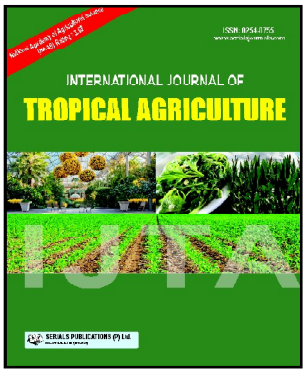

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UHPLC Analysis and Antioxidant Activities of Hexane fraction from *Acacia catechu* WILLD.

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Abstract: The plants synthesize variety of secondary metabolite during their life time. These secondary metabolites due to their structural diversity are categorized under different classes such as phenols, alkaloids, tannins so on and so forth. These secondary metabolites as a virtue of their chemical structure offer numerous health benefits. They stabilize free radicals generated in our body during normal metabolic activities and under various stress conditions. Thus, they help us restrict progression of dreadful diseases. In the present study, we selected a medicinal plant *Acacia catechu*. It leaves were used to prepare hexane fraction (AHF). This fraction was analyzed for antioxidant activity using various established protocols. Further, the AHF was subjected to UHPLC analysis to find out presence of polyphenol compounds. Ellagic acid was found to be the most abundant polyphenol in AHF. Further studies are in progress to study the *in vivo* mechanism of AHF in curbing free radicals.

INTRODUCTION

Natural plant products, especially secondary metabolites (SM), offer infinite prospects for new drug development due to matchless disposal of biochemical range [1,2]. They exhibit wide array of pharmacological effects in human beings by scavenging free radicals [3,4]. SM represents the most prized yet under-utilized pool of natural remedial measure against numerous dreadful diseases [5]. Polyphenols, non-enzymatic group of plant SMs, represent one such group of metabolites [6]. They being the structurally diverse, due to hydroxylation, glycosylation and acylation of phenol ring, are

present in numerous forms in various plant parts [7].

The plants being rich source of disease preventive phenols, their dietary intake is often linked to alternative mode of disease prevention. The phenols have reported for numerous health benefits due to their antioxidant, anticancer, anti-ageing, antimicrobial, carioprotective, ant-inflammative, hepatoprotective activities [8]. Carrying such immense curative properties, the researchers are exploring medicinal plants to eke out bioactive polyphenols. The *Acacia catechu* Willd. (Fabaceae) is an important medicinal plant growing in drier regions of India. It is rich in SMs and has numerous medicinal properties mentioned in Ayurveda. Earlier we have reported hepatoprotective activity of ethyl acetate fraction of leaves of *A. catechu*. It's leaves contain numerous polyphenols like ellagic acid, rutin, gallic acid, quercetin and catechin [9]. The present work was designed to estimate the *in vitro* antioxidant activity of hexane fraction prepared from leaves of *A. catechu* (AHF).

MATERIALS & METHODS

Extract preparation

The leaves from *A. catechu* plant harvested in the month of June Guru Nanak Dev University campus, Amritsar. The leaves of plant were thoroughly washed with tap water and powder. The powdered leaves were extracted with 80% methanol to get methanol extract (ME). The ME was dissolved in water to get aqueous methanol extract which was extracted thrice with hexane. The hexane fraction was filtered, pooled and concentrated to get hexane fraction of *A. catechu* (AHF).

UHPLC Analysis

For UHPLC analysis AHF (10 mg) was dissolved in 1 ml methanol (HPLC grade) and filtered through 0.22 mm syringe filter (PALL Life Sciences). Sample was analyzed on Shimadzu UHPLC Nexera system (Shimadzu, MA, USA), provided with a photodiode array (PDA) detector using C18 column. The gradient mobile phase consisting of 0.1% acetic acid aqueous as solution A and Methanol as solution B was used. The gradient elution is: 0–1 min, 30% B; 1–10 min, 65% B; 10–14 min, 80% B; 14–16 min, 80% A, 16–17 min: 40% B, 17– 20 min: 35% B and 20–21 min: 30% B. The flow rate was set as 1 ml/min and the injection volume was 5 ml. Quantification of peaks was also done using software provided with Shimadzu UHPLC Nexera system.

Quantitative Analysis

The total phenol and flavonoid content of AHF was estimated using methods of Yu *et al.* [10]. and Kim *et al.* [11] respectively.

In Vitro Antioxidant Assays

The Hydrogen donating potential of AHF was estimated by DPPH and ABTS assay following methods of Blois *et al.* [12] and Re *et al.* [13]. The ion reducing activity of AHF was estimated by Ferric ion reducing (FIR) and Cupric anion reducing capacity (CUPRAC) assays using the methods of Oyaizu [14] & Apak *et al.* [15] respectively. The superoxide scavenging (SS) and peroxide radical scavenging (PRS) assays were also carried out employing well established protocols of Nishikimi *et al.* [16] and Ohkawa et al [17] respectively.

RESULTS & DISCUSSION

The total phenolic and flavonoid content of AHF was found to 60.00 mg GAE/100mg fraction and 15.53 mg RE/100mg respectively. The UHPLC analysis of AHF showed presence of nine polyphenol compounds. The Ellagic acid was found in highest amount followed by quercetin, rutin, kaempferol, epicatechin, catechin, chlorogenic acid, umbelliferone, gallic acid and coumaric acid (Figure 1 and table 1).

