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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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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 $(CH_3)_4Si$ 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_3COONa, CH_3COONa/H_3BO_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 (1.0Kg) 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 C_6H_6, Et_2O, EtOAc and EtCOMe. The C_6H_6 fraction after concentration yielded a pale yellow needle, recrystallized from MeOH and designated as compound **I** (910mg). The Et_2O 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 $NaHCO_3$ solution, decolourized Br_2 water and green colour with Fe^{3+} [16]. It was blue under UV changing to bright blue under UV/NH_3; R_f similar to hydroxy cinnamic acid. UV λ_{max}(MeOH): 233, 296, 319nm; (+NaOMe): 234, 304sh, 346; (+ CH_3COONa): 227, 284sh, 323nm; (+CH_3COONa / H_3BO_4): 224, 296sh, 322nm; (+$AlCl_3$): 237, 302sh, 331nm; (+$AlCl_3$/HCl): 234, 297sh, 323nm(fig-2-7); ^{1}H NMR (500MHz), DMSO-d_6; δ 7.46 (d, J=16.0 Hz, 1H, H-α); δ 7.24 (d, J=1.55 Hz, 1H, H-2); δ 7.05 (dd, J=1.55 & 1.50 Hz, 1H, H-6); δ 6.76 (d, J=8.4 Hz, 1H, H-5); δ 6.34 (d, J=16.05 Hz, 1H, H-β)(fig-8). ^{13}C NMR (500MHz), DMSO-d_6; 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 116.01 (C-8); δ 56.18 (C-10)(fig-9). MS(-ve): (*m/z*, rel. int. %) 193(M+, 100%) calculated for $C_{10}H_{10}O_4$; 194(M+H); 192 (M-H);(Fig-10). IR (γmax, 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).

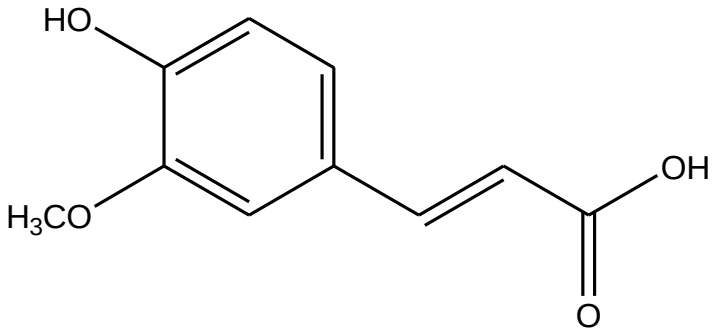


Figure: Structure of Ferulic acid (4-hydroxy-3-methoxy cinnamic acid)

