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GC-MS analysis and antibacterial activity of *Cuscuta reflexa* against bacterial pathogens

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ABSTRACT

Objective: To find out the antibacterial efficacy of *Cuscuta reflexa* (*C. reflexa*) and examine the chemical composition of the essential oil from *C. reflexa* by gas chromatography and mass spectrometry (GC-MS).

Methods: Antibacterial potency of *C. reflexa* plant extracts has been tested against *Bacillus subtilis* IFO 3026, *Sarcina lutea* IFO 3232, *Xanthomonas campestris* IAM 1671, *Escherichia coli* IFO 3007, *Klebsiella pneumoniae* ATTC 10031, *Proteus vulgaris* MTCC 321 and *Pseudomonas denitrificans* KACC 32026 by disc diffusion assay. The chemical compositions of the essential oil were analyzed by GC-MS.

Results: GC-MS analysis identified sixteen compounds from essential oil of *C. reflexa*, of which piperitone (15.19%), caryophyllene (12.55%), β-elemene (10.35%), elemol (6.20%), α-selinene (4.49%), α-humulene (3.98%) and thujone (5.18%) were the major compounds. α-Bergamotene and juniper camphor were identified as the minor compounds. Dichloromethane and petroleum ether extracts of *C. reflexa* showed a great potential of antibacterial activity against all tested bacteria. Minimum inhibitory concentrationvalues of various extracts ranged from 16 to 512 μg/mL.

Conclusions: These extracts showed good antibacterial activity and could lead to new antibacterial drug designing.

1. Introduction

Bacterial infectious diseases are one of the important causes of mortality and morbidity[1]. These are the second leading cause of death worldwide and the biggest threat for child and young adults[2]. Gram-positive bacteria are mainly responsible for various disease including nosocomial infection, skin infection, pruritic eruption, endocarditis, pneumonia and *etc*. Gram-negative bacterium such as *Escherichia coli* (*E. coli*) is present in human intestine and causes lower urinary tract infection, gastrointestinal tract, wound infections, bacteriaemia, pneumonia, septicaemia and meningitis[3].

Multiple drug resistance in human pathogenic microorganisms has also been developed due to unscientific and impractical uses of commercial antimicrobial drugs[4]. Multiple factors are responsible for the development of antibiotic resistance including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors[5]. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents[6].

Natural plant products may offer a new source for developing new antibacterial agents[3]. Regarding this issue over the last few

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years, numbers of studies have been conducted to identify plant derived substances for the treatment of bacterial disease[4,7-10]. According to Pradhan *et al*. (2013), 80% of world's population relies on plant derived medicines for combating disease and maintenance health[11]. Plant derived medicines are relatively cheap, environmental friendly and low adverse effect, which increase its acceptability to consuming public[12,13].

Various research demonstrated that plants contain some bioactive phytochemical constituents which are mainly responsible for combating against disease[14]. In addition, different types of secondary metabolites are produced in plants which show their potentiality against different ailments.

Cuscuta reflexa (*C. reflexa*) (dodder) is a perennial parasitic herb belongs to the Convolvulaceae family[15]. It is leafless, rootless, green yellowish and thread like twining parasitic herb[16] and has no photosynthetic system. In the traditionally medicine, this herb is used to cure tumours, body ache, body pain, oedema, skin infection, melancholy, insanity, control blood sugar and jaundice[17-20]. It is also used to treat impotence, premature ejaculation, sperm leakage, frequent urination, ringing in the ears, lower back pain, sore knees, white discharge from the vagina (leucorrhoea), dry eyes, blurred vision, tired eyes and itching[21].

In this study, we tested the antibacterial efficacy of various organic extracts of *C. reflexa* and examined the chemical composition of the essential oil from *C. reflexa* by gas chromatography and mass spectrometry (GC-MS), with emphasis for the possible future use of the extracts as an alternative to chemical bactericides.

2. Materials and methods

2.1. Plant material

Whole plant of *C. reflexa* was collected from the Jessore region of Bangladesh in February 2014 and identified by Bushra Khan, Principal Scientific Officer, National Herbarium, Mirpur, Dhaka, Bangladesh where a voucher (DACB 38572) has been deposited.

2.2. Preparation of plant extracts

Collected plant materials were washed under running tap water, then distilled water and chopped into small pieces, and air dried under shade at room temperature for fifteen to twenty days. The dried plant materials were pulverized into the powder form. Different extracts including ethanol, methanol, ethyl acetate, *n*-hexane, dichloromethane and petroleum ether were prepared by using this powder at 37 ℃. Then, the solvents were evaporated at 37 ℃ by keeping in incubator. Finally, all the extracts were concentrated into 4 mg/mL and were stored in refrigerator at 4 ℃ in sterile container for further use.

