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Studies on Phytochemical Screening, Antimicrobial, Antioxidant and Cytotoxic Activities of *Caragana Jubata* of Nepal

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Research Article

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Abstract

Objective: Phytochemical screening as well as several bioassays was performed on the extracts of aerial parts of *Caragana jubata*.

Methods: Extraction of *C. jubata* was carried out by solvents of increasing polarity. The Phytochemical screening of extracts was carried out using standard procedures. Furthermore, extracts were also evaluated for (i) antibacterial assay, (ii) brine shrimp lethality bioassay and (iii) antioxidant assay. American Type Culture Collection(ATCC) strains six bacteria, one gram-positive (*Staphylococcus aureus* ATCC 25525), five gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsella pneumonia* ATCC 700603, *Serratia marcescens* ATCC 13880 and *Salmonella Typhimurium* ATCC 14028 were used to evaluate its antimicrobial activity. Brine shrimp lethality assay was done on the plants extracts. For the antioxidant test, 2, 2-Diphenyl 1, picrylhydrazyl (DPPH) free radicals scavenging assay was done.

Results: The phytochemical analysis indicated the presence of alkaloids, flavonoids, saponins, and terpenoids in aerial parts of *C. jubata*. In the case of the antibacterial, different extracts showed 9-13 mm and 0-15 mm zone of inhibition in diameter against gram positive bacteria *S. aureus* and gram negative bacteria (*E. coli, P. aeruginosa, K. pneumonia, S. marcescens* and *S. Typhimurium*). This inhibition was found less in comparison to the standard compounds, Chloramphenicol (26±0.5mm to 32±0.5mm) and Gentamicin (26±0.5mm to 30±0.5mm) antibiotics. Similarly, in brine shrimp lethality assay, methanol extracts showed a potent lethal effect against the brine shrimp nauplii with LC_{50} of 50.93±7.8µg/ml. In the DPPH free radical scavenging assay, the extracts showed strong antioxidant activity in the order: methanol extract> dichloromethane extract> hexane extract, however all the extracts little less active than the standard compound, ascorbic acid. Methanol extract showed the highest DPPH free radical scavenging with IC_{50} value of 6.1µg/ml.

Conclusion: The present study suggests the potentiality of *C. jubata* to become a natural source of antioxidant for the protective as well as prevention of diseases.

Keywords: *Caragana jubata;* Antibacterial; Antioxidant and Lethality assay.

Introduction

Natural products have been one of the important sources to obtain useful drugs since last two centuries. Plants form the basis for the treatment in the traditional medicinal systems and practices such as the Ayurveda, Chinese Traditional Medicine, the Unani, Kampo Yaku and others. Folk medicine also employs a wide variety of the plants as medicines. Therefore it can be safely said that plants still hold promise for providing useful medicines [1,2]. Plants produce a multitude of secondary metabolites that have antimicrobial activity. The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in case of food-borne illness and nosocomial infections [3-5].

Naturally occurring antimicrobials are also being sought as replacements for synthetic preservatives such as parabens (ethyl, methyl, butyl and propyl parabens), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are deemed as the cancer causing agents [6,7].

The genus *Caragana* belongs to the family Fabaceae and comprises over 80 species distributed in Asia and Europe [8]. Among them 15 species are available in the central and western Nepal [9]. Dense populations of *Caragana* species have been reported in the lower and upper Mustang, Annapurna Conservation Area of Nepal. Heart wood of *C. jubata* is used to treat blood pressure and menstrual disorder in the Mustang area [10]. Many species of this genus have been used in the Traditional Chinese Medicine for the treatment of hypertension, irregular menstruation and fatigue. The major constituents of *Caragana* species were reported to be the flavonoids and stilbenoids [11]. Several novel compounds have also been isolated from species of *Caragana* and they are shown to possess anti-tumor, antivirus, anti-inflammation, sedative, acetyl cholinesterase inhibitory, immunosuppressant activities phytoestrogenic and anti HIV activities [12-14]. The ethnopharmacological importance and wide traditional therapeutic values of *Caragana* genus have prompted us to carry out the phytochemical studies on *C. jubata* found in Nepal. Among the *Caragana* species, *C. jubata* is found generally in open dry slopes; 2500- 4200 m, in the Himalayan region. Whole plant of *C. jubata* has been widely used in the treatment of some cardiovascular diseases such as atherosclerosis, hyper lipidemia, hypertension, blood circulation disorder, blood stasis in the Amchi Medicine. It has also been used to treat arthritis and abnormal menstruation and muscle pain [15,16]. Though the shrub is used for different purposes, it's phytochemical as well as bioactivity studies are still lacking. Because of this, we began phytochemical as well as biological studies with this plant. The present study describes the phytochemical screening as well as the antimicrobial, antioxidant and brine shrimp lethality test of various fractions from *C. jubata*.

