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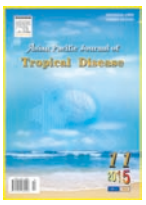

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Proximate composition, phytochemical screening, GC-MS studies of biologically active cannabinoids and antimicrobial activities of *Cannabis indica*

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ABSTRACT

Objective: To investigate the proximate composition, minerals analysis, phytochemical screening, gas chromatography-mass spectrometry (GC-MS) studies of active cannabinoids and antimicrobial activities of *Cannabis indica* (*C. indica*) leaves, stems, and seeds.

Methods: Standard qualitative protocols of phytochemical screening were accomplished for the identification of biologically active phytochemicals. Minerals in plant samples were analyzed by using atomic absorption spectrophotometer. The resins of *C. indica* were analyzed for medicinally active cannabinoid compounds by GC-MS. The sample for GC-MS study was mixed with small quantity of *n*-hexane and 30 mL of acetonitrile solution for the identification of cannabinoids. Agar well diffusion method was used for antibacterial activity. For antifungal activity, the tested fungal strains were sub-cultured on Sabouraud's dextrose agar at 28 ℃.

Results: Mineral analysis revealed the presence of sodium, potassium, magnesium and some other minerals in all parts of *C. indica*. Phytochemical investigation showed the presence of alkaloids, saponins, tannins, flavonoids, sterols and terpenoids. *C. indica* divulged wide spectrum of antibacterial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. The extracts of plant leaves, seeds and stems showed significant antifungal activities against *Aspergillus niger*, *Aspergillus parasiticus*, and *Aspergillus oryzae*. The biologically active cannabinoids of delta-9-tetrahydrocannabinol (25.040%) and cannabidiol (resorcinol, 2-p-mentha-1,8-dien-4-yl-5-pentyl) (50.077%) were found in *Cannabis* resin in high percentage.

Conclusions: The findings of the study suggested that the existence of biologically active remedial cannabinoids in elevated concentrations and antimicrobial bioassays of *C. indica* make it a treasured source to be used in herbal preparation for various ailments.

1. Introduction

Cannabis indica (*C. indica*) (hemp) belonging to the family Cannabaceae is an angiosperm and *Cannabis* is the genus. In temperate climates, hemp plant itself is easy to nurture which requires fertilizer, fertile soil, and water but no herbicides and pesticides. It is harvested usually in 120 days after attaining a height of 10–15 feet[1]. Leaves of *Cannabis* are bitter, aphrodisiac, alterative, astringent, tonic, abortifacient, intoxicating, analgesic and stomachic. They are used in otalgia, diarrhoea, convulsions, abdominal disorders, tetanus, skin diseases, malarial fever, hysteria, dysentery, hydrophobia, insomnia, gonorrhoea and colic. Its excessive use can cause cough, melancholy, dyspepsia, impotence, insanity, dropsy and restlessness[2]. Cannabinoid drugs and *Cannabis* are extensively used to treat disease or lighten symptoms, but their effectiveness for specific indications is not obvious[3]. Globally in 2012, it is one of the most well-liked recreational drugs with an estimated 178 million people (15 to 64 years of age) used *Cannabis* at least once[4]. Medical *Cannabis* refers cannabinoids or *Cannabis* was used as a medical therapy to alleviate symptoms or treat a disease. Administration of cannabinoids can be executed sublingually and orally. They can be inhaled, can be smoked, made into tea or mixed with food. They also can be taken in herbal form, naturally extracted from the plant, gained by cannabidiol

