Vol 5 | Issue 2 | 2014 | 67-72.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF STEM BARK OF PLANT *TOONA CILIATA M.ROEM* (MELIACEAE)

Sonam Soni, Vinay S Verma, Hemendra Swarnkar, Sanjay Vaishnav, Md.Nazir Nasim Khan, [1]Gautam Jana, Mukesh Sharma*

Rungta College of Pharmaceutical Science and Research, Kohka, Bhilai (C.G) 490023, India.
1 Gayatri College of Pharmacy sambalpur Orissa, India.

ABSTRACT

Toona is a large deciduous tree with a spreading crown, commonly attaining a height of 20-30 m and a girth of 1.8-3 m. *Toona ciliata* (Family: Meliaceae) is commonly known as Toonee, Tuni in Hindi; Red ceder in English and Nandi in Sanskrit. It is reported to have good medicinal values in traditional system of medicines. The stem of *Toona ciliata* is reported the antioxidant, analgesic, antiulcer, antifungal, antimicrobial, antifeedant, cytotoxicity properties, flowers used as emmenagogue, used in menstrual disorders, and Bark is bitter, acrid, powerful astringent, tonic, expectorant, anthelmintic. The phytoconstituents present in the plants including triterpenoids, cedrelone, polyynes, limonoids, siderin etc. of *Toona ciliata*. In the present study, an attempt was made to carry out the extraction of chemical constituents. The Extracted compound was identified by TLC and chemical analysis and the crude drug was passes from different test which shows their purity, solubility and extractive values. The present study aims to determine the antipyretic activity of methanolic extract of *Toona ciliata* in brewer's yeast induced pyrexia model in albino rats. From different parameters the crude drug and extract show different result such as Water soluble extractive 20%, Alcohol soluble extractive 21%, Moisture content11.5%, Total ash 6%, Rf value 0.78.

Keywords: *Toona ciliata*, Antipyretic, Cytotoxicity, Pharmacological activities.

INTRODUCTION

Food itself is a part of health thus vegetable resources are the first ever known substance for health and healing. Plant provides Food, clothing, shelter and medicine. Several great changes have taken place in medicine during the second half of the 20th century .Different source of the drugs are used in Ayurveda –in general are of vegetable ,animal , mineral origin. Among vegetable drugs include leaves, root, barks, Flower, wood, fruits, and gums. The drugs of biological origin continue to remain the backbone of the drug therapy [1]. Fever is common medical sign characterized by an elevation in temperature. Above the normal range of 36.5 -37.5°C (98-100°F) due to an increase in the body temperature regulatory set point Fever is of four type's Continuous fever, intermittent fever, remittent fever, Pel-Ebstein fever [2-3]. The currently available antipyretic drugs in allopathic system of medicine are not so effective in combating wide variety of complications. Ayurvedic formulations contain number of ingredients in which one ingredient may act to enhance the action of other ingredient. Also as a result of so many ingredients present in the particular ayurvedic formulation it helps in combating other diseases in addition to antipyretic activity. *Tooona ciliate* belongs to the family meliaceae possess numerous medicinal properties but the activity was not extensively reported. This promote us to extend the studies of the whole plant of *Tooona ciliate* as it may leads to the discoveries of the new moieties and therapeutic value for the new existing and emerging diseases The present study aims to determine the Antipyretic properties , of stem bark of *Toona ciliate* [4].

Distribution

A tree of subtropical climates, T. ciliata grows in moist localities such as ravines, banks of streams and even swamps. It grows best in fire-protected savannah, abandoned cultivation and in small gaps in forest, and does not do well on dry hillslopes. Native : Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Thailand, Vietnam Exotic : Australia, Kenya, Mauritania, Sierra Leone, South Africa, Tanzania, Uganda, United States of America, Zambia, Zimbabwe[5-6]

Corresponding Author: **Mukesh Sharma Email:-** mukesh.rcpsr@gmail.com

Morphology [7-8]

Trunk & Bark: Bark reddish brown, exfoliating in large flakes when old; blaze deep pink with white streak.

Branches and branchlets: Branchletsterete, lenticellate, glabrous.

Leaves: Leaves compound, paripinnate, 23-90 cm long, clustered at twig ends

Inflorescence / Flower: Inflorescence terminal panicles, drooping; flowers white.

