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ORIGINAL ARTICLE

Isolation and characterization of constituents from areal part of *Cuscuta reflexa* Roxb.

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ABSTRACT
*In present study, phytochemical investigation of the areal part of Cuscuta reflexa Roxb.(Convolvulaceae) yields three new phytoconstituents namely, 2,3-dihydro-3,5,7-trihydroxy-2-(3-hydroxy-4-methoxyphenyl) chromen-4-one (**A**), 2,3-dihydro-3,7dihydroxy-2-(3,4dihydroxyphenyl) chromen-4-one (**B**) and 6-methoxy-2H-chromen-2-one (**C**) together with two known compounds N-(4-methoxyphenethyl)-3-(3,4-dihydroxyphenyl)acrylamide (**D**) and N-(4-butylphenethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (**E**). Structures of these compounds were determined by spectral analysis. The structures of the isolated new compounds have been confirmed on the basis of UV, FT-IR, ^{1}H NMR and mass spectral studies.*
Key words: *Cuscuta reflexa Roxb., Convolvulaceae, FT-IR, ^{1}H NMR.*

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INTRODUCTION

Cuscuta reflexa is ordinarily called as dodder plant, furthermore known as devil's hair, witch's hair, love vine, amarbel. *Cuscuta* belongs to the *Cuscutaceae* family and on the basis of Angiosperm phylogeny group it is accepted as belonging to family, *Convolvulaceae* [1, 2]. *Cuscuta reflexa* is a parasitic weed plant and also an extensive climber. *Cuscuta* develops as homoparasite, it has low level of chlorophyll and photosynthesis action; totally depends over the host plant for its survival. Dodder plant has the capacity to perceive its host plant as well as to move towards its prey with critical exactness and effectiveness. Dodder plant can likewise pick a fitting host between numerous plants on the premise of unpredictable mixes discharge by the host plant as their typical procedure of transpiration [3, 4]. Parasitism of *Cuscuta reflexa* is wrapping around itself over the host plant after connection with host. *Cuscuta* makes haustorial association with the vascular tissue of the host plant. This haustorium can infiltrate the xylem and phloem of the host plant and connected with tissues of the host plant [5]. *Cuscuta reflexa* differs in the shade of flowers created from white to pink. Flowers for the most part created in the early summer and harvest time additionally rely on upon the species. Seeds are created in the expansive amounts. Seeds of *Cuscuta reflexa* can get by in the dirt for a long time in the hunt of suitable host, as of now it relies on upon the nourishment save in endosperm of the seed [6].

Phytochemical examined on *Cuscuta reflexa* have reported the nearness of kaempferol-3-Oglucoside, myrecetin, astragallin [7], myrecetin, benzopyrones [8], glucopyranosides [9], quercetin and quercetin-3-O-glucoside [10], β-sitosterol, bergenin and propenamide, flavonols [11]. The present examination of concoction constituents of *C. reflexa* was embraced as a major aspect of a more extensive study to discover the dynamic constituents present in this plant. Different parts of this plant are utilized as a part of tribal drug for the disease like antibacterial [12], anti-epileptic [13], antitumor activity [14], anti-

inflammatory [15], lower back pain leucorrhea, impotence, premature ejaculation, sperm leakage, frequent urination, lower back pain and sore knees [16].

The present investigation of chemical constituents of *C. reflexa* was undertaken as part of a wider study to find out the active constituents present in this plant. In present study, we describe the isolation and structural elucidation of three new chemical compounds namely, *2,3-dihydro-3,5,7-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)chromen-4-one* (**A**), *2,3-dihydro-3,7dihydroxy-2-(3,4-dihydroxyphenyl) chromen-4-one* (**B**) and *6-methoxy-2H-chromen-2-one* (**C**) together with two known compounds *N-(4-methoxyphenethyl)-3-(3,4-dihydroxyphenyl)acrylamide* (**D**) and *N-(4-butylphenethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide* (**E**) reported for the first time from *C. reflexa.*

EXPERIMENTAL

The melting points were determined in open capillary tubes and were uncorrected. The purity of all the isolated compounds were checked by TLC on precoated silica gel-G aluminum sheets (Type 60 GF_{254}, Merck) and the spots were detected by exposure to iodine vapors. The infrared (FT-IR) spectra were recorded on 470-Shimadzu infrared spectrophotometer using the KBr disc prepared by pressed pellet technique and υ_{max} is expressed in cm^{-1}. NMR spectra were measured in DMSO-d_6 as solvent at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) on a BRUKER AVANCE-300 spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are given in parts per million (ppm). Spin multiplicities are given as s (singlet), d (doublet) and m (multiplet). Mass spectra were obtained on Shimadzu 2010A LC-MS spectrometer. All the solvents were distilled and dried with usual desiccant.

