



## Phytochemical Analysis of Methanolic Extract of *Cuscuta reflexa* Grown on *Cassia fistula* and *Ficus benghalensis* by GC-MS

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### ABSTRACT

The objective of present study was to analyze the phytochemicals present in the *Cuscuta reflexa* parasite grown on two different plant species. A comparative GC-MS analysis was done on methanol extract of *Cuscuta reflexa* grown on *Cassia fistula* and *Ficus benghalensis*. This analyses revealed that benzofuran 2, 3, dihydro-, 2-methoxy-4-vinylphenol and 2-propenoic acid, 3-(4-hydroxyphenyl)-methyl ester were present in both *Cuscuta reflexa* grown on *Cassia fistula* and *Cuscuta reflexa* grown on *Ficus benghalensis*. It was found that urea N, N'-bis(1-methylethyl)-, 6-Chloro-4-phenyl-2-propylquinoline and benzo[h]quinoline 2, 4-dimethyl- were present only in *Cuscuta reflexa* grown on *Cassia fistula* while propanedioic acid, propyl, 2-[2-thienyl]-4-acetyl quinoline and L-alanine-4-nitroanilide were found to be present only in *Cuscuta reflexa* grown on *Ficus benghalensis*. This variation in phytochemicals present in *Cuscuta reflexa* parasite grown on *Cassia fistula* and *Ficus benghalensis* confirms that chemical constituents of parasite depends on nature of host.

**Keywords:** *Cuscuta reflexa*, *Cassia fistula*, *Ficus benghalensis*, GC-MS analysis.

### INTRODUCTION

Medicinal plants have been used since ages in traditional medicines due to their therapeutic potential and the search in medicinal plants have led to the discovery of novel drug candidates used against various diseases.<sup>1</sup> The treatment of various diseases with indigenous medicinal plants generates considerable health and economic benefits. Traditional knowledge in this regard has been conserved for generations in different tribal communities in several parts of India.<sup>2</sup>

Knowledge of potential chemical constituents is important for the discovery of therapeutic agents, which may be of great value as the source of economical phytocompounds for the synthesis of complex chemical substances.

*Cuscuta reflexa* is rootless, leafless perennial parasitic twining herb of convululaceae family, commonly known as amarbel or dodder. It has no chlorophyll and cannot make its own food by photosynthesis. It spread from one host plant to another, and on each victim, they twin and cling tightly with special branching organs called haustorium. Haustorium penetrate the host and connect to the host xylem as well as to the host phloem and absorb from it both water and elaborated food stuff.<sup>3</sup> It is believed that the parasitic herbs extract healthy and potential sap from host plant and if their host plants are medicinal plants then these parasitic herbs show many similar properties to host plants. *Cuscuta* species feeding on commonly used medicinal herbs are given special attention by traditional healers.

*Cuscuta reflexa* is the valuable medicinal herb. Stem of this plant is antibacterial and used externally to treat it

and internally in fever.<sup>4</sup> It is useful in treatment of androgen induced alopecia.<sup>5</sup> It also gives anti inflammatory and anti cancer activity.<sup>6</sup> The aqueous and alcoholic extract of *C. reflexa* has diuretic activity.<sup>7</sup> The crude water extract of the *C. reflexa* also shows the anti HIV activity.<sup>8</sup>

### MATERIALS AND METHODS

#### Collection of plant material

*Cuscuta* stem were collected from the tree of *Cassia fistula* and *Ficus benghalensis* tree near village areas of Gokulpur in Jabalpur Madhya Pradesh, India in the month of September and November 2010 respectively. Collected plant material was authenticated by Dr. Indu Gupta Botany Dept. Govt. Model Science College Jabalpur (M.P), India. Stems were washed thoroughly with water. Immense care was taken to avoid the mixing of host plant with that of targeted *Cuscuta* stem. Stems of *Cuscuta* were cleaned and completely separated from the stems of host plant.

#### Solvent extraction

Thoroughly washed stems of *Cuscuta reflexa* from both the host trees were shade dried for 15 days and the powdered in the grinder. The shade dried powder was extracted with petroleum ether, ethyl acetate, methanol and water in increasing polarity. The extracts were filter with Whatman's filter paper. Filtrates were concentrated under reduced pressure and preserved at 5°C in dark air tight bottles

#### Sample preparation for GC-MS analysis

50 µl of sample was dissolve in 2ml of methanol and kept in ultrasonic bath for 15 min and centrifuged for 10 min at

6000 rpm and supernatant was injected in GC-MS for analysis.

