SUPPLEMENTARY MATERIAL

'Evaluation of the Antioxidant and Anti-Inflammatory Activities of Solvent Extracts of *Tricholepis chaetolepis* (Boiss) Rech. f. Whole Plant'

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Abstract

The whole plant, *Tricholepis chaetolepis*, powder was investigated using proximate and fluorescence analysis along with determining the extractive values. Total phenolics, flavonoids and total protein content of n-hexane, chloroform and methanolic extracts of the whole plant were also determined. The anti-diabetic activity of all the three extracts of the plant was determined by *in vitro* alpha-amylase inhibition assay. The anti-oxidant potential was evaluated using Phosophomolybdenum and DPPH methods. The anti-inflammatory potential of all extracts was determined by carrageenan-induced rat paw oedema model. The evaluation of the plant extracts exhibited the anti-diabetic, anti-oxidant, and anti-inflammatory activities in dose dependent fashion. The research concludes that *Tricholepis chaetolepis* extracts contain phenol, flavonoids, and tannins that show observable anti-oxidant and anti-diabetic potential. It is also concluded that the methanol extract of the plant showed the maximum effect against inflammation induced by carrageenan in rat paw oedema as compared with n-hexane and chloroform extracts.

Key words: *Tricholepis chaetolepis*, anti-diabetic, anti-oxidant, inflammation

1. EXPERIMENTAL

1.1 MATERIALS AND METHODS

Tricholepis chaetolepis was purchase form the local nurseries in Quetta (Baluchistan) and authenticated by Prof. Zaheer-ud-Din of Botany Department, GC University Lahore, Pakistan. A voucher specimen number GC.Herb.Bot.3323 was kept in Department of herbarium for further reference.

The chemicals and reagents used were of analytical grade while the water used was glass distilled.

1.2 Preparation of plant extracts

The plant was washed under the tap to remove all contaminants and grounded into a fine powder. The 100 grams plant powder was taken in a cellulose extraction thimble and the thimble was inserted into the glass Soxhlet assembly fitted with 250 ml flask. Plant powder was extracted by n-hexane followed by chloroform and

methanol. Rotary evaporator was utilized to evaporate the surplus solvent and then 3 extracts were obtained and dried.

1.3 Phytochemical screening

The standard methods were used to access the phytochemical tests of each extract of the plant (Akhtar et al. 2016)

1.4 Physicochemical properties

The standard methods were used to access the physicochemical parameters of the finely divided plant powder The physicochemical parameters include foaming index, extractive values (methanol, n-hexane, and chloroform), ash values (acid insoluble ash, water soluble ash), swelling index, moisture content (loss on drying) and fluorescence studies (Ghosh et al. 2017).

1.5 Proximate analysis

The standard methods were used to access the proximate analysis in the plant with slight modifications The proximate analysis involves the calculation of crude protein percent, total ash percent, crude fiber percent, moisture content percent and crude fat (Goyeneche et al. 2015).

1.6 Determination of total phenol content

The standard methods were used to access total phenol content within all extracts of the plant with slight modifications (Russo et al. 2015). Total phenolic content was estimated by Folin Ciocalteu's method. 0.2 ml from the aliquots and standard gallic acid (10, 20, 40, 80, 100, 120 ug/ml) was positioned into test tube and 0.2 ml of Folin-Ciocalteau reagent was mixed and shaken. After 5 minutes, 1 ml 15% Na_2CO_3 solution was added and volume was made to 3 ml by methanol.

It was allowed to incubate for 2 hours at room temperature. Intense blue color was developed. After incubation, absorbance was measured at 760 nm using UV-visible spectrophotometer. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents present in each extract was expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass.