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isomerisation, or unnaturally manufactured[5]. The essential oils and fatty acids of plants were investigated by gas chromatography-mass spectrometry (GC-MS) analysis[6]. Due to the presence of essential oils and fatty acids, different extract of plant species revealed noteworthy antibacterial and antifungal activities[7]. Typical human diet consists of carbohydrates, proteins, fats, water, minerals and vitamins. Human health constantly depends on balanced mineral contents in the body and if the balance is below or above the limits, it causes abnormalities in human health[8]. For man and animals, plant is one of the sources of mineral. To find out the mineral composition of medicinal plant and to correlate it for the treatment of diseases, researchers are trying[9]. For chronically sick patients, few countries have legally recognized medicinal grade *Cannabis*. Specialized companies of the Netherlands and Canada have government-run programs, in which they supply quality-controlled herbal *Cannabis*[10]. To permit the medical use of *Cannabis*, laws have been introduced in the United States (23 states) and Washington, DC (May 2015)[11]. For several life threatening diseases, the major responsible bacterial pathogens are *Salmonella, Staphylococcus*, *Pseudomonas* and *Bacillus*[12]. Due to subcutaneous, deep inside and superficial infections, pathogenic fungi are responsible for various diseases. Pathogenic fungi infected humans significantly increased in all parts of the world[13].

This study was therefore, designed to investigate the proximate composition, minerals analysis, phytochemical screening, GC-MS studies of active cannabinoids and antimicrobial activities of *C. indica* leaves, stems, and seeds.

2. Materials and methods

2.1. Collection of plant

Mature plants of *C. indica* were collected from the Medicinal Botanical Garden of PCSIR Laboratories Complex Peshawar, KPK, Pakistan. The plants were cleaned and separated to stems, seeds and leaves parts. The leaves and stems were dried and grinded by laboratory mill into powder form.

2.2. Proximate composition and mineral analysis

For ash value determination of the plant, the method of Premnath *et al*. was used[14]. Furnace was used for the determination of ash value. For the determination of moisture value of the plant, the method of Ashutosh *et al*. was used[15]. Standard procedures were followed for the determination of fats, crude fibers and crude proteins. Minerals in plant samples were analyzed by using atomic absorption spectrophotometer (Shimadzu, Japan) equipped with graphite furnace and flame.

2.3. Phytochemical screening

For the screening of alkaloids, carbohydrates, tannins, flavonoids, saponins, terpenoids, steroids *etc*., qualitative tests were performed on different extracts by using standard procedures[16-18].

2.4. GC-MS studies of active cannabinoids

2.4.1. Method of analysis and GC-MS conditions

A gas chromatograph hyphenated to a mass spectrometer QP 2010 plus (Shimadzu, Tokyo, Japan) outfitted with an auto injector (AOC-20i) and auto sampler (AOC-20S) was used for medicinally active cannabinoid compounds. The conditions of the GC-MS used during analysis were: ion source temperature (280.0 ℃), interface temperature (280.0 ℃), and solvent cut time (2.0 min). Resin (10 g) from sample was grinded and extracted with 200 mL of *n*-hexane under reflux by maintaining the temperature between 40–50 ℃ for 90 min. The extract was then evaporated under vacuum by rotary evaporator [PLC/MBC (Phy. Std.)/011 Eyela] and stored in refrigerator.

2.4.2. Analysis of resin extract by GC-MS

Sample (0.1–0.2 g) was taken from *n*-hexane extract and stirred with small quantity of *n*-hexane and 30 mL of acetonitrile solution was added and shacked in separating funnel. To the acetonitrile layer, 600 mL of solution (water + sodium chloride + *n*-hexane) was added and shacked. To the organic layer, anhydrous Na_2SO_4 was added and solution was filtered and evaporated to dryness. To the residue, 1 mL of internal standard was added and diluted with cyclohexane. This solution was filtered to obtain a clear solution. This clear solution (1 μL) was injected to GC-MS. Analysis was carried out using Shimadzu QP-2010 Plus equipped with DB-5 ms column (0.25 μm, 0.255 mm and 30 m) with helium as the carrier gas. Injector temperature was 300 ℃ and injector mode was split.

