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# Chemical investigation and anti-microbial study of *Cleome gynandra*

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## ABSTRACT

The systematic chemical investigation of aerial parts of *Cleome gynandra* were found to contain 1-flavone, 2-flavonols,1-flavanone and a flavanone glycoside confirming the general characteristics of *Capparidaceae* family. The flavonoids are fully characterized as Ferulic acid, Apigenin, Kaempferol, Quercetin, Naringenin and Naringenin-7-O-neohesperidoside. The identification of all these compounds were unambiguously established by chemical and spectral methods including UV, IR, Mass, $^{1}$H NMR and $^{13}$CNMR. All these compounds were reported for the first time from the aerial parts of *Cleome gynandra* except Kaempferol and Apigenin. The occurrence of these compounds, has been already reported from the leaves and seeds of this species[5].

The screening of antimicrobial activity of ethanolic extract of the plant *Cleome gynandra* was carried out against five test pathogens - *Staphylococcus aureus, Streptococcus mutans, Bacillus cereus, Escherichia coli* and *Salmonella abony*. 

## KEYWORDS

*Cleome gynandra*;
Flavonoid;
Naringenin.

## INTRODUCTION

*Cleome Gynandra* is commonly called "VelaiKeerai" in Tamil. It belongs to the family *Capparidaceae*. It is widely distributed in tropical and sub-tropical parts of the world including India. The leaves and seeds are used as an indigenous medicine in the treatment of several diseases including neuralgia, rheumatism and other local pain. The leaves of *Cleome gynandra* are applied to boils to prevent the formation of pus. The plant is reported in literature for its use against snake bite and its leaf extract asremedy of nostalgia[2],[6].

In the absence of any report on a systematic chemical examination of this plant and in continuation of our studies on the flavonoids of Indian medicinal plants the aerial parts of *Cleome gynandra* were investigated for polyphenolics and the results leading to the isolation and characterization of Ferulic acid, Apigenin, Kaempferol, Quercetin, Naringenin and Naringenin -7 - 0 - neohesperidoside are presented here.

## RESULTS AND DISCUSSION

From the alcoholic extracts of the air dried aerial parts, six compounds were isolated.

**Compound (1)**

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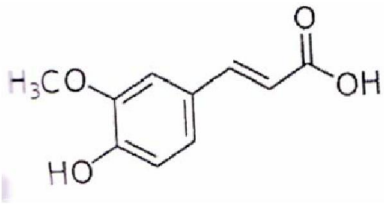
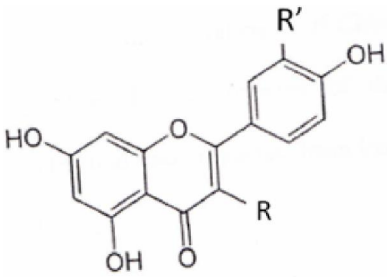
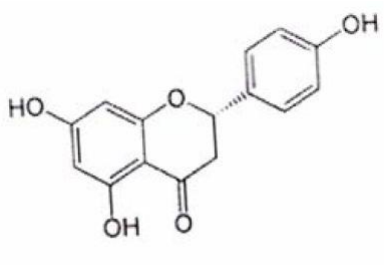



Ferulic Acid (Compound 1)

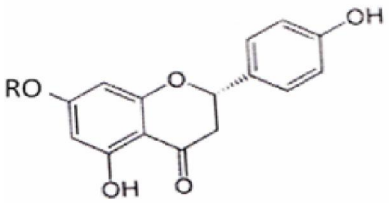



Apigenin : Compound (2) R , R'=H

**Kaempferol : Compound (3) R = OH,R'=H**
**Quercetin : Compound (4) R,R'= OH**




Naringenin: Compound 5




Naringenin 7-O- neohesperidoside
Compound(6)

R= neohesperidoside

Compound (1) had fluorescence blue under UV, changing bright blue under $NH_3$, colourless crystals from MeOH. Melting point was 210-230°C. gave brisk effervescence with $HCO_3^-$ solution, decolorized $Br_2$ water and green with $Fe^{3+}$: HPLC. Retention time of this compound as well as the authentic trans-Ferulic acid had $R_t$= 5.1 min. and 5.2 min. in two columns respectively (MeOH : 11%, HOAC : 6.4). Thus compound (1) was identified as (E)-4-hydroxy-3-methoxy cinnamic acid: (E) - Ferulic acid [7].

**Compound (2)**

Compound (2) had fluorescence and UV λ max characteristic of an aglycone of flavone. $^1$H NMR spectrum showed evidence of a 5, 7, 4'-trioxygenated flavone as well as the presence of protons at 6,8,2',6',3',5',3 by a typical doublet pattern and a characteristic singlet for protons at C-3. The $^{13}$CNMR spectrum of the compound further supported the above findings. Thus the structure of the flavone was established as 5,7,4'-trihydroxy flavone or Apigenin.

