

Chemical Characterization and Pharmacological Evaluation of Essential Oil from Leaves of *Casearia tomentosa*

Ritu Tyagi^{1*}, Abha Shukla¹, Rishi Kumar Shukla², Swati Vats¹, Chakit Tyagi³, Anchal Choudhary¹

¹Department of chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand-249407, India,

²Department of chemistry, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand-249404, India.

³Analytical Department, Jubliant Generics Ltd., C-26, Sector-59, Noida, Uttar Pradesh- 201301, India.

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ABSTRACT

The present work deals with the isolation of essential oil from the leaves of *Casearia tomentosa* as well as its chemical characterization by GC-MS and evaluation of *in vitro* pharmacological activities. The crude essential oil was obtained using Clevenger apparatus. Thirteen compounds were identified representing 77.62% of the total oil composition. The oil was mainly composed of 9,12-Octadecadienoic acid, ethyl ester (31.45%) followed of the 9,12,15-Octadecatrienoic acid, ethyl ester (20.11%), Phytol (10.70%), Di-epi-alpha-cedrene (3.74%), β -bisabolene (1.87%), β -Caryophyllene (0.83%) and some other trace components. Antidiabetic activity estimated by α - amylase and α - glucosidase inhibitory assay and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant potential (FRAP) method while antibacterial activity was performed by disk diffusion method. The results demonstrated prominent α - amylase ($IC_{50} = 184.47 \pm 1.06 \mu\text{g/mL}$), α - glucosidase ($IC_{50} = 91.10 \pm 0.80 \mu\text{g/mL}$) inhibition, DPPH radical scavenging ($IC_{50} = 75.50 \pm 0.60 \mu\text{g/mL}$) and FRAP activity ($37.55 \pm 0.66 \mu\text{M/mL}$, FRAP value 1.636). Antimicrobial activity performed towards the selected five human pathogenic bacterial cultures like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Salmonella typhi*. Highest inhibitory activity was seen against *Staphylococcus aureus* (zone of inhibition 12.2 mm) while the weakest activity was demonstrated against *Pseudomonas aeruginosa* (zone of inhibition 8.9 mm). Overall results revealed that *C. tomentosa* leaves (CTL) essential oil exhibits potent antidiabetic, antioxidant and antibacterial activities. This is the first study regarding the isolation of essential oil from the leaves of *Casearia tomentosa* and its pharmacological activities.

Keywords: Antidiabetic activity, Antioxidant activity, Antibacterial activity, *Casearia tomentosa*, DPPH radical scavenging, essential oil, FRAP activity, α - amylase, α - glucosidase etc.

INTRODUCTION

Essential oils are the odorous and volatile products of numerous plants. These are also known as volatile oils or ethereal oils. Nowadays essential oils and some of their compounds have been used in day to day life products such as cosmetics, air fresheners and household cleaning products, hygiene products, agriculture, and food, as well as in medicinal product^{1,2}. Chemically volatile oil comprises a mixture of numerous compounds and mostly the trace constituents which are solely responsible for attributing its characteristic flavor, fragrance and their bioactivities³. Essential oils have recently began to receive much attention as possible sources of safe and natural alternative medicines because they have been known to possess various pharmacological activities, including antidiabetic⁴, antioxidant⁵, anti-inflammatory⁶, antimicrobial⁷, antiviral⁸, and anticarcinogenic⁹ etc. The genus *Casearia* (*Salicaceae*) has more than 160 described species. Some species of *Casearia* like *Casearia decandra*, *Casearia sylvestris*, *Casearia grandiflora*¹⁰⁻¹³,

have been reported for their therapeutically important essential oils. *Casearia tomentosa* is one of the important species of this genus. According to literature, it has large number of traditional uses such as in diabetes, skin diseases, peptic ulcer, and malarial fever etc.¹⁴, but till date no chemical study is reported on *C. tomentosa* leaves essential oil. The present study aimed to estimating pharmacological potential and chemical characterization of leaves essential oil of leaves of this plant.