2.5. Antibacterial susceptibility assay

The antibacterial activity of *C. indica* extracts was determined by agar well diffusion method[19]. Six bacterial strains *i.e*. *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae (K. pneumoniae)*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Proteus mirabilis* (*P. mirabilis*) were used for this activity. The stock solution was prepared by the addition of 1 mg of plant extracts in 10 μL dimethylsulfoxide. Mueller-Hinton agar media (2.8 g/100 mL) and broth media (1.3 g/100 mL) were prepared in distilled water and sterilized in autoclave at 121 ℃ temperature and 1.5 pounds pressure for 20 min. The media was poured into the Petri plates and placed in an incubator at 37 ℃. The microbial cultures were inoculated in nutrient broth and incubated in shaking water bath for 18 h at 250 r/min at 37 ℃. And 100 μL of standardized inoculum (10^6 CFU/mL; 0.5 McFarland) of each test bacterium were spread on sterile Mueller-Hinton agar plate. The extracts solution (10 μg/μL) was introduced in the wells of agar plates and then incubated at 37 ℃ for 24 h. Levofloxacin antibiotic (5 μg/μL) was used as positive control,

while dimethylsulfoxide was taken as negative control. The zone of inhibition (mm) was recorded to the nearest size[20].

2.6. Antifungal susceptibility assay

Five fungal strains including *Aspergillus niger* (*A. niger*), *Aspergillus parasiticus* (*A. parasiticus*), *Aspergillus oryzae* (*A. oryzae*), *Aspergillus fumigatus* (*A. fumigatus*), and *Candida albicans* (*C. albicans*) were used for antifungal susceptibility bioassay. Tested fungal strains were sub-cultured on Sabouraud's dextrose agar at 28 ˚C for 3–5 days. For fungal strains refreshment, nutrient broth media (28 g/1 000 mL) was prepared in distilled water, sterilized in autoclave at 121 ˚C temperature and 1.5 pounds pressure for 20 min and distributed evenly to 4 volumetric flasks (250 mL). Inoculation of fungal strains was executed to each volumetric flask and placed in incubator at 30 ˚C for 3 days. Sterilized Sabouraud's dextrose agar plates were taken and a sterile cork borer (6 mm diameter) was used to bore wells in the agar. Plant extracts (10 μg/μL) were then introduced into each well. The plates were then incubated at 28 ˚C. Clotrimazole antibiotic (5 μg/μL) was used as positive control, while dimethylsulfoxide was taken as negative control. The zone of inhibition (mm) was recorded to the nearest size[21].

3. Results

3.1. Proximate composition and mineral analysis

The proximate composition and mineral analysis of *C. indica* was given in Table 1. The moisture value of the leaves [(8.46 ± 0.92)%], seeds [(11.90 ± 0.46)%] and stems [(6.86 ± 0.31)%] of *C. indica* was recorded. Plant seeds exhibited significant ash value [(12.74 ± 0.52)%] comparatively. High percentage of crude fiber [(13.49 ± 0.77)%] was found in leaves of the plant followed by crude protein [(8.63 ± 0.69)%] and fat [(5.81 ± 0.84)%] contents. In plant seeds, fats were presented in high percentage [(28.33 ± 0.78)%] in comparison with crude proteins [(17.42 ± 0.61)%] and crude fibers [(16.59 ± 0.38)%]. High content of crude fiber [(23.61 ± 0.52)%] were analyzed in plant stems. The mean concentration levels of minerals found in *C. indica* were given in Table 2. The highest sodium [(12.35 ± 0.23)%], potassium [(10.54 ± 0.55)%], iron [(9.46 ± 0.24)%], and calcium [(5.94 ± 0.36)%] contents were found in plant leaves. The lowest copper contents were found in seeds [(0.84 ± 0.17)%] and magnesium contents [(0.88 ± 0.10)%] were found in stems of the *C. indica*.

Table 1
Proximate composition of various parts (leaves, seeds and stems) of *C. indica*. %.

