Original Article

Phytochemical Investigation of High Altitude Medicinal Plants *Cinnamomum tamala* (Buch-Ham) Nees and Eberm and *Rhododendron arboreum* Smith

Shagun Gill[1], Preeti Panthari[2] and Harsha Kharkwal*[3]

[1]Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida-201303
[2]Amity Institute of Phytomedicine and Phytochemistry, Amity University Uttar Pradesh, Noida-201303
[3]Amity Center for Carbohydrate Research, Amity University Uttar Pradesh, Noida-201303

Address for Correspondence

Amity Center for Carbohydrate Research, Amity University Uttar Pradesh, Noida-201303
E-mail: hkharkwal@amity.edu

ABSTRACT

The present review provides a detailed information about two high altitude medicinal plants, namely *Cinnamomum tamala* and *Rhododendron arboretum* used in curing a number of ailments since ancient time. It highlights the work done since last one decade on chemical constituents, uses, formulations and pharmacological activities of these important medicinal species. These medicinal plants have therapeutic value that aids for alleviating various ailments of humankind. Both these plants are known to have antioxidant, antifungal, antimicrobial activities, anti-inflammatory and anti- diarrhoeal activites.

Keywords: Pharmacology, Phytochemical, Antioxidant, Antimicrobial, Anti-inflammatory, Anti-diarrhoeal.

INTRODUCTION

High altitude medicinal plants are used worldwide in both traditional and Western medicine systems. India is known for its vast biological diversity and knowledge rich ancient traditional systems of medicine, which provides a strong base for utilization of a large number of plants in general health care. The high altitude ecosystem is considered to be hotspots of medicinal plant diversity and are most neglected regions for research in view of their inaccessibility and harsh climatic conditions. In a survey, it was found that only 20% high altitude medicinal plants are used in Indian drug trade which is collected from wild sources[1].

Proper methodologies for the research and development are the need of the day for tapping the full therapeutic potential of plants. To find out the efficacy

of medicinal plants one needs to analyze the phytochemicals present in it. Scientists and pharmaceutical companies are working out for the development of new and effective drugs all over the world. Phytochemicals are the natural chemical compounds which are present in plants, having various characterized property such as antioxidant, anti-bacterial, anti-fungal, anti-diabetic activity. The main purpose of photochemical screening is to detect the presence of secondary metabolites such as phenols, saponins, coumarins, alkaloids, terpenoids and flavonoids which have great medicinal importance. Any drug is mostly derived from the phytochemicals present in such medicinal plants, which has been used since time immemorial. Alkaloids helps in controlling development system of living organisms, plays some metabolic role and have protective property[2]. They are used as steroidal alkaloids as a medicine. While phenolic compounds and flavonoids are known to possess biological activity such as antioxidant, anti-inflammatory, anti carcinogenic etc[2]. According to Okuda *et al* 1983 tannins have the reducing power which prevents liver injury by inhibiting the formation of lipid peroxides[3]. Flavonoids have been reported to exhibit the anti-oxidative, antiviral, antimicrobial and anti-platelet activities[4]. Polyphenols, particularly flavonoids, which are present in considerable amounts in fruits, vegetables, spices, medicinal herbs and beverages, have been used to treat many human diseases, such as diabetes, cancer and coronary heart disease[5-6].

To determine bioactive compounds from plant parts successfully, it is necessary to use different solvent in extraction method which highlights the need to use as much solvent as possible in the screening of biologically active phytochemicals[7].

Cinnamon tamala and *Rhododendron arboretum* are the two medicinal plants present at altitude of 900-2500m in tropical and sub-tropical Himalayas[8] and above 1500m to 6000m in Himalayan forests or Nilgiri Hills in Southern India[9] respectively and both have therapeutic and economical value.

THE POPULAR CHEMICAL CONSTITUENTS ISOLATED

Chemicals constituents which have been widely reviewed and investigated are Eugenol, Cinnamaldehyde from leaves of *C. tamala.* Leaves from Gorakhpur were analyzed for oleoresins present in them, it was found that the oleoresins varied with respect to the solvent used. With Methanol, ethanol, iso-octane and carbon tetrachloride 28, 34, 42 and 41 components were obtained respectively. Eugenol (66.1%), spathulenol (4.8%), viridiflorene (2.4%), methyleugenol (1.9%), aromadendrene (1.5%), with other minor compounds were detected[10]. Chemical structure of some isolated compound of *Cinnamomum tamala* (Nees and Eberm) is given in Fig. 1

While in case of *Rhododendron arboreum* isolated compounds are Taraxerol, Betulinic acid, Ursolic acid acetate from bark; Arbutin, Hyperoside, Amyrin, Epifridilenol from leaves; and Quercetin, Rutin, Coumaric acid from flower part[11]. The chemical structure of some chemical constituents of *Rhododendron arboreum* is given in Fig. 2

The present review highlights the work done till now on the phytochemical and pharmacological aspect of these two medicinal plants and also gives a brief on the recent research and future prospects which will help in finding new lead compounds for drug discovery. The phytochemical screening test is given in Table 4.

