

Phytochemical and antimicrobial activities of Himalayan *Cordyceps sinensis* (Berk.) Sacc.

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This study evaluated the phytochemical and antimicrobial activities and also quantified bioactive nucleoside using high performance thin layer chromatography (HPTLC) of five extracts of Indian Himalayan *Cordyceps sinensis* prepared with different solvents employing accelerated solvent extraction (ASE) technique. The phytochemical potential of these extracts was quantified in terms of total phenolic and total flavonoid content while antioxidant activities were determined by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and ferric-reducing antioxidant power (FRAP) assays. Total reducing power (TRP) was determined by converting iron (III) into iron (II) reduction assay. CS_{50%Aic} (15.1 ± 0.67mg/g of dry extract) and CS_{100%Aic} (19.3 ± 0.33mg/g of dry extract) showed highest phenolic and flavonoid content, respectively while CS_{Aq} extract showed maximum antioxidant activity and the highest concentration of the three nucleosides (adenine 12.8±0.49 mg/g, adenosine 0.36±0.28 mg/g and uracil 0.14±0.36 mg/g of dry extract) determined by HPTLC. The evaluation of extracts for antimicrobial activity against gram-negative and gram-positive bacterial strains showed CS_{25%Aic}, CS_{75%Aic} and CS_{100%Aic} extract to be more effective against *E. coli*, *P. aeruginosa* and *B. subtilis* giving 9, 7 and 6.5 mm of zone of inhibition (ZOI) in 93.75, 93.75 and 45 µg concentration, respectively, whereas CS_{Aq} extract showed minimal inhibition against these.

Keywords: Antimicrobial activity, Antioxidant, Bioactive nucleoside, Caterpillar fungus, Herbal

Oxidative stress is associated with increased production of oxidizing species or significant decrease of antioxidants and is involved in various human diseases such as in cellular necrosis, cardiovascular disease, cancer, neurological disorder and even aging¹. Non-toxic antioxidants from natural sources, especially medicinal plants are known to prevent oxidative damage caused by free radical and are rich in polyphenolics and bioactive compounds². Phenolic compounds are one of the most widely available groups of phytochemicals found in plants and possess antioxidant and several health promoting activities. There are studies which have clearly shown that medicinal plants can protect the human body

against both oxidative stress and pathogenic bacteria due to their inherent antioxidant and antimicrobial properties³. Hence, it is important to characterize and evaluate the medicinal herbs responsible for antioxidant and antimicrobial potential.

Medicinal mushrooms or mushroom extracts have been traditionally used for centuries in China and Japan as herbal medicines because they possess large amount of essential amino acid, nucleotides, important minerals, vitamins and large contingent of enzymes etc. *Cordyceps sinensis* is one of such medicinal fungi, belongs to *clavicipitaceae* family and genus *ascomycete*. It is an entomogenous fungus that parasitizes the larva of the moth and this entire fungus and larva combination results in unique profile of secondary metabolites which is used for medicinal purposes⁴. *C. sinensis* is found in the alpine region of the Himalayan at an altitude of more than 3500 meters⁵ in India, China, Tibet, and Yunnan. The known pharmacological and biological actions of *C. sinensis* are mainly due to its bioactive polysaccharides, modified nucleosides

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Abbreviations: ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); ASE, Accelerated Solvent Extraction; CSE, *Cordyceps sinensis* extracts; CS_{Aq}, *C. sinensis* aqueous extract; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; FRAP, ferric reducing antioxidant power; TE, trolox equivalent; TPTZ, 2,4,6-tripyridyl-s-triazine; TRP, Total reducing power; ZOI, zone of inhibition.

and polyphenolic compounds⁶. Bioactive constituents such as cordycepin, ergosterol and peptide containing alpha aminoisobutyric acid produced by this fungus are responsible for its antimicrobial effects⁷, while nucleosides such as adenine increases the cellular adenosine triphosphate (ATP), play important role in cellular respiration⁸ and adenosine have been used for modulation of immunoregulatory diseases⁹ as well as act as modulators of neurotransmission for promoting sleep¹⁰.

Though there are numerous studies on the preventive and therapeutic applications of Chinese *C. sinensis* for the various diseases, the Indian variety of *C. sinensis* is hardly studied. Since, the geographical origin changes the bioactivity pattern of the plant due to the difference in the presence of type of the secondary metabolites, they may behave differently towards therapeutic effect for many chronic pathological disorders. Therefore, the present study is aimed to carry out the phytochemical and antimicrobial activities along with HPTLC analysis of different extracts of Indian species of *C. sinensis*.

