Indian Journal of Ecology (2022) 49(3) (SI): 1113-1121
DOI: https://doi.org/10.55362/IJE/2022/3639

Manuscript Number: 3639
NAAS Rating: 5.79

Effect of Solvents Extraction on Chemical Profile and Biological Potential of *Isodon coetsa* Seeds

Shaweta Sharma, Amita Kumari*, Jyoti Dhatwalia, Ishita Guleria, Ajit Sharma[1], Vedpriya Arya[2] and Ashwani Kumar[2]

School of Biological and Environmental Sciences, Faculty of Sciences, Shoolini University, Solan-173 212, India
[1]Dr. Yashwant Singh Parmar University of Horticulture and Forestry, College of Horticulture and Forestry Thunag, Mandi-175 048, India
[2]Patanjali Herbal Research Department, Patanjali Research Institute, Haridwar-249 405, India
**E-mail: amitabot@gmail.com*

***Abstract*:** *Isodon coetsa* is a perennial medicinal herb of *Lamiaceae* family. Traditionally, root and leaf juices have been reported to treat fever and gastrointestinal disorders. The present study dealt with phytochemical screening and biological potential characterization of *I. coetsa* seeds. The results showed the presence of a higher quantity of secondary metabolites in the methanolic extract of seeds (ME). The antioxidant activity through DPPH analysis was higher in ethyl acetate extract of seeds (94.58% scavenging at 40 µg mL^{-1}) as compared to other extracts. The antimicrobial assays showed the higher antimicrobial potential of ME followed by chloroform extract of seeds (CE) against tested bacteria and fungi with agar disk diffusion method, food poison method, and MIC. The GC-MS analysis of chloroform seed extract revealed octadecanoic acid, 2-(2hydroxyethoxy) ethyl ester (33.75%); dimethylsulfoxonium formylmethylide (21.40%); 9,12,15-octadecatrienoic acid (Z,Z,Z) (12.12 %); 12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z) (7.17%); 9,12-octadecadienoic acid (Z,Z) (6.75%), and hexadecanoic acid, methyl ester (3.69%) as major chemical constituents, whereas methanolic extract of seeds was characterized with bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl (49.93%); phenol,2-methoxy-3-(2-propenyl) (14.63 %); 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (9.63%); caryophyllene oxide (2.57%), and caryophyllene (2.46%). The present study reported good antioxidant and antimicrobial potential of *I. coetsa* seeds and thus it can be explored further as a source of natural antioxidants and medicinally important phytocompounds for utilization in food as well as pharmaceutical industries.

***Keywords*:** *Isodon coetsa,* Phytoconstituents, Antioxidants, Antimicrobial activity, Gas chromatography/mass spectrometry

Plants with medicinal values have been used for the cure of several diseases since prehistoric times. Their biological potential as well as role in the treatment of various diseases was well documented with the whole plant and sometimes with plant components such as leaves, stems, roots, seeds, and barks, etc. (Petrovska 2012, Kumari et al 2018, Dhatwalia et al 2021, Sharma et al 2021, Balkrishna et al 2021, Sonam et al 2021). Leaves, roots, rhizome, and barks are the most common herbal components in the traditional medicinal system. Seeds are the nutrient powerhouse and are generally utilized for daily food and nature has developed them as a reproductive unit (Rindt 2008). Because of their high nutrient value, they are used to reduce blood pressure, cholesterol, and blood sugar, etc. (Marwat et al 2011) and also carry medicinal properties like anti-inflammatory, diuretic activities, etc. (More et al 2013, Afshari et al 2016). The genus *Isodon* formerly known as *Rabdosia* is one of the major group of Angiosperms. More than 150 species of this genus are distributed worldwide throughout tropical and subtropical Asia and southwestern China (Sun et al 2006). The most common species of the genus *Isodon* are *I. rubescens*, *I. ternifolia*, *I. lophanthoides,* and *I. megathyrsa*. These species have been documented in the Chinese traditional medicine system for the cure of diseases like gastrointestinal and respiratory bacterial infections, enteritis, jaundice, inflammation, and cancer, etc. (Sun et al 2006,Park 2011). Among them, *I. coetsa* (Buch.-Ham. ex D. Don) Kuso (syn. *Plectranthus coesta* Buch.-Ham. ex D. Don) is a medicinal perennial herb mainly distributed in Tropical and Subtropical Asia. The plant is native plant of Bangladesh, Nepal, India, China, Myanmar, Philippines, Jawa, Sri Lanka, Sumatera, and Thailand (KewScience-Plants of the World online 2021). Morphologically, the plant is a perennial herb with opposite ovate or ovate lanceolate leaves and lavender-blue cymes bearing flowers arranged in spreading panicles. This species can be easily differentiated by its thinner leaves, sub-equal lobes, and strongly decurved corolla tube (The Plant List 2013). Traditionally, the roots and leaf juices of *I. coetsa* are used for the cure of fever and gastrointestinal disorders (Sun et al 2006). Additionally, antibacterial and anticancer activities of leaves of *I. coetsa* are also reported in the literature (Sun et al 2006, Zhao et al 2011, Xu et al 2012).

Although none of the literature available on the traditional medicinal property of seeds of *I. coetsa*, therefore, the current investigation was focused to evaluate the phytochemicals and biological properties of the unexplored part (seeds) of the *I. coetsa*. The seeds of *I. coesta* are morphological very much similar to the seeds of *Ocimum* species reported to have medicinal value.

MATERIAL AND METHODS

Seed Source and Seed Extracts Preparation

The seeds of *I. coetsa* were procured from Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). For the preparation of seeds extracts, 20 g of *I. coetsa* seeds were converted to a coarse powder using an electric grinder and with the cold maceration method, the coarse powder was then extracted using different solvent systems viz., methanol, ethyl acetate, chloroform, pet ether followed by filtration using Whatman filter paper no. 1 (Kumar et al 2018, Kumar et al 2020). All crude seed extracts were dried at room temperature, labeled as methanol seeds extract (ME), ethyl acetate seeds extract (EE), chloroform seed extract (CE), and petroleum ether seed extract (PE), and stored at 4ºC for further use.