PHYTOCHEMISTRY

The compounds present in *C. tamala* belonging to different localities varies widely due to environmental conditions prevailing over that region. The chemical constituents of essential oils present in the leaves and bark of *C. tamala* reported from North East India are given in Table 1.

While the Chemical constituents of *Rhododendron arboretum* from Himalayan region are listed in Table 2.

PHARMACOLOGICAL ACTIVITIES OF CINNAMOMUM TAMALA AND RHODODENDRON ARBOREUM

Antibacterial activity

Pankaj *et al* (2009) showed that the stem bark of *Cinnamomum tamala* has antibacterial activity. To prove it further they prepared aqueous, ethanol, methanol, ethyl acetate and hexane extract in a final concentration of 100mg/ml and performed Agar well diffusion assay to evaluate the antibacterial potential of *C. tamala* bark extract, and concluded that almost all extract of the stem bark showed variable degrees of inhibition zone against different bacterial species except hexane which was found inactive. Ethanol, methanol & ethyl acetate fraction were found to have significant activity against *Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes, Bacillus (subtilis, cereus)* except *E.coli.* Overall methanolic extract was found to be more effective with zone size ranging from 11.26 mm to 20.77 mm while ethanolic extract showed inhibition zone ranging from 11.83 mm to 17.90 mm; mild activity was shown by the ethyl acetate extract ranging from 12 mm to 15 mm. Aqueous extract was found to be effective against *Staphylococcus aureus* and Bacillus cereus with zone size 11 mm approx. against each bacterium[27]. Vardar *et al* (2003) showed that volatile oil was 100% effective against *Fusarium moniliforme*, *Aspergillus niger*, *A. oryzae* and *A. solani* but not for *A. awamori* in inverted patri-plate method[28]. Md. Hemayet *et al* showed that the ethanolic extract of the leaves of *C. tamala* (500 µg/disc) showed moderate anti-microbial activity against *Staphylococcus epidermidis* (10mm), *Vibrio cologet* (Zone of inhibition 11mm), *Streptococcus agalactiae (*9mm*), Shigella sonnei* (9 mm), *Streptococcus pyogenes* (9mm), *Staphylococcus saprophyticus* (11mm), *Staphylococcus aureus* (8 mm).

Mohammad *et al* (2013) reported the antibacterial activity of the methanolic extract of various parts such as such, as flowers, leaves, bark, stem and roots of *Rhododendron arboreum* by determining the zone of inhibition against the tested bacterial strains such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilus and Salmonella typhi.*

Antifungal activity

A.K *et al*, 2012 reported that *C. tamala* has fungicidal characteristic. It was shown that oil extracted had the potent antifungal efficacy against *Aspergillus niger, A. fumigatus, Candida albicans, Rhizopus stolonifer and Penicillium spp.,* which was determined by measuring the diameter of the zone of inhibition (ZOI) surrounding wells, in agar diffusion assay and concluded that *Candida albicans* showed the maximum ZOI of 25 mm in comparison to other fungi.

The MFC values of oil against all the test fungi were found to be 230µg/ml. While the Minimum fungicidal concentration (MFC) of oil extracted was evaluated at four different concentrations of oil (115,230,460 and 690µg/ml) and was concluded that higher concentration of oil showed complete fungicidal activity and MFC values of oil against all fungal isolates tested were found to be 230µg/ml[29].

Nisar *et al* (2013) in his study reported that the various fractions of *Rhododendron arboreum* bark showed anti-fungal activity in agar well diffusion method. It was shown that higher concentration of extracts, i.e. 50 mg/ml exhibited maximum inhibition zone. For methanolic extract it was recorded to be 25 ±1.19, 29±1.02, 28±1.98, 17±1.44, 28±1.56, 32±1.66 mm against fungi (*A. niger, C. albacan, C. flavus, F. solani, M. canis* and *D. glaberata* respectively; while for ethyl acetate it was reported to be 22±1.92, 28±1.02, 25±1.32, 16±1.16, 24±1.11 and 28 ±1.00 respectively. The chloroform extract was reported to produce an antifungal effect which was lesser than ethyl acetate and methanolic extract while n- hexane extract failed to produce an outstanding fungicidal effect.