Parameters	Leaves	Seeds	Stems
Moisture	8.46 ± 0.92	11.90 ± 0.46	6.86 ± 0.31
Ash	10.67 ± 0.68	12.74 ± 0.52	7.37 ± 0.47
Fat	5.81 ± 0.84	28.33 ± 0.78	2.67 ± 0.23
Crude fiber	13.49 ± 0.77	16.59 ± 0.38	23.61 ± 0.52
Crude protein	8.63 ± 0.69	17.42 ± 0.61	4.21 ± 0.26

Table 2
Minerals analysis of various parts (leaves, seeds and stems) of *C. indica*. %.

Minerals (ppm)	Leaves	Seeds	Stems
Sodium	12.35 ± 0.23	3.53 ± 0.29	3.83 ± 0.23
Potassium	10.54 ± 0.55	7.62 ± 0.32	6.58 ± 0.37
Iron	9.46 ± 0.24	8.38 ± 0.46	7.51 ± 0.29
Magnesium	3.78 ± 0.22	1.58 ± 0.10	0.88 ± 0.10
Phosphorus	4.85 ± 0.27	3.63 ± 0.12	2.67 ± 0.15
Calcium	5.94 ± 0.36	4.55 ± 0.27	2.24 ± 0.25
Zinc	2.26 ± 0.18	1.52 ± 0.13	1.89 ± 0.14
Copper	1.86 ± 0.10	0.84 ± 0.17	1.24 ± 0.12

3.2. Phytochemical screening

Phytochemical screening of leaves, seeds and stems of *C. indica* showed the presence of alkaloids, saponins, tannins, phenols and flavonoids (Table 3). Glycosides were absent in all parts of the plant. Sterols and terpenoids were present in leaves and seeds of the plant.

Table 3
Phytochemical analysis of methanolic extract of *C. indica*.

Phytochemicals	Leaves	Seeds	Stems
Alkaloids	++	+	++
Saponins	+	+	+
Tannins	+++	+	++
Phenols	+++	++	+
Flavonoids	++	+	+
Glycosides	ND	ND	ND
Sterols	+	++	ND
Terpenoids	+++	++	ND

+: Small quantity; ++: Average quantity; +++: Large quantity; ND: Not detected.

3.3. Cannabinoids studies by GC-MS

Mature plants of *C. indica* were collected from Medicinal Botanical Garden of PCSIR Laboratories Complex Peshawar and dried under shed. Its resin was prepared and active cannabinoid compounds were analyzed medicinally by GC-MS. The biologically active cannabinoids were delta-9-tetrahydrocannabinol and cannabidiol was found in *Cannabis* resin in high percentage (Table 4). The highest percentage of delta-9-tetrahydrocannabinol present was 25.040% and that of cannabidiol (resorcinol, 2-p-mentha-1,8-dien-3-yl-5-pentyl) was 50.077%. Other canabinoids quantified were p-heptylacetophenone (2.071%), delta 9-tetrahydrocannabinovarin (2.032%), cannabivarin (0.450%), cannabigerol (0.703%), 6,6,9-trimethyl-3-pentyl-6H-dibenzo(b,d)pyran-1-ol (15.420%) and cannabichromene (3.509%).

Table 4
Cannabinoids identified by GC-MS analysis.

Identified cannabinoids	Retention time (Min)	Peak area (mV.S)$^{\beta}$	Conc. (%)$^{\#}$	SD*
p-Heptylacetophenone	17.417	34 751	2.071	0.004
THCV	18.297	24 124	2.032	0.001
CBV	18.803	10 827	0.450	0.006
CBD	19.184	846 058	50.077	0.013
CBC	19.245	75 661	3.509	0.008
CBG	19.626	11 790	0.703	0.016
THC	20.007	407 242	25.040	0.003
CBN	20.506	252 936	15.420	0.005

THCV: Delta-9-tetrahydrocannabinovarin; CBV: Cannabivarin; CBD: Cannabidiol (resorcinol, 2-p-mentha-1,8-dien-4-yl-5-pentyl); CBC: Cannabichromene; CBG: Cannabigerol; THC: Delta-9-tetrahydrocannabinol; CBN: 6,6,9-Trimethyl-3-pentyl-6H-dibenzo(b,d)pyran-ol. $^{\beta}$: Average of three measurements; $^{\#}$: Percent concentration; *: Standard deviations.

