Indian Journal of Natural Products and Resources
Vol. 13(4), December 2022, pp. 505-515
DOI: 10.56042/ijnpr.v13i4.39187

Phytochemical analysis, biological activities, and GC profiling of extracts of some medicinal plant growing in Nepal

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Received 08 August 2020; revised received 23 September 2022; accepted 21 November 2022

The present study was aimed to determine the antioxidant, α amylase inhibition, and antibacterial activities on ten traditionally used medicinal plant extracts, namely *Cyperus rotundus*, *Citrus medica*, *Gaultheria fragrantissima*, *Jasminum humile*, *Osyris wightiana*, *Buddleja asiatica*, *Berberis aristata*, *Robus ellipticus*, *Schima wallichii*, and *Smilax ovalifolia* growing in Nepal. The bioactive fraction of *J. humile* was subjected for GC analysis. The free radical scavenging properties of plant extracts were assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and antibacterial activity was performed by the well diffusion method. The antidiabetic activity was assessed α amylase inhibition assay. The chemical compounds were isolated from the active plant fraction by silica column chromatography, and the collected fractions were analyzed by GC and FTIR. The phytochemical analysis showed that plant extracts were rich sources of secondary metabolites. The *in vitro* antioxidant activity showed IC_{50} ranging from 30.57±0.02 to 155.65±0.10 µg/mL. The promising antioxidant activity was demonstrated by *S. wallichii* of IC_{50} 30.57±0.02 µg/mL and *J. humile* 35.28±0.54 µg/mL, respectively whereas, the *S. ovalifolia*, exhibited the moderate antioxidant activity of IC_{50} 155.65±0.10 µg/mL. The *J. humile* showed significant antidiabetic activity of IC_{50} 59.4±23.47 µg/mL. The antidiabetic activities exhibited ranged from IC_{50} of 77.29±2.05 (*S. wallichii* to 608.28±71.50 µg/mL (*C. rotundus*). The *R. ellipticus* showed maximum ZOI (22 mm) against the *Staphylococcus aureus* (ATCC 25923), whereas *J. humile* (20 mm), *O. wightiana* (18 mm), and *G. fragrantissima* (16 mm) showed moderate antibacterial activity against the *S. aureus*. The *C. rotundus*, *J. humile*, *S. ovalifolia*, *O. wightiana*, and *B. asiatica* showed promising antibacterial activity against *E. coli* (ATCC 25922) with ZOI 15, 17, 14, 17, and 18 mm respectively. These findings provide partial scientific support for traditional uses of these medicinal plants against diabetes and infectious diseases. Therefore, the *J. humile* could be a promising source of natural antidiabetic and antioxidant compounds that may be drug candidates for future drug development. To the best of our knowledge, this work is the first attempt to perform all these biological activities and phytochemical analyses growing in the particular region of Nepal.

Keywords: Antimicrobial, Antioxidant, Diabetes, DPPH, *Jasminum humile*, Medicinal plants.

IPC code; Int. cl. (2021.01)- A61K 36/00, A61P 3/00, A61P 3/10, A61P 31/00, A61P 39/00

Introduction

Nepal is rich with the most varied diverse soil and climate conditions suitable for the growth of plant species[1]. The indigenous people are well familiar with the properties and uses of plants in their surroundings to cure simple to life-threatening diseases. Until the middle of the 19th century, medicinal plants were the main therapeutic agents used by human beings. About 60% of the world population and 60-90% of the people of developing countries rely on traditional remedies for primary health care derived from plants[2]. Traditional medicine in Nepal consists of those practices based on beliefs that were in existence often for many years before the development and spread of modern medicine[3]. The government of Nepal aims to promote medicinal plant use and conservation programs for livelihood improvement and poverty alleviation through various policies[4]. It has been reported that 56% of high-altitude plants were traditionally used as medicine in the Nepal Himalayas. The Himalayas' topography has resulted in various ecological levels that host diverse medicinal plants[3].

In Nepal, People of different communities have been using medicinal plants for many years to treat various diseases. Human diseases such as diabetes, oxidative cell damage, and infection caused by microorganisms are growing worldwide. Synthetic oral hypoglycemic and antioxidant agents have many side effects, due to which there is growing interest in search of the natural compound which possesses a

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dual role for treating diabetes and oxidative stress with least or without side effects[5]. Antioxidants are chemical compounds capable of deactivating free radicals before they attack cells. The reactive species are major of either oxygen-derived reactive species (ROS) or nitrogen-derived reactive nitrogen species (RNS). ROS are highly reactive oxygen-containing molecules, including the hydroxyl radical, superoxide anion radical, hypochlorite radical, and various lipid peroxides capable of reacting with membrane lipids, nucleic acids, proteins, and other small molecules, resulting in cellular damage[6,7]. These ROS are generated in the body due to normal aerobic metabolism, oxidative burst from phagocytes, and xenobiotic metabolism[8]. Antioxidants are one such chemical compound that is capable of neutralizing free radicals generated in the human body and their effects[9]. Antioxidants transfer a hydrogen atom or electron to DPPH free radical to neutralize its free radical character and become a stable diamagnetic molecule[10].