Antidiabetic activity

Palanisamy *et al* described the anti-diabetic activity of *Cinnamomum tamala* Linn, with special reference to its curative and protective role in streptozotocin-induced diabetic animal model. The efficacy of 50% ethanolic extract of the leaves of *Cinnamomum tamala* showed significant decrease in the blood glucose level and increase in the antioxidant efficacy in streptozotocin induced diabetes. It was demonstrated that the oral administration of the 50% ethanolic extract of leaves of *C. tamala* extract to streptozotocin induced diabetic rats showed significant positive changes in the biochemical and physiological parameters related to carbohydrate, protein and lipid metabolism[30]. It has been shown by Subash *et al*. that oral administration of cinnamaldehyde produces a significant antihyperglycemic effect which lowers both total cholesterol and triglyceride levels and, at the same time, increases HDL-cholesterol in STZ induced diabetic rats[31]. Mega *et al* (2010) showed the antidiabetic activity using α-amylase inhibition assay of methanolic and successive water extract of bark of *C. tamala* and concluded that methanol extract have high potent activity than later[32]. While Usha *et al* (2010) demonstrated that the administration of 250 mg/kg aqueous extract of *C. tamala* in streptozotocin induced diabetic rats showed significant decrease in levels of blood glucose and urine sugar[33].

M.R. Bhandary and J. Kuwabata (2008) examined anti-diabetic property in *R.arboreum* and isolated active compounds from it. Administration of the aqueous methanolic extract of the flower was found to exhibit inhibitory activity on the rat intestinal α-glucosidase. Both the water soluble and ethyl acetate-soluble portions from the aqueous methanolic extract showed inhibitory activities on α- glucosidase, revealing higher activity by the ethyl acetate soluble portion. α- glucosidase inhibitor quercetin-3-O-β-D-galactopyranoside (hyperin) was isolated from the ethyl acetate-soluble portion, through enzyme-assay guided separation. The isolated compound showed a dose dependent α-glucosidase inhibitory activity with IC50 values of 1.66 mM and 0.76 mM for sucrase and maltase, respectively. From this study, they could conclude that the flower possessed antidiabetic potential which could be utilized for medicinal preparations, nutraceutical or functional food for diabetes.

Anti-diarrhoeal activity

Md. Hemayet *et al* performed the castor oil induced diarrheal method in mice of ethanolic extract of *C. tamala* and showed that an extract inhibited the mean number of defecation which were 24.49% and 40.82% at doses of 250mg/kg and 500mg/kg respectively.

N. Verma *et al*, 2011 reported that the ethyl acetate fraction of *Rhododendron*

arboretum (flowers) has potent antidiarrheal activity. High performance thin layer chromatography (HPTLC) method was used for the determination of hyperin. Using HPLC the concentration of hyperin in flowers of *R. arboreum* was found to be 0.148%. Oral administration of EFRA in 100, 200 and 400 mg/kg showed dose-dependent and significant (P<0.05- 0.001) antidiarrhoeal potential in castor oil and magnesium sulfateinduced diarrhoea. EFRA was found to possess an anti enteropooling in castor oil-induced experimental animals by reducing both weight and volume of intestinal content significantly. It is also reported that the ethyl acetate fraction of *R. arboreum* flowers reduce magnesium sulfate-induced diarrhoea significantly, which could be due to increased absorption of water and electrolytes. The extract inhibits gastrointestinal motility in diarrhoea through anticholinergic effect. Phytochemical screening exhibit the presence of numerous constituents such as flavonoids, saponins, tannins, phytosterols, reducing sugars and phenolic compounds. Hence tannins, reducing sugars and sterols may be responsible for the mechanism of antidiarrhoeal activity of EFRA[34].

Antioxidant activity

The antioxidant potential of Cinnamomum oil and oleoresins for mustard oil has been evaluated by different methods such as peroxide, p- anisidine, thiobarbituric acid and total carbonyl value method. For determining antioxidant activity of essential oil and oleoresins of *Cinnamomum tamala* linoleic acid system and scavenging effect on DPPH[35-36]. An experiment was conducted to evaluate *in-vitro* antioxidant activity of Indian Bay Leaf on rat by S. Lakshmi Devi *et al* (2007) and reported increases in the levels of lipid and lipid peroxidation products and a decline in antioxidant potential in diabetic rat brain synaptosomes when induced with methanolic extract of bay leaf. Extract of Bay leaf displayed scavenging activity against superoxide and hydroxyl radical in a concentrate dependent manner. It can be concluded that synaptosomes from diabetic rats are susceptible to oxidative damage[37].

