

Original Article

PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF DIFFERENT EXTRACTS OF PLANT *CASEARIA TOMENTOSA* LEAVES

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ABSTRACT

Objective: In present study different extracts of the leaves of plant *Casearia tomentosa* was investigated for the phytochemical screening, total phenolic content and various pharmacological activities like antioxidant, antidiabetic, antibacterial.

Methods: Leaves of *C. tomentosa* were successively extracted by soxhlet extraction method using petroleum ether, chloroform, ethyl acetate and ethanol. The preliminary phytochemical investigation of different extracts was carried out according to standard methods. Antioxidant activity evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant potential (FRAP) method. Total phenolic content was evaluated by Folin-ciocalteu method. Antidiabetic activity estimated by α -amylase and α -glucosidase inhibitory assay while antibacterial activity was performed by disk diffusion method.

Results: *C. tomentosa* leaves show the presence of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, protein etc. Results of antioxidant studies revealed that the ethanol extract possessed highest 2, 2-diphenyl-1-picryl-hydrazyl (IC₅₀ 31.87±0.65 μ g/ml) activity, ferric reducing antioxidant power (43.78±0.65 μ M/ml, FRAP value=2.30). Among different extracts, ethanol extract shows highest (228.17±0.63 mg gallic acid equivalents (GAE)/gram of dry mass) amount of total phenolic content. The ethyl acetate extract exhibited highest inhibition on α -amylase (IC₅₀ 262.08±1.26 μ g/ml) and α -glucosidase (IC₅₀ 459.25±1.80 μ g/ml) enzymes. Results of antibacterial activity revealed that all extracts show inhibition against selected bacterial strains like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Salmonella typhi*.

Conclusion: The present study suggests that *Casearia tomentosa* leaves can be used as a potential source of natural antioxidant and natural antidiabetic, antibacterial agents.

Keywords: *Casearia tomentosa*, Phytochemical, DPPH, FRAP, α -amylase, α -glucosidase etc

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INTRODUCTION

Ayurveda, the ancient healing system of India, grow luxuriantly from the Vedic period in India. In history, the classical texts of Ayurveda like Charaka samhita and Sushruta samhita were written around 1000BC. Medicinal plants like turmeric, ashwagandha, ginger, brahmi, manjistha and tulsi are integral part of ayurvedic medicines. All these plants have been used for the treatment of diseases, traditionally and their secondary metabolite constituents are the sources of important modern drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine. According to one estimate nearly 70 % of the synthetic drugs have been derived from medicinal plants [1].

Casearia is one of the genus of *Salicaceae* family with huge pharmacological importance. In South American and Asian countries, different *Casearia* species have been used as folk medicines since ancient times. The pharmacological studies proved that the crude extracts and isolated compounds from this genus showed hypoglycemic, antioxidant, antiulcer, anti-inflammatory activities, cytotoxic, antimicrobial as well as anti-snake venom property [2]. Investigations on *C. sylvestris* revealed its antimicrobial, antioxidant and cytotoxic potential and presence of many phenolic compounds and their biological activities [2-4]. Another species *C. grayi* and *C. multinervosa* exhibited the potent antioxidant and antibacterial potency [5]. Furthermore, Clerodane diterpenes isolated from the bark of *C. grewiiifolia* have been showed moderate antimycobacterial effects [6]. Similarly *C. esculenta* root extracts and its isolated chemical constituents have been reported exclusively for the excellent hypoglycemic effect [7, 8].

The literature survey revealed that *Casearia tomentosa* is still an under explored species with a diverse range of folk uses [2]. In an

effort to provide the accurate scientific information of the plant *C. tomentosa*, this article presents the results of an extensive investigation of the secondary metabolites, total phenolic content, biological activities like antioxidant, antidiabetic and antibacterial which would assist further researches and potential applications of this plant. There is no report on these pharmacological activities from *Casearia tomentosa*. The present study gives a clue towards the immense medicinal properties of this plant.

MATERIALS AND METHODS

Plant material

Leaves of plant *Casearia tomentosa* were collected from Lachhiwala forest Dehradun, Uttarakhand (India) (latitude 30.1950000 °and longitude 77.9944400 °), identified and authenticated by Botanical Survey of India, (BSI) Dehradun with accession No.115689. A voucher specimen has been deposited in medicinal plants herbarium in Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar, India with registry no. 1/3. The collected fresh leaves were washed with distilled water, dried under shade for 30 d, crushed in a grinder to powder and finally stored in an air tight container for further extraction and various processes.

