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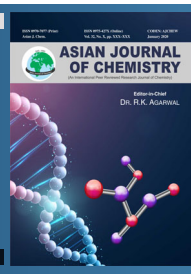

Isolation and Identification of Pure Bioactive Compounds from *Cuscuta reflexa* Grown on *Nerium oleander* Host Plant by Flash Column Chromatography and GC-MS/MS Analysis

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In this work, an ethyl acetate extract of *Cuscuta reflexa* grown on *Nerium oleander* host plant was used for the isolation and identification of pure bioactive compounds. Chloroform:methanol was used as mobile phase for flash column chromatography. Five pure bioactive compounds were isolated and two compounds were identified using GC-MS/MS technique. Glycerol-1-palmitate and squalene were identified in *Cuscuta reflexa* using NIST library on the basis of area percentage.

Keywords: *Cuscuta reflexa*, Column chromatography, Bioactive compounds, GC-MS/MS Scanning, Glycerol-1-palmitate, Squalene.

INTRODUCTION

Therapeutic potential of medicinal plants are due to the active phytoconstituents having different pharmacological activities which formed the basis for the use of these bioactive compounds as alternative medicines, in natural therapies and also in food preservation. These alternative medicines are more economic and easy to get comparatively to allopathic medicines [1]. The herbal medicines are affordable and easily accessible treatment in primary health care system. Previous studies showed that around 25% drugs contain compounds obtained from plants [2]. Moreover, investigations are running on for development of herbal drugs to treat AIDS, cancer, malaria, arthritis and asthma [3].

In the current scenario of health science, herbal medicines are gaining more concern then pharmaceutical drugs because of lesser side effects of herbal medicines. Medicinal plants are the only source for the search of herbal medicines. *Cuscuta reflexa* plant also known as dodder plant, is a class of parasitic plants which has 100-170 different species of red, orange, yellow and green parasitic plants. On the basis of the work of angiosperm phylogeny group, *Cuscuta* is now accepted as belonging of morning glory family *Convolvulaceae,* which was formerly treated as an only genus in the family of *Cuscutaceae* [4]. *Cuscuta* is commonly found at the temperate and tropical region of the world with the greatest species diversity in tropical and subtropical regions [5]. *Cuscuta* is used for medicinal purpose from ancient times, at the age of Ayurveda; juice of stems of *Cuscuta* was used for many medicinal purposes as to treat jaundice, warm paste is used to cure rheumatism and paste of whole plant was used to cure headache [6].

Bioactive constitutents of *Cuscuta reflexa* have anti-steroidogenic, heamodynamic and anticonvulsant activities. *Cuscuta* plant was also used as antispasmodic, bradycardia, antihypertensive and antiviral drug [7]. It's seeds are used for the treatment of bilious disorder, and also have anthelmintic and carminative properties [8]. Jha and Shelke [9] studied the hepatoprotective activity of *C. reflexa* in Swiss albino rats against paracetamol induced hepatic injury and concluded that *C. reflexa* hydroalcoholic extract possess hepatoprotective activity. Ethanolic and ethyl acetate extract have shown a remarkable antioxidant activity as comparable to the standard antioxidant agents [10]. Dandopani *et al*. [11] investigated the antitumor activity of *C. reflexa* chloroform and ethanol extracts against *Ehrlich ascites* carcinoma tumor in mice at the dose of 200 and 400 ppm body weight orally.

On the basis of UV-visible and FT-IR spectral analysis, Ramya *et al*. [12] reported the chemical constitution such as alkaloids, flavonoids, carbohydrates, phytosterols, glycosides, fixed oil and fats, proteins, phenolics compounds and saponins

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in the ethanolic extract of *Cuscuta reflexa*. Flowers and stems of *Cuscuta reflexa* were also studied using HPLC-DAD which revealed the presence of 16 phenolic compounds including 13 phenolic acids and 3 aldehydic derivatives. Results have shown that caffeic acid and *p*-coumaric acid are the main constitutents in flower and stem and five phenolics acids *viz*. gallic acid, gentisic acid, 2,4,6-trihydroxybenzoic acid, β-resorcinolic acid and vanillic acid were present [13].