Diabetes mellitus is a metabolic disease recognized as a collection of disorders with chronic hyperglycemia, and alteration of carbohydrate, protein, and lipids metabolism leads to abnormal secretion and activity of insulin[11]. The disease is spread worldwide today due to rapid and uncontrolled urbanization and the modernization of lifestyle and dietary habits. The major complication that can be experienced is eye cataracts and retinopathy, cardiovascular diseases and stroke[7]. Inhibition of α-amylase therapy is responsible for delaying absorption and lowering the level of glucose in the blood after a meal. Several α-amylase inhibitors have been isolated from medicinal plants to develop new drugs with increased potentiality and reduce adverse side effects than synthetic drugs[9].

Antibacterial agents play a significant role in reducing the global burden of infectious diseases. But, the multidrug-resistant bacterial strain becomes a critical public threat because no effective antibacterial agents are available for the infection caused by pathogenic bacteria from natural sources[12,13]. Plants are a rich source of obtaining a variety of biological drugs. Many medicinal plants have been noticed as the source of an antibacterial compound that can potentially be effective in treating bacterial infections[14].

Traditional knowledge of using medicinal plants has to be studied to develop an appropriate method that plays a role in establishing scientific and local knowledge[15]. Due to the changing perception of the local people and the ever-increasing influence of synthetic drugs and socio-economic transformation, traditional knowledge on plant resource use is constantly diminishing[16]. Regarding the vast potentiality of medicinal plants as sources for antioxidant, antibacterial, and antidiabetic drugs, this study aimed to investigate the antioxidant, antibacterial, and antidiabetic activities of ten selected medicinal plant extracts growing Kavre district of Nepal.

Materials and Methods

Collection and identification of plant materials

The plant materials were collected from the Kavre district of Nepal, growing at different altitudes in June 2019. The photographs of the collected plant samples are shown in Fig. 1. The collected plants were identified at the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal. The herbarium of the plants was submitted to the same department and the voucher specimen of the identified plants has been preserved in the same department. The list of voucher specimen numbers, scientific names, local names, parts used, and the traditional uses of the studied plants are shown in Table 1.

Extraction of plant materials

The powder form of each plant (100 g) was dipped in methanol (300 mL) for cold percolation at room temperature for 48 h with frequent agitation. The mixture was filtered through clean cotton by repeating about 8-10 times as required for the complete extraction. The filtrate was concentrated at low temperature by a rotary evaporator under reduced pressure. The concentrated filtrates were collected in the beaker and left for drying. The dry filtrates were stored at 4°C until use.

Phytochemical analysis

The analysis of the absence or presence of leading groups of natural constituents in the different plant extracts was performed by the colour differentiation methods following the standard protocol[25].

Antioxidant activity

The antioxidant activity of plant extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay with a slight modification[26]. Briefly, different plant extracts/standard solution concentrations were mixed

