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Phytochemical analysis and antibacterial activity on seed and pod extracts of *Crotalaria incana*

[Análisis fitoquímico y actividad antibacteriana de extractos de semillas y vainas de *Crotalaria incana*]

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Abstract

Context: Crotalaria incana L. (*Fabaceae*) is used for various traditional medicinal treatments such as astringent, jaundice and palpitation, inflammation, skin disease and purgative.

Aims: To investigate the phytochemical constituents and evaluate the antibacterial activity of seed and pod extracts of *C. incana*.

Methods: Phytochemical screening tests were conducted to identify the class of compounds present in the dichloromethane/methanol (1:1) extracts of seed and pod. Silica gel column chromatographic technique was applied to separate the constituents of the extracts. Various spectroscopic techniques (IR, NMR (^{1}H, ^{13}C and DEPT-135) were applied to determine the structures of isolated compounds. Antibacterial activities were evaluated via disc diffusion method.

Results: Preliminary phytochemical screening of seed and pod extracts of *C. incana* L. revealed the presence alkaloids, flavonoids, triterpenes, tannins and steroids and absence of anthraquinones. Silica gel column chromatography separation of the extract yielded a known steroid β-sitosterol **(1)** and 1-nonadecanol **(2)**. Evaluation of antibacterial activity of crude extract via disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Salmonella typhi* showed moderate susceptibility of this selected Gram-positive and Gram-negative bacteria strains, comparable to the activity of the antibiotic (gentamicin).

Conclusions: The compound **(2)** (1-nonadecanol) was isolated for the first time from the *C. incana*. These results proved that polar extracts of the seeds and pods of *C. incana* possess antibacterial activity.

Keywords: Antibacterial; *Crotalaria*; *Fabaceae*; 1-nonadecanol; steroid.

Resumen

Contexto: Crotalaria incana L. (*Fabaceae*) es usada en varios tratamientos medicinales tradicionales como astringente, ictericia y palpitaciones, inflamación, enfermedades de la piel y purgante.

Objetivos: Investigar los constituyentes fitoquímicos y evaluar la actividad antibacteriana de extractos de semillas y vainas de *C. incana*.

Métodos: Se llevaron a cabo pruebas de tamizaje fitoquímico para identificar la clase de compuestos presentes en extractos de semilla y vaina en diclorometano/metanol (1:1). La técnica cromatográfica en columna de gel de sílice se aplicó para separar los constituyentes de los extractos. Diversas técnicas espectroscópicas (IR, RMN (1H, 13C y DEPT-135) se aplicaron para determinar las estructuras de los compuestos aislados. Las actividades antibacterianas fueron evaluadas mediante el método de difusión en disco.

Resultados: El tamizaje fitoquímico preliminar de los extractos de semillas y vainas de *C. incana* L. reveló la presencia de alcaloides, flavonoides, triterpenos, taninos y esteroides y ausencia de antraquinonas. Mediante la separación por cromatografía en columna en gel de sílice del extracto se aisló un conocido esteroide β-sitosterol **(1)** y 1-nonadecanol **(2)**. La evaluación de la actividad antibacteriana de extracto crudo mediante el método de difusión en disco frente a *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* y *Salmonella typhi* mostraron susceptibilidad moderada a estas cepas de bacterias Gram-positivas y Gram-negativas seleccionadas, comparable a la actividad del antibiótico (gentamicina).

Conclusiones: El compuesto **(2)** (1-nonadecanol) fue aislado por primera vez de *C. incana*. Estos resultados demostraron que los extractos polares de las semillas y las vainas de *C. incana* poseen actividad antibacteriana.

Palabras Clave: Antibacteriana; *Crotalaria*; esteroide; *Fabaceae*; 1-nonadecanol.

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INTRODUCTION

Medicinal plants have played a pivotal role in the primary healthcare and formed the basis of traditional systems of medicines as well as known to provide a rich source of raw materials of natural products. Plants have been bestowed us with food, spices, flavors, fragrances, medicines, etc. Plants are being used to treat many diseases or ailments viz. infectious diseases, inflammatory disorders, and skin diseases, among others. In Africa, Asia and other parts of developing world since ancient time (Balandrin et al., 1985; Houghton, 1995). Particularly those living in rural areas of the developing countries, it is continued to be used as the primary source of medicine. As in many African countries, the plants in religious ceremonies as well as for magic and medicinal purpose are also common in Ethiopia.