Fruit and Seed: Capsule, elliptic, 5-valved, to 2 cm long, with white patches; seeds many, papery winged.

Constituents

Main constituents (Stem Bark) -Triterpenoids, Alkaloids, glycosides. Other constituents are Resin, extractive matter, gum, a bitter substance nyctanthin.

Part Used

Bark, Gum, and Flowers part of the plant mainly used in the formulation.

Traditional uses

Flowers-Traditionally the flowers used as emmenagogue, used in menstrual disorders.

Bark- Bark is bitter, acrid, powerful astringent, tonic, expectorant, anthelmintic, aphrodisiac and antiperodic. It is useful in chronic dysentery, ulcer, leprosy, cures fever, headache, blood complaints (Ayurveda), cardiotonic, aphrodisiac, anthelmentic; good for scabis and expectorant (Yunani) [9].

MATERIAL AND METHODS

Pharmacognostical study

Morphological Character

Morphology is the description of that form where the material is known to occur in a particular form. Morphological features and organoleptic features viz. colour, odor, taste, shape and sizes were observed and evaluated botanically.

Microscopical Characters

Transverse section of the stem Bark

The section of the stem Bark were taken .Transferred the section into watch glass containing water, and the section were stained with saffaranin and then mounted in glycerin and observed under low power.

Powder Microscopy

In this study the dry leaves were powdered. The cleared powder mounted on slide with the help of glycerin. Then stain the cleared powder with the staining reagents such as phloroglucinol and conc. hydrochloric acid (1:1). Various identified characters were observed**.**

Proximate value:

The followings proximate value were determined for the drug of plant *Toona ciliate.*

Extractive value [10]

Alcohol soluble extractive value

5gm of shade-dried *Toona ciliate* stem bark coarse powder was macerated with 100ml alcohol in a closed flask, shaking frequently during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly taking precaution against loss of alcohol .Evaporate 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105^{o}C and weighed. Percentage alcohol soluble extractive was calculated with reference to the shade – dried plant powder.

Water soluble extractive value

5gm of shade-dried *Toona ciliate* stem bark coarse powder was macerated with 100ml water in a closed flask, shaking frequently during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly taking precaution against loss of water .Evaporate 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105^{o}C and weighed. Percentage water soluble extractive was calculated with reference to the shade – dried leaves powder.

Moisture content [11]

An Accurately weighed quantity of the shade dried coarsely powered stem bark of toona *ciliate* was taken in a tarred glass bottle and the initial weight was taken.

The crude drug was heated at 105^{o}C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of the sample was calculated as percentage with reference to the shade dried material.

Ash value [12]

Total ash

2g of accurately weighed quantity of the shade dried coarsely powered stem bark of *Toona ciliate* was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^{o}C until free from carbon, cooled and weighed .The percentage of total ash was calculated with reference to shade dried bark powder.

Acid insoluble ash

Total ash obtained was boiled for five minutes with 25ml of dil. Hydrochloric acid. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited, cooled and weighed. The percentage of acid insoluble ash was calculated with reference to shade dried bark powder.

Water soluble ash

Total ash obtained was boiled for five minutes with 25ml of distilled water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes, cooled and weighed. The percentage of water soluble ash was calculated with reference to shade dried bark powder.

Phytochemical Study [13]

The stem bark of *Toona ciliate* M.Roem was collected from the local Market of Bhilai (C.G).The bark is

dried in sun .The dried Stem bark was coarsely powdered, weighed and filled in Soxhlet apparatus for extraction. The solvent used was 95% methanol. The extract was dried at vaccum dryer and then the extract was identified by the TLC [10] and chemical analysis.
Phytochemical examinations were carried out for all the extracts as per the standard methods

1.Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.
Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

2.Detection of phytosterols
Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled.
Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates
the presence of phytosterols.

3.Detection of phenols
Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

4.Detection of tannins
Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

5.Detection of flavonoids
Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids

TLC Of Extract [14]

Thin-layer chromatography (TLC) is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition and adsorption, depending on the particular type of support,its preparation and its used with different solvent. The Rf values are the ration between the distance of the solute from the starting spot to the solvent front.