MATERIALS AND METHODS

The areal part of plant were collected from the herbal garden of Acharya Narendra Deo College of Pharmacy, Babhnan, Gonda, Uttar Pradesh, India in the month of December and identified by an expert taxonomist in Department of Taxonomy & Pharmacognosy, National Botanical Research Institute, Lucknow. The plant specimens were authenticated (Ref. No NBRI/CIF/413/2013). The areal part of plant was shade dried, reduced to coarse powder and stored in airtight container till further use.

EXTRACTION AND ISOLATION

The air-dried areal part (20 kg) of *C. reflexa* were coarsely powdered and extracted in a Soxhlet apparatus with ethanol for 72 hr. The ethanol extract was concentrated under reduced pressure and give a viscous dark green mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

The viscous dark green mass was adsorbed on silica gel (60-120 mesh) for column after being dissolved in little quantity of ethanol for preparation of slurry. The slurry (200 gm) was air dried and chromatographed over silica gel column packed in *n*- hexane. The column was eluted successively with *n*-hexane, mixture of *n*-hexane and chloroform (9:1, 3:1, 1:1, 1:3, 1:9), pure chloroform, mixture of chloroform and ethyl acetate (5:5), pure ethyl acetate and mixture of ethyl acetate and ethanol (9:1, 8:2, 6:4, 5:5, 4:6, 2:8, 1:9). Various fractions were collected separately and match by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The following isolated compounds were recrystallized to get the pure compound.

RESULT

Compound A

Elution of column with ethyl acetate-ethanol (9:1) (fraction 11-16) compound **A** was obtained as amorphous powder; brown solid; R_f = 0.74; Yield 27%; mp 265-267 ^{0}C; UV λ max (nm): 283;

The FTIR (KBr, ν, cm^{-1}) spectrum of compound **A** showed absorption bands at: 3609.14 (O-H, free hydroxyl group), 3294.81(O-H, Hydrogen bonding), 3034.23 (Cyclic C-H, str), 2914.81 (Ali- C-H, str), 1690.38 (C=O, ketone), 1424.48 (C-C ring, str), 1219.17 (C-O-C asym. str), 1070.23 (C-O-C sym str);

The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ (ppm) of compound **A** displayed the characteristic signals at 2.23 (s, 1H, OH, D_2O exchangeable), 3.72 (s, 3H, OCH_3), 5.02-5.14 (s, 3H, OH, D_2O exchangeable), 6.92-6.35 (m, 4H, Ar-H), 7.03 (s, 1H, Ar-H), 7.66-7.90 (d, 2H, Ar-H);

The ^{13}C NMR (75 MHz, DMSO-d_6) spectrum of compound **A** displayed the characteristic signals at: δ 56.7, 72.2, 84.5, 94.6 (2), 101.9, 112.8, 115.2, 120.8, 134.4, 145.5, 150.2, 158.6, 162.3, 165.8, 194.8

The mass data EIMS (m/z) which showed m/z = 318.07 $[M]^+$, 319.09 $[M+1]^+$. (Calcd for $C_{16}H_{14}O_7$).

Compound **A** was isolated and its molecular formula was determined as $C_{16}H_{14}O_7$ {m/z = 318 (100) $[M^+]$}. The structures of the flavone were identified on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature.

Compound B

Elution of column with ethyl acetate-ethanol (2:8) (fraction 21-25) compound **B** was obtained as amorphous powder; white solid; $R_{f=}$ 0.67; Yield 48%; mp 276-278 ^{0}C; UV λ max (nm): 265;

The FTIR (KBr, ν, cm^{-1}) spectrum of compound **B** showed absorption bands at: 3639.41 (O-H, free hydroxyl group), 3287.18 (O-H, Hydrogen bonding), 3032.92 (Cyclic C-H, str), 2821.25 (CH_2 symmetric str), 1678.43 (C=O, ketone), 1418.08 (C-C ring, str); 1210.27 (C-O-C asym, str), 1065.33 (C-O-C sym, str);