A.K *et al* 2012 showed that a different extract of C. tamala (Petroleum ether, Benzene, Chloroform, Ethyl acetate, Acetone, ethyl alcohol, water) has antioxidant activity 66.3%, 34.8%, 38.9%, 5.2%, 31.1%, 28.5%, 4.0% respectively; after 3 hours using β-carotene linoleate model system[38].

Research carried out by Swamidasan *et al* (2008) on mice and rats had shown that ethanolic extract of *Rhododendron arboreum* exhibit adaptogenic property. For the evaluation of adatogenic activity, anoxia stress tolerance, swimming endurance, immobilization stress models was used. Related treatment with ethanolic extract at doses 250 and 500 mg/kg, showed markedly increased in anoxia stress tolerance and swimming endurance time as compared to control group. Similarly, pre-treatment with extract showed marked decrease in blood glucose, cholesterol, triglyceride level as compared to stress control group in immobilization stress. Weights of the liver and adrenal glands are markedly decreased, but no weight changes in the spleen and testes were observed [39]. The secondary flavonoids isolated from the leaves of *R. arboreum* were found to have potent antioxidant property[40].

Anti-ulcer activity

Lima *et al* (2010) showed that the hydro alcoholic extract of *C. tamala* leaves have the potential to protect the gastric mucosa from chemical, stress and physically induced ulcers. They inhibit gastric acid secretion probably by blocking H+ K+-ATPase action and offer antioxidant

protection against oxidative stress-induced gastric damage. The findings of this experimental study lead to further isolation, and pharmacological activity of new therapeutic compounds effective against ulcer [41].

Anti-inflammatory activity

Thamizhselvam *et al* (2012) in his study evaluated that the methanolic extract of C. tamala has antiinflammatory activity in the carrageenan induced paw edema in Wistar albino rats. The percentage inhibition of edema formation was 66.75% and 73.71% at 250 and 500 mg/kg dosage respectively[42].

N. Verma *et al* (2011) has observed that the ethyl acetate extract fraction of *R. arboreum* exhibit remarkable anti-inflammatory and anti-nociceptive potential in animal models. Oral administration of *Rhododendron arboreum* extract (EERA) (100, 200 and 400 mg/kg) showed dose dependent and significant anti-inflammatory activity in arachidonic induced hind paw edema ($p<0.01$), cotton pellet granuloma model of inflammation ($p<0.01$) and Freund's adjuvant-induced paw arthritis ($p<0.01$). A significant ($p<0.05$) anti-nociceptive activity was evidenced in mice, protected in acetic acid-induced writhing. EERA at the dose of 100, 200 and 400 mg/kg showed significant ($p<0.001$) resistance against analgesymeter induced pain in mice. The anti-inflammatory effect of the extract may be due to the presence of flavonoids (hyperin), tannins, saponins and other phytochemicals present either as single or in combination. The significant level of anti-inflammatory activity of the ethyl acetate extract could be attributed to the high amount of flavonoids present in the extract [43].

Alzheimer prevention Potential

Alzheimer is the most common form of dementia among older people, which affects person's ability to carry out daily activities. In a recent clinical study, it was found that two compounds (Cinnamaldehyde & Epicatechin) have potential to ward off or even prevent Alzheimer disease. The stated compound present has a protective power which can protect component protein of neuron, called tau, from oxidative stress, thus allowing neuron to function properly[44].

Unlike *C. tamala*, there is no report on Anti-Alzheimer activity for *Rhododendron arboreum*.

Hepatoprotective activity

Selvam *et al* (2010) reported the hepatoprotective activity of methanolic extract of *Cinnamomum tamala* (nees) against paracetamol intoxicated swiss albino mice.

N. Verma *et al* (2011) showed the hepatoprotective potential of the ethyl acetate fraction of *Rhododendron arboretum* against CCl_4-induced liver damage in preventive and curative models. Fraction at a dose of 100, 200 and 400 mg/kg was administered orally once daily for 14 days in CCl_4-treated groups (II, III, IV, V and VI). The serum levels of glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (SALP), γ- glutamyltransferase (γ-GT), and bilirubin were estimated along with the activities of glutathione S-transferase (GST), glutathione reductase, hepatic malondialdehyde formation, and glutathione content. The substantially elevated serum enzymatic activities of SGOT, SGPT, SALP, γ-GT, and bilirubin due to CCl_4 treatment were restored to normal in a dose-dependent manner. Meanwhile, the decreased activities of GST and glutathione reductase were also restored to normal. In

addition, ethyl acetate fraction also significantly prevented the elevation of hepatic malondialdehyde formation and depletion of reduced glutathione content in the liver of CCl_4 -intoxicated rats in a dose-dependent manner[45]. Some