Chemicals and reagents

Ascorbic acid (Rankem), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ)(Sigma Aldrich), sodium carbonate (CDH), gallic acid (Merck), Folin-ciocalteu phenol reagent (Merck), dimethyl sulphoxide (DMSO)(Merck), α -amylase (SRL Pvt. Ltd), 3,5-dinitrosalicylic acid (DNSA)(SRL Pvt. Ltd.), tris buffer (Lobal Chemie), p-nitrophenyl- α -D-glucopyranoside (p-NPG)(SRL Pvt. Ltd.), α -glucosidase (SRL Pvt. Ltd), methanol (Merck), acarbose (Bayer India Limited), Ofloxacin (5

mcg) (Hi-Media), Muller Hinton agar (Hi-Media), Muller Hinton broth(Hi-Media) were purchased. All other chemicals and solvents were used of analytical grade.

Extraction of plant material

Two hundred grams of the dried powdered leaves of *C. tomentosa* were loaded and extracted in a soxhlet apparatus using 1250 ml each of petroleum ether, chloroform, ethyl acetate and ethanol successively in accordance to the hierarchy of polarity of solvents. Extraction was continued until the solvent coming out of the siphoning tube was colorless [9]. Extracts were concentrated under reduced pressure in rotary vacuum evaporator and refrigerated for further use.

Preliminary phytochemical screening

The phytoconstituents present in different extract were analyzed by using standard qualitative method [10, 11]. All extracts were analyzed for the presence of phytoconstituents like alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids, fat and oil, saponins, protein etc.

Antioxidant activity

Antioxidant activity of extract was determined by DPPH free radical scavenging assay and by ferric reducing antioxidant potential assay.

DPPH free radical scavenging assay

The free radical scavenging assay of different successive extracts of *C. tomentosa* leaves were evaluated by stable DPPH free radical according to the method of Brand-Williams with some modification [12]. A working solution of 0.004% was freshly prepared by dissolving 4 mg of DPPH in 100 ml of methanol. One milliliter of each extracts dilution of different concentration (1-1000 µg/ml) was added to 3 ml working solution of DPPH, Keep this reaction mixture in dark for 30 min. After 30 min the absorbance of the reaction mixture were taken at 517 nm with UV-VIS spectrophotometer (Systronic, UV-VIS 117) which were compared with the corresponding absorbance of standard ascorbic acid of similar concentrations (1-1000 µg/ml). One milliliter of methanol with 3 ml of working DPPH solution serves as blank. Then the percent radical scavenging activity or % inhibition was evaluated by following equation-

$$\% \text{ Inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample or standard}}{\text{Absorbance of blank}} \times 100$$

IC₅₀ of different extracts and standard ascorbic acid were calculated by graphical method by plotting % inhibition vs. concentration.

Ferric reducing antioxidant potential assay (FRAP)

The FRAP assay was done according to Benzie and Strain with some modification [13]. The stock solution includes 300 mmol acetate buffer pH 3.6, 10 mmol TPTZ (2,4,6-tri-(2-pyridyl)-1,3,5-triazine) solution in 40 mmol HCl, and 20 mmol FeCl₃.6H₂O solution. The working FRAP reagent was freshly prepared by mixing acetate buffer, TPTZ solution and FeCl₃.6H₂O solution in proportion of 10:1:1 (v/v) and then warmed at 37 °C before using it. Antioxidant potential were determined by reacting a mixture 1 ml of each extract (50 µg/ml) and 10 ml of working FRAP reagent. Absorbance (abs) of colored solution (ferrous tripyridyl triazine complex) was then taken at 593 nm after 5 min of incubation at 37 °C. Ascorbic acid standard (std) solutions were tested in a similar way. The standard curve was linear between 10-100 µM ascorbic acid. Working FRAP reagent serves as blank and 1 ml of methanol with 10 ml of working FRAP reagent act as control. Ferric reducing antioxidant power calculations were made by calibration curve. Results were expressed as µM/ml. FRAP value of sample was calculated by following equation-

$$\text{FRAP value } (\mu\text{M}) = \frac{\text{Change in abs of sample from 0 to 5 min}}{\text{Change in abs of std from 0 to 5 min}} \times \text{FRAP value of std}$$