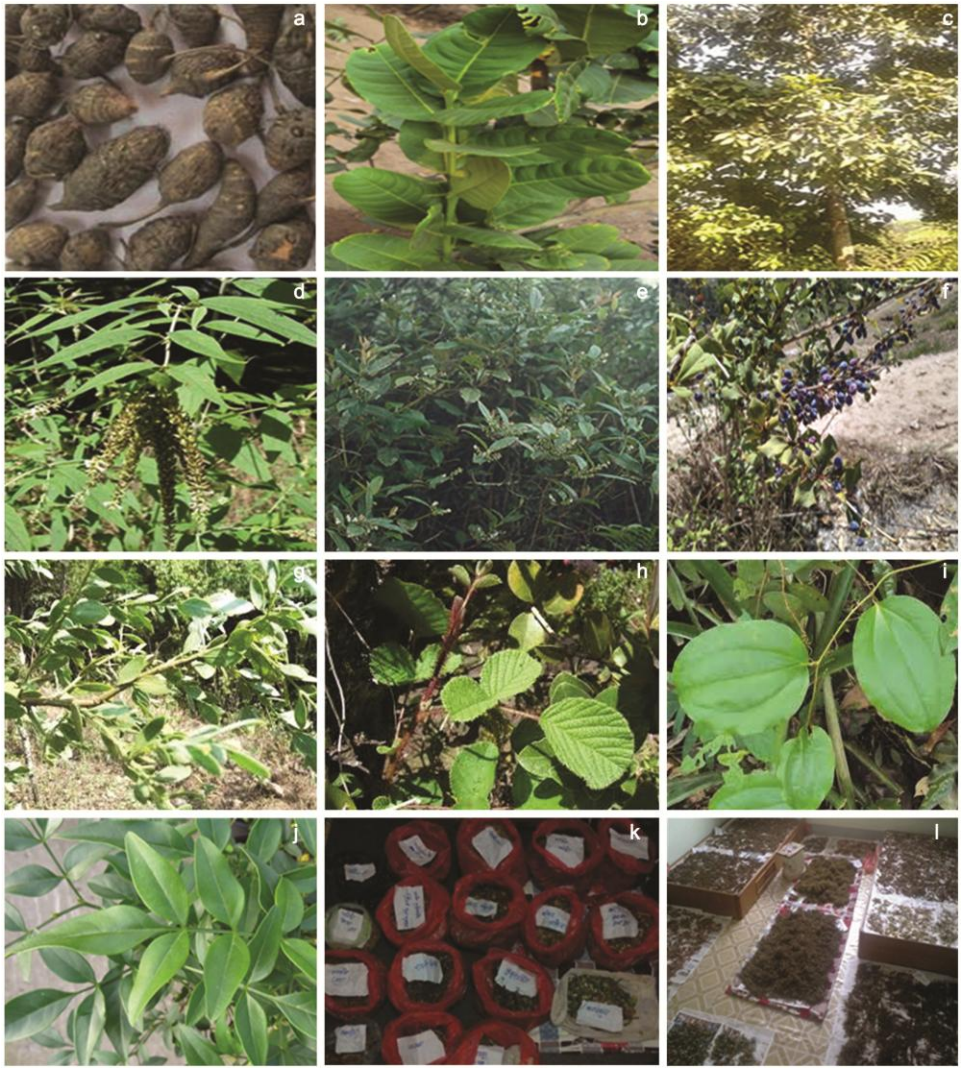

Fig. 1 — Photographs of plant samples collected from the study area and harvesting plants. a) *Cyperus rotundus*; b) *Citrus medica*; c) *Schima wallichii*; d) *Buddleja asiatica*; e) *Gaultheria fragrantissima*; f) *Berberis aristata*; g) *Osyris wightiana*; h) *Robus ellipticus*; i) *Smilax ovalifolia*; j) *Jasminum humile*; k) Sample preparation; and l) shade drying samples.

Table 1 — List of selected plants with voucher number, scientific name, plant parts, and traditional uses

Voucher No.	Scientific name	Part used	Altitude (m)	Traditional uses
RB_1	*Cyperus rotundus*	Rhizome	1300-1600	Anti-arthritis, diarrhoea, chemopreventive[17]
RB_2	*Citrus medica*	Leaf	1000-1900	Indigestion, rheumatic pains[18]
RB_3	*Gaultheria fragrantissima*	Aerial part	1200-2400	Sprain, joint pains, arthritis[19]
RB_4	*Jasminum humile*	Aerial part	1000-2500	Throat problem, treat fever, headache[20]
RB_5	*Osyris wightiana*	Bark	900-2500	Stop bleeding and joint pains[21]
RB_6	*Buddleja asiatica*	Leaf	2000-3000	Swelling, skin disease[22]
RB_7	*Berberis aristata*	Root	2000-3000	Skin disease, wound healing, and jaundice[23]
RB_8	*Robus ellipticus*	Root	1000-1500	Treating fever, colic, coughs, and sore throat[20]
RB_9	*Schima wallichii*	Bark	900-2100	Anthelmintic, rheumatism, wound healing[23]
RB_{10}	*Smilax ovalifolia*	Aerial part	300-1800	Rheumatism, venereal diseases, wounds[24]

with DPPH solution. The mixture was incubated for 30 min in the dark at room temperature, and the absorbance was recorded at 517 nm using a UV spectrophotometer. The percentage of the DPPH free radical scavenging activity was calculated by using the following equation:

$$\text{Radical scavenging (\%)} = [(A_o\text{-}A_s)/A_o] \times 100$$

where A_0 = Absorbance of the control (DPPH solution + methanol), A_s = Absorbance of the test sample.

The IC_{50} (50% inhibitory concentration) value is indicated as the sample's effective concentration required to scavenge 50% of the DPPH free radicals. IC_{50} values were calculated using the inhibition curve by plotting extract concentrations versus the corresponding scavenging effect. Linear regression plots calculated the IC_{50} values. The X-axis represents the concentration of the tested plant extracts, and the y-axis the average per cent of scavenging capacity from three replicates.

Antidiabetic activity

The α-amylase enzyme inhibition activity was performed through the starch iodine procedure with a slight modification. In brief, the solution of plant extracts/standard with different concentrations was added to the phosphate buffer containing α-amylase solution and incubated at 37°C for 10 min. Then, to the mixture, the starch solution was added, and the content was kept in incubation at 37°C for 1 h. The reaction was stopped by adding hydrochloric acid, followed by the addition of iodine reagent[27]. The absorbance of the content was measured at 630 nm where the undigested starch due to enzyme inhibition was detected. The assay was carried out by measuring the absorbance of triplicates reading using a UV spectrophotometer. The percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = (1-[Abs_2-Abs_1/Asb_4-Abs_3]) *100$$

where, Abs_1 is the absorbance of the incubated mixture containing plant sample, alpha-amylase, and starch; Abs_2 is the absorbance of an incubated mixture of sample and starch; Abs_3 is the absorbance of the incubated mixture of starch and α-amylase; and Abs_4 is the absorbance of an incubated solution containing starch.

Antibacterial activity

Antibacterial screening of the plant extracts was performed by agar well diffusion method. The effectiveness of antimicrobial substance was evaluated by measuring the zone of inhibition (ZOI) following the standard protocol[21]. In brief, a sterile cotton swab was used to evenly distribute bacterial culture drawn from respective inoculums over the Petri plate containing MHA. The wells of 6 mm diameter were then made in the inoculated plates using a sterile cork borer. Then, 100 µL of each plant sample (50 mg/mL in 50 % DMSO) was introduced into the well with 50% DMSO as negative control and 1 mg/mL of neomycin antibiotic as a positive control. The plates were left in upright condition with lids closed for half an hour so that the test solutions were diffused into the media. The inoculated plates were then incubated at 37°C for 24 h. Finally, the zone of inhibition was measured using a scale ruler.

