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HPLC Screening of Phenolic Acids of an Exotic Weed Croton Bonplandianum. Baill

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ABSTRACT - There are many plants considered as weed and waste, people do not know the effectiveness of those plants though they are frequently used by tribes and villagers. Croton bonplandianum Bail . is also one of them. They grow in wild and waste lands and ignored by people as useful plants. Though, traditionally, it is used to cure skin, liver, lung diseases, also wound healing, antimicrobial. Recently this plant attracted the few scientists and explored by them. The plant is considered as an exotic weed, phytotoxic to its associates; show some allelopathy by destroying photosynthetic pigments. It is also toxic to another weed Parthenium hysterophorus. It is also an example of hyper accumulator, accumulates copper, rich in essential fatty acids as palmitic acid, linoleic acid and linolenic acid. Plant extract is carcinogenic if person touches and eat it, contains sesquiterpenes though studies show that it checks the tumour in potato tubers. Present study deals with High performance liquid chromatographic screening of phenolic acid in Crton bonplandianum. Baill and its larvicidal activity. Study is helpful in detecting biopotent chemicals as such.

Keywords :- Weed, HPLC, Phenols

Introduction -: *Croton bonplandianum* a tropical shrub, belonging to family Euphorbiaceae (spurge family). The plant is very common, found along the way sides, in waste places and bank of river and ponds. Plant is herbaceous, much branched, leaves ovate to lanceolate; Flowers raceme, greenish, male flower above, female flower fascicled in the axil of minute bracts; male outer tepals obovate, minute; inner tepals linear oblong. Female tepals lanceolate; Stamens 10-15; ovary densely stellate-hairy; stigma 2-fid. Fruit; capsule, stellate-hairy and with caruncle. Milky latex is the characteristic of plant and show fungitoxic properties (Asthana *et al*, 1989). Its aqueous extract shows the allelopathic effect on some crop plants (Swapnil and Siddiqui, 2010) and on weed like *Parthenium* (Thapar and Singh, 2006). Present study deals with HPLC based identification and quantification of cretain phenolic acids in different parts of *Croton bondplandianum*. Plant phenolics are secondary metabolites that consist of several types of structurally diverse group of natural products biogenetically arising from the shikimate-phenylpropanoids-flavonoids pathways. Plants need phenolic compounds for their growth, pigments, reproduction, resistance to pathogens and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution. Plants synthesize a greater array of secondary compounds than animals because they cannot rely on physical mobility to escape their predators and have therefore evolved a chemical defence against such predators. Plant phenols and polyphenols act as UV sunscreens, signal compounds, pigments, internal physiological regulators

or chemical messengers, the role of phenolics in the resistance mechanisms of plants against fungal pathogens and phytophagous insects.

Antioxidant activity shown is shown by Cinnamic acid (Khan et al. 2000), Ferulic acid (Graf et al. 1992), Caffeic acid (Nardini et al.), (Kahkonen et al.) (Kono et al.), (Rhodes et al.) Antioxidant activity of Gallic Acid protects the human cells against the oxidative damage and also shows cytotoxicity against cancer cells. It also used in treatment of albuminuria, Diabetes, psoriasis, ex ternal haemorroids. It has good antibacterial activity Repoted in *Caesalpinia* (A. chanwitheesuk et al. 2007)

Ferulic acid is one of the most abundant phenolic phytochemical found in plant cell wall. It is related to trans cinnamic acid and also act as precursor of aromatic compounds. It is synthesized by caffeic acid due to the action of enzyme o- methyl transferase. Ferulic acid shows antioxidant activity like other phenols (Graf et al 1992.) It may reduce oxidative stress and formation of thymine dimers in skin. In vitro studies shows that Ferulic acid havedirect antitumor activity against treat cancer.

Materials and Methods

Collection of Plants

Plants have been collected from Allahabad then different part of plant viz root, leaves andinflorescence have been separated

Drying of Plants and Extraction

Different separated parts of the plants were air dried and powdered and extracted separately with Methanol and Sterile water (1:1) using soxhlet apparatus.

