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Research article

Comparative phytochemical analysis of *Cuscuta reflexa* Roxb. Parasite grown on north India by GC-MS

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Abstract: The present study was aimed to determine the phytochemicals present in *Cuscuta reflexa* parasite grown on two different areas. The comparative GC-MS analysis of extract of *Cuscuta reflexa* the plant grown on North India was performed. The extract of plant sample was dissolved in 75 ml of methanol for 24 hrs. Then the filtrates were collected and evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a Mass detector Turbo mass gold-Perking Elmer Turbomas 5.1 spectrometer with an Elite-1 (100% Dimethy1 ply siloxane), 30 m x 0.25 mm ID x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C min^{-1}, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml min^{-1}. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 25–400 mhz. The chemical constituents were identified by GC-MS. The result of the GC-MS analysis of *C. reflexa* shows the presence nitrogen (13.56%), aromatic compound (7.88%), fluro (28.40%), alkaloid (7.64%), silica (5.66%), phosphorus (16.31%) and chlorine compounds (6.26%). In general the isolated compounds are reported to possess antimicrobial, antitumor, anticarcinogenic and anti-inflammatory properties. This comparative study confirms that phytochemicals present in *Cuscuta reflexa* parasite depends on nature of plants.

Keywords: *Cuscuta reflexa* - Phytochemistry - Parasitic Plants - GC-MS analysis.

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INTRODUCTION

Cuscuta reflexa Roxb. (Convolvulaceae) is an extensive climber parasite. It occurs throughout the plains of India. It is more often called dodder in English. Traditional healers called in Hindi Akash bel in Tamil Akashavalli. Other names include hell weed, devil's gut, and beggar weed, strangle tare, scald weed, dodder of thyme, greater dodder, and lesser dodder. In Chinese, *Cuscuta* seeds are called *tu si zi*. It has no chlorophyll and cannot make its own food by photosynthesis. Some research studies say that the plant has very low levels of chlorophyll and can slightly photosynthesis. But other species of *Cuscuta* are entirely dependent on the host plants for nutrition. The stem is thread like filaments it is begin to grow and attach themselves to nearby host plants. The nature plants lives its entire life without attachment to the ground. It has long history of ethnomedicinal use. *Cuscuta* is a genus of about 100–170 species.

In North India *Cuscuta reflexa* is usually associated with parasitism in ornamental plants and its occurrence in medicinal crops is unusual. This species is originally from India, is common over the Northern region of the country from the state of Uttar Pradesh and Uttarakhand. *Cuscuta reflexa* Roxb. is a valuable medicinal herb (Fig. 1). Stem of this plant is antibacterial and used externally to treat itch and internally in fever (Pal *et al.* 2006). It is useful in treatment of androgen induced alopecia (Pandit *et al.* 2008). It also gives anti-inflammatory and anti-cancer activity (Suresh *et al.* 2011). The aqueous and alcoholic extract of *C. reflexa* has diuretic property (Sharma *et al.* 2009). The crude water extract of *C. reflexa* also shows anti HIV activity (Mahmood *et*

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al. 1997). It is a parasitic plant completely dependent on host plant for food and nutrition. The organic matter is transported from the phloem of the host to the parasite through the hostorium (Kumar *et al.* 2012). It is believed that the parasitic herbs extract healthy and potential sap from host plant and if their host plant is medicinal plants then these parasitic herbs how many similar properties to host plants. *Cuscuta* species feeding on commonly used medicinal herbs are given special attention by traditional healers. Present work evaluates the comparative study of phytochemical activity of extract of *Cuscuta reflexa* grown on Muzaffarnagar and Pantnagar, North India.

MATERIAL AND METHOD

Collection of plant material

Cuscuta reflexa plant leaves were collected from village area of district Muzaffarnagar, Uttar Pradesh and area of Pantnagar, Uttarakhand, India. The plants were authenticated by Dr. Anju Pal, Dept. of Horticulture, G.B. Pant University of Agriculture and Technology, Uttarakhand, India.

Figure 1. *Cuscuta reflexa* Roxb. on his host *Euphorbia tirucalli* L.

Preparation of aqueous extract

Hundred grams each of dried leaves of *C. reflexa* collected from different location of North India were macerated with 100 ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 120°C for 30 min. The extracts were preserved aseptically in brown bottles at 4°C until further use.

