



Plant Archives

Journal home page: www.plantarchives.org

DOI Url: <https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.258>

PHYTOCONSTITUENTS FROM THE ROOTS OF *CUSCUTA CHINENSIS*

Srinivasarao Pandy¹, Sunitha Katta², Ganapaty Seru^{2*} and Krishna Nallamothu¹

¹Research and Development Wing, Divis Laboratories Ltd, Visakhapatnam, India

²Department of Pharmacognosy and Phytochemistry, GITAM Institute of Pharmacy, GITAM University, Rushikonda, Visakhapatnam, India

*Email: ganapatyseru@gitam.edu

(Date of Receiving-25-12-2020; Date of Acceptance-21-03-2021)

ABSTRACT

Cuscuta chinensis, a parasitic vine, was used in several traditional medicine systems, and it demonstrated a wider range of pharmacological activities in various diseases. The chemical components from *C. chinensis* consist mainly of flavonoids, steroids, volatile constituents, lignans, alkaloids, and polysaccharides. In view of its wider pharmacological properties, the authors have attempted to re-investigate the whole plant for its phytoconstituents and reported flavones salvigenin, chrisimaritin for the first time apart from the earlier reported quercetin and triterpenoid betulinic acid.

Keywords: *Cuscuta chinensis*, Convolvulaceae, Salvigenin, Cirisimaritin, Quercetin

INTRODUCTION

Cuscuta chinensis (Convolvulaceae), commonly called a dodder plant, is a parasitic perennial vine found in temperate and tropical regions like China and India (Ch.P, 2015). It is one of the essential Chinese medicinal plants recorded in Shennong's Herbal 2000ago and used by TCM practitioners for its regulatory effects on ovulation and hormonal balance (Ke *et al.*, 2013; Ma *et al.*, 2008). It was extensively studied for its therapeutic potential in various diseases like cancer (Wang *et al.*, 2013; Ghazanfari *et al.*, 2013), inflammation (Liao *et al.*, 2013; Kang *et al.*, 2014), and neural disease (Lin *et al.*, 2013). It also has an anti-aging (Sun *et al.*, 2014) effect and facilitates osteoporosis (Yang *et al.*, 2014). Phytochemicals reported from the plant include 4-Hydroxy benzoic acid, paracoumaric acid, coumarin, vanillic acid, α -resorcylic acid, rutin, naringenin-7-rhamnoglucoside, marsileagenin A, 3-hydroxy triacontane-11-one, protocatechuic acid, caffeic acid, and ferulic acid.(Shekarchi *et al.*, 2014) Keeping in view of its pharmacological properties, the authors have attempted to re-investigate the whole plant for its phytoconstituents and reported flavones salvigenin, chrisimaritin for the first time apart from the earlier reported quercetin and triterpenoid betulinic acid.

MATERIALS AND METHODS

Column chromatography was done using silica gel (60-120 mesh) and TLC using silica gel (60- silica gel G (Acme). Visualization of the plates was done by using UV Chamber or by spraying 5% methanolic sulphuric acid. Melting points were recorded using the Boietus melting point apparatus. UV spectra were obtained on Shimadzu UV spectrophotometer IR spectra were recorded on

BUCK Scientific-500 spectrophotometer using KBR pellets. NMR spectral data were obtained using BRUKER AM 400 with TMS as an internal standard.

Plant material

The whole fresh plant of *Cuscuta chinensis* (1.5 kg) was collected during September 2017, and the identity was established by Dr.M.Venkaiah, Department of Botany, Andhra University, Visakhapatnam.

Extraction and isolation of phytoconstituents

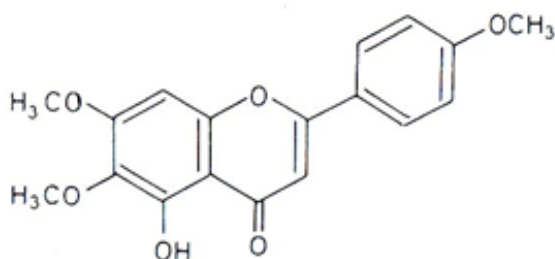
The dried material was powdered and extracted with ethyl acetate and concentrated under a vacuum, which gave a gummy residue. The whole fresh plant of *Cuscuta chinensis* (1.5 kg) was collected, washed thoroughly, and air-dried. The dried material was powdered and extracted with ethylacetate. The extracts obtained were subjected to phytochemical screening followed by chromatographic separation. Phytoconstituents isolated from the chloroform and methanolic extracts of the roots of *C. chinensis* were identified by spectral studies.

RESULTS AND DISCUSSION

The ethyl acetate extract (19 g) upon concentration under reduced pressure left a dark gummy residue. It gave positive Liebermann- Burchard reaction (Pink-Blue-Green) and ferric and Shinoda tests for flavonoids. In TLC over silica gel, it showed four prominent spots. The residue was separated on column chromatography over silica gel, which afforded four compounds named CCW-01, CCW-02, CCw-03, and CCW-04.