MATERIAL AND METHODS

Plant material

Fresh leaves of plant *Casearia tomentosa* were collected from Lachhiwala forest Dehradun, Uttarakhand (India), 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 with registry no. 1/3. The collected leaves were washed with distilled

water and finally cut into small pieces for extraction of essential oil.

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), 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 (Hi-Media), Muller Hinton Agar (Hi-Media), Muller Hinton broth (Hi-Media) were purchased. All other chemicals were used of analytical grade.

Extraction

Fresh leaves (280.00 g) of *C. tomentosa* were extracted over 5 hours by steam distillation in a Clevenger apparatus. Obtained essential oil was dried over anhydrous sodium sulphate and stored at 4°C.

Characterization of essential oil

The oil was characterized by GC-MS using a TG 5MS (30m X 0.25mm, film thickness 0.25 μ m) capillary column. GC chromatograms were obtained on a TRACE 1300 GC gas chromatograph equipped with an MS TSQ 8000 detector and an injector (S/SL). These analyses were performed by injecting 1.0 μ L of a 1.0mg/mL solution of volatile oil employing helium as the carrier gas (1 mL/min) under the following conditions: ion source and injector temperatures of 230°C and 250°C, respectively; oven programmed temperature from 60–250°C at 2°C/min, holding 5 min at 250°C. MS conditions were as follows: Ionization voltage 70eV, acquisition mass range (m/z) 40-800. The identification of the individual compounds was performed by comparison of mass spectra of pure compound¹⁵, as well as from NIST 08 MS library of pure compounds.

Antidiabetic Activity

Postprandial blood sugar control is one of the possible six pathways that can be efficiently used for regulating diabetes^{16,17}. Enzymes α -amylase and α -glucosidase are involved in the digestion of carbohydrates. Inhibitors of these enzymes can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet¹⁸⁻²⁰. Thus Anti-diabetic activity of essential oil of plant *C. tomentosa* leaves was assessed by α -amylase and α -glucosidase inhibitory methods.

Alpha-Amylase Inhibition Activity

The α -amylase inhibitory activity of essential oil was performed using 3,5-Dinitrosalicylic acid method with a little modification²¹. Briefly, 1mL of each solution of different dilutions (1-1000 μ g/mL) of essential oil or standard acarbose in DMSO was incubated with 1mL of α -amylase (concentration 0.25 mg/mL in 20 mM phosphate buffer containing 6.7mM NaCl, pH 6.9) for 30 min at 37°C. In another set of tubes 1mL of alpha amylase was pre incubated with 1mL of phosphate buffer. After pre incubation, 1mL of 1% starch solution in 20mM 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 1mL of DNSA color

reagent (96 mM 3,5-dinitrosalicylic acid and 5.315M sodium potassium tartrate in 2M NaOH) was added. The tubes containing reaction mixture were then placed in a boiling water bath for 5 minutes and then cooled to room temperature. The absorbance was taken at 540 nm with a UV-Vis spectrophotometer (Systronic, UV-VIS 117) after diluting each tube with 9mL of distilled water. The α -amylase inhibitory activity was calculated by equation (1). α -amylase inhibitory activity (% Inhibition) =

$$\frac{[(A_{C^+} - A_{C^-}) - (A_S - A_B)]}{(A_{C^+} - A_{C^-})} \times 100$$

Where, A_{C^+} = Absorbance of pure control having 100% enzyme activity (DMSO and Enzyme). A_{C^-} = Absorbance of blank for pure control having 0% enzyme activity (DMSO and Buffer). A_S = Absorbance of sample or standard (sample/standard and Enzyme). A_B = Background absorbance due to sample and standard (sample/standard and Buffer). IC_{50} value (concentration at which sample shows 50% enzyme inhibition) of essential oil and standard acarbose were determined graphically by plotting % inhibition vs. concentration.