Preparation of plant Solvent extracts

Soxhlet extraction will be the method used for plant extraction. A portion of dried leaves (100 g) of *Cuscuta reflexa* was placed in a Soxhlet apparatus. Extraction was performed with 500 ml of an appropriate solvent (Ethanol, Methanol, Chloroform) with increased polarity for 24 h at 95^{0C} temperature not exceeding the boiling point of the solvent. The extract was filtered through a 45 µm filter paper and concentrated under vacuum. In this experiment three solvents were used: Ethanol, Chloroform and methanol. The resulting three solutions were concentrated in vacuoum to dryness to give Ethanol (4 g), Chloroform extract (10 g) and methanol extract MeOHE (12 g). The stock solutions were kept at 4°C until further use.

Sample preparation

The extract of plant sample was dissolved in 75 ml of methanol for 24 hrs. Then the filtrates were collected and evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a Mass detector Turbo mass gold-Perking Elmer Turbomas 5.1 spectrometer with an Elite-1 (100% Dimethy1 ply siloxane), 30 m x 0.25 mm ID x 1 µm df

capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 25-400 mhz. The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology - Mass Spectral database (NIST-MS). The percentage of each component was calculated from the relative peak area of each component in the chromatogram.

RESULTS

The phytochemical compounds present in the methanolic extract of *Cuscuta reflexa* were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) in the extracts of *C. reflexa* were presented. From Muzaffarnagar sample, totally 15 compounds were identified table 1. The prevailing compounds were lauric acid (2.46%), ester compound (0.05%), alkanes (0.05), phenolic compound (0.08%), myristic acid (2.77%), plasticizer compound (4.15%), palmitolic acid (2.27%), palmitic acid (13.97%), diterpene (2.31%), stearic acid (1.68%), mono unsaturated fatty (5.19%), chlorine compound (2.16%), steroid (11.6%), alkaloid (1.78%), triterpenes (3.56%) and amino compound (39.27%).

Table 1. Qualitative and quantitative determination of biochemical constituents of plants sample collected from Muzaffarnagar.

S.No.	RT	Name of the Compound	Molecular Formula	MW	Peak Area (%)	Compound Nature	Activity
1	8.13	Tetradecane	$C_{14}H_{30}$	198	0.05	Alkane	No activity reported
2	9.07	Phenol, 3,5-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.08	Phenolic Compound	Analgesic, Anesthetic, Antioxidant, Antiseptic, Antibacterial, Antiviral Cancer preventive, Fungicide
3	10.50	Dodecanoic acid	$C_{12}H2_4O_2$	200	2.46	Lauric acid	Antipyretic, Antiinflammatory, Analgesic, Antiseptic, Pesticide, Cancer preventive, Carminative
4	13.56	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.77	Myristic acid	Antioxidant, Cancer preventive, Nematicide, Hypocholesterolemic
5	14.84	1,2-Benzenedi carboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	278	1.67	Plasticizer Compound	Antimicrobial, Antifouling
6	16.30	Hexadecenoic acid, Z-11-	$C_{16}H_{30}O_2$	254	2.27	Palmitolic Acid	Hypocholesterolemic
7	16.77	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.97	Palmitic acid	Antioxidant, Flavor, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor
8	18.45	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	2.16	Alcoholic Compound	Antimicrobial
9	19.01	Phytol	$C_{20}H_{40}O$	296	2.31	Diterpene	Antimicrobial, Anticancer Anti-inflammatory, Diuretic
10	19.50	Oleic Acid	$C_{18}H_{34}O_2$	282	5.19	Mono unsaturated fatty acid	Antiinflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic,
11	22.29	Tris (1,3-dichloro isopropyl) phosphae	$C_9H_{15}C_{l6}O_4P$	428	2.16	Chlorine Compound	Antimicrobial
12	25.66	1,2Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	4.15	Plasticizer compoud	Antimicrobial, Antifouling

13	30.53	Squalene	$C_{30}H_{50}$	410	3.56	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive,
14	32.59	Tetrazol-5-amine, N-(3,4-dimethoxybenzyl)-	$C_{10}H_{13}N_5O_2$	239	39.37	Amino compoud	Antimicrobial
15	34.19	Cholestan-3-one, Cyclic1,2 ethanediyl	$C_{29}H_{50}O_2$	430	11.61	Steroid	Antimicrobial, Antiarthritic Antiasthma, Anti-inflammatory

The mass spectrum and structures of these above mentioned compounds were shown in figure 2. They are suggested to be the medicinally important compounds which can be used as antimicrobial, anti-inflammatory, cancer preventive, antioxidant, antiviral, antidiabetic, antifouling and hepatoprotective agent.