Materials and Methodology

Plant material and preparation of extracts

The aerial part of *C. jubata* was collected from Muktinath temple area of Annapurna Conservation Area (2920 m), Nepal in July 2012. The plant was identified by a plant taxonomist, Rita Chhetri. The plant was given voucher number 201272 and was deposited in National Herbarium and Plant Laboratory, Godavari, Lalitpur, Nepal. The aerial parts were dried under shade for one month. The plant material was grounded using a grinder into a fine powder. The powder sample was subjected to the successive extraction using different solvents with increasing polarity-hexane, dichloromethane, methanol two times heating each for 12-18 hr respectively. The extracts were then filtered through a cotton plug. The filtrates were dried under vacuum in a rotary evaporator. The hexane, dichloromethane, methanol extracts were collected, stored at 4^{o}C and used for further investigation for the phytochemical as well as bioassay studies.

Phytochemical screening

The different extracts collected after successive extraction of the aerial part of *C. jubata* were subjected to qualitative chemical analysis for the identification of different phyto-constituents [17,18].

Antimicrobial Activity

Microbial strains

The collected extracts of *C. jubata* were tested for their antibacterial activities against six bacterial strains namely *Staphylococcus aureus* ATCC 25923), *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsella pneumonia* ATCC 100603, *Serratia marcescens* ATCC 13880 and *Salmonella Typhimurium* ATCC 14028. These strains of bacteria were gift from National Public Health Laboratory, Teku, Nepal and Institute of Medicine, Tribhuvan University, Maharajgunj, Nepal.

Microbial assay

The agar well diffusion assay was used to determine the antimicrobial activity of the extracts of *C. jubata* [19,20]. Mueller Hinton agar (Himedia) was prepared as per the manufacturer's protocol. The sterile Mueller Hinton agar was poured into sterile petri dishes and seeded with test microorganisms. The concentration of microorganism used was equivalent to McFarland solution (standard). Seven holes (6mm diameter) were made by cutting out from agar plates using a sterilized stainless steel borer of 6mm. They were bore and filled with 20µl (50 mg/ml) of the fraction. The plates inoculated with different bacteria were incubated at 37°C up to 48 h. The diameter of any resultant zone of inhibition was measured. For each combination of extracts and the bacterial strain, the experiment was performed in duplicate and repeated thrice. The antibacterial activity of different plant extracts was compared with two commonly employed antibiotics was (i) Chloramphenicol (30µg/ml) and (ii) Gentamicin (10µg/ml) as positive control and DMSO as negative control.

Brine shrimp lethality assay

About 1gm of brine shrimps (*Artemia salina*) eggs (Ocean Star International, Inc., USA) was allowed to hatch in one liter of artificial sea water. The temperature was maintained at 22-28°C in presence of fluorescent lamp with continuous aeration for 48 hrs.

This assay was performed by using the method described by Meyer et al. [21,22]. Stock solutions were prepared by dissolving 2mg of extract in 2ml of DMSO. Different concentrations of extracts: 10µg/ml, 100µg/ml and 1000µg/ml in DMSO were used against brine shrimp larvae. The experiment was in triplet. These vials were incubated for 24 hr. The survival rate of these larvae was observed against all concentration of test samples. For this purpose, 5 ml of brine and 10 shrimp in each vials containing of three replicates solutions with 0.5ml of each fraction was applied. These vials were incubated for 24 hr.

