of ethanolic extract of *Fagonia indica* at various concentrations. Where Ciprofloxacin (5µg/ml) as a standard. The results are expressed in between Microorganism Vs Zone of inhibition in mm.

**Table 3.** Effects of various extracts of *Fagonia indica* on thermally induced pain in mice

Treatment/ Dose (mg kg <sup>-1</sup> )	0 min	15 min.	30 min.	45 min.	60 min.	120 min.
Control (I) (5 ml kg <sup>-1</sup> )	3.110 ±0.0058	3.187±0.018	3.237±0.020	3.203±0.026	3.223±0.026	3.403±0.049
Standard ( 5 mg kg <sup>-1</sup> )	3.180±0.038*	7.170±0.091*	9.297±0.050*	11.25±0.23*	13.28±0.092*	12.20±0.071*
Ethanolic Extract (200 mg kg <sup>-1</sup> )	3.127±0.037	6.22±0.012	8.263±0.090	9.147±0.029	10.36±0.026	8.79±0.036
Ethanolic Extract (400 mg kg <sup>-1</sup> )	3.237±0.019	6.70±0.12	8.677±0.096	10.683±0.10	11.16±0.102	9.25±0.097
Aqueous Extract (200 mg kg <sup>-1</sup> )	3.19±0.015	5.923±0.020	8.22±0.067	9.88±0.040	9.813±0.059	8.797±0.026
Aqueous Extract (400 mg kg <sup>-1</sup> )	3.067±0.009	6.317±0.064	8.703±0.064	10.383±0.062	10.900±0.12	9.667±0.084

Results are expressed as Mean±SEM. p<0.05(Dunnett's test), \* p<0.01, when compared with control

For determination of the analgesic activity we have used tail flick method. The analgesic activity profile of ethanolic extract and aqueous extract at different doses (200mg/kg and 400 mg/kg) showed significant ( $P < 0.05$ ) analgesic activity when compared to control. But ethanolic extract was slightly more effective against algesia compared to aqueous extract (Table 3) (fig.2).



**Fig.2** Analgesic activity of various solvent extracts of *Fagonia indica*. Where test (200mg/kg) and test (400mg/kg)=aqueous extract, test-2 (200mg/kg) and test-2 (400 mg/kg)= ethanolic extract and standard= pethidine. The results are expressed in between response in sec Vs time in min. Each value represents mean  $\pm$ S.E.M.

### Discussion

The ethnobotanical approach assumes that the popular uses of plants can offer strong clues to the biological activity of plants. The high percentage of positive results found in this and previous studies (20) shows that this approach is also promising for antimicrobial activity. The result of the present study reveals the fact that the organic solvent extract (Ethanolic extract) exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (21-22). Research into the effects of local medicinal plants is expected to boost the use of these plants in the therapy against disease caused by the test bacterial species and other micro-organisms. It is possible that better therapy for many microbial diseases can be found in the leaves extracts. The preliminary results of this investigation indicates that *Fagonia indica* leaves have good potential of antimicrobial activity.



Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain (23-24). The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic. The study suggests that flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception (25). The ethanol and aqueous extracts of *Fagonia indica* leaves exhibited a significant and dose dependent suppression of pain induced by thermally induced pain by analgesiometer.

Thus, we concluded that the crude leaves extracts of *Fagonia indica* produced significant antibacterial activity and analgesic activities in dose dependent manner.

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