2.3. Preparation of essential oil

The fresh plant samples were subjected to solvent extraction process using a Clevenger type apparatus. Chloroform was used as a solvent to extract essential oil. Then, the oil was dried over anhydrous Na_2SO_4 and preserved at 4 ℃ in a sealed vial for further analysis[22].

2.4. Test organism

Bacillus subtilis IFO 3026 (*B. subtilis*), *Sarcina lutea* IFO 3232 (*S. lutea*), *Xanthomonas campestris* IAM 1671 (*X. campestris*), *E. coli* IFO 3007, *Pseudomonas denitrificans* (*P. denitrificans*), *Proteus vulgaris* MTCC 321 (*P. vulgaris*), *Klebsiella pneumoniae* ATTC 10031 (*K. pneumoniae*) were used in this study, obtained from the Microbiology laboratory of Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh.

2.5. Determination of antimicrobial activity

In vitro antimicrobial activity assay was performed by using disc diffusion method[23]. Whatman No. 1 filter paper disc of 5.5 mm diameter were prepared by impregnated with extract. Each disc contains 300 μg respective extract. For Minimum inhibitory concentration (MIC) calculation, blank filter paper discs were impregnated with different concentration of each extracts including 16, 32, 64, 128, 256 and 512 μg/mL. After drying, paper discs were placed on nutrient agar culture media inoculated with the test bacteria and incubated at 37 ℃ for 24 h. After incubation, the culture plates were examined and zone of inhibition measured in millimeter scale. Standerd antibiotic discs were used as positive control and blank disc impregnated with solvents followed by evaporation was used as negative control.

2.6. GC-MS analysis

GC-MS analysis was carried out in Bangladesh Council of Scientific & Industrial Research. GC-MS was performed by using Agilent Technologies 7890A (GC Model) and Agilent Technologies 5975C (MS model) inert XL EI/CI MSD with triple axis detector. One microliter of extract was injected in splitless mode in injection port of GC column. The inlet temperature was set at 250 ℃ and oven temperature was programmed as 60 ℃ for 1 min then 3 ℃ min^{-1} to 240 ℃ min^{-1} for 4 min. Total run time was 60 min. Helium gas was used as the carrier gas at a constant flow rate of 1.0 mL/min. The

interface temperature (GC to MS) was set to 280 ℃.

MS was set in scan mode. MS quad temperature was 150 ℃, MS source temperature was 230 ℃. Ions were obtained by electron ionization mode. Molecular ions (mass range) were monitored for identification which was set at 50-550 m/z. Peak area denoted the relative percentage of oil constituents.

2.7. Statistical analysis

Each experiment was run in triplicate and mean values were calculated with SD. SPSS version 11.0 was used for the data analysis.

3. Results

3.1. Antibacterial activity of plant extracts

The *in vitro* antibacterial activities of various organic extracts of *C. reflexa* against the tested bacteria were assessed by the presence or absence of inhibition zone. The organic extracts exhibited antibacterial activity against two Gram-positive and four Gram-negative bacteria (Figure 1). Dichloromethane and petroleum ether extracts of *C. reflexa* revealed great potential of antibacterial activity against all bacteria (zone ranging from 8 to 14 mm) (Figure 2). The highest zone of inhibition is measured by petroleum ether extract against *P. vulgaris* (14 mm). Methanol and *n*-hexane showed moderate antibacterial activity against *K. pneumoniae* and *X. campestris* (8 to 9 mm). Ethanol extract also showed moderate activity against *K. pneumoniae* (8 mm). Table 1 shows the MIC values of various extract against the tested bacteria. The lowest MIC value of dichloromethane extract is 16 μg/mL against *S. lutea*. The second lowest MIC value of petroleum ether and methanol extract were measured with 32 μg/mL against *S. lutea* and *X. campestris*, respectively.

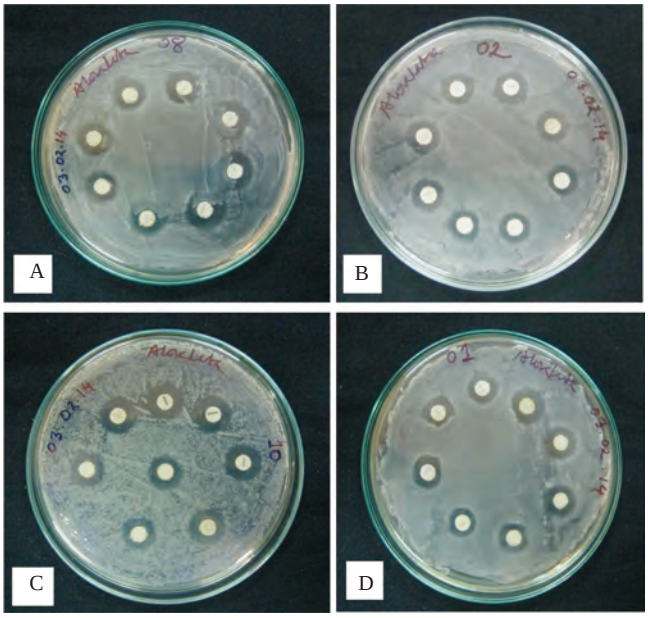

Figure 2. Different concentrations of petroleum ether extract of *C. reflexa* produce zones of inhibition against (A) *B. subtilis*, (B) *S. lutea*, (C) *P. vulgaris*, and (D) *P. denitrificans*.