1.7 Determination of total flavonoid content

The standard methods were used to access the total flavonoid content within all the extracts of the plant with slight modifications (Ullah et al. 2016). Total flavonoid content was measured with the aluminium chloride colorimetric assay. 0.2 ml of aliquots and 0.2 ml standard quercetin solution (10, 20, 40, 80, 100, 120 ug/ml) was positioned into test tube and 0.8 ml of methanol. After 5 minutes, 0.1 ml of 10% Aluminium chloride solution, 0.1 ml of 1 M Potassium acetate solution and 3.8 ml of distilled water were added. Orange yellowish color was developed. The absorbance

was measured at 415 nm using UV-visible spectrophotometer. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin. The data of total flavonoids present in each extract were expressed as mg of quercetin equivalents/ 100 g of dry mass.

1.8 Determination of total protein content

The standard methods were used to access the total protein content within all the extracts of the plant with slight modifications (Pandey et al. 2015)[6].

1.9 Phosphomolybdenum method

The standard phosphomolybdenum complex formation methods were used to access antioxidant potential of all extracts of the plant with slight modifications (Woźniak et al. 2015). 0.1 ml of sample (100 ug) solution is combined with 4 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95°C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 695 nm against in UV spectrophotometer. A typical blank solution contained 4 ml of reagent solution and the appriate volume of the same solvent used for the sample and it is incubated under same conditions as rest of the sample. Ascorbic acid was used as reference standard. The antioxidant activity is expressed as the number of equivalents of ascorbic acid (AscAE).

1.10 Evaluation of antioxidant activity by DPPH radical scavenging method

The standard methods of DPPH scavenging assay were used to access in-vitro antioxidant potential of all extracts with slight modifications (Ahmed et al. 2017). In brief, 0.1mM solution of DPPH in methanol was prepared. This solution (2 ml) was added to 1 ml of different extracts in methanol at different concentration (125, 250, 500, 1000 ug/ml). The mixture was shaken vigorously and allowed to stand at room temperature for half an hour, then, absorbance was measured at 517 nm by using UV-VIS spectrophotometer. Reference standard compound being used was ascorbic acid and an experiment was done in triplicates. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. The lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using the following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$.

Where A_0 was the absorbance of control reaction and A_1 was the absorbance in the presence of test or standard sample.

1.11 *In vitro* inhibitory alpha amylase assay

The standard methods were used to access the *in vitro* inhibitory alpha amylase assay of all the extracts of the plant with slight modifications (Franco et al. 2017). The 1 ml of plant extracts was incubated with 1 ml of alpha amylase (Sigma Aldrich) and 2 ml of phosphate buffer solution at room temperature (32°C) for about 30 minutes. After incubation, 1 ml of of 1 % starch solution (dissolving 1 g of potato starch in 100 ml of distilled water with boiling and stirring for 15 minutes) was added and was incubated at room temperature (32°C) for about 15 minutes. To the above, 1 ml of DNSA reagent was added to stop the reaction and was incubated in hot water bath (85°C) for 5-8 minutes. After 5 minutes, reaction mixture color changed to orange-red and was removed from water bath and cooled to room temperature.

It was diluted up to 10 ml of distilled water. Extracts at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mg/ml) were performed in triplicates. Individual blank was performed by replacing enzyme with buffer. Control was performed by replacing extract with solvent. Acarbose (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mg/ml) was used as standard. Absorbance was measured at 540 nm in spectrophotometer. Enzyme unit is defined as one unit of enzyme will liberate 1mg of maltose from 1 % starch in 5 minutes under defined condition i.e. room temperature. Logarithmic regression curve was established by plotting percentage of alpha amylase inhibition against sample concentration in order to calculate IC50 (inhibitory concentration) value. This represents sample concentration (mg/ml) required to decrease the absorbance by 50 % of alpha amylase

The inhibition percentage of alpha amylase was calculated by the following formula.