CCW-01 (Salvigenin)

It was obtained from benzene-chloroform as lemon yellow rectangular crystals, m.p. 196-197⁰, analyzed for C₁₈H₁₆O₆ (m/e 328 M⁺). It gave olive green ferric reaction, orange color in Shinoda's test, and yellow color in Wilson's-boric and citric acid, indicating a 5-OH flavone. On paper chromatography, purple and intense purple spots appeared under UV and UV/NH₃. On treatment with dimethyl sulphate and potassium carbonate, it gave a tetramethyl ether m.p. 162-63⁰ and gave acetate (m.p. 170-172⁰) with acetic anhydride and sodium acetate. The UV spectrum of the compound showed peaks at 275, 329 nm. With AlCl₃/HCl, a 22 nm bathochromic shift in Band I is observed, which indicated the presence of a free 5-hydroxyl group and a shift of 40 nm in Band I of NaOMe spectrum suggested 4'-substitution. With NaOAc, there was no bathochromic shift in Band II of the methanol spectrum, which implies that position C-7 was substituted. IR spectrum exhibited bands at 3450 (OH) and 1660 cm⁻¹ (C=O). The ¹H NMR spectrum showed three methoxyl groups at δ 3.90, 3.92, and 3.98 and two doublets (J = 9 Hz each) centered at δ 7.73 and 6.93, integrating for 4 protons assigned to 2', 6; and 3', 5' protons of the Ring B. The two other singlets at δ 6.50 and 6.53 were attributed to C₈ and C₃ protons of Ring A. From the previous data, the compound MML-01 was identified as 5-hydroxy 6,7,4'-trimethoxy flavone (Salvigenin). The identity was further confirmed by comparison with an authentic sample.



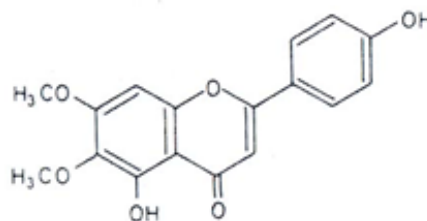
Salvigenin

Mass spectrum showed molecular ion at m/e 328 (M⁺, 100%), 313 (82%, M-CH₃), characteristic for 6-OCH₃ [382], 299 (22% M-Co-H), 285 (13%, M-CH₃-Co), 288 (15%), 269 (6%, M-Co-OCH₃), 253 (4%), 214 (5%), 181 (16%), 167 (51%, A-ring, tri-O-substitution) [383,384], 153 (22%), 135 (19%), 133 (20%), 132 (20%), and 117 (13%).

CCW-02 (Cirisimaritin)

It was obtained from methanol as lemon yellow crystals, m.p. 248-250⁰, C₁₇H₁₄O₆ (m/e 314 M⁺). A positive ferric reaction and orange-red color with Mg+HCl suggested the flavone nature of the compound. It formed a diacetate, m.p. 202-203⁰ and a tetramethyl ether, m.p. 162-163⁰. UV showed absorption bands at (nm): 325, 270. A 35 nm bathochromic shift in Band I of AlCl₃/HCl spectrum

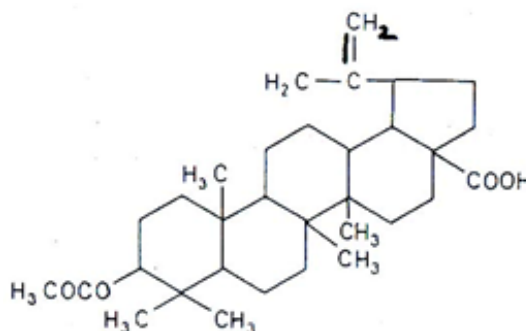
indicated a free 5-hydroxyl group and 6-OCH₃ [385], and a shift of 53 nm in Band I of NaOMe spectrum showed the presence of a free 4'-hydroxyl. The absence of Band II shift with NaOAc suggested that C-7 is substituted. IR exhibited bands at 3450 (OH), 2880 (CH stretch), 1640 (C=O), 1500, 835 and 765 cm⁻¹. The ¹H NMR spectrum showed a clear A₂B₂ pattern with doublets centered at δ 7.00 and 8.01 (J = 8.5 Hz), two methoxyls at δ 3.75 and 3.92, and flavone proton singlet at δ 6.82 (C-3H). The data coincided well with that of Cirisimaritin, and the identity was confirmed by comparison with an authentic sample.



Cirisimaritin

CCW-03 (Betulinic acid)

It crystallized from chloroform-methanol as shining silky needles, m.p. 294-296⁰, + 8.6⁰ (chloroform) and analyzed for the formula C₃₀H₄₈O₃. It is pink color in L.B. Test. IR showed absorptions at 3460 (-OH), 1690 (carbonyl of COOH), 1640 (double bond), 1380 and 1390 cm⁻¹ (gem dimethyl). It formed a monoacetate, m.p. 287-290⁰, + 12.2⁰ (chloroform) with Ac₂O/Py, a monoester with diazomethane m.p. 220-221⁰, + 8.5⁰ (chloroform), a methyl ester acetate, with Ac₂O/Py, m.p. 195-198⁰, + 14.2⁰ (chloroform). The above data agreed well with that of betulinic acid, and the identity was further confirmed by comparison with an authentic sample (mmp and Co-TLC).

