



## Phytochemical screening and antioxidant activity of the leaves of plant *Casearia tomentosa*

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### Abstract

In the present study petroleum ether extract of the leaves of plant *Casearia tomentosa* were investigated for phytochemical screening and antioxidant activity. *Casearia tomentosa* leaves were extracted by soxhlet apparatus and phytochemical screening was evaluated using standard methods. The antioxidant activity was performed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method and Ascorbic acid used as standard antioxidant. Phytochemical screening revealed that the presence of various medicinal active phytoconstituent such as terpenoids, steroids, phytosterol, fat and oil etc. This extract shows good antioxidant activity with IC<sub>50</sub> value 280 µg/ml. All these experimental analysis established a good support to the use of this plant in herbal medicine and can be used to prevent oxidative stress.

**Keywords:** *Casearia tomentosa* / Phytochemical / Antioxidant activity / Ascorbic acid / Terpenoids

### Introduction

Search for drugs to improve the quality of life and cure diseases has been a part of human life right from its beginning. In many of the well developed ancient civilizations this knowledge was evaluated and formed an essential part of the texts of their traditional systems of medicine, such as *Ayurveda*, *Siddha* and *Unani* (Singh *et al.*, 2009). *Ayurveda* has enriched with numerous plants introduction, their medicinal importance and usage. Plants have been used and still are using as a rich source of many natural products. In India most of which have been extensively used for traditional human health care system (Rex and Lyla, 2009).

The Indian Himalayan region alone supports about 18,440 species of plants of which about 45% are having medicinal properties. According to Samant *et al.*, out of the total species of vascular plants, 1748 species are medicinal. Uttarakhand is a storehouse of a rich variety of herbs and medicinal and aromatic plant species (Samant, *et al.*, 1998).

Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to easy availability, no side-effects, and better patient compliance. These contain dozens of active constituents such as alkaloids, flavonoids,

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glycosides, saponins, terpenoids, phytosterol, steroids, tannins etc which combine to give the plant its therapeutic value (Vijayaraghavan *et al.*, 2013). Natural antioxidants play a key role in health maintenance and prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Jayasri *et al.*, 2009; Uddin *et al.*, 2008). In the last few decades, the demand for natural antioxidants has been increased due to consumer concerns about the safety of synthetic antioxidants (Shukla *et al.*, 2014).

One of such natural source is *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 *Casearia 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 (Rao *et al.*, 2014); Adhikari *et al.*, 2010; Maurya and Seth, 2014). Therefore, the present investigation was undertaken to study the antioxidant potential of this plant and to put forward the evidence of the fact that this plant is having good antioxidant activity.

## Material And Methods

### Plant material

*Casearia tomentosa* leaves were collected from Lachhiwala forest Dehradun, Uttarakhand (India) in the month of August and September, 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. 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

(DPPH)1,1-diphenyl-2-picrylhydrazyl (Sigma Aldrich), Ascorbic acid (Rankem, India), Petroleum ether (Merck), Ethanol (Merck). All the other solvents and chemical used were of analytical grade.

### Extraction

150 gm air dry powderd leaves of *Casearia tomentosa* was treated with 1250 ml of petroleum ether by soxhlet extraction technique for 18 hr. It was concentrated to dryness under reduced pressure and controlled temperature using rotary evaporator. The petroleum ether extract yielded a greenish yellow waxy mass. The collected leaves extract was stored in a refrigerator.

### Phytochemical screening

The phytoconstituents present in petroleum ether extract was analysed by using standard qualitative method (Evans, 2009; Harborne, 1998). The leaves extract was screened for the presence of biologically active compounds like alkaloids, flavonoids, glycosides, saponins, terpenoids, phytosterol, steroids, tannins, fat and oils etc.

### Alkaloids

Five milligrams of extract was dissolved in twenty milliliters of dilute HCl and then filtered.

**Mayer's test:-** 5 milliliters of filtrate was treated with Mayer's reagent. *Yellow colour* precipitate indicates presence of alkaloid.

**Wagner's test:-** 5 milliliters of filtrate was treated with Wagner's reagent. *Brown reddish* precipitate indicates presence of alkaloids.

**Dragendroff's test:-** 5 milliliters of filtrate was treated with Dragendroff's reagent. *Red* precipitate indicates presence of alkaloids.

**Hager's test:-** 5 milliliters of filtrate was treated with Hager's reagent. *Yellow* precipitate indicates presence of alkaloids.

### **Flavonoids**

**Alkaline Reagent test:-** Extract was treated with few drops of NaOH solution. Formation of intense Yellow color which becomes colourless on addition of dilute acid (HCl or H<sub>2</sub>SO<sub>4</sub>) indicates the presence of Flavanoids.

**Lead Acetate test:-** Extract was treated with few drops of Lead Acetate solution. Formation of intense Yellow coloured precipitates indicates the presence of flavanoids.