Quantitative Determination of Phytochemicals

Estimation of alkaloids: The alkaloids content in the various seeds extracts of *I. coetsa* was checked following the method of Harborne (1973). The crude seed extract (1g) was treated with 100 mL of acetic acid in ethanol (1:9) and covered with aluminum foil. The solution was kept as such for 4 hours, filtered, and kept on a water bath (60ºC) until the volume is reduced by 1 quarter. The ammonium hydroxide (concentrated) was added dropwise to the extract for complete precipitation. After settling down, the solution was filtered and precipitates were collected, washed with ammonium hydroxide (diluted), and filtered again. The obtained alkaloid residues were dried, weighed, and expressed in percentage.

Estimation of flavonoids: The flavonoids content of *I. coetsa* seeds were checked spectrophotometrically using the method of Chang et al (2002)**.** The seed extract (100 mg) was mixed with methanol (4.5 mL) and to the mixture, 0.1 mL of aluminum chloride (10 %) and Sodium acetate (1M) solutions were added. Absorbance was taken after 30 min at 415 nm. The flavonoids content was computed from the rutin (RUT) calibration curve and represented as mg RUT/g of extract.

Estimation of terpenoids: The total terpenoid content of seeds of *I. coetsa* was calculated as per the method of Ghorai et al (2012). To 100 mg of the seed extract, chloroform (3 mL) was added and mixture was thoroughly vortexed and left for 3 min. To the solution concentrated H_2SO_4 (200 µL) was added. The solution was then incubated for $1^{1/2}$-2 hours at room temperature in dark condition and reddish-brown precipitates were formed after incubation. The supernatant was decanted carefully and to the precipitates, 3 mL of methanol (95%) was added and until all the precipitates completely dissolved in methanol the solution was vortexed thoroughly. The absorbance was measured at 538 nm. Terpenoids were calculated from linalool (LIN) calibration curve and expressed as mg LIN/g of extract.

Total tannin content: The total tannin content was estimated using Tambe and Bhambar (2014) method. To 100 mg of the crude extract distilled water (7.5 mL) and Folin-Ciocalteu phenol reagent (0.5 mL) were added. The 1 mL of Na_2CO_3 solution (35%) was added to the mixture and again diluted with distilled water (10 mL). The mixture was dissolved properly and kept for 30 min at room temperature. The standard solutions of Gallic acid (20-100 µg/mL) were prepared and absorbance at 725 nm was measured. The total tannin content was further calculated from the calibration curve of Gallic acid (GAE) and represented as mg GAE/g of extract.

Saponins content: The saponins content of *I. coetsa* seeds was determined following the procedure of Madhu et al (2016). The seed extract (100 mg) was added to 2 mL of vanillin solution (1g of vanillin in 70 mL of ethanol). To this solution, 2 mL of sulfuric acid solution (72 %) was added, properly mixed and the solution was heated at 100ºC using a water bath for 10 min. Absorbance was recorded at 544 nm. The diosgenin (DIO), as a standard material was used and saponins content was measured from the diosgenin calibration curve, and presented in mg DIO/g of extract.

Total phenolic content: Total phenolic content present in the seeds of *I. coesta* was calculated using the Folin Ciocalteu reagent method given by McDonald et al (2001). Briefly, from the stock solution (100 mg extract in 9 mL distilled water), 1 mL was taken, mixed with Folin Ciocalteu reagent (0.5 mL) and 20% Na_2CO_3 solution (1.5 mL). The whole volume of the solution was made up to 8 mL by adding distilled water, followed by forceful shaking. The solution for 2 hours was allowed to stand as such and then absorbance was taken at 765 nm. The total phenolic content was calculated from standard calibration curve of gallic acid and expressed in mg GAE/g of extract.

Total glycosides content: Total glycosides content was observed by following Solich et al (1992) method. The seed extract (100 mg) was mixed with freshly prepared Baljet's reagent (10 mL) and after an hour, the mixture was diluted with distilled water (20 mL) and the absorbance at 495 nm was observed. As a standard, digitoxin (DIG) was used and

glycosides content was calculated in mg DIG/g of extract.

Steroids: The steroid content from seeds of *I. coesta* was checked using Madhu et al (2016) methodology. To the seed extract (100 mg), 4 N sulfuric acid (2 mL) and 0.5 % iron (III) chloride solution (2 mL; 0.5 % w/v) were added followed by 0.5 mL of potassium hexacyanoferrate (III) solution (0.5 mL; 0.5 % w/v). On a water bath (70±20ºC) the mixture was heated for 30 minutes and further diluted with distilled water with shaking. The absorbance was taken at 780 nm against the blank solution. As a standard, Cholesterol was used and steroids content was represented in mg/g CHO of extract.

***In vitro* Antioxidant Activity of *I. coesta* Seeds**

***2,* 2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity:** Different seed extracts of *I. coetsa* were evaluated for their antioxidant activity using DPPH assay (Sharma and Bhat 2009) and ascorbic acid was taken as a control.

$$\text{Scavenging activity \%} = \frac{^{A}(\text{control})^{A}\text{-(sample)}}{^{A}\,(\text{control})} \times 100$$

where, $A_{(control)}$ was the absorption of the DPPH and $A_{(sample)}$ was the absorption of the plant extracts. The graph was plotted between % radical scavenging activity and different concentrations. From the graph, half-maximal inhibition (IC_{50}) value (µg mL^{-1}) was calculated using the regression method.

Antimicrobial Potential of Different Extracts of *I. coetsa* Seeds

Microbial strains and growth conditions: Pathogenic bacterial (*Bacillus subtilis* MTCC 5521*, Staphylococcus aureus* MTCC73, *Escherichia coli* MTCC739, and *Klebsiella pneumoniae* MTC109] and fungal strain (*Fusarium oxysporum* SR266-9) were used to observe the antimicrobial potential of *I. coetsa* seeds. All pathogens were taken from the School of Microbiology, Shoolini University, Solan. Bacteria were maintained on nutrient agar (NA) plates while fungus was grown on potato dextrose agar (PDA) at 37ºC and 25ºC, respectively.