Alpha-Glucosidase Inhibition Activity

The α -glucosidase inhibitory activity of *C. tomentosa* leaves (CTL) essential oil was determined according to Shukla et al. with little modification²¹. Briefly, 1mL of each dilution of (1-1000 μ g/mL) of sample or standard acarbose in DMSO was pre-incubated with 1mL of α -glucosidase (1U/mL in 100mM phosphate buffer pH 6.8) for 30 min at 37°C. In another set α -glucosidase was pre-incubated with 1ml of phosphate buffer. After pre incubation, 1mL of, 5mM p-NPG (in 100mM 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 4mL 0.5M Tris buffer was added to stop reaction. The absorbance was taken by UV-VIS spectrophotometer (Systronic, UV-VIS 117) at 410 nm. The % inhibition and IC_{50} was calculated in similar way as mentioned in α -amylase activity.

Antioxidant Activity

Specifically free radicals produce cell damages, tissue injuries and increased levels of reactive oxygen species (ROS). The source of oxidative stress is a cascade of ROS leaking from the mitochondria. This process has been associated with the onset of type 1 diabetes via apoptosis of pancreatic beta cells and the onset of type 2 diabetes via insulin resistance. Furthermore, insulin deficiency also promotes β -oxidation of fatty acids, which results in increased formation of hydrogen peroxide. As such, under the diabetic condition the increased levels of ROS will damage the pancreas and liver cells²². So the role of antioxidant agents in treatment of diabetes has been crucial. Thus antioxidant activity of CTL essential oil 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 *C. tomentosa* leaves essential oil was evaluated by stable DPPH free radical with some modification²³. A working solution of 0.004%

was freshly prepared by dissolving 4 mg of DPPH in 100 ml of methanol. 1ml of sample solution of different concentration (1, 5, 10, 50, 100, 500,1000µg/mL) was added to 3ml working solution of DPPH, Keep this

reaction mixture in dark for 30 min. After 30 minute the absorbance of the reaction mixture were taken at 517nm with UV-VIS spectrophotometer (Systronic, UV-VIS 117)

Table 1: Chemical composition of *Casearia tomentosa* leaves essential oil.

S. No.	Name of the compound	RT ^a (min)	Molecular Formula	Peak Area %	Reported Biological Activities ²⁶
1.	β-Caryophyllene	18.30	C15H24	0.83	Antitumor, Analgesic, Antibacterial, Anti-inflammatory, Antimelanogenesis activity, Perfuming agent, Skin whitening agent, Food additive
2.	Di-epi-alpha-cedrene	18.67	C15H24	3.74	Alpha-Amylase-Inhibitor, Alpha-Glucosidase-Inhibitor, Alpha-Reductase-Inhibitor, Testosterone-5-Alpha-Reductase-Inhibitor
3.	Beta-bisabolene	19.90	C15H24	1.87	Beta-glucuronidase-Inhibitor Food additive, Natural sweetener
4.	Not identified	26.79	-	2.17	-
5.	Thymol methyl ether	28.41	C16H22O4	1.44	Catechol-O-Methyltransferase-Inhibitor, Methyl-Guanidine-Inhibitor, Antimicrobial, Antibacterial, Antitumor, Antimutagenis,
6.	4-Terpineol	29.92	C10H18O	1.04	Antiasthmatic , Antioxidant, Insectifuge, Antibacterial, Antifungal, Perfumery, Diuretic
7.	Not identified	30.29	-	2.28	-
8.	Not identified	30.88	-	1.67	-
9.	Phytol	33.06	C20H40O	10.70	Antimicrobial, Anti-inflammatory, Anticancer,
10.	n-Propyl 9cis.,11.trans.-octadecadienoate	33.76	C22H38O2	1.49	Glucosyl-Transferase-Inhibitor, Increase Glyoxalate Transamination, Reverse-Transcriptase-Inhibitor, Antitumor, Decrease Norepinephrine Production, Arylamine-N-Acetyltransferase-Inhibitor, Anaphylactic
11.	9,12-Octadecadienoic acid, methyl ester	33.89	C19H34O2	1.43	Antialopecic, Antioxidant, Antifibrinolytic, Antimicrobial, Urinary-Acidulant, Arachidonic-Acid-Inhibitor, Catechol-O-Methyltransferase-Inhibitor and as Food additive.
12.	(-)-Isoaromadendrene-(V)	34.02	C15H24	1.50	-
13.	Not identified	34.51	-	0.80	-
14.	Not identified	34.89	-	1.23	-
15.	9,12-Octadecadienoic acid (Z,Z)-, ethyl ester	36.25	C20H36O2	31.45	Antimicrobial, Antioxidant, Antibacterial, Increase zinc bioavailability, Increase Aromatic Amino Acid Decarboxylase Activity,
16.	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	36.38	C20H34O2	20.11	Anti-inflammatory, Hypocholestromic, cancer preventive, Hepetoprotective, Nematicide, Antihistaminic, Antieczemic, Antiacne,5-Alpha Reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary and Perfuming emollient
17.	Not identified	37.89	-	4.16	-