Total phenolic content

Total phenolic content of different successive leaves extracts of *Casearia tomentosa* were determined by using Folin-ciocalteau

method [14] with little modification. Extract was diluted with methanol to form a concentration of 1000 µg/ml. Gallic acid dilutions range of 25µg/ml to 300 µg/ml is used for making standard calibration curve. One milliliter of each extracts was added to 10 ml of 10 % folin-ciocalteau reagent in 25 ml of volumetric flasks, after 8 min 8 ml of 7.5 % sodium carbonate was added. Further, total volume is made up to mark by adding distilled water. The complete reaction mixtures were incubated for about 45 min in the dark and at room temperature of about 25±2 °C. After incubation, the absorbance was measured at 765 nm with UV-VIS spectrophotometer (Systronic, UV-VIS 117). The same procedure was followed with gallic acid standard dilutions and also with blank where methanol is used in place of extract. Calibration curve of gallic acid was use for calculations. The total phenolic content of extracts was expressed as mg gallic acid equivalents (GAE)/gram of dry mass by following equation-

$$T = C \times V / M$$

Where, T= Total phenolic content mg GAE/gm of plant extract, C= Concentration of Gallic acid from the calibration curve, V = Volume of the extract in ml, M =Weight of the plant extract in gm.

Antidiabetic activity

In recent diabetic treatments, α-amylase and α-glucosidase inhibitors are most warranted because these enzymes increase post-prandial hyperglycemic conditions. Thus Anti-diabetic activity of different extracts of plant *C. tomentosa* leaves were assessed by α-amylase and α-glucosidase inhibitory methods.

Alpha-amylase inhibition activity

The α-amylase inhibitory activity of different extracts performed using 3,5-Dinitrosalicylic acid (DNSA) method with a little modification [15]. Briefly, One milliliter of each solution of different dilutions (10-5000 µg/ml) of extracts or standard acarbose in DMSO was incubated with 1 ml of α-amylase (concentration 0.25 mg/ml in 20 mmol phosphate buffer containing 6.7 mmol NaCl, pH 6.9) for 30 min at 37°C. In another set of tubes 1 ml of alpha amylase was pre incubated with 1 ml of phosphate buffer. After pre incubation, 1 ml of 1% starch solution in 20 mmol phosphate buffer, pH 6.9, was added to both sets of reaction mixtures to start the reaction. The reaction mixtures were then incubated for 15 min at 37 °C and 1 ml of DNSA color reagent (96 mmol 3,5-dinitrosalicylic acid and 5.315M sodium potassium tartrate in 2M NaOH) was added. The tubes containing reaction mixture were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The absorbance was taken at 540 nm with a UV-VIS spectrophotometer after diluting each tube with 9 ml of distilled water and compared with the corresponding absorbance of standard acarbose of similar concentrations (10-5000 µg/ml). The α-amylase inhibitory activity was calculated by equation-

$$\% \text{ Inhibition} = \frac{\{[(Ac\#) - (Ac*)] - (As - Ab)\}}{\{(Ac\#) - (Ac*)\}} \times 100$$

Where "Ac#" represents absorbance of pure control having 100% enzyme activity (DMSO and enzyme), "Ac*" is used for absorbance of blank for pure control having 0% enzyme activity (DMSO and buffer), "As" represents absorbance of sample or standard (sample/standard and enzyme) and "Ab" is used for background absorbance due to sample or standard (sample/standard and buffer).

IC₅₀ value (concentration at which sample shows 50% enzyme inhibition) of different extracts and standard acarbose were determined graphically by plotting % inhibition vs. concentration.

Alpha-glucosidase inhibition activity

The α-glucosidase inhibitory activity of successive extracts was determined according to Shukla et al. with little modification [15]. Briefly, 1 ml of each dilution of (10-5000µg/ml) of samples or standard acarbose in DMSO was pre-incubated with 1 ml of α-glucosidase (1U/ml in 100 mmol phosphate buffer pH 6.8) for 30 min at 37 °C. In another set α-glucosidase was pre-incubated with 1 ml of phosphate buffer. After pre incubation, 1 ml of 5 mmol p-NPG (in 100 mmol phosphate buffer pH 6.8) was added to both sets of reaction

mixtures to start the reaction. The reaction mixtures were then incubated for 15 min at 37°C and 4 ml 0.5M Tris buffer was added to stop reaction. The absorbance was taken by UV-VIS spectrophotometer (Systronic, UV-VIS 117) at 410 nm and compared with the corresponding absorbance of standard acarbose of similar concentrations (10-5000 µg/ml). The % inhibition and IC50 was calculated in similar way as mentioned in α-amylase activity.