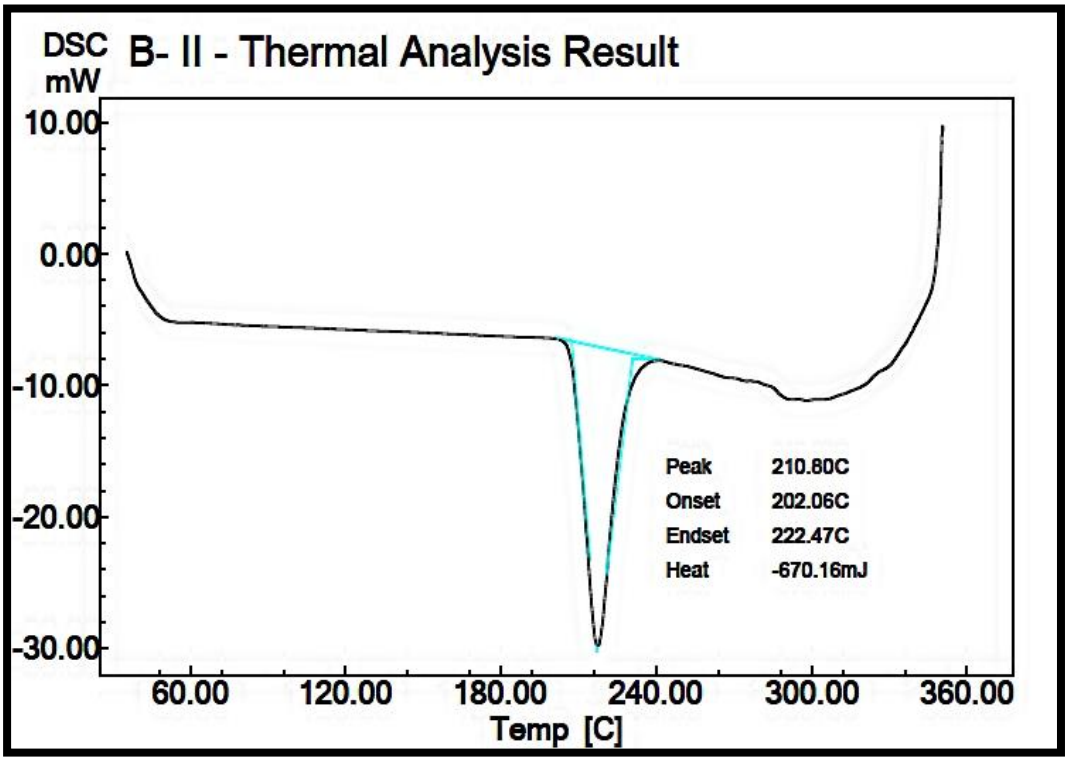


Figure 1: Melting Point of compound B

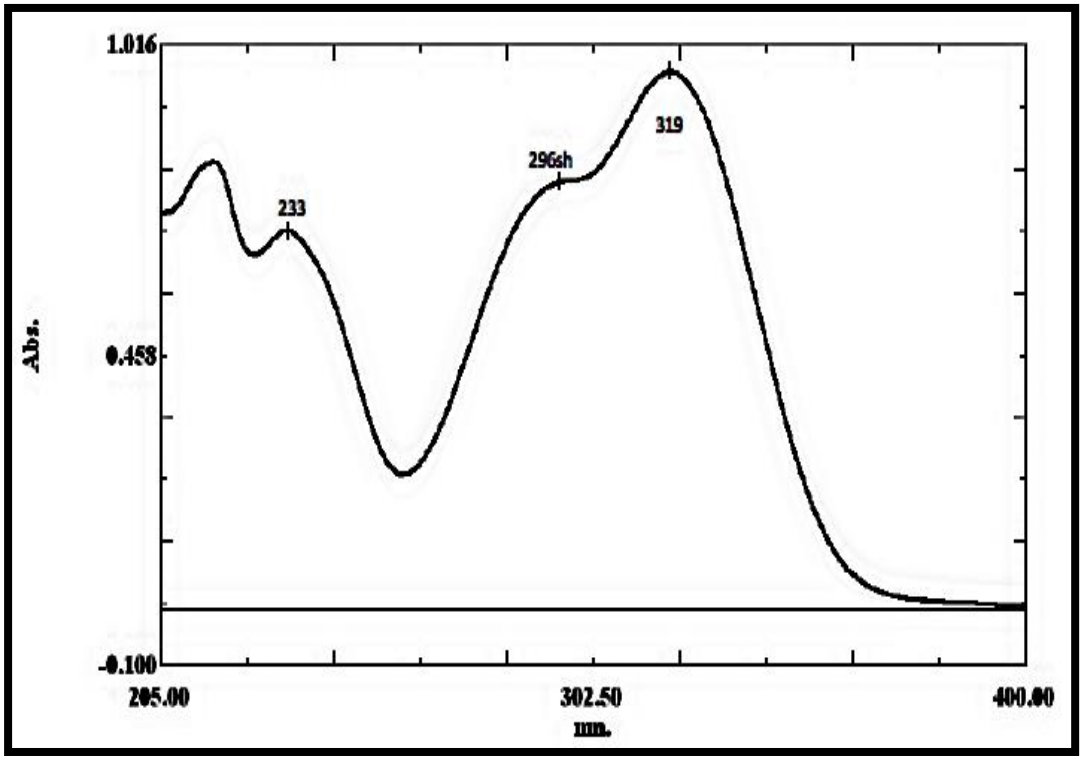


Figure 2: UV Spectrum of Compound B in MeOH

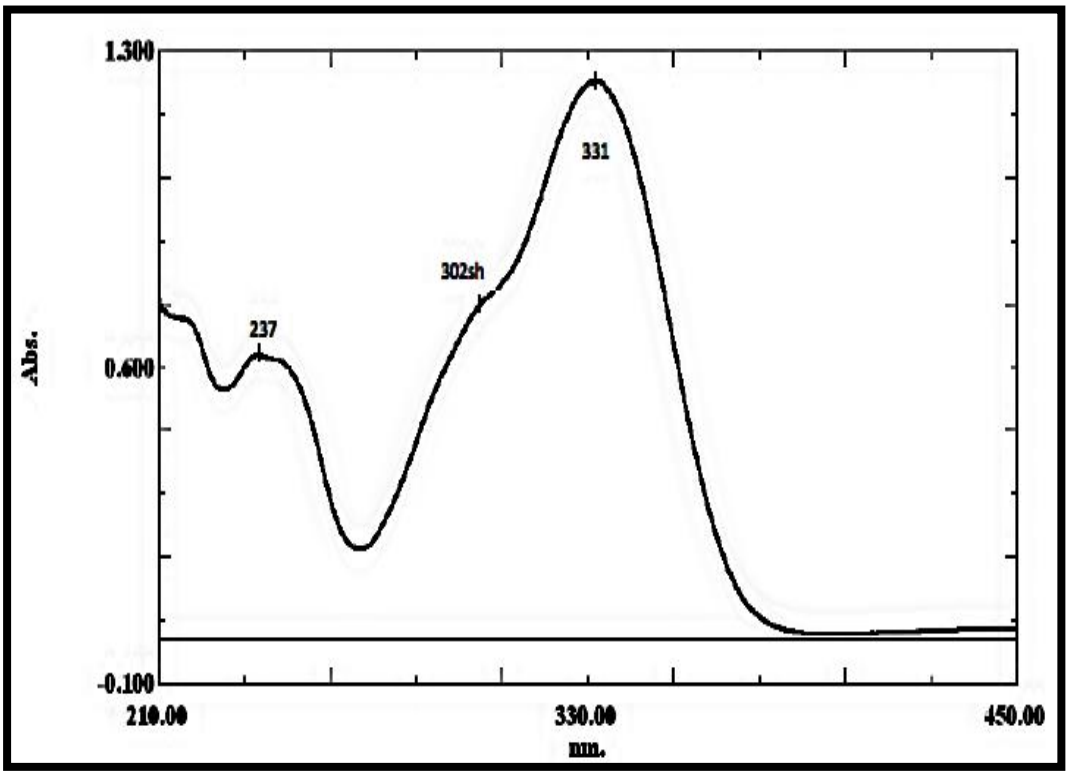