Preparation of chromatoplate

Cleaned and dried glass plates were taken. Uniform slurry of silica Gel-G in water was prepared in the ratio of 1:2. The slurry was then poured into the chamber of the TLC applicator, which was fixed and the thickness was set to 0.5mm. Glass plates were moved under the applicator smoothly to get a uniform coating of slurry on the plates. The plates were dried first at room temperature and then kept for activation at 110^0c for 1 hour.

Preparation of Solvent System and saturation of Chamber

The solvent system used for the development of chromatogram was prepared carefully by mixing. "Petroleum ether: Hexane: ethyl acetate: formic acid (10:30:15:1)"

Application of sample

The solution of the parent compounds and its derivatives were taken in small bored capillary tube and spotted at 2 cms from the base end of the plate. After spotting the plate were allowed to dry at room temperature and plates were transferred to chromatographic chamber containing solvent system for development.

Development of Chromatogram

Plates were developed by ascending technique when solvent front had reached a distance of 10-12cms; they were taken out and dried at room temperature.

Detection of spots

Examination of developed plates done by using spraying with vanillin sulphuric acid reagent and heating the plate at 105ºC for about ten minutes.

Pharmacological Study
Animals: Male Albino rats (70-100g) and albino mice of either sex (22-30g) were used in experiments. The animals were housed in polypropylene cages under standard conditions (12hr light; 12 hr dark cycle; 25± 5^0 C; 35-60% humidity). They were fed with standard rat pellet diet and water . Experimental protocol was approved by the Institutional Animal Ethical Committee. Animal ethical norms were strictly followed during all experimental procedures [15].

The animals were divided into two groups and each group consists of five mice. The defined or fixed dose level of methanolic extracts (2000 mg/kg) was given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death [16].

Antipyretic activity [17-19]

Animals were selected for the experiment after confirmation of approximate constant rectal temperature for 7 days. The antipyretic activity of the extracts was evaluated based on Brewer's yeast-induced pyrexia in rats Pyrexia was induced by intraperitonial injection of 3mg/kg of 5% w/v Brewer's yeast. The rectal temperature of each rat was measured at time, 0 h, using a telethermometer and before injection of the yeast. At 18 h following yeast injection, the different groups were treated with the vehicle, extracts (200 mg/kg) and standard drug, paracetamol (150 mg/kg). The rectal temperature was then recorded over a period of 3h.

RESULT AND DISCUSSION

Pharmacognostical Investigation

Crude drug was morphologically and microscopically studied and the result was tabulated in Table 1, Table 2, Table 3 .

Extraction

The dried powder of *Toona ciliata* stem bark parts (400 gm) was extracted with 95% (v/v) Methanol by soxhlet extraction. The yield of methanolic extract was 26.4 gm.

Proximate values:

Ash values

Different ash values like, total ash, acid insoluble ash, water-soluble ash of *Toona ciliata* were determined separately for stem bark. The values are presented in table 4.

Extractive values

Extractive values were determined for stem bark of *Toona ciliata* by extracting with various organic solvents successively by maceration. Percentage of extractive values was calculated with reference to air dried drug and result are presented in Table 4

Moisture Content

Moisture content of Toona ciliate stem bark was determined and the result are presented in Table 4

Qualitative Phytochemical Screening

All extracts obtained during successive extraction of *Toona ciliata* were examined for the presence of various phytoconstituents by performing qualitative phytochemical tests and the results are recorded in table 6.

Antipyretic Activity

Antipyretic activity of *Toona ciliata* on Brewer's yeast induced pyrexia in rats as shown in table 7 and 8. From the tabulation 8 the methanolic extract of *Toona ciliata* has significant antipyretic activity.

ANTIPYRETIC ACTIVITY

Antipyretic activity of the extract of *Toona ciliate* was performed by using albino rats animal model.