The genus *Crotalaria* belongs to the *Fabaceae* family and contains approximately 600 species that grow wild in tropical and subtropical areas (Polhill, 1981). In Ethiopia, 85 species of *Crotalaria* have been recorded. Whilst about 15 of these species are endemic to the country, the remaining are known to occur in other tropical countries, mainly in Africa. Only a few members of the genus have been studied, and the degree and pattern of toxicity were found to vary (Asres et al., 2004).

Crotalaria incana L. subsp. *purpurascens* (Yeayte Misir in amharic local language of Ethiopia) is included in *Fabaceae* family and used for various traditional medicinal practices such as astringent, jaundice and palpitation, inflammation, skin disease and purgative (Wagner et al., 1999). In the Northern part of Ethiopia, where present study were carried out, the root of the plant is taken crushed and mixed with water as traditional medicine for rabies (Evans, 2002), and the seed of *C. incana* L. is taken to treat of wound traditional by local people (Lulekal et al., 2013).

The present study aims to isolate and characterize bioactive compounds and evaluate antibacterial activities via disc diffusion of the seed and pod crude extracts of *C. incana* L. subsp. *purpurascens*.

MATERIAL AND METHODS

General experimental materials

Melting point was determined with Mettler Toledo Model FP62 machine. Infrared (KBr pellet) spectrum was recorded Perkin-Elmer BX infrared spectrometer in the range 4000-400 cm^{-1}. Nuclear magnetic resonance (NMR) spectra (^{1}H-NMR, ^{13}C-NMR and DEPT-135) were recorded on a Bruker Avance 400 MHz spectrometer. Column chromatography was performed on silica gel 60 (60-120 mesh).

Plant material

The seed and pod of *C. incana* were collected in December 2014 from Amahara region, South Gondar Zone from Debre Tabor University garden, located around Debre Tabor city, located 666 km from Addis Ababa, Ethiopia. The plant material was identified by Melaku Wondafrash from the National Herbarium, Department of Biology, and Addis Ababa University, where voucher (MA-249/2015) specimens were deposited.

Extraction and isolation

The pod and seed of the plant were dried and powdered with a mechanical grinder separately. The dried seed and pod powder of *C. incana* (250 g and 180 g, respectively) were extracted by cold percolation with CH_2Cl_2/CH_3OH (1:1) three times for 24 h while shaking at speed of 220 rpm and temperature controlled at 25.4°C separately. The extracts were concentrated using a rotary evaporator to yield brown 13.32 g (7.4%) and 23.25 g (9.3%) crude extracts of seed and pod from *C. incana,* respectively.

A 12 g portion of seed extract was subjected to column chromatography and eluted with increasing gradient of ethyl acetate in *n*-hexane. A total of 25 fractions (50 mL each) were collected. Fraction 11 (20% ethyl acetate in *n*-hexane) yielded a precipitate with a pink color on TLC by single spot on TLC SiGel (60-120) F_{254} which was further washed with *n*-hexane using suction filtration to give 15 mg of compound **(1)** as a white powder.

Moreover, 20 g of the CH_2Cl_2/CH_3OH (1:1) pod extract was chromatographed on a silica gel column and elution was carried out using *n*-hexane 100%, *n*-hexane + ethyl acetate (1-10, 20, 30, 40, 50 60 and 80%), ethyl acetate 100%, ethyl acetate + methanol (10, 20, 30 and 50%) and finally with methanol 100%. A total of 22 fractions (50 mL each) were collected. Fractions 11-15 (20-60% hexane in ethyl acetate) were re-fractionated using mixtures of hexane and ethyl acetate in ratios 99:1 to 90:10. Sub-fraction 3 was crystallized and showed a pale pink color by single spot on TLC SiGel (60-120) F_{254} which was further washed with *n*-hexane using suction filtration to give 15 mg of compound **(2)** as colorless needle crystals.

Preliminary phytochemical screening

Phytochemical screening tests were done to determine the class of secondary metabolites present in the crude extract following the standard protocols (Pradeep et al., 2014, Saleem et al., 2014). The results were reported as (+Ve) for presence and (-Ve) for absence in (Table 1).

