Anti-Microbial Activity of Ferulic Acid Isolated From Cansjera Rheedii J.Gmelin (Opiliaceae)


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ABSTRACT

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) isolated from the aerial parts of Cansjera rheedii J.Gmelin (Opiliaceae) has been tested for its antimicrobial activity The antimicrobial activity have been studied with ferulic acid against Stapylococcus aureus, Bacillus substilis, Escherichia coli, Salmonilla paratyphi, Candida albicans, Aspergillus fumigates which gave significant results of activity against Ciprofloxacin & Clotrimazole as standards.

Keywords: Cansjera rheedii, Anti-microbial activity, Ferulic acid, 4-hydroxy-3-methoxy cinnamic acid, Ciprofloxacin, Clotrimazole

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Received 20 April 2018, Accepted 26 April 2018

Please cite this article as: Mounnissamy VM et al., Anti-Microbial Activity of Ferulic Acid Isolated From Cansjera Rheedii J.Gmelin (Opiliaceae). American Journal of PharmTech Research 2018.
INTRODUCTION

_Cansjera rheedii_ J Gmelin (Opiliaceae) is a climbing shrub, sometimes armed, generally found in India through Malaya to Hong Kong and North Australia[1-2]. The tribes of Nilgiris in Tamil Nadu, India using the plant extract for the treatment of post-natal pain [3], intermittent fever [4] and poisonous bites and skin diseases [5]. In our earlier studies, the ethanol extract of aerial parts of _C. rheedii_ has been reported to have hepatoprotective [6], cytotoxic [7], anthelmintic [8], anti-inflammatory and membrane stabilizing property [9], antipyretic [10], anti-nociceptive [11] and diuretic [12] activities. The safety of this plant has also been proved by studying acute and sub-acute toxicity studies[13]. The compounds such as 3, 4-dihydroxy cinnamic acid (Caffeic acid), 4-hydroxy 3-methoxy cinnamic acid (Ferulic acid), 3, 5, 7, 3’, 4’-pentahydroxy flavone (Quercetin), 5, 7, 3’, 4’-tetrahydroxy -3-O-β-D-glucopyranosyl flavones (Quercetin-3-O-β-glucoside) and 5, 7, 3’, 4’-tetrahydroxy-3-O-(6-O-α-L-rhamnopyanosyl)-β-D-glucopyranosyl flavone (Quercetin-3-O-β-rutinoside (or) Rutin). Structures of all these compounds were established by spectral and chemical methods[14]. This was the first report of the above 5 compounds from the plant. The present study is focused on evaluation of anti-microbial activity[15] of ferulic acid isolated from aerial parts of _Cansjera rheedii_ J.Gmelin (Opiliaceae).

MATERIALS AND METHOD

**General experimental procedures**

1D and 2D NMR spectra were recorded on a JEOL 600 MHZ spectrometer, chemical shifts (ppm) are related to (CH$_3$)$_3$Si as TMS as internal standard. Optical rotations were determined on a JASCO P-1020 Polarimeter in MeOH. Elemental analysis by CHNSO Analyser (Thermofinnigan -Flash EA 1112 series). IR spectra were recorded on a Perkin-Elmer FTIR spectrometer. UV-Visible spectrophotometer (Shimadzu-UV-2500PC series) of each compound was determined in MeOH and after addition of different shift reagents such as AlCl$_3$, AlCl$_3$/HCl, CH$_3$COONa, CH$_3$COONa/H$_3$ BO$_4$ and NaOMe at 190-500nm. Mass spectra were recorded on GCMS-Celuras-500 (Perkin-Elmer). Melting point determinations by Differential Scanning Calorimeter (DSC-60) (Shimadzu Co., Japan). Open column chromatography was carried out on Sephadex LH-20 (Amersham Pharmacia Biotech Co., UK) as packing material and Whatmann No.1 filter paper and TLC-Silica gel 60 F$_{254}$ sheets( Merck Co., Germany)

**Plant material**

The aerial parts of the plant _C. rheedii_ (Opiliaceae) were collected from Auroville, Puducherry in June 2006 and it was identified and authenticated by Auro Herbarium Sakthi Botanical Survey
Department, Auroville, India. A Voucher specimen has been kept in our laboratory for future reference (VS-12).