Figure 1. Microscopy of *Toona ciliate* stem bark

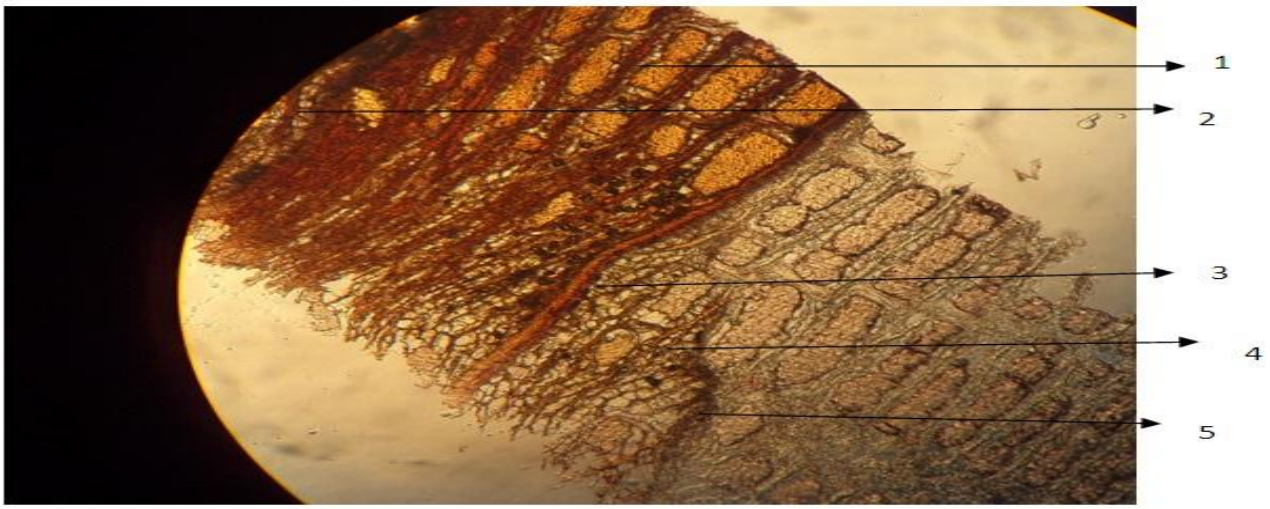

Table 1. Morphological Evaluation of *Toona ciliate* stem bark

S. No.	Features	Observation
1	Outer surface	Brown or grey, scaly, shed in oblong or irregular, leaving slight depressions in the surface of the bark
2	Inner surface	red, laminated and fibrous
3	Odour	Strong Odour
4	Taste	Bitter Taste
5	Shape	Channeled to quill
6	Size	Variable
7	Fracture	Short and fibrous

Table 2. Microscopy of *Toona ciliate* stem bark

S. No.	Features	Observation
1	Cork	Multi layer thick and thin walled
2	Phellogen	2 to 3 layers of thin walled cells without any cellular content
3	Sclerides	Sclerenchymatous cells, pitted inner and radial walls more thick
4	Medullary rays	▪ Narrow at inner side, wider in the scleride band side
5	Phloem fibers	▪ Single, isolated, circular, lignified with stratification.

Table 3. Micro chemicals tests performed on powder of stem bark of *Toona ciliate*

S. No.	Reagents	Observation	Diagnosis
1	Phloroglucinol:con.HCl	Pink	Lignified fibers, Stone cells
2	Dilute iodine	Blue-black	Starch grains

Table 4. Proximate values

S. No	Parameters	Determined value(%w/w)
1	Water soluble extractive	20%
2	Alcohol soluble extractive	21.5%
3	Moisture content	11.5%
4	Total ash	**6%**
5	Acid insoluble ash	**1.9%**
6	Water soluble ash	**2.7%**

Table 5. TLC Profile for evaluation methanolic extract of stem bark of Plant *Toona Ciliata* M.Roem

Phyto constituents	Solvent system	Spray reagent	No.of spot	Rf value
Triterpenoids	petroleum ether : hexane : ethyl acetate : formic acid (10:30:15:1)	Vanillin sulphuric acid	**1**	**0.78**

Table 6. Qualitative chemical analysis of extract of *Toona ciliate* M.Roem

Phytoconstituents	Methanolic extract
Carbohydrates	+
Coumarin Glycoside	+
Flavonoids	+
Phenols	+
Tannins	+
Phytosterols	+

Table 7. Rectal Temperature of different group after administration of Yeast for inducing pyrexia

Rectal temperature after inducing Brewer yeast for Pyrexia							
S. No.	Dose (mg/kg) Of 5% Brewer yeast	-18hr	0hr	30 min	1hr	2hr	3hr
Group 1	3	96.54	96.54	96.88	97.23	98.45	98.87
Group 2	3	96.67	96.67	97.23	97.54	98.86	98.67
Group 3	3	96.87	96.87	97.21	97.45	98.98	98.88