**Compound (3)**

Compound (3) was yellow under UV and UV-$NH_3$ characteristic of flavonol with free 3 – OH. The $^1$H NMR spectrum showed signals for 7-OH, 5 -OH,H-3' 5', H-2' 6',H-8 and H-6 confirming the above structure. It was further supported by $^{13}$C NMR spectrum of this compound. From these data, compound (3) was characterized as 3,5,7,4' - tetrahydroxy flavones-Kaempferol. The identity was further confirmed by co-PC with an authentic sample [10].

**Compound (4)**

Compound (4) had fluorescence and UV maxima characteristic of aglycone of flavanol. $^1$HNMR spectrum showed the evidence of 3,5,7,3',4'-penta oxygenated flavone as well as the presence of protons at 2', 6', 5', 6, 8 by a typical doublet pattern. The $^{13}$CNMR spectrum of the compound further supported the above findings. The structure of the flavonoid was established as 3, 5, 7, 3', 4'-pentahydroxy flavone or Quercetin.

**Compound (5)**

Compound (5) was dull violet under UV and yellow under UV/$NH_3$. It gave magenta red color with Mg-HCl, pink with alcoholic $NaBH_4$ and HCl. It had λ max inMeOH characteristic of a typical flavanone. The presence of free OH at 5, 7, 4' was characterized by UV visible spectrum of this compound with shift reagents. The $^1$H and $^{13}$C NMR spectral signals were in good agreement with reported values for dihydroflavones. Further the appearance of peak at m/z 272 ($M^+$,63) in EIMS was in good

agreement with molecular formula $C_{15}H_{12}O_5$ of compound (5). Based on these observations, the compound (5) was characterized as 5, 7, 4' - trihydroxyflavanoneor Naringenin,[1, 9].

**Compound (6)**

Compound (6) was dull violet under UV and yellow under UV/$NH_3$. It gave magenta red color with Mg-HCl, pink with alcoholic $NaBH_4$ and HCl. It had λmax in MeOH characteristic of a typical flavanone. The presence of free OH at 5, 4' was characterized by UV visible spectrum of this compound with shift reagents. The absence of free 7-OH was indicated by no shift in the band II of MeOH, + NaOAc spectrum. It gave positive Molisch test indicating that it should be a glycoside. On acid hydrolysis with 2N HCl yielded Naringenin and two sugars, D-glucose and L-rhamnose identified by co-chromatography with authentic samples. On EIMS, it gave a peak at m/z 580 ($M^+$, 10) which was in good agreement with the molecular formula, $C_{27}H_{32}O_{14}$. Based on these observations, the flavanone glycoside was identified as Naringenin-7-O-neohesperidoside.

## EXPERIMENTAL

**Plant material**

Fresh aerial parts were collected from Tirukanoor village, Puducherry in April 2013 and authenticated by the department of Botany KMCPGS, Puducherry where a voucher specimen was deposited.

**Extraction and isolation**

The air dried aerial parts of the plants were extracted thrice with boiling 95% EtOH (3x5L) and concentrated in vacuo to 300ml. The aqueous extract was then fractionated into Benzene, ether, EAc and MEK solubles. The benzene fraction gave no characteristic spots on PC for polyphenolics and was not worked up further. The ether fraction was concentrated and chromatographed over a column of Sephadex LH-20. Twenty seven fractions each of 10 ml were collected. Compound (1) 20mg from fractions 4 to 6, compound (2) 20mg from fractions 14 to 27 were obtained. The EAc and MEK fractions were found to be identical on PC (15 % AcOH). These two fractions were mixed, concentrated and chromatographed over a column of Sephadex LH-20. 49 fractions each of 10 ml were collected. compound (3) 25 mg from fractions 5 to 10, compound (4) from fractions 7 to 14, compound (5) from fractions 17 to 25 and compound (6) from fractions 27 to 49 were obtained.

**(a) E - Ferulic acid (1)**

Colourless crystals from MeOH, mp.210-213°C, gave brisk effervescence with $HCO_3^-$ solution, decolourised $Br_2$ water and green with $Fe^{3+}$, blue under UV changing to bright blue under UV/$NH_3$ ; UV (max,nm) (+MeOH) 227,287,312; (+NaOAc) 227,284,310; (+NaOAc/$H_3BO_3$) 224, 288sh,312; (+$AlCl_3$) 237,308sh,327; (+$AlCl_3$/HCl) 228,306sh,324 ; (+NaOMe) 234,304, 344 ; IR (max, $cm^{-1}$,KBr) 3430, 2900, 2860,1680,1660, 1610, 1590, 1510, 1460,1425,1370,1320,1270,1200,1170,1110, 1030, 970, 940, 850, 800, 750 ; HPLC: retention time of the acid as well as the authentic trans-Ferulic acid had $R_t$=5.1 min and 5.2 min in the two columns respectively (MeOH;11% HOAc ; 6:4).