Phytochemical Analysis

Qualitative phytochemical analysis of the aqueous, methanol, ethanol, ethyl acetate, chloroform and hexane extracts of *Cinnamomum tamala* was carried out to test the presence of phytochemicals such as alkaloid, flavonoids, terpenoids, sterols, tannins, glycosides (Table no. 4). Phytochemical screening of different solvent extracts of *Cinnamomum tamala* (Kasargod district of Kerela) and *Rhododendron arboreum* (Western ghats) is listed in the Table no. 5 and 6 respectively.

Future Prospects

Cinnamon tamala and *Rhododendron arboretum* have beneficial effects with wider uses. Numerous studies have been conducted in different parts of both medicinal plants, but these plants have not yet developed as a drug by pharmaceutical industries. Newer concepts aligning with industrial norms for extracting oil, which has tremendous utility in the food industry can be developed.

The field of nanotechnology has evolved rapidly and this technology in the area of medicine can be considered as a boon to treat human disease in near future. Katti, director of Cancer Nanotechnology Platform (2010), discovered that phytochemicals present in Cinnamomum have the ability to treat cancer when combined with gold nanoparticles because active pharmaceutical ingredients are carried by gold nanoparticles into cancer cells and assist in the destruction or imaging of malignancies. Chanda *et al* (2011) showed that cinnamon coated gold nanoparticles have the ability to detect cancer cells[51].

This review elicits on all the aspects of the herb and throws attention to set the mind of the researchers to carry out the work for developing various formulations, which can ultimately be beneficial for the human beings as well as animals. Further research is required to isolate the bioactive principle of this plant as well as further studies on its bioefficacy against human pathogens. Moreover, chemistry of genus *Cinnamomum* is quite interesting as this medicinal plant exhibits several chemotypes within a species and very little attention has been paid to nonvolatile compounds and also a very little work has been reported in the bark and root oil composition of *C. tamala*, so this is an area worth exploring.

CONCLUSION

The review suggests that *Cinnamomum tamala* and *Rhododendron arboreum* both are high altitude medicinal plant having wide application in the fields like pharma, medicines, cosmetics, etc. Various parts are exported due to their high medicinal values, hence the protection of these endemic species should be stressed upon. *Rhododendron arboretum* is an ornamental tree and can be used in avenue decoration. *Cinnamomum tamala* a miraculous plant should be explored in the pharmaceutical industry for drug development. The plant has traditional importance and an in-depth systematic study of the different aspects of the plant is the need of the hour. The detailed catalouging will help the researchers to carry out their research on this highly medicinal plant which will lead to new formulations both to man and animals. The essential oil and oleoresins possess high antioxidant and antimicrobial activity against bacterial and fungi. It can be concluded that there is a

huge scope of the oil in food industry as natural food additive [10].

Rhododendron arboretum extracts have high antioxidant potential which can replace synthetic antioxidants in pharma field. In the treatment of cancer, suitable extract of different parts of the tree can be used. The review suggests that this plant is one of the important plants of the Himalayan region and needs protection and have a high commercial utility of different part extract.

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Table 1. Some examples of Chemical compounds with content percentage in *Cinnamomum tamala*(Nees and Eberm)

Plant Parts	Compound	Precentage
Leaves[12]		
	β- caryophyllene	0.50%
	α- pinene	2.25%
	β- pinene	0.50%
	camphene	0.34%
	Linalool	1.20%
	Cinnamaldehyde	0.20%
	Eugenol	68.10%
	p-cymene	4%
Bark	Cinnamic aldehyde	70-80%
Roots	Eugenol	-
	Safrole	-
	Benzaldehyde	-
	Terpine	-

Table 2. Different compounds isolated from *Rhododendron arboreum* Smith

Plant parts	Compound present	Percentage
Wood (Stem)[13]	Ash content	0.75%
	Extractives	4.39%
	Homocelluloses	69.82%
	Hemicelluloses	22%
	Alpha-cellulose	47.98%
	Pentosans	16.84%
	Lignins	24.88%
Leaves[14]		Molecular formula
	Ericolin(arbutin)	$C_{12}H_{16}O_7$
	Ursolic acid	$C_{30}H_{48}O_4$
	Alpha-amyrin	$C_{30}H_{50}O$
	Epifriedelinol	$C_{30}H_{52}O$
	Triterpenoids(Campanulin, quercetin & hyperoside)	$C_{21}H_{20}O_{12}$
Flowers[14]		
	Quercetin-3-rhamnoside	$C_{21}H_{20}O_{11}$
Phenolic compound	Quecetin Rutin Coumaric acid	$C_{15}H_{10}O_7$ $C_{27}H_{30}O_{16}$ $C_9H_8O_3$

