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# Separation and identification of four phenolic acids from some selected medicinal plants of South India

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## ABSTRACT

Four phenolic acids-P-Coumaric acid, Ferulic acid, Chlorogenic acid and Tannic acid have been isolated from the aerial parts of four medicinal plants such as *Merremia turpethum*, *Erythrina variegata*, *Merremia gangetica and Anisomeles malabarica* respectively. They were unambiqueously identified using chemical and spectral methods including UV, IR, Mass, $^{1}H$ NMR and $^{13}C$ NMR. All these compounds were reported for the first time from these plants. These compounds were subjected to antioxidant studies by various methods and also screened for antimicrobial activities against certain Gram positive and Gram negative bacteria.



## KEYWORDS

P-Coumaric acid;
Ferulic acid;
Chlorogenic acid and
tannic acid.

## INTRODUCTION

*Merremia turpethum* is a medicinal plant of convolvulaceae family. It is commonly called civatai in Tamil. The whole plant is used as astringent, carminative, febrifuge and tonic[3, 8, 1, 7, 10].

*Erythrina variegata* is a species of Leguminaceae family. The name "Coral tree" is used as a collective term for 110 species of the genus Erythrina. The plant Erythrina varigata have been used in traditional medicine as nervine sedative febrifuge, aniti-asthmatic and antiepileptic. It is also used against fever, inflammation, bacterial infection, insomnia, cough, cuts and wounds[11, 2, 14].

*Merremia gangetica* is a twining or prostrate herb. (9) The plant is used as deobstruent, diuretic and also incurring cough, headache, neuralgia, rheumatism and stomach problems[4, 5, 6, 15, 16].

*Anisomales malabarica* ia a plant of Laminaceae family native to india, Bangaladesh, Srilanka, Andaman and Nicobar Islands. It is used against amentia, anorexia, dyspepsia, colic, flatulence, epilepsy and hysteria. It is essential oil is used in rheumatism[12, 17, 13].

In the absence of any report on phytochemicals in the literature these medicinal plants, they were taken up for chemical investigations and results leading to the isolation and characterization of four phenolic acids viz. P-Coumaric acid, Ferulic acid, Chlorogenic acid and Tannic acid are presented here apart from flavonoids.

## RESULTS AND DISCUSSION

From the alcoholic extract of the air dried aerial

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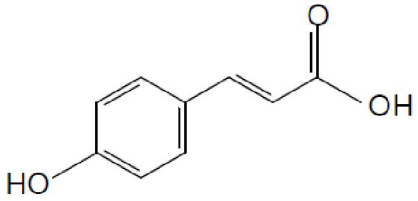
parts of four medicinal plants four compounds were isolated.

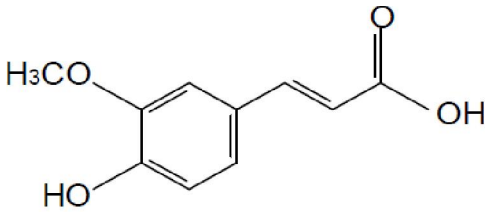
**Compound (1)**

It crystallized from MeoH as colourless needles, $C_9H_8O_3$, mp. 218-220°C. It produced pale yellow colour with alkalis, greenish brown with $Fe^{3+}$ and decoloured bromine water. It gave effervescence with $NaHCO_3$. It was colourless under UV changing to blue under UV/$NH_3$ and had $\lambda max$ (MeOH) 224, 304 nm and the EIMS exhibited molecular ion peak at m/z 164. The fragmentation pattern was found to be in close agreement with reported values. This was further supported by HPLC, $^1$H NMR and $^{13}$C NMR spectrum. Thus the compound (1) was identified as p-hydroxy cinnamic acid (p-coumaric acid).




**Compound (2)**

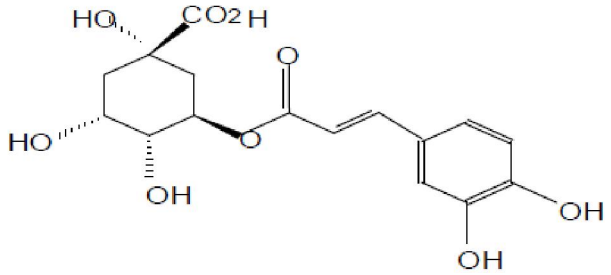
Compound, colourless needles from MeOH $C_{10}H_{10}O_4$, mp. 210-212°C, decolourised $Br_2/H_2O$, gave effervescence with $NaHCO_3$ solution and developed green colour with $Fe^{3+}$ suggesting that it is an unsaturated phenolic acids. It was blue under UV changing to bright blue under UV/$NH_3$. It had (MeOH) 225, 285, and 310 nm and had if Rf values similar to hydroxy cinnamic acid. EIMS exhibited molecular ion peak at m/z 194 along with other fragment ions at m/z 179 and 166. The fragmentation pattern was found to be in close agreement with reported values. This was further supported by HPLC, $^1$H NMR and $^{13}$C NMR spectrum. Thus the compound (2) was identified as (E)-4-hydroxy- methoxy cinnamic acid (ferulic acid).