The alpha-amylase inhibitory activity = Ac – Ae or A_s /Ac x100

Where,

Ac= Absorbance of the control

A_e = Absorbance of extract

A_s = Absorbance of the standard

1.12 Anti-inflammatory activity

The standard methods for carrageenan-induced rat paw oedema were used to access anti-inflammatory potential of all extracts with slight modifications (Akhtar et al. 2016), (Saleem et al. 2017). Rats used in this experiment were divided into eleven groups of six animals each and were treated with DMSO, n-hexane extract (250, 500 and 1000 mg/kg, p.o.), chloroform extract (250, 500 and 1000 mg/kg, p.o.), methanol extract (250, 500 and 1000 mg/kg, p.o.), and diclofenac (100 mg/kg, p.o.) respectively. Half an hour after administration of the various agents, oedema was induced by injection of carrageenan (0.1 ml, 1 % w/v in saline) into the sub-plantar

tissue of the right hind paw. The paw oedema was measured by using digital plethysmometer at 1 h interval for 5 h.

$$\% \text{ Inhibition} = \frac{\text{Increase in paw oedema control} - \text{Increase in paw edema test}}{\text{Increase in paw oedema control}} \times 100$$

2. Results

2.1 Calculation of phenolic content:

The amount of total phenolic content was expressed in µg / ml. Phenolic content were determined using following formula.

$y = 0.016x + 0.0306$ $R^2 = 0.9963$;

linear equation obtained from standard curve of gallic acid

For example, the absorbance for methanol is 0.705

$x = 0.705 – 0.0306 / 0.016$

$x = 42.15$

Table S1: Absorbance of gallic acid by Folin –Ciocalteu's Reagent method at λmax =760nm

Gallic acid Conc. (µg/ml)	**Absorbance**
10	0.12 ± 0.010
20	0.40 ± 0.010
40	0.70 ± 0.017
80	1.31 ± 0.017
100	1.65 ± 0.010
120	1.92 ± 0.017

TableS2: Absorbance of *Tricholepis chaetolepis* n-hexane, chloroform and methanol extracts by Folin – Ciocalteu's Reagent method at λmax =760nm.

Extracts		Mean
n-Hexane extract	1	0.293
	2	0.296
	3	0.295
Chlorofrom extract	1	0.564
	2	0.566
	3	0.567
Methanol extract	1	0.706
	2	0.695
	3	0.713

TableS3: Total phenolic contents in n-hexane, chloroform and methanol extracts of *Tricholepis chaetolepis* by Folin – Ciocalteu's Reagent method

Extracts	Gallic acid content (mgGA/g)
n-Hexane extract	16.525 ± 0.104
Chloroform extract	33.462 ± 0.110
Methanol extract	42.15 ± 0.579

2.2 Calculation of flavonoid content:

The amount of flavonoid content were calculated in µg / ml. Flavonoid content were determined using following formula.

$y = 0.0103x + 0.0179$ $R^2 = 0.9966$;

linear equation obtained from standard curve of Quercetin (standard)

For example the absorbance for methanol is 0.439

$x = 0.439 – 0.0179 / 0.0103$

Table S4: Absorbance of standard Quercetin at λmax = 415 nm

Quercetin Conc. (µg / ml)	Absorbance
10	0.144 ± 0.003
20	0.193 ± 0.001
40	0.416 ± 0.021
80	0.861 ± 0.001
100	1.076 ± 0.007
120	1.220 ± 0.014

Table S5: Absorbance of *Tricholepis chaetolepis* n-hexane, chloroform and methanol extracts by aluminum chloride calorimetric method at λmax = 415 nm

Extract		Absorbance			Mean
		1	2	3	
n-Hexane extract	1	0.148	0.148	0.150	0.148
	2	0.150	0.151	0.154	0.151
	3	0.152	0.153	0.150	0.151
Chloroform extract	1	0.221	0.221	0.223	0.221
	2	0.221	0.225	0.224	0.223
	3	0.222	0.220	0.221	0.221
Methanol extract	1	0.440	0.439	0.438	0.439
	2	0.439	0.438	0.440	0.439
	3	0.440	0.441	0.442	0.441

Table S6: Flavonoid contents in n-hexane, chloroform and methanol extracts of *Tricholepis chaetolepis* by Aluminum chloride calorimetric method

Extracts	**Quercetin content (µg/ml)**
n-Hexane extract	12.889 ± 0.168
Chloroform extract	19.815 ± 0.116
Methanol extract	40.948 ± 0.112

2.3 Calculation of total protein content:

The amount of total protein content was calculated in µg / ml. Protein content were determined using following formula.

