Hepatoprotective Potential of Selected Medicinal Plants: Evaluating their Antioxidant and Antibacterial Activities

Khem Raj Joshi[1*] and Sabina Parajuli[1]

[1]School of Health and Allied Sciences, Faculty of Health Sciences, Pokhara University, Nepal.

***CORRESPONDING AUTHOR:**

Khem Raj Joshi

Email:khemraj_pu@yahoo.com

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ABSTRACT

From the survey, a total of 41 plant species belonging to 30 families were found to be useful in the treatment of hepatic disorders. The mostly used were the whole plant of Cuscuta reflexa (85%) and fruits of *Carica papaya* (50%), *Saccharum officinarum* (46.5%) and *Cucurbita pepo* (44.5%). Herbal remedies were mostly prepared from freshly collected plants and used alone or with water. Among the documented species, six plant parts, that were least studied previously, were selected for the study of antioxidant and antibacterial activities. The extracts of *Diplazium stoliczkae,* rhizomes and leaves both, with IC_{50} value, 5.54 and 5.49 µg/mL respectively, close to that of standard, ascorbic acid (4.80 µg/mL) showed potent antioxidant activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Also, the extracts of rhizomes and leaves of *D. stoliczkae* and bark of *Bombax Ceiba* showed antibacterial activity comparable to that of standard drugs at a concentration of 1 mg/mL.

Keywords: Hepatoprotective, Antioxidant, Antibacterial activity, *Diplazium stoliczkae, Bombax ceiba, Cuscuta reflexa*

1. Introduction

The use of wild plants or plant parts to fulfill the needs such as food, medicine and agricultural tools was base for indigenous people living in particular areas, so they have considerable knowledge on the uses of medicinal plant. Various ethnobotanical surveys on medicinal plants have been performed in various parts of Nepal (Bhattarai *et al*. 2006; Burlakoti & Kunwar 2008; Joshi & Joshi 2000; Kunwar *et al*. 2006; Manandhar 2002). However, there is a sparseness of documentation and a limited repository of knowledge regarding the utilization of wild medicinal plants in the country, particularly in the Kaski district, with a focus on specific diseases, such as liver disorders. The present research attempted to document the medicinal plants used by the people in the villages and the surrounding areas of the Kaski district.

Liver is a major organ attacked by reactive oxidative species or free radicals (Sanchez-Valle 2012). Oxidative stress is one of the pathological mechanisms that results in initiation and progression of various liver diseases, steatosis, fibrosis, cirrhosis, chronic hepatitis and hepatocellular carcinoma (Cichoz-Lach & Michalak 2014; Sha *et al*. 2015). So, various anti-oxidative therapy and antioxidants are

proposed to prevent and treat liver diseases. The clinical effects of antioxidants as adjuvants including vitamin E/C, mitoquinone, N-acetylcysteine, polaprezinc silymarin, silibinin and some antioxidant cocktail on chronic hepatitis C patients have been examined has shown clear benefit of antioxidants to interferon based therapy of hepatitis C virus (HCV) (Esrefoglu 2012). Due to presence of hydroxyl and carboxyl group, phenols and flavonoids can bind to the free radicals and deactivate them along with the ability to donate electron or the hydrogen atom to the unpaired free radicals. By donating the electron, phenolic compounds do not become the reactive species but possess the ability to donate to other free radicals (Michalak 2006).

Bacterial infections are very common in patients with cirrhosis and a major challenge for physicians caring for patients with liver diseases. Despite the recent improvements in the knowledge of pathogenesis, prevention, and management, bacterial infections still represent a major cause of morbidity and mortality among patients with cirrhosis (Jalan *et al*. 2014). Plants are the sources of various phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activities (Madhuri & Pandey 2009). Various ethnobotanical surveys on medicinal plants have been performed in various parts of Nepal and several papers were published on different aspects of ethnobotany by different researchers. However, there is lack of documentation and store of knowledge on the uses of wild medicinal plants of the country, specifically, of Kaski district, based on specific diseases, such as liver disorders. The present research attempts to document the medicinal plants used by the people in the villages and the surrounding areas of the Kaski district.