**Extraction and isolation**

The air dried and coarsely powdered aerial parts (1.0 Kg) were extracted with boiling 95% ethanol (3 X 5l) and the extract was concentrated to about 250 ml. The insoluble green residue was removed by filtration and the soluble in the filtrate (150 ml) were fractioned into C₆H₆, Et₂O, EtOAc and EtCOMe. The C₆H₆ fraction after concentration yielded a pale yellow needle, recrystallized from MeOH and designated as compound I (910mg). The Et₂O concentrate was column chromatographed over sephadex LH-20 using MeOH. 35 fractions of 50ml each were collected. Fraction 4-29 gave colourless needles, recrystallized from MeOH and designated as compound II (800mg). The EtOAc concentrate was column chromatographed over Sephadex LH-20 using MeOH. 44 fractions of 50ml each where collected, fractions 7-32 gave yellow needles, recrystallized with MeOH and designated as Compound-III (1.1g). The EtCOMe concentrate was chromatographed on a column of Sephadex LH-20 using MeOH as eluent. 107 fractions of 50ml each were collected, fractions 6-35 deposited a homogenous yellow solid recrystallized from MeOH and designated as compound-IV (89mg). Fractions 36-98 gave a pale yellow homogenous solid, recrystallized from MeOH and were designated as compound-V (530mg).

**RESULTS AND DISCUSSION**

**Characterization of Ferulic acid (4-hydroxy 3-methoxy cinnamic acid):**

Colourless needles, mp. 210.8°C (Fig-1) gave effervescence with NaHCO₃ solution, decolourized Br₂ water and green colour with Fe³⁺. It was blue under UV changing to bright blue under UV/NH₃; Rᵣ similar to hydroxy cinnamic acid. UV λ<sub>max</sub>(MeOH): 233, 296, 319nm; (+NaOMe): 234, 304sh, 346; (+ CH₃COONa): 227, 284sh, 323nm; (+CH₃COONa / H₃BO₄): 224, 296sh, 322nm; (+AlCl₃): 237, 302sh, 331nm; (+AlCl₃/HCl): 234, 297sh, 323nm(fig-2-7); <sup>1</sup>H NMR (500MHz), DMSO-d₆; δ 7.46 (d, J=16.0 Hz, 1H, H-α); δ 7.24 (d, J=1.55 Hz, 1H, H-2); δ 6.76 (d, J=8.4 Hz, 1H, H-5); δ 6.34 (d, J=16.05 Hz, 1H, H-β)(fig-8).<sup>13</sup>C NMR (500MHz), DMSO-d₆; δ 168.55 (C-9); δ 148.42 (C-7); δ 149.59 (C-3); δ 145.05 (C-4); δ 126.28 (C-1); δ 123.37 (C-6); δ 116.12 (C-5); δ 111.60 (C-2); δ 115.60 (C-8); δ 56.18 (C-10)(fig-9). MS(-ve): (m/z, rel. int. %) 193(M+, 100%) calculated for C₁₀H₁₀O₄; 194(M+H); 192 (M-H);(Fig-10). IR (γ<sub>max</sub>, cm⁻¹, KBr): 3436, 2903, 2841, 1686, 1609, 1514, 1424, 1277, 1173, 1116, 1033, 941, 852, 803, 749.(fig-11) Thus, compound (II) was identified as 4-hydroxy-3-methoxy cinnamic acid (Ferulic acid) (Figure).
Figure: Structure of Ferulic acid (4-hydroxy-3-methoxy cinnamic acid)

Figure 1: Melting Point of compound B
Figure 2: UV Spectrum of Compound B in MeOH

Figure 3: UV Spectrum of Compound B in MeOH +AlCl₃
Figure 4: UV Spectrum of Compound B in MeOH +AlCl₃ + HCl

Figure 5: UV Spectrum of Compound B in MeOH +NaOAc
Figure 6: UV Spectrum of Compound B in MeOH + NaOAc + H$_3$BO$_3$