$y = 0.0025x + 0.0448$ $R^2 = 0.9967$;

linear equation obtained from standard curve of Bovine serum albumin (standard)

Table S7: Absorbance of Bovine serum albumin (BSA) standard curve at λmax = 660nm

BSA Conc. (µg/ml)	**Absorbance**
5	0.050 ± 0.001
10	0.079 ± 0.001
25	0.110 ± 0.002
40	0.142 ± 0.001
55	0.182 ± 0.002
70	0.224 ± 0.002
85	0.256 ± 0.003
100	0.299 ± 0.002

Table S8: Absorbance of *Tricholepis chaetolepis* n-hexane, chloroform and methanol extracts at λmax = 660 nm

Extract		Mean
n-Hexane extract	1	0.294
	2	0.295
	3	0.298
Chloroform extract	1	0.346
	2	0.345
	3	0.343
Methanol extract	1	0.369
	2	0.370
	3	0.370

Table S9: Total protein contents in n-hexane, chloroform and methanol extracts of *Tricholepis chaetolepis* by Folin – Ciocalteu's Reagent method

Extracts	**BSA content (µg/ml)**
n-Hexane extract	0.010 ± 0.0005
Chloroform extract	0.012 ± 0.0005
Methanol extract	0.013 ± 0.0005

2.4 Determination of total antioxidant activity by phosphomolybdenum method:

Table S10: Absorbance of *Tricholepis chaetolepis* n-hexane, chloroform and methanol extracts for total antioxidant activity by phosphomolybdenum method.

Extracts		Mean
n-Hexnae extract	1	0.071
	2	0.071
	3	0.070
Chloroform extract	1	0.115
	2	0.117
	3	0.118
Methanol extract	1	0.163
	2	0.165
	3	0.162
Ascorbic acid		0.636

Table S11: Antioxidant activity of *Tricholepis chaetolepis* n-hexane, chloroform and methanol extracts

Extracts	**Total antioxidant activity**
n-Hexane extract	0.070 ± 0.007
Chloroform extract	0.117 ± 0.001
Methanolic extract	0.163 ± 0.001
Ascorbic acid	0.636 ± 0.024

Each value of antioxidant activity is expressed as means ± S.D. (n=3) of three separate replicates

2.5 Determination of antioxidant activity by DPPH method:

The percentage inhibition was calculated by the following formula.

% inhibition = Positive control – standard or extract / Positive control x 100

For example; if the absorbance of the n-hexane is 0.600

n-hexane extract = 0.600 Control = 1.02

% inhibition = 1.02 – 0.600 / 1.02 x 100

% inhibition = 41.176 %

Table S12: Absorbance of *Tricholepis chaetolepis* n-hexane extract at λmax = 517nm

n-Hexane extract concentration (µg/ml)		Mean
125	1	0.601
	2	0.606
	3	0.599
250	1	0.562
	2	0.560
	3	0.564
500	1	0.512
	2	0.513
	3	0.511
1000	1	0.474
	2	0.473
	3	0.474

Table S13: Absorbance of *Tricholepis chaetolepis* chlororform extract at λmax = 517nm

Chloroform extract concentration (µg/ml)		Mean
125	1	0.426
	2	0.431
	3	0.431
250	1	0.349
	2	0.350
	3	0.347
500	1	0.327
	2	0.326
	3	0.325
1000	1	0.304
	2	0.301
	3	0.301

Table S14: Absorbance of *Tricholepis chaetolepis* methanol extract at λmax = 517nm

