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Evaluating the efficacy of *Tribulus terrestris* on pH dependent uric acid crystallization under experimental condition

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Supplementary Data

Phenolic content estimation

The total phenolic content (TPC) of *Tribulus* extract was determined using a modified Folin-Ciocalteu assay[1]. Folin-Ciocalteu reagent is a mixture of phosphomolybdic and phosphotungstic acids. When the phenolic content in the extract reacts with this mixture under alkaline condition it will result in the formation of blue coloured complex which can be quantified by spectrophotometry[2]. A 200 µL aliquot of the extract was mixed with 5 mL of 10% (w/v) Folin-Ciocalteu reagent[3]. After 5-minute incubation, 4 mL of 7% Na_2CO_3 solution was added, followed by 2 mL of distilled water. The mixture was then stored in the dark at 40°C for 60 min, after which the absorbance was recorded at 765 nm[1,3,4]. Gallic acid (1 mg/mL) was used as the reference standard[1,5]. To construct the standard curve, varying concentrations of gallic acid solutions (50–500 µg/mL) were prepared, and the same procedure was followed. The TPC of the extracts was expressed in milligrams of gallic acid equivalents (GAE) per gram of dry weight sample (mg/g), calculated according to the formula outlined in[1].

$$C = x \times \left(\frac{V}{m}\right) \quad \ldots \quad (1)$$

Where,

C= TPC (mg GAE/g dry extract)
x = concentration from calibration curve
V= extract volume
m = mass of extract (g)

The coefficient of determination (R^2) for the standard curve obtained ($y = 0.0012x$) was 0.9946, indicating good linearity in the investigated concentration range.

Suppl. Table S1 — Phytochemical analysis of *Tribulus* extract

Phytoconstituents	Aqueous extract
Phenol	+
Alkaloid	-
Flavonoid	+
Saponin	+
Coumarin	-
Sterols	+
Glycoside	-

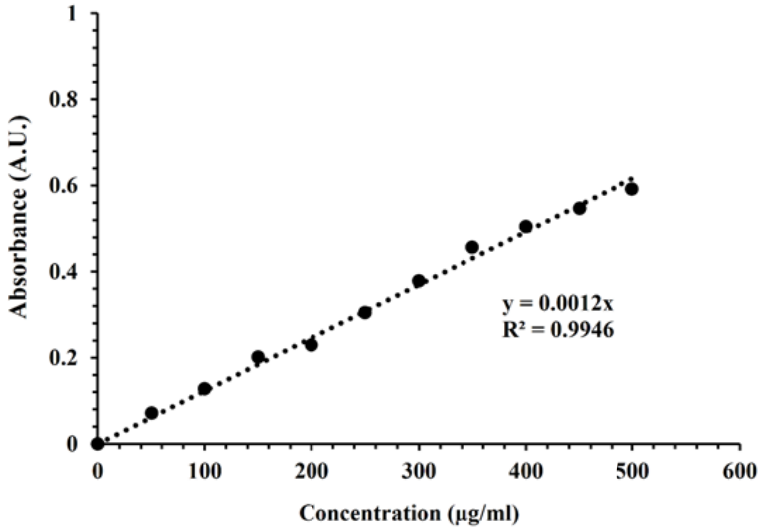

Fig. S1 — Standard curve for estimation of total phenolic content. The absorbance of the solution was measured at 765 nm

Flavonoid content estimation

The total flavonoid content (TFC) of *Tribulus* extract was measured using the aluminium chloride ($AlCl_3$) method[6]. Quercetin served as the reference standard[7], with quercetin solutions ranging from 100–500 µg/mL prepared to construct the standard curve. The principle of $AlCl_3$ method for flavonoid content determination is in the creation of a complex between $AlCl_3$ and flavonoids, specifically flavones and flavonols resulting in colour change. In the assay, 500 µL of the extract was combined with 400 µL of ethanol, followed by the addition of 500 µL of 2% $AlCl_3$ ethanolic solution. The mixture was thoroughly mixed and incubated at room temperature (25°C) for 40 min. Absorbance was then measured at 510 nm, and the TFC was expressed in milligrams of quercetin equivalents per gram of dry weight sample (mg/g). The coefficient of determination (R^2) for the standard curve obtained (y = 0.0014x) was 0.9729.