The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ (ppm) of compound **B** displayed the characteristic signals at 2.16 (s, 1H, OH, D_2O exchangeable), 5.12-5.34 (s, 3H, OH, D_2O exchangeable), 6.67-6.98 (m, 5H, Ar-H), 7.02 (s, 1H, Ar-H), 7.09-7.42 (d, 2H, Ar-H);

The ^{13}C NMR (75 MHz, DMSO-d_6) spectrum of compound **B** displayed the characteristic signals at: δ 72.6, 84.3, 101.6, 107.9, 114.8 (2), 117.6, 120.0, 130.4, 133.5, 145.8, 148.2, 158.3, 162.3, 195.8;

The mass data EIMS (m/z) which showed m/z = 288.25 $[M]^+$, 289.17 $[M+1]^+$ (Calcd for $C_{15}H_{12}O_6$).

Compound **B** was isolated and its molecular formula was determined as $C_{15}H_{12}O_6$ {m/z = 288 (100) $[M^+]$}. The structures of the flavone were identified on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature.

Compound C

Elution of column with *n*-hexane-chloroform (1:9) (fraction 5-8) compound **C** was obtained as amorphous powder; white solid; $R_{f=}$ 0.53; Yield 18%; mp 243-244 ^{o}C; UV λ max (nm): 247;

The FTIR (KBr, ν, cm^{-1}) spectrum of compound **C** showed absorption bands at: 3022.42 (Ar C-H, str), 1645.23 (C=O, ketone), 1428.08 (C-C ring str), 1267.57 (C-O-C asym, str), 1055.36 (C-O-C sym, str);

The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ (ppm) of compound **C** displayed the characteristic signals at 3.72 (s, 3H, OCH_3), 6.89-6.98 (m, 2H, Ar-H), 7.03-7.12 (m, 2H, Ar-H);

The ^{13}C NMR (75 MHz, DMSO-d_6) spectrum of compound **C** displayed the characteristic signals at: δ 56.6, 111.6, 113.3 (2), 122.6, 123.0, 145.8, 148.2, 158.3, 162.3;

The mass data EIMS (m/z) which showed m/z = 176.17 $[M]^+$, 177.16 $[M+1]^+$ (Calcd for $C_{10}H_8O_3$);

Compound **C** was isolated and its molecular formula was determined as $C_{10}H_8O_3$ {m/z = 176 (100) $[M^+]$}. The structures of the coumarin were identified on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature.

Compound D

Elution of column with *n*-hexane-chloroform (5:5) (fraction 9-13) Compound **D** was obtained as amorphous powder; colourless solid; $R_{f=}$ 0.63; Yield 23%; mp 252-253 ^{0}C; UV λ max (nm): 296;

The FTIR (KBr, ν, cm^{-1}) spectrum of compound **D** showed absorption bands at: 3575.24 (O-H, free hydroxyl group), 3376.24 (N-H, str), 3042.44 (Cyclic C-H, str), 1647.65 (C=O, ketone), 1412.43 (C-C ring str), 1195.86 (C-N, str), 1202.42 (C-O-C asym. str.), 1158.45 (C-O-C sym, str), 689 (C-H bending, monosubstituted), 819 (C-H bending, disubstituted);

The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ (ppm) of compound **D** displayed the characteristic signals at 3.56 (s, 3H, OCH_3), 3.82-3.96 (m, 4H, CH_2), 5.12-5.34 (s, 2H, OH, D_2O exchangeable), 6.67-6.88 (m, 3H, Ar-H), 7.02 (s, 2H, CH_2), 7.09-7.42 (m, 4H, Ar-H); 8.06 (s, 1H, NH, D_2O exchangeable);

The ^{13}C NMR (75 MHz, DMSO-d_6) spectrum of compound **D** displayed the characteristic signals at: δ 35.3, 44.1, 56.3, 113.2, 115.3, 118.3 (2), 121.3, 127.3 (2), 130.1, 132.4, 145.3, 147.4, 148.9, 152.23, 156.5, 167.8

The mass data EIMS (m/z) which showed m/z = 313.12$[M]^+$, 314.14$[M+1]^+$ (Calcd for $C_{18}H_{19}O_4$);

Compound E

Elution of column with *n*-hexane-chloroform (2:8) (fraction 8-12) Compound **E** was obtained as amorphous powder; colourless solid; $R_{f=}$ 0.56; Yield 36%; mp 267-268 ^{o}C; UV λ max (nm): 289;