Statistical analysis

Data were recorded as a mean of three determinations of absorbance for each concentration, from which the linear correlation coefficient (R^2) value was calculated. The data were expressed as mean±standard deviation. The regression equation is given as, Y = mx + c, where, Y = absorbance of extract, m = slope from the calibration curve, x = concentration of extract and c = intercept. Using this regression equation concentration of extracts was calculated. All the determinations were conducted at least 3 times (n = 3); the statistical mean was calculated with ±SD using Microsoft Excel 2013.

Analytical conditions for FTIR and GC-MS

The FTIR instruments send infrared radiation of about 400 to 4000 cm^{-1} through a sample, with some radiation absorbed and passed through. The sample molecules convert the absorbed radiation into rotational and/or vibrational energy. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis an excellent tool for chemical identification.

GC-MS analysis was performed on the gas chromatography-mass spectrometer GC-MS 2010 under the following conditions: injection volume 1 µL with slit ratio 1:90:Helium as a carrier gas with an Rtx-5Ms column of dimension 30 m × 0.25 mm × 0.25 µm, temperature-programmed at 50, 160, and 300°C with a hold time of 0.0 and 2.00 min identification was accomplished by comparison of MS with those reported in NAST QP 2010 ULTR SHIMADZU.

Results

Phytochemical analysis

The phytochemical analysis of the different extracts revealed the presence of a diversity of chemical families, including flavonoids, terpenoids, saponins, alkaloids, glycosides, reducing sugars, quinones, coumarins, and polyphenols. However, flavonoids and primary alkaloids were absent in most plant extracts, and terpenoids were present in all ten

Table 2 — Phytochemical screening of plant extracts

Phytochemical Constituents	RB_1	RB_2	RB_3	RB_4	RB_5	RB_6	RB_7	RB_8	RB_9	RB_{10}
Alkaloids	-	-	+	-	-	-	+	+	-	-
Coumarins	+	+	+	+	+	+	-	+	+	-
Flavonoids	+	-	+	-	+	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	-	+	+	-	+
Quinones	-	+	+	+	+	+	-	+	-	+
Reducing sugars	-	+	+	-	-	-	+	+	-	+
Saponins	+	-	+	-	+	+	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+	+	+

(+) represents presence and (-) represents absence

RB_1 = *Cyperus rotundus*, RB_2 = *Citrus medica*, RB_3 = *Gaultheria fragrantissima*, RB_4 = *Jasminum humile*, RB_5 = *Osyris wightiana*, RB_6 = *Buddleja asiatica*, RB_7 = *Berberis aristata*, RB_8 = *Robus ellipticus*, RB_9 = *Schima wallichii* and RB_{10} = *Smilax ovalifolia*

Table 3 — Percentage of free radical scavenging against concentrations of plant extracts

Plant extracts	% Radical scavenging (mean±SD)/ Concentration				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
Jasminum humile	32.12±0.25	58.48±0.45	51.78±0.08	55.39±0.49	82.25±0.15*
Berberis aristata	26.60±0.50	13.18±0.36	32.27±0.63	33.38±0.17	72.92±0.60
Schima wallichi	35.16±0.01	75.63±0.09	40.79±0.22	79.32±0.17	63.50±0.02
Robus ellipticus	11.16±0.04	34.31±0.04	41.18±0.01	81.08±0.02	83.68±0.59*
Smilax ovalifolia	5.68±0.02	10.74±0.03	9.71±0.02	16.11±0.03	38.40±0.04
Cyperus rotundus	55.09±0.15	79.44±0.19	58.04±0.09	77.52±0.58	72.87±0.85
G. fragratissima	73.26±0.15	77.95±0.30	79.84±0.30	81.46±0.30	83.33±0.17*
Buddleja asiatica	57.83±0.87	76.27±0.06	80.08±0.76	82.44±0.33	76.31±5.44
Osyris wightiana	72.53±0.58	68.99±0.09	50.38±1.65	68.31±0.39	78.25±0.47

Note: The values were calculated in the triplicates reading with mean±SD, the significance level is 0.05 (P <0.05), and the corresponding confidence level is 95%; the number of observations included.

plant extracts. The results of the phytochemical analysis are shown in Table 2.

The results presented here are slightly different from the data reported by the previous researcher. It is due to the variation in altitude of the plant habitat, different environmental conditions, method and time of sample collection, extraction procedure, and lab setup and chemical grades used in the research[19].

Free radical scavenging activity

The results of the plant extracts' DPPH free radical scavenging activities are expressed as IC_{50} (µg/mL), shown in Table 3. The results showed high radical scavenging exhibited by the five plant extracts out of nine study samples. The plant extracts that have a high scavenging effect are highlighted with asterisks. The DPPH radical scavenging assay measures the reduction of DPPH radical by hydrogen-donating or electron-transferring antioxidants due to the formation of the non-radical form, DPPH-H. The concentration required to attain a 50% radical-scavenging effect (IC_{50}) was calculated from a series of concentrations tested results. A lower IC_{50} value corresponds to a larger scavenging activity. The results of antioxidant activity expressed in IC_{50} are shown in Table 4.