18.	n-Tetratriacontane	39.43	C34H70	0.82	Antitumor, Anaphylactic, Arylamine-N-Acetyltransferase-Inhibitor, NADH-Oxidase-Inhibitor
19.	Not identified	40.25	-	9.05	-
20.	Not identified	41.00	-	1.03	-
21.	Hexatriacontane	42.86	C36H74	1.20	NADH-Ubiquinone-Oxidoreductase-Inhibitor, Myo-neuro-stimulant, Antitumor, Anaphylactic

^aRT: Retention time of the compounds based on GC-MS peaks; Compounds are listed in order of their elution from a TG 5MS column;

which was compared with the corresponding absorbance of standard ascorbic acid of similar concentrations (1-1000µg/mL). One millilitre of methanol with 3 mL of working DPPH solution serves as blank. Then the % radical scavenging activity or % inhibition was evaluated by equation (2)-

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample/ standard}) \text{ after 30 min}}{(\text{Absorbance of blank}) \text{ after 30 min}} \times 100$$

IC₅₀ of essential oil and standard ascorbic acid was calculated by graphical method by plotting percent inhibition vs. concentration.

Ferric reducing antioxidant potential assay (FRAP)

The FRAP assay was done according to Benzie and Strain with some modification²⁴. The stock solutions includes 300mM acetate buffer pH3.6, 10mM TPTZ (2,4,6-tri-(2-pyridyl)-1,3,5-triazine) solution in 40 mM HCl, and 20mM 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 was determined by reacting a mixture of 1mL of sample (50µg/mL) and 10mL of working FRAP reagent. Absorbance of colour solution (ferrous tripyridyl triazine complex) was then taken at 593 nm after 5 min of incubation at 37°C. Ascorbic acid standard solutions were tested in a similar way. The standard curve was linear between 10 - 100µM ascorbic acid. Working FRAP reagent serves as blank and 1mL of methanol with 10mL of working FRAP reagent act as control. Calculations were made by calibration curve. Results were expressed as µM/mL. FRAP value of sample was calculated by equation (3):

$$\text{FRAP value of Sample } (\mu\text{M}) = \frac{(\text{Change in absorbance of Sample from 0 to 5 minute})}{(\text{Change in absorbance of Standard from 0 to 5 minute})} \times \text{FRAP value of Standard}$$

Antibacterial activity

The determination of antibacterial screening of *C. tomentosa* leaves essential oil was carried out by agar well diffusion technique²⁵.

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 2h 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 7mm diameter were punched with the help of cork borer in agar medium and filled with 40µl of essential oil 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. Essential oil 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 (sigmastat 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<0.05.

RESULTS AND DISCUSSION

Extractive Yield of Oil

The oil obtained from *C. tomentosa* leaves is pale yellow in color with a yield of 0.057 % (w/w).