Methanol extract concentration (µg/ml)		Mean
125	1	0.331
	2	0.331
	3	0.332
250	1	0.249
	2	0.247
	3	0.250
500	1	0.207
	2	0.206
	3	0.205
1000	1	0.192
	2	0.193
	3	0.190

Table S15: Absorbance of standard ascorbic acid at λmax = 517nm

Ascorbic acid concentration (µg/ml)		Mean
125	1	0.089
	2	0.091
	3	0.090
250	1	0.059
	2	0.058
	3	0.059
500	1	0.038
	2	0.037
	3	0.039
1000	1	0.027
	2	0.027
	3	0.027
Control		1.020

Table S16: % Inhibition of n-hexane, chloroform, methanol and standard ascorbic acid by DPPH method

Concentration (µg/ml)	% Inhibition			
	n-Hexane extract	Chloroform extract	Methanol Extract	Ascorbic Acid
125	40.947 ± 0.385	57.908 ± 0.283	67.516 ± 0.056	91.144 ± 0.065
250	44.869 ± 0.181	65.784 ± 0.130	75.588 ± 0.149	94.216 ± 0.032
500	49.771 ± 0.065	68.006 ± 0.130	79.771 ± 0.117	96.242 ± 0.098
1000	53.529 ± 0.056	70.359 ± 0.172	81.176 ± 0.163	97.320 ± 0.032

2.6 Determination of in vitro alpha amylase inhibitory assay:

The percentage inhibition was calculated by the following formula.

% inhibition = Control – Standard or Extract / Control x 100

For example; if the absorbance of the n-hexane is 0.045

n-hexane extract = 0.045 Control = 0.07

% inhibition = 0.07 – 0.045 / 0.07 x 100

% inhibition = 35.714 %

Table S17: Absorbance of *Tricholepis chaetolepis* n-hexane extract at λmax = 540nm

n-Hexane extract concentration (µg/ml)		Mean
100	1	0.045
	2	0.045
	3	0.044
200	1	0.041
	2	0.040
	3	0.041
300	1	0.039
	2	0.039
	3	0.038
400	1	0.037
	2	0.037
	3	0.036
500	1	0.034
	2	0.034
	3	0.033

	1	0.031
600	2	0.030
	3	0.031
	1	0.029
700	2	0.029
	3	0.028

Table S18: Absorbance of *Tricholepis chaetolepis* chloroform extract at λmax = 540nm

Chloroform extract concentration (µg/ml)		Mean
100	1	0.029
	2	0.028
	3	0.029
200	1	0.026
	2	0.026
	3	0.025
300	1	0.021
	2	0.020
	3	0.021
400	1	0.018
	2	0.017
	3	0.018
500	1	0.015
	2	0.016
	3	0.016
600	1	0.014
	2	0.015
	3	0.014
700	1	0.013
	2	0.013
	3	0.012

Table S19: Absorbance of *Tricholepis chaetolepis* methanol extract at λmax = 540nm

Methanol extract concentration (µg/ml)		Mean
100	1	0.035
	2	0.036
	3	0.035
200	1	0.031
	2	0.031
	3	0.029
300	1	0.026
	2	0.025
	3	0.023
400	1	0.021
	2	0.023
	3	0.024
500	1	0.019
	2	0.020
	3	0.020
600	1	0.016
	2	0.017
	3	0.016
700	1	0.014
	2	0.015
	3	0.015

Table S20: Absorbance of standard acarbose at λmax = 540nm

Acarbose concentration (µg/ml)		Mean
100	1	0.025
	2	0.024
	3	0.024
200	1	0.023
	2	0.021
	3	0.021
300	1	0.016
	2	0.017
	3	0.017
400	1	0.013
	2	0.011
	3	0.012
500	1	0.008
	2	0.010
	3	0.010
600	1	0.006
	2	0.007
	3	0.007
700	1	0.005
	2	0.005
	3	0.004
Control		0.070

Table S21: % Inhibition of n-hexane, chloroform, methanol and standard acarbose by in-vitro inhibitory alpha amylase assay