### GC-MS analysis

GC-MS of methanol extract was performed using Agilent 7890A. Compounds were separated on Agilent 1909S-433: 2065.49541 HP -5MS. 5% phenyl methyl silox column (30m x 250 $\mu$ m x 0.25 $\mu$ m). Oven temperature was programmed as follows: isothermal temperature of 50 $^{\circ}$ C for 2 min then increased to 150 $^{\circ}$ C at the rate of 5 $^{\circ}$ C /min and held for 1.75 min then increased to 280 $^{\circ}$ C at the rate of 8 $^{\circ}$ C /min and kept constant for 5min. The run time was 45 min. ionization of sample components were performed on EI mode (70 eV). The carrier gas was helium at 1.0ml/min flow rate. 0.5 ml of sample was injected in split mode of 20:1. The mass spectrum scan range was set at 29.0 to 500(m/z).

### Identification of compounds

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and

Technology (NIST). The mass spectrum of phytochemicals was compared with the spectrum of known compounds stored in the NIST library.

### RESULTS

Phytochemicals present in methanol extract in *Cuscuta reflexa* grown on *C. fistula* and *F. benghalensis* are summarized in table 1 and table 2.

Figure 1 represents the GC chromatogram of methanol extract of *C. reflexa* grown on *C. fistula*. It shows many peaks out of which six major peaks were characterized and identified on comparison of mass spectra with NIST library. Fig 3 represents the mass spectrum of components present in *C. reflexa* grown on *C. fistula*.

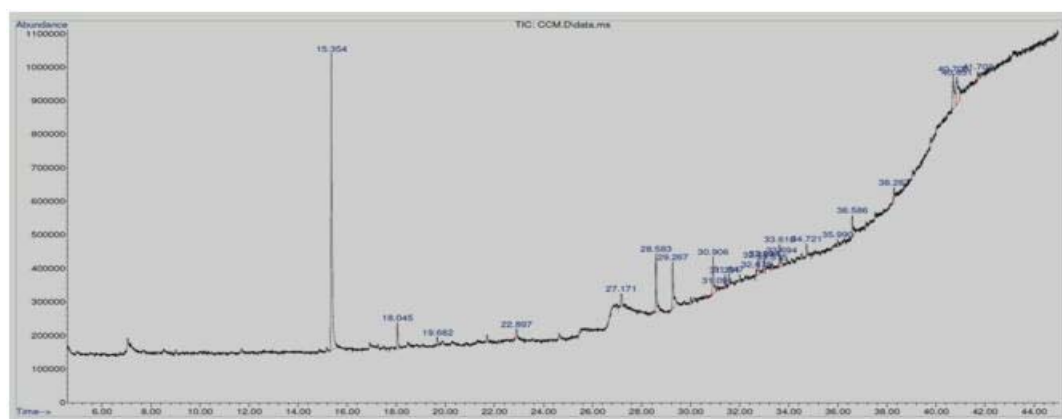
Fig 2 shows the GC chromatogram of methanol extract of *C. reflexa* grown on *F. benghalensis*. Out of many peaks six important peaks were characterized. Fig 4 shows the mass spectrum of phytochemicals identified in *C. reflexa* grown on *F. benghalensis*.

**Table 1:** Phytochemicals identified in methanol extract of *C. reflexa* grown on *C. fistula*

S.No	RT	Name of Compound	MF	MW	Total peak %
1.	15.354	Benzofuran,2,3-dihydro-	C <sub>8</sub> H <sub>8</sub> O	120.14	38.024
2.	18.045	2-Methoxy-4-vinylphenol	C <sub>9</sub> H <sub>10</sub> O	150.17	3.473
3.	19.682	Urea, N, N'- bis(1-methylethyl)-	C <sub>7</sub> H <sub>16</sub> N <sub>2</sub> O	144.21	0.866
4.	28.583	2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester	C <sub>10</sub> H <sub>10</sub> O <sub>3</sub>	178.18	7.585
5.	29.267	6-Chloro-4-phenyl-2-propylquinoline	C <sub>18</sub> H <sub>16</sub> ClN	281.77	7.445
6.	31.547	Benzo[h]quinoline,2, 4-dimethyl-	C <sub>15</sub> H <sub>13</sub> N	207.27	1.234

**Table 2:** Phytochemicals identified in methanol extract of *C. reflexa* grown on *F. benghalensis*

S.No	RT	Name of Compound	MF	MW	Total peak %
1.	15.354	Benzofuran,2,3-dihydro-	C <sub>8</sub> H <sub>8</sub> O	120.14	24.971
2.	18.045	2-Methoxy-4-vinylphenol	C <sub>9</sub> H <sub>10</sub> O	150.17	2.400
3.	19.682	Propanedioic acid, propyl	C <sub>6</sub> H <sub>10</sub> O <sub>4</sub>	146.14	8.021
4.	28.583	2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester	C <sub>10</sub> H <sub>10</sub> O <sub>3</sub>	178.18	3.809
5.	29.267	2-[2-Thienyl]-4-acetyl quinoline	C <sub>15</sub> H <sub>11</sub> NOS	253.31	15.241
6.	31.547	L-Alanine-4-nitroanilide	C <sub>9</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	209.2	1.733