Studies have shown that *Cuscuta reflexa* is a huge source of various phytochemicals that have many different bioactive potentials. Being a parasitic plant *Cuscuta reflexa* sucks nutrients from its host plant so the phytochemical constitution of *Cuscuta* also varies with different host plants making this plant more important in herbal medicines. In the present investigation, *Cuscuta reflexa* plant grown on *Nerium oleander* host plant ethyl acetate extract was isolated using flash column chromatography and then isolated pure compounds were identified using GC-MS/MS technique.

EXPERIMENTAL

Collection of plant: *Cuscuta reflexa* grown on *Nerium oleander* (CRN) host plant was collected from C-scheme area of Jaipur, Rajasthan, India. Plant sample of *Cuscuta reflexa* was identified and submitted in Ethnomedicinal Herbarium, Centre with potentials of Excellence funded by Department of Science and Technology, JECRC University, Jaipur, India. Further, voucher specimens of *Cuscuta reflexa* was deposited at herbarium of University of Rajasthan, Jaipur, India and verified by senior taxonomist of department and provided with accession no. RUBL211577. Collected plant samples were shade dried and were screened for foreign matter and then pulverized.

Selection of solvent system: Solvent system was decided on the basis of thin layer chromatography of the mixture of compounds. Solvent system which gives R_f value of 0.35 on TLC plate was used as mobile phase to carry out column chromatography.

Quantity of silica gel: Quantity of silica gel required to pack glass column depends upon the difference between R_f value of the compounds to be separated and on the amount of sample. Usually silica gel requires 30-100 times of weight of sample. A ratio of 30:1 of silica gel and sample was used to perform column chromatography of *Cuscuta reflexa* extracts.

Slurry preparation of crude: Crude sample was mixed with silica gel and diluted with the solvent in which crude can be dissolved easily. This mixture was dried on rotavap to make fine dried powder of slurry.

Preparation of plant extract: Pulverized plant material was extracted using different amount of ethyl acetate as extraction solvent for 12 h with continues stirring (Table-1). After extraction process, extraction solvent was evaporated using rotary evaporated to get dried crude extract. Dried crude extract was further subjected to column chromatographic analysis.

GC-MS/MS analysis: GC-MS/MS analysis of isolated compounds were performed using Thermo Scientific, TSQ 8000 triple quardrupole MS with Trace 1300 gas chromatograph. Instrumental method for GC-MS/MS scan was set by ramping of temperature, initial temperature was 50 ºC, 150- 240 ºC at 5-35 min with hold time 2.0 min, 240-280 ºC at 35-52 min with hold time 8.0 min with total rum time of 52 min using helium as carrier gas, flow rate 1.0 mL/min and sample volume was 1.0 µL; ion source temperature: 250 ºC and MS transfer line temperature: 280 ºC; acquisition mode scan: DB-5MS column of 30 m length, 0.25 µm film coating and 0.25 mm diameter was used for GC-MS/MS analysis.

TABLE-1
LIST OF PURE COMPOUNDS ISOLATED FROM *Cuscuta reflexa* GROWN ON *Nerium oleander* HOST PLANT ETHYL ACETATE EXTRACT BY FLASH COLUMN CHROMATOGRAPHY

Isolated compound name	Colour of spot on TLC plates	Quantity (mg)
CRN-EA1	Orange colour	30
CRN-EA2	Pink colour	70
CRN-EA3	Purple colour	50
CRN-EA4	Dark pink colour	250
CRN-EA5	Purple colour	130

RESULTS AND DISCUSSION

Five compounds named as CRN-EA1, CRN-EA2, CRN-EA3, CRN-EA4 and CRN-EA5 were isolated from column chromatography on the basis of single spot on TLC plate using chloroform and methanol as mobile solvent (Table-2). All the five compounds were subjected to GC-MS/MS analysis, where only two compounds CRN-EA4 and CRN-EA5 were identified by GC-MS/MS spectra. Other three compounds were found to be impure and could not identified.