Concentration (mg/ml)	% Inhibition			
	n-Hexane extract	Chloroform Extract	Methanol extract	Acarbose
0.1	35.714 ± 1.259	58.571 ± 0.824	48.571 ± 0.824	64.761 ± 0.952
0.2	41.428 ± 0.476	62.857 ± 0.476	55.714 ± 1.649	68.571 ± 1.259
0.3	44.285 ± 0.476	70.000 ± 0.952	64.285 ± 1.716	75.714 ± 0.824
0.4	47.142 ± 0.476	74.285 ± 0.824	67.142 ± 2.075	82.380 ± 1.428
0.5	51.428 ± 1.259	77.142 ± 0.476	71.428 ± 0.824	85.714 ± 1.649
0.6	55.714 ± 0.476	79.047 ± 0.476	75.714 ± 0.824	90.000 ± 0.952
0.7	58.571 ± 0.476	81.428 ± 0.824	78.571 ± 0.476	92.857 ± 0.824

Each value of in vitro alpha amylase inhibitory assay is expressed as means ± S.D. (n=3) of three separate replicates.

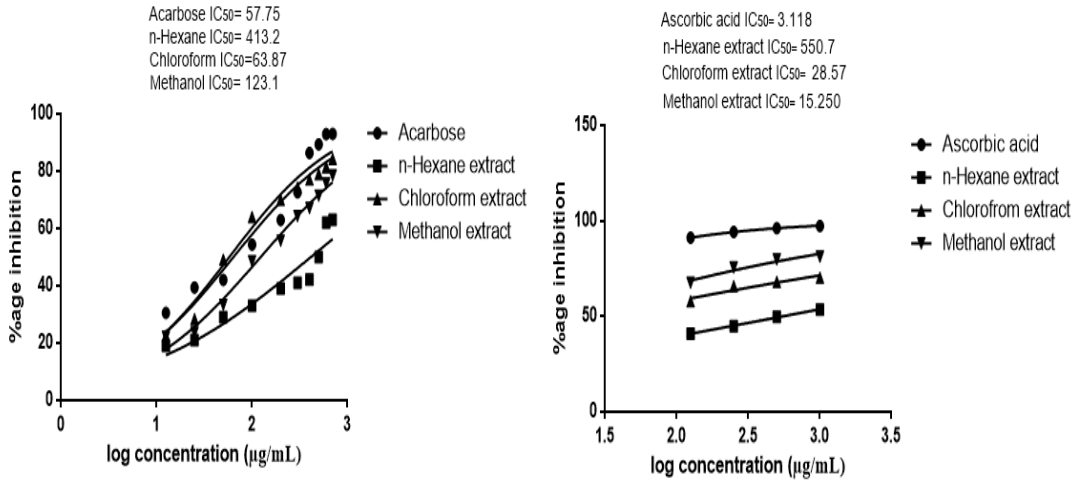

Figure S1: Graphical representation of IC_{50} of n-hexane, chloroform, methanol extracts of *Tricholepis chaetolepis* and (A) standard acarbose (B) standard ascorbic acid

2.7 Determination of anti-inflammatory activity:

The anti-inflammatory potential of the extracts of *Tricholepis chaetolepis* was accessed by carrageenan-induced rat paw oedema.

Table S22: Carrageenan-induced paw oedema: Paw volume of the rats treated with vehicle i.e DMSO (1 ml/kg)

Control group	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.49	0.73	0.97	1.08	1.00	0.92
Rat 2	0.48	0.72	0.96	1.09	1.01	0.94
Rat 3	0.46	0.72	0.95	1.11	1.02	0.92
Rat 4	0.48	0.73	0.96	1.08	1.01	0.93
Rat 5	0.47	0.72	0.97	1.09	0.99	0.92
Rat 6	0.49	0.71	0.96	1.09	0.99	0.92

Table S23: Carrageenan-induced paw oedema: Paw volume of the rats treated with standard Diclofenac sodium (100 mg/kg)