Figure 3: UV Spectrum of Compound B in MeOH +$AlCl_3$

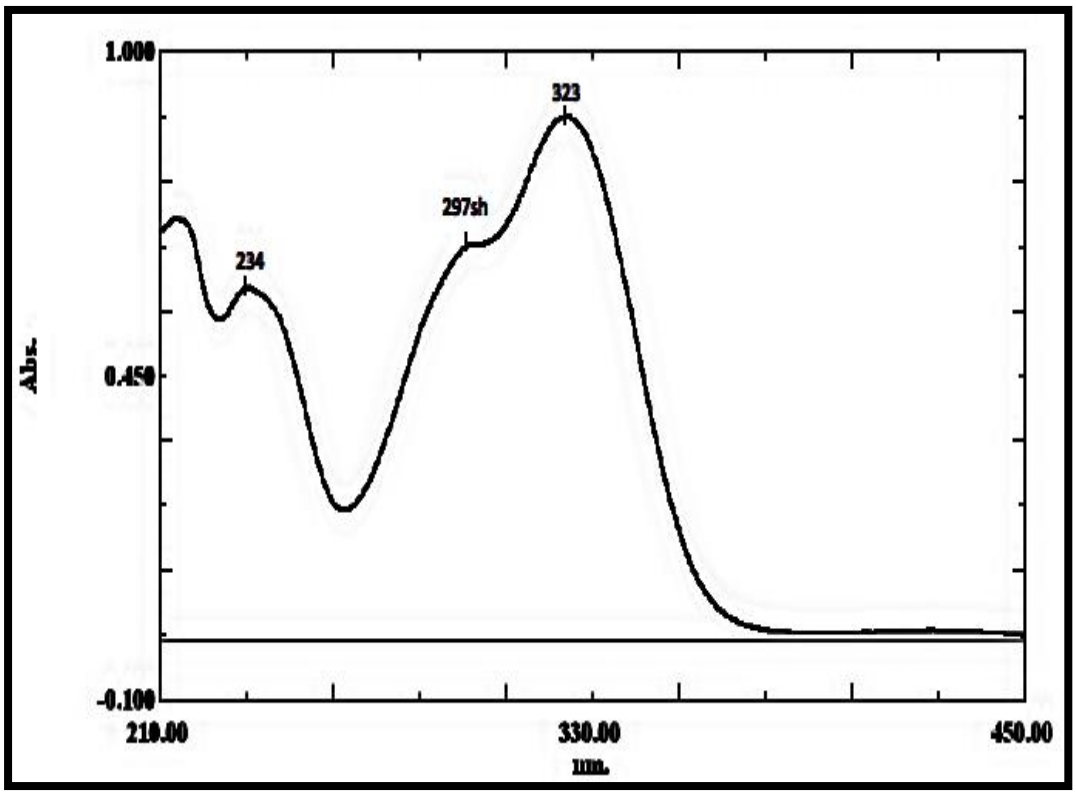


Figure 4: UV Spectrum of Compound B in MeOH +$AlCl_3$ + HCl

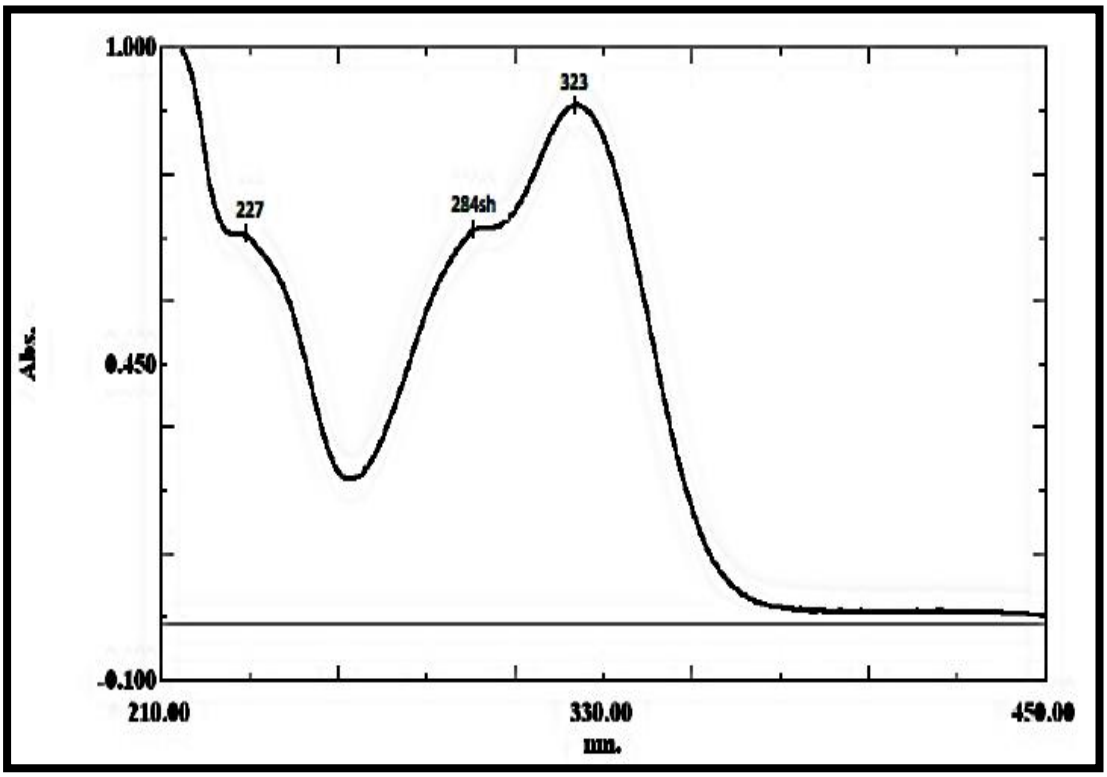


