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PHYTOCHEMICAL ANALYSIS AND BIOACTIVITIES OF DIFFERENT ORGANS OF *BUNIUM FERULACEUM* SM.

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Anti-inflammatory activity

Bovine serum protein denaturation assay

Effect of hydro-methanolic extracts of *B. ferulaceum* different organs on heat-induced bovine serum albumin (BSA) denaturation assay was carried out using a method described by [23] with minor modifications. Briefly, 0.45 mL of a 0.5% BSA solution was combined with 0.05 mL different concentrations of extracts, or standard drug diclofenac sodium (150, 250, 350 and 500 µg/mL). The reaction mixtures were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 70°C for 5 min. After cooling, 2.5 mL of phosphate buffer (pH= 6.4) was added. The absorbance was measured at 660 nm using UV-Vis spectrophotometer (Camspec M550). The test control signifies total denaturation of the protein. The percentage inhibition of BSA denaturation was calculated using Eq.(1).

$$\% \text{ Inhibition of BSA denaturation} = 100 \times [\, 1 - (A_2 / A_1)]. \quad (1)$$

where, A_1 - absorbance of the control;
A_2 - absorbance of the test sample.

Haemolytic assay

The effect of extracts on hypotonicity-induced erythrocyte membrane haemolysis assay was performed following the method described by [24]. A healthy donor provided fresh blood (5 mL), which was collected in a heparinized tube and subjected to centrifugation at 3000 rpm (rpm) for 10 min and the supernatant was carefully removed while the packed red blood cells were washed in freshly prepared isohaline solution (0.85% NaCl). Afterwards, the blood was washed and centrifuged repeatedly until the supernatant became clear. Stock red blood cell (10% v/v) was prepared in isosaline solution. The assay mixture contained 1 mL sodium phosphate buffer (pH= 7.2–7.4), 2 mL hyposaline solution (0.36% w/v NaCl), 0.5 ml stock red blood cell suspension (10%, v/v) with 0.5 mL of extract or diclofenac sodium of varying doses (300, 500, 750 and 1000 µg/mL) in test tubes. For the control, distilled water replaced hyposaline solution to induce 100% haemolysis. The different test tubes were incubated at 56°C in a water bath for 30 min and then centrifuged at 5000 rpm. Each tube's absorbance reading is recorded with a UV-visible spectrophotometer (Camspec M550) at 560 nm. The rate of haemolysis is calculated as a percentage relative to the haemolysis total, according to the the Eq.(2).

$$\text{Haemolysis rate (\%)} = 100 - [(\text{optical density of extract/optical density of control}) \times 100]. \quad (2)$$

Antibacterial activity

The extracts were tested against 7 conditional-pathogenic bacteria, of which three were Gram- positive; *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus cereus* (ATCC11778) and four were Gram-negative; *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (MTCC 1588), *Enterobacter cloacae* (ATCC 23355) and *Klebsiella pneumonia* (MTCC 7162).

The antibacterial effectiveness of the extracts was assessed using the disk diffusion method and agar dilution assays. On Mueller–Hinton (MH) agar plates, 100 µL of a bacterial suspension containing approximately 10^8 CFU/mL was spread evenly. Sterile paper discs (6 mm in diameter) were then impregnated with 10 µL of each extract (25 mg/mL) and placed on the agar surface. Ciprofloxacin (10 µg/disc) was used in its pure commercial form as a positive control, following the [25] recommendations. The plates were incubated at 37°C for 24 hours, and antibacterial activity was expressed as the diameter (mm) of the inhibition zone around each disc. The minimum inhibitory concentration (MIC)

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was determined by the direct contact method [26]. The extracts were serially diluted to obtain final concentrations ranging from 0.1 to 25 mg/mL. After 24 hours of incubation at 37°C, the MIC was defined as the lowest concentration of extract showing no visible bacterial growth. The lowest concentration of extract at which the incubated bacteria were entirely killed was deemed as the minimum bactericidal concentration (MBC).

Table S1

Correlation analysis and significance between phytochemical contents and anti-inflammatory activity (IC_{50} values).

Phytochemical	*r (BSA denaturation)*	*p (BSA)*	*r (Haemolysis)*	*p (Haemolysis)*
Phenolics	0.16	0.895	–0.43	0.714
Flavonoids	0.18	0.887	–0.42	0.723
Tannins	0.23	0.850	–0.37	0.759
Triterpenes	–0.97	0.154	–0.93	0.237

r: Correlation coefficients.
p: Significance values.
r between the two assays: A significant positive correlation was observed (r= 0.82).