GC-MS Analysis of Leaves oil

This analysis allowed the identification of 13 chemical entities (Table 1) representing 77.62% of the total oil composition. Leaves essential oil is a mixture of terpenoids, diterpenoids, sesquiterpenoids, fatty acid esters, hydrocarbons etc. The oil is mainly composed of 9,12-Octadecadienoic acid, ethyl ester (31.45%) followed of the 9,12,15-Octadecatrienoic acid, ethyl ester (20.11%), Phytol (10.70%), Di-epi-alpha-cedrene (3.74%), Beta-bisabolene (1.87%), β-Caryophyllene (0.83%) and some other trace components. Most of these have important biological activities such as Antioxidant, Alpha Amylase

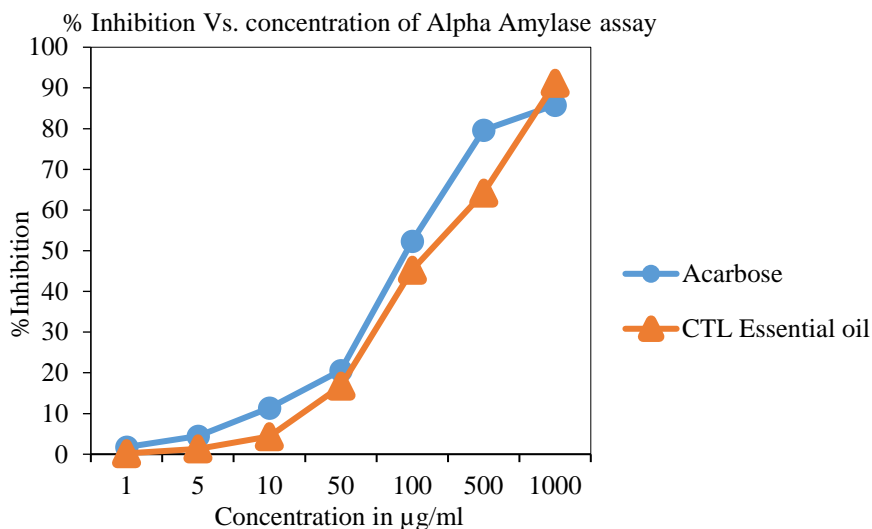


Figure 1: Alpha amylase inhibitory activity and estimation of IC₅₀ value of *C. tomentosa* leaves Essential oil and acarbose.

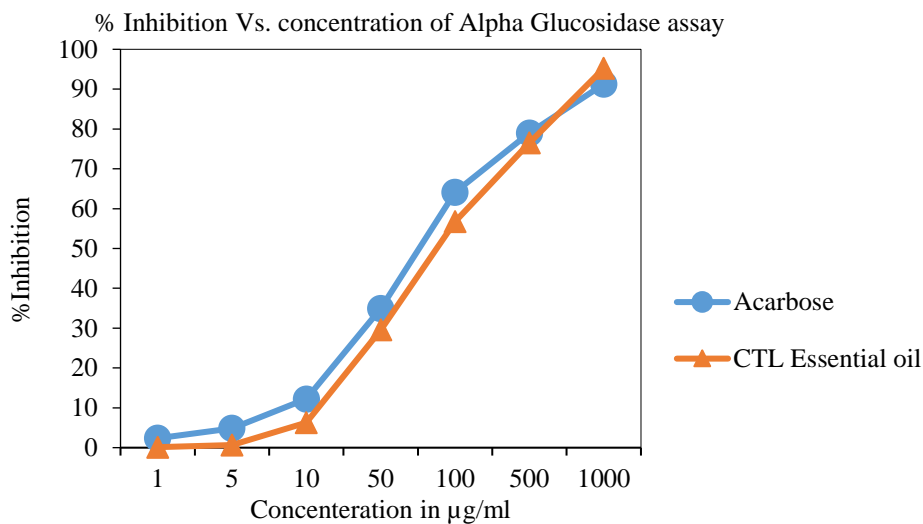


Figure 2: Alpha glucosidase inhibitory activity and estimation of IC₅₀ value of *C. tomentosa* leaves Essential oil and acarbose.