Agar disc diffusion method for antibacterial and poison food method for antifungal activity*:* For the evaluation of the antimicrobial activity of different seed extracts of *I. coetsa,* the agar disc diffusion method was used *(*Bayer et al 1966). The extracts were taken in different concentrations (2-8 mg mL^{-1} in DMSO, w/v) and antimicrobial activity was expressed in terms of zone of inhibition (ZOI) towards the growth of microorganisms after incubation. As a positive and negative control, Ampicillin and DMSO, respectively were used. A similar procedure was repeated thrice for each bacterial strain. The poison food technique (Ramaiah and Garampalli 2015) was performed to evaluate the antifungal potential of *I. coetsa* against *F. oxysporum* using different concentrations (20-80 mg/mL in DMSO, w/v). As a positive and negative control, Hygromycin B (2 mg mL^{-1}) and DMSO (4 mg mL^{-1}) were used.

$$\text{Inhibition \%} = \frac{(C - T)}{C} \times 100$$

Where, C represents the diameter of the control and T represents the treated colony.

Determination of minimal inhibitory concentrations (MIC): The broth dilution method described by the Clinical and Laboratory Standard Institute (CLSI) was used to investigate the MIC of different *I. coetsa* seeds extracts. Nutrient broth (NB) was used for antibacterial activity, while potato dextrose broth (PDB) was used to observe the antifungal activity. The geometric dilutions of extracts ranged from 50-0.098 µg mL^{-1} was prepared in a microtiter plate (96-welled), including one growth control (only NB/PDB containing DMSO), one with the positive control (NB/PDB inoculated with bacterial and fungal culture) and one with antibiotics (ampicillin/hygromycin B). Inoculums containing 2×10^{6} CFU/mL) were added into each well and the plates were kept at 37°C for 24 h (for bacterial strains) and 25°C for 48 h for fungi (Mohr et al 2017).

Gas chromatography and mass spectrometry (GC-MS) analysis: The GC-MS analysis of the essential oil samples of *M. piperita* and *M. longifolia* leaves was performed as per the standard method of Ladhim et al (2016) in the SERF laboratory of Punjab University, Chandigarh, India. The analysis was done using a GC-MS instrument (Thermo Trace 1300 GC coupled with Thermo TSQ 8000 Triple Quadrupole MS) fitted with a TG 5MS capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness) for qualitative determination. The essential oil was diluted 1/10 in n-hexane (v/v) before analysis. The Autosampler was built for the injection of extract, injector temperature: 250°C, ion-source temperature: 230°C. Oven temperature: 60°C for 2 min, 60-250°C (10°C/min to 250°C), 250°C for 5 min. Carrier gas: helium gas (99.999%), flow rate: 50 mL/min, injection volume of 3.0 µl, split ratio: 33:3 Mass spectra: 70eV; a scan interval: 0.5 s and fragments rate: 45 to 450 Da. Total GC running time was 5 min. For analysis 1 µl of a sample was used. The components were recognized based on their retention times (R_T). Further, the percentage of constituents was measured based on peak area and phytoconstituents were identified by comparing their mass spectra with those recorded in NIST 2.0 electronic Library and Wiley 275 libraries by considering Match Factor (SI) and Reverse Match Factor (RSI) higher than 600 and also with mass spectrum from the literature.

Data analysis: All the experiments were carried out in triplicate, statistically analyzed with PRISM software, and results were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Quantitative phytochemical analysis: The quantitative phytochemical estimation revealed a higher amount of phenols (819.39 mg GAE/g), flavonoids (261.98 mg RUT/g), saponins (124.58 mg DIO/g), glycosides (729.23 mg DIG/g), terpenoids (181.69 mg LIN/g) and alkaloid (28.06%) in ME, whereas, total steroid content was higher in CE of *I. coetsa* seeds (444.71 mg CHO/g) (Table 1).

Antioxidant potential of *I. coetsa* seeds: The antioxidant potential of seed extracts of *I. coetsa* increased with an increase in the concentration of seeds extract (5-40 µg mL^{-1}) (Fig. 1). In DPPH assay, the highest free radical scavenging activity (%) was observed with EE followed by ME and CE (with IC_{50} <5 µg mL^{-1}) as compared to PE (IC_{50} ranged between 5-10 µg mL^{-1}). Whereas, Ascorbic acid showed IC_{50} value 29.87 µg mL^{-1} (Fig. 1).

Antimicrobial activity of *I. coetsa* seeds: The antimicrobial activity of extracts depends on concentration and activity was increased with an increase in extract concentration from 2-8 mg mL^{-1} against bacteria and 20-80 mg mL^{-1} against fungi (Fig. 2). Agar disc diffusion assays revealed a higher zone of inhibition with ME and CE against both bacterial and fungal strains. Similarly, the poison food method also showed higher % inhibition with ME and CE (Fig. 3). Broth dilution method showed the maximum antimicrobial activity of ME for *E. coli* (MIC-1.25 mg mL^{-1}), *K. pneumoniae* (MIC-1.25 mg mL^{-1}), *F. oxysporum* (MIC-2.5 mg mL^{-1}), *S. aureus* (MIC-2.5 mg mL^{-1}), and *B. subtilis* (MIC-5 mg mL^{-1}) (Fig. 4).

