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Research Article

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Total phenolic content, antioxidant activity and phytochemical screening of hydroalcoholic extract of *Casearia tomentosa* leaves

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ABSTRACT

In present study hydroalcoholic extract of the leaves of plant Casearia tomentosa was investigated for the phytochemical screening, total phenolic content and antioxidant activity.The preliminary phytochemical investigation of leaves extract revealed the presence of active phytoconstituents such as alkaloids, glycosides, steroids, saponins, flavonoids, terpenoids and tannins etc.Total phenolic content was evaluated by Folin-Ciocalteau method which revealed the presence of very good amount of phenolic content (170 mg GAE/g of dry weight*) in extract. The extract was also analyzed for antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant potential(FRAP) assay. In vitro DPPH radical scavenging assay showed potent antioxidant power with IC_{50} values of 60 µg $mL^{-1}$and Ferric reducing antioxidant power for extract(43.12 µM/ml, FRAP value =2.1) which arecomparable toascorbic acid. The present study reveals that Casearia tomentosa can be used as a potential source of natural antioxidant which may be treat various oxidative stress related diseases.*

Keywords:*Casearia tomentosa, Phytochemical, DPPH assay, FRAP assay, Phenolic content etc.*

INTRODUCTION

India has several medical systems such as Ayurveda, Siddha and Unani Systems which are prominent from decades .In different civilizations the contribution of floral biodiversity to health care has been well documented [1].Historically plants have been used as the richest source of drugs for traditional systems of medicine. These plants are played important role in modern medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs[2]. Due to easy availability, no side-effects, and better patient compliance, the demand for medicinal plants is increasing all over the world. Medicinal plants contain dozens of active constituents such as alkaloids, flavonoids, glycosides, saponins, terpenoids, steroids and tannins etc. which are responsible for therapeutic efficacy of any medicinal plant [3].

Oxygen is essential for the survival of all living creatures on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals [4, 5]. These radicals if not scavenged effectively in time, they may damage important bio molecules like lipids, proteins including those present indifferent cell membranes, mitochondria and the DNA resulting in abnormalities leading to disease conditions [6].Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing [6,7]. The demand for natural antioxidants has been increased due to side effect associated with synthetic antioxidants[8].

In the last few decades, plants has received much attention as sources of bioactive substances such as antioxidants, antimutagens and anticarcinogens[9,10]. In this regard present study is based on plant named *Casearia tomentosa*, it is a small tree up to 50-80 cm girth and 7 m tall belongs to the family *Salicaceae*. Its common name is *Chilla*. Different parts of *C. tomentosa* is traditionally claimed for its medicinal importance like in ulcers, dropsy, fissures, colic pain in the abdomen, malarial fever, tonsillitis pain, wounds, and in severe bone fractures as a plaster[11].

Therefore, the present study consists of investigation of the antioxidant potential of *Casearia tomentosa* plant and to put forward the evidence of the fact that this plant has immense therapeutic power. This is the first report regarding antioxidant activity, total phenolic content and phytochemicals study of the leaves of this plant.

EXPERIMENTAL SCETION

Plant material

Casearia tomentosa leaves were collected from Lachhiwala forest Dehradun, Uttarakhand (India) in the month of August 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 register no.1/3. The collected leaves were washed, dried in shade and finally grinded to powdered form and stored in polythene bags for further use.

Chemicals and reagents

2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) (Sigma Aldrich),1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), Folin's ciocalteau phenol reagent(Merck), Gallic Acid (Loba chemie), Ascorbic acid (Rankem, India),Petroleum ether (Merck), Ethanol (Merck)were purchased. All the other solvents and chemicals used were of analytical grade.

Defatting of leaves of *Casearia tomentosa:*

150 gm. Of air dried powdered leaves of *C. tomentosa* was treated with 1250 ml of petroleum ether by soxhlet extraction technique for 18 hours. Defatted leaves was taken out from soxhlet thimble spread as a bed on a clean paper and dried to 30 to 45 minutes so as to completely evaporate petroleum ether from leaves surface. Marc so obtained was used for hydroalcoholic extraction purpose.