Antibacterial activity

The determination of antibacterial screening of different leaves extracts of *C. tomentosa* were carried out by agar well diffusion technique [16].

Bacterial strains

Five human pathogenic bacterial cultures like *Staphylococcus aureus* (MTCC-737), *Escherichia coli* (ATCC-433), *Pseudomonas aeruginosa* (MTCC-430), *Klebsiella pneumonia* (ATCC-109) and *Salmonella typhi* (ATCC-733) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India and used in the present study. The bacteria revived in Muller Hinton broth at 37 °C for 18 h and then stocked at 4 °C in Muller Hinton agar (MHA). Subcultures were prepared from the stock for bioassay. The bacterial culture was inoculated into sterile Muller Hinton broth and incubated at 37 °C for 2 h until the culture attained a turbidity of 0.5 McFarland units so as to obtain the cell suspension between 10⁵ to 10⁸ CFU/ml.

Antibacterial assay

Prepared Muller Hinton agar plates were inoculated with 100µl of the inoculums. After solidification wells of 7 mm diameter were punched with the help of cork borer in agar medium and filled with 40µl of each successive extract (50 mg/ml in DMSO) and antibiotic

drug. The plates were incubated for 24 h at 37 °C. For each bacterial strain, pure DMSO was used as negative control, whereas ofloxacin was used as positive control. After incubation at 24 h the results were recorded for the zone of inhibition. The antibacterial activity was interpreted from the diameter size of zone of inhibition, measured in millimeters with the help of zone reader or vernier calipers. Different extracts and standard drug assayed in triplicate and the mean value is calculated.

Statistical analysis

Results were expressed as mean±standard deviation of triplicate measurements and analyzed as one-way analysis of variance (sigma stat ver. 2.0). The significant differences between means were calculated by a one way analysis of variance (ANOVA) using dunnett multiple-range test at P<0.01.

RESULTS

Extractive yield

The extractive yield (w/w), appearance and consistency of *C. tomentosa* different extracts with petroleum ether, chloroform, ethyl acetate and ethanol respectively was shown in table 1.

Phytochemical screening

The results for preliminary phytochemical investigation of *C. tomentosa* leaves extracts are shown in table 2. The result of phytochemical investigation shows that *C. tomentosa* leaves contain a number of active principles like alkaloids, flavonoids, carbohydrates, glycosides, protein, amino acid, steroids, saponin, terpenoids, fixed oils, fats, tanins and phytosterol. The presence of these phytoconstituents reveals medicinal importance of the leaves of this plant.

Table 1: Extractive yield of *C. tomentosa* leaves extracts

S. No.	Extracts	Appearance	Consistency	% yield (w/w)
1.	Petroleum ether	Greenish yellow	Waxy	1.515
2.	Chloroform	Dark Green	Dried Wax	3.223
3.	Ethyl acetate	Green	Dried Wax	2.410
4.	Ethanol	Dark Brown	Crystalline solid	6.118

Table 2: Phytochemicals present in leaves extracts of *C. tomentosa*.

Phytoconstituents and test performed		Extracts			
		Petroleum ether	Chloroform	Ethyl acetate	Ethanol
Alkaloids	Mayer's test	-	-	+	+
	Wagner's test	-	-	+	+
	Hager's test	-	-	+	+
	Dragendroff's test	-	-	+	+
Flavonoids	Alkaline test	-	-	+	+
	Lead acetate test	-	-	+	+
	Shinoda test	-	-	+	+
	Sulphuric acid test	-	-	+	+
Tannins	Ferric chloride test	-	-	-	+
Carbohydrate	Molisch's test	-	-	+	+
	Fehling's test	-	-	+	+
	Benedict's test	-	-	+	+
	Barfoed's test	-	-	+	+
Glycosides	Keller-Killiani test	-	-	+	+
	Legal's test	-	-	+	+
	Borntrager's test	-	-	+	+
Terpenoids	Liebermann burchard test	+	+	+	+
	Salwoski test	+	+	+	+
	Salwoski test (Triterpenes)	-	+	+	-
Steroids	Liebermann burchard test	+	+	+	+
Fat and Oil	Saponification test	+	+	+	-
	Filter paper test	+	+	+	-
Saponin	Foam test	-	-	+	+
Protein and amino acid	Ninhydrin test	-	-	+	+
	Biuret test	-	-	+	+
Phytosterol	Salwoski test	+	+	+	+
	Liebermann burchard test	+	+	+	+
Gums and Mucilage		-	-	-	-