3.4. Antibacterial susceptibility assay

In vitro antibacterial activities of various extracts of different plant parts (leaves, seeds and stems) of *C. indica* were given in Tables 5–7. The leaves, seeds and stems extracts of *C. indica* were tested against six multidrug resistant bacterial strains. The methanol extract of plant leaves showed significant activities against *P. mirabilis* [(19.00 ± 0.83) mm], *S. aureus* [(18.00 ± 0.41) mm], *K. pneumoniae* [(17.00 ± 0.65) mm], and *B. cereus* [(16.00 ± 0.53) mm]. Chloroform extracts were active against *K. pneumoniae* [(16.00 ± 0.74) mm], *P. aeruginosa* [(15.00 ± 0.66) mm] and completely inactive against *S. aureus* and *P. mirabilis*. Plant leaves *n*-butanol extract revealed prominent activities against *B. cereus* [(15.00 ± 0.70) mm] and *S. aureus* [(14.00 ± 0.49) mm] (Table 5). Antibacterial activities of methanol extract of plant seeds were prominent against *P. aeruginosa* [(16.00 ± 0.46 mm)] and *K. pneumoniae* [(15.00 ± 0.53) mm]. Chloroform and *n*-hexane extracts of plant seeds showed significant activities against *S. aureus* [(16.00 ± 0.53) and (17.00 ± 0.56) mm], *B. cereus* [(15.00 ± 0.63) and (14.00 ± 0.50) mm] and *P. aeruginosa* [(15.00 ± 0.66) and (12.00 ± 0.53) mm] respectively (Table 6). Plant stems ethyl acetate and methanol extracts were active against *S. aureus* [(16.00 ± 0.76) and (12.00 ± 0.41) mm], *B. cereus* [(17.00 ± 0.83) and (16.00 ± 0.70) mm], *E. coli* [(17.00 ± 0.57) and (15.00 ± 0.63) mm] and *P. mirabilis* [(16.00 ± 0.67) and (15.00 ± 0.72) mm] respectively (Table 7). All the plant parts and extracts revealed a range of inhibitory concentrations.

3.5. Antifungal susceptibility assay

In vitro antifungal activities of various extracts of different plant parts (leaves, seeds and stems) of *C. indica* were given in Tables 8–10. Plant leaves methanol, *n*-hexane, chloroform and ethyl acetate extracts revealed significant antifungal activities against *A. niger* [(14.00 ± 0.64), (13.00 ± 0.45), (12.00 ± 0.71) and (12.00 ± 0.53) mm], *A. parasiticus* [(16.00 ± 0.52), (13.00 ± 0.47), (12.00 ± 0.45), (17.00 ± 0.62) mm], and *A. fumigatus* [(17.00 ± 0.58), (15.00 ± 0.63), (11.00 ± 0.27) and (14.00 ± 0.55) mm] respectively (Table 8). Prominent antifungal activities were recorded by plant seeds methanol, *n*-hexane and chlorform extracts against *A. niger* [(12.00 ± 0.28), (14.00 ± 0.43) and (15.00 ± 0.57) mm]. Seeds ethyl acetate

Table 5
Antibacterial activity of the extracts of *C. indica* leaves. mm.

