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Qualitative Analysis of Phenolic Constituents from Leaves of Some Plants of Family Meliaceae

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Abstract: *Screening of phenolic constituents in leaves of Cedrela serrata, Toona ciliata, Azadirachta indica and Melia azedarach was done by reversed phase high performance liquid chromatography (RP-HPLC). The phenolic compounds were identified by comparison with authentic standards. The phenolic acids identified in all plants leaves extract were gallic acid, protocatechuic acid, p-hydroxy benzoic acid, chlorgenic acid, syringic acid, vanilic acid, o-coumaric acid and ferulic acid while, none plant sample were having p-coumaric acid in its leaf extracts*

Keywords: *Cedrela serrata; Toona ciliata; Azadirachta indica; Melia azedarach; Phenolics; HPLC*

1. INTRODUCTION

Plants have been widely used in folk medicines and to extend the shelf life of foods. Phenolic compounds are secondary metabolites which are synthesized by the plants for their defence purpose. They are used as antimicrobial/antifeedant by plants to defend themelves from microbes and insects attack. Phenolics also posses various biological properties such as: antioxidant, antiapoptosis, anti - aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities [1]. Crude extracts of plant materials are rich in phenolics. Phenolics plays important role in plant disease management and imparts resistance to plants against many diseases/pest attack. Interests in phenolics are increasingly in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [2]. Plants contain scores of functional phytochemicals and their consumption has long been associated with physical wellbeing. Among phytochemicals with health benefit are phenolic acids, flavonoids, and other polyphenols, which have been demonstrated to exhibit positive effects on health. Medicinal and aromatic plants have been widely used in folk medicines to cure various ailments and are also used to extend the shelf life of foods.

The genus *Toona* (Meliaceae) consists of upland trees that are widely distributed at the higher altitude eastwards from India, Nepal, China, Burma, Thailand, Malaysia, and Java to Europe. *Toona ciliata* M. Roem commonly called Toon in hindi is mainly timber yielding plant. It is semi-deciduous trees, upto 30 m high, found mostly in Indo-Myanmar region It is also cultivated in Sri Lanka, Afghanistan, Africa and the Hawaiian Islands. *Toona* species are and well known for their medicinal properties like antibacterial, antioxidant, antiulcer and antimicrobial [3]

Cedrela serrata Royleis a moderate-sized deciduous tree, native of India, Indonesia, Myanmar, Sri Lanka. The leaves and young shoots are lopped for cattle fodderand the wood is used for furniture, bridges, poles, packing cases, plywood, door and window shutters, ceiling boards, planking, toys and musical instruments. Bark of the plant is used as anti-leishmanicial, antiperiodic, tonic, astringent; externally applied to ulcers; also used in chronic infantile dysentery [4]

Melia azedarach L., commonly known as chinaberry tree, is a deciduous tree in the mahogany family that is an evergreen tree, cultivated in various parts of the Indian subcontinents. Leaves have been used as a natural insecticide to keep with stored grains. A diluted infusion of leaves juice has been used to induce uterus relaxation anthelmentic, diuretic and emmenagogue. Extracts of leaf, seed and bark possess a wide spectrum of antibacterial action [5, 7]

Azadirachta indica A. Juss commonly known as neem is native plant of India. Neem tree have been used as traditional Ayurvedic medicine in India. It use as anti-inflammatory, antiarthritic, antipyretic; hypoglycaemic; antigastric ulcer, spermicidal, antifungal antibacterial, diuretic, antimalarial, antitumour and immunomodulatory have been reported. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. Neem oil finds use to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis [8].

2. MATERIALS AND METHODS

2.1. Plant material

The plants were collected from Districts US Nagar and Nainital (Uttarakhand). The plant was identified by Prof. Y. P. S. Pangtey, Taxonomist, Department of Botany, Kumaun University, Nainital. A voucher specimen was identified and deposited in the Herbarium of the Department of Chemistry (Division Agricultural Chemicals) G.B. Pant University of Agriculture and Technology.

2.2. Extraction of Phenolics

The extraction of the phenolics from the leaves of *C. serrata, T. ciliate, A. indica* and *M. azedarach* was determined according to [9-10], with some modification. The fresh leaves (200 g) of plant materials were shade dried and crushed to coarse powder. The powder (20 g) was macerated with 25 ml distilled water of 2 N-HCl and heated in water bath for 1 h at 100^0 C using air condenser and filtered. The filtrate was extracted with diethyl ether using separating funnel. The diethyl ether layer was washed with distilled water, dried over anhydrous sodium sulphate and evaporated using rotary vacuum evaporator at 25°C to obtain extract. The extract collected was re-dissolved in known amount of (5 ml) HPLC grade methanol, prior to the injection into HPLC column the sample was filtered through 0.22µm organic filter (Millipore).

2.3. Analysis of Phenolics

The qualitative analysis of thirteen phenolic acids compounds viz., caffeic acid, syringic acid, vanillic acid, p-hydroxybenzoic acid, ferulic acid, chlorgenic acid, protocatechuic acid, gallic acid, cinnamic acid, p-coumaric acid, o-coumaric acid, benzoic acid, 3,5-dihydroxybenzoic acid were used for study and performed by reverse phase high performance liquid chromatography (RP-HPLC) under following conditions: Apparatus: HPLC-Beckman model-322 equipped with 100 A model pump, 420 controller, mixer, 210 injector and BD-40 recorder, Column: Ultrasphere C18 column 5µm, (25 cm x 4.6 mm length), Mobile phase: Methanol: Water (1% acetic acid in 20: 80 v/v); the mobile phase was degassed prior to use in HPLC, Flow rate 1 ml min^{-1}, Chart speed 1 cm min^{-1}, UV Detector, λ max 254 nm, 0.02 aufs Attenuation, isocratic mode. The detector response for individual phenolics was calibrated with authentic phenolic acids used for analysis. All the standard phenolics were procured from Sigma-Aldrich Chemical Company, USA.

