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LC/ TOF/ ESI/ MS based detection of bioactive compounds present in leaf and bark extract of *Acacia arabica*

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Abstract

An HPLC / MS/MS was employed to investigate bioactive principles present in methanolic leaf and bark extracts of *Acacia arabica* . The compounds commonly present in leaf and bark extracts were Oleic acid ($C_{18}H_{34}O_2$), Myristic acid ($C_{14}H_{28}O_2$), Palmitic acid ($C_{16}H_{32}O_2$), Ferulic acid ($C_{10}H_{10}O_4$), *p*-Coumaroyl-glucoside ($C_{15}H_{19}O_7$), *p* -Coumaroylquinic acid ($C_{16}H_{18}O_8$), Quercetine 3-O- (4'-O-acetyl)-rhamnopyranoside ($C_{28}H_{30}O_{16}$) and Steroidal sapogenin. The other compounds observed in leaf extract were Caffeic acid phenethyl ester (CAPE) ($C_{17}H_{16}O_4$), Epi catecine-3-gallate ($C_{22}H_{18}O_{10}$) and Methyl 3,4,5 tri hydroxyl benzoate ($C_8H_8O_5$). One compound seperately identified from bark extract was 3, 4, 5-trihydroxybenzoate ($C_7H_6O_5$).

Keywords: *HPLC / MS/MS, Acacia arabica*

INTRODUCTION

Fabaceae is one of the largest family and large number of trees and plants belongs to the same family. *Acacia arabica* belongs to a complex species and has a number of subspecies. The leaves, bark, gums and fruits of the plant are used for various medical purposes. Leaves are used for treatment of gonorrhea, dropsy and leucorrhea. The present study deals with investigation of bioactive components present in methanolic leaf and bark extracts of *Acacia arabica* using HPLC / MS/MS. The tree is rich in polyphenolic compounds including flavonoids and tannins. Phenolic compounds are widely distributed in trees and plants having numerous medicinal properties. Therefore, it is necessary to detect and identify valuable bioactive components. Shohaib *et al.* (2011) documented flavonoids as Vitamin P which plays a very important role in photosynthesizing plant cells. *Acacia arabica* is rich in antioxidants due to the presence of flavonoids, tannins and polyphenolic compounds. Olbinri *et al*. (2010) reported that phenolic antioxidants are persuasive free radical terminators.

MATERIALS AND METHODS

Plant material collection and extraction: Leaves and barks of *Acacia arabica* were collected and shade dried after washing. Finely macerated plant parts were extracted in 100 % MeOH.

Isolation and Purification: The most active methanolic crude extracts were fractioned using silica gel 60-120 mesh column and sephadex LH-20 column. Fractionation of methanolic leaf extract was done by method recommended by Saleem *et al.* (2002) using sephadex LH-20 column whereas fractionation of stem bark extract was done according to Sundaram and Mitra (2006) using Silica gel 60-120 mesh.

HPLC and Preparative HPLC conditions: HPLC (LC-10A/AAA, Shimadzu, Japan) was used to detect the compounds in the extracts using 10 µl of the injected sample into a RP- C18 column with particle size of 5µM and a flow rate of 1ml min^{-1}(CFTRI, Mysore). Detection was done by the UV detector. Multiple wavelengths selected were 280 nm, 294 nm and 289 nm. Saponin was detected at 294 nm with mobile phase of acetonitrile: acidified water (70: 30) at 40 ^oC. Gallic acid was detected at 280 nm with 0.1% phosphoric acid: acetonitrile as mobile phase while tannic acid was detected at 289 nm when mobile phase of glacial acetic acid: acidified water (25: 975) was used. Separation of bioactive compounds was effected through Preparative High Performance Liquid Chromatography (LC-18, Shimadzu, Japan) with C-18 column (20mm x 250mm) and mobile phase acetonitrile: water (7:3) as isocratic solvent system at a flow rate of 10 ml/min. UV detector at 280, 289 and 294 nm was used for detecting the peaks. Fractions collected for each peak were concentrated and used for LC-MS analysis.