GC-MS Characterization of chloroform and methanol extracts of *I. coetsa* seeds: The CE and ME showed highest antioxidants and antimicrobial activity among all extracts of *I. coetsa* seeds, therefore, these two extracts were

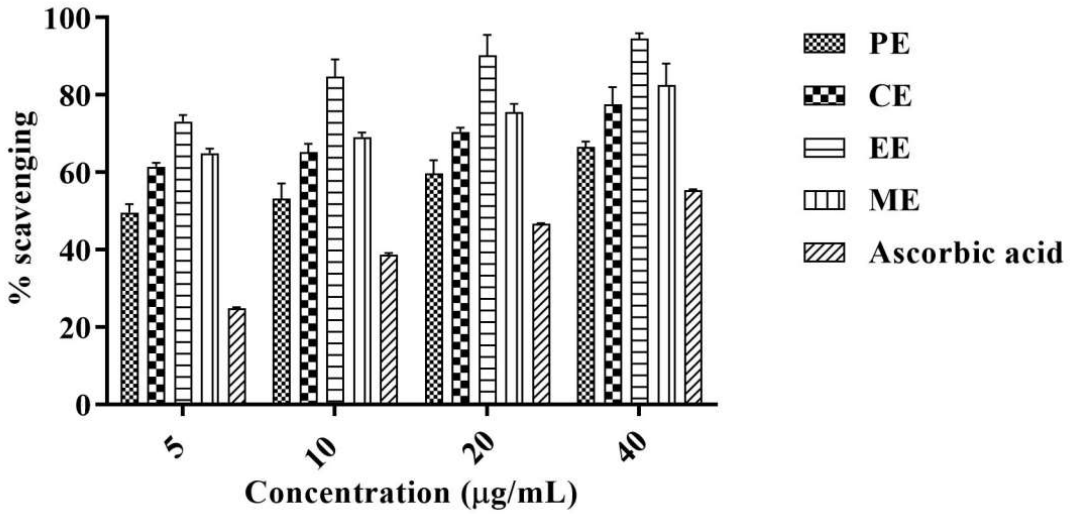

Fig. 1. DPPH radical scavenging activity of *I. coetsa* seed extracts. ME: Methanolic extract; CE: Chloroform extract; EE: Ethyl acetate extract; PE: Petroleum ether extract

Table 1. Quantitative phytochemical analysis of different seed extracts of *I. coetsa*

Phytochemicals	*I. coetsa* seeds (mean±SD)			
	ME	CE	PE	EE
Tannins (mg GAE/g)	198.98±2.71	108.04±0.81	42.67±0.57	77.30±1.47
Phenols (mg GAE/g)	819.39±1.60	346.14±3.16	152.91±2.88	186.26±1.49
Flavonoids (mg RUT/g)	261.98±0.59	127.60±1.01	26.81±0.93	172.55±1.66
Saponins (mg DIO/g)	124.58±0.87	100.72±0.58	67.78±0.44	32.03±0.39
Glycosides (mg DIG/g)	729.23±2.93	473.68±3.34	234±2.30	212.54±3.93
Terpenoids (mg LIN/g)	181.69±0.34	99.14±0.44	43.21±1.11	43.21±1.11
Steroid (mg CHO/g)	403.5±1.63	444.71±0.79	80.55±0.83	262.86±1.58
Alkaloid (%)	28.06±0.05	25.22±0.06	21.26±0.041	19.90±1.20

SD: Standard deviation; ME: Methanol seeds extract; PE: Petroleum ether seeds extract; EE: Ethyl acetate seeds extract; CE: chloroform seeds extract

further characterized through GC-MS analysis. GC-MS chromatogram showed the presence of total 19 compounds in CE, and 9 compounds in ME of *I. coetsa* (Fig. 5 a and b). CE was characterized with octadecanoic acid, 2-(2hydroxyethoxy) ethyl ester (33.75%), dimethylsulfoxonium formylmethylide (21.40%); 9,12,15-octadecatrienoic acid (Z,Z,Z) (12.12%); 12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z) (7.17%); 9,12-octadecadienoic acid (Z,Z) (6.75%), and hexadecanoic acid, methyl ester (3.69%) as major chemical constituents (Table 2), whereas ME was characterized by bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl (49.93%); phenol,2-methoxy-3-(2-propenyl) (14.63%); 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (9.63%); caryophyllene oxide (2.57%) and caryophyllene (2.46%) as major phytocompounds (Table 3).

Plants are the richest source of metabolites, viz., alkaloids, phenols, glycosides, flavonoids, steroids, saponins, and tannins. (Dillard and German 2000). Most of these metabolites have health effects on people in terms of free radical scavengers, substrates for biochemical process, selective inhibition to the hazardous intestinal bacterium, etc. (Webb 2013). The present work is possible the first report on the quantitative estimation of phytocompounds in *I. coesta* seeds. Similar to this study, several studies have reported the presence of alkaloids, glycosides, saponins, flavonoids, tannins, steroids in various plant parts extracts (*Plectranthus hadiensis*, *Coleus aromaticus, Pogestemon patchouli, Ocimum sanctum,* and *Mentha spicata*) of family Lamiaceae (Menon and Sasikumar 2011, Rai et al 2013, Soni and Sosa 2013).

The quantitative phytochemical analysis results showed a higher amount of phytocompounds in ME, indicating the maximum extraction capacity of methanol solvents in comparison to other solvents. Similar to the present study, Swamy et al (2017), Menon and Sasikumar (2011), Rai et al (2013), and Vimala et al (2014) also observed higher phenolic, flavonoids, and tannin content in the methanol extract of *P. amboinicus, P. hadiensis, Leucas linifolia, O. sanctum,* and *O. basilicum,* respectively. The current study also showed higher steroid content in CE followed by ME of *I. coetsa*, similar to the results of Rai et al (2013) on *Pogestemon patchouli* and *Leucas linifolia*. Antioxidants are substances that can protect the cell from free radical's damage and reduce harm (Santos-Sanchez et al 2019). Maximum antioxidant activity was observed in EE and ME of *I. coetsa* with DPPH assay. Onder et al (2020) also observed higher antioxidant potential or lower IC_{50}value in ethyl acetate extracts of leaves of *Thymus sipyleus, Phlomis armeniaca*, and *Sideritis galactica* as compared to the methanol extract. In addition to these, EE of *Mentha officinalis* leaves also