Material and Methods

Standards and reagents—1,1'-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethyl-enzothiazoline-6-sulphonic acid) (ABTS), 3,4,5-trihydroxybenzoic acid (Gallic Acid), 2,4,6-tripyridyl-s-triazine (TPTZ), 6 hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), Rutin, Adenosine, Adenine, Uracil were procured from Sigma Aldrich Chemicals, USA. Folin-Ciocalteu reagent and ascorbic acid (Vitamin C) were from Sisco Research Laboratories, India. *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus subtilis* ATCC 6051 were obtained from NuLife Consultants and Distributors Pvt. Ltd., Lajpat Nagar, New Delhi, India, while *Escherichia coli* strain was received from National Institute of Cholera and Enteric Diseases, Kolkata, India.

Plant material—*C. sinensis* was collected from the hilly region of western Himalayas at an altitude, above 3200 meters where the plant grows widely in natural condition. Voucher specimen is preserved in Defence Institute of Bio Energy Research, Pithoragarh, after ethno-botanical identification of species and established as ICS-1 gene sequence, deposited in NCBI vide accession no. JQ 357559. The whole *C. sinensis* was washed, dried in shade and powdered.

Preparation of extracts—Five different extracts of *C. sinensis* were prepared i.e., aqueous (CS_{Aq}), 25% alcoholic (CS_{25%Aic}), 50% alcoholic (CS_{50%Aic}), 75% alcoholic (CS_{75%Aic}) and 100% alcoholic (CS_{100%Aic}) employing different solvents composition, 100% water, 25% ethanol, 50% ethanol, 75% ethanol and 100% ethanol respectively using accelerated solvent extraction system (ASE 350) equipped with a solvent controller unit from Dionex Corporation (Sunnyvale, CA, USA). Extraction was carried out in three complete cycles at 1500 psi pressure for 15 min. Ethanolic portion of extracted solutions except aqueous extract was evaporated using rotavapour (Buchi Rotavapour R-124) and then concentrated solutions were lyophilized in Lyophilizer (Allied frost FD-5, India). The collected extracts were stored at 4 °C until use.

Phytochemical analysis

(i) **Determination of total phenolic content**—Total phenolic content of the extracts was determined using Folin-Ciocalteu reagent¹¹. 150 µL extract (1mg/mL of each extract), 2400 µL nanopure water and 150 µL of 0.25 N Folin-Ciocalteu reagents were mixed well together. The mixture was allowed to react for 3 min followed by addition of 300 µL of 1N Na₂CO₃ solution. The solution was incubated at room temperature in the dark for 2 h. The absorbance was measured at 725 nm using spectrophotometer (JENWAY 6705) and the results are expressed in mg of gallic acid/g of dry extract.

(ii) **Determination of total flavonoid content**—Total flavonoid content was determined according to the method of Zou *et al.*¹². Briefly, 1.0 mL of extract (1mg/mL of each extract) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a 5% NaNO₂ solution. After about 6 min, 0.15 mL of a 10% AlCl₃ solution was added and allowed to stand for next 6 min, then 2 mL of 4% NaOH solution was added to the mixture. The solution so obtained was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm. All values are expressed as mg of rutin/g of dry extract.

Antioxidant activity analysis

(i) **DPPH assay**—DPPH assay was carried out as per Thaipong *et al.*¹³. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and stored at -20 °C until needed. The working solution was obtained by mixing 10 mL of stock solution with

45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using the spectrophotometer. 150 μ L of extract (1 mg/mL of each extract) was allowed to react with 2850 μ L of the DPPH solution for 2 h in the dark. The absorbance of the solution so obtained was measured at 515 nm. The standard curve was linear between 0.1 and 0.4 μ M Trolox. Results are expressed in μ M Trolox equivalent (TE)/g of dry extract.