"+= Present; -= Absent"

Antioxidant activity

Antioxidants protect cells against the adverse effects of free radicals which results oxidative stress leading to cellular destruction. Now these days, many researchers have been investigating the antioxidant activity of different plants in search of safe natural antioxidants.

DPPH free radical scavenging assay

DPPH radical scavenging activity is one of the most widely used method for evaluation of antioxidant activity of plant extracts. The

principle of DPPH method is based on the reduction of DPPH radical in the presence of a hydrogen donating antioxidant. Antioxidants reduce the color of DPPH due to the power of hydrogen donating capability [17]. *C. tomentosa* leaves extracts were investigated for their possible DPPH radical scavenging activity. The free radical scavenging activity (DPPH assay) was found maximum for ethanol extract, followed by ethyl acetate, petroleum ether and chloroform. The free radical scavenging activity of ethanol extract is found very close to standard ascorbic acid. Results are expressed in terms of IC_{50} in table 3.

Table 3: IC_{50} of *C. tomentosa* leaves extracts in DPPH assay

S. No.	Extracts/Standard	IC_{50} values in $\mu\text{g/ml}$
1.	Ascorbic acid	20.00±0.26
2.	Petroleum ether	280.00±0.01
3.	Chloroform	381.00±0.87
4.	Ethyl acetate	81.25±0.50
5.	Ethanol	31.87±0.65

Results are expressed as mean of three values±standard deviation.

Ferric reducing antioxidant potential assay (FRAP)

The ferric reducing antioxidant potential (FRAP) assay is a protocol that tells about the total antioxidant levels in plants [17]. The results were expressed as $\mu\text{M/ml}$ using the standard curve equation: $y = 0.0032x - 0.0212$, $R^2 = 0.9982$, where y is the absorbance at 593 nm and x is the ferric reducing antioxidant ability in 50 $\mu\text{g/ml}$ of extracts shown in table 4. The ferric reducing antioxidant potential

(FRAP) was found maximum for ethanol extract, followed by ethyl acetate, petroleum ether and chloroform. Ethanol extract shows excellent results even better than standard ascorbic acid, while ethyl acetate nearly comparable to ascorbic acid.

The unit $\mu\text{M/ml}$ means the quantity of Fe^{3+} in μM that can be reduced to Fe^{2+} by per ml of potential antioxidant. The higher the FRAP value the greater is the antioxidant activity [18].

Table 4: Ferric reducing antioxidant potential (FRAP Assay) of *C. tomentosa* leaves extracts compared with ascorbic acid

S. No.	Standard/Extracts	Ferric reducing antioxidant power ($\mu\text{M/ml}$)	FRAP value (μM)
1.	Ascorbic acid	39.45±0.14	2.00
2.	Petroleum ether	24.17±1.18	0.90
3.	Chloroform	18.33±0.38	0.60
4.	Ethyl acetate	29.67±1.42	1.80
5.	Ethanol	43.78±0.65	2.30

Results are expressed as mean of three values±standard deviation.

Total phenolic content (TPC)

The total phenolic content of *C. tomentosa* leaves extracts were determined by Folin-ciaocalteau method. The TPC was expressed as milligram gallic acid equivalent GAE/gram dry weight using the

standard curve equation $y = 0.0042x + 0.058$, $R^2 = 0.9976$ where y is the absorbance at 765 nm and x is the total phenolic content in 1000 $\mu\text{g/ml}$ of the extracts. TPC was found highest in ethanol, followed by ethyl acetate, petroleum ether and in chloroform. The total phenolic content of *C. tomentosa* leaves extracts are shown in table 5.