Test for alkaloids

A small portion of the sample was stirred with few drops of dilute hydrochloric acid and was tested with Dragendroff's reagent for the presence of alkaloids; a white to buff precipitate was observed which proves the presence of alkaloids.

Tests for steroids and terpenoids (Salkowski test)

About 0.2 g of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated sulphuric acid (red color at lower layer indicates the presence of steroids and formation of yellow colored lower layer indicates the presence of triterpenoids).

Test for tannins

A small quantity of the sample was taken in water, and test for the presence of tannins was carried out with the dilute Ferric chloride solution (5%) resulted in a characteristic violet color.

Test for flavonoids (alkaline reagent test)

About 5 mL of dilute aqueous ammonia solution was added to a 0.2 g of the aqueous filtrate of the plant extract, followed by addition of concentrated H_2SO_4. The instant disappearance of yellow coloration indicated the presence of flavonoids in the crude extract.

Test for free anthraquinones

About 0.5 g of the extract was boiled with 10% HCl for few minutes in a water bath and filtered. The filtrate was allowed to cool, and an equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% ammonia were added to the mixture and heated. The formation of rose-pink color was taken as an indication of the presence of anthraquinones.

Microorganism strains

The antibacterial activity of extracts of *C. incana* was evaluated by using bacterial strains of one Gram-positive *(Staphylococcus aureus)* and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi)*. These microorganisms were cultured in Microbiology Laboratory of Department of Biology, Bahri Dari University, Ethiopia.

Antibacterial activity

Antibacterial activity of extracts was evaluated by the disc diffusion method in the accordance with the guidelines of National Committee for Clinical Laboratory Standards (2002) with minor modification. Gentamicin was used as a standard antibacterial agent. With a loop, touch the top of 24 hours old culture individual selected bacteria and transfer to a tube of saline (0.85%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 [~10^6 colony forming units (CFU) per milliliter]. To avoid further growth before inoculation, the media was sterilized in a flask and cooled to 40-45°C Petri dishes containing 20 mL of Mueller-Hinton agar were used to inoculate bacterial suspension and distributing the medium in Petri dishes homogeneously. Filter paper discs (Whatman no. 1, 6 mm diameter) impregnated with the extract solution

prepared in DMSO (25, 50, 75, and 100 mg/mL) were placed on the inoculated plates and Petri dishes were incubated for 24 h at 37°C. A filter paper disc impregnated with gentamicin prepared in DMSO (50 mg/mL) was used as positive control. The inhibition zone diameters were measured in millimeters.

Statistical analysis

All data are presented as the mean ± SD of three measurements. The comparisons between the control group (gentamicin) and the test groups (antibacterial activity extracts of *C. incana*) were performed by statistical package software like one-way analysis of variance (ANOVA) with non-parametric post-hoc Dunnett's test using KyPlot (version 2.0 beta 15). Statistical significance p-level was selected as 0.05.

RESULTS

The CH_2Cl_2/CH_3OH (1:1) seed and pod extracts of *C. incana L.* were subjected to various qualitative tests for phyto-constituents such as alkaloids, flavonoids, triterpenoids, tannins, steroids and anthraquinones. The phytochemical screening of seed and pod extracts of *C. incana* presumed the presence of alkaloids, triterpenoids, flavonoids, tannins and steroids and the absence of anthraquinones (Table 1).

Compound **(1)** was isolated from seed extract of *C. incana* as a white powder (melting point 136-137°C) with R_f value of (0.67) in 30% ethyl acetate in *n*-hexane. IR spectrum (in KBr) exhibited a characteristic absorption band at 3455 cm^{-1} (due to O-H stretching), absorption at 2935 and 2866 cm^{-1} (due to aliphatic C-H stretching), absorption at 1653 cm^{-1} (due to C=C stretching), absorption at 1458 cm^{-1} (due to cyclic $(CH_2)_n$ and 1375 cm^{-1} attributed to C-H bending.

