

## Phytochemical and GC-MS Analysis of *Cuscuta reflexa* Roxb: a parasitic plant collected from host *Vitex negu*

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### ABSTRACT

Plants are an essential part of life on the planet earth and their products directly or indirectly support the life processes. Wild plants constitute an integral part of the customs and cultures of different ethnic communities. *Cuscuta reflexa* Roxb commonly known as Amarbell, Swarnlatta belongs to the family Convolvulaceae. It is a parasitic plant that depends on various host plants. In the present work, *Cuscuta reflexa* was collected from the host plant i.e. *Vitex negundo* to investigate the phytoconstituents of the plant such as Alkaloids, Protein, Carbohydrate, Flavonoid, Steroid, Tannin, Glycosides, Terpenoid, Phenol. The result of GC-MS profiling of the plant identifies 79 bioactive compounds such as *n*-Hexadecanoic acid, Phytol, Vitamin E, Phenol, 2,4-bis (1,1)-dimethyl ethyl, 2,3 dihydro benzofuran, 9,12,15-Octadecatrienoic acid (Z, Z), Stigmasterol, Benzofuran, etc. The isolated bioactive compounds are reported to be effective as antioxidant, antibacterial, anti-inflammatory, antidiabetic, antipyretic, antitumor, hepatoprotective, anti-cancerous, antihypertensive, anti-hair fall, and anti-arthritis properties. In the present study, Phytochemical Analysis and GC-MS profiling of *Cuscuta reflexa* Roxb is performed.

**Keywords:** *Cuscuta reflexa* Roxb, Phyto-chemical, GC MS analysis, a bioactive compound

### INTRODUCTION

For an eternity, the use of herbal medicine is the basis of traditional medicine as well as an integral part of all customs. The knowledge of ethnomedicine from prehistoric texts and various indigenous folklore to modern preparation has verified the presence of bioactive compounds with therapeutic potential. *Cuscuta* species are prevalent in folk medicines and rich in bioactive constituents that have pharmacological activity. Studying *Cuscuta reflexa* on different host plants can help record essential knowledge of its phytochemical presence, pharmacological and biological significance, and folk medicinal uses, which will enhance its value as a potent pharmaceutical precursor and drug discovery (Noureen Shazia, 2019). Due to the development and advancement in scientific approaches and the pharmaceutical sector, new therapeutic and improvement of drugs for different health issues have been observed in present times. Treatment of patients and development of medicine is the objective of ethnopharmacology and ultimately the verification of the folklore practices (Bagghi, 2021). *Cuscuta reflexa* Roxb belongs to the family Convolvulaceae. It is commonly known as Amarbel means immortal vine. It is leafless,

yellowish green, rootless, and an obligate parasitic plant dependent on the host with the inter-twined stem. The presence of haustoria i.e. modified root penetrates the host stem to absorb water and nourishment from vascular tissue. Some of the bioactive components present in plants are flavonoids, alkaloids, steroids, terpenoids, phenol, tannin, glycosides, etc (Dokuparthi K.S, 2014; S. & B, 2020; Ulhas, 2015). This plant is of many pharmacological activities like anti-oxidants, anti-bacterial, anti-inflammatory, anti-tumor, anti-cancer, anti-viral, hepatoprotective, anti-hypertensive, anti-hair fall, anti-arthritis, anti-coronary, a-glucosides inhibition, androgen-induced alopecia, etc(Saini Puja, 2015; Tanruean K, 2017; Patel, 2012; Vijikumar.S, 2011).The main objective of this study is to determine the qualitative phytochemicals analysis such as alkaloid, tannin, flavonoid, phenol, etc, and identify the phytoconstituent through GC-MS profiling.

### MATERIALS AND METHODS

#### Samples Collection

*Cuscuta reflexa* Roxb stems were collected from the host plant *Vitex negundo* in the area of Ranchi district, Jharkhand, India in the month of March 2023 (Kavita, 2023).

### Authentication and Taxonomical Identification

The plant was authenticated by the Taxonomist University Professor Dr Kunul Kandir Mam. Department of Botany, Ranchi University, Ranchi, and based on Flora's Book "The Botany of Bihar and Orissa Vol I. II, III" and the plants were also identified by BSI, Central National Herbarium reference no.CNH/Tech.II/2023/132, Howrah, Kolkata.