Sample Preparation for HPLC analysis of phenolic compounds

Samples of every part have been prepared. Phenolic acids were extracted as per the method of Singh *et al*., 2002. One gram from each sample was macerated and suspended in 5 ml ethanol-water (80:20; v/v). The collected samples were subjected to ultrasonication (Branson Sonifier, Danbury, CT, USA) for 15 min at 40^0C followed by centrifugation at 12500 x g for 15 min and supernatant is filtered. The clear yellowish supernatant was subjected to charcoal treatment. The residue was re-extracted twice with the same extracting solution and the supernatant was pooled prior to evaporation under vacuum (Buchi Rolavapor Re Type, Labco, India; Ambala Cant. India). Dried samples were resuspended in 1.0 ml high- performance liquid chromatography (HPLC)-grade methanol by vortexing and filtered through ultra membrane filter (pore size 0.45 mμ Milipore) before HPLC analysis.

HPLC analysis

Quantitative analysis of the sample was performed according to the method of Singh *et. al* 2002. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pump, a variable Shimadzu SPD-10 AVP UV-VIS detector and a Rhedodyne model 7725 injector with a loop size 20 μl. The peak area was calculated with a Winchrom integrator. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250x4.6 mm i.d., particle size 5 μm, Luna 5μ C-18 (2); phenomenex, Torrance, CA USA) at 25ºC. Running conditions included injection volume 5μl;

mobile phase, methanol 0.4% acetic acid (80:20 v/v); flow rate, 1 ml/min and detection at 290nm. Samples were filtered through an ultra-membrane filter (pore size 0.45 μm; E-Merc, Darmstdt, Germany) prior to injection in the sample loop. Tannic, Gallic, Vanillic, Caffeic, Ferulic, Chlorogenic and Cinamic acids were used as internal and external standards. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention tiem(R_t) of individual standards and further confirmed by co-injection with isolated standards.

Result and Discussion

HPLC based analysis of different parts of plant *Croton bonplandianum* Bail. reveal the presence of variety of phenolic acids *viz* Tannic acid. Gallic acid, `Caffeic acid, Benzoic acid, Ferrulic acid, Cinnamic acid. These phenolic acids show diverse activity as they are anti-inflammatory, antibacterial, antifungal, antiviral, antioxidant, provide protection against different pathogenic microorganisms, caffeic acid is inhibitory to plant pathogenic bacteria and fungi even in in-vitro condition, thus can be used in therapy. Therapeutic Uses are given in Table–1.

The amount of each phenolic acid is expressed as micrograms per gram of fresh weight given in table-2. Peaks of HPLC analysis of different part of plant *viz.* roots, leaves, inflorescence are given in Fig-2 (A, B & C) respectively.

Conclusions

Among various phenolic acids, Benzoic acid is present in highest amount in all the parts of plant *viz* roots, leaves and inflorescence, while cinnamic acid is present in least amount in roots and leaves while inflorescence does not show the presence of cinnamic acid but Ferrulic acid is present in least amount.

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Table-1 Amount of each phenolic acid is expressed as micrograms per gram of freshweight.

Plant	Phenolic Acid					
Croton bondplandianum	Tannic acid	Gallicacid	Caffeicacid	Benzoic acid	Ferrulicacid	Cinnamic acid
Root	14.165438	4.4213407	-	17.197817	0.2883931	0.0226058
Leaves	22.9000415	11.584243	-	207.04229	2.0674352	0.47
inflorescence	24.31	6.25	1.16484	29.35607	0.76974	-