Table 4 — The IC_{50} values for the DPPH free radical scavenging activities of the plant extracts

Samples/plant extracts	IC_{50} (µg/mL)
Jasminum humile	**35.28±0.54**
Berberis aristata	85.29±0.45
Schima wallichi	**30.57±0.02**
Robus ellipticus	59.70±0.13
Smilax ovalifolia	155.65±0.10

Among the nine medicinal plants, only five exhibited potential antioxidant activity compared to the standard ascorbic acid. The most potent activities were observed in the plant extracts of *J. humile* with IC_{50} 35.28±0.54 µg/mL and *S. wallichi* with 30.57±0.02 µg/mL. The plant extracts *Robus ellipticus* (IC_{50} 59.70±0.13 µg/mL) and *Berberis aristata* (85.29±0.45 µg/mL) exhibited mild antioxidant activity, whereas the least activity was observed in *Smilax ovalifolia* IC_{50} 155.65±0.10 µg/mL. All the plant extracts showed antioxidant activity in a concentration-dependent manner. The

results of the present study almost resembled to the previous results of some medicinal plants. The antioxidant activities were observed in the plant extracts of *Ludwigia octovalvis* with IC_{50} 25.9 µg/mL, *Vitis thunbergii* 58.4 µg/mL, *Prunella vulgaris* 113 µg/mL, *Saurauia oldhamii* 124 µg/mL, *Rubus parvifolius* 151 µg/mL, and *Jussiaea repens* 159 µg/mL[28]. The antioxidant activity exhibited by the plant extracts used in the present study was found to be potent as compared to the previously reported results. It may be due to the altitudinal variation of medicinal plants, the nature of the experiment, and the season of the sample collection. The results reflect that the medicinal plants used in this study are found rich in the potential source of natural antioxidant compounds.

α-Amylase inhibition activity

The results of the α-amylase enzyme inhibitory activity against the different concentrations of plant extracts are shown in Fig. 2a and b. In this study, α-amylase enzyme inhibition activity of the plant extracts revealed that the methanol extracts of all plant extracts were found to be inhibitors against α-amylase inhibition activity. The plant extracts of *J. humile* (IC_{50} 59.4±23.47 µg/mL), *S. wallichii* (IC_{50} 77.29±2.05 µg/mL), and *C. rotundus* (IC_{50} 608.28±71.50 µg/mL) showed significant inhibition of α amylase enzyme activity. The rest of the plant extracts were found to have poor α-amylase enzyme inhibitors. The results of the present study were compared to the previously reported results against the crude extract of *C. prophetarum* fruits fractionated in chloroform, basic fraction, and neutral fraction by acid-base extraction. The water-soluble fraction exhibited effective α-amylase enzyme inhibition with an IC_{50} 20.6 µg/mL; crude aqueous fraction exhibited significant antidiabetic activity with IC_{50} 85.17 µg/mL. The chloroform, basic, and neutral fractions showed mild antidiabetic activity with IC_{50} values of 715.27, 542.88, and 95.71 µg/mL, respectively[29].

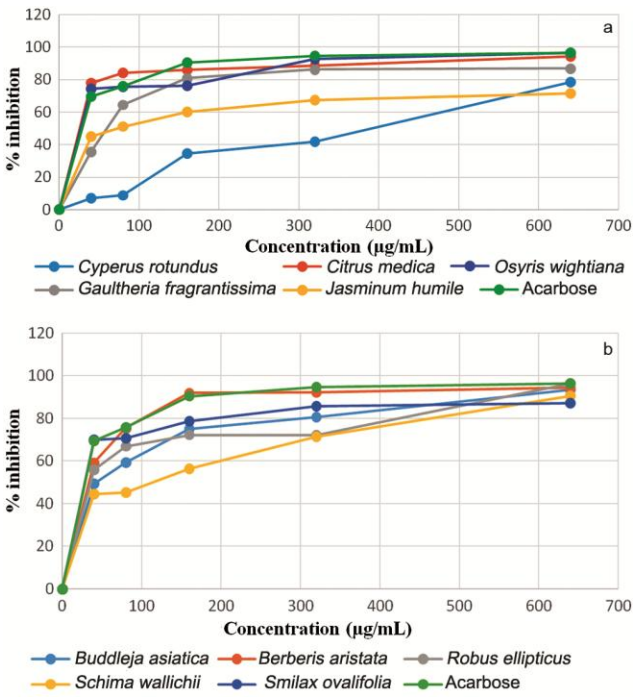

Fig. 2 — a) Percentage inhibition of α amylase by the plant extracts and acarbose; and b) Percentage inhibition of α amylase by plant extracts and acarbose.