(ii) *ABTS assay*—For ABTS assay, the procedure followed the method of Arnao *et al.*¹⁴ with some modification. Firstly, to produce the radical cation $ABTS^+$, 7.4 mM ABTS diammonium salt and 2.6 mM potassium persulfate were mixed in a ratio of 1:1. The reaction mixture was allowed to stand in dark for 12 h at room temperature. The solution was then diluted by mixing 1.0 mL $ABTS^+$ solution with 60 mL methanol to obtain an absorbance of 1.10 ± 0.02 units at 734 nm using the spectrophotometer. $ABTS^+$ solution was freshly prepared for each assay. 150 μ L of the extract (1mg/mL) was then allowed to react with 2850 μ L of the $ABTS^+$ solution and the absorbance was measured at 734 nm after 2 h incubation in dark using the spectrophotometer. The standard curve was linear between 0.04 and 0.24 μ M Trolox. Results are expressed in mM TE/g of dry extract.

(iii) *FRAP assay*—FRAP assay was carried out using the earlier reported method as described by Kumar *et al.*¹⁵. The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37 °C before using. 150 μ L of extract (1mg/mL of each extract) solution was allowed to react with 2850 μ L of the FRAP solution for 30 min in the dark condition. Absorbance of the coloured product (ferrous tripyridyltriazine complex) were then measured at 593 nm. The standard curve was linear between 0.04 and 0.24 μ M Trolox. Results are expressed in μ M TE/g of dry extract.

(iv) *Determination of total reducing power*—power was determined, using the method of Negi *et al.*¹⁶. 1.0 mL of extract solution (1mg/mL of each extract) was mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in

a water bath at 50 °C for 20 min afterward, 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added and the mixture was centrifuged at 3000 rpm for 10 min. A 2.5 mL aliquot of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1% (w/v) solution of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. Ascorbic acid was used as standard and results are expressed in μ M ascorbic acid equivalent (AAE)/g of dry extract.

Antimicrobial activities—(i) *Microorganisms tested*: Antimicrobial activity and Minimum inhibitory concentration (MIC) value of the CSEs were evaluated against gram negative bacteria- *P. aeruginosa* ATCC 27853, *E. coli* and gram positive bacteria *B. subtilis* ATCC 6051. Strains of bacteria were maintained at 4 °C on Luria Bertani plates and were sub-cultured (24 h, 37 °C) prior to use. Purity of the cultures were checked at regular intervals as described by Farahani *et al.*¹⁷.

Determination of antimicrobial activity and minimum inhibitory concentration—Sensitivity of different bacterial strains to various extracts was determined in terms of zone of inhibition (ZOI) using disc-diffusion assay¹⁸. Sterile Whatman paper discs (6 mm in diameter) were soaked with different amount of plant extracts and placed on the 100 μ L (0.5 McFarland turbidity standard equivalents to 5×10^8 cfu/mL) bacterial inoculums spread plates. The plates were incubated at 37 °C for 24 h and diameter of resultant zone of inhibition was measured in millimeter (mm). MIC was calculated based on the minimum amount of plant extract exhibiting hair line growth inhibition around the extract containing disc. Absolute ethanol and autoclaved nanopure water was used as a negative control against the pathogens. All experiments were performed in triplicates.

HPTLC analysis

Standard stock solution preparation and calibration—Stock standard solution was prepared by dissolving 8 mg of adenosine, 1 mg of adenine and 1 mg of uracil in 10 mL methanol. Different volumes of stock solution (0.1, 0.5, 1.0, 5.0 and 10.0 μ L) were applied in duplicate on respective TLC plate to furnish 0.08 μ g to 8 μ g of adenosine and 0.01 μ g to 1 μ g of adenine and uracil, respectively. Calibration curve was prepared by plotting peak area vs. corresponding concentrations using linear least square regression analysis.

Sample solution preparation—The lyophilized CSEs solutions were prepared in known concentration of 20 mg/mL in methanol followed by sonication (15 min) and centrifugation (3000 rpm for 10 min) and was analyzed for adenosine, adenine and uracil content. The supernatant solution (15 μ L) was applied to a TLC plate followed by development in mobile phase and scanning.

Chromatography—Chromatography was performed on 20 \times 10 cm aluminium backed silica gel 60 F₂₅₄ TLC plates. Before use, the plates were washed with methanol then dried in an oven. Samples were applied to the plates as, 5 mm bands, using Camag Linomat V sample applicator equipped with 100 μ L syringe (Hamilton). Ascending development of the plate with the migration distance of 85 mm was performed at 25 \pm 2 $^{\circ}$ C with chloroform, methanol and formic acid in the ratio of 8:2:0.8 (v/v), as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 10 min. The average development time was 25 min. After development the plate was dried and scanned at 254 nm using Camag TLC scanner III equipped with

winCats software and deuterium light source with the slit 5.00 \times 0.45 mm.