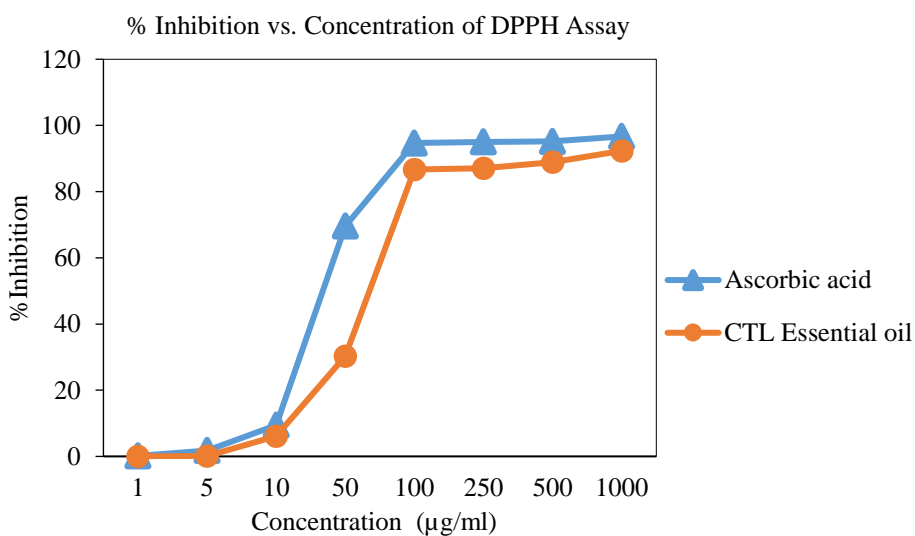


Figure 3: DPPH Radical Scavenging activity and estimation of IC₅₀ value of *C. tomentosa* leaves Essential oil and ascorbic acid.

inhibitory, Alpha Glucosidase inhibitory, Antitumor, Analgesic, Antibacterial, Anti-inflammatory Hypocholesterolemic, cancer preventive, Hepatoprotective, Nematicide, Antihistaminic, Antieczemic, Antiacne, 5-Alpha Reductase inhibitory, Antiandrogenic etc. (Table 1)²⁶⁻³⁸.

Antidiabetic Activity

Alpha-amylase Inhibition Activity

The in vitro α -amylase inhibition studies demonstrated that *C. tomentosa* leaves essential oil had significant inhibitory activity which is compared with acarbose (positive control) illustrated in Table 2. Figure 1 graphically shows the α -amylase inhibition on changing the concentration of sample and standard and also help in the estimation of IC_{50} value of both. Results showed that the inhibition activity of the essential oil is a concentration dependent process. Essential oil showed percentage α -amylase inhibition of 0.200% - 91.206% on varying concentration from 1-1000 $\mu\text{g/ml}$ with an IC_{50} value $184.47 \pm 1.06 \mu\text{g/ml}$ while α -amylase inhibition due to acarbose was 1.760% - 85.82% on similar concentrations with an IC_{50} value $100.20 \pm 0.96 \mu\text{g/ml}$. Lower IC_{50} value indicates greater therapeutic efficacy. The IC_{50} values indicate that α -amylase inhibition activity of *C. tomentosa* essential oil and acarbose is nearly comparable. Thus essential oil can be regarded as an excellent α -amylase inhibitor.

Alpha-Glucosidase Inhibition Activity

The in vitro α -glucosidase inhibitory activity of essential oil compared with acarbose is shown in Table 2. Figure 2 graphically indicate the α -glucosidase inhibition on varying concentration of sample and also help in estimation of IC_{50} value of oil as well as standard acarbose. Results revealed that α -glucosidase inhibitory activity of essential oil ($IC_{50} = 91.10 \pm 0.80 \mu\text{g/mL}$) is nearly

comparable with acarbose ($IC_{50} = 76.60 \pm 0.78 \mu\text{g/mL}$). Thus essential oil can be regarded as potent α -glucosidase inhibitor. Therefore, leaves essential oil could be well tolerated as antidiabetic agent in comparison to synthetic inhibitors. While synthetic inhibitors have a number of side effects like bloating, flatulence, diarrhea and abdominal problem²¹. GC-MS analysis of oil also supports its antidiabetic activity. Chemical constituent Di-epi-alpha-cedrene is mainly responsible for CTL essential oil immense antidiabetic activity²⁶.