2. Materials and Methods

2.1 Study area

Field study was carried out in the villages of Naudanda, Armala, Furse khola, Kotre, Ghandruk and in the surrounding areas of the Kaski district of Western Region of Nepal from September to December, 2015. The selection was random and the study design was cross-sectional. Key informants were identified after preliminary discussion with the people. Information on uses of the plants was collected by interviewing key informants of total 200 respondents of five different villages using a semi-structured questionnaire. The plants were identified in the field with the help of informants and their nomenclatures were found out with the help of literature. Those who have no idea about the medicinal plants were excluded.

Six selected plant extracts were taken for antioxidant and antibacterial activity. The plants selected were *Diplazium stoliczkae* (rhizomes), *D. stoliczkae* (leaves), *Cuscuta reflexa* (whole plant), *Bombax ceiba* (bark), *Ocimum basilicum* (seeds) and *Cirsium verutum* (roots). Those plants were identified and collected from survey area with the help of local people. The herbarium and voucher specimen (*Diplazium stoliczkae* (rhizomes) 13-2015, *D. stoliczkae* (leaves) 16-2025, *Cuscuta reflexa* (whole plant) 17-2015, *Bombax ceiba* (bark) 24-2015, *Ocimum basilicum* (seeds) 25-2015 and *Cirsium verutum* (roots) 28-2015). of those plants were stored Department of Pharmaceutical Sciences, School of Health and Allied Sciences, Pokhara University.

2.2 Extract Preparation

The collected plants were finely powdered and extracted using 70% methanol. Each plant sample of 50 g was mixed with 400 mL of 70% methanol by maceration for 4 days (96 hours). The 70% methanolic extracts were then filtered and the filtrates were dried using rotatory vacuum evaporator.

2.3 Antioxidant activity determination2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical free assay was measured according to the method of Kim *et al*. 2007 with some modifications (Kim *et al*. 2007). In brief, 2 mL of different extract solution (0.1 µg/mL, 1 µg/mL, 10 µg/mL and 100 µg/mL) of each plant sample were mixed with 2 mL of 60 µM DPPH solution. The mixture was allowed to stand for 30 minutes. The absorbance was measured at 517 nm using UV spectrophotometer. Radical scavenging activity of each sample was calculated by using following formula:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_S)/ A_0] \times 100$$

Where, A_0 = absorbance of control and A_S= absorbance of sample. Control is the test solution without sample. Similar process was done with ascorbic acid solution of concentrations (0.1 µg/mL, 1 µg/mL, 10 µg/mL and 100 µg/mL). Ascorbic acid was taken as standard solution.

2.4 Antibacterial activity determination

Pathogenic strains of *Staphylococcus aureus*, *Proteus vulgaris*, and *Escherichia coli* with their antibiotic resistance profiles were obtained from the National Public Health Laboratory (NPHL), Kathmandu. The Well diffusion method assay was according to Sen *et al*. 2012 with slight modifications (Sen & Batra 2012). An inoculum suspension was swabbed uniformly to solidified 20 mL Mueller-Hinton agar (MHA) for bacteria, and the inoculum was allowed to dry for 5 minutes. Holes of 6 mm in diameter were made in the seeded agar using Glass Pasteur pipettes. Aliquot of 50 µL from each plant crude extract (50 mg/mL) was added into each well on the seeded medium and allowed to stand on the bench for 2 hours for proper diffusion and thereafter incubated at 37 °C for 24 hours. Ofloxacin and cefpodoxime (1 mg/ml) were used as standards. 10% DMSO was used as positive control. The resulting inhibition zones were measured in millimeters (mm). Larger the zone of inhibition, higher is the antibacterial activity.

2.5 Determination of total phenol

Total phenols were determined by Folin Ciocalteu reagent with some modifications (Pourmorad *et al*. 2006). In brief, 200 µL of each extract solution (1 mg/mL) was mixed with 1800 µL of distilled water and 2 mL of Folin reagent. After standing for 3 minutes, 2 ml of 10% sodium carbonate was mixed and shaken. The mixture was allowed to stand for 1 hour and the absorbance was measured at 760 nm. Gallic acid was taken as a standard phenolic compound. Different concentrations of gallic acid (500 mg/L, 400 mg/L, 300 mg/L, 200 mg/L, 100 mg/L, and 50 mg/L) were prepared. Total phenol values were expressed as mg gallic acid equivalent per gram dry extract weight.