Statistical analysis—All standard curves were analyzed using a linear regression of data calculating the coefficient of correlation (r). Data is expressed in mean \pm SE of three replicates.

Results

Total phenolic and flavonoid content—CSEs were found to have substantial amount of total phenolic and flavonoid content. CS_{50%Aic} extract was found to have maximum phenolic content (15.1 \pm 0.67mg/g of dry extract) while CS_{100%Aic} extract contained highest value of flavonoids (19.3 \pm 0.3 mg/g of dry extract) and the maximum yield of 37.41% was obtained for CS_{50%Aic} extract. Results are given in Table 1.

Antioxidant activity—The results of the free radical scavenging activity of CSEs studied by ABTS, DPPH and FRAP assays as presented in Table 1 show that all the CSEs are good scavenger for free radicals however, CS_{Aq} extract was found to have maximum free radical scavenging ability amongst the all extracts.

Table 1—Phytochemical analysis and Antioxidant activity of CSEs as determined by the, DPPH, ABTS, FRAP and TRP assay. [Values are mean \pm SE of 3 replicates except % of yield].

Extracts	% of yield	Phenolic content mg/g	Flavonoid content mg/g	Antioxidant activity			
				DPPH μ M TE/g	ABTS mM TE/g	FRAP μ M TE/g	TRP μ MAAE/g
CS _{Aq}	32.25	13.8 \pm 0.28	11.3 \pm 0.33	4.02 \pm 0.14	31.9 \pm 0.07	1.56 \pm 0.04	379.8 \pm 20.1
CS _{25%Aic}	36.33	11.6 \pm 0.11	7.10 \pm 0.17	3.31 \pm 0.16	19.9 \pm 0.67	1.19 \pm 0.01	360.7 \pm 10.8
CS _{50%Aic}	37.41	15.1 \pm 0.67	17.0 \pm 0.33	2.63 \pm 0.02	21.6 \pm 0.10	1.22 \pm 0.02	321.5 \pm 4.86
CS _{75%Aic}	29.20	8.80 \pm 0.23	13.0 \pm 0.33	2.86 \pm 0.07	14.4 \pm 0.87	0.89 \pm 0.06	315.2 \pm 17.4
CS _{100%Aic}	22.64	5.40 \pm 0.11	19.3 \pm 0.33	0.82 \pm 0.05	6.96 \pm 0.43	0.58 \pm 0.01	167.0 \pm 1.74

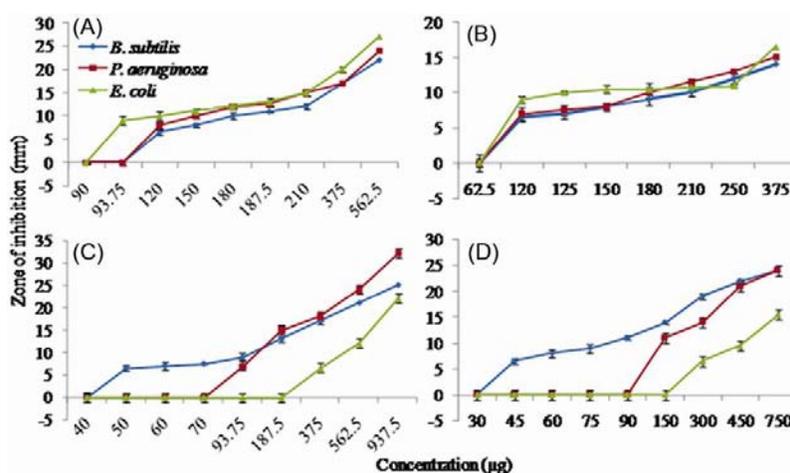


Fig. 1—MIC of extracts (A) CS_{25%Aic}; (B) CS_{50%Aic}; (C) CS_{75%Aic}; and (D) CS_{100%Aic} against bacterial strains.

Table 2—MIC determination of CSEs against bacterial strain using disc-diffusion assay. ZOI is a zone of inhibition and (-), indicates zone of inhibition less than 6.5 mm. [Values are mean \pm SE of 3 determinations.]