LC-MS analysis: Each peak collected separately was lyophilized and redissolved in known volume of 100% MeOH for further using in LC-MS (CFTRI, Mysore). For LC analysis Perkin Elmer Series (Japan) was used with ESI- TOF (Electrospray Ionization –Time- Of –Flight) and LC conditions were same as used in RP-HPLC with methanol flow rate of 1 ml min^{-1}. Both positive and negative ESI was used for detection of mass of every individual peaks *i.e* sub- fractions. Positive ESI was used for detection of saponins and aldehyde groups and negative ESI was used for estimation of organic acids and OH containing organic bioactive components. For negative ESI mass ranges from 200 to 900 *m/z*, scan speed 60-700 *m/z* (cycle time: 330 m sec), dry gas N_2, dry gas temperature 360 ^oC , capillary voltage 4000 V (Theerasin and Baker, 2009). For positive ESI mass ranges from 400 to 1000 *m/z*, scan speed 400-1122 *m/z* (cycle time: 447 m sec), dry gas N_2, dry gas temperature 230 ^oC, capillary voltage 4500 V. Data acquisition was performed from the company

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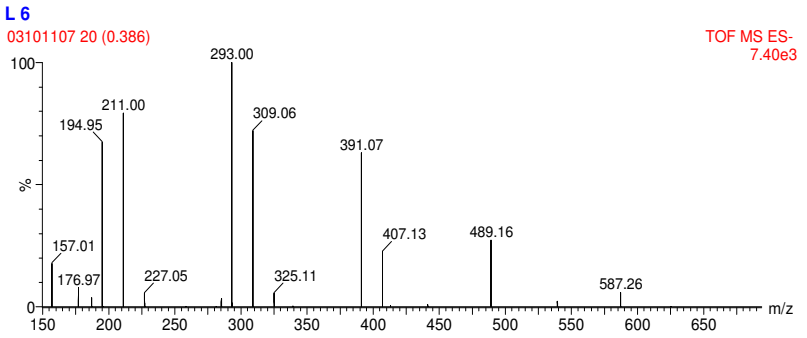
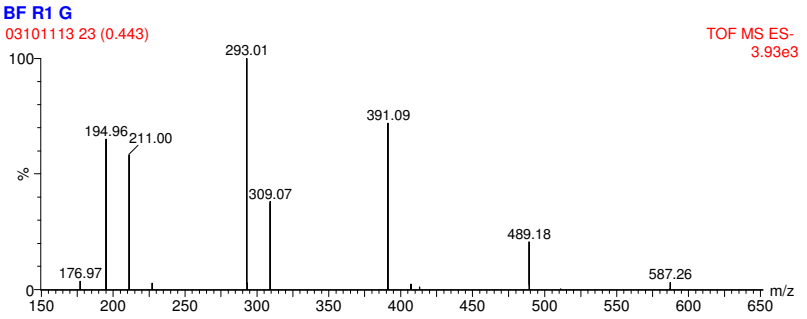
supplied software with the instrument. The injection volume was 1 µl. The data was collected and compared with mass spectra of data from literatures and softwares. The fragmentation was used to detect and identify few of the compounds present in the extracts.