Table 2. GC-MS analysis of chloroform extract of *I. coetsa* seeds

Compound name	Retention time (min)	Peak area (%)	RSI	SI
Butane, 2-ethoxy-2-methyl	3.08	0.21	943	659
1,3,5-Cycloheptatriene	3.62	1.36	926	906
Dimethylsulfoxonium formylmethylide	6.00	21.40	979	979
Dimethyl sulfone	9.69	0.58	841	830
2,4-Heptadien-1-ol, (E,E)-	10.34	0.31	608	586
Dodecane, 2,6,11-trimethyl	13.81	0.35	852	822
Cyclohexasiloxane, dodecamethyl	14.47	0.23	745	631
Caryophyllene	15.75	0.75	878	869
1-Iodo-2-methylundecane	16.60	0.38	865	760
Isoaromadendrene epoxide (Ledene oxide-(II))	16.94	0.75	806	803
Andrographolide	18.39	0.77	785	719
10,12-Tricosadiynoic acid, methyl ester	18.46	0.22	782	778
Hexadecanoic acid, methyl ester	21.28	3.69	903	903
Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	21.95	33.73	747	744
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	22.97	7.17	904	904
Methyl stearate	23.15	0.58	858	858
9,12-Octadecadienoic acid (Z,Z)	23.43	6.75	851	851
Butyl 9,12,15-octadecatrienoate	23.60	1.20	854	806
9,12,15-Octadecatrienoic acid, (Z,Z,Z)	23.95	12.12	864	859

RSI: Reverse match factor; SI: Match factor

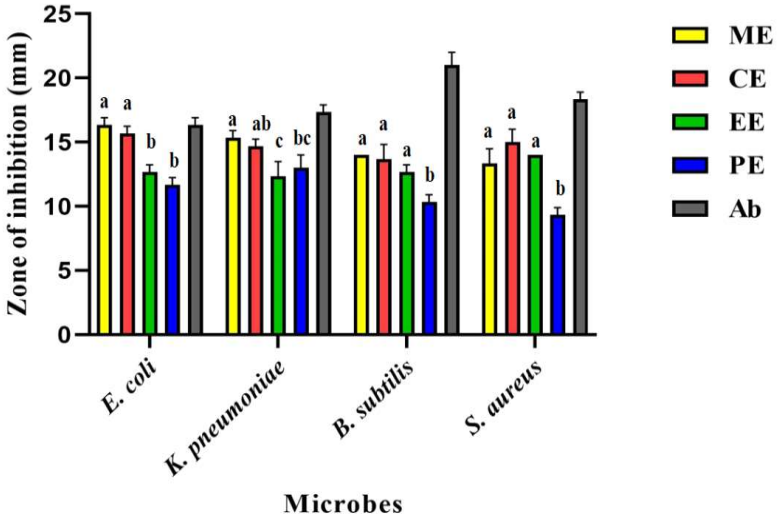

Fig. 2. Antibacterial activity of *I. coetsa* seed extracts (8 µg mL^{-1}) in terms of zone of inhibition against bacterial strains. Ab: Ampicillin (50 µg mL^{-1}); ME: Methanolic extract; CE: Chloroform extract; EE: Ethyl acetate extract; PE: Petroleum ether extract. Different letters in same column indicate statistical significant differences ($p<0.05$) Tukey's test)

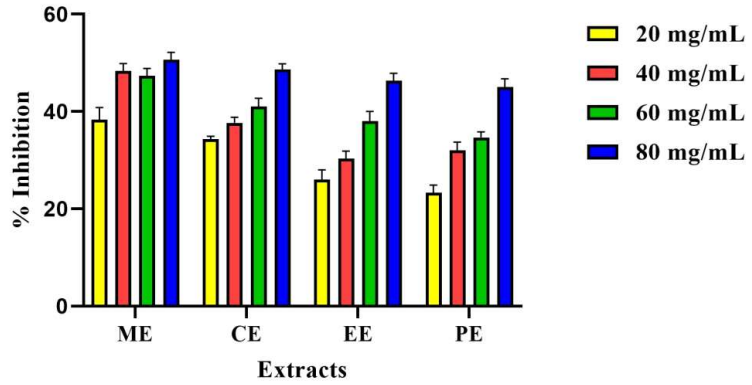

Fig. 3. Anti-fungal activity of *I. coetsa* seed extracts at varying concentrations against *F. oxysporum.* ME: Methanolic extract; CE: Chloroform extract; EE: Ethyl acetate extract; PE: Petroleum ether extract

Table 3. GC-MS analysis of methanolic extract of *I. coesta* seeds

Compound name	Retention time (min)	Peak area (%)	RSI	SI
Ethane, 1,1-diethoxy	3.36	2.81	946	946
Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-, (1S)-	11.84	49.93	960	913
Glycerin	12.13	10.22	940	940
Nonadecan-1-ol trimethylsilyl ether	14.46	2.55	591	534
Phenol, 2-methoxy-3-(2-propenyl)	15.04	14.63	889	889
Caryophyllene	15.75	2.46	790	745
3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimeth ylsiloxy) tetrasiloxane	16.64	1.54	714	604
Caryophyllene oxide	17.77	2.57	827	764
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	22.98	9.63	824	824

showed the highest antioxidant potential followed by butanol, methanol, and petroleum ether extracts (Hassan et al 2019). Swamy et al (2017) observed the highest ferric ion reduction potential in methanolic leaves extract of *Plectranthus amboinicus* [849.63 M of Fe (II)/g dry weight] followed by hexane and acetone extract.