Preparation of Extract

147.788 gm. of defatted leaves of *C. tomentosa* was extracted with ethanol: water (1:1 v/v) in soxhlet apparatus for 21hours and the extract was collected. It was concentrated to dryness under reduced pressure and controlled temperature 40^0C using rotary evaporator.

Phytochemical screening

The phytoconstituents present in hydroalcoholic extract was analyzed by using standard qualitative methods[12,13]. Extract was screened for the presence of biologically active compounds like alkaloids, flavonoids, tannins, glycosides, saponins, protein, terpenoids, steroids, fat and oil etc.

Total phenolic content

Total phenolic content of hydroalcoholic extract of leaves of *Casearia tomentosa* was determined by using Folin-Ciocalteau method [14] with some 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. 1 ml of extract was added to 10 ml of 10 % Folin Ciocalteau reagent in 25 ml of volumetric flask, after 8 minutes 8 ml of 7.5 % sodium carbonate was added. Further, total volume is made up to mark by adding distilled water. The complete reaction mixture was incubated for about 45 min in the dark and at room temperature of about 25°C±2. The same procedure was followed with gallic acid standard dilutions and also with blank where methanol is used in place of extract. After incubation, the absorbance was measured at 765 nm with UV-VIS spectrophotometer. 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.

$$\mathbf{T = C \times V/M}$$

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

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 hydroalcoholic leaves extract of *C. tomentosa leaves* was evaluated by stable DPPH free radical according to the method of Brand-Williams with some modification[15]**.**A working solution of 0.004%was freshly prepared by dissolving 4 mg of DPPH in 100 ml of methanol. 1ml of each extract solution of different concentration (1, 5, 10,50, 100, 500,1000 µg mL^{-1}) 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 which was compared with the corresponding absorbance of standard ascorbic acid of similar concentrations (1-1000 µg mL^{-1}). 1 ml of methanol with 3ml of working DPPH solution serves as blank. Then the % radical scavenging activity or % inhibition was evaluated by equation:

$$\% \text{ Inhibition} = \frac{\text{(Abs of blank – Abs of sample/ standard) after 30 min}}{\text{(Abs of blank) after 30 min}} \text{ X100}$$

IC50 of extract and standard ascorbic acid was 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[16]**.** The stock solutions includes 300mM acetate buffer P^H3.6, 10mM TPTZ (2,4,6-tri-(2-pyridyl)-1,3,5-triazine) solution in 40mMHCl, 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 37^0C before using it. Antioxidant potential was determined by reacting a mixture 1ml of extract (50 µg mL^{-1}) and 10 ml of working FRAP reagent. Absorbance of colored solution (ferrous tripyridyl triazine complex) was then taken at 593 nm after 5 min of incubation at 37^0C.Ascorbic acid standard 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 1ml of methanol with 10ml of working FRAP reagent act as control. Calculations were made by calibration curve. Results were expressed as µM mL^{-1}. FRAP value of sample was calculated by equation:

$$\text{FRAP value of Sample (µM)} = \frac{\text{(Change in abs of Sample from 0 to 5 minute)}}{\text{(Change in abs of Standard from 0 to5 minute)}} \text{X FRAP value of STD}$$

Statistical analysis

The experimental results are expressed as mean ± standard deviation of triplicate measurement and the results are processed using Microsoft Excel 2010 and sigmastat variance.

RESULTS AND DISCUSSION

Extractive yield

The extractive yield (in % w/w) of hydroalcoholic extract was 39.311%.After complete removal of the solvent the extract is dark brown semisolid.