**Figure 1:** GC-MS Chromatogram of methanol extract of *C. reflexa* grown on *C. fistula*

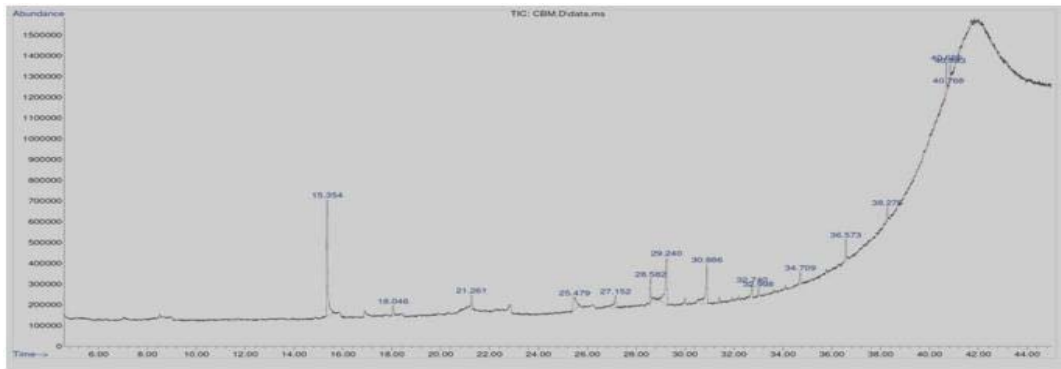


Figure 2: GC-MS Chromatogram of methanol of *C. reflexa* grown on *F. benghalensis*



Figure 3: Mass spectrum of phytochemicals identified in *C. reflexa* grown on *C. fistula*



Figure 4: Mass spectrum of phytochemicals identified in *C. reflexa* grown on *F. benghalensis*

Retention times of phytochemicals identified in methanol extract of *C. reflexa* grown on *C. fistula* were found to be 15.354, 18.045, 19.682, 28.583, 29.267 and 31.547.

Phytochemicals identified in *C. reflexa* grown on *C. fistula* were benzofuran 2, 3-dihydro- (38.024%), 2-methoxy-4-vinylphenol (3.473%), Urea, N, N'- bis (1-methylethyl)- (0.866%), 2-propenoic acid, 3-(4-hydroxyphenyl)-methyl ester (7.585%), 6-Chloro-4-phenyl-2-propylquinoline (7.445%), benzo[h]quinoline, 2, 4-dimethyl- (1.234%).

From GC-MS analysis of methanol extract of *C. reflexa* grown on *F. benghalensis* six compounds were identified. Retention times of these compounds were found to be 15.354, 18.046, 25.479, 28.582, 29.240 and 32.998. The phytochemicals identified were benzofuran 2, 3-dihydro- (24.971%), 2-methoxy-4-vinylphenol (2.400%), propanedioic acid, propyl (8.021%), 2-propenoic acid, 3-(4-hydroxyphenyl)-methyl ester (3.809%), 2-[2-Thienyl]-4-acetyl quinoline (15.241%), L-alanine-4-nitroanilide (1.733%).

## DISCUSSION

From table 1 and Table 2 it was revealed that benzofuran 2, 3, dihydro-, 2-methoxy-4-vinylphenol and 2-propenoic acid, 3-(4-hydroxyphenyl)-methyl ester was common to both *C. reflexa* grown on *C. fistula* and *C. reflexa* grown on *F. benghalensis*. It was found that urea N, N'-bis(1-methylethyl)-, 6-Chloro-4-phenyl-2-propylquinoline and benzo[h]quinoline, 2, 4-dimethyl- were present only in *C. reflexa* grown on *C. fistula* while Propanedioic acid, propyl, 2-[2-Thienyl]-4-acetyl quinoline and L-alanine-4-nitroanilide were found to be present only in *C. reflexa* grown on *F. benghalensis*.

Benzo[h]quinolines are found to have wound healing, antibacterial, DNA binding and antioxidant property.<sup>9</sup> Quinolines and its derivatives possess anticancer, antimicrobial, anticonvulsant, anti-inflammatory and cardiovascular activity.<sup>10</sup> 2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester is the derivative of p-coumaric acid which is worked out for its anti oxidant and antimicrobial activity.<sup>11</sup>

## CONCLUSION

It is revealed from this study that *C. reflexa* from both the host trees is rich in secondary metabolites which possess wide range of biological activities. Different compounds are present in *C. reflexa* on two different host thus it is

concluded that variation in phytochemicals in *C. reflexa* is host dependent. Further study need to be undertaken to investigate the biological activity and other phytochemicals present in *C. reflexa* grown on *C. fistula* and *F. benghalensis*.

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