The ^{1}H-NMR spectrum of the isolated compound **(1)** showed a series of proton signal at δ 1.0 - 1.8 due to overlapping of methylenes and methines, a characteristic frame work of steroid. The ^{1}H-NMR showed two protons, one-proton multiplet at δ 3.54 and δ 5.35 typical for H-3 and H-6 of a steroidal nucleus. The presence of six methyl groups at δ 0.68, 0.93, 0.83, 0.81, 0.84 and 1.01 is also in agreement with the steroidal nucleus.

The ^{13}C-NMR spectrum of compound **(1)** revealed the presence of 29 carbon signals including an oxymethine carbon signal at δ 71.8 and two olefinic carbons at δ 140.8 and δ 121.7 (Fig. 1). DEPT-135 experiment displayed six methyl (CH_3) groups, eleven methylene (CH_2) groups, nine methine (CH) groups and three quaternary carbons (C). On the basis this evidence as well as a comparison with literature (Chaturvedula and Venkata, 2012) (Table 2), it was deduced that compound **(1)** was β-sitosterol.

Table 1. Preliminary phytochemical screening of extracts from *C. incana*.

Chemical components	Reagents	Results of extracts	
		Seed	Pod
Alkaloids	Dragendroff's reagent	+	+
Steroids	Chloroform and concentrate H_2SO_4	+	+
Terpenoids	Chloroform and concentrate H_2SO_4	+	+
Tannis	$FeCl_3$	+	+
Flavonoids	Dilute ammonia solution	+	+
Anthraquinones	10% Ammonia solution	_	_

(+) indicates Present (-) indicates absent.

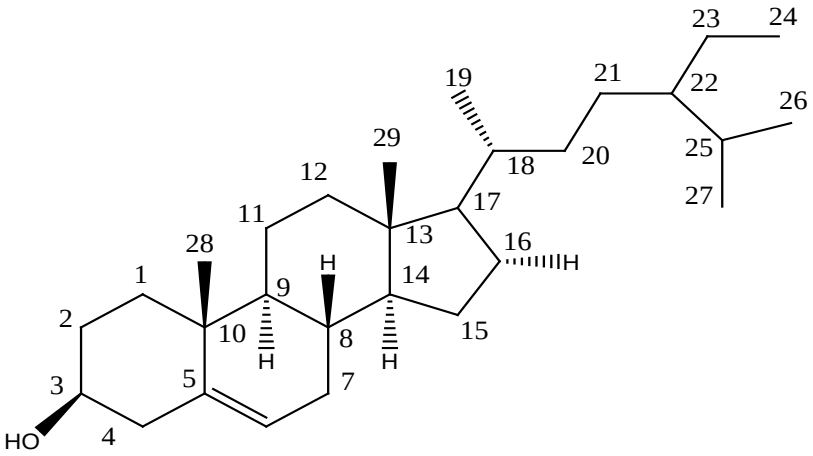

Figure 1. Compound **(1)**, β-sitosterol isolated from seed extract of *C. incana*.

Table 2. Observed ^{1}H-, ^{13}C-NMR spectra data along with reported ^{13}C-NMR data of β- sitosterol isolated from *C. incana*.

Position	Compound (1)		Reported Data (Chaturvedula and Venkata, 2012)	
	^{1}H	^{13}C	^{1}H	^{13}C
1		37.3		37.5
2		31.9		31.9
3	3.54 (tdd, 1H, *J* = 4.5, 4.2, 3.8 Hz	71.8	3.53 (tdd, 1H, *J* = 4.5, 4.2, 3.8 Hz	72.0
4		42.3		42.5
5	5.35 (t, 1H, *J* = 6.1 Hz)	140.8	5.36 (t, 1H, *J* = 6.4 Hz)	140.9
6		121.7		121.9
7		32.0		32.1
8		33.1		32.1
9		50.1		50.3
10		36.2		36.7
11		21.1		21.3
12		39.8		39.9
13		42.8		42.6
14		56.8		56.0
15		25.9		26.3
16		28.3		28.5
17		56.1		56.3
18		36.2		36.3
19	0.92 (d, 3H, *J* = 6.2 Hz)	19.4	0.91 (d, 3H, *J* = 6.2 Hz)	19.2
20		35.5		34.2
21	5.14 (m, 1H)	26.1	5.14 (m, 1H)	26.3
22		45.9		46.1
23		23.1		23.3
24	0.85 (t, 3H, *J* = 7.1 Hz)	11.9	0.84 (t, 3H, *J* = 7.2 Hz)	12.2
25		29.2		29.4
26	0.83 (d, 3H, *J* = 6.2 Hz	19.8	0.83 (d, 3H, *J* = 6.4 Hz)	20.1
27	0.82 (d, 3H, *J* = 6.6 Hz)	19.2	0.81 (d, 3H, *J* = 6.4 Hz)	19.6
28	0.67 (s, 3H)	18.8	0.68 (s, 3H)	19.0
29	1.01 (s, 3H)	11.9	1.01 (s, 3H)	12.1