The FTIR (KBr, ν, cm^{-1}) spectrum of compound **E** showed absorption bands at: 3564.62 (O-H, free hydroxyl group), 3388.62 (N-H, str), 3064.42 (Cyclic C-H, str), 2942.22 (Aliphatic C-H str), 1642.55 (C=O, ketone), 1187.02 (C-N, str), 1203.88 (C-O-C asym, str), 1180.23 (C-O-C sym str.), 692 (C-H bending, monosubstituted), 805 (C-H bending, disubstituted);

The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ (ppm) of compound **E** displayed the characteristic signals at 3.68 (s, 3H, OCH_3), 3.62-3.88 (m, 5H, CH_2), 5.44-5.65 (s, 2H, OH, D_2O exchangeable), 6.78-6.88 (m, 3H, Ar-H), 7.24 (s, 2H, CH_2), 7.34-7.67 (m, 4H, Ar-H), 8.18 (s, 1H, NH, D_2O exchangeable);

The ^{13}C NMR (75 MHz, DMSO-d_6) spectrum of compound **E** displayed the characteristic signals at: δ 23.3, 24.1, 36.3(2), 42.2, 58.3, 112.2, 116.3 (2), 121.3, 127.3(2), 128.4, 134.5, 136.4, 137.5, 141.7, 144.2, 144.3, 145.4, 153.9, 167.8;

The mass data EIMS (m/z) which showed m/z = 353.45 $[M]^+$, 354.48 $[M+1]^+$. (Calcd for $C_{22}H_{27}NO_3$);

DISSCUSION

Compound **A** was assigned the molecular formula $C_{16}H_{14}O_7$ by high resolution LC-MS, which showed a $[M]^{+1}$ peak at *m/z* 318.075. The IR spectrum suggested the presence of free hydroxyl group at 3294.81 and hydrogen bonded hydroxyl group at 3034.23, Carbonyl group (ketone) at 1690.38 and methoxy asymmetric and symmetric stretch at 1219.17 and 1070.23 cm^{-1} respectively. The UV spectrum showed strong absorption maxima at λ max 283 nm, indicating a highly conjugated system. The MS of **A** displayed an ion peak at *m/z* 318 which was due to the loss of $C_{16}H_{14}O_7$ from the molecular ion. The fragment peak was observed at *m/z* 196, 124, 116, 110 and 78.The ^{1}H-NMR spectrum of **A** showed singlet peak at 2.23 due to a hydroxyl group. The singlet peak was appeared at 3.72 for three proton of methoxy group. The presence of hydroxyl group was confirmed by D_2O exchangeable signals for three protons at 5.02-5.14.

Compound **B** was assigned the molecular formula $C_{15}H_{12}O_6$ by high resolution LC-MS, which showed a $[M]^+$ peak at *m/z* 288.25. The IR spectrum suggested the presence of free hydroxyl group at 3639.41 and hydrogen bonded hydroxyl group at 3287.18, Carbonyl group (ketone) at 1678.43 and methoxy asymmetric and symmetric stretch at 1210.27 and 1065.33 cm^{-1} respectively. The UV spectrum showed strong absorption maxima at λ max 265 nm, indicating a highly conjugated system. The MS of **B** displayed an ion peak at *m/z* 288 which was due to the loss of $C_{15}H_{12}O_6$ from the molecular ion. The fragment peak was observed at *m/z* 180, 116, 110, 107 and 78. The ^{1}H-NMR spectrum of **B** showed a signal singlet at 2.16 due to a hydroxyl group. The presence of hydroxyl group was confirmed by D_2O exchangeable signals for three protons at 5.12-5.34. The eight aromatic protons were observed at 6.67-7.42.

Compound **C** was assigned the molecular formula $C_{10}H_8O_3$ by high resolution LC-MS, which showed a $[M]^+$ peak at *m/z* 176. The IR spectrum suggested the presence of Carbonyl group (ketone) at 1645.23 and methoxy asymmetric and symmetric stretch at 1267.57 and 1055.36 cm^{-1} respectively. The UV spectrum showed strong absorption maxima at λ max 247 nm, indicating a highly conjugated system. The MS of **C** displayed an ion peak at *m/z* 176 which was due to the loss of $C_{10}H_8O_3$ from the molecular ion. The fragment peak was observed at *m/z* 146, 96 and 78. The ^{1}H-NMR spectrum of **C** showed a singlet for three protons at 3.72 due to a methoxy group and four aromatic protons showed multiplet at in the range of 6.89-7.12.