Diclofenac sodium	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.45	0.60	0.68	0.72	0.62	0.54
Rat 2	0.46	0.59	0.69	0.73	0.62	0.55
Rat 3	0.43	0.58	0.68	0.72	0.61	0.56
Rat 4	0.44	0.60	0.69	0.71	0.60	0.56
Rat 5	0.45	0.59	0.67	0.73	0.61	0.55
Rat 6	0.46	0.60	0.68	0.74	0.61	0.56

Table S24: Carrageenan-induced paw oedema: Paw volume of the rats treated with n-hexane extract (250 mg/kg) of *Tricholepis chaetolepis*

n-Hexane extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.50	0.73	0.89	0.96	0.86	0.77
Rat 2	0.48	0.70	0.88	0.94	0.86	0.78
Rat 3	0.47	0.69	0.87	0.91	0.85	0.76
Rat 4	0.46	0.64	0.88	0.93	0.82	0.74
Rat 5	0.45	0.64	0.85	0.91	0.84	0.76
Rat 6	0.44	0.63	0.87	0.90	0.86	0.79

Table S25: Carrageenan-induced paw oedema: Paw volume of the rats treated with n-hexane extract (500 mg/kg) of *Tricholepis chaetolepis*

n-Hexane extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.50	0.71	0.87	0.94	0.82	0.79
Rat 2	0.45	0.67	0.87	0.90	0.82	0.77
Rat 3	0.47	0.68	0.86	0.90	0.84	0.75
Rat 4	0.46	0.66	0.88	0.92	0.83	0.75
Rat 5	0.44	0.65	0.85	0.91	0.79	0.74
Rat 6	0.46	0.66	0.86	0.93	0.85	0.76

Table S26: Carrageenan-induced paw oedema: Paw volume of the rats treated with n-hexane extract (1000 mg/kg) of *Tricholepis chaetolepis*

n-Hexane extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.46	0.68	0.84	0.89	0.80	0.76
Rat 2	0.47	0.67	0.84	0.90	0.80	0.74
Rat 3	0.47	0.68	0.85	0.89	0.81	0.76
Rat 4	0.46	0.67	0.83	0.90	0.84	0.76
Rat 5	0.46	0.66	0.85	0.92	0.85	0.74
Rat 6	0.45	0.65	0.83	0.89	0.80	0.74

Table S27: Carrageenan-induced paw oedema: Paw volume of the rats treated with chloroform extract (250 mg/kg) of *Tricholepis chaetolepis*

Chloroform extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.46	0.67	0.85	0.90	0.81	0.72
Rat 2	0.44	0.64	0.84	0.90	0.8	0.73
Rat 3	0.45	0.66	0.83	0.91	0.78	0.71
Rat 4	0.47	0.68	0.86	0.90	0.79	0.74
Rat 5	0.46	0.66	0.84	0.92	0.78	0.73
Rat 6	0.45	0.66	0.85	0.91	0.79	0.72

Table S28: Carrageenan-induced paw oedema: Paw volume of the rats treated with chloroform extract (500 mg/kg) of *Tricholepis chaetolepis*

Chloroform extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.43	0.64	0.81	0.87	0.76	0.68
Rat 2	0.45	0.67	0.82	0.87	0.74	0.69
Rat 3	0.46	0.65	0.80	0.89	0.75	0.69
Rat 4	0.43	0.66	0.80	0.87	0.76	0.67
Rat 5	0.42	0.62	0.82	0.86	0.75	0.66
Rat 6	0.46	0.64	0.83	0.88	0.72	0.68

Table S29: Carrageenan-induced paw oedema: Paw volume of the rats treated with chloroform extract (1000 mg/kg) of *Tricholepis chaetolepis*

Chloroform extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.44	0.63	0.79	0.83	0.72	0.67
Rat 2	0.43	0.62	0.78	0.82	0.75	0.68
Rat 3	0.45	0.64	0.79	0.84	0.73	0.65
Rat 4	0.42	0.63	0.80	0.85	0.75	0.66
Rat 5	0.45	0.64	0.82	0.83	0.72	0.65
Rat 6	0.44	0.63	0.80	0.85	0.74	0.66