**(b) Apigenin (2)**

Light yellow needles, melting point 348-350°C (MeOH-$Me_2CO$); purple under UV changing to yellow with $NH_3$; UV max (MeOH) 267,296sh, 336; (+NaOMe) 275,324,392; (+NaOAc), 274,301,376; (+NaOAc / $H_3BO_3$) 268,302sh, 338; (+$AlCl_3$) 276,301,348,384; (+$AlCl_3$/HCl) 276,299,340, 381nm ; $^1$HNMR (200.13 MHz, DMSO-$d_6$): 13.39 (1H,s,OH-5), 10.98 (1H,s, OH-7), 8.34 (2H,d,J,7.8Hz,H-2',H-6'), 7.34 (2H,J 7.9 H,H-3',H-5'), 7.20(1H,s,H-3), 6.89 (1H d, J 2.4 Hz,H-8), 6.60 (1H,d,J2.45Hz,H-6); $C^{13}$ NMR (50.32MHz,DMSO-$d_6$) : 181.8 (s, C-4) 164.3 (s,C-7), 163.8 (s,C-2),161.5(c-4') 161.39 (s,C-5), 157.34 (s,C-9), 128.5 (d, C-2,C-6), 121.2 (s, C -1), 116.0 (d,C-3,C-5), 103.7 (s,C-10), 102.9(s,C-3), 98.9(s,C-6), 94.0 (s, C – 8) ; ESIMS (positive) : m/z (relint.) 271(M + $H^+$,100).

**(c) Kaempferol (3)**

Crystallized as yellow needles from $Me_2CO$,

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mp.277-279°C, gave pink colour with Mg-HCl, yellow with alkalis and green with $Fe^{3+}$. Yellow under UV/$NH_3$ ; UV (max,nm); (+MeOH) 266,369; (+NaOAc)274,306sh,384;(+NaOAc/$H_3BO_3$) 266, 370; (+$AlCl_3$) 267, 347,423; (+$AlCl_3$/HCl)255, 349,420;(+NaOMe)281,320sh, 425;IR(max,$cm^{-1}$, KBr)3400br,1650,1600,1525,1425,1350, 1300, 1250, 1160,1070,1025,845,775; $^1$H NMR (200MHz, DMSO-$d_6$, ppm) 12.5 (s,1H,OH-7),10.1(brs,1H,OH-5), 8.04 (d, J= 8.76 Hz,2H,H-2'6'),6.92 (d,J=8.76Hz,2H, H-3'5'),6.44 (d, J=1.72 Hz,1H,H-8), 6.19 (d, J = 1.74Hz, 1H, H-6) ; $^{13}$C NMR (50 MHz,DMSO-$d_6$, ppm) 176.00 (C-4), 164.0 (C-7), 168.81 (C-7),159.28 (C-4'), 156.27 (C-9), 146.88 (C-2), 135.77 (C-3), 129.62 (C-2'6'), 121.78 (C-1'), 150.54 (C-3'5'), 103.1 4 (C-10), 98.31 (C-6), 93.59 (C-8).

**(d) Quercetin (4)**

Yellow needles from $Me_2CO$, mp.305-306°C (5mg), gave pink colour with Mg-HCl, olive green with $Fe^{3+}$ and yellow with alkalis. Yellow under UV as well as UV/$NH_3$; UV (max,nm); (+MeOH) 256, 271sh, 300sh,370; (+NaOAc) 272,330, 389 (dec); (+NaOAc /$H_3BO_3$) 260, 302sh, 388; (+$AlCl_3$) 272, 304, 332,457; (+$AlCl_3$/HCl) 265,301sh, 328,427; (+NaOMe) 245sh, 321, 412 (dec); IR (max, $cm^{-1}$ Nujol) 3410 br, 1650, 1612, 2535,1512, 1370, 1320, 1250,1210, 1160, 1091, 875, 850, 790 ; $^1$H NMR (300 MHz, DMSO – $d_6$, ppm) 12.22 (s, 1H,OH-5)10.20 (s, 1H,OH - 7) 8.90 (s,1H, OH - 4') 8.70 (s, 1H, OH-3) 8.50 (s, 1H, OH-3') 7.75 (d, J=2.2 Hz,1H, H-2') 7.59 (d, J=8.3 Hz, 1H,H-6') 6.92 (d, J = 8.4 Hz, 1H,H-5'), 6.37 (d,J=2.2 Hz, 1H, H-8) 6.20 (d, J = 2.2 Hz, 1H,H-6); $^{13}$C NMR (75.48 MHz, DMSO - $d_6$ – $CDCl_3$, ppm) 175.66 (C- 4), 164.00 (C - 7), 160.98 (C- 5), 156.54 (C-9), 147.37 (C - 4 '), 146.35 (C-2), 144.81 (C - 3'), 135.81 (C -3), 122.57 (C -1'), 120.32 (C-6'),115.45 (C-5'), 115.11 (C- 2 '),103.25 (C-10),98.47 (C-6), 93.55 (C- 8) ; MS (EIMS, relative intensity as %) 303 ($MH^+$,5), 302 ($M^+$,19),300 ($M^+$-2$H^+$, 100), 301(M-H, 3),286 ($M^+$- OH,6),272 (8), 136 (10).