### Preparation of plant extracts for phytochemical analysis

The fresh stem of *Cuscuta reflexa* Roxb collected from host *Vitex negundo* was washed thoroughly under running tap water and then sterilized with the help of distilled water. The stem is dried under shade for 15 to 20 days and fine powdered with the help of a mixer grinder separately. Extracts were prepared by mixing 10 gm of stem powder and 100ml of ethanol. And left for 72 hours in an incubator shaker at 42 degrees Celsius. The Whatman filter paper was used to filter the solution. The extract was kept for 4 days to evaporate the solvent completely and obtain dried extract. Now the final concentration was prepared by adding ethanol to the dried extract. Phytochemical analysis of *Cuscuta reflexa* Roxb stem was done by following standard protocols of (Harborne, 1998; RAMYA, 2024) to identify the various constituents present.

### GC-MS Profiling

10mg of stem powder of *Cuscuta reflexa* Roxb was taken and mixed in 1000ul of Ethanol. The solution was amalgamated in a thermomixer. After that the solutions were centrifuged and filtered in a tube and the impurities settled at the bottom. For further texting the prepared solution was sent to Advanced Instrumental Research Facility, Jawaharlal Nehru University, New Delhi, India. For the identification of various bioactive chemical compounds found in the sample was done from online relevant literature, Pubmed, Google Scholar, Pub Chem, Springer, Science Direct, etc.

## RESULTS AND DISCUSSION

### Phytochemical Analysis

In the present study, phytochemical analysis of *Cuscuta reflexa* Roxb plant stem

collected from host *Vitex negundo* shows the presence of bioactive components such as Alkaloids, Carbohydrate, Protein, Flavonoid, Steroid, Tannin, Glycosides, Terpenoid, Phenol. Only Saponin is absent.

Table 1: Phytochemical Analysis of secondary metabolite of *Cuscuta reflexa* collected from 4+ host *Vitex Negundo*

Ethanollic Extract			
S No.	Phytochemical Test	Observation	Result
1	Alkaloids	Brownish red	Present
2	Protein	White ppt	Present
3	Carbohydrate	Greenish Blue	Present
4	Flavonoid	Yellow	Present
5	Steroid	Dark Green	Present
6	Tannin	Dark Green	Present
7	Glycosides	Orangish Yellow	Present
8	Terpenoid	Redish Deep Brown	Present
9	Phenol	Dark Green	Present
10	Saponin	Foam Not Formed	Not Present



Fig 1: *Cuscuta reflexa* grown on *Vitex negundo*  
**GC-MS Profiling**

For the GC-MS profiling, the prepared ethanollic solution of *Cuscuta reflexa* stem collected from host *Vitex Negundo* was sent to the Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India. The information on phytoconstituents of plants is beneficial because such knowledge will be important for the synthesis of alternative and complementary drugs (Raza Asam Muhammad,

2015). Analysis of bioactive compounds by GC-MS has been reported by different researchers (Perveen, 2013; Rai D.K, 2016). The findings of GC-MS profiling of *Cuscuta reflexa* extracts were efficacious and helpful. *Cuscuta reflexa* extract identified the 79 chemical compounds that show various pharmacological potential. The highest was reported for 2-3 Dihydro BenzoFuran with an area percentage of 32% and retention time of 12.260s. And lowest was reported for 9, 12, 15-Octadecatrienoic acid having a peak area of 0.01% and retention time of 22.709s. The result of the GC-MS analysis showed the presence of 16 bioactive compounds having more than 1% peak area. Compounds that are present in the majority are cis-9-Hexadecenal (6.32%), n-Hexadecanoic acid (4.75%), 5-Hydroxymethyl furfural (5.33%) and Octadecanoic acid (1.30%). In Table 2, the bioactive chemical compound has been identified in GC-MS analysis. Pyrrodine alpha, alpha is present and shows Antimicrobial, Antiviral, Anticancer, Anti-inflammatory, and Antidiabetic activity, and treats Parkinson's (Bais Neetu, 2013; Li Petri, 2021). N-Hexadecanoic acid has Antioxidant, Antiandrogenic, flavor, Nematicide, pesticide, hemolytic, and anticancer (Aparna, 2012). Behenic Alcohol (C<sub>22</sub>H<sub>46</sub>O) has anti-viral protective activity, hair conditioning, moisturizer, lubricant, and hair oil. (Amudha, 2018). Benzofuran is anti-microbial, anti-inflammatory, anti-fungal, anti-hyper glycaemic, anti-parasitic, and anti-tumor (Khanam, 2015; Miao, 2019). 2-3 Dihydro Benzo Furan has anti-oxidant, anti-fungal, anti-proliferate, anti-cancer, anti-diabetic activity (Nath, 2021). Tetra decanoic acid commonly known as myristic acid possesses anti-cancerous, anti-oxidant, nematicide, anti-hypo cholesterolic (Suresh, 2011). Hexadecanoic Acid, Ethyl Ester has anti-microbial, anti-hypo-cholesterolic, and anti-androgenic properties (Sakshy, 2009). Phytol is an Acyclic Diterpene alcohol that has anti-microbial, anti-cancer, diuretic, and anti-diabetic activity and promotes hair growth. (Kumar, 2019). Gamma-tocopherol has anti-oxidant and antifungal activity (Essien, 2012). Vitamin E has antioxidant, anti-inflammatory, immune-enhancing anti-aging, and dermatic compounds (Mitchel, 2003). Cyclopent-4-ene-1, 3-dione has anti-bacterial, antifungal effects, anti-inflammatory, cytostatic, and specific enzyme inhibitory activities (Sevcikova, 2014). Ergost-5-