Table 3. Various Activities reported on *Cinnamomum tamala* (Nees and Eberm) and *Rhododendron arboreum* Smith

Species	Part ustilized	Activity
C.tamala (Nees& Eberm)	Leaves(ethanolic extract)	Hypoglycaemic activity[15].
C.tamal(Nees& Eberm)	Leaves(aqueous extract)	Antidiabetic and anti-oxidant activities [16].
C.tamala(Nees& Eberm)	Leaves (ethanol extract)	Antidiabetic [17].
*C.tamala(*Nees& Eberm))	Leaves (methanolic extract)	Analgesic, anti- inflammatory and antipyretic activity [18].
C.tamala (Nees& Eberm)	Leaves(ethanolic extract)	Reno-protective properties [19].
Rhododendron arboreum(Smith)	Leaves(flavonoids)	Anti-oxidant activity [20].
Rhododendron arboreum(Smith)	Flower(ethyl acetate fraction)	Anti-inflammatory and anti-nociceptive activity [21].
Rhododendron arboreum(Smith)	Flower(ethyl acetate fraction)	Antidiarrheal, hepatoprotective potential[20], Antihyperglycemic & antihyperlipidemic activity [21].
Rhododendron arboreum(Smith)	Flower & leaves(alcoholic extract)	Anticancer activity [24].
Rhododendron arboreum(Smith)	Flower juice	Hypolipidemic effect [25].
Rhododendron arboreum(Smith)	Bark(n-hexane, chloroform, ethyl acetate, methanol extract)	Antifungal activity [26].

Table 4. Preliminary test

S. No	Experiment	Observation	Inference
1.	20 mg powder was dissolved in 2 ml distilled water and filtered then 2 ml $FeCl_3$ was added to the filtrate[46].	Blue – black precipitate	Indicates the presence of **Tannin**
2.	20 mg extract was dissolved in 2 ml distilled water and filtered. To the filtrate, 2–4 drops of 1% HCl was added and steam was passed through it. To the 1 ml of this solution 6 drops of Wagner's reagent was added[46].	Brownish red ppt	Indicates the presence of **Alkaloids**
3.	0.5ml of filtrate obtained from alkaloid test 5ml distilled water was added(Wall et al,1954)	Honey comb froth persistence for 15-30 minutes	Indicates the presence of **Saponins**
4.	20 mg extract were dissolved in 10 ml ethanol and filtered. 0.5 ml conc. HCl and magnesium ribbon were added to 2 ml filtrate [46].	Pink or magenta red or crimson colour	Indicates presence of Flavonoids
5	Extract were dissolved individually in 5ml distilled water , filtered and the filtrate was tested for the presence of **carbohydrate** using the procedures described by Harper (1975) and Balbaa (1986).		
a)	**Molisch's test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in test tube.	Bluish violet ring at the junction	Indicates the presence of **carbohydrates** and or **glycosides**.
b)	**Fehling's test:** To 5ml filtrates were treated with 5ml Fehling's(A & B) and heated	Formation of red precipitate	Indicates presence of **reducing sugars.**
c)	**Benedict's test:** To 1ml of filtrate,5ml of Bendict's reagent were added and heated	Red precipitate	Indicates the presence of **reducing sugar**.
6)	**Glycosides test:** Extract were hydrolysed with dil.HCl, and subjected to glycoside test		
a)	**Legal's Test:** Extract were treated with sodium nitroprusside in sodium hydroxide and pyridine.	Formation of pink to blood red colour	Presence of cardiac **glycosides**.
7. a)	**Phytosterols test: Salkowski's test:** Extracts were treated with chloroform and filtered. To the filtrates few drops	Golden yellow colour	Indicates presence of **triterpenes**