Compound **D** was assigned the molecular formula $C_{18}H_{19}NO_4$ by high resolution LC-MS, which showed a $[M]^+$ peak at *m/z* 313.12. The IR spectrum suggested the presence of free hydroxyl group 3575.24 and hydrogen bonded amine group at 3376.24, carbonyl group (ketone) at 1647.15 and methoxy asymmetric and symmetric stretch at 1202.42 and 1158.45 cm^{-1} respectively. The CH bending for monosubstituted at the paraposition is confirmed by the peak at 689.23 and for disubstituted observed at 819.45 cm^{-1}. The UV spectrum showed strong absorption maxima at λ max 296 nm, indicating a highly conjugated system. The MS of **D** displayed an ion peak at *m/z* 178 which was due to the loss of $C_{18}H_{19}NO_4$ from the molecular ion. The fragment peaks were observed at *m/z* 283, 220, 164, 132 and 78. The ^{1}H-NMR spectrum of **D** showed a signal at three protons singlet for methoxy at 3.56. The presence of hydroxyl group was confirmed by D_2O exchangeable signals for two proton in the range of 5.22-5.44 and for one proton NH at 8.06. Seven aromatic protons showed multiplet at 6.67-7.42. A singlet was observed for methylene two aromatic protons at 7.02.

Compound **E** was assigned the molecular formula $C_{22}H_{27}NO_3$ by high resolution LC-MS, which showed a $[M]^+$ peak at *m/z* 353.45. The IR spectrum suggested the presence of free hydroxyl group 3564.62 and hydrogen bonded amine group at 3388.62, carbonyl group (ketones) at 1642.55 and methoxy asymmetric and symmetric stretch at 1203.88 and 1180.23 respectively. The CH bending for monosubstituted at the proposition is confirmed by the peak at 692.34 and for disubstituted observed at 805.67 cm^{-1}. The UV spectrum showed strong absorption maxima at λ max 289 nm, indicating a highly conjugated system. The MS of **E** displayed an ion peak at *m/z* 178 which was due to the loss of $C_{22}H_{27}NO_3$ from the molecular ion. The fragment peak was observed at *m/z* 297, 221, 178, 132, 78 and 57. The ^{1}H-NMR spectrum of **E** showed a signal at three protons singlet for methoxy at 3.68. The presence of hydroxyl group was confirmed by D_2O exchangeable signals for two protons in the range of 5.44-5.65 and for one proton NH at 8.18. Seven aromatic protons showed multiplet at 6.78-7.67. A singlet peak was observed for methylene two aromatic protons at 7.24.

The three new compounds were isolated by column chromatography from ethanol extract of the areal part of *C. reflexa*. On the basis of TLC, UV, IR, ^{1}H NMR, ^{13}C NMR and LC-MS spectroscopic analysis and by chemical transformation, structures and molecular formula of compounds A, B and C were elucidated as *2,3-dihydro-3,5,7-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)chromen-4-one* ($C_{16}H_{14}O_7$), *2,3-dihydro-3,7dihydroxy-2-(3,4-dihydroxyphenyl) chromen-4-one* ($C_{15}H_{12}O_6$) and *6-methoxy-2H-chromen-2-one* ($C_{10}H_8O_3$) respectively.

CONCLUSION

The three new compounds were obtained on the basis of TLC, UV, IR, NMR and LC-MS spectroscopic analysis and by chemical transformation, structures and molecular formula of compounds A and B were elucidated as *2,3-dihydro-3,5,7-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)chromen-4-one*, *2,3-dihydro-3,7dihydroxy-2- (3,4-dihydroxyphenyl) chromen-4-one* and *6-methoxy-2H-chromen-2-one* respectively.

LIST OF ABBREVIATIONS

Ar = Aromatic
UV = Ultra violet spectroscopy
FTIR = Fourier transform infrared spectroscopy
NMR = Nuclear magnetic resonance spectroscopy
str = Stretching
IR = Infrared spectroscopy
ppm = Parts per million
TLC = Thin layer chromatography

CONFLICT OF INTEREST

None of the author has any conflict of interest in the context of this work.

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