Table -2 Therapeutic Uses

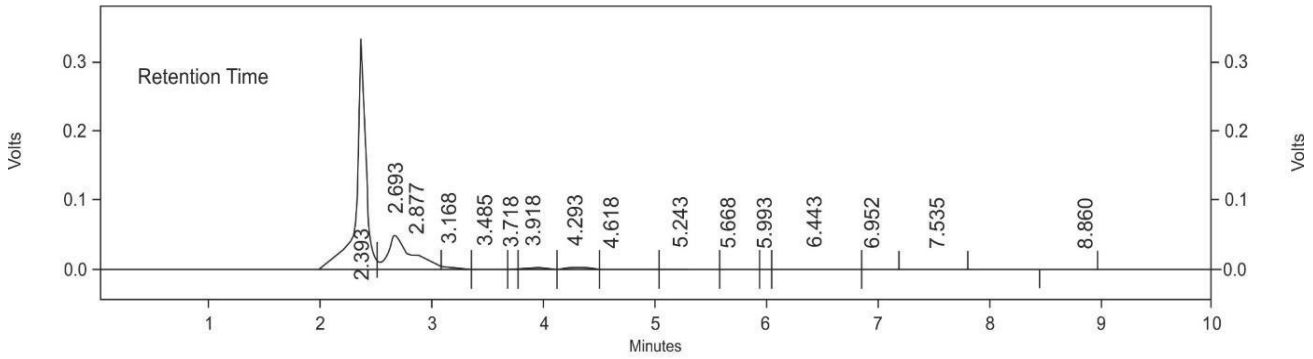
Therapeutic Uses	Phenolic acids
Antioxidant	Cinnamic acid (Khan ***et.al***2000.) ,Ferulic acid(Graf *et. al* 1992*)*, Caffeic acid (Nardini.*et al.*) (Kahkonen *et al.*1999)(Kono *et al.*1997) (Rhodes *et.al.*1997)
Antinflammatory	Gallic acid (kros *et al.*1992), Ferulic acid, Caffeic acid((Fernandez *et al.*1998)
Antifungal	Ferulic acid, Cinnamic acid (Tawata *et al.*1996), (Mehrotra *et al.*,1997)
Antibacterial	Gallic acid (Binute *et al.*2000)
Anti-viral activity	Cinnamic acid (Galbove *et al.*1998)

Croton bonplandianum Baill in its Natural Habitat

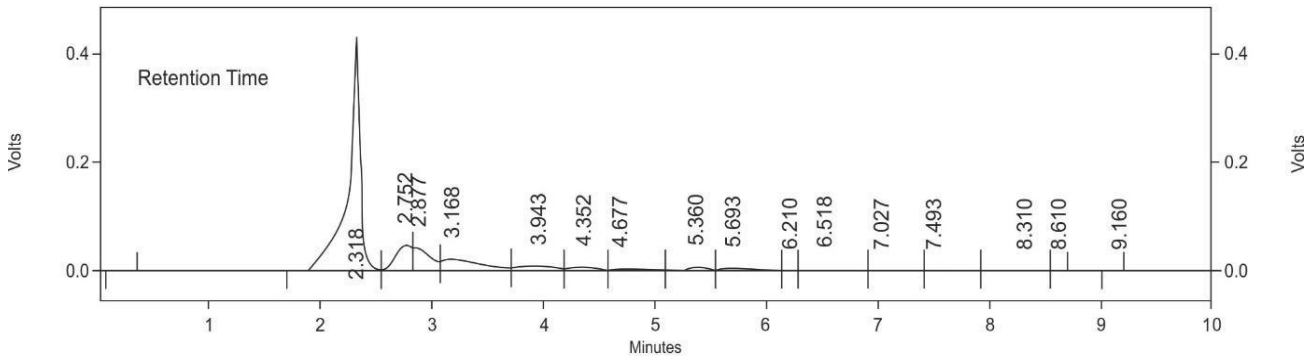
Flower and Fruit of *Croton bonplandianum* Baill

Flower Inflorescence of *Croton bonplandianum* Baill

HPLC ANALYSIS OF ROOTS



HPLC ANALYSIS OF LEAVES



HPLC ANALYSIS OF INFLORESCENCE