(a)



(b)

Fig 1. LC/ TOF/ ESI/ MS spectrum of bioactive compounds of a) leaf and b) bark extract of *Acacia arabica*

Table 1. Identification of Bioactive compounds in fractions of methanolic leaf and bark extract by using Preparative HPLC, LC- MS data

Compounds	Plant Part	Molecular weight	*m/z* value	Wave length	Ion (+/-)	References
3, 4, 5-trihydroxybenzoate $C_7H_6O_5$	Bark	170.12	169.04	289	$[M-H]^-$	Ma *et al.*(2004)
Oleic acid $C_{18}H_{34}O_2$	Bark and leaf	282	281.13	289	$[M-H]^-$	Houjou *et al.*(2007)
Myristic acid $C_{14}H_{28}O_2$	Bark and leaf	228	227.6	294	[M-H]-	Houjou *et al.* (2007)
Palmitic acid $C_{16}H_{32}O_2$	Bark and leaf	256	255.33	289	[M-H]-	Houjou *et al.* (2007)
Ferulic acid $C_{10}H_{10}O_4$	Bark and leaf	194	194.95	294, 289	$[M]^+$, $[M+H]^+$	Giusti *et al.* (1999)
p-Coumaroyl-glucoside $C_{15}H_{19}O_7$	Bark and leaf	326	325.11, 325.33, 325.34, 325.35	289, 294	[M-H]-	Seeram *et al.* (2006), Aaby *et al.* (2007)
p -Coumaroylquinic acid $C_{16}H_{18}O_8$	Bark and leaf	340	339.37, 339.38, 339.39, 339.40	289, 294	[M-H]-	Seeram *et al.* (2006), Aaby *et al.* (2007)
Ascorbic acid $C_6H_8O_6$	Bark and leaf	176	176.97	294	$[M]^+$	Aaby *et al.* (2007)
Epi catecine-3-gallate $C_{22}H_{18}O_{10}$	leaf	442.37	441.36	289	[M-H]-	Ma *et al.* (2004)
Quercetine 3-O- (4'-O-acetyl)-rhamnopyranoside $C_{28}H_{30}O_{16}$	leaf	490	489.16,	289	[M-H]-	Laponen *et al.*(2001)
Caffeic acid phenethyl ester (CAPE) $C_{17}H_{16}O_4$	leaf	284	285.06	289	[M+H]-	Kudugunti *et al.*(2011)
Methyl 3,4,5 tri hydroxyl benzoate $C_8H_8O_5$	leaf	184.5	183.07	294	[M-H]-	Mahajan and Pai, (2010)
Steroidal sapogenin	Bark and leaf	700-1500	900.15	289,294	$[M+H]^+$	Berhow *et al.* (2002)