Compound **(2)** was isolated from pod extract of *C. incana* as colorless needle crystalline (melting point 62-63°C) with R_f value of (0.54) in 30% ethyl acetate in *n*-hexane. The IR spectrum (in KBr) exhibited characteristic absorption band at 3425 cm^{-1} that is O-H stretching. Absorption at 2935 and 2866 cm^{-1} is due to aliphatic C-H stretching. The weak frequencies were observed at 1458 cm^{-1} and 1375 cm^{-1} for C-H bending. ^{13}C-NMR spectrum showed nine signals, which were discriminated into CH_3 resonance at δ 14.1 and a signal due to a primary alcoholic group at δ 60.40. The other signals were assigned to aliphatic CH_2 groups, which resonated at δ 22.6-27.2. DEPT-135 experiment displayed one methyl (CH_3) groups, eighteen methylene (CH_2) groups including more deshielded methylene carbon due to the primary alcohol (CH_2OH) group. These data suggested compound **(2)** to be an aliphatic straight chain primary alcohol.

^{1}H-NMR spectrum revealed a signal at δ 3.47 (t, *J* = 6.8 Hz, 2H) assigned for CH_2OH group that was confirmed by absorption band at 3425 cm^{-1} (alcoholic O-H stretching) and 1080 cm^{-1} (C-O stretching of primary alcohol) in IR spectrum (Silverstein et al., 2005), in addition to a signal at δ 0.78 (t, *J* = 7.4 Hz, 3H) assigned for the terminal CH_3 group. And at δ 1.64 (m, 2H) assigned for methylene proton (CH_2) that neighboring proton for the carbon attached to electronegative element. A singlet peak at δ 2.00 confirmed the existence of O-H. An upfield broad signal resonated at δ 1.16 (34 H, m) was assigned to H3-H18 and confirmed by absorption band at 720 cm^{-1} (C-H bending of repeated methylene groups of straight chain paraffins) in IR spectrum (Silverstein et al., 2005). On the basis of this evidences as well as comparison with literature (Yhiya et al., 2013) (Table 3), it was deduced that compound **(2)** is 1-nonadecanol (Fig. 2), which was previously isolated from *Convolvulus lanatus*, family *Convolvulaceae* (El-Nasr et al., 1984), and isolated from *T. tipu* (*Fabaceae*). The best of our knowledge the compound **(2)** (1-nanodecanol) was isolated for the first time from the *C. incana.*

Antibacterial activities via disc diffusion method were presented in (Table 4), determined by measuring the 'inhibition zone' for seed and pod the crude extract of C. *incana*. The activity of seed and pod crude extracts of *C. incana* has also been compared with the broad spectrum commercially available antibiotic (gentamicin). Gentamicin showed the inhibition zone for *E. coli* (18 ± 0.8 mm), *S. aureus* (17 ± 0.1 mm), *K. pneumonia* (16 ± 0.1 mm) and *S. typhi* (16 ±0.3. mm) at the concentration 50 mg/mL; while seed extract of *C. incana* recorded for *E. coli* (11.4 ± 0.6 mm), *S. aureus* (12.0 ± 1 mm*) K. pneumoniae* (10.2 ± 7.2 mm) and *S. typhi* (10.0 ± 0.6 mm) at the concentration of 100 mg/mL. The seed extract of *C. incana* recorded for *E. coli* (5.7 ± 0.4 mm), *S. aureus* (6.0 ± 0.1 mm*), K. pneumoniae* (5.0 ± 0.1 mm) and *S. typhi* (4.4 ± 0.5 mm) at the concentration of 25 mg/mL.