Antioxidant activity

Antioxidants protect cells against the deleterious effects of reactive oxygen species or free radicals which results oxidative stress leading to cellular damage. Now these days, many researchers have been investigating the antioxidant activity of different essential oils in search of safe natural antioxidants.

DPPH free radical scavenging assay

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant essential oil. The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. Essential oil reduces the colour of DPPH due to the power of hydrogen donating ability^{39,40}. *C. tomentosa* leaves essential oil was investigated for their possible DPPH radical scavenging power. Figure 3 shows the graphical estimation of IC_{50} value, which is a measure of inhibitory concentration. The antioxidant activity of essential oil ($IC_{50} = 75.50 \pm 0.60 \mu\text{g/mL}$) is found very close to standard ascorbic acid ($IC_{50} = 20.00 \pm 0.26 \mu\text{g/mL}$).

Ferric reducing antioxidant potential assay (FRAP)

The ferric reducing antioxidant potential (FRAP) assay is a simple and inexpensive protocol that tells the total antioxidant levels in plants⁴¹. Frap assay, particularly

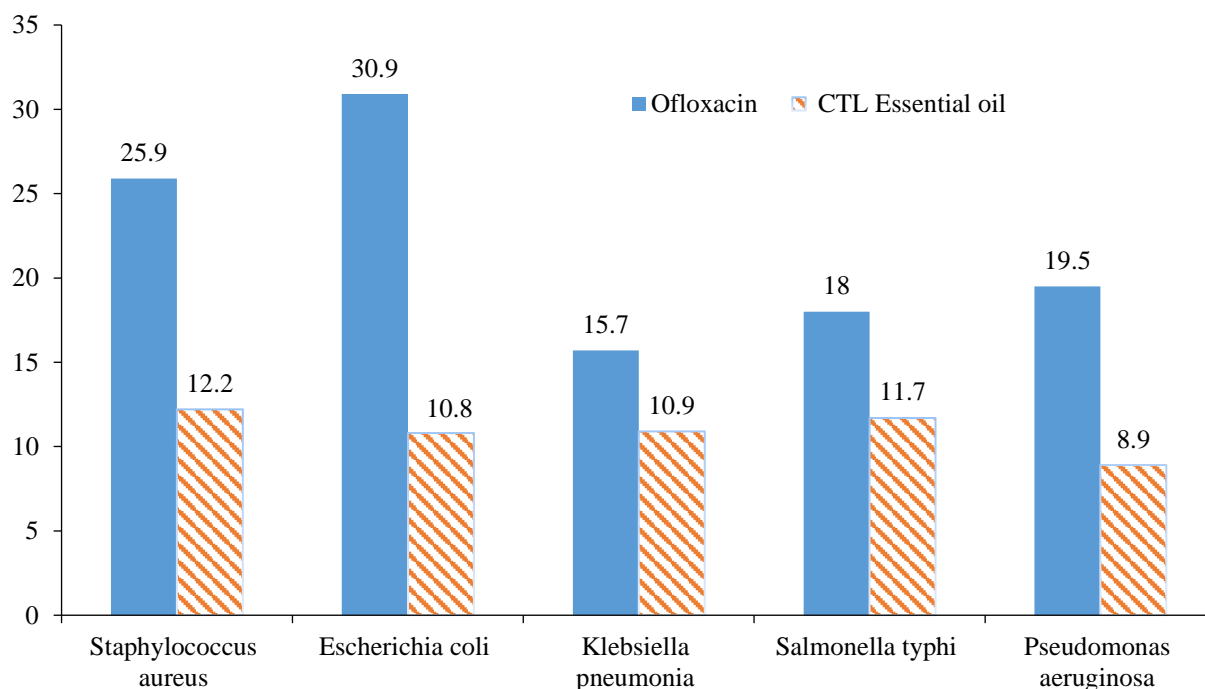


Figure 4: Graphical representation of zone of inhibition of *C. tomentosa* leaves essential oil and ofloxacin against the selected pathogens.