**Thus compound (2) was identified as (E) Ferulic acid.**

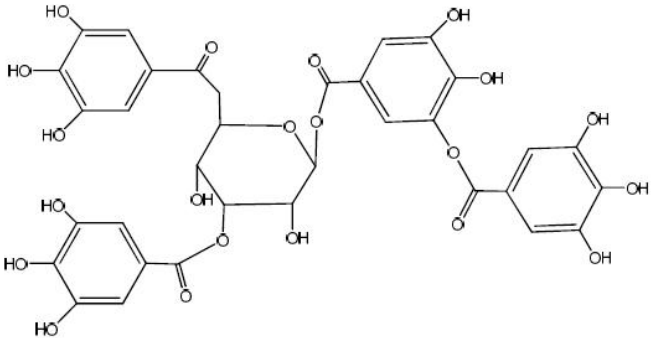
**Compound (3)**

Colourless needles, $C_{16}H_{18}O_3$, mp. 199-200°C, gave deep blue with $Fe^{3+}$, rosyred with phenol red reagent and blue UV flurescence changing to yellow green with UV/$NH_3$. It had (MeOH) 241, 327 nm of suggesting it to be a phenyl propanoid Alkali hydrolysis yielded caffeic acid along with an alphatic acid. EIMS exhibited molecular ion peak at m/z 354 along with other fragment ions 336(32), 180(60) and 163(100). The peak at m/z 180 was due to caffeic acid there by suggesting it to be a caffeoyl ester of quinic acid and the fragmentation pattern found to be in close agreement with the reported values[18]. The occurrence of M+ and M+_18 were due to the formation lactone in quinic acid moiety and the peak at m/z 163(100) was that of caffeoyl part[19]. The quinic acid moiety was further supported by HPLC, $^1$H NMR and $^{13}$C NMR spectra. The $^1$H NMR spectrum of compound (3) gave further evidence by exhibiting signals at aromatic region due to caffeoyl protons. The trans stereochemistry was deduced from the peaks at δ 7.40 and 6.12 (d,J=16Hz). The signals for hydroxyl protons at δ 9.57, 9.14, 4.95, 4.77 and 3.90 disappeared on addition of $D_2O$. The aliphatic regions showed signals for three hydroxyls (1, 4 and 5-OH) and two –$CH_2$ groups (2, 6-$CH_2$) and a multiplet (2H, H-4, 5). The singlet at δ 5.05 (m, 1H) was in agreement with 3-O-Caffeoyl quinic acid. Finally $^{13}$C NMR supported the characterization of the compound (3) to be 3-O-Caffeoyl quinic acid (chlorogenic acid) and the identity was further confirmed by CO-PC with an authentic sample.




**Compound (4)**

Colourless needles from MeOH, $C_{34}H_{28}O_{21}$, mp. 223-300°C, decolourised $Br_2/H_2O$, gave effervescence with $NaHCO_3$ solution and developed green colour with $Fe^{3+}$. It was blue under UV and chang-



ing to bright blue under UV/$NH_3$. On hydrolysis it gave glucose and gallic acid. EIMS exhibited molecular ion peak at m/z 772. This was further supported by HPLC, $^1$H NMR and $^{13}$C NMR spectrum. Thus the compound (4) was identified as tannic acid.

## EXPERIMENTAL

### Plant material

Fresh aerial parts were collected from Marakanam forest near Pondicherry India in May 2014 and authenticated by the department of Botany Tagore Arts College, Puducherry where a voucher specimen of each plants were deposited.

## EXTRACTION AND ISOLATION

The air dried aerial parts of each plants were extracted thrice with boiling 95% EtOH (3X5L) and concentrated in vacuo to 500 ml. The aqueous extract of each plants were then fractionated into Benzene, Ether ethylacetate and methyl ethyl ketone solubles. The benzene fraction, of each plants containing more chlorophyll and gave no characteristic spots on paper chromatography for polyphenolics are were not worked up further. The ether fractions gave characteristic spots on paper chromatography for phenolic acids. Hence they were concentrated and chromatographed over sephadex LH 20 sucessively. Fractions were collected in 10 ml aliquots.

Ether concentrate of *Merremia turpethum* deposited a collourless solid from 1-4 Column fractions designated as compound (1) similarly ether column fractions 3-7 of *Erythrina varigata*, 1-4 of *Merremia gangetica* and 4-9 fractions of *Anisomeles malabarica* deposited collourless needles designated as compound (2), compound (3) and compound (4) respectively.

