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In Vivo Anti-Inflammatory and in Vitro Antioxidant Activities of Toona Ciliata Leaves Native to Bangladesh

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Abstract

The study was performed to assess the antiinflammatory, antioxidant activities and identify the polyphenols of Toona ciliata grown in Bangladesh. Antiinflammatory activity was examined by the methods of carrageenan and histamine-induced paw edema. At the dose of 400 mg/kg, effective anti-inflammatory activity (P<0.01) was observed in rats for both the test models of carrageenan and histamine-induced paw edema, compared to indomethacin. In ABTS scavenging assay, IC50 value was found significant (5.50?g/ml) compared to ascorbic acid (12.01?g/ml). The maximum absorbance of reducing power was obtained 0.4939 at 250?g/ml relative to ascorbic acid (1.1115?g/ml). Total antioxidant capacity, total phenolic and flavonoid content were found to be 357.1 mg/g ascorbic acid, 239.2 mg/g gallic acid, and 98.36 mg/g quercetin equivalent, respectively. During HPLC analysis, catechin and ellagic acid were determined in considerable amounts (825.95 and 416.70 mg/100g extract, respectively). The findings suggest that Toona ciliata could be a potential source of natural antioxidant.

Index terms— toona ciliata, free radical scavenging, antiinflammatory, hplc, epicatechin, p-coumeric acid, rutin hydrate.

including toonacilin and the leaves hold a considerable glycoside, tannins, flavonoids, phenolic compounds, triterpenoids and steroids. (5) In addition, three new norlimonoids, two new tirucallane-type triterpenoids, and a new pimaradiene-type diterpenoid, along with two known limonoids and eight known tirucallane-type triterpenoids, were isolated from the leaves and twigs of T. ciliata. (5,6) The plant T. ciliata possess many important biological properties that account for it's traditional uses in medicinal treatments, construction purpose, dye preparation, etc. (7) The flowers are used to produce dye, which are worn around Asia as color silk. T. ciliata barks are useful in chronic dysentery, ulcer, leprosy, fever, headache, blood complaints, etc. (8) The plant has been reported to exhibit significant antibacterial, antifungal, anticancer, antiulcer, anti-tumor, analgesic, anti-microbial, gastro protective and cytotoxic activity. (9,10,11) The ethanol leaf extract of T. ciliata was studied for its inhibitive effects on protein non-enzymatic glycation. (12) The aim of the present work is to determine the anti-inflammatory, antioxidant activities and identify the bioactive polyphenolic compounds by HPLC in the ethanol extract of Toona ciliata leaves grown in Bangladesh.

1 II.

2 Materials and Methods

3 a) Plant material

Fresh leaves were collected in May 2013 from Khulna, Bangladesh. Leaves of T. ciliata were washed, dried in the shade to minimize loss of volatile constituents and reduced to powder with a grinder.

4 b) Extraction

Collected fresh leaves were separated from undesirable materials and washed with water before letting it stand under the sun for a week. The dried leaves were coarsely powdered with the help of a grinder (Capacitor start motor, Wuhu motor factory, China). About 400g of the powered material was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of ethanol. The container along with its contents Introduction oona ciliata (T. ciliata), also commonly known as the red cedar, toon or toona, Burma cedar, Indian cedar or Indian mahogany, is a forest tree in the mahogany family (Meliaceae). It grows widely in the regions of southern Asia and Australia. (1, 2) These are usually large plants that grow up to a height of 25 to 35 m and the leaves are alternate and pinnetely veined with assymetrical base and an acute apex. (3) Studies on the transverse section of the bark of T. ciliata revealed the presence of periderm, cortex, sclerides, mednllary rays and phloem fiber. (4) The barks were also found to contain tetranortriterpenoids, T amount of aromatic compounds like coumarin, , was sealed and left to stand for a period of 7 days with continuous stirring by an orbital shaker. The mixture was first filtered in a clean cotton plug to remove any plant debris, and then through Whatman filter paper no. 1 (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated by rotary vacuum evaporator (R-210, Buchi, Switzerland) and dried. The sample rendered 51g of greenish gummy concentrate (12.75%) and was designated as the crude ethanol extract.