Figure 5: UV Spectrum of Compound B in MeOH +NaOAc

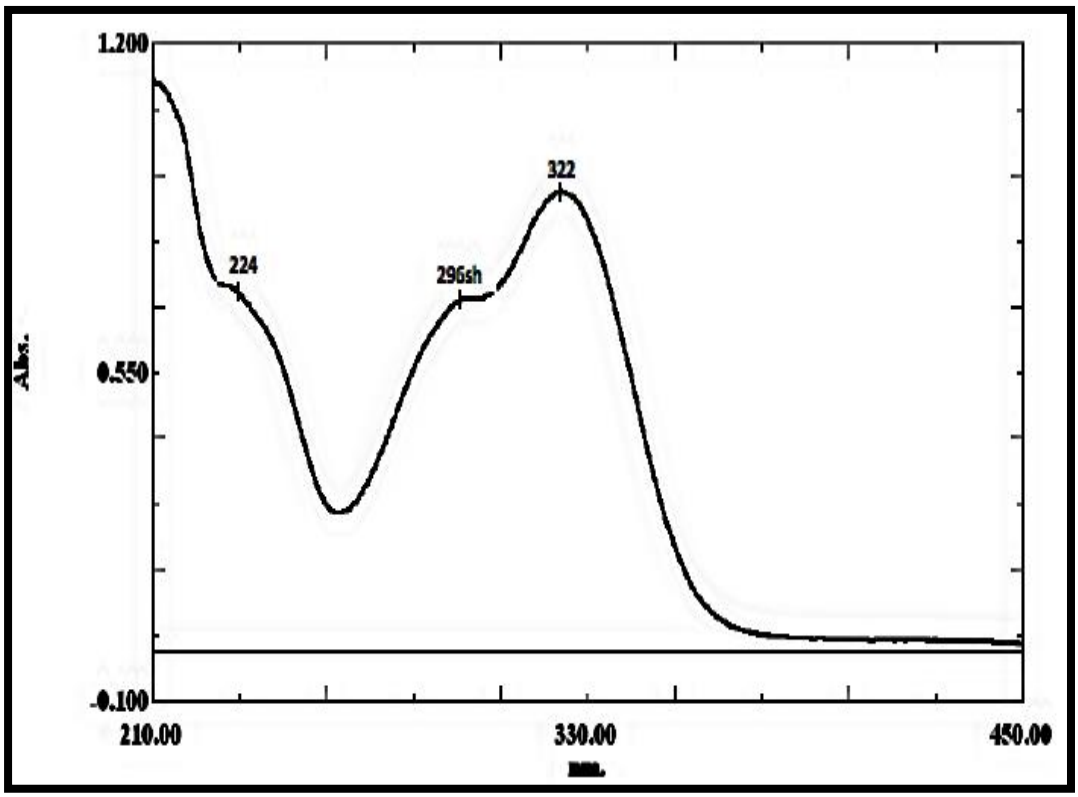


Figure 6: UV Spectrum of Compound B in MeOH +NaOAc+ H_3BO_3