Extracts	<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>		<i>Bacillus subtilis</i>	
	Conc. of sample (μ g)	ZOI (mm)	Conc. of sample (μ g)	ZOI (mm)	Conc. of sample (μ g)	ZOI (mm)
CS _{Aq}	-	-	-	-	-	-
CS _{25%Alc}	93.75	9.0 \pm 0.45	120	8.0 \pm 0.24	120	6.5 \pm 0.42
CS _{50%Alc}	125	9.0 \pm 0.53	120	7.5 \pm 0.51	120	6.5 \pm 1.23
CS _{75%Alc}	375	6.5 \pm 1.52	93.75	7.0 \pm 1.24	50	6.5 \pm 0.34
CS _{100%Alc}	300	6.5 \pm 0.24	150	11.0 \pm 0.85	45	6.5 \pm 0.17

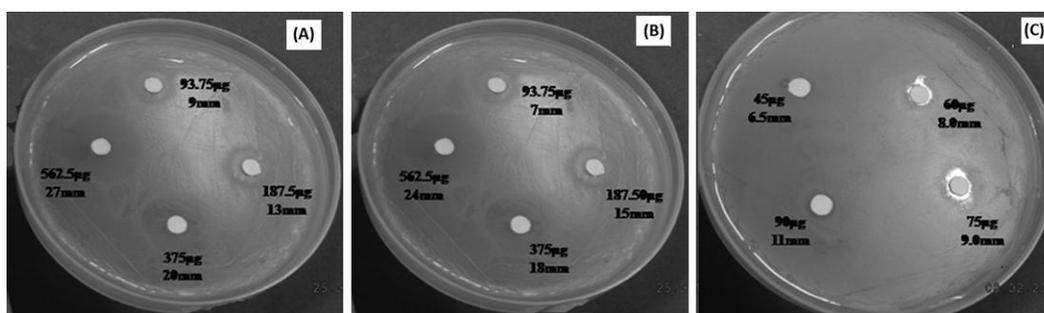


Fig. 2—ZOI of CS_{25%Alc} (A), CS_{75%Alc} (B) and CS_{100%Alc} (C) against *E. coli*, *P. aeruginosa* and *B. subtilis* strain, respectively.

Total reducing power—The total reducing potential of CSEs was determined using a modified iron (III) to iron (II) reduction assay spectrophotometrically at 700 nm. The reducing potential decreased in the order as Ascorbic Acid > CS_{Aq} > CS_{25%Alc} > CS_{50%Alc} > CS_{75%Alc} > CS_{100%Alc} extract given in Table 1.

Antibacterial activity—Antibacterial activity of CSEs was determined by using disc-diffusion assay in terms of zone of inhibition. MIC of CS_{25%Alc}, CS_{50%Alc}, CS_{75%Alc} and CS_{100%Alc} extract against all the three bacterial strains are shown in Fig. 1 (A-D) and given in Table 2. CS_{25%Alc}, CS_{75%Alc} and CS_{100%Alc} extract were more effective against *E. coli*, *P. aeruginosa* and *B. subtilis* giving 9, 7 and 6.5 mm of zone of inhibition in 93.75, 93.75 and 45 μ g concentration, respectively as shown in Fig. 2 (A-C). However, aqueous extract i.e., CS_{Aq} has shown minimal inhibition against these strains.

HPTLC analysis—Different concentrations of standard stock solution and CSEs were subjected to HPTLC analysis using the mobile phase chloroform, methanol and formic acid (8:2:0.8, v/v). HPTLC fingerprints were developed for aqueous and alcoholic extracts of *C. sinensis* (Fig. 3 A). The HPTLC

chromatograms of adenosine, adenine and uracil obtained from standards and CSEs are shown in Fig. 3 (B-G). These nucleosides were also quantified and the results (Table 3) showed the maximum concentration in CS_{Aq} amongst all other extracts. The R_f value of adenosine, adenine and uracil was found to be 0.28, 0.40 and 0.54, respectively. The correlation coefficient of 0.98 for adenosine and 0.99 for adenine as well as for uracil, respectively was indicative of good linear relationship between peak area and the concentration.