5 c) Chemicals

Gallic acid (GA), (+)-catechin hydrate (CH), vanillic acid (VA), caffeic acid (CA), (-)-epicatechin (EC), p-coumaric acid (PCA), rutin hydrate (RH), ellagic acid (EA), quercetin (QU), ascorbic acid, ABTS, folinciocalteu's phenol reagent, carrageenan and histamine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC), methanol (HPLC), acetic acid (HPLC), ethanol, trichloroacetic acid (TCA), phosphate buffer (pH 6.6), potassium ferricyanide [K3Fe(CN)6], ferric chloride (FeCl3), sodium phosphate, EDTA, ammonium molybdate and sodium carbonate were of analytical grade and purchased from Merck (Darmstadt, Germany).

6 d) Test animals & drugs

For the screening of in vivo anti-inflammatory activity, male rats of Wister strain weighing 175-205 g were used. The animals were housed under standard laboratory conditions maintained at 25 ± 1°C and under 12/12 h light/dark cycle and feed with Balanced Trusty Chunts and water ad libitum. All experimental protocols were in compliance with Bangladesh Council of Scientific and Industrial Research (BCSIR) ethics committee on Research in animals as well as internationally accepted principles for laboratory animal use and care.

The standard drug, Indomethacin was used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

7 III. Anti-Inflammatory Activity Test a) Carrageenan-induced oedema

The activity of T. ciliata ethanol leaf extract was evaluated using the carrageenan induced hind paw edema model. (13) The rats were divided into four groups (five rats per group). Group I (control) was given 1% tween 80 in normal saline (10 ml/kg), while Group II (positive control) received 10 mg/kg body wt. of indomethacin orally. Group III and IV were injected with 200 and 400 mg/kg body wt. of T. ciliata orally, respectively. Acute inflammation was induced in all the four groups by sub plantar injection of 0.1 ml of its suspension of carrageenan with 1% tween 80 in normal saline in the right paw of the rats, 1 h after the oral administration of the tested materials. The paw volume was measured with a micrometer screw gause at 1-hour interval after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression:

Percentage inhibition of inflammation = [(Vc-Vt)/Vc] × 100, where Vc is the average degree of inflammation by the control group and Vt is the average degree of inflammation by the test group.

8 b) Histamine-induced oedema

The activity of the T. ciliata extract was evaluated with histamine-induced paw edema model. (14) The paw oedema was generated by injecting 0.1% histamine solution sub-plantarly into the left hind paw of each mice at a dose of 0.1 ml. Twenty rats were divided into four groups of five animals each. Group I (control) was supplied with 1% tween 80 in normal saline (10 ml/kg). Group II (positive control) received 10 mg/kg body wt. of indomethacin orally. Group III and IV were given 200 and 400 mg/kg body wt. of T. ciliata orally, respectively. Acute inflammation was induced in all the four groups by sub plantar injection of 0.1 ml of histamine with 1% tween 80 in normal saline in the right hind paw of the rats, 1 h after the oral administration of the tested materials. The paw volume was measured with a micrometer screw gause at 1, 2, 3 and 4 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the same formula as for calculating the carrageenan-induced paw oedema.