	of conc. Sulphuric acid was added.		
b)	**Libermann Burchard's test:** Extract were treated with chloroform and filtered. To the filtrate few drops of acetic anhydride were added followed by conc. Sulphuric acid[47].	Formation of brown ring at the junction	Indicates the presence of **phytosterol.**
8)	To 1ml extract 10 ml chloroform were added followed by addition 10 ml of conc. Sulphuric acid.	Change of color from violet to blue /green	Presence of **steroids**
9)	To 1gm of sample in test tube covered with filter paper moistened with dil. NaOH and heated on water bath for few minutes. (El-Tawil , 1983).	Yellow fluorescence on filter paper observed under U.V light	Indicates the presence of **coumarins**:
10. **a)**	Plant extract were dissolved in 10 ml distilled water, filtered and subjected to various test. **Millon's Test-** Few drops of Millon's reagent were added to 2ml filtrate	White precipitate	Indicate presence of **Protein**
b)	**Biuret Test-** Drop of 2%$CuSO_4$ were added to 2ml filtrate followed by 1ml 95% ethanol and excess potassium hydroxide solution 60%	Appearance of pink colour in ethanol layer	Indicates presence of **Protein**[48]
11)	Methanolic extract of plant were dissolved in distilled water and 0.5ml Folin-ciocal teau reagent was added followed by 2ml 20% Na_2CO_3 solution.	Formation of bluish colour	Indicates the presence of **Phenol**[49].

Table 5. Preliminary screening of phytochemicals present in *Cinnamomum tamala* (Nees and Eberm)[48]

Phytochemical constituents	Ethanol	chloroform	Ethyl acetate	Methanol	water
Flavonoids	+	-	-	+++	-
Terpenoids	-	++	+	-	-
Steroids	-	++	++	-	-
Alkaloids	++	+	++	-	-
Tannins	-	++	-	-	-
Glycosides	+	-	-	+++	-
Carbohydrates	-	-	-	+	++
Saponins	-	+	-	-	-
Protein	-	-	-	+	+

(--) = Not present;
(+) = Present but low in abundance;
(++) = Present in moderate amount;
(+++) = Present in high amount.

Table 6. Preliminary screening of phytochemicals obtained in Rhododendron arboreum Smith[50]

Phytochemical constituents	Acetone	benzene	chloroform	Ethanol	Petroleum ether	water
Alkaloids	-	-	-	-	-	-
Phenol	+++	++	+	+++	+	++
Flavonoids	-	-	-	-	-	-
Saponins	-	+++	+++	-	+++	-
Protein	-	-	+	+	-	-
Steroids	-	+	-	+++	-	-
Tannins	+++	-	-	+++	-	+++
Xanthoprotein	+++	-	-	-	-	-
Carboxylic acid	-	-	-	-	-	-
Coumarins	+++	-	+	-	+	-
Carbohydrates	-	+	+++	+	+	-

(-)= absent;
(+) = low;
(++)= average;
(+++)= high.

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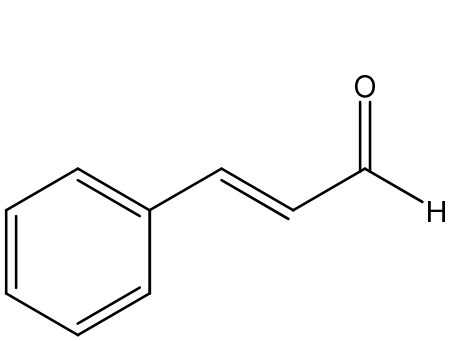
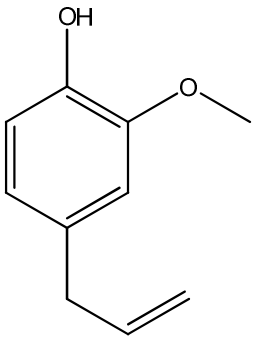
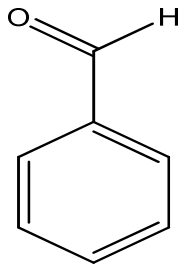


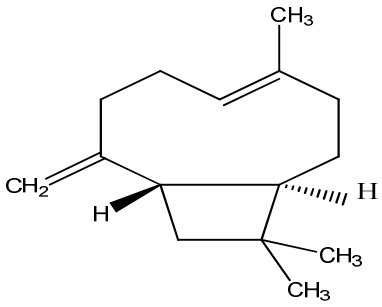
cinnamaldehyde



Eugenol



Benzaldehyde



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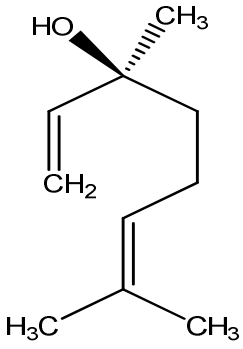