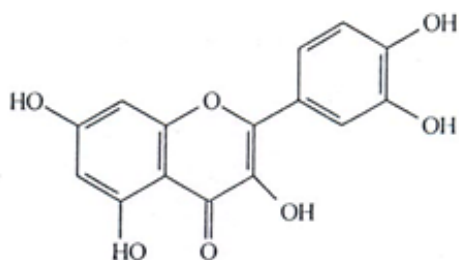


Betulinic acid

CCW-04 (Quercetin)

It was obtained from methanol as a yellow crystalline solid, m.p. 312-314⁰ and analyzed for the formula C₁₅H₁₀O₇. In PC, it was yellow under UV and intense yellow under UV/NH₃. With ferric chloride, it gave green color, and with magnesium + HCl (Shinoda test), magenta color

characteristics for flavonoids. An orange, red precipitate with neutral lead acetate confirmed the presence of 3-hydroxyl. The presence of the 5-hydroxyl group was inferred through Wilson's boric and citric acid reaction. It formed a Penta acetate, m.p. 194-196° and a pentamethyl ether, m.p. 150-151°. UV showed absorption at 257, 267sh, 301sh, 370nm. A 55 nm Band, I bathochromic shift with AlCl₃/HCl, suggested the presence of 5-hydroxyl. The presence of B ring ortho-dihydroxyl grouping was indicated by a 15nm Band II bathochromic shift with NaOAc/H₃BO₃ reagent. A bathochromic shift of 18 nm in Band II with NaOAc suggested the presence of 7-hydroxyl. ¹H NMR (Fig. 3.1.4) exhibits peaks at δ 6.15 (d, 6H), 6.4 (d, 8H), δ 6.90 (d, 5'H), δ 7.60 (d, 6'H) and δ 7.75 (d, 2'H). The elemental analysis of the compound and its acetate and UV spectral data indicated that the compound is quercetin, and the identity was confirmed by comparison with an authentic sample (mmp and Co-PC).



Quercetin

CONCLUSION

The chemical examination of the ethylacetate extract of the whole plant of *C. chinensis* afforded four compounds- salvigenin, cirisimaritin, quercetin, and betulinic acid.

ACKNOWLEDGMENTS

The authors are grateful to the GITAM Management for providing Laboratory facilities. They are also thankful to the University Scientific Instrumentation Centre (USIC), Andhra University, Visakhapatnam, for their help in providing the spectra of the compounds.

REFERENCES

- C. P. Commission (2015). Pharmacopoeia of the People's Republic of China, vol. 1, *Medical Science Press*, Beijing, China.
- Lin, S., S. Ye, J. Huang, *et al.*, (2013). How do Chinese medicines that tonify the kidney inhibit dopaminergic neuron apoptosis?. *Neural Regeneration Research*, 8(30): 2820–2826.
- Liao, J.C., W.-T. Chang, M.-S. Lee *et al.*, (2014). Antinociceptive and anti-inflammatory activities of *Cuscuta chinensis* seeds in mice. *American Journal of Chinese Medicine*, 42(1): 223–242.
- Kang, S.Y., H. W. Jung, M.-Y. Lee, H. W. Lee, S. W. Chae, and Y.-K. Park, (2014). Effect of the semen extract of *Cuscuta chinensis* on inflammatory responses in LPS-stimulated BV-2 microglia, *Chinese Journal of Natural Medicines*, 12, (8): 573–581.
- Wang, J., X. Li, and L. Gao (2013). Study on the extraction process of tannins from *Semen Cuscutae* and their anti-papilloma activity., *African journal of traditional, complementary, and alternative medicines: AJTCAM / African Networks on Ethnomedicines*, 10(3): 469–474.
- Ghazanfari, T., M. Naseri, J. Shams, and B. Rahmati, (2013). Cytotoxic effects of *Cuscuta* extract on human cancer cell lines. *Food and Agricultural Immunology*, 24(1): 87–94.
- Sun, S. L., L. Guo, Y.-C. Ren *et al.*, (2014). Anti-apoptosis effect of polysaccharide isolated from the seeds of *Cuscuta chinensis* Lam on cardiomyocytes in aging rats. *Molecular Biology Reports*, 41 (9): 6117–6124.
- Yang, H.M., Shin, H., Kang, Y., and Kim, J., (2009). *Cuscuta chinensis* extract promotes osteoblast differentiation and mineralization in human osteoblast-like MG-63 cells. *Journal of Medicinal Food*. 12 (1): 85–92.
- Shekarchi, M., Kondori, B., Hajimehdipoor, H., Abdi, L., Naseri, M., Pourfarzib, M. and Amin, G. (2014) Finger Printing and Quantitative Analysis of *Cuscuta chinensis* Flavonoid Contents from Different Hosts by RP-HPLC. *Food and Nutrition Sciences*, 5, 914-921.