9 IV.

10 Antioxidant Activity Test a) ABTS radical scavenging activity test

The method of decolourisation of free radical ABTS + was performed according to Fan et al. with some modifications. (15) ABTS radical cation was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate. The mixture was allowed to stand for 12-16 h at room temperature in the dark until reaching a stable oxidative state. The ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 with pH 7.4 phosphate buffered saline (PBS) solution at 734 nm, before use. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm was immediately recorded. The ABTS scavenging activity was calculated as follows: The reducing power of T. ciliata was studied using the method of Hemayet et al. and Dehpour et al. (16, 17) The extract at different concentrations was mixed with 1 ml ethanol, 2.5 ml phosphate buffer (0.2 M, pH 6.6), and 2.5 ml potassium ferricyanide [K 3 F e (CN) 6] (1%). The sample solutions were next incubated at 50°C for 20 min and a 10% solution of trichloroacetic acid (2.5 ml) was added to them. They were then centrifuged at 3000 rpm for 10 min. The top layer of the mixture (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% FeCl 3 . The absorbance was measured at 700 nm with a spectrophotometer. All determinations were carried out in triplicate.ABTS scavenging effect = I (%) = (A o -A s / A o) x

11 c) Total antioxidant capacity

The total antioxidant capacity was measured by the method of Prieto et al. (18) The ethanol extract was prepared in its respective solvent and mixed with 1 ml of the reagent solution (0.6M H 2 SO 4 , 28 mM sodium phosphate, 4 mM ammonium molybdate mixture). The tubes were incubated for 90 min at 95ºC. The mixture was cooled to room temperature and the absorbance was read at 695 nm against a blank sample. Ascorbic acid equivalents were calculated using the standard graph for ascorbic acid. The experiment was conducted in triplicates and values were expressed as equivalents of ascorbic acid in mg per gram of extract.

12 D. Total phenolic content

Total phenolic content of the extract was determined using the modified Folin-Ciocaltu method. (19, 20) After reacting 0.5 ml of extract (1 mg/ml), 5 ml Folin-Ciocaltu reagent (1:10 v/v distilled water) and 4 ml (75 g/l) of sodium carbonate, the sample solutions were mixed and left to stand at 40°C for the next 30 min for color development. The absorbance was read at 765 nm. The total phenolic content was calculated and expressed as mg of gallic acid equivalent per gram using the equation obtained from the standard gallic acid calibration curve, y = 6.993x + 0.0379, R2 = 0.9995.

13 d) E. Total flavonoid content

The total flavonoid content was determined by reactions of the aluminium chloride colorimetric method with some modifications. (21,22) The absorbance of the reaction mixture was measured at 430 nm with a double beam Analykjena UV/Visible spectrophotometer (Model 205, Jena, Germany). Quercetin was used for calibration of a standard curve (y = 6.2548x + 0.0925; R 2 = 0.998) and the results were expressed as mg of quercetin equivalent per gram dry weight of sample.

V.

14 Hplc Detection of Polyphenolics

Detection of selected polyphenolic compounds in the extract was carried out by HPLC as described by Ismet et al. (23) The analysis was performed on a Dionex UltiMate 3000 system equipped with quaternary rapid separation pump (LPG-3400RS) and photodiode array detector (DAD-3000RS). Separation was done using Acclaim® C 18 (5µm) Dionex column (4.6 x 250 mm) at 30 ºC with a flow rate of 1 ml/min and an injection volume of 20 µl. The mobile phase consisted of acetonitrile (solvent A), acetic acid solution pH 3.0 (solvent B), and methanol (solvent C) with the gradient elution program of 10%A/80%B/10%C (0-9 min), 20%A/60%B/20%C (10-19 min) and 100%A (20-30 min) with post run equilibration of the system with 5%A/95%B (5 min). The UV detector was set to 280 nm for 18.0 min, changed to 320 nm for 6 min, and finally to 380 nm and held for the rest of the analysis period while the diode array detector was set at an acquisition range from 200 nm to 700 nm. For the preparation of calibration curve, a standard stock solution was prepared in methanol containing gallic acid (GA), vanillic acid (VA), (+)catechin (CH), (-)-epicatechin (EC), p-coumaric acid (PCA), rutin (R), ellagic acid (EA) (20 µg/ml each), caffeic acid (CA) (8 µg/ml) and quercetin (QU) (6 µg/ml).