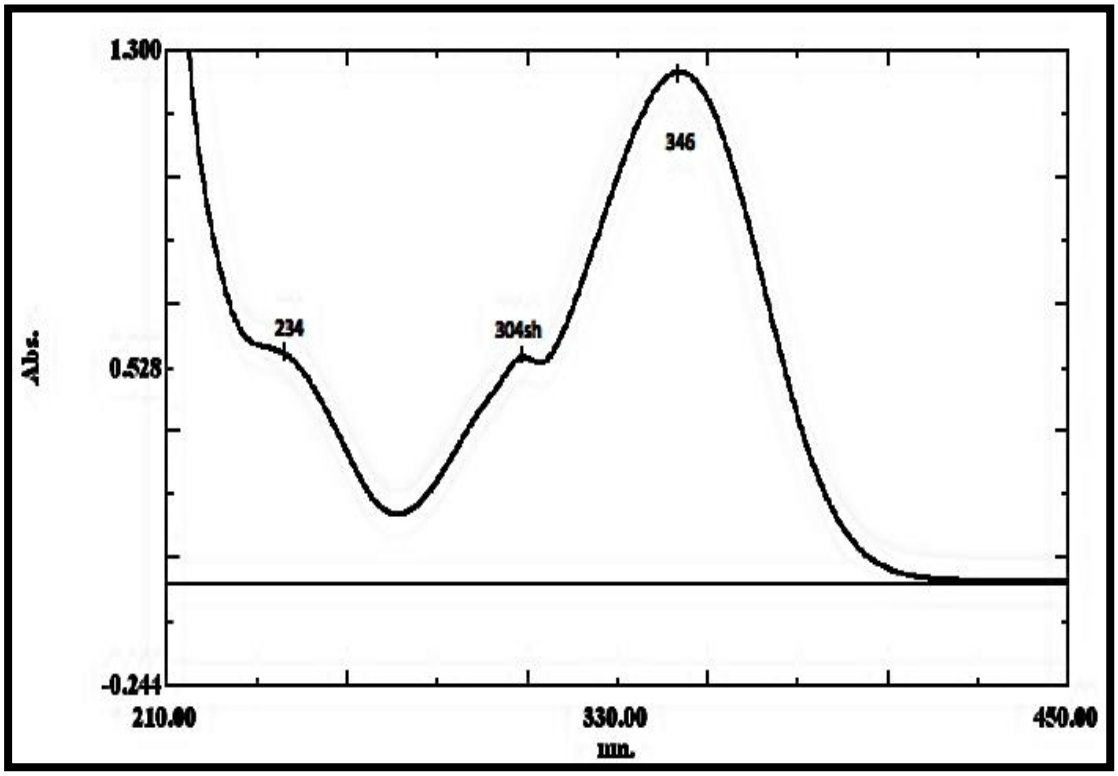


Figure 7: UV Spectrum of Compound B in MeOH +NaOH

IR (γmax, cm^{-1}, KBr) (fig.5.22)

3436, 2903, 2841, 1686, 1609, 1514, 1424, 1277, 1173, 1116, 1033, 941, 852, 803, 749.