Plant part and standard	Plant extracts and standard	Zone of inhibition ± SD					
		S. aureus	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*
Leaves	Methanol	18.00 ± 0.41	16.00 ± 0.53	13.00 ± 0.77	17.00 ± 0.65	15.00 ± 0.56	19.00 ± 0.83
	n-Hexane	13.00 ± 0.34	-	-	9.00 ± 0.52	13.00 ± 0.47	10.00 ± 0.39
	Chloroform	-	10.00 ± 0.74	14.00 ± 0.40	16.00 ± 0.74	15.00 ± 0.66	-
	Ethyl acetate	12.00 ± 0.52	17.00 ± 0.63	15.00 ± 0.65	-	16.00 ± 0.63	12.00 ± 0.52
	n-Butanol	14.00 ± 0.49	15.00 ± 0.70	13.00 ± 0.47	14.00 ± 0.48	13.00 ± 0.52	12.00 ± 0.44
	Aqueous	-	10.00 ± 0.72	-	-	11.00 ± 0.39	10.00 ± 0.29
Standard	Levofloxacin	28.00 ± 0.67	25.00 ± 0.93	23.00 ± 0.96	24.00 ± 0.58	27.00 ± 0.69	26.00 ± 0.73

Zone of inhibition represented sensitivity. - : No inhibition zone (resistance). Levofloxacin: 5 μg/μL.

Table 6
Antibacterial activity of the extracts of *C. indica* seeds. mm.

Plant part and standard	Plant extracts and standard	Zone of inhibition ± SD					
		S. aureus	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*
Seeds	Methanol	14.00 ± 0.34	11.00 ± 0.46	13.00 ± 0.53	15.00 ± 0.53	16.00 ± 0.46	-
	n-Hexane	17.00 ± 0.56	14.00 ± 0.50	11.00 ± 0.37	-	12.00 ± 0.53	-
	Chloroform	16.00 ± 0.53	15.00 ± 0.63	-	14.00 ± 0.52	15.00 ± 0.66	13.00 ± 0.47
	Ethyl acetate	-	15.00 ± 0.49	-	-	14.00 ± 0.43	12.00 ± 0.52
	n-Butanol	12.00 ± 0.38	16.00 ± 0.37	12.00 ± 0.42	14.00 ± 0.48	-	13.00 ± 0.44
	Aqueous	9.00 ± 0.32	-	9.00 ± 0.36	-	10.00 ± 0.39	10.00 ± 0.24
Standard	Levofloxacin	28.00 ± 0.67	25.00 ± 0.93	23.00 ± 0.96	24.00 ± 0.58	27.00 ± 0.69	26.00 ± 0.73

Zone of inhibition represented sensitivity. - : No inhibition zone (resistance); Levofloxacin: 5 μg/μL.

Table 7
Antibacterial activity of the extracts of *C. indica* stems. mm.

Plant part and standard	Plant extracts and standards	Zone of inhibition ± SD					
		S. aureus	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*
Stems	Methanol	12.00 ± 0.41	16.00 ± 0.70	15.00 ± 0.63	14.00 ± 0.69	13.00 ± 0.48	15.00 ± 0.72
	n-Hexane	15.00 ± 0.73	-	-	13.00 ± 0.76	-	-
	Chloroform	10.00 ± 0.58	-	-	16.00 ± 0.82	14.00 ± 0.62	-
	Ethyl acetate	16.00 ± 0.76	17.00 ± 0.83	17.00 ± 0.57	-	-	16.00 ± 0.67
	n-Butanol	13.00 ± 0.45	15.00 ± 0.54	11.00 ± 0.41	12.00 ± 0.72	13.00 ± 0.58	12.00 ± 0.48
	Aqueous	-	9.00 ± 0.29	10.00 ± 0.42	-	-	11.00 ± 0.59
Standard	Levofloxacin	28.00 ± 0.67	25.00 ± 0.93	23.00 ± 0.96	24.00 ± 0.58	27.00 ± 0.69	26.00 ± 0.73

Zone of inhibition represented sensitivity. - : No inhibition zone (resistance); Levofloxacin: 5 μg/μL.