Antibacterial activity

The results of antibacterial activity are shown in Table 5. The present study revealed that out of the ten tested medicinal plants; *J. humile*, *S. wallichii* and *Robus ellipticus* possess potent antibacterial activity against Gram-positive bacteria strain *Staphylococcus aureus* (ATCC 25923), showing ZOI 20, 20, and 20 mm respectively. At the same time, the rest of the plant extracts possess mild antibacterial activity against *S. aureus*. The plant extracts of *B. asiatica*,

Table 5 — Results of antibacterial screening of different plant extracts

Plant extracts	Organisms	ZOI (mm) plant extract 100 mg/mL	ZOI (mm) Positive control 100 mg/mL
Cyperus rotundus	*E. coli*	15	14
	S. aureus	16	35
Jasminum humile	*E. coli*	17	14
	S. aureus	20	35
Smilax ovalifolia	*E. coli*	14	14
	S. aureus	12	35
Osyris wightiana	*E. coli*	17	14
	S. aureus	18	35
Buddleja asiatica	*E. coli*	18	14
	S. aureus	12	35
Citrus medica	*E. coli*	14	14
	S. aureus	14	18
Schima wallichii	*E. coli*	-	14
	S. aureus	20	35
Gaultheria fragratissima	*E. coli*	-	14
	S. aureus	16	35
Robus ellipticus	*E. coli*	-	14
	S. aureus	22	35
Berberis aristata	*E. coli*	-	14
	S. aureus	15	35

Negative control DMSO ZOI =0, (-) = No effective antibacterial activity, ZOI = Zone of Inhibition, *E. coli*: Gram-negative organism, *S. aureus*: Gram-positive organism

J. humile, and *O. wightiana* exhibited strong antibacterial activity against Gram-negative bacterial strain *E. coli* (ATCC 23922), showing ZOI 18, 17, and 17 mm respectively. In comparison, the rest of the plant extracts exhibit mild activity against *E. coli*. The preliminary ethnobotanical study showed that these medicinal plants are extensively used by local peoples for many years in the treatment of infectious diseases are shown in Table 1.

Out of the two strains, Gram-positive bacteria were more inhibited effectively than Gram-negative bacteria. This fact can be described by the presence of a unique outer membrane that excludes the extract from penetrating the cell in Gram-negative bacteria, which is absent in the case of Gram-positive type.

GC analysis

Based on the different biological activities and the TLC results, the hexane fraction of *J. humile* was selected for the GC analysis. The list of the compounds detected in GC is given in Table 6, and the chromatogram is shown in Fig. 3. The results of GC analysis on the hexane fraction of *J. humile* revealed the presence of thirty-four compounds. The identified compounds with their retention time (RT), molecular formula, molecular mass, and concentration (peak area %) are shown in Table 6. Out of thirty-four compounds, the major compounds were 4',5-dihydroxy-7-methoxyflavanone, D-Limonene, styrene and heptane were known through the database and the library matching (Fig. 4).

Based on the peak area percentage, 4',5-Dihydroxy-7-methoxyflavanone and heptane are the major chemical compounds present in the biologically active hexane fraction of the *J. humile.*

Isolation of chemical compounds

Based on antioxidant, α-amylase inhibition, and antibacterial activities, the bark extract of *J. humile* was selected as the potent extract for isolating the chemical constituents by using a silica column. After column chromatography, the purity of isolated compounds was tested by TLC. Fractions showing a single spot in TLC were mixed and subjected to FTIR to analyze functional groups. The different fractions collected in column chromatography are listed in Table 7.

The fractions (36-40) were mixed based on the TLC report and were subjected to FTIR analysis to analyze the possible functional groups present in the

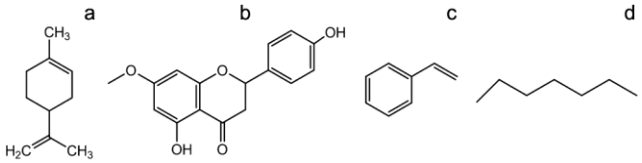

Fig. 4 — Structures of the major compounds detected in GC. a) D-Limonene; b) 4',5-Dihydroxy-7-methoxyflavanone; c) Styrene; and d) Heptane.

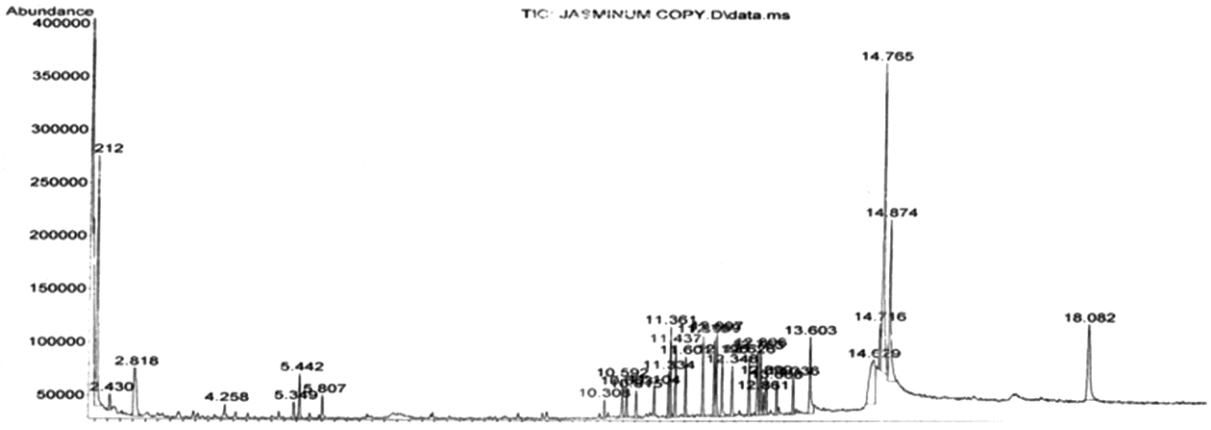

Fig. 3 — Chromatogram of hexane fraction *Jasminum humile*.