Discussion

The maximum concentration of flavonoid compounds was found in CS_{100%Alc} extract while other extracts also contained considerable quantity of these compounds which confirms the good antioxidant behaviour of the different extracts of Indian Himalayan *C. sinensis*. Among all these different extracts, CS_{50%Alc} extract was found to have maximum quantity of phenolic compounds as well as maximum yield of the extract suggesting 50% ethanol as a best solvent system for the extraction. The earlier studies have also indicated that phenolics are the

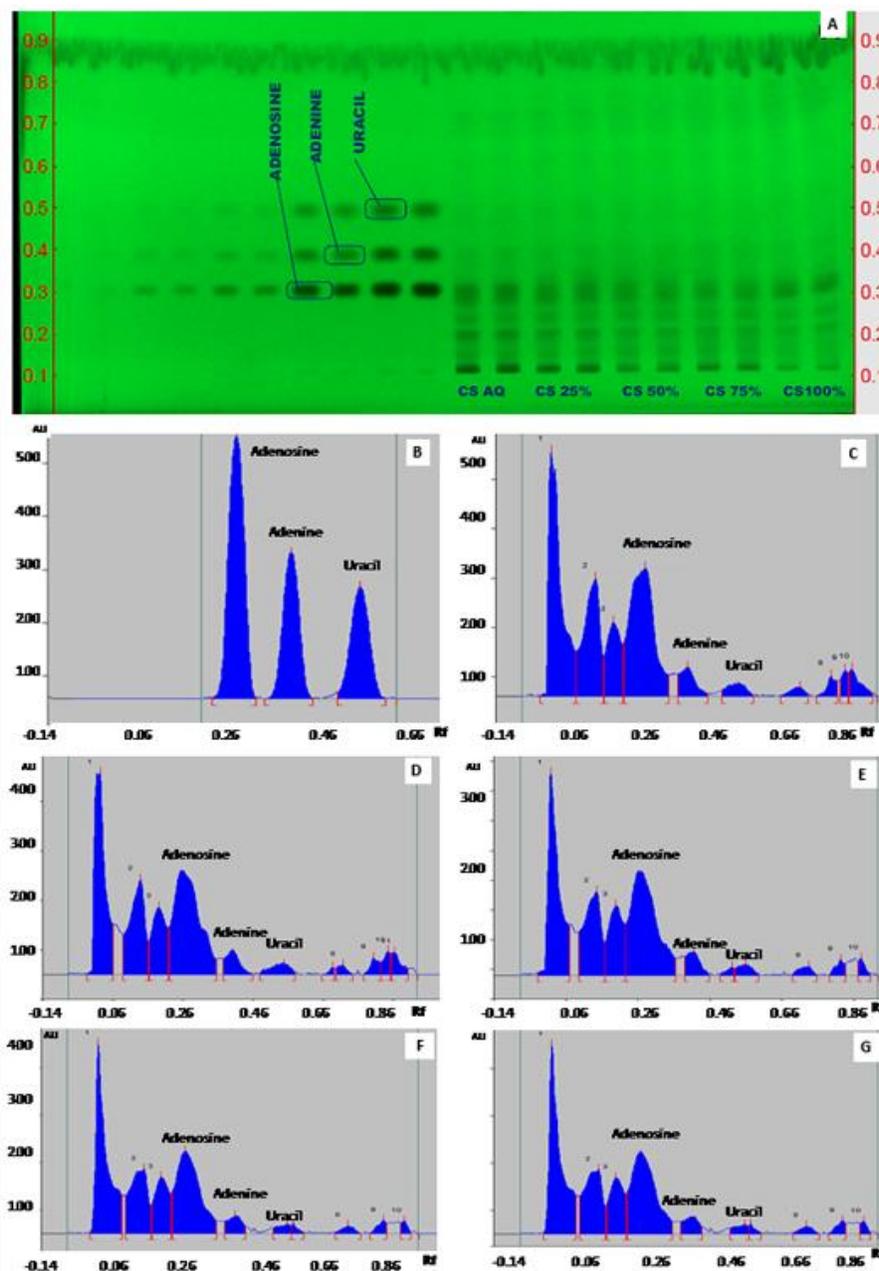


Fig. 3—Chemical structure of nucleosides quantified by HPTLC (A), HPTLC profiling at λ 254 showing the presence of adenosine, adenine and uracil in different concentration of standard and CSEs (B), HPTLC chromatogram of adenosine, adenine and uracil standard (C), HPTLC chromatogram of adenosine, adenine and uracil in CS_{Aq} (D), CS_{25%Alc} (E), CS_{50%Alc} (F), CS_{75%Alc} (G) and CS_{100%Alc} (H).