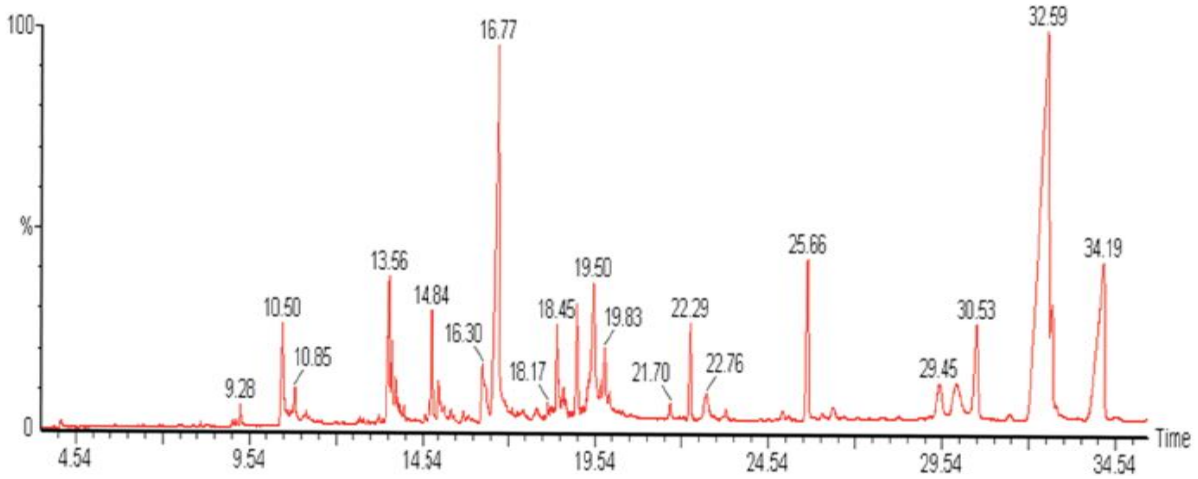

Figure 2. GC-MS chromatogram of methanolic extracts of *Cuscuta reflexa* (Muzaffarnagar Sample).

The result of the GC-MS analysis of *C. reflexa* from Pantnagar plant samples is presented in the table 2. The GC-MS chromatogram of these medicinal plant extracts was shown in the figure 3. Nearly 12 compounds were indentified in the Pantnagar sample. They were nitrogen (13.56%), aromatic (7.88%), fluro (28.40%), alkaloid (7.64%), silica (5.66%), phosphorus (16.31%) and chlorine compounds (6.26%). In general the isolated compounds are reported to possess antimicrobial, antitumor, anticarcinogenic and anti-inflammatory properties.

Table 2. Qualitative and quantitative determination of biochemical constituents of plants sample collected from Pantnagar.

S.No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)	Compound Nature	
1	2.15	1,4 Bis*4' 5' Bis (Trimethylfluromethyl) 1', 3'- Dithiac	C10 N4 F12 S4	532	28.40	Fluro compound	Antimicrobial
2	2.29	Dihydrofuranoartobilochromen A	C25H22O7	434	10.28	Pigment	No activity
3	2.49	Tetra (Diphenylphosphinyl) Allene	C51H40P4	76	16.31	Phosphorus compound	No activity
4	2.80	Methyl-2-hydroxy-2-cyclohexylacetate	C9H16O3	172	8.30	Acetate compound	No activity
5	3.51	Methyl-6,8 dioxo-2,C4Diphenyl-7-oxa-3-Azabicyclo(3	C20H17O5 N	351	7.68	Nitrogen compound	No activity
6	3.69	Isopropyl(P-metho xyphenyl)malononitrile	C13H14O N2	214	11.62	Nitrogen compound	No activity
7	4.18	Dimetilan	C10H16O3 N4	240	13.56	Nitrogen compound	No activity
8	4.44	TMS-8,11-Di – OHTetrahydrocannabinol	C30H54O4 Si3	562	5.66	Silica compound	No activity
9	22.62	1,2 Di-Hydroxy anthroquinone DITMS	C20H24O4 Si2	384	7.88	Aromatic compound	No activity

10	4.71	Tetramethyl 5 (Hexachloro-2,4,6 Cycloheptatrien	C20H12O8 Cl6	590	6.26	Chlorine compound	Antimicrobial
11	5.18	N [1,2,2,2 tetrafluro-1-(Trifluromethyl)ethyl]SU	C6H9O2N 2F7S	306	5.68	Fluro compound	Antimicrobial
12	5.82	Ditetrazolo1,5- A:5',1'-C Pyracine	C4 H2 N8	162	7.64	Alkaloid	Antimicrobial

Figure 3 shows mass spectrum and structures of these compounds, and also are suggested to be the medicinally important compounds which can be used as antimicrobial, anti-inflammatory, cancer preventive, antioxidant, antiviral, antidiabetic, antifouling and hepatoprotective agent.