**Shinoda's test:-** Small quantity of extract was dissolved in alcohol. To that few piece of magnesium with concentrated hydrochloric acid was added dropwise and heated. Appearance of magenta colour indicates the presence of flavonoids.

**Sulphuric acid test:-** Extract was treated with few drops of concentrated sulphuric acid . Yellow orange colour indicates the presence of flavonoids.

### **Tannins**

**Ferric Chloride Test:-** 100 mg of the extract was boiled with 20 ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered. Filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicates the presence of tannins.

### **Carbohydrate**

50 mg extracts were dissolved in 20 ml of distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

**Molisch Test:-** 5 ml filtrate was treated with a drop of alcoholic naphthol solution in a test tube. Formation of Violet ring at the junction indicates the presence of carbohydrates.

**Fehling's Test:-** 5 ml filtrate was boiled on water bath with 1 ml of each Fehling solution A and B. A red precipitate indicates the presence of sugar.

**Benedict's Test:-** 5 ml filtrate was treated with Benedict's reagent and heated gently in water bath. An orange red precipitate indicates the presence of reducing sugar.

**Barfoed's Test:-** 5 ml filtrate was treated with Barfoed's reagent and heated gently in water bath. An orange red precipitate indicates the presence of reducing sugar.

### **Glycosides**

Five milligrams of extract was dissolved in twenty milliliters of dilute HCl and then filtered.

**Modified Borntrager's test:-** Extract was treated with 5 % Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was then cooled and extracted with equal amount of chloroform. The chloroform layer was separated and treated with Ammonia solution. Formation of pink colour in the ammonical layer indicates the presence of glycosides.

**Legal test:-** Five milligrams of extract was treated with sodium nitroprusside in Pyridine and NaOH. Formation of red colour indicates the presence of glycosides.

**Keller killiani test:-** Five milligrams of extract mixed with chloroform and evaporate to dryness. Add 1 ml glacial acetic acid containing trace amount of ferric chloride. Transfer it to test tube and add carefully 0.5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> by the side of the test tube. Acetic acid layer shows blue colour indicates the presence of glycosides.

### Terpenoids and Steroids

**Salvoskii Test:-** 20 mg of extract was mixed with chloroform followed by 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of terpenoids.

**Libermann Buchard's Test:-** Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid followed by few drops of acetic acid, 3 ml of acetic anhydride. Formation of brown ring at the junction shows the presence of terpenoids and upper layer turn green which shows presence of steroids.

### Fats and Oils

**Saponification Test:-** A few drops of 0.5 N alcoholic KOH are added to small quantity of extract along with drop of phenolphthalein. The mixture was heated on water bath for 1 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

**Filter paper spot Test:-** Extracts rubbed between filter paper and if a spot resist after boiling of that filter paper confirm the presence of fixed oil and fats.

### Saponins

**Foam test:-** 100 mg of the extract was diluted with 10 ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable foam.

### Phytosterols

**Salvoskii Test:-** 5 mg of extract was mixed with 10 ml of chloroform followed by 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> to form a layer. A brown precipitate coloration at the interface formed indicated the presence of phytosterols.

**Libermann Buchard's Test:-** Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid followed by few drops of acetic acid, 3 ml of acetic anhydride. A bluish green colour indicates the presence of phytosterols.

### Protein and Amino acid

50 mg extracts were dissolved in 20 ml of distilled water and filtered.

**Millon's Test:-** Few drops of Millon's reagent was added to 2 ml of aqueous filtrate. A white precipitate shows the presence of protein.

**Biuret Test:-** In 2 ml of aliquot of filtrate few drops of 2% copper sulphate solution was added. To this, 1 ml of ethanol (95%) is added followed by excess of KOH pellets. Pink colour in the ethanol layer indicates the presence of protein.

**Ninhydrin Test:-** 2 drops of ninhydrin solution are added to 2 ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acid.

### Anthraquinone

**Benzene test:-** 10 mg extract was shaken with 20 ml of benzene and filtered. Few drops of 10% ammonia solution was added to the filtrate and the mixture was shaken well and the presence of violet colour in the aqueous phase in the presence of the anthraquinone.

### Antioxidant activity

Antioxidant activity of petroleum ether extract was performed by DPPH free radical scavenging assay (Brand - Williams *et al.*, 1995).

### DPPH free radical scavenging assay

The free radical scavenging assay of petroleum ether leaves extract of *Casearia tomentosa* was evaluated by stable DPPH

free radical according to the method of Brand-Williams *et al.* with some modification (Brand - Williams *et al.*, 1995). 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, 250, 500, 750, 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 preparations were taken at 517 nm with an UV-VIS spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid of similar concentrations (1-1000 µg/ml). 1 ml of methanol with 3 ml of working DPPH solution serves as blank. Then the % inhibition or % anti radical activity was calculated by equation:

$$\% \text{ inhibition} = (\text{Absorbance of control} - \text{Absorbance of sample}) / (\text{Absorbance of control}) \times 100$$

IC<sub>50</sub> of extract and standard ascorbic acid was calculated by graphical method by plotting % inhibition vs concentration.