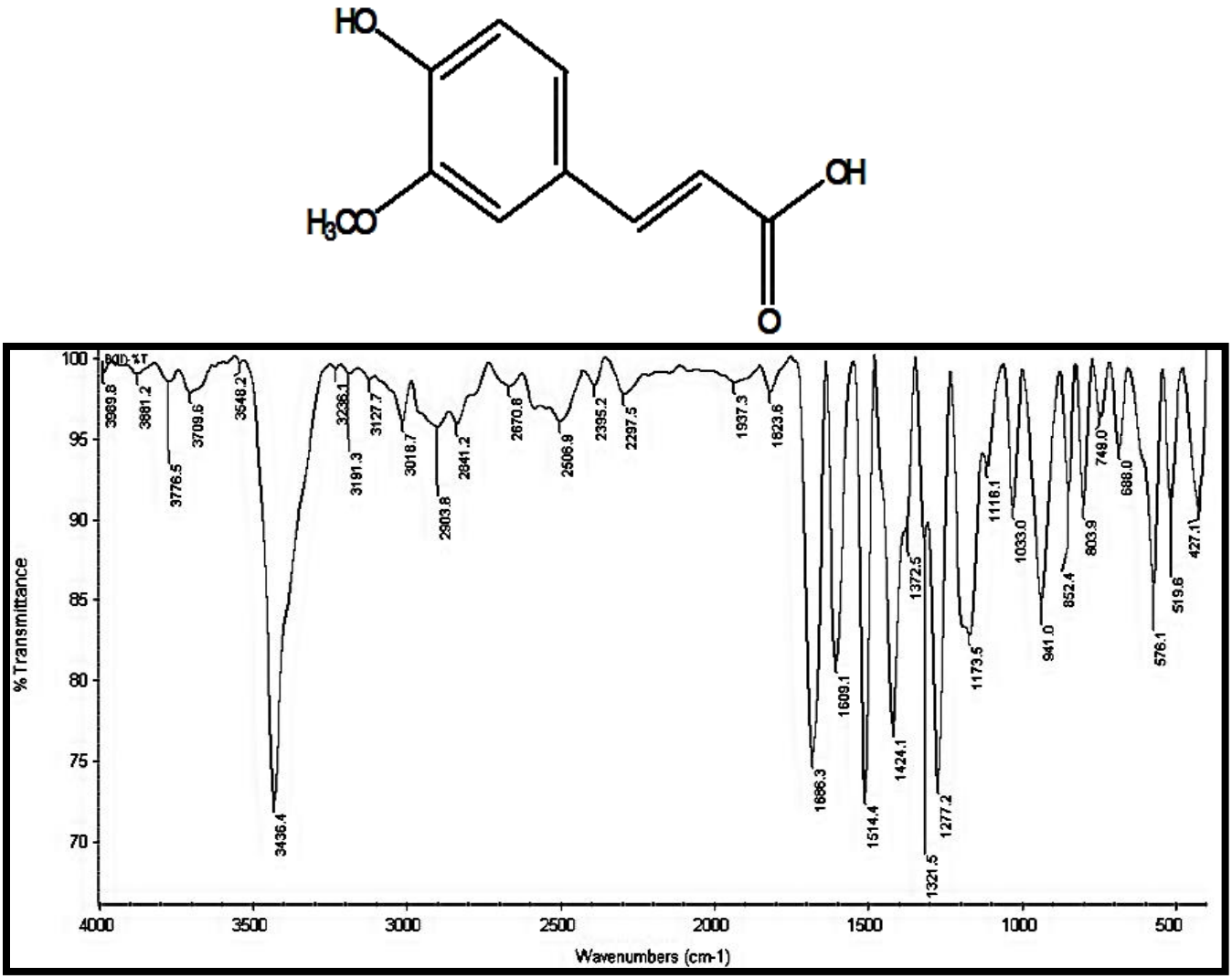


Figure 11: IR Spectrum of the Compound B

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