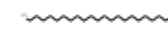
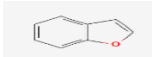
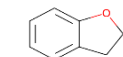


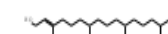
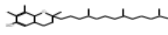
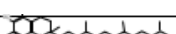
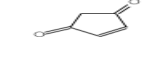

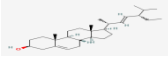
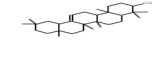





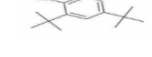
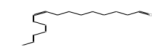
en-3-ol, (3-beta) commonly called campesterol has anti-oxidant and anti-cancer properties (Bradford, 2007). Stigmasterol is anti-cancer, anti-diabetic, anti-inflammatory, anti-fungal, anti-bacterial, anti-stereo arthritis, antiparasitic, antioxidant, neuroprotective, immunomodulatory (Kaur, 2011) beta-Amyrin having anti-inflammatory, gastro protective (Wen, 2018). 9, 12, 15-Octadecatrienoic acid, ethyl ester, Z, Z,-having anti-diabetic, anti-hypertensive, anti-cancer, anti-inflammatory, HEPA protective, anti-acne, anti-chronic, anti-atherosclerotic (Pandit, 2008; Huang, 2010). Dodecanoic Acid, Silver (1+) Salt commonly known as Lauric acid has anti-pyretic, anti-septic, anti-inflammatory, anti-analgesic, and anti-cancer (Malayaman, 2019). Pentadecanoic Acid is anti-microbial, anti-oxidant (Rai, 2016), 9-Octadecenoic Acid (Z)-Ethyl Ester is anti-oxidant, acute neurological disorder, respiratory failure (Nimbeshaho, 2020). Phytol palmitates help in hair growth (Patel, 2014; Amin & Hosseinzadeh, 2015). Phenol, 2, 4-Bis (1, 1-Dimethylethyl) - having anti-oxidant, anti-fungal, anti-cancer, anti-bacterial, analgesic, analagestic (Wang, 2005). cis-9-Hexadecenal has pheromone (Chhabra, 2023).

## DISCUSSION

The pharmacological tenor is determined in medicinal plants due to the secondary metabolite present on it. In the current investigation, the Phytochemical present in the ethanolic extract of *Cuscuta reflexa* grown on *Vitex negundo* were Alkaloid, Carbohydrate, Protein, Flavonoid, Steroid, Tannin, Phenol, and Glycoside but Saponins are absent. In the study of Shika et.al. (2013), *C. reflexa* showed the presence of alkaloids, saponins, tannins, and phenols but glycosides, steroid, and flavonoids are absent. In the research work of Akarsh S and Thippeswamy B. (2020) absence of Alkaloids, Saponin, and Steroids but the presence of flavonoids, terpenoids, glycosides, tannins, and phenols. In the work of Permesha et.al. (2014) on the phytochemical analysis of *C. reflexa*, the methanolic extract shows the presence of alkaloids, flavonoids, steroids, saponins, glycosides, phenol, and tannins. In Nahid et.al (2019) a total of 21 n-hexane soluble compounds were identified from the whole plant of *Cuscuta reflexa* by GC-MS analysis. The