## Results and Discussion

### Extractive yield

The extractive yield (in % w/w) of petroleum ether extract was 1.515 %. After complete removal of the solvent its consistency is waxy and greenish yellow in colour.

### Phytochemical screening

The result for phytochemical screening of *Casearia tomentosa* leaves are summarized in Table.1. The Preliminary phytochemical screening of extract of this plant revealed the presence of active phytoconstituent such as steroids, phytosterol, terpenoids and fats and oils etc. Out of which terpenoids are among the most widespread and chemically diverse groups of natural products. It is reported that

plant derived terpenoids possess activities like antioxidant, anticancer, anti-inflammatory, sedative, cytotoxic activity *etc.* Plant steroids also referred to as 'cardiac glycosides' are one of the most naturally occurring plant phytoconstituents and numerous reports support their use as cardiac drugs and as antioxidant (Mooradian, 1993). Beside these phytosterols was also present in this plant extract, which is also responsible for antioxidant activity[16]. Various reports support that plants fixed oil have variety of biological activity such as cytotoxic and antioxidant *etc* (Muna *et al.*, 2014)

From above discussion, we can interpret that the presence of these phytochemicals in petroleum ether extract shows medicinal importance of leaves of *Casearia tomentosa*.

### DPPH free radical scavenging assay

DPPH radical scavenging activity is one of the most widely used method for evaluation of 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 (Blosis, 1958). DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (Yamaguchi *et al.*, 1998). Petroleum ether extract of leaves shows DPPH anion scavenging power. The IC<sub>50</sub> 280 µg/mL, and 20µg/mL were evaluated for petroleum ether extract and ascorbic acid respectively (Fig.1). Antioxidants may guard against reactive oxygen species (ROS) toxicities by scavenging reactive metabolites and converting them to less reactive molecules (Shukla *et al.*, 2014).

**Conclusion**

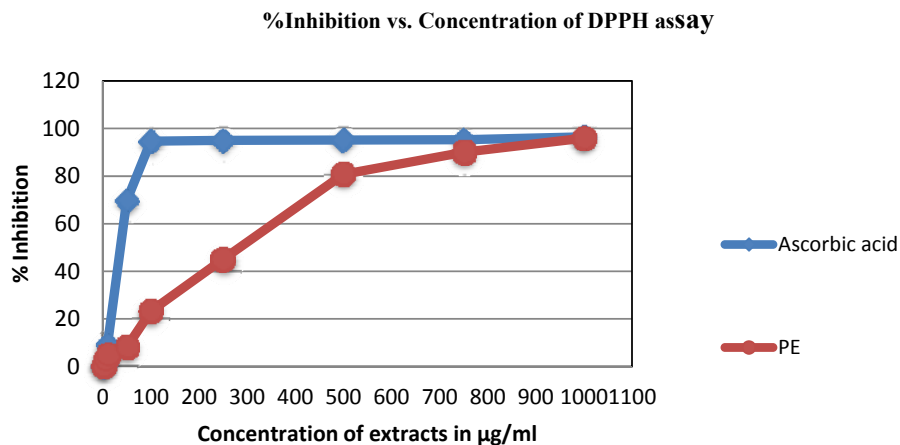
The present study was aimed to perform phytochemical evaluation and antioxidant activity of *Casearia tomentosa* leaves. From the study it is concluded that this plant is a good sources of antioxidants as observed in DPPH scavenging assay. Phytochemical

studies in leaves laid down a platform in search for a lead molecule that could be a potent antioxidant agent of natural origin. The present finding partially validates the traditional knowledge of the tribals about the goodness of consumption of this plant and required more research works.

Phytoconstituents and Test performed		Petroleum ether Extract
<b>Alkaloids</b>	Mayer's Test	-
	Wagner's Test	-
	Hager's Test	-
	Dragendroff's test	-
<b>Flavonoids</b>	Alkaline test	-
	Lead acetate test	-
	Shinoda's Test	-
	Sulphuric acid test	-
<b>Tannins</b>	Ferric chloride test	-
	Molisch's Test	-
<b>Carbohydrate</b>	Fehling's Test	-
	Benedict's Test	-
	Barfoed's test	-
	Keller-Killiani Test	-
<b>Glycosides</b>	Legal's Test	-
	Borntrager's test	-
	Liebermann burchard test	+
<b>Terpenoids</b>	Salvoski test	+
	Salvoski test (Triterpenes)	-
	Liebermann burchard test	+
<b>Steroids</b>	Saponification test	+
	Filter paper test	+
<b>Fat and Oil</b>	Foam test	-
	Millon's test	-
<b>Saponin</b>	Ninhydrin	-
	Biuret	-
	Salvoski test	+
<b>Protein and amino acid</b>	Liebermann burchard test	+
	Benzene test	-

(+)-Present;(-)-Absent

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



**Fig 1:** DPPH radical scavenging activity of leaves extract of *Casearia tomentosa*

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