Phytochemical screening

The result of phytochemical screening of *C. tomentosa* leaves extract are summarized in Table.1.Whichrevealed the presence of active phytoconstituents such as alkaloids, glycosides, steroids, saponins, flavonoids, terpenoids and tannins etc. Out of which flavonoids are one of the largest group of phytochemical which led to the antioxidant activity and numerous reports support their use as antioxidants or free radical scavengers [17].Alkaloids were also present in this plant and it is reported that Plant-derived alkaloids possess activities like antioxidant, analgesics, muscle relaxant, antibiotics, anticancer and also responsible for antiprotozoal, cytotoxic and antimicrobial properties [18, 19].Tannin are the large groups of phenolic compound, used as healing agents in a number of diseases like leucorrhoea, rhinorrhea and diarrhea. Saponins are important phytochemicals as they are shown to have hypolipidemic, antidiabetic, and anticancer activity which was present in appreciable amount in this extract. Glycosides were also present and these are important class which are useful against cardiac arrhythmia and other heart related diseases [20].Some of the glycosides are used as astringents, anticancer, and as flavoring agents in many pharmaceutical preparations [21].

From above discussion, we can interpret that the presence of these phytochemicals in hydroalcoholic extract reveals medicinal importance of plant *Casearia tomentosa*.

Table 1: Phytoconstituents present in leaves extract of *Casearia tomentosa*

Phytoconstituents and Test performed		Hydroalcoholic Extract
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	-
	Filterpaper test	-
Saponin	Foam test	+
Protein and amino acid	Ninhydrin	+
	Biuret	+
Phytosterol	Salwoski test	+
	Liebermann burchard test	+

+: Present, -: Absent

Total Phenolic Content (TPC)

The total phenolic content of C. tomentosa leaves extract was 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 1000µg/ml of the extract. Hydroalcoholic extract contain very good amount of phenolic content which was found to be 170 ± 1.22 mg GAE/g of dry weight. Generally Phenolic constitutes are well known for their various biological activities such as antioxidant, antimutagenic and anticariogenic activity etc. They are located in different parts of plant's tissues and cells, such as vacuoles, cell walls, cell nuclei and also play a key role in plant defense mechanisms to counteract reactive oxygen species in order to survive and prevent molecular damage and damage by microorganism, insect, and herbivores [4].

DPPH free radical scavenging assay

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. Extracts reduce the colour of DPPH due to the power of hydrogen donating ability [22, 23]. Hydroalcoholic extract of C. tomentosa was subjected for their possible DPPH radical scavenging power. Figure 1 shows the graphical estimation of IC_{50} value, which is a measure of inhibitory concentration. A lower IC_{50} value would reflect the greater antioxidant property of the sample. The antioxidant activity of hydroalcoholic extract (IC_{50} 60±0.06 µg mL^{-1}) is found very close to standard ascorbic acid (20±0.26 µg mL^{-1}). Antioxidants may guard against reactive oxygen species (ROS) toxicities by scavenging reactive metabolites and converting them to less reactive molecules [24, 25].