After 24 hr, the survivors were counted with the help of magnifying glass. Percentage mortality was determined for three replications. From this data, the % lethality of fraction was calculated for each concentration. The LC_{50} of the extracts were obtained by plotting percentage of shrimp killed against the logarithm of sample concentration.

Antioxidant activity evaluation using DPPH free radical scavenging activity

The DPPH radical-scavenging assay was performed as described by Blois et al. [23] with some modification. DPPH solutions (50 µL, 100µg/ml) were added to the methanol solution of sample (200 µL, 12.5-100 µg/ml). After storage at the room temperature for 30 min in dark, the absorption of the reaction mixture was recorded at 517 nm in the spectrophotometer against the solvent. Inhibition of DPPH radical scavenging activity in percent (I %) was calculated by using the following equation [24].

$$\% \text{ scavenging activity} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the test sample})}{\text{Absorbance of the control}} \times 100$$

The inhibition curve was plotted for triplicate experiments and represented as % of mean inhibition with standard deviation. IC_{50} value was determined by interpolation from linear regression of plot of percentage of inhibition against the logarithm concentration of extracts, as the amount of extract needed to scavenge 50% of DPPH radicals.

Statistical Analysis

All assays were carried out in triplicate. Results are expressed as means ± SD. The LC_{50} and IC_{50} values were calculated by linear interpolation between values above and below 50% activity by Microsoft excel analysis.

Result and Discussion

Qualitative phytochemical tests of *C. jubata* were performed on the different extracts of the aerial parts. The results of various chemical tests for the detection of group of secondary metabolites are summarized in the Table 1.

Plants were selected on the basis of a potentially useful phytochemical composition by consulting ethnopharmacological and ecological information [25,26] therefore *C. jubata* was selected as well. Acceptance of medicines from such plant origin as an alternative form of healthcare is increasing because they are serving as promising sources of novel antibiotic prototypes [27]. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer [28]. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipas. The presence of these phenolic compounds in this plant contributed to their antioxidative properties [29].

Flavonoids serve as health promoting compound as a results of its anion radicals [30] and this property may explain the mechanisms of antioxidative action of *C. jubata*. These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores. Also, the plant extract was revealed to contain saponins, known to produce inhibitory effect on inflammation and are major ingredients in traditional Chinese medicine [31]. This may be reason that most of the observed biological effects are good and this tends to the use of *C. jubata* in traditional medicine. The result of phytochemical screening of plants indicated the presence of flavonoids, terpenoids, tannins, alkaloids and saponins are response for the activity.

Plant Extracts	Phytochemicals						
	Phenol	Alkaloid	Flavonoid	Saponin	Tannin	Terpenoid	Cardiac glycoside
Hexane	+	-	+	-	-	+	-
Dichloro-methane	+	+	+	-	-	+	-
Methanol	+	+	+	+	+	+	+
Aqueous	+	-	-		+	-	+

Note: "+" present; "-" absent

Table1: Classes of phytochemicals present in *Caragana jubata.*

Aerial part of *C. jubata* showed antibacterial activity against different ATCC culture bacteria (Table 2) as measured by zone of inhibition. In antibacterial screening, the extracts showed less zone of inhibition (10-12 mm in diameter) against gram positive *S. aureus* and zone of inhibition (0-15 mm in diameter) against gram-negative *S. Typhimurium, K. pneumonia, S. marcescens, P. aeruginosa* in comparison to standard Gentamicin and Chloramphenicol showed zone of inhibition 26-30mm. Methanol extract showed more active against tested bacteria than other extracts.

Bacteria		Inhibition zone diameters (mm) Antibiotics Extracts				
		Gentamicin	Chloramphenicol	Hexane	Dichloromethane	Methanol
Gram Negative	[a]*E.coli* ATCC25922	26.0±0.5	30.0±0.5	-	-	-
	[a]*K.pneumonia* ATCC100603	30.0±1.2	26.0±0.5	9.0±1.0	11.3±0.6	10.0±0.5
	[a]*S.Typhimurium* ATCC 14028	30.0±0.5	30.0±0.5	-	-	-
	[a]*S. marce-scens* ATCC 13880	30.0±1.2	26.0±0.5	-	-	10.7±1.2
	[a]*P. aeruginosa* ATCC 27853	30.0±0.5	26.0±0.5	11.7±1.5	9.3±0.6	13.0±0.5
Gram positive	[a]*S. aureus* ATCC 25923	26.0±0.5	32.0±1.2	-	10.3±0.6	10.3±0.6

Note: (-) absent. ± Standard Deviation (SD), *n* = 3.[a]Well size was 6mm, Inhibition zone diameter (mm)

Table 2: Zones of growth inhibition (mm) by the extracts against six bacteria.