### P-Coumaric acid

**UV (λ max., nm)**

MeOH : 243, 323
$AlCl_3$ : 233sh, 263, 318, 358
$AlCl_3$/HCl : 232sh, 298sh, 318
NaOMe : 304sh, 332

**$^1$H NMR 350 MHz (DMSO-$d_6$ δ ppm):**

C2-6.27 (d: 15.0 Hz), C3-7.48 (d: 15.0 Hz), C5 & C9-6.79 (d: 8.0 Hz), C6 & C9-6.78 (d: 8.0Hz)

$^{13}$C NMR (67.89 MHz, broad band decoupled, DMSO-$d_6$ δ ppm):

C1-168.41, C2-115.79, C3-144.63, C4-125.73, C5 & C9-130.54, C6 & C8-116.20, C7-60.75

**Mass: EI-MS m/z (rel. int.):**

164 ($M^+$, 100).

### Ferulic acid

**UV (λ max., nm)**

MeOH : 225, 285, 310
NaOAc : 225, 282, 308
NaOAc/$H_3BO_3$ : 220, 286sh, 310
$AlCl_3$ : 235, 306sh, 325
$AlCl_3$/HCl : 226, 304sh, 322
NaOMe : 232, 302, 342

**$^1$H NMR 350 MHz (DMSO-$d_6$ δ ppm)**

3.81 (3H, s, H-4'), 6.34 (1H, d, J=15 Hz, H-2'), 6.79 (1H, d, J=9 Hz, H-6), 7.08 (1H, dd, J=8 and 2 Hz, H-5), 7.08 (1H, d, J=2 Hz, H-3), 7.50 (1H, d, J=15 Hz, H-1').

**$^{13}$C NMR (67.89 MHz, broad band decoupled, DMSO-$d_6$ δ ppm)**

56.1 (C-4'), 111.06 (C-5), 115.0 (C-2), 116.09 (C-2'), 147.05 (C-6) 148.37 (C-1), 168.44(C-3')

**Mass: EI-MS m/z (rel. int.):**

194[M]+100, 179 (21), 166 (7), 137 (32), 89, 87 (15), 77 (27).

### Chlorogenic acid

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**UV (λ max., nm)**

MeOH : 241,303 sh, 327;
$AlCl_3$ : 258 sh, 308, 356;
$AlCl_3$/HCl : 238, 298 sh, 325,
NaOMe : 258 sh, 298sh, 378;

**$^1$H NMR 350 MHz (DMSO-$d_6$ δ ppm)**

9.57, 9.14, (each brs, each 1H, OH-7', 8'), 7.40 (d, J = 16Hz, 1H', H-3), 7.03 (d, J =2Hz, 1H, H-9'), 7.00 (dd, J=8 & 2 Hz, 1H, H-5'),

**$^3$C NMR (67.89 MHz, broad band decoupled, DMSO-$d_6$ δ ppm).**

175.37 (C-7), 166.13 (C-1),148.79 (C-7') 145.40 (C-6'), 126.05 (C-4'), 121.81 (C-9'), 115.82 (C-2') 114.74 (C-5'/8'), 73.91 (C-1), 71.32 (C-3), 68.51 (C-4), 68.40 (C-5) 36.77 (C-2).

**MS/(EIMS, m/z Intensity as %)**

354 ($M^+$ 5%), 336 (32), 225 (20), 180 (Caffeic acid$^+$, 60), 150 (18), 103 (38), 89 (42), 75 (48).

**Tannic acid**

**$^1$H NMR 350 MHz (DMSO-$d_6$ δ ppm):**

7.11 (C2'), 7.10 (C2), 7.09 (C4), 7.06 (C1), 7.03 (C3 & C5), 7.01 (C1'), 6.9 (C5), 6.8 (C3' & C4'), 4.9 (C6).

**$^{13}$C NMR (67.89 MHz, broad band decoupled, DMSO-$d_6$ δ ppm):1**

109.17 (C2' & C6'), 119.44 (C2' & C5'), 120.90 (C61), 145.86 (C3' & C4'), 146.14 (C3' & C5'), 151.03 (C3 & C5), 164.44 (C5 & C7)

**Mass: EI-MS m/z (rel. int.)**

772 (M+100).

## CONCLUSION

In the present study we report isolation and characterization of *para*-coumaric acid from *Merremia turpethum*, Ferulic acid from *Erythrina varigata*, Chlorogenic acid from *Merremia gangetica* and Tannic acid from *Anisomeles malabarica* respectively. All these four phenolic acid from were reported for the first time from the aerial parts of these plants. The structures were fully established using chemical and spectral methods.

## ACKNOLEDGEMENT

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