Table 1
MIC of various extracts of *C. reflexa* (μg/mL).

Bacteria	Dichloro-methane	Methanol	Ethanol	Ethyl acetate	*n*-Hexane	Petroleum ether
B. subtilis	128	-	-	512	-	128
S. lutea	16	-	-	512	-	32
X. campestris	512	32	-	256	128	64
E. coli	512	512	-	-	128	64
K. pneumoniae	256	64	256	-	256	512
P. vulgaris	128	-	-	-	128	512
P. denitrificans	512	512	-	-	64	512

3.2. Chemical composition of essential oil

GC-MS analysis of the essential oil led to the identification of

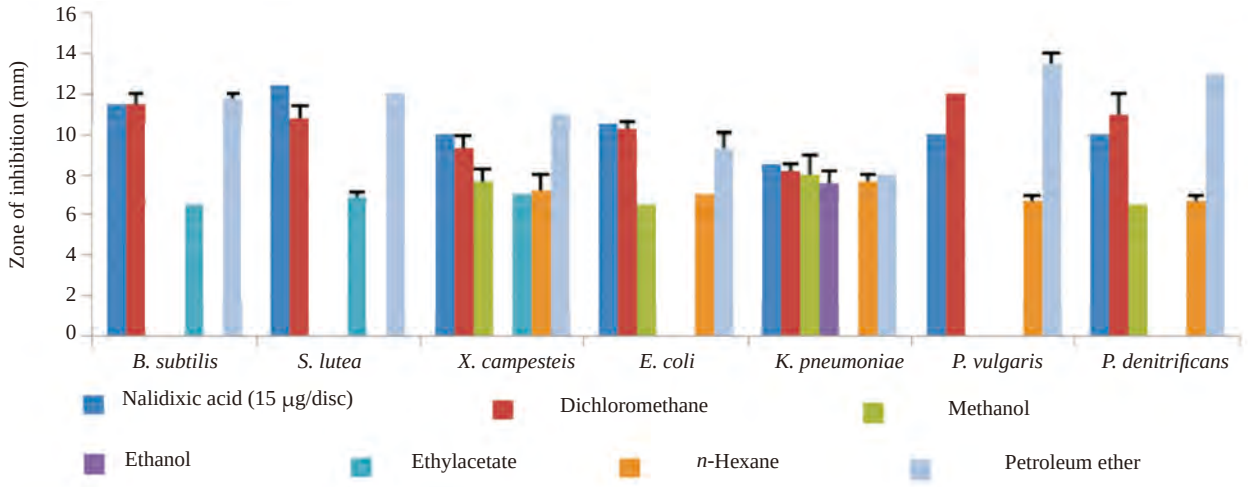

Figure 1. Antibacterial activity of various extracts of *C. reflexa*. Values are given as mean ± SD (n = 3).