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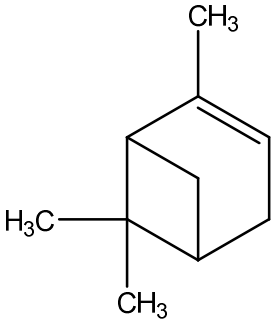
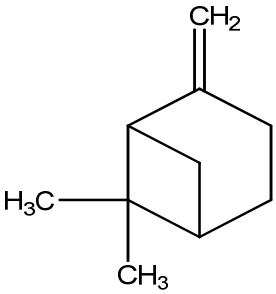


α-pinene



β-pinene

Figure 1. Chemical structures of some compounds found in different parts of *Cinnamomum tamla*(Nees and Eberm)

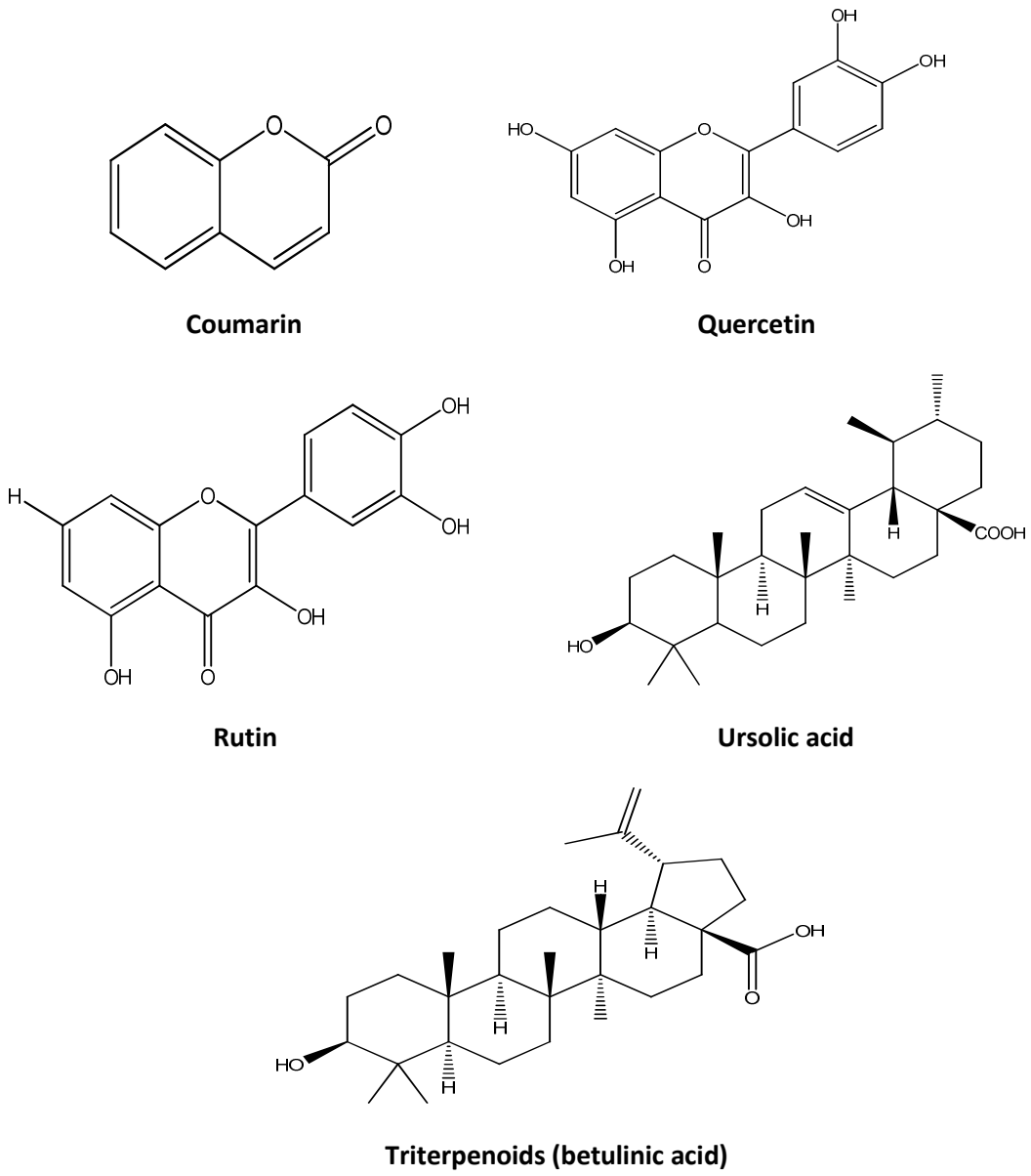


Figure 2. Chemical Structures of some isolated compounds of *Rhododendron arboreum* Smith