2.6 Determination of flavonoids content

Aluminum chloride colorimetric method was used for flavonoid determination (with some modifications (Chang *et al*. 2002). In brief, 1 mL of each plant extract solution (1 mg/mL) was mixed with 4 mL of distilled water. Then, 300 µL of 5% sodium nitrite was added. After 5 minutes, 300 µL 20% aluminum chloride was added and allowed to stand for 6 minutes. Then after, 2 mL of 1M sodium hydroxide was added. The mixture was shaken and the absorbance was measured at 510 nm using UV spectrophotometer. Quercetin was taken as standard flavonoid compound. Different concentrations of quercetin (500 mg/L, 400 mg/L, 300 mg/L, 200 mg/L, 100 mg/L, and 50 mg/L) were prepared. Total flavonoid values were expressed as mg quercetin equivalent per gram dry extract weight.

3. Results

From the survey, a total of 41 plant species belonging to 30 families were found to be useful in the treatment of jaundice and hepatitis. The mostly used plants for hepatic ailments are shown in Fig. 1. Eighty five percent of the total respondents have knowledge about the whole plant of *Cuscuta reflexa.* The modes of uses were documented and herbal remedies were mostly prepared from freshly collected plants used alone or with water. The preferred method of preparation was crushing while doses of most plants were as per required till cure. The knowledge about the medicinal plants were mostly found to be transformed from ancestors and other by local practitioners, learnt from secondary sources such as studying books, literatures, friends, relatives, etc.

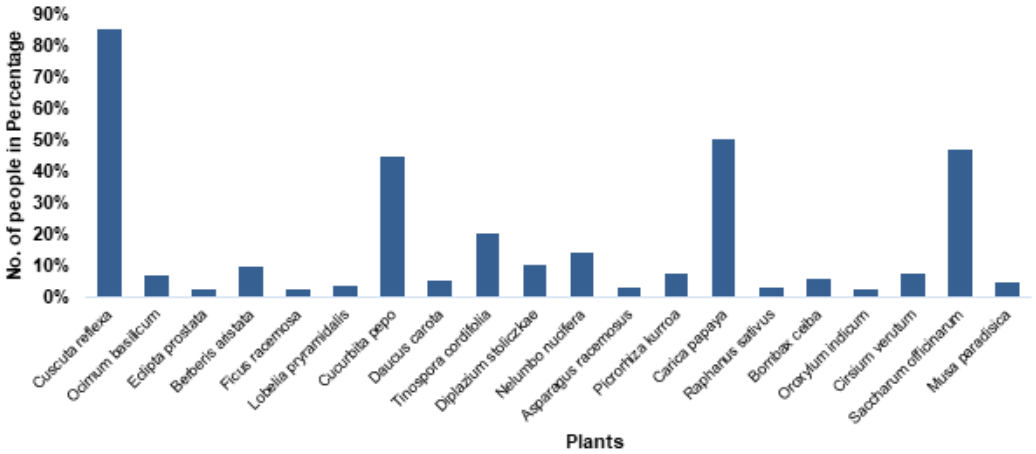

Fig. 1: Graph representing the widely used plants in hepatic disorders by local people

According to the survey conducted in this study, in addition to *Cuscuta reflexa, Cucurbita pepo, Carica papaya,* and *Sacaharum officinarum* were seen as highly preferred plants for treating hepatic disorders by local people. Among the documented species, six plant parts, that were least studied previously, were selected for the study of antioxidant and antibacterial activities.

3.1 Antioxidant activity determination

The hydrogen atom or electron donation ability of each plant extract against DPPH free radical was measured from the bleaching of violet colored methanol solution of DPPH. The DPPH radical absorbs at 517 nm and antioxidant activity was determined by monitoring the decrease in absorbance. DPPH radical scavenging activity of each sample at different concentrations is shown in Fig. 2. Results were reported as IC_{50} which is defined as the amount of antioxidant required to inhibit 50% of DPPH free radicals under the experimental conditions. Among the studied plant extracts, the extract of *D. stoliczkae* (rhizomes), *D. stoliczkae* (leaves) and *B. ceiba* (bark) showed IC_{50} value close to that of standard, ascorbic acid (Fig. 3).