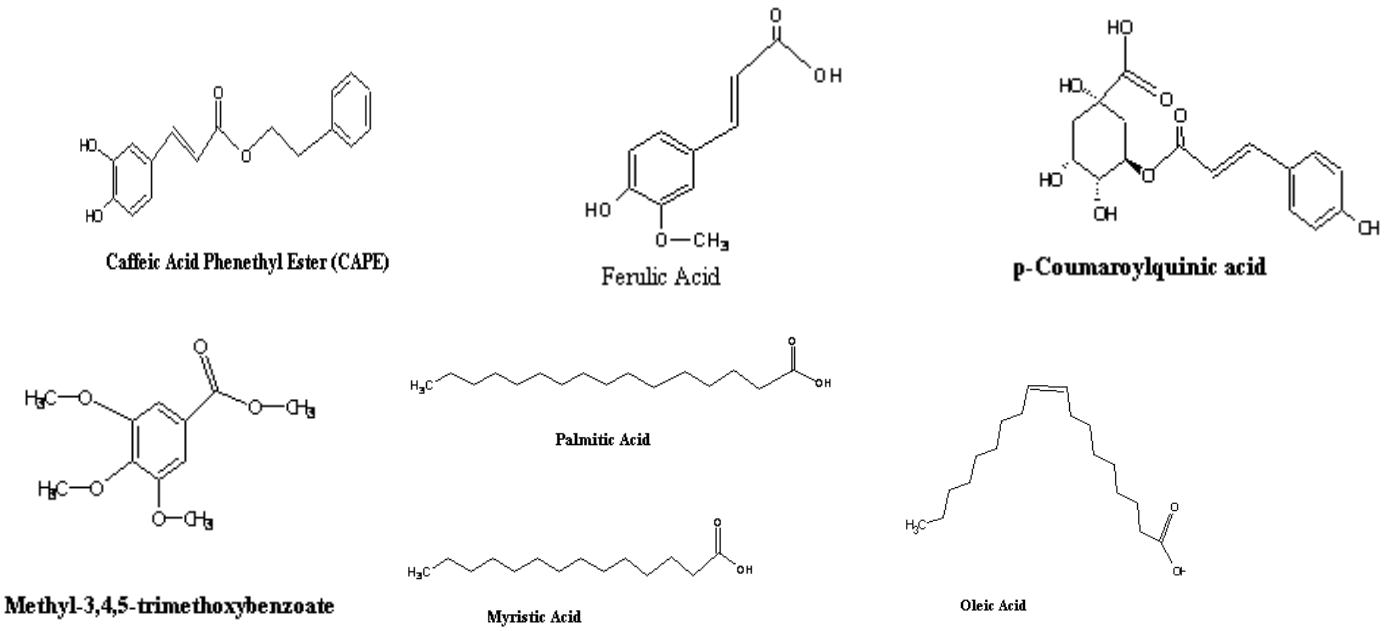


Fig 2. Structures of some compounds present in leaf and bark extracts of *Acacia arabica*

RESULTS AND DISCUSSION

The experimental methods described by using chromatographic separation and mass spectrophotometric detection, by using ESI give a large number of fragmentation pattern. Both positive and negative ESI were used to analyze the bioactive compounds. The negative ESI mode are characterized by the formation of [M-H]- ion and positive ESI mode are characterized by formation of $[M+H]^+$ion. Table-1 represents typical fragmentation pattern of detected bioactive compounds from leaf and bark extracts of *Acacia arabica.* Wen *et al*.(2004) documented that HPLC coupled with LC-MS is one of the most powerful tool for detection of bioactive principles from botanical extracts

In HPLC ESI separation the compounds detected with *m/z* value of 169.04 with [M-H]- ion mode was 3, 4, 5-trihydroxybenzoat ($C_7H_6O_5$) from methanolic bark extract. The identity of Oleic acid ($C_{18}H_{34}O_2$), Myristic acid ($C_{14}H_{28}O_2$) and Palmitic acid ($C_{16}H_{32}O_2$) was confirmed from *m/z* values of 281.13, 227.6 and 255.33 respectively from both methanolic leaf and bark extracts. In addition Ferulic acid ($C_{10}H_{10}O_4$) with *m/z* value of 194.95 was also identified from both plant parts. Using MS-MS, Ascorbic acid ($C_6H_8O_6$) with *m/z* value of 176.97 was detected. Epi catecine-3-gallate ($C_{22}H_{18}O_{10}$), Caffeic acid phenethyl ester (CAPE) ($C_{17}H_{16}O_4$) and Methyl 3,4,5 tri hydroxyl benzoate ($C_8H_8O_5$) with *m/z* values of 441.36, 285.06 and 183.07 respectively were detected only from methanolic leaf extract. *p*-Coumaroyl-glucoside showed fragmentation patterns with *m/z* of 325.11, 325.33, 325.34 and 325.35. In addition, *p* -Coumaroylquinic acid ($C_{16}H_{18}O_8$) showed four different *m/z* values of 339.37, 339.38, 339.39 and 339.40. Both *p*-Coumaroyl-glucoside + *p* -Coumaroylquinic acid were present in combined form in leaf and bark extracts. Steroidal sapogenin were also detected from leaf and bark extracts with positive ion mode. Chou *et al.* (1998) reported the presence of Caffeic acid, Ferulic acid, Gallic acid, *m*-hydroxy benzoic acid and *m*-hydroxyphenyl acetic acid from aqueous extracts of various parts of *Acacia confuse* which is in agreement with the findings obtained from present study using TOF- ESI/ MS.

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