Produced around the well by adding 20 µl of extracts. Brine shrimp lethality is a general preliminary bioassay which is indicative of some form of pharmacologic activities.

The LC_{50} value (50.93±7.8µg/ml) of the methanol extract showed the highest active against experimental shrimps in comparison to other extracts. Similarly, dichloromethane and hexane extracts of *C. jubata* showed brine shrimp lethality with LC_{50} (141.99±1.0 µg/ml and 248.24±2.8 µg/ml) respectively.

Extracts	Hexane	Dichloromethane	Methanol
LC_{50} (µg/m)	248.24±2.8	141.99±1.0	50.93±7.8

Note: ± SD (*n* = 3).

Table 3: Brine shrimp mortality of extracts

The antioxidant activity of the extract was assessed by the DPPH free radical scavenging assay and their scavenging activity was compared with the standard antioxidant ascorbic acid (Figure 1).

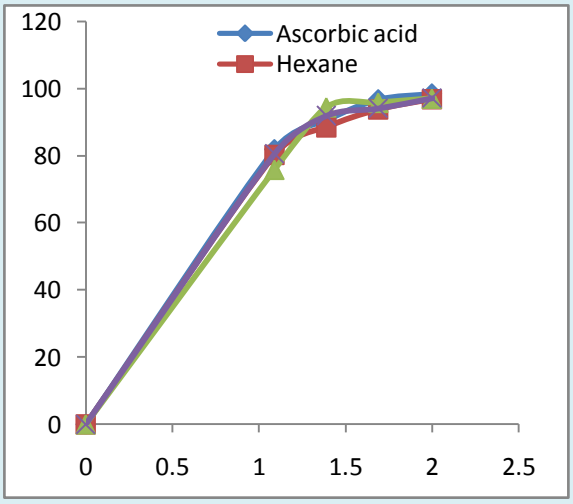

Figure1: Percentage of inhibition verses conc in log (µg/ml) of different extracts and Ascorbic Acid.

DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to be decolorized in the presence of antioxidants. The methanol extract showed the highest DPPH free radical scavenging with LC_{50} of 6.1µg/ml. Similarly, dichloromethane and hexane extract showed IC_{50} value of 6.20µg/ml and IC_{50} value of 6.3µg/ml respective as compared to the standard ascorbic acid was 5.16µg/ml. Free radicals from oxidative stress are involved in many disorders like atherosclerosis, angina pectoris, neurodegenerative diseases and cancer. Antioxidants due to their scavenging activity are useful for the management of these diseases. The IC_{50} values showed that methanol extract of *C. jubata* is more potent than to other extract (Table 4).

Standard/Extracts	Ascorbic acid	Hexane	Dichloromethane	Methanol
$IC_{50}$of DPPH(µg/ml)	5.16±0.27	6.3±0.01	6.2±0.12	6.1±0.06

Note ±: SD, $n = 3$.

Table 4: DPPH scavenging free radical of extracts.

Conclusion

Phytochemical screening and biological activities of aerial parts of *C. jubata*'s different extracts have been studied here for the first time. Further work demand isolation of biologically active components and testing them in advanced enzymatic bioassays. Furthermore, the activity exhibited by the extracts may offer scientific justification for the ethno medicinal uses of these plants. The result of this study signifies the potential of *C. jubata* as a source of therapeutic agent.

Competing Interest

We have declared no competing interest.

Author's Contributions

Reeta Mandal has been participated in collection of the plant material, performed the experiments, analyzed the data and prepared the manuscript. Mohan Bikram Gewali and Kanti Shrestha helped selecting the plant, designing experiments, interpreting the data and finalizing manuscript.

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