**(e) Naringenin (5)**

Yellow coloured needles (MeOH), mp. 246-248°C gave magenta – red colour with Mg-HCl, pink with alcoholic $NaBH_4$ and HCl purple under UV and yellowish green under UV/$NH_3$ ; UV (max,nm); (+MeOH) 289, 326 sh; (+NaOAc / $H_3BO_3$) 293, 330 sh ; (+$AlCl_3$) 305,373 ; (+$AlCl_3$/HCl) 305, 371; (+ NaOMe) 245,273 sh,323 ; $^1$HNMR (200 MHz, DMSO - d6, ppm) 12.15 (s,1H,OH-5), 10.81 (s,1H,OH – 7), 9.61 (s,1H,) H-4'), 7.41 (d, 2H,J = 8.89 Hz, H – 2 ',6'), 6.94 (d,2H,J = 8.43 Hz, H - 3', 5'), 5.88 (s,1H,H – 8), 5.46 (dd, J= 2.2 & 12 Hz, 1H, H - 2), 3.41 (dd, J = 12 & 15Hz, 1H,Hax – 3), 2.72 (dd,J = 2.8 &15 Hz,1H,Heq – 3),$^{13}$CNMR (50 MHz, DMSO - $d_6$, ppm) 196.42 (C – 4), 166.87 (C – 7), 163.77 (C -5), 163.13 (C-9), 157.96 (C -4 '),129.11 (C - 1), 128.51 (C – 2',6'), 115.47 (C - 3',5',101.99 (C- 10), 96.14 (C – 6), 95.25 (C - 8), 78.69 (C - 2), 42.23 (C – 3) ; MS(EIMS, relative intensity as %) 273 ($MH^+$,5), 272 ($M^+$,63), 271 (M - H,37), 255 ($M^+$ - OH,5), 153 (72) 124 (35) 120 (56), 43(100).

**(f) Naringenin -7-0-neohesperidoside (6)**

yellowcoloured needles (MeOH), mp. 166-168°C gave magenta –red colour with Mg-HCl, pink with alcoholic NaBH4 and HCl. Purple under UV & yellowish green under UV/NH3; UV (max,nm); (+MeOH) 284,326;(+NaOAc)284,323;(+NaAc/ $H_3BO_3$)293, 330; (+AlCl3) 300,373 (+AlCl3/HCl) 300, 371 ; (+NaOMe) 284,323; MS (EIMS, relative intensity as %) 580 ($M^+$,6),579 (M – H, 5), 563 ($M^+$-OH,7),167, (12), 90 (22), 72 (26), 58 (100).

The screening of antimicrobial activity of ethanolic extract of the plant *Cleome gynandra* was carried out against five test pathogens - *Staphylococcus aureus NCIM (2079), Streptococcus mutans WCIM (2611), Bacillus cereus NCIM (2106), Escherichia coli NCIM (2005)* and *Salmonella abony NCIM (2577).* The inhibition zone of ethanolic extract was found to be maximum for *Bacillus cereus* at 100 µl concentration (10mm). The inhibitory zone was found to be less than 10mm for *Bacillus cereus* at 50 µl concentration, *Staphylococcus aureus*at 100 µl concentration and *Salmonella abony* at 100 µl concentration respectively. Among the test organisms, *Bacillus cereus* was found to be very sensitive to the ethanolic extract of the plant *Cleome*

*gynandra*.

The antibacterial and pharmacological activity exhibited by the aerial parts of the plant may be attributed to the presence of Ferulic acid and Naringenin whose pharmacological activities were well established earlier[8, 3].

## CONCLUSION

In the present study, we reported isolation and characterization of 1-phenolic acid and 5-Flavonoids from *Cleome gynandra*, of family *Capparidaceae*. The isolation of Kaempferol and Apigenin has been already reported from leaves and seeds of this species[4]. The remaining four compounds Ferulic acid, Quercetin, Naringenin, Naringenin-7-o-neohesperidoside were reported for the first time from the aerial parts of this plant. *Bacillus cerus* was found to be very sensitive to the ethanolic extract of this plant.

## ACKNOWLEDGEMENT

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