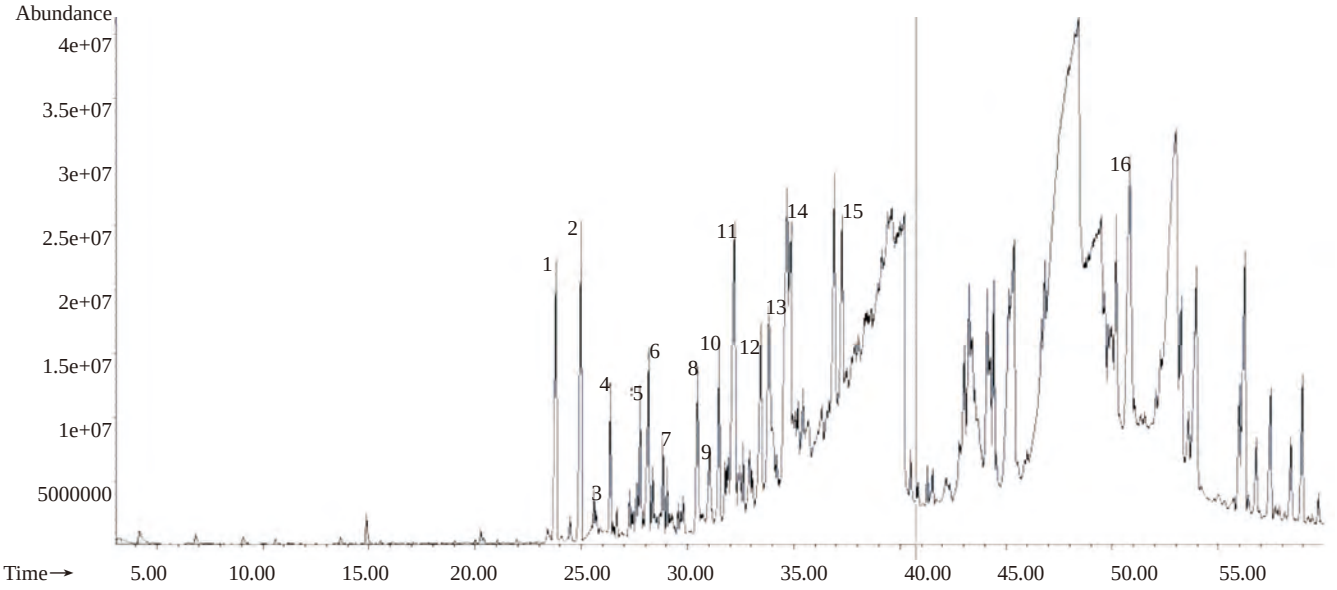

Figure 3. GC-MS chromatograph of essential oil extract of *C. reflexa*.

16 different compounds. List of identified were shown in Table 2. GC-MS chromatograph of oil extracts showed sixteen peaks, each peak indicating the presence of sixteen compounds (Figure 3). The major compounds detected in oil were piperitone (15.19%), caryophyllene (12.55%), β elemene (10.35%), elemol (6.20%), α-selinene (4.49%), α-humulene (3.98%) and thujone (5.18%). The component in lower amount α-bergamotene and juniper camphor, were identified as minor compound.

Table 2

Chemical composition of essential oil of *C. reflexa*.

Peak No.	Retention time	Area %	Name of the compound	Formula
1	23.819	10.35	β Elemene	$C_{15}H_{24}$
2	25.008	12.55	Caryophyllene	$C_{15}H_{24}$
3	25.597	0.87	α-Bergamotene	$C_{15}H_{24}$
4	26.378	3.98	α-Humulene	$C_{15}H_{24}$
5	27.777	3.86	β-Selinene	$C_{15}H_{24}$
6	28.167	4.49	α-Selinene	$C_{15}H_{24}$
7	28.855	2.66	Widdrol	$C_{15}H_{26}O$
8	30.493	6.20	Elemol	$C_{15}H_{26}O$
9	31.041	2.42	Nerolidol 1	$C_{15}H_{26}O$
10	31.496	5.18	Thujone	$C_{15}H_{24}$
11	32.230	15.19	Piperitone	$C_{10}H_{16}O$
12	33.460	7.05	2,6,10-Trimethylundecan-(5E)-2,5,9-trien-4-one	$C_{14}H_{22}O$
13	33.839	1.14	Juniper camphor	$C_{15}H_{26}O$
14	34.707	1.89	β-Eudesmol	$C_{15}H_{26}O$
15	37.301	1.51	Farnesol	$C_{15}H_{26}O$
16	50.852	2.58	Phytol	$C_{20}H_{40}O$

4. Discussion

Due to the enormously increasing of the antibiotic resistant pathogen, it has became inevitable to find out the new drugs in pharmaceutical industries. Plant extract can be the best alternative for antibiotic against pathogen particularly with the presence of essential oil[22]. Previous study demonstrated methanol extract of *C. reflexa* showed antibacterial activity against *E. coli* and *K. pneumoniae*. The result of present study is persistent with previous studies[15,16]. This study showed the various organic extract exhibited great potential inhibitory effect against the tested bacteria.

The constituents of volatile matter, long and branched chain hydrocarbons, alcohols, acids and esters can be identified by GC-MS. GC-MS is one of the best techniques by which more precise analysis is performed[24]. The GC-MS analysis of essential oil of *C. reflexa* revealed the presence of oxygenated mono and sesquiterpene hydrocarbons like, piperitone, caryophyllene, α-selinene, β-selinene, α-humulene and phytol, *etc*. Previous research showed that these components play major role in antibacterial activities[22,25,26]. On the other hand the compound in lower amount such as α-bergamotene and juniper camphor also showed antibacterial activity possibly by synergism with the other active compounds[22].

This study is preliminary step which indicates the further study is necessary to investigate the toxicology of this plant extracts for finally formulating and synthesizing a new antibacterial drug.

Conflict of interest statement

We declare that they have no conflict of interest.

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