Figure 7: UV Spectrum of Compound B in MeOH + NaOH

IR ($\gamma_{\text{max}}$, cm$^{-1}$, KBr) (fig.5.22)

3436, 2903, 2841, 1686, 1609, 1514, 1424, 1277, 1173, 1116, 1033, 941, 852, 803, 749.
Figure 11: IR Spectrum of the Compound B

MS (EIMS, m/z, rel. intensity as %) (Fig.10)

194(M+, 100%), 195(M+H), 193 (M-H), 177 (M⁺-17), 136 (M⁺-44), 116, 63
**Figure 10:** Mass Spectrum of Ferulic acid

$^1$H NMR (500MHz, DMSO-$d_6$, $\delta$, ppm) (Fig 8)

7.46 (d, J=16.0 Hz, 1H, H-\(\alpha\)); 7.24 (d, J=1.55 Hz, 1H, H-2); 7.05 (dd, J=1.55 & 8.40 Hz, 1H, H-6); 6.76 (d, J=8.4 Hz, 1H, H-5); 6.34 (d, J=16.05 Hz, 1H, H-\(\beta\)).
Anti-Microbial Activity:\nThe invitro antibacterial and antifungal activity of ferulic acid isolated from *C.rheedii* was carried out against *Stapylococcus aureus, Bacillus substilis, Escherichia coli, Salmonilla paratyphi, Candida albicans, Aspergillus fumigates* using serial dilution technique in double strength nutrient broth for Antibacterial and Sabouraud dextrose broth as medium for antifungal. The isolated compound was dissolved in DMSO to the concentration of 100µg/disc.

**Antibacterial assay**:
2 petridishes for Gram-positive organisms (*Stapylococcus aureus* and *Bacillus substilis*) and 2 petridishes for Gram-negative organisms (*Escherichia coli* and *Salmonilla paratyphi*). Each dish is divided into 2 quadrants and name one quadrant of the disc as test (Ferulic acid) (100µg/disc) and 1 quadrant for standard Ciprofloxacain (10µg/disc). The ferulic acid was placed in each plate with the help of sterile swabs, then petridishes were placed in refrigerator for diffusion at 4º C for 1 h and incubate at 37ºC for 2hrs. Observe the zone of inhibition produce by isolated compound and standard (Table-1).
Table 1: In-vitro antibacterial activity of Ferulic acid (MIC in µg/ml)

<table>
<thead>
<tr>
<th>Compounds code</th>
<th>Antibacterial activity (Zone of inhibition)</th>
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<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
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<tr>
<td>Standard [Ciprofloxacin (10µg/disc)]</td>
<td>31</td>
</tr>
<tr>
<td>Ferulic acid (100 µg/disc)</td>
<td>30</td>
</tr>
</tbody>
</table>

**Antifungal assay**

2 petridishes for antifungal organisms (*Candida albicans* and *Aspergillus fumigates*). Each dish is divided into 2 quadrants and name one quadrant of the disc as test (Ferulic acid) (100µg/disc) and 1 quadrant for standard Clotrimazole (10µg/disc), then ferulic acid was placed in each plate with the help of sterile swabs then petridishes were placed in refrigerator for diffusion at 4º C for 1 h and incubate at 37ºC for 2hrs. Observe the zone of inhibition produce by by isolated compound and standard(Table- 2).

Table 2: In-vitro antifungal activity of ferulic acid (MIC in µg/ml)

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Antifungal activity (Zone of inhibition)</th>
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<tbody>
<tr>
<td></td>
<td><em>Candida albicans</em></td>
</tr>
<tr>
<td>Std [Clotrimazole (10µg/disc)]</td>
<td>11</td>
</tr>
<tr>
<td>Ferulic acid (100 µg/disc)</td>
<td>15</td>
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**CONCLUSION**

In this paper, we have explored the isolation of Ferulic acid from the medicinal plant *Cansjera rheedii* J.GMelin (Opiliaceae) and evaluated its antimicrobial activities using disk diffusion method against bacteria and fungi showed excellent anti-microbial activity.

**REFERENCES**