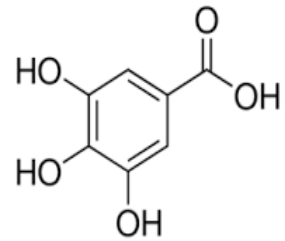
3. RESULTS AND DISCUSSION

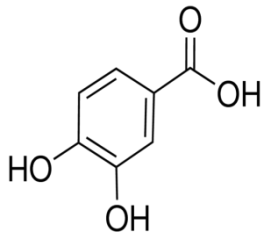
HPLC analysis of phenolics in leaves of *C. serrata, T. ciliata, A. indica* and *M. azedarach* revealed that leaves were rich in phenolic contents. Phenolic acid compounds were identified with the standard compounds while, compounds which could not be identified are reported as unidentified. The extract of the leaves of *C. serrata, T. ciliata, A. indica* and *M. azedarach* showed the presence of 9, 11, 11 and 10 phenolic acids, respectively (Table 1). Some compounds remained unidentified in leaves samples. All phenolic acids tested were present in leaves samples except p-coumaric acid which was not present in any plant samples. Cinnamic acid was present only in *M. azedarach* leaves. Phenolic acids which were present in leaves of *C. serrata, T. ciliata, A. indica* and *M. azedarach* are shown in figure 1.

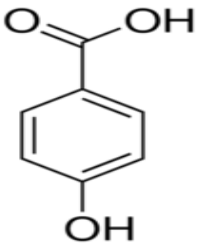
Table1. *Phenolics analyzed from the leaves, C. serrata, T. ciliata, A. indica and M. azedarach*

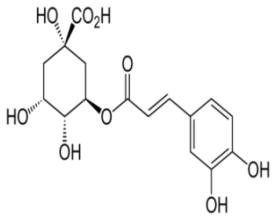
S.No	**Phenolics (Standards)**	***C. serrata***	***T. ciliata***	***A. indica***	***M. azedarach***
		Leaves	Leaves	Leaves	Leaves
1.	Gallic acid	+	+	+	+
2.	Protocatechuic acid	+	+	+	+
3.	p-Hydroxy benzoic acid	+	+	+	+
4.	Chlorgenic acid	+	+	+	+
5.	Syringic acid	+	+	+	+
6.	Vanilic acid	+	+	+	+
7.	o-Coumaric acid	+	+	+	+
8.	p-Coumaric acid	-	-	-	-
9.	Caffeic acid	+	tr	+	+
10.	Benzoic acid	-	+	+	-
11.	Ferrulic acid	+	+	+	+
12.	Cinnamic acid	-	-	-	+
13.	3,5-dihydroxybenzoic acid	-	+	+	-
14.	Unidentified	8	10	7	9

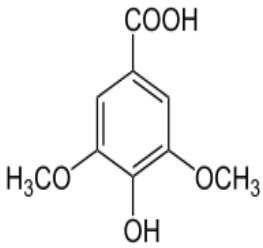
+ =Present; - =Absent; tr= traces

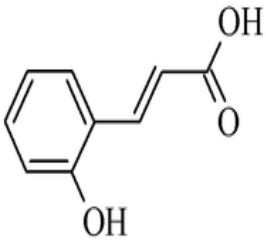

Gallic acid

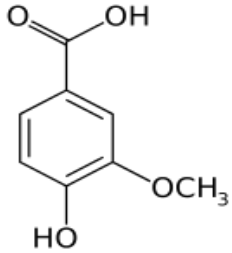

Protocatechoic acid

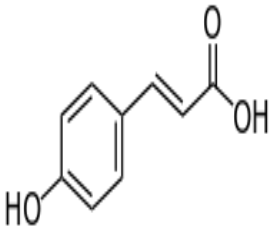

p-Hydroxy benzoic acid

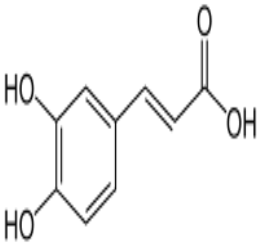

Chlorgenic acid


Syringic acid


o-Coumaric acid


Vanilic acid


p-Coumaric acid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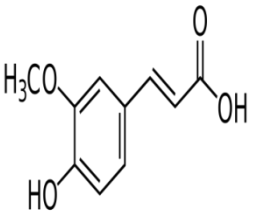
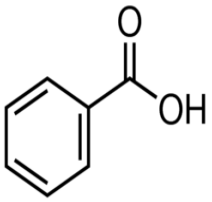
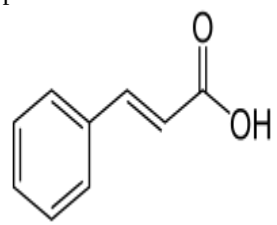

Ferrulic acid


Benzoic acid


Cinnamic acid

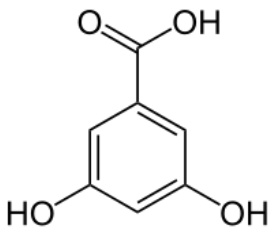

3,5-dihydroxybenzoic acid

Figure1. *Phenolic acids*

Phenolic compounds are of different origins and functions, most of them belong to principal biologically highly active components of plant origin and play vital role of protecting organisms against harmful effects [11]. Phenolics are the dietry constituents and are used as chemo-preventive

agents. Phenolic compound(s) were present in the ethanolic and methanolic extracts, which exhibited anticonvulsant properties in the picrotoxin-induced convulsions model [12]. Thus, the phenolic compounds present in this study could be cause for biological activity of these plants.

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