Table S30: Carrageenan-induced paw oedema: Paw volume of the rats treated with methanol extract (250 mg/kg) of *Tricholepis chaetolepis*

Methanol extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.45	0.67	0.79	0.89	0.75	0.69
Rat 2	0.44	0.65	0.78	0.89	0.75	0.68
Rat 3	0.46	0.66	0.81	0.90	0.77	0.69
Rat 4	0.45	0.64	0.80	0.88	0.74	0.68
Rat 5	0.46	0.66	0.79	0.89	0.75	0.68
Rat 6	0.47	0.67	0.79	0.91	0.74	0.67

Table S31: Carrageenan-induced paw oedema: Paw volume of the rats treated with methanol extract (500 mg/kg) of *Tricholepis chaetolepis*

Methanol extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.46	0.63	0.79	0.84	0.73	0.63
Rat 2	0.45	0.64	0.78	0.85	0.72	0.62
Rat 3	0.46	0.63	0.77	0.84	0.69	0.60

Rat 4	0.44	0.61	0.76	0.82	0.68	0.61
Rat 5	0.45	0.65	0.78	0.84	0.73	0.64
Rat 6	0.46	0.65	0.77	0.83	0.72	0.64

Table S32: Carrageenan-induced paw oedema: Paw volume of the rats treated with methanol extract (1000 mg/kg) of *Tricholepis chaetolepis*

Methanol extract	Paw volume					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Rat 1	0.45	0.62	0.75	0.80	0.68	0.6
Rat 2	0.46	0.61	0.76	0.80	0.67	0.59
Rat 3	0.44	0.62	0.77	0.79	0.69	0.58
Rat 4	0.46	0.61	0.74	0.78	0.68	0.60
Rat 5	0.44	0.62	0.76	0.80	0.69	0.59
Rat 6	0.45	0.60	0.75	0.79	0.67	0.57

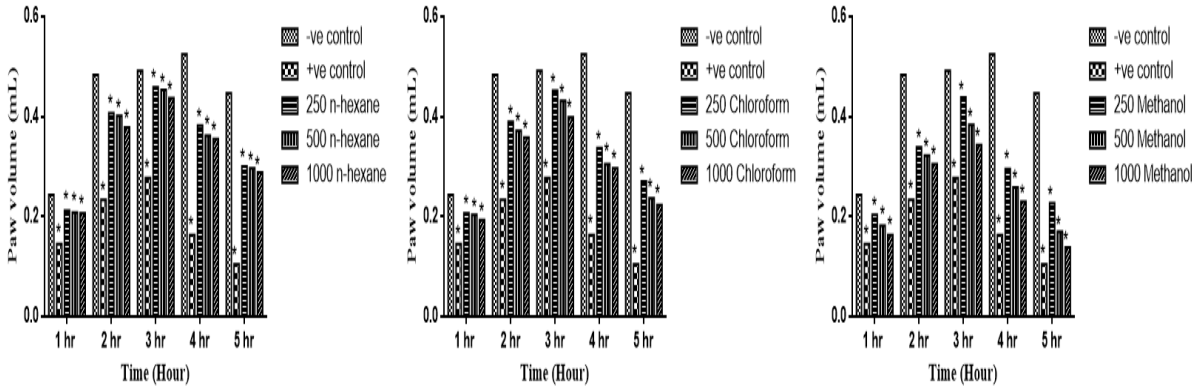

Figure S2: Paw volume of n-hexane, chloroform and methanol extract of *Tricholepis chaetolepis* respectively on carrageenan-induced paw oedema. Data are expressed as Mean ± SEM, n=6 animals in each group. One-way ANOVA was carried out using Tukey's multiple comparison tests. The symbol represents statistical significance: **P<0.0001*

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