extract were active against *A. parasiticus* [(14.00 ± 0.49) mm], *A. oryzae* [(11.00 ± 0.57) mm] and *A. fumigatus* [(15.00 ± 0.68) mm] and were completely inactive against *A. niger* and *C. albicans* (Table 9). Plant stems *n*-hexane, chloroform, ethyl acetate and *n*-butanol extracts revealed significant antifungal activities (11–15 mm) against *A. fumigatus*. Methanol extracts of stems were active against *A. oryzae* [(17.00 ± 0.84) mm] and *A. parasiticus* [(16.00 ± 0.47) mm] (Table 10). *C. indica* all parts and extracts revealed a range of inhibitory concentrations during antifungal susceptibility bioassay.

Table 8
Antifungal activity of the extracts of *C. indica* leaves. mm.

Plant extracts	Zone of inhibition ± SD				
	A. niger	*A. parasiticus*	*A. oryzae*	*A. fumigatus*	*C. albicans*
Methanol	14.00 ± 0.64	16.00 ± 0.52	-	17.00 ± 0.58	-
n-Hexane	13.00 ± 0.45	13.00 ± 0.47	14.00 ± 0.51	15.00 ± 0.63	13.00 ± 0.46
Chloroform	12.00 ± 0.71	12.00 ± 0.45	9.00 ± 0.65	11.00 ± 0.27	15.00 ± 0.35
Ethyl acetate	12.00 ± 0.53	17.00 ± 0.62	16.00 ± 0.71	14.00 ± 0.55	-
n-Butanol	15.00 ± 0.64	12.00 ± 0.38	-	13.00 ± 0.62	10.00 ± 0.38
Aqueous	10.00 ± 0.38	-	8.00 ± 0.36	11.00 ± 0.39	-
Clotrimazole	22.00 ± 0.83	25.00 ± 0.71	21.00 ± 0.69	26.00 ± 0.89	23.00 ± 0.73

Clotrimazole was used as standard control. - : No inhibition zone (resistance); Clotrimazole: 5 μg/μL.

Table 9
Antifungal activity of the extracts of *C. indica* seeds. mm.

Plant extracts	Zone of inhibition ± SD				
	A. niger	*A. parasiticus*	*A. oryzae*	*A. fumigatus*	*C. albicans*
Methanol	12.00 ± 0.28	13.00 ± 0.52	-	15.00 ± 0.43	-
n-Hexane	-	11.00 ± 0.38	-	13.00 ± 0.51	-
Chloroform	14.00 ± 0.43	-	16.00 ± 0.72	12.00 ± 0.70	13.00 ± 0.46
Ethyl acetate	-	14.00 ± 0.49	11.00 ± 0.57	15.00 ± 0.68	-
n-Butanol	15.00 ± 0.57	-	13.00 ± 0.60	-	15.00 ± 0.78
Aqueous	-	11.00 ± 0.53	-	10.00 ± 0.37	-
Clotrimazole	22.00 ± 0.83	25.00 ± 0.71	21.00 ± 0.69	26.00 ± 0.89	23.00 ± 0.73

Clotrimazole was used as standard control. - : No inhibition zone (resistance); Clotrimazole: 5 μg/μL.

Table 10
Antifungal activity of the extracts of *C. indica* stems. mm.

Plant extracts	Zone of inhibition ± SD				
	A. niger	*A. parasiticus*	*A. oryzae*	*A. fumigatus*	*C. albicans*
Methanol	14.00 ± 0.52	16.00 ± 0.47	17.00 ± 0.84	-	13.00 ± 0.46
n-Hexane	-	12.00 ± 0.60	-	15.00 ± 0.59	14.00 ± 0.53
Chloroform	-	13.00 ± 0.53	14.00 ± 0.58	11.00 ± 0.70	-
Ethyl acetate	12.00 ± 0.63	-	13.00 ± 0.71	15.00 ± 0.63	11.00 ± 0.47
n-Butanol	15.00 ± 0.49	-	14.00 ± 0.60	12.00 ± 0.57	13.00 ± 0.65
Aqueous	-	11.00 ± 0.37	-	10.00 ± 0.49	-
Clotrimazole	22.00 ± 0.83	25.00 ± 0.71	21.00 ± 0.69	26.00 ± 0.89	23.00 ± 0.73

Clotrimazole was used as standard control. - : No inhibition zone (resistance); Clotrimazole: 5 μg/μL.