A solution of T. ciliata leaf extract was prepared in ethanol at 5 mg/ml. All solutions were filtered through 0.20 µm nylon syringe filter (Sartorius, Germany) and degassed in an ultrasonic water bath (Hwashin, Korea) for 15 min. Data acquisition, peak integration, and calibrations were performed with Dionex Chromeleon software (Version 6.80 RS 10).

15 VI.

16 Statistical Aanalysis

Data were presented as mean ± Standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using SPSS Data Editor for Windows, Version 11.5.0 (SPSS Inc., U.S.A.). The results obtained were compared with the control group. P values < 0.05 were considered to be statistically significant.

17 VII.

18 Results

19 a) Carrageenan-induced paw edema

The anti-inflammatory effect of the T. ciliata using carrageenan induced oedema test is expressed in b) Histamine-induced paw edema Table 2 gives information on the effect of T. ciliata extract on acute inflammation using histamineinduced paw edema test. A maximum edema paw volume of 1.59 ± 0.08 mm was observed in the control group at 4 h after histamine was injected. Rats that were pre-treated with 400 mg/kg body weight of the extract significantly compressed ($p<0.05$; $p<0.01$) the histamine-induced edema paw volume, in comparison to that by indomethacin. The percentage inhibition of the edema paw volume at 1, 2 and 3 h by the 400 mg/kg body weight of the extract was also found effective ($p<0.05$; $p<0.01$). The maximum reduction in the paw volume by the 400 mg/kg body weight of T. ciliata at 4 h was 56.60%, while that by the indomethacin declined to 65.41%, respectively.

20 c) ABTS radical scavenging activity

At minimum concentration (10 µg/ml), the highest activity obtained by the extract of T. ciliata was 98.22 ± 0.04 µg/ml (Table 3). The IC50 value of the extract was found to be 5.50 ± 0.16 ?g/ml, which was similar to that of the ascorbic acid (12.01 ± 0.12 ?g/ml). The values are expressed as mean ± standard deviation (n=3).

21 d) Reducing power assay

The reducing power assay was determined based on the relative maximum absorbance of the extract of T. ciliata and was observed to increase with an increase in concentration (Table 4). At 250 ?g/ml, the maximum absorbance for the ethanolic leaf extract of T. ciliata was found to be 0.4939 ± 0.029, while the standard ascorbic acid showed an absorbance of 1.1115 ± 0.009. The values are expressed as mean ± standard deviation (n=3).

22 e) Total antioxidant capacity

The ethanol extract of T. ciliata possessed a high total antioxidant capacity (Table 5). The total antioxidant capacity of the extract was obtained in significant quantity relative to the standard ascorbic acid per gram of extract (357.10 ± 2.02). The values are expressed as mean ± standard deviation (n=3).

f) Total phenolic content Table 6 demonstrates the total phenolic content in the ethanol leaf extract of T. ciliata. High phenolic content was determined in the extract (239.2 ± 2.53 mg/g of gallic acid equivalent). The values are expressed as mean ± standard deviation (n=3).

g) Total flavonoid content Table 6 demonstrates the total flavonoid content in the leaf extract of T. ciliata. A considerably large amount of flavonoid was observed in the extract (98.36 ± 1.07 mg/g of quercetin).

23 h) HPLC assay of T. ciliata

The contents of the phenolic compounds in the leaf extract of T. ciliata were analyzed by RP-HPLC (Table 7). Based on the comparison of the retention times with those of the standard peaks, seven phenolic compounds: (+) catechin, vanillic acid, epicatechin, pcoumeric acid, rutin hydrate, ellagic acid and quercetin were identified, respectively (Figure 1). The most abundant phenolic compound obtained from the extract of T. ciliata was catechin (825.95 ± 5.39 mg/100 g dry extract) followed by ellagic acid (416.70 ± 3.58 mg/100 g dry extract). Next, there was epi-catechin, p-coumeric acid and rutin hydrate, which were also obtained in significant quantities, but in lower amounts than that of the first two (211.7 ± 2.36, 102.20 ± 1.87 and 77.57 ± 1.49 mg/100 g dry extract, respectively). Other polyphenolic compounds like vanillic acid and quercetin were also obtained in similar concentrations (34.05 ± 0.83 and 29.13 ± 0.65 mg/100 g dry extract).