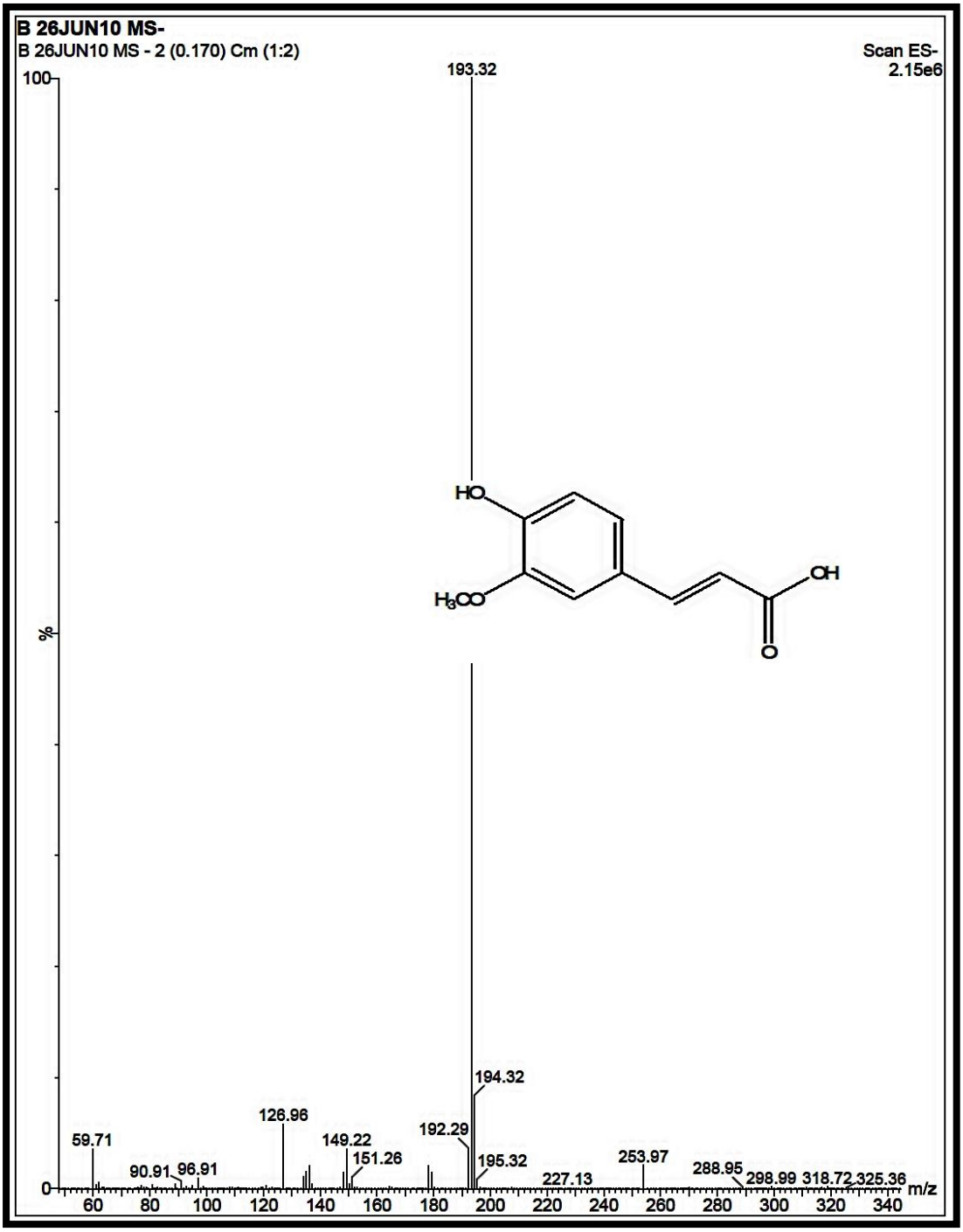
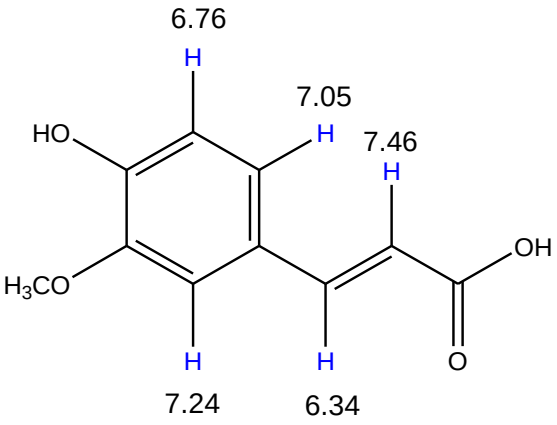