Table 6 — The major chemical constituents detected in hexane fraction of *Jasminum humile*

Compounds	Retention time (RT)	Peakarea (%)	Molecular mass	Molecular formula
D-Limonene	5.807	0.92	136.24	$C_{10}H_{16}$
Heptane	2.212	10.81	100	C_7H16
Styrene	4.258	0.82	104.15	C_8H_8
4',5-Dihydroxy-7-methoxyflavanone	21.54	73.04	286.08	$C_{16}H_{14}O_5$

Table 7 — Different fractions collected after column chromatography of hexane fraction of *Jasminum humile* and TLC results

Elution solvent system	Fraction number	Volume of eluent (mL)	Solvent system TLC	Remarks, TLC spots
100 % hexane	1 to 5	250	1 % EtOAc in hexane	No spots
1% EtOAc in hexane	6 to 10	250	3 % EtOAc in hexane	No spots
3% EtOAc in hexane	11 to 15	250	5 % EtOAc in hexane	Two spots
5% EtOAc in hexane	16-20	250	5 % EtOAc in hexane	Single spots
10% EtOAc in hexane	21-25	250	15% EtOAc in hexane	No spots
10% EtOAc in hexane	26-30	250	20% EtOAc in hexane	No spots
10% EtOAc in hexane	31-35	250	20% EtOAc in hexane	Multiple spots
10% EtOAc in hexane	36-40	250	20% EtOAc in hexane	Single spot
10% EtOAc in hexane	41-45	250	20% EtOAc in hexane	Tailing
15% EtOAcin hexane	46-50	250	20% EtOAc in hexane	Single spot
15% EtOAcin hexane	51-55	250	20% EtOAc in hexane	Tailing
20% EtOAc in hexane	56-60	250	20% EtOAc in hexane	Tailing
25% EtOAc in hexane	61-65	250	20% EtOAc in hexane	Multiple spots
50% EtOAc in hexane	66-70	250	40% EtOAc in hexane	Tailing
80% EtOAc in hexane	71-73	150	80% EtOAc in hexane	Tailing
100% EtOAc	74-78	250	1% MeOH in EtOAc	Tailing
1% MeOH in EtOAc	85-90	250	15% MeOH in EtOAc	Tailing
2% MeOH in EtOAc	91-95	250	20% MeOH in EtOAc	Tailing
5% MeOH in EtOAc	96-98	150	20% MeOH in EtOAc	Two spots
10% MeOH in EtOAc	99-100	100	20% MeOH in EtOAc	Multiple spots
20% MeOH in EtOAc	101	50	20% MeOH in EtOAc	Tailing
25% MeOH in EtOAc	102-103	100	25% MeOH in EtOAc	Tailing

EtOAc = Ethyl acetate, MeOH = Methanol

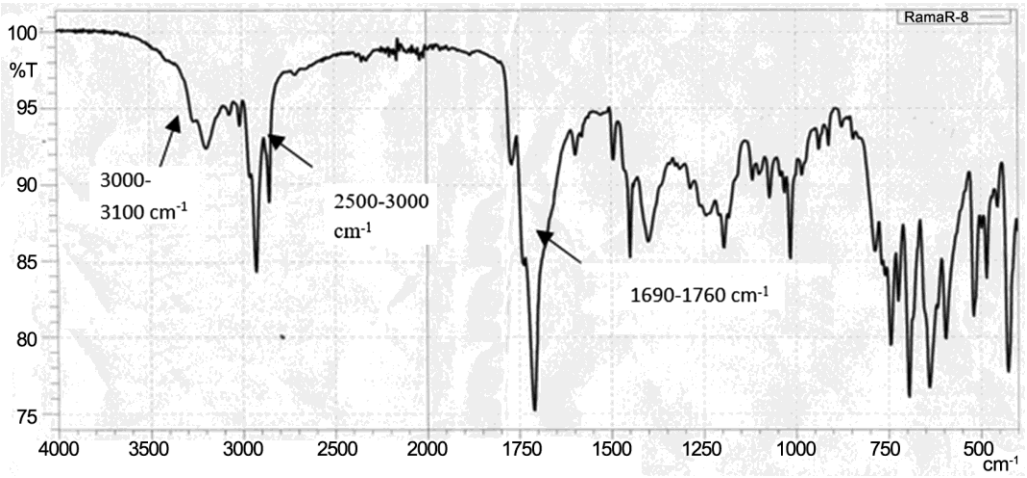

Fig. 5 — FTIR of a semi-purified fraction collected in 10% EtOAc in hexane through column chromatography.

sample. A sharp peak at 1750 cm^{-1} indicates the carbonyl group (C=O) may be of aldehyde, ketone, oic acid, ester, etc. Similarly, a broad peak in the range of 3200-3500 cm^{-1} generally shows the presence of an O-H bond, i.e., H-bonded alcohol or phenol group in the sample. A small peak arises at 3000 cm^{-1} indicating the presence of an aromatic C-H bond. The functional group shown in the semi-purified fractions justified the similar functional groups that have been reported in the same plant by previous researchers. The list of the assigned regions in FTIR is shown in Fig. 5 and Table 8.