The antimicrobial activity of seeds of *I. coetsa* was higher in ME followed by CE, EE, and PE against *E. coli, K. pneumoniae*, *B. subtilis*, *S. aureus,* and *F. oxysporum*. Similar to our report, methanolic leaves extract of *P. amboinicus* also showed greater antimicrobial activity (*B. subtilis*, *P. aeruginosa*, *S. aureus*, *E. coli*, and *C. albicans*) (Swamy et al 2017). Rauf et al (2012) also revealed the higher antibacterial potential of methanolic extract of *I. rugosus* leaves against *E. coli, K. pneumoniae,* and *S. aureus,* followed chloroform, ethyl acetate, and n-hexane

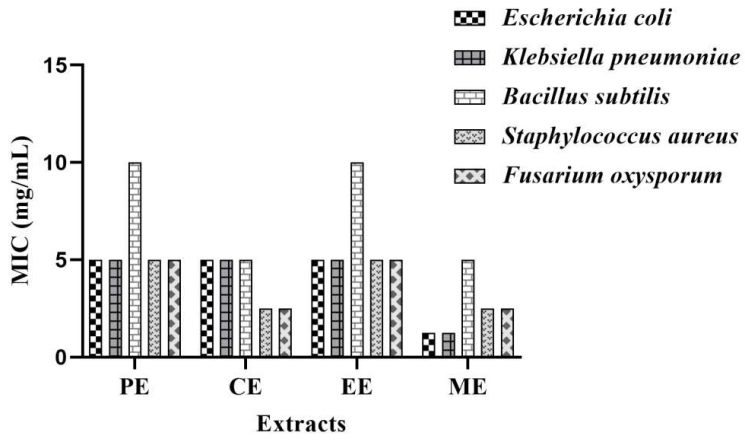

Fig. 4. MIC values of different seed extracts of *I. coetsa* against bacterial and fungal strains. ME: Methanolic extract; CE: Chloroform extract; EE: Ethyl acetate extract; PE: Petroleum ether extract

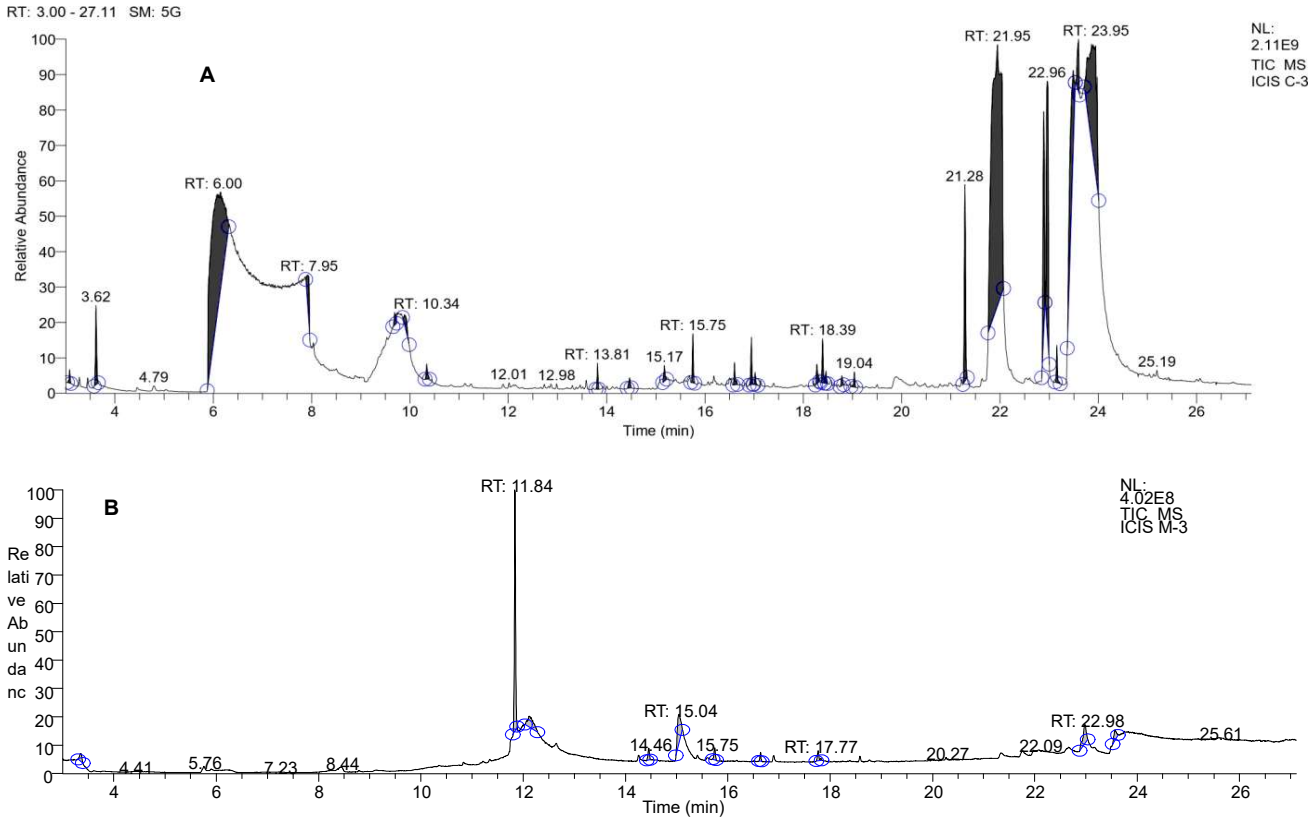

Fig. 5. GC-MS chromatogram of chloroform (a) and methanolic seed extract (b) of *I. coesta*

extract, whereas methanol leaves extract of *I. rugosus* showed higher antimicrobial activity against *Salmonella typhi* and *Klebsiella pneumoniae* than that of ethyl acetate and chloroform extracts (Zeb et al 2017). Further, the chemical characterization of chloroform extract of *I. coetsa* seeds showed the presence of more phytocompounds as compared to that of methanol extract (Tables 3 and 4). Similar to the present study, methanolic extract of *O. sanctum* showed the presence of eugenol (3.31%), caryophyllene (3.61%), germacrene (1.52%), 9,12,15-octadecatrienoic acid methyl ester (1.88%), hexadecanoic acid methyl ester (2.30%), and phenol-2-methoxy-4-(1-propenyl) (3.31%) through GC-MS analysis (Arulraj et al 2014). All the compounds (9,12,15-octadecatrienoic acid, caryophyllene oxide, bicyclo[2.2.1]heptan-2- one,1,7,7-trimethy, dimethylsulfoxonium formylmethylide, octadecanoic acid, and hexadecanoic acid) observed in ME and CE of *I. coetsa* have been reported in the literature as biologically active compounds (Al-Marzoqi et al 2015, Mujeeb et al 2014, Guerrero et al 2017, Paude et al 2019). Therefore, it can be concluded that the biological activities in the CE and ME of *I. coetsa* could be due to the presence of these observed biologically active phytocompounds.