Figure 10:Mass Spectrum of Ferulic acid

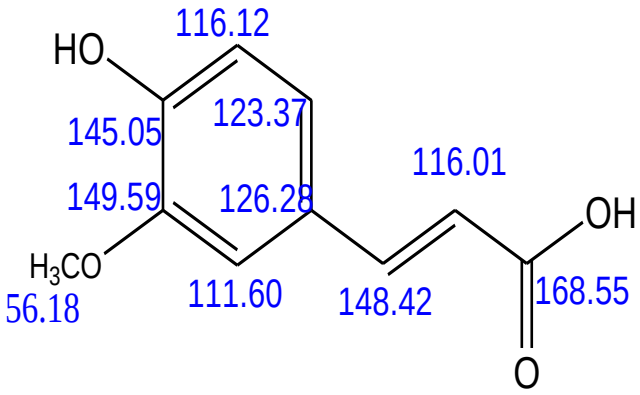
^{1}H NMR (500MHz, DMSO-d_6, δ, ppm) (Fig 8)

7.46 (d, J=16.0 Hz, 1H, H-α); 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-β).



^{13}C NMR (500MHz, DMSO-d_6, δ, ppm) (Fig 9)

168.55 (>C=O); 148.42 (C-β); 149.59 (C-3); 145.05 (C-4); 126.28 (C-1); 123.37 (C-6); 116.12 (C-5); 111.60 (C-2); 116.01 (C-α); 56.18 (-OCH_3).



Anti-Microbial Activity[15]:-

The 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[17]. 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 Ciprofloxacin (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)

Compounds code	**Antibacterial activity (Zone of inhibition)**			
Standard [Ciprofloxacin (10µg/disc)]	***Staphylococcus aureus***	***Bacillus substilis***	***Escherichia coli***	***Salmonella paratyphi***
	31	34	28	35
Ferulic acid (100 µg/disc)	30	29	29	33

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)

Compound code	**Antifungal activity (Zone of inhibition)**	
	Candida albicans	***Aspergillus fumigates***
Std [Clotrimazole (10µg/disc)]	11	18
Ferulic acid (100 µg/disc)	15	34

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.

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