24 Discussion

Carrageenan and histamine induced paw oedema were evaluated for their anti-inflammatory effect in T. ciliata. The carrageenan induced inflammatory response in rats is a biphasic response, which causes marked oedema formation that results from the rapid production of several inflammatory mediators such as histamine, serotonin, and bradykinins. The second step is the release of prostaglandins and nitric oxide with a peak at 3 h, which is produced by an inducible is of orm of cyclooxygenase (COX-2) and nitric oxide synthase Volume XIV Issue

VII Version I Year 2014 ( ) B (iNOS). (24) The present investigation was carried out in an attempt to reduce the oedomatogenic response in rats evoked by carrageenan. Results show that pretreated oral administration of the extract was effective in the reduction of the response. Thus, a relationship can be inferred between the anti-inflammatory properties of the extract and the inhibition of intracellular signalling pathways in inflammatory mediators.

On injection, histamine acts as an inflammation mediator. (25) The liquid spreads out inside the body of the rat like a wheal and increases the permeability of the host capillary venules in the skin. Substances that inhibit the activity of histamine receptors shrink that particular area where the wheal was formed. This could be because the anti-inflammatory activity of the extract is supported by its anti-histamine activity. The antihistaminic effect of the extract increases with the concentration of the extract. The extract inhibits the formation and action of the inflammatory mediators, effectively suppressing the production of oedema by histamine. This study shows that T. ciliata has significant anti-oedematogenic effect ($P<0.01$) on paw oedema in rats induced by both carrageenan and histamine.

When subjected to reducing power assay, the extract causes the oxidation of ferricyanide complex to its ferrous form. This results in the extract to donate a hydrogen atom, which in turn helps to break the free radical chain and exert an antioxidant response. (26) The high phenolic content in the ethanol leaf extract of T. ciliata might be a reason for this reduction of Fe3+ to Fe2+, exhibiting stronger reducing power ability.

The total antioxidant capacity depends on the reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of green phosphate/Mo (V) complex at an acidic pH. (27) Studies on the antioxidant activities of the leaf and flower extracts of Toona ciliata have previously been carried out using several different solvents i.e. petroleum ether, chloroform, ethyl acetate and methanol. Based on the results, all the extracts showed significant DPPH and ABTS radical scavenging activity in comparison with the standard, BHT (butylatedhydroxytoluene). The petroleum ether, chloroform, ethyl acetate and methanol extracts of Toona ciliata showed DPPH and ABTS significant activity with IC50 value of 150, 135.5, 105 and 92.5 ?gml-1 for DPPH and 145, 120, 120.5 and 95 ?gml-1 for ABTS scavenging activity, respectively, in comparison to standard BHT with an IC50 of 8 ?gml-1 and 11.5 ?gml-1, respectively. (12) Better results for the ABTS radical scavenging activity of T. ciliata ethanol extract relative to ascorbic acid were obtained from the present investigation (IC50 5.50 $\pm$ 0.130). Another study on the methanol and hexane fractions of T. ciliata leaves compared the plant's reducing power activity, which suggests a better activity for the methanol extract with a maximum relative absorbance of 0.440 $\pm$ 0.0001, which was very close to the absorbance of the standard drug, ascorbic acid (0.461 $\pm$ 0.0030). (28) HPLC analysis of the ethanol leaf extract of T. ciliata was used to determine and quantify the phenolic compounds present in the extract. Several studies have shown that vanillic acid and quercetin possess antioxidant properties. In addition, catechin and ellagic acid compounds have been found to play a role in the anti-inflammatory activity (29,30,31) and rutin hydrate and quercetin are known to demonstrate good antiinflammatory properties. (32,33,34) HPLC studies confirm the presence of relatively high concentration of these antioxidant chemicals in T. ciliata, which helps to explain the significant anti-inflammatory and antioxidant activity of this plant extract.