CONCLUSIONS

This study is the first attempt to quantify the phytochemicals of different solvent extracts of *I. coetsa* seeds. The current study concluded the highest extraction capacity of methanol and chloroform solvents for several chemical constituents of *I. coetsa* seeds which are responsible for various biological activities. The results from the present study showed higher antioxidant and antimicrobial potential of *I. coetsa* seeds, therefore, can be utilized in the food industry as a source of natural antioxidants and in pharmaceutical industries for the development of safer drugs. However, further study needs to be done to identify phytocompounds of *I. coetsa* seeds and also their biological potential with *in-vivo* studies.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

All authors have made a substantial and direct contribution to the work.

ACKNOWLEDGEMENTS

The authors are thankful to Shoolini University, Solan for providing funds for GC-MS analysis.

REFERENCES

Afshari AR, Sadeghnia HR and Mollazadeh H 2016. A review on potential mechanisms of *Terminalia chebula* in Alzheimer's disease. *Advances in Pharmacological Sciences* **2016**: 1-14.

Al-Marzoqi AH, Hameed IH and Idan SA 2015. Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). *African Journal of Biotechnology* **14**(40): 2812-2830.

Arulraj J, Shanmugaiah V and Lakshmanan N 2014. Studies on phytochemical analysis and antimicrobial activity of Tulsi (*Ocimum sanctum* Linn) leaf extract against human and fish pathogens. *International Journal of Advanced Life Sciences* **7**(1): 27-34.

Balkrishna A, Rohela A, Kumar A, Kumar A, Arya V, Thakur P, Oleksak P, Krejcar O, Verma R, Kumar D and Kuca K 2021. Mechanistic insight into antimicrobial and antioxidant potential of *Jasminum* species: A herbal approach for disease management. *Plants* **10**(6): 1-25.

Bayer AW, Kirby WM, Sherris JC and Turck M 1966. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Pathology* **45**(4): 493-496.

Benzie IF and Strain JJ 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power, the FRAP assay. *Analytical Biochemistry* **239**(1): 70-76.

Chang CC, Yang MH, Wen HM and Chern JC 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* **10**(3): 178-182.

Dhatwalia J, Kumari A, Verma R, Upadhyay N, Guleria I, Lal S, Thakur S, Gudeta K, Kumar V, Chao CJ, Sharma S, Kumar A, Manicum AE, Lorenzo JM and Amarowicz R 2021. Phytochemistry, pharmacology, and nutraceutical profile of *Carissa* species: An updated review. *Molecules* **26**(22): 1-34.

Dillard CJ and German JB 2000. Phytochemicals: Nutraceuticals and human health. *Journal of the Science of Food and Agriculture* **80**(12): 1744-1756.

Ghorai N, Chakraborty S, Gucchait S, Saha SK and Biswas S 2012. Estimation of total terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. *Research Square* 1-5.

Gohari AR, Hajimehdipoor H, Saeidnia S, Ajani Y and Hadjiakhoondi A 2011. Antioxidant activity of some medicinal species using FRAP assay. *Journal of Medicinal Plants* **10**(37): 54-60.

Guerrero RV, Vargas RA and Petricevich VL 2017. Chemical compounds and biological activity of an extract from *Bougainvillea* x *buttiana* (var. Rose) Holttum and Standl. *International Journal of Pharmacy and Pharmaceutical Sciences* **9**(3): 42-46.

Harborne, JB (ed.). 1973. *Phytochemicals Methods*. Chapman and Hall Ltd. London. **1**: 49-88.

Hassan RA, Abotaleb ST, Hamed HB and Eldeen MS 2019. Antioxidant and antimicrobial activities of *Melissa officinalis* L. (Lemon Balm) extracts. *Journal of Agricultural Chemistry and Biotechnology* **10**(9): 183-187.

Kadhim MJ, Mohammed GJ and Hussein H 2016. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. *International Journal of Pharmaceutical and Clinical Research* **8**(7): 655-670.

Kumar R, Sharma N, Rolta R, Lal UR, Sourirajan A and Dev K 2020. *Thalictrum foliolosum* DC: An unexplored medicinal herb from north western Himalayas with potential against fungal pathogens and scavenger of reactive oxygen species. *Biocatalysis and Agricultural Biotechnology* **26**: 101621.

Kumar V, Sharma N, Sourirajan A, Khosla PK and Dev K 2018. Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas, India.

Journal of Traditional and Complementary Medicine **8**(1): 100-106.

Kumari A, Verma R, Sharma M, Chauhan P and Kumar A 2018. Evaluation of phytochemical, antioxidant, antibacterial and anti-cancerous activity of *Ficus auriculata* Lour. and *Osyris wightiana* Wall. ex Wight. *Bulletin of Environment, Pharmacology and Life Sciences* **7**(8): 64-70.

Madhu M, Sailaja V, Satyadev TN, and Satyanarayana MV 2016. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *Journal of Pharmacognosy and Phytochemistry* **5**(2): 25-29.

Marwat SK, Khan MS, Ghulam S, Anwar N, Mustafa G and Usman K 2011. Phytochemical constituents and pharmacological activities of sweet Basil- *Ocimum basilicum* L. (Lamiaceae). *Asian Journal of Chemistry* **23**(9): 37-73.