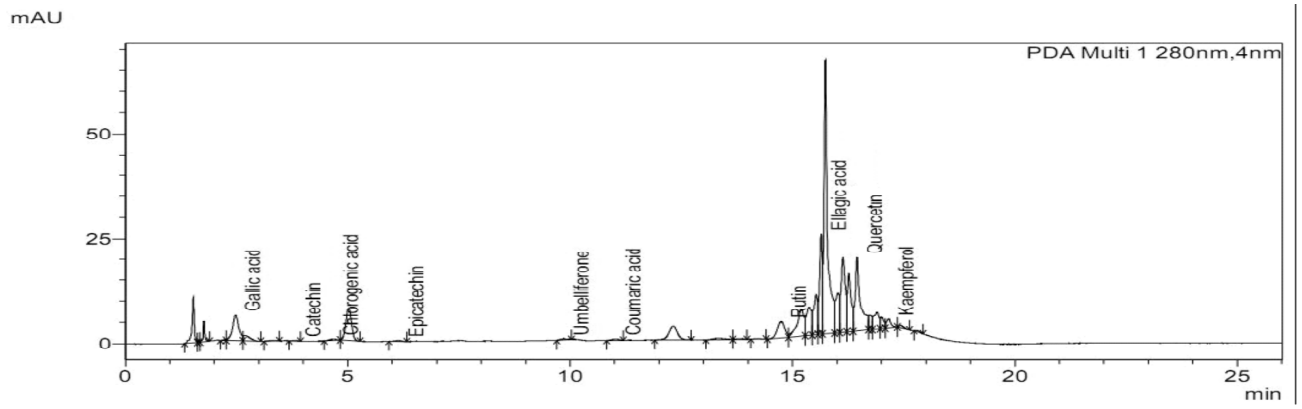

Figure 1: UHPC chromatogram of AHF

Table 1
Chromatographic profile of polyphenolic compounds present in AHF

S. No.	*Compound*	*Peak*	*Retention Time*	*Area*	*Height*	*Concentration (ppm)*
1.	Gallic Acid	5	2.477	58987	5857	4.137
2.	Catechin	8	3.817	1545	200	0.647
3.	Chlorogenic acid	9	4.671	3827	354	0.501
4.	Epicatechin	11	6.147	2634	233	0.692
5.	Coumaric acid	13	11.024	3560	340	0.121
6.	Rutin	18	14.744	44734	3793	11.525
7.	Ellagic acid	22	15.646	104283	23968	**22.935**
8.	Quercetin	27	16.450	143404	17345	19.709
9.	Kaempferol	31	17.161	14327	2092	6.105

The *in vitro* antioxidant assays were performed to check antioxidant activity of AHF. The IC_{50} value (µg/ml) was calculated by preparing regression equation. The DPPH, ABTS assays were employed for checking hydrogen donating ability. The AHF showed IC_{50} value of 499.45 and 132.55 in DPPH and ABTS assay respectively (table 2). Both hydrophilic and hydrophobic phytochemicals are exploited in ABTS assay unlikely in DPPH assay which only uses hydrophilic compounds to assess hydrogen donating ability.

The anion reducing potential of AHF was estimated by FIRA and CUPRAC assay. The IC50 value was found to be 359.02 and 251.09 in FIRA and CUPRAC assay respectively. The radical scavenging activity of AHF was assesses by SSA and PRSA. The IC50 value was found to be 416.03 and 278.80 for SSA and PRSA respectively.

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Table 2
Antioxidant activity of AHF in various *in vitro* antioxidant assays.

Conc.(µg/ml)	*Antioxidant assays (Antioxidant potential ± S.E.)*					
	DPPH	*ABTS*	*FIRA*	*CUPRAC*	*SSA*	*PRSA*
20	0.960 ± 2.500	06.65 ± 1.410	01.94 ± 0.667	01.19 ± 0.366	01.23 ± 0.739	01.07 ± 0.545
40	03.53 ± 0.472	15.21 ± 0.335	04.10 ± 0.568	02.87 ± 0.480	03.49 ± 0.586	03.05 ± 0.666
60	06.23 ± 0.660	23.11 ± 0.419	08.06 ± 0.312	07.19 ± 0.554	06.77 ± 0.736	07.73 ± 0.651
80	08.24 ± 0.680	27.53 ± 2.590	10.70 ± 0.341	11.27 ± 0.831	09.21 ± 0.447	10.27 ± 1.207
100	08.81 ± 0.492	38.44 ± 0.887	14.73 ± 0.811	13.18 ± 0.771	12.26 ± 0.635	13.61 ± 1.528
120	12.17 ± 0.796	46.15 ± 0.777	16.86 ± 0.667	15.11 ± 0.719	14.23 ± 0.870	19.14 ± 0.854
140	14.35 ± 0.772	53.46 ± 1.031	18.87 ± 0.433	21.42 ± 1.194	16.89 ± 0.500	24.90 ± 1.077
160	16.19 ± 0.682	59.04 ± 1.074	22.09 ± 0.322	29.09 ± 1.178	19.01 ± 0.582	28.51 ± 0.545
180	17.67 ± 0.767	69.30 ± 0.838	24.99 ± 0.381	36.69 ± 0.969	21.91 ± 0.315	31.84 ± 0.573
200	19.33 ± 0.503	75.30 ± 1.154	26.69 ± 0.407	42.68 ± 1.183	22.27 ± 0.255	32.97 ± 0.502
Reg. Eq.	y = 0.101 x-0.445	y = 0.382 x-0.635	y = 0.141 x-0.622	y = 0.227 x-6.998	y = 0.122 x-0.756	y = 0.194 x-4.089
R^2 value	0.991	0.997	0.994	0.953	0.991	0.987
IC_{50} (µg/ml)	499.45	132.55	359.02	251.09	416.03	278.80

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