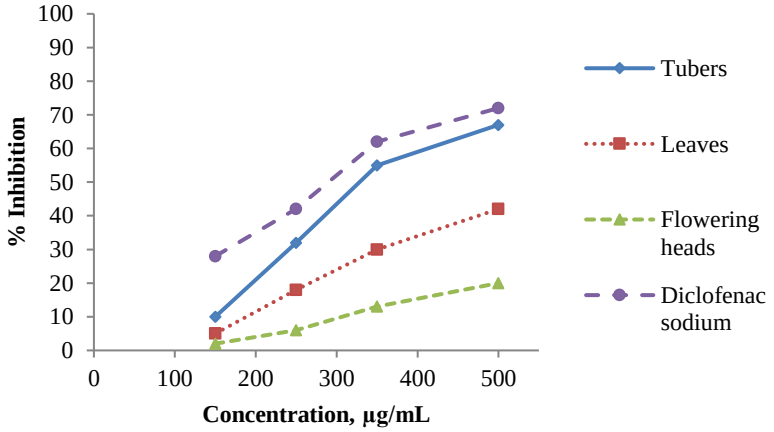

Figure S1. Effect of hydro-methanolic extracts of *B. ferulaceum* parts and standard on heat-induced BSA denaturation (mean±SD, n= 3): Leaves, flowering heads, tubers and diclofenac sodium.

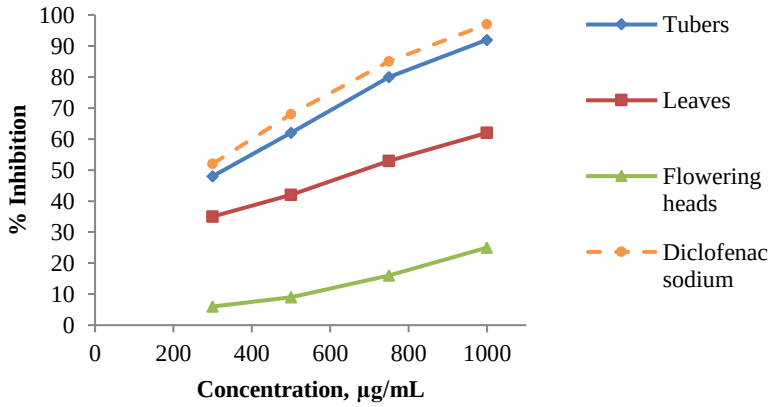

Figure S2. Effect of hydro-methanolic extracts of *B. ferulaceum* parts and standard on inhibition of haemolysis (mean±SD, n= 3): Leaves, flowering heads, tubers and diclofenac sodium.

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Table S2

Inhibition zones, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of hydro-methanolic extracts of *B. ferulaceum* against bacteria tested.

	Leaves			*Tubers*			*FH*			*Ciprofloxacin*		
Bacteria	IZ*	MIC	MBC	IZ*	MIC	MBC	IZ*	MIC	MBC	IZ*	MIC	MBC
					Gram+ bacteria							
S. aureus	25.15±0.12	0.75	1.02	18.16±0.44	1.95	2.22	10.15±0.17	9.04	>25	28±0.30	0.03	0.30
E. faecalis	18.26±0.51	0.92	1.75	14.54±0.18	2.12	2.45	11.39±0.33	8.55	>25	32±0.04	0.02	0.30
B. cereus	12.40±0.62	3.67	5.12	10.17±0.20	5.25	5.93	6.55±0.38	>25	>25	22±0.12	0.06	0.50
					Gram– bacteria							
P. aeruginosa	18.65±0.21	11.9	15.2	11.65±0.21	15.65	>25	na	na	na	12±0.22	1	1.2
E. coli	8.33±0.16	>25	>25	na	na	na	na	na	na	9±0.13	0.06	0.65
E. cloacae	9.84±0.16	>25	>25	na	na	na	na	na	na	10±0.55	0.3	1
K. pneumoniae	8.14±0.19	>25	>25	na	na	na	na	na	na	8±0.08	0.25	0.90

na: no antibacterial activity. IZ: Inhibition zone in diameter (mm) around, MIC and MBC values given as mg/mL.
*Means are statistically different at a level of significance of 5%.