On the basis of retention area, area percentage and library search of GC-MS/MS data, CRN-EA4 (Fig. 1) and CRN-EA5 (Fig. 2) were identified as squalene ($C_{30}H_{50}O$, m.w. 410 g/mol) and glycerol 1-palmitate ($C_{19}H_{38}O_4$, , m.w. 330 g/mol).

Glycerol-1-palmitate is a component of chemical group monoacylglycerols, which may be used as a substrate for detection or differentiation of enzymes that hydrolyze monoacylglycerols and plays a role in the hydrolysis of fat. Whereas squalene is natural polyunsaturated hydrocarbon which is an important precursor of different hormones in flora and fauna.

Conclusion

Cuscuta reflexa grown on *Nerium oleander* host plant has been studied for the isolation and identification of pure compounds by flash column chromatography and GC-MS/MS analysis and concluded that *Cuscuta reflexa* plant contains glycerol-1-palmitate and squalene in its phytoconstitution. *Cuscuta reflexa* plant is a natural source of squalene which has both medicinal and commercial values. Further studies can also be performed on *Cuscuta reflexa* plant grown on other medicinally important host plants, which may lead in the development of new herbal drugs for the treatment of various diseases.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-2
CONCENTRATION OF METHANOL AND CHLOROFORM FOR *Cuscuta reflexa* GROWN ON *Nerium oleander* HOST PLANT ETHYL ACETATE EXTRACT FLASH COLUMN CHROMATOGRAPHY 10% H_2SO_4 AS SPRAYING REAGENT

Methanol (%)	Volume of chloroform (mL)	Result
0	300.0	–
0.5	298.5	–
0.5	298.5	CRN-EA1
1.0	297.0	CRN-EA1
1.0	297.0	CRN-EA1
1.0	297.0	CRN-EA1
1.0	297.0	Mixed compound
1.5	295.5	CRN-EA2
1.5	295.5	Mixed compound
2.0	294.0	Mixed compound
2.5	292.5	Mixed compound
3.0	292.0	Mixed compound
3.5	289.5	CRN-EA3
3.5	289.5	CRN-EA3 + impurity
4.0	288.0	Mixed compound
4.5	286.5	Mixed compound
5.0	285.0	Mixed compound
5.5	283.5	CRN-EA4
5.5	283.5	CRN-EA4
5.5	283.5	CRN-EA4
6.0	282.0	Mixed compound
6.5	280.5	Mixed compound
7.0	279.0	Mixed compound
7.5	277.5	CRB-EA3
8.0	276.0	CRN-EA5
8.0	276.0	No Result
8.5	274.5	Product end

CRN = *Cuscuta reflexa* grown on *Nerium oleander* host plant; EA = Ethyl acetate

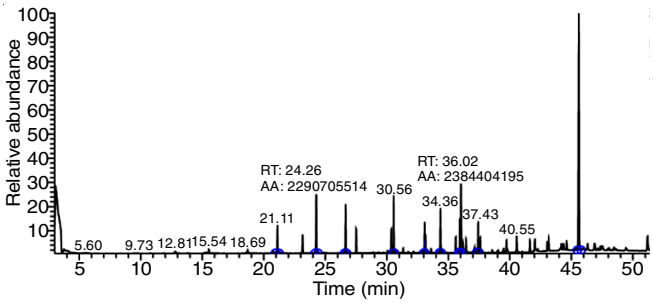

Fig. 1. GC-MS/MS spectra of CRN-EA4

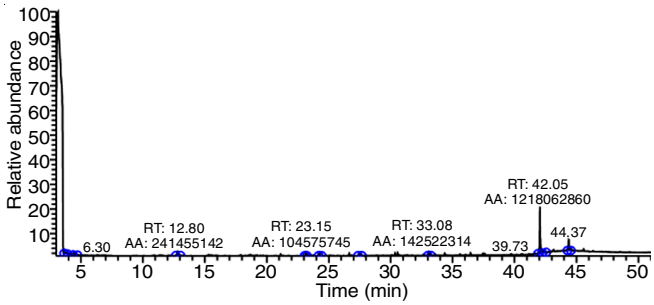

Fig. 2. GC-MS/MS spectra of CRN-EA5

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