Table 8. Rectal Temperature of different group after administration of Drugs

Drugs	Dose (mg/kg)	Group 1			Group 2			Group 3		
		1hr	2hr	3hr	1hr	2hr	3hr	1hr	2hr	3hr
Control	200	00	00	00	-	-	-	-	-	-
Paracetamol	150	-	-	-	97.88	97.33	96.88	-	-	-
Extract	200	-	-	-	-	-	-	98.22	97.87	97.21

CONCLUSION

The antipyretic property of the *Toona ciliata* was found to be almost equal to that of paracetamol, This crude drug may form an substitute to paracetamol in battling common fever. Accidental over dose of paracetamol may result in liver injury. From the phytochemical investigation of the *Toona ciliate* stem bark and evaluation of the same for Antipyretic activity, the following conclusion can be made: *Toona ciliate* stem bark was found to contain glycosides, flavonoids, steroids, alkaloids, tannins, as the chemical constituents. The total extract shown the Antipyretic activity. So we can conclude that the total extract of bark due to synergistic effect of several constituents the extract give more than one effects.

REFERENCES

1. Butler MS. Natural products to drugs, natural product-derived compounds in clinical trials. *Nat. Prod. Rep*, 25, 2008, 475–516.
2. Mehlisch DR. The efficacy of combination analgesic therapy in relieving dental pain. *J Am Dent Assoc*, 133(7), 2002, 861–71.
3. Murnion B. Combination analgesics in adults. Australian Prescriber (33), 113–5. Retrieved 12 August 2010.
4. Da Saliva M, Fatima Das GF,Agasinho SMM, De Paula JR, Neto JO, Castro- Gamboa LF, Rodrigues FE, Fernandes JB, Vieira PC. Chemistry of Tonna ciliata & Cedrela odorata graft (Meliaceae), Chemosystematic & ecological significance. *Pure Appl Chem*, 1999, 71, 1083-1087.
5. The Ayurvedic pharmacopeia of India part-1. Vol.5. Govt of India Ministry of Health and Family welfare Department of Ayush, 179.
6. http,//en.wikipedia.org/wiki/Toon ciliate
7. Evans WC. Trease and Evans Pharmacognosy. WB Saunders Ltd. London, 2002, 32, 33, 95 - 99, 512, 547.
8. Wallis TE. Textbook of Pharmacognosy. Published by SK Jain, 1985, 572-575.
9. Kiritikar KR, Basu BD, Indian Medicinal Plants. International Book distributors, Dehradhun 248, 001, 1995, 562.
10. Wallis TE. Textbook of Pharmacognosy 5th ed, New Delhi CBS Publisher & Distributor, 1985.
11. Kokate CK, Purohit AP, Gokhle SB. Practical Pharmacognosy, 2nd ed.Pune, Mumbai, Nirali Prakashan, 1994.
12. World Health Organization. Quality Control Method for Medicinal plant material. Geneva, AITBS Publishers & Distributors, Delhi, 1998.
13. Monohara KP. Phytochemical Investigation & Pharmacological Screening of Targetes erecta Linn for Kidney disorder.
14. The Ayurvedic pharmacopeia of India part -1. Vol.5. Govt of India Ministry of Health and Family welfare Department of Ayush, 179.
15. Jain BB, Rathi BS, Thakurdesai PA, Bodhankar SL. Antipyretic activity of aqueous extract of leaves of *Cocculus hirsutus*. *Indian J Nat Prod*, 23, 2007, 26-29.
16. Metowogo K, Agbonon A, Eklu-Gadegbeku K, Aklikokou AK, Gbeassor M. Anti-ulcer and Antiinflammatory Effects of Hydro-alcohol Extracts of Aloe buettneri A. Berger (Lilliaceae). *Trop J Pharm Res*, 7, 2008, 907-912.
17. Gupta M, Mazumder UK, Kumar RS, Gomathi P, Rajeshwar Y, Kakoti BB, Selven VT. Antiinflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J Ethnopharmacol*, 98(3), 2005, 267-73.
18. Kirtikar KR and Basu BD. Indian Medicinal Plants. Bishen Singh Mahendra Pal Singh, Dehradun, India, International book distributor Vol.2. 2nd edn. 1975, 894-895.
19. Jain BB et al. Antipyretic activity of aqueous extract of leaves of cocculus hirsutus. *Indian Journal of Natural Product*, 23, 2000, 26-29.