Table 5: Total phenolic content of *C. tomentosa* leaves extracts

S. No.	Extracts	Total phenolic content (mg GAE/gm of dry weight)
1.	Petroleum ether	62.17±0.80
2.	Chloroform	50.75±0.87
3.	Ethyl acetate	71.92±1.01
4.	Ethanol	228.17±0.63

Results are expressed as mean of three values±standard deviation, Antioxidant activities of *C. tomentosa* extracts were corresponding to their total phenolic content. In other word higher phenolic content leads higher antioxidant activity.

Antidiabetic activity

For diabetic patients blood glucose stabilization is important, because it prevents hyperglycemia and the difficulties associated with diabetes. α -amylase and α -glucosidase are such enzymes that catalyzes the breakdown of starch to maltose and then to glucose. The inhibition of these enzymes leads to a decrease in blood glucose level [19].

Alpha-amylase inhibition activity

The *in vitro* α -amylase inhibition studies suggested that *C. tomentosa* leaves extracts had significant inhibitory activity which is compared with acarbose (positive control) illustrated in table 6. Results showed that the inhibition activity of the

extracts is a dose dependent process. Lower IC_{50} value indicates greater therapeutic efficacy. Ethyl acetate extract reflects the highest alpha amylase inhibitory activity followed by petroleum ether extract, ethanol and chloroform. The IC_{50} value of ethyl acetate and petroleum ether extracts are nearly comparable with acarbose and thus can be regarded as alpha amylase inhibitor.

Alpha-glucosidase inhibition activity

The *in vitro* α -glucosidase inhibitory activity of successive extracts compared with acarbose is shown in table 7. Alpha-glucosidase inhibitory activity of extracts was found highest in ethyl acetate, followed by petroleum ether and ethanol. The IC_{50} value of ethyl acetate is nearly comparable with acarbose. Results show that *C. tomentosa* leaves extracts can be regarded as moderate α -glucosidase inhibitor.

Table 6: Alpha amylase inhibitory activity of *C. tomentosa* leaves extracts compared with acarbose

S. No.	Standard/Extracts	IC ₅₀ value of alpha amylase assay in (µg/ml)
1.	Acarbose	100.20±0.96
2.	Petroleum ether	421.83±1.18
3.	Chloroform	3852.50±1.75
4.	Ethyl acetate	262.08±1.26
5.	Ethanol	950.25±1.64

Results are expressed as mean of three values±standard deviation.

Table 7: Alpha glucosidase inhibitory activity of *C. tomentosa* leaves extracts compared with acarbose

S. No.	Standard/Extracts	IC ₅₀ value of alpha glucosidase assay in (µg/ml)
1.	Acarbose	76.60±0.78
2.	Petroleum ether	1621.42±1.61
3.	Chloroform	Out of concentration limit
4.	Ethyl acetate	459.25±1.80
5.	Ethanol	4253.08±2.02

Results are expressed as mean of three values±standard deviation.

Antibacterial activity

Results show that all extracts are active against all five pathogenic bacterial strains. The zones of inhibition for bacterial strains were determined and results were shown in table 8. Results reveal that ethyl acetate extract shows best inhibition against *Staphylococcus*

aureus, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* except *Salmonella typhi*. *Salmonella typhi* was best inhibited by chloroform extract.

In the present study, the growth of all pathogenic bacteria was remarkably inhibited by different extracts of plant *C. tomentosa*.

Table 8: Antibacterial activity of different extracts from *Casearia tomentosa* leaves compared with ofloxacin

S. No.	Bacteria (Gram strain)	Zone of inhibition in millimeters (mm)				
		Standard drug and extracts				
		Ofloxacin	Petroleum ether	Chloroform	Ethyl acetate	Ethanol
1.	<i>Staphylococcus aureus</i> (+)	25.9±0.29	11.9±0.49	11.8±1.37	21.01±1.31	17.9±0.46
2.	<i>Escherichia coli</i> (-)	30.9±0.24	12.2±1.06	10.9±1.14	18.4±0.72	11.7±0.50
3.	<i>Klebsiella pneumonia</i> (-)	15.7±0.87	12.9±1.43	11.1±0.99	20.2±0.101	9.8±1.16
4.	<i>Salmonella typhi</i> (-)	18.0±0.91	15.7±1.13	18.7±1.52	16.2±1.06	12.9±1.06
5.	<i>Pseudomonas aeruginosa</i> (-)	19.5±0.95	13.3±1.65	13.8±0.95	18.7±0.44	12.7±0.68

Results are expressed as mean of three values±standard deviation.