Table 3—Quantification of bioactive components in CSEs.
[Values are mean \pm SE of 3 determinations.]

Bioactive Component Content	Aqueous Extract (mg/g dry extract)		Alcoholic Extracts (mg/g dry extract)		
	CS _{Aq}	CS _{25%Alc}	CS _{50%Alc}	CS _{75%Alc}	CS _{100%Alc}
Adenosine	12.8 \pm 0.49	10.2 \pm 0.12	8.13 \pm 0.51	7.45 \pm 0.46	8.52 \pm 0.45
Adenine	0.36 \pm 0.28	0.25 \pm 0.36	0.17 \pm 0.56	0.18 \pm 0.18	0.21 \pm 0.35
Uracil	0.14 \pm 0.36	0.13 \pm 0.42	0.04 \pm 0.26	0.06 \pm 0.46	0.08 \pm 0.26

major plant constituents responsible for antioxidant activity¹⁹. The flavonoids being a class of polyphenolic compounds possesses fifteen carbon atoms; two benzene rings joined by a linear three carbon chain. Flavonoids constitute one of the most characteristic classes of compounds in the plants. The six major subgroups of this class of compounds are: chalcones, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Flavonoids are responsible for various pharmacological and biological actions such as in prevention of cardiovascular disease²⁰, improve cognitive ability²¹, hepatoprotective potential²², and antimicrobial activities along with antioxidant activities²³.

The free radical scavenging activity of CSE_s was also measured by ABTS, DPPH assay in terms of their ability to bleach the stable free radical. In FRAP assay, the antioxidant potential of CSEs was estimated by their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). CS_{100%Aq} extract was found to have highest free radical scavenging ability as DPPH, ABTS and FRAP assay, showing highest antioxidant activities amongst the all extracts but no significant correlation was observed between the quantity of phenolics, flavonoid content and antioxidant activities of these extracts. Antioxidant capacities of the phenolic compounds vary from compound to compound because of their structural differences²⁴. Therefore, the high antioxidant activity of aqueous extracts may be attributed to the presence of some other known phytochemicals such as tocopherol, vitamins and other pigments as well as nucleosides and polysaccharides etc in *Cordyceps sinensis*^{25,26}.

TRP of CSEs was determined in terms of their capacity to reduce the Fe³⁺/Ferricyanide ion to the Fe²⁺ form, depending upon the concentration of the antioxidant compounds present in the extracts. The maximum reducing potential observed in CS_{Aq} extract may be directly correlated to its maximum antioxidant power²⁷.

All the extracts showed antimicrobial activity against *E. coli*, *P. aeruginosa* and *B. subtilis*. The variation in the sensitivity of these extracts against different pathogens may be due to the difference in the genotype of these strains²⁸. Since phenolic constituents of the plant extracts have shown potent antimicrobial properties in earlier studies²⁹, observed antibacterial activity in the present CSEs could also be attributed to the presence of phenolic compounds.

However, CS_{Aq} extract has shown minimal inhibition against these strains despite having maximum antioxidant activity. This suggests that the antimicrobial activity may not be directly related to the antioxidant activity of the plant extract which is also supported by the previous study³⁰. However, it needs further investigation on the relationship of secondary metabolites and their antimicrobial activity in particular extract.

Different nucleosides (adenine, adenosine and uracil) were also quantified by HPTLC and the results showed maximum concentration in CS_{Aq} extract amongst all other extracts.

The presence of nucleosides together with antioxidants and antimicrobial activity of as reported in this study definitely suggest that CSEs may be used in prevention and treatment of inflammatory and free radical mediated diseases associated with pathogenic infection.

Conclusion

It is concluded that CS_{50%Aic} was found to have maximum phenolic content along with the highest yield of the plant extract while level of flavonoid compounds was considerable in CS_{100%Aic}. Further, aqueous extract (CS_{Aq}) possessed the highest content of three individual nucleosides (energy molecules) as quantified by HPTLC compared to hydro-alcoholic extracts and considerable correlation has been observed for the aqueous extract in terms of both antioxidant potential and nucleoside content. Also, all the hydro-alcoholic and alcoholic CSEs were found to have good antimicrobial activity against gram negative as well as gram positive bacterial strains.

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Conflict of interest

The authors declare that there is no conflict of interest.

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