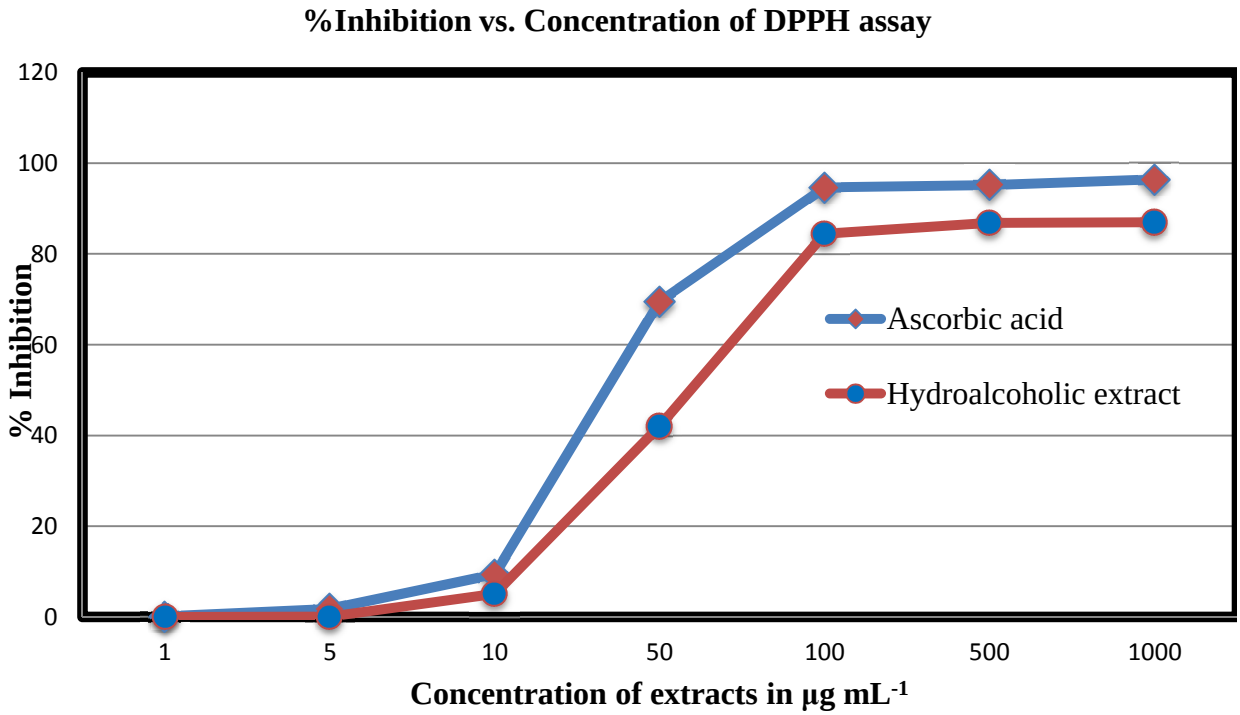

Fig1. DPPH radical scavenging activity of leaves extract of C*asearia tomentosa* and ascorbic acid

Ferric reducing antioxidant potential assay (FRAP)

The ferric reducing antioxidant potential (FRAP) assay is another a simple and inexpensive procedure that tells the total antioxidant levels in plants [26]. FRAP-method was initially developed to assay plasma antioxidant capacity, but can be used to measure the antioxidant capacity from a wide range of biological samples and pure compounds [27]. Frap assay, particularly helps in assessing the antioxidant behavior of extracts in which those phytoconstituents are present which acts by reducing ion or by donating an electron and not by radical quenching mechanism [19]. The results were expressed as µM mL^{-1}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 $mL^{-1}$of extracts. The unit µM mL^{-1}means the quantity of Fe3+ in µM that can be reduced to Fe2+ by per ml of extract or ascorbic acid. The higher the FRAP value the greater is the antioxidant activity [22].

Table 2: Ferric reducing antioxidant potential (FRAP Assay) of *C. tomentosa* extract compared with ascorbic acid

S.No.	Extract/Standard	Ferric reducing antioxidant power (µM/ml)	FRAP Value
1.	**Hydroalcoholic**	43.12 ± 0.60	2.1
2.	**Ascorbic acid**	39.45 ± 0.14	2.0

Even though the DPPH radical scavenging activity of ascorbic acid is better than hydroalcoholic extract, the FRAP activity of latter is superior to the former. Thus it is indicative that contains such constituents which act other than by radical quenching mechanism.

CONCLUSION

The present study was aimed to perform phytochemical evaluation, total phenolic content and antioxidant activity of hydroalcoholic extract of *Casearia tomentosa* leaves. Since this is the first report regarding phytochemical screening and antioxidant activity along with total phenolic content on leaves of this plant and provide referential information for correct identification. The antioxidant activity of leaves established a scientific proof for its traditional claim. Phytochemical studies in leaves laid down a platform in search for a lead molecule that could be a potent antioxidant agent of natural origin.