McDonald S, Prenzler PD, Antolovich M and Robards K 2001. Phenolic content and antioxidant activity of olive extracts. *Food Chemistry* **73**(1): 73-84.

Menon DB and Sasikumar JM 2011. Pharmacognostic study and phytochemical investigation of *Plectranthus hadiensis*. *International Journal of Pharmacy and Pharmaceutical Sciences* **3**(5): 300-304.

Mohr FB, Lermen C, Gazim ZC, Gonçalves JE and Alberton, O 2017. Antifungal activity, yield, and composition of *Ocimum gratissimum* essential oil. *Genetics and Molecular Research* **16**(1): 1-10.

More SK, Lande AA, Jagdale PG, Adkar PP and Ambavade SD 2013. Evaluation of anti-inflammatory activity of *Solanum xanthocarpum* Schrad and Wendl (Kaṇṭakāri) extract in laboratory animals. *Ancient Science of Life* **32**(4): 222-225.

Mujeeb F, Bajpai P and Pathak N 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed Research International* **2014**: 497-606.

Onder A, Çinar AS, Gençaslan G and Çoban T 2020. Antioxidant potentials of the extracts from 14 selected medicinal plants. *Journal of Medicinal Herbs and Ethnomedicine* **6**(19): 17-20.

Park, S 2011. Research on *Isodon* species: still going strong. *Archives of Pharmacal Research* **34**(12): 1999-2001.

Paudel MR, Chand MB, Pant B and Pant B 2019. Assessment of antioxidant and cytotoxic activities of extracts of *Dendrobium crepidatum*. *Biomolecules* **9**(9): 478-480.

Petrovska, BB 2012. Historical review of medicinal plants' usage. *Pharmacognosy reviews* **6**(11): 1-5.

KewScience-Plants of the World online 2021. *Isodon coetsa*. Available online: http://www.plantsoftheworldonline.org (accessed on 18 November 2021).

Rai VM, Pai VR, Kedilaya PH and Hegde S 2013. Preliminary phytochemical screening of members of Lamiaceae family: *Leucas linifolia*, *Coleus aromaticus* and *Pogestemon patchouli*. *International Journal of Pharmaceutical Science Review and Research* **21**(1): 131-137.

Ramaiah AK and Garampalli RK 2015. *In-vitro* antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*. *Asian Journal of Plant Science and Research* **5**(1): 22-27.

Rauf A, Muhammad N, Khan A, Uddin N and Atif M 2012. Antibacterial and phytotoxic profile of selected Pakistani medicinal plants. *World Applied Sciences Journal* **20**(4): 540-544.

Rindt, A 2008. *Consumer acceptance of cranberry seed oil in several food formulations*. Master Dissertation, University of Wisconsin-Stout, Menomonie, Wisconsin.

Santos-Sánchez NF, Salas-Coronado R and Villanueva-Cañongo C 2019. *Antioxidant compounds and their antioxidant mechanism*. *In Antioxidants* London, UK. IntechOpen. 418pp.

Sharma, OP and Bhat, TK 2009. DPPH antioxidant assay revisited. *Food Chemistry* **113**(4): 1202-1205.

Sharma S, Kumari A, Dhatwalia J, Guleria I, Lal S, Upadhyay N, Kumar V and Kumar A 2021. Effect of solvents extraction on phytochemical profile and biological activities of two *Ocimum* species: A comparative study. *Journal of Applied Research on Medicinal and Aromatic Plants* **25**: 100348.

Solich P, Sedliakova V and Karlicek R 1992. Spectrophotometric determination of cardiac glycosides by flow-injection analysis. *Analytica Chimica Acta* **269**(2):199-203.

Sonam, Kumari A, Kumar V, Guleria S, Sharma M, Kumar A, Alruways MW, Khan N and Raina R 2021. Antimicrobial potential and chemical profiling of leaves essential oil of *Mentha* species growing under North-West Himalaya conditions. *Journal of Pure and Applied Microbiology* **15**(4): 2229-2243.

Soni A and Sosa S 2013. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *Journal of Pharmacognosy and Phytochemistry* **2**(4): 22-29.

Sun HD, Huang SX and Han QB 2006. Diterpenoids from *Isodon* species and their biological activities. *Natural Product Reports* **23**(5): 673-698.

Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM and Sinniah UR 2017. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. *Evidence-Based Complementary and Alternative Medicine* **2017**: 1-10.

Tambe VD and Bhambar RS 2014. Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts. Journal of Pharmacognosy and Phytochemistry **2**(4): 41-47.

The Plant List 2013. *Isodon coetsa*. Available online: http://www.theplantlist.org (accessed on 1 January 2021).

Vimala V, Rebecca Mathew P, Deepa S and Kalaivani T 2014. Phytochemical analysis in *Ocimum* accessions. *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(1): 555-557.

Webb D 2013. Phytochemicals' role in good health. *Today's Dietitian* **15**(9): 70.

Xu LI, Yong-Bo XU, Ke DO, Xiao-Nian LI, Yan LI, Jian-Xin PU, Ji-Zhou WU and Han-Dong SU 2012. Three new entkaurane diterpenoids from *Isodon rubescens* and their cytotoxicities. *Journal of Natural Medicines* **10**(6): 464-470.

Zeb A, Ullah F, Ayaz M, Ahmad S and Sadiq, A 2017. Demonstration of biological activities of extracts from *Isodon rugosus* Wall. ex Benth: Separation and identification of bioactive phytoconstituents by GC-MS analysis in the ethyl acetate extract. *BMC Complementary and Alternative Medicine* **17**(1): 284-290.

Zhao W, Pu JX, Du X, Wu YL, Zhao Y, He F, Zhang HB, Xue YB, Xiao WL, Chen GQ and Sun HD 2011. Chemical constituents from the aerial parts of *Isodon coetsa* and their cytotoxicity. *Archives of Pharmacal Research* **34**(12): 2007-2014.

Received 12 January, 2022; Accepted 22 April, 2022