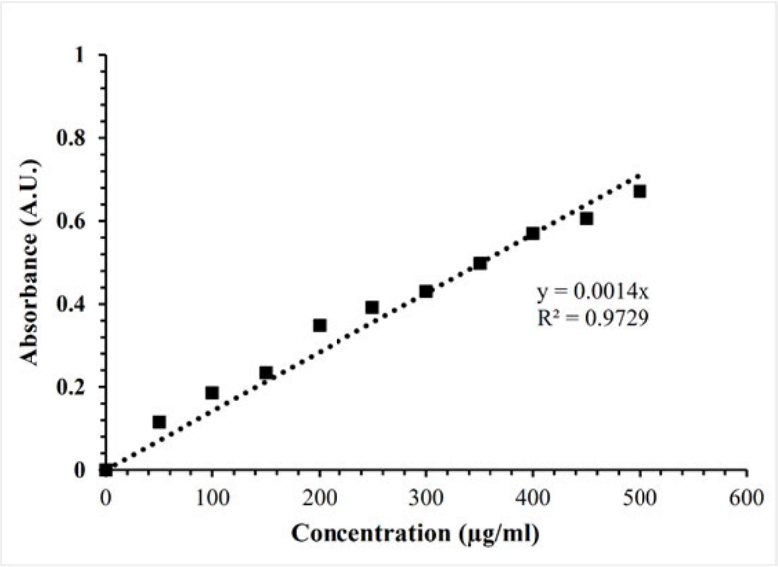

Fig. S2 — Standard curve for estimation of total flavonoid content. The absorbance of the solution was measured at 510 nm

Total antioxidant capacity

The total antioxidant capacity (TAC) was determined using a modified phosphomolybdate assay[8]. The TAC by phosphomolybdate method is based on the principle that the antioxidants present in the extract will reduce the molybdenum (VI) to molybdenum (V) which gives a green coloured complex[9]. The phosphomolybdenum reagent was prepared by dissolving 0.168 g of NaH_2PO_4, 1.6 mL of H_2SO_4,

and 0.247 g of ammonium molybdate, and adjusting each solution to a final volume of 50 mL. The resulting solutions were combined to obtain a total volume of 150 mL. Subsequently, 600 µL of the extract was mixed with 3 mL of the reagent and incubated in a water bath at 90°C for 90 min. After cooling, the absorbance of the mixture was measured at 690 nm. Ascorbic acid was used as the standard, and the calibration curve was constructed using absorbance values from ascorbic acid concentrations ranging from 100 to 1000 µg/mL. The TAC was expressed in milligrams of ascorbic acid equivalents (AAE) per gram of dry sample, reported as mg AAE/g. The coefficient of determination (R^2) for the standard curve obtained (y = 0.001x) was 0.949.

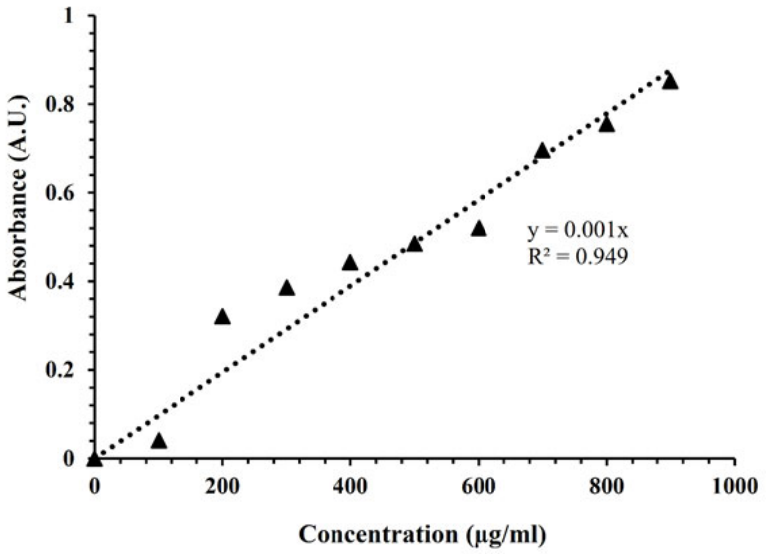

Fig. S3 — Standard curve for estimation of total antioxidant capacity. The absorbance of the solution was measured at 690 nm

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