DISCUSSION

The search of potent natural drugs from plants cannot be complete without knowing the usefulness of phytochemicals. Present phytochemical analysis reveals that most of phytoconstituents are present in ethyl acetate and ethanol extracts (table 2). Out of these phytoconstituents flavonoids are one of the largest group of phenolic compounds, numerous reports support their use as primary antioxidant and possess antimicrobial, anti-inflammatory, antiallergic, anticancer activities etc [20]. Plant derived alkaloids are the biggest class of phytochemical and exhibit many therapeutic effects like antioxidant, analgesics, muscle relaxant, antibiotics, anticancer and also responsible for antiprotozoal, cytotoxic and antimicrobial properties [21]. Tannin are water-soluble phenolic compound, used as healing agents in a number of diseases like leucorrhoea, rhinorrhea, diarrhea and also inhibit the growth of many fungi, yeasts, bacteria, and viruses [22]. The presence of glycosides improves cardiac arrhythmia and heart related diseases. Some glycosides are used as astringents, anticancer, and as flavoring agents in many pharmaceutical preparations [23, 24]. Saponins are a special class of glycosides which have soapy characteristics and possess antibacterial, hypolipidemic, antidiabetic, and anticancer activity [25]. Out of these phytochemicals steroids are known to be important against cardio tonic activity and antimicrobial activities [26]. Steroids and tannins were also found interest in pharmacy due to their relationship with such compounds as sex hormones [27]. It is reported that terpenoids are among the most widespread chemically diverse groups of natural products and possess activities like antioxidant, anticancer, anti-inflammatory, sedative, cytotoxic activity etc [28]. Beside these

phytosterol and fixed oil was also present in this plant extracts, which is also responsible for cytotoxic and antioxidant activity [29, 30]. It is now established that phytochemicals of medicinal plants account for their medicinal value.

Different plants extract exhibit efficient antioxidant properties due to their phytoconstituents, including phenolic compound [31]. Hydrogen donating abilities of phenolic compounds for DPPH radical were found in same ratio to the number of hydroxyl group and the amount of inactivated DPPH radical directly related to the antioxidant capability of the plant extracts [32]. The above statement clearly justify the results as TPC was found higher in ethanol extract of leaves of *C. tomentosa* and also the IC₅₀ was lower for the same extract. On the other hand FRAP assay, particularly helpful in evaluating the antioxidant power of sample in which those compounds are present which acts by reducing ion or by donating an electron and not by radical quenching pathway [14]. The FRAP activity of sample may be due to the availability of phenolic hydroxyl or methoxy group, ketone groups, free carboxylic groups, flavones hydroxyl, triterpenes and their derivatives [33]. The statement fairly justify the results as most of phytochemicals were present in ethanol and ethyl acetate extracts, due to this FRAP values of these extracts were quite good (table 4).

According to previous studies, it was found that polyphenols and various flavonoids are among the natural active antidiabetic agents. In general, the enzyme inhibitory activities of the plant extracts not only depend on the polyphenols quantity but also might depend on the quality [34]. That is the reason due to which ethyl acetate extract shows better alpha amylase and alpha glucosidase enzyme

inhibition than ethanol while ethyl acetate extract having lower total phenolic contents (table 6 and 7).

The literature revealed that the presence of secondary metabolites including alkaloids, terpenoids, flavonoids in plants are responsible for its potential antibacterial activity [31]. On correlating the results an inference can be drawn that presence of majority of phytochemicals and good quality of phenolic compounds in ethyl acetate extract may be responsible for its prominent activity against all the bacterial strain. The present study not only clarified the pharmacological applications of *C. tomentosa* leaves, but also introduced novel sources for the prevention of non-communicable diseases in India and in other countries.

CONCLUSION

This study suggests that *C. tomentosa* leaves are an excellent source of active principals that can prevent oxidative stress, development of diabetes mellitus and bacterial infections and their complications. However these active principles need to be isolated, identified and characterized, and the structure need to be elucidated. While these *in vitro* results are of a preliminary nature, further investigation of *C. tomentosa*, in particular, *in vivo* pharmacological testing is warranted. These studies will provide a clearer picture on the potential of this interesting traditional plant.

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CONFLICT OF INTERESTS

Authors declare no conflict of interest

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