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REFERENCES

[1] RK Shukla; D Painuly; A Shukla; V Kumar; J Singh; A Porval1 and S Vats, *Int. J. Pharm .Pharm .Sci.*,**2015**, 7(1), 191-197.
[2]K Vijayaraghavan;SMohamed Ali;R Maruthi, *Int. j. innov. res. sci. eng. technol.,* **2013**, 2(12),7315-7321.
[3]A Shukla; S Vats; RK Shukla,*Int J PharmTech Res*, 2013, 5(4), 1670-1678.
[4]RK Shukla; D Painuly; A Porvall; A Shukla, *Journal of Herbs, Spices & Medicinal Plants*, **2014**, 20 (2), 196-208.
[5]RK Shukla; A Porvall; D Painuly; A Shukla, *'Natural Products: An Indian Journal*, **2013**, 9(4), 133-137.
[6]MA Jayasri; L Mathew;A Radha, *Int J Integr Biol.*,**2009**, 5(1), 20-26.
[7]RK Shukla; D Painuly; A Porvall; A Shukla, *'Natural Products: An Indian Journal*, **2012**, 8(9), 361-369.
[8]H MillogoKone; M Lompo;F Kini;SAsimi;IP Guissou; O Nacoulma, *Research Journal of Medical Science.*, **2009**, 3(2), 70–74.
[9]MI Yousef; AA Saad; LK Shennawy, *Food Chem.Toxicol.*,**2009,** 47, 1176-1183.
[10]M Alothman; R Bhat; AA Karim,*Food Chem.*,**2009,**115, 785-788.
[11]KT Rao; K Sreedevi; A Veerabhadrappa,MK Chetty,L Ramesh, *Ind. J. Pl. Sc.*,**2014**, 3 (1), 136-144.
[12]WC Evans: Trease and Evans' Pharmacognosy.16th Edition, Elsevier Health Sciences publishers. UK; **2009**.
[13]JB Harborne, Phytochemical Methods A Guide To Modern Techniques of Plant Analysis, 4th Edition, Springer publishers;**1998**.
[14]VL Singleton; R Orthofer; MR Raventos, *MethodsEnzymol*.,**1999**,299,152-177.
[15]W Brand-Williams; ME Cuvelier; C Berset, *Food Science and Technology*,**1995**,28, 25-30.
[16]FF Benzie; JJ Strain, *MethodsEnzymol*.,**1999,**299, 15-23.
[17]A Kar, Pharmaocgnosy and Pharmacobiotechnology ,Revised-Expanded 2nd Edition,New Age International LimtedPublishres, New Delhi,**2007**; 332-600.
[18]S Nagalingam;CS Sasikumar;KM Cherian,*Asian J. Pharm. Clin. Res.*, **2012**,5,179-181.
[19]AShukla;S Vats;RKShukla, *Int. J. Pharm .Pharm .Sci.*, **2014**, 6(11), 360-364.
[20]SP Malu;GO Obochi;CA Edem;BE Nyong, Global *J. Pure App. Sci.*,**2009**, 15, 373-376.
[21]A Doss;V Parivuguna;M Vijayasanthi;S Surendran , Ind.*J. Sci. Tech.*, **2011**, 4, 550-552.
[22]RK Shukla; D Painuly; A Shukla; J Singh; A Porval1 and S Vats, *J. Chem. Pharm. Res.*,**2014**, 6(11),200-210.
[23]MS Blois, *Nature*,**1958**, 181, 1199-1200.
[24]T Yamaguchi;H Takamura; T Matoba; J Terao, *Biosci. Biotechnol. Biochem*,**1998**, 62, 1201-1204.
[25]HN Krishna Kumar; SN Navyashree; HR Rakshitha; JB Chauhan, *Int J Pharm. Biomed. Res.*, **2012**, 3(2), 81-84.
[26]P Chorage;DA Kadam; AS Kadam;YA Ghuleand VT Aparadh,*Int J of Biomed Res*,**2013**, 3(2), 34-36.
[27]A Ghiselli; M Nardini; A Baldi; C Scaccini,*J. Agr. Food Chem.*, **1996**; 46, 361–367.