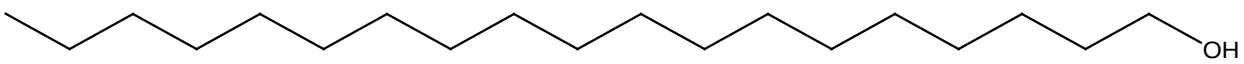

Figure 2. Compound **(2)**, 1-nonadecanol isolated from pod extract of *C. incana.*

Table 3. Observed ^{1}H-, ^{13}C-NMR spectra data along with reported ^{1}H, ^{13}C-NMR data of 1-nonadecanol isolated from *C. incana*.

Position	Compound (2)		Reported data (Yhiya et al., 2013)	
	^{1}H	^{13}C	^{1}H	^{13}C
1	3.47 (*t*, *J* = 6.9 Hz, H-1)	60.5	3.47 (*t*, *J* = 6.9 Hz, H-1)	62.5
2	1.44 (m, H-2)	33.0	1.44 (m, H-2)	32.5
3		31.9		31.9
4-16	1.16 (m, H-3 to H-18)	29.4 – 27.2	1.16 (m, H-3 to H-18)	29.4 – 29.7
17		25.8		25.8
18		22.7		22.7
19	0.78 (*t*, *J* = 7.3 Hz, H-19)	14.1	0.78 (*t*, *J* = 7.3 Hz, H-19)	14.1

Table 4. Antibacterial activities of seed and pod extracts of *Crotalaria incana* L.

Bacteria	Diameter of zone of inhibition (mm)								
	Seed extract (mg/mL)				Pod extract (mg/mL)				Gen (mg/mL)
	25	50	75	100	25	50	75	100	50
E. coli	5.7 ± 0.4	6.5 ± 0.5	7.6 ± 0.6	11.4 ± 0.6	5.0 ± 0.1	6.5±0.8	7.0 ± 0.2	12.0± 1.0	18 ± 0.8
S. aureus	6.0 ± 0.1	7.7 ± 0.4	9.0 ± 0.6	12.0 ± 0.6	6.0 ± 0.3	7.0 ± 0.1	9.0 ± 0.6	12.5 ± 0.9	17 ± 0.1
K. pneumoniae	5.0 ± 0.1	6.0 ± 0.1	8.7 ± 0.3	10.2 ± 0.7	4.5 ± 0.5	6.0 ± 0.3	8.0 ± 0.5	10.0 ± 0.5	16 ± 0.1
S. typhi	4.4 ± 0.5	6.0 ± 0.1	7.0 ± 0.2	10.0 ± 0.6	4.4 ± 0.5	6.0 ±0.2	7.0 ± 0.1	11.0 ± 1.0	16 ±0. 3

The data represent the mean ± SD, n=3. No significantly differences were observed with respect to the reference antibiotic compound at 0.05 level of Dunnett test, ($p > 0.05$), Gen = Gentamicin.

The pod extract of *C. incana* recorded for *E. coli* (12.0 ± 1.0 mm), *S. aureus* (12.5 ± 0.9 mm*) K. pneumoniae* (10.0 ± 0.5 mm) and *S. typhi* (11.0 ± 1.0 mm) at the concentration of 100 mg/mL. The pod extract of *C. incana* also recorded for *E. coli* (5.0 ± 0.1 mm), *S. aureus* (6.0 ± 0.3 mm*), K. pneumoniae* (4.5 ± 0.5 mm) and *S. typhi* (4.4 ± 0.5 mm) at the concentration of 25 mg/mL. Thus, the antibacterial activity of the seed and pod extract of *C. incana* (10.0 ± 0.6 mm to 12.5 ± 0.9 mm) was comparable to the standard gentamicin (18.0 ± 0.8 mm to 16.0 ± 1.0 mm)

ANOVA test of data on the antibacterial activity of crude extract of *C. incana* on *E. coli, S. aureus, K. pneumoniae,* and *S. typhi* revealed had not significant effect ($p > 0.05$) on the level of inhibition respect to reference compound (gentamicin).