Table 2: Identified phytochemicals and their bioactivities of *Cuscuta reflexa* grown on *Vitex Negundo*

Name of compound	Nature of Compound	Structure	Pharmacological Activity
Pyrrrodine alpha, alpha	Tetrahydropyrrole		anti-microbial, anti-viral, anti-cancer, anti-inflammatory, anti-diabetic, treat Parkinson
n-Hexadecanoic acid	Palmitic acid (Fatty Acid)		antioxidant, antiandrogenic, flavor, nematicide, pesticide, hemolytic anti-cancer, anti-inflammatory
Behenic Alcohol	Behenic Alcohol		anti-viral protective activity, hair conditioning, moisturizer, lubricant, hair oil
Benzofuran			antitumor, antimicrobial, antihyperglycemic, analgesic, anti-inflammatory, antiviral, antipyretic activities, antioxidant
2,3 dihydro benzofuran			antitumor, antimicrobial, antihyperglycemic, analgesic, anti-inflammatory, antiviral, antipyretic activities
Tetradecanoic acid	Myristic acid		antioxidant and cancer preventive
Hexadecanoic acid, Ethyl ester	Ester		anti-microbial, anti-hypo-cholesterolic, anti-androgenic properties, antioxidant
Phytol	Acyclic Diterpene Alcohol		anticancer, antioxidant, anti-inflammatory, diuretic, antitumor, chem preventive, antimicrobial, use in vaccine formulations, promote hair growth
Gamma-Tocopherol	Tocopherol		anti-oxidant, antifungal activity
Vitamin E	Fat Soluble Vitamin		anti-oxidant, anti-inflammatory, immune enhancing, anti-aging, anti dermatic compound
Cyclopent-4-ene-1,3-dione			antibacterial, antifungal effects, anti-inflammatory, cytostatic
Ergost-5-en-3-ol, (3-beta)	Sterol		anti-oxidant, anti-cancer properties
Stigmasterol			Anti-Estero, arthritis, anti-inflammatory, anti-diabetic, anti-microbial, immuno-modulatory, anti-cancer, anti-mutagenic, anti-tumor, cytotoxicity, anti-inflammatory, gastro protective
Beta-Amyrin	Triterpenes		
9,12,15-Octadecatrienoic acid (Z, Z)			antipyretic, anti-septic, anti-inflammatory, anti analgesic, anti-cancer
Dodecanoic Acid, Silver (1+) Salt	Lauric acid		anti-pyretic, anti-septic, anti-inflammatory, anti analgesic, anti-cancer
Pentadecanoic acid			antioxidant, anti-microbial
9-Octadecenoic Acid (Z)-, Ethyl Ester	Stearic acid		antioxidant and cancer preventive
Phytol Palmitate			hair growth
Phenol, 2,4-bis (1,1)-dimethyl ethyl	Phenolic Compound		analgesic, antioxidant, antiseptic, antibacterial, anticancer, antifungal
cis-9-Hexadecenal			pheromone



phyto constituents of this parasitic plant *Cuscuta reflexa* Roxb are dependent on the host diversity, which is recommended by many previous studies (Tanruean et.al 2017).

## CONCLUSION

The present study gives an overview of the presence of a large number of secondary metabolites including alkaloids, flavonoids, terpenoids, tannin, phenol, etc. GC -MS identifies the presence of various bioactive compounds that show pharmacological importance such as anti-microbial, antibacterial,

anti-oxidant, anti-cancer, anti-diabetic, anti-diuretic, hepatoprotective, anti-inflammatory, anti-arthritic, promotes hair growth, etc and confirm all medicinal practices used by earlier studies. Conducting an analysis of the GC-MS phytochemical profiles of *Cuscuta reflexa* grown on the host plant *Vitex negundo* provides a detailed understanding of the secondary metabolites produced. *Cuscuta reflexa* is an obligate parasitic plant and it absorbs nutrients from its host plants, potentially leading to variations in its phytochemical composition based on the host. In our investigation, GC-MS is the synergistic combination of two analytical methods to separate, identify, and quantify the different compounds in the test sample. The present phytochemical analysis identifies *Cuscuta reflexa* as very valuable; they can be used in pharmacology, pharmacognosy, phytochemistry, natural products and the pharmaceutical industry for the development of new drugs or modification of existing drugs. Thus, the present study will act as an add-on to the existing knowledge of the pharmacological uses of ethnomedicinal plants and helpful in further studies.

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