Table 2: Alpha amylase and Alpha glucosidase inhibitory activities of *C. tomentosa* leaves essential oil compared with Acarbose.

S. No.	Standard/Essential oil	IC ₅₀ value of alpha amylase assay in (µg/ml) ^a	IC ₅₀ value of alpha glucosidase assay in (µg/ml) ^a
1.	Acarbose	100.20 ± 0.96	76.60 ± 0.78
2.	CTL essential oil	184.47 ± 1.06	91.10 ± 0.80

^aResults are expressed as mean of three values ± standard deviation

Table 3: Ferric reducing antioxidant potential (FRAP Assay) of *C. tomentosa* leaves oil compared with Ascorbic acid.

S. No.	Standard/Essential oil	Ferric Reducing antioxidant Power(µM/ml) ^a	FRAP Value (µM)
1.	Ascorbic acid	39.45±0.14	2.00
2.	CTL essential oil	37.55±0.66	1.636

^aResults are expressed as mean of three values ± standard deviation

Table 4: Antibacterial activity of essential oil from *Casearia tomentosa* leaves compared with Ofloxacin.

S. No.	Bacteria (Gram strain) (code)	Zone of Inhibition in millimeters (mm ^a)	
		Ofloxacin	CTL Essential oil
1.	<i>Staphylococcus aureus</i> (+) (MTCC-737)	25.9±0.29	12.2±0.94
2.	<i>Escherichia coli</i> (-) (ATCC-433)	30.9±0.24	10.8±0.17
3.	<i>Klebsiella pneumonia</i> (-) (ATCC-109)	15.7±0.87	10.9±0.61
4.	<i>Salmonella typhi</i> (-) (ATCC-733)	18.0±0.91	11.7±0.99
5.	<i>Pseudomonas aeruginosa</i> (-) (MTCC-430)	19.5±0.95	8.9 ±0.67

^aResults are expressed as mean of three values ± standard deviation

helpful in assessing the antioxidant power of sample in which those constituents are present which acts by reducing ion or by donating an electron and not by radical quenching mechanism⁴². The results were expressed as µM/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 µg/ml of oil shown in Table 3. The unit µM/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 activity⁴⁰. GC-MS analysis of oil supports its excellent antioxidant activity. Chemical constituents 9, 12-Octadecadienoic acid (Z, Z)-, ethyl ester, 9,12-Octadecadienoic acid, methyl ester, 4-Terpeneol are excellent antioxidants and responsible for CTL essential oil antioxidant activity²⁵.

Antibacterial activity

Results shows that leaves essential oil (concentration 1mg/ml) is active against all five pathogenic bacterial strains. The zones of inhibition for bacterial strains were determined and results were shown in Table-4 and graphical representation is shown in Figure-4. The gram positive *S. aureus* is more sensitive than the all gram negative bacterial strain (*Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Pseudomonas aeruginosa*). Highest inhibitory activity was seen against *S.aureus* (zone of inhibition 12.2 mm) while the weakest activity was demonstrated against *P. aeruginosa* (zone of inhibition 8.9 mm). This shows that the constituents present in the potent essential oil are versatile in nature and can have wide range of antimicrobial spectrum. GC-MS analysis of oil also supports its immense antibacterial activity. Chemical constituents β-Caryophyllene, Thymol methyl ether, Phytol, 9, 12-Octadecadienoic acid (Z, Z)-, ethyl ester, 9,12-Octadecadienoic acid, methyl ester, 4-Terpeneol are

excellent antibacterial agents and responsible for CTL essential oil antibacterial activity²⁶.

CONCLUSION

C-MS analysis of oil demonstrated that *C. tomentosa* leaves essential oil contain various therapeutically valuable components. According to literature these components have vast list of their biological activities. Therefore, this oil has a large scope for research to explore more biological activities. The present pharmacological study suggests that leaves essential oil is rich sources of natural antidiabetic, antioxidant and antibacterial agents and can be used to replace synthetic ones.

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