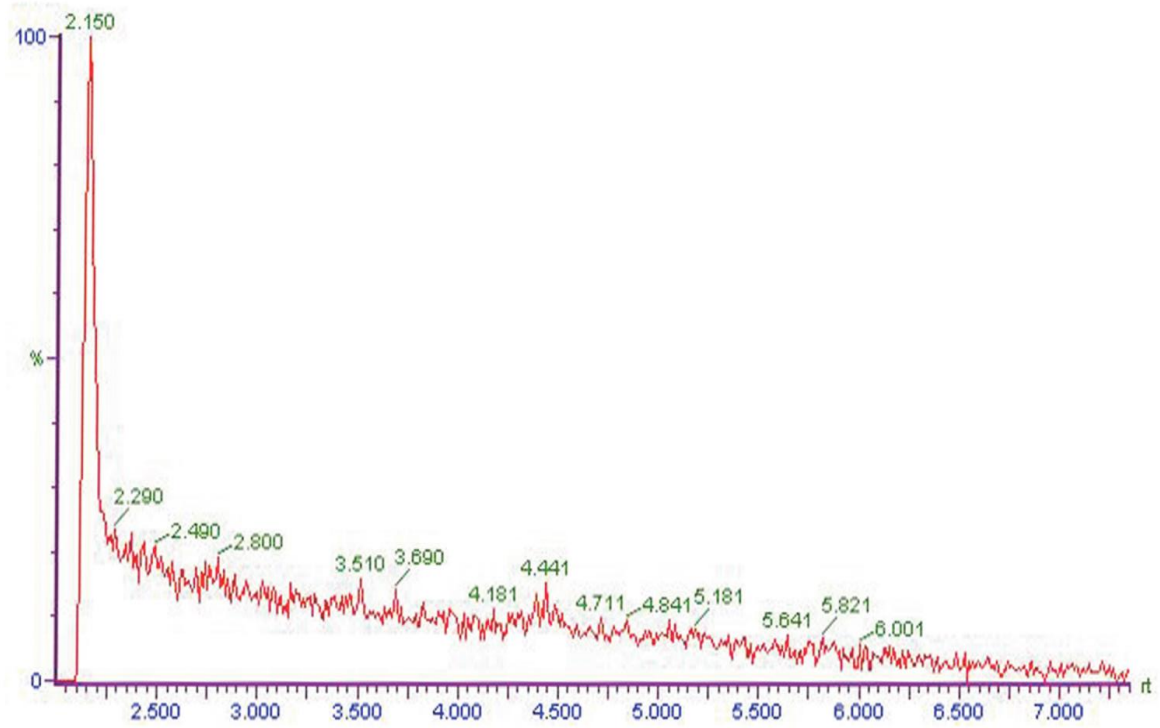

Figure 3. GC-MS chromatogram of methanolic extracts of *Cuscuta reflexa* (Pantnagar Sample).

DISCUSSION

In the present study the GC-MS analysis of ethyl acetate extract of *C. reflexa* grown on North India .showed the fifteen and three compounds respectively.Tetradecane ,Phenol,3,5-bis (1,1 dimethylethyle,1,2 Benzenedi carboxile acid bis (2 methyle propyle ester), n Heaxadecenoic acid ,1 hexadecanol, 2 methyle, phytol, tetrazol – 5- amine N (3,4- dimethoxybenzyl,cholestan 3- one cyclic 1,2 ethanediyle. Are present in Muzaffarnagar village area sample. And 1,4 Bis 4,5 trimethyleflurоment and other 11 types of compound nature are present in Pantnagar area sample .tetramethyle and its derivatives are known to have bacterial inhibiting effect.

CONCLUSION

It is revealed from this study that *C. reflexa* from both the region is rich in secondary metabolites which possess wide range of biological activities. Different compounds are present in *C. reflexa* on two different region thus it is concluded that variation in phytochemicals in *C. reflexa* is different region dependent. Further study need to be undertaken to investigate the biological activity and other phytochemicals present in *C. reflexa* grown on North India.

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