The detailed analysis of the antibacterial activity of the CH_2Cl_2/CH_3OH (1:1) pod and seed extracts of *C. incana* was showed moderate activity shown at an amount of 100 mg/mL while less activity was shown at 25 mg/mL concentration for both tested bacteria strains with respect to standard antibiotic compound (gentamicin). Hence the extracts of *C. incana* are having higher antibacterial activity against *S. aureus* followed by *E. coli, K. pneumoniae and S. typhi* at both lower and high concentrations in all extracts. Gram-negative bacteria were more susceptible towards this extract than tested Gram-positive ones.

Previous studies on the genus *Crotalaria* revealed significant antimicrobial activity that is supposed to be linked to their constituents' viz. phenolic, protein (Naseem et al., 2006; Bhakshu et al.,

2008). The maximum antibacterial activity of *C. juncea* flower was seen in *C. juncea* petroleum ether fractioned ethanol extract against *K. pneumonia* (18 mm) and *S. aureus* (18 mm) at 500 µg/disc concentration (Chouhan and Singh, 2010).

The presence of the compound β-sitosterol (**1**) and the existence of β-hydroxyl group might have enabled the seed extract of *C. incana* to enhance activity. *C. incana* pod and seed extracts showed moderate activity against Gram-positive and Gram-negative bacteria, comparable to the activity of typical antibiotic (gentamicin), indicating the possibility of the presence of strong, active compounds, the results obtained with this plant may be considered clinically promising, and it may be a valuable source for obtaining new antimicrobial agents. It is of some consideration that this extracts might contain other kinds of bioactive components in addition to the isolate, which might be direct acting.

Compound (1): White crystalline compound (melting point 136-137°C) (Rf 0.67), IR (KBr) υ_{max}/cm^{-1}: 3455, 2935, 2866, 1651, and 1082. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.35 (1H, d), 3.54 (1H, m), 5.14 (m, 1H), 1.01, 0.67 (3H, s), 0.92 (3H, d), 0.85 (3H, *d*), 0.83 (3H, *d*) and 0.79 (3H, *d*). ^{13}C-NMR (400 MHz, $CDCl_3$) δ: 37.3 (CH_2,C-1), 31.9 (CH_2,C-2), 71.8 (CH,C-3), 42.3 (CH2,C-4), 140.8 (Cq,C-5), 121.7 (CH,C6), 32.0 (CH_2,C-7), 33.1 (CH,C-8), 50.1 (CH,C-9), 36.2 (Cq,C-10), 21.1 (CH_2,C-11), 39.8 (CH_2,C 12), 42.8 (Cq,C-13), 56.8 (CH,C-14), 25.9 (CH_2,C-15), 28.3 (CH_2,C-16), 56.1 (CH,C-17), 11.9 (CH_3,C-18), 19.4 (CH_3,C-19), 36.5 (CH,C-20), 19.0 (CH_3,C-21), 36.2(CH_2,C-22), 26.1 (CH_2,C 23), 45.9 (CH,C-24), 29.2 (CH,C-25), 19.8 (CH_3,C-26), 18.8 (CH_3,C-27), 23.1 (CH_2,C-28), 11.9 (CH_3,C-29).

Compound (2): Colorless needle crystals (melting point 61-62°C) (R_f 0.54) on precoated silica gel plates F_{254} using 30% EtOAC /hex. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ ppm): 2.47 (*t*, *J* = 6.8Hz, H-1), 2.0 (s, O-H) 1.64 (m, H-2), 1.16 (m, H-3 to H-18), 0.78 (*t*, *J* = 7.4 Hz, H-19).^{13}C-NMR (CDCl3, 400 MHz, δ ppm): 60.4 (C-1), 33.7 (C-2), 31.9 (C-3), 29.07 – 29.70 (C-4 to C-14), 28.9(C-15), 27.2(C-16), 25.8 (C-17), 22.7 (C-18), 14.1 (C-19).

CONCLUSION

This work is the first attempt to phytochemically analysis the seed and pod extracts of *C. incana*. The antibacterial activities of crude seed and pod extracts of *C. incana* showed moderate susceptibility of selected Gram-positive and Gram-negative bacteria strains. The present investigation helps the discovery of plant-based drugs to human welfare. Further studies are recommended in various parts of the plant so as to isolate and test more bioactive compounds in support of its traditional use.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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