Discussion

The secondary metabolites produced in plants are reported to exhibit many health-inducing activities against different human diseases, including antibacterial, antioxidant, anticancer, and antidiabetic activity[30-31]. The major class of organic compounds which are produced in plants, such as alkaloids,

Table 8 — Functionality of the semi-purified fraction collected in column chromatography

Functionality	Assignment regions (cm^{-1})
Aldehydes, ketones, carboxylic acids, esters	1690-1760
Alkanes	1350-1470
Aromatic C-H bond	3000-3100
Alcohols, ethers, carboxylic acids, esters	1080-1300

flavonoids, terpenes, and polyphenols, are included as an essential part of the human diet owing to their various medicinal properties[32]. The present research reveals the antioxidant activities of the ten medicinal plants in which five show the significant antioxidant activity of IC_{50} ranging from 30.57±0.02 µg/mL (*S. wallichi*) to 155.65±0.10 µg/mL (*S. ovalifolia*). An earlier investigation reported that the methanol extract of *J. humile* was 70.43 µg/mL[33]. The plant *J. humile* growing in Kavre of Nepal is a potential source of the natural antioxidant compounds showing the IC_{50} of 35.28±0.54 µg/mL. In this study, the plant extracts show moderate α-amylase inhibition. The plant extracts of *J. humile*, *S. wallichii*, and *C. rotundus* are potential α-amylase enzyme inhibitors. In contrast, the rest of the plant extracts are poor inhibitors of the α-amylase enzyme. The extracts of plant growing in Kavre of Nepal are potential source of natural antidiabetic compounds that could be drug candidates in future drug development. The phytochemicals study of these plant extracts revealed that alkaloids, coumarins, glycosides, polyphenols, saponins, and terpenoids are helpful for the prevention and management of diabetes mellitus[34-36]. A recent study shows that the plant extracts rich in polyphenols, terpenoids, and saponins showed a strong inhibitory effect against the α-glucosidase enzyme inhibition but a mild inhibitory activity on α-amylase. Thus suggesting the use of these plants' secondary metabolites for treating and managing diabetes[37].

The inhibition of the α-amylase enzyme slows down the activity of carbohydrates metabolizing enzymes, such as α-glucosidase or α-amylase, which are potential therapeutic agents in diabetes[38]. The antibacterial activity of hydro-methanol extracts of some medicinal plants against Gram-positive and Gram-negative bacteria frequently encountered in infectious diseases[31]. The plant extract of *Beberis vulgaris* root barks exhibited potent activity against *S. aureus* with ZOI 23.0 mm, *E. faecalis* 13.0 mm, *Cistus monspeliensis* 16.0 mm, and *Punica granatum* 17.0 mm. The plant extracts of *Cinnamomum cassia* peels, *Rhus tripartite* aerial parts, and *Withania frutescens* leaves exhibited low activity with ZOI 11.0 and 12.0 mm against *S. aureus* and 11.0, 12.0, and 10.0 mm against *E. coli*, *E. cloacae*, and *P. aeruginosa* respectively. The present study showed comparable ZOI against the microorganisms *S. aureus* and *E. coli* with the results reported by the earlier researcher[31]. The plant's secondary metabolites often show considerable activity against Gram-positive but not Gram-negative bacteria. The Gram-positive bacteria have an effective permeability barrier. The action of the plant extracts against the Gram-positive, and Gram-negative bacteria indicate the presence of broad-spectrum antibiotic compounds[39,40]. The bioactive hexane fraction of the *J. humile* was subjected to GC profiling that shows the four compounds as the major based on the peak area percentage. The hexane fraction was subjected to silica gel column chromatography to isolate the chemical compounds. The semi-purified fractions were collected through the column, TLC tested purity, and finally, the functionality was analyzed by FTIR.

Conclusion

Based on the results of the present study, it can be concluded that the methanolic extracts of the medicinal plants growing in Kavre of Nepal were found to be active and efficacious towards the antibacterial activity as compared to the positive control. This study reveals that the methanol extracts of *J. humile*, *S. wallichii* and *R. ellipticus* are found active against the *Staphylococcus auresus* .Whereas the plant extracts of *B. asiatica*, *J. humile*, and *O. wightiana* are found active against the *E. coli*. The results also indicate that the plant extracts of *J. humile*, *S. wallichii*, and *C. rotundus* have potent antioxidant activity. From the results of α-amylase enzyme inhibitory activity, it can be concluded that the plant extracts of *C. rotundus, S. wallichii*, and *J. humile* exhibited the highest α amylase inhibition activity among the tested plant extracts, whereas the rest of the plants exhibit moderate activity. This study can recommend that the medicinal plants used in this study are good sources of natural antioxidants, antidiabetic and antibacterial compounds. These plant samples could be used to isolate the pure natural compounds to perform the *in-vivo* biological activities to assure the future drug candidates in the drug discovery process. The results of this study impart the

scientific supports in the traditional uses of these medicinal plants against diabetes and the infectious diseases.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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