4. Discussion

The results of the study showed the presence of significant cannabinoids in *C. indica* and exhibited noteworthy antimicrobial activities due to these important biologically active compounds. There are wide variations in the relative amounts of cannabinoids in *Cannabis*[22]. Many factors have been considered responsible for these variations. These include the genetic characteristics, environment, maturity, sex, part of harvested plant and the time which has elapsed between harvesting and chemical analysis, as well as the conditions of storage of the plant. The chemical composition of different parts of the plant also varies. In present study, plant resins were collected because delta-9-tetrahydrocannabinol was found in plant's flowering or fruit tops, leaves and resins. It has been reported that *Cannabis* resin has a higher tetrahydrocannabinol contents than rest of the plant material[23]. *Cannabis* species seeds are main the sources of fibers, proteins and carbohydrates. The whole seed of *Cannabis* contains roughly 15% insoluble fiber, 25% protein, and 30% carbohydrates[2]. Terpenoids, tannins and reducing sugars are the major phytochemicals present in methanol, ethanol and benzene extracts of leaves of *Cannabis sativa*, which has good antibacterial potential[24]. The antibacterial activities of crude alkaloid extracted from the leaves of *Cannabis* against bacterial strains representative of skin, mouth and ear micro-flora and also against *E. coli* bacteria responsible for urinary tract infection in human beings were 2.1 cm, 2.2 cm, 1.7 cm and 2.5 cm respectively[25]. Antimicrobial and phytochemical analysis of plant extracts exhibited significant activity against multidrug resistant bacteria and fungi[26]. Seventeen studies were appraised for nabiximols, a specific extract of *Cannabis*, four for smoked tetrahydrocannabinol, five for nabilone, three for tetrahydrocannabinol oromucosal spray, two for dronabinol, one for vaporized *Cannabis*, one for ajuvenic acid capsules, and one for oral tetrahydrocannabinol. Nabilone was compared by one trial and compared with amitriptyline[27], while all further studies were placebo controlled. One of these studies assessed nabilone as an adjunctive cure to gabapentin[28]. Cannabidiol blocks the metabolism of delta 9-tetrahydrocannabinol to 11-hydroxy-delta(9)-tetrahydrocannabinol and may produce dysphoria because it is more psychoactive than delta-9-tetrahydrocannabinol. Cannabidiol has various remedial effects including muscle relaxant, anti-inflammatory, anti-emetic, anxiolytic, anti-convulsant and analgesic properties[29]. *Cannabis* has been classified on the basis of cannabidiol to tetrahydro cannabinol ratio into fiber and drug type[30].

The main objective of the study was to investigate the proximate composition, phytochemical content, minerals analysis, GC-MS studies of its active cannabinoids and antimicrobial activities of the *C. indica* leaves, stems, and seeds. The results of the study revealed that *C. indica* not only mean frivolous rationales but the existence of biologically active remedial compounds in elevated concentrations making it a treasured source to be used in herbal preparation for various ailments. The noteworthy advances in cannabinoid study have opened innovative frontiers and are foremost to a better understanding of *Cannabis* effect in humans. *Cannabis* based medicines are more complex containing a mixture of cannabinoids, flavonoids and terpenes, therefore medicinal *Cannabis* must be of reliable quality. A medicine must be effective and secure for its purpose because these compounds synergistically interact with one another. Therefore, for the growth of herbal medicine, the plant material chosen must be systematically analyzed.

Conflict of interest statement

We daclare that we have no conflict of interest.

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