IX.

25 Conclusion

The study demonstrates significant antioxidant and anti-inflammatory activity of the ethanol leaf extract of T. ciliata. Moreover phenolic compounds were detected with HPLC and a correlation can be suggested between the plant's antioxidant and anti-inflammatory properties and the high level of polyphenolic compounds present in its extract. Nevertheless, activity varies depending on several conditions including the plant type, its biome, growing conditions, etc. However, based on the results obtained, it can be asserted that the plant T. ciliata, grown in Bangladesh can be of great medicinal value in physiological processes and other cures, relieves and preventions.

26 X.

27 Conflict of Interest

We declare that we have no conflict of interest.

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Year 2014 (B) [1] [2] [3]

[1] © 2014 Global Journals Inc. (US)

[2] © 2014 Global Journals Inc. (US) reduction in the paw volume by the 400 mg/kg body weight of the extract at 4 h was 55.42%, while that by indomethacin was 64.46%, respectively.

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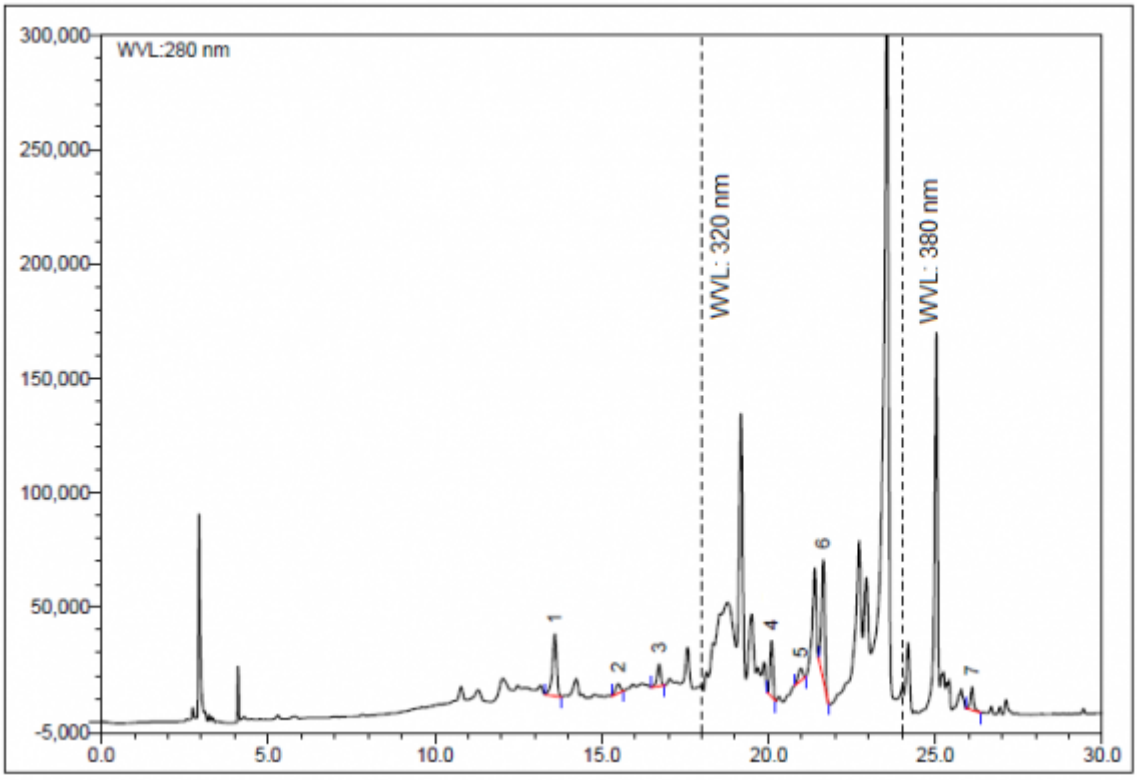
Figure 1:



1

Figure 2: Figure 1 :

1

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. The paw edema was highly reduced by indomethacin ($p<0.05$; $p<0.01$) between the first and forth hour (50.48% to 64.46% inhibition). A maximum edema paw volume of 1.66 ± 0.08 mm was observed in the control group, four hours after the carrageenan injection. Rats which received 400 mg/kg body weight of the extract were observed to significantly decrease ($p<0.05$; $p<0.01$) the carrageenan-induced oedema paw volume between the 1 to 4 hour time interval, in comparison to that of the standard drug, indomethacin, at a dose of 10 mg/kg body weight. The highest

Figure 3: Table 1

1

Values are expressed as mean±SD; Values are calculated as compared to control using one way-ANOVA
followed by Dunnet's Test; * indicates $P < 0.05$; ** indicates $P < 0.01$ vs. control; $n = 5$.

Figure 4: Table 1

2

		volume in male wistar rats			
Treatment Groups	Doses (mg/kg body weight)	Right hind paw volume (mm) 1 h	2 h	3 h	4 h
Control	2 ml/kg	1.09 ± 0.06	1.29 ± 0.07	1.40 ± 0.05	1.59 ± 0.08
Positive Control (Indomethacin)	10	0.48 ± 0.08** (55.96)	0.56 ± 0.03* (56.59)	0.53 ± 0.08* (62.14)	0.55 ± 0.03** (65.41)
Extract	200	0.84 ± 0.05* (22.94)	0.87 ± 0.09* (32.56)	0.92 ± 0.07** (34.29)	0.99 ± 0.06* (37.73)
Extract	400	0.60 ± 0.04* (44.95)	0.67 ± 0.07** (48.06)	0.71 ± 0.05* (49.29)	0.69 ± 0.05** (56.60)

Values in brackets denote percentage inhibition of the oedema paw volume.
Values are expressed as mean±SD; Values are calculated as compared to control using one way-ANOVA followed by Dunnet's Test; * indicates $P < 0.05$; ** indicates $P < 0.01$ vs. control; $n = 5$.

Figure 5: Table 2 :

3

Concentration (µg/ml)	T. ciliata leaf extract	Ascorbic acid
10	98.22 ± 0.04	48.60 ± 0.17
20	98.60 ± 0.12	85.79 ± 0.25

Figure 6: Table 3 :

4

Concentration (µg/ml)	T. ciliata leaf extract	Ascorbic acid
10	0.0190 ± 0.028	0.3801 ± 0.012
20	0.0695 ± 0.071	0.4577 ± 0.017
40	0.0819 ± 0.017	0.5398 ± 0.023
60	0.1056 ± 0.041	0.6345 ± 0.037
80	0.1699 ± 0.062	0.7125 ± 0.013
100	0.1986 ± 0.041	0.7811 ± 0.029
250	0.4939 ± 0.029	1.1115 ± 0.009

Figure 7: Table 4 :

5

Extract	Total antioxidant capacity mg of ascorbic acid equivalent (AAE) per g of dry extract
T. ciliata leaf extract	357.1 ± 2.02

Figure 8: Table 5 :

6

Extract	Total phenolic content mg of gallic acid equivalent (GAE) per g of dry extract	Total flavonoid content mg of quercetin equivalent (QE) per g of dry extract
T. ciliata leaf extract	239.2 ± 2.53	98.36 ± 1.07

Figure 9: Table 6 :

7

Polyphenolic compound	Ethanol extract of T. ciliata leaf Content (mg/100 g of dry extract)	% RSD
CH	825.95	5.39
VA	34.05	0.83
EC	211.7	2.36
PCA	102.2	1.87
RH	77.57	1.49
EA	416.7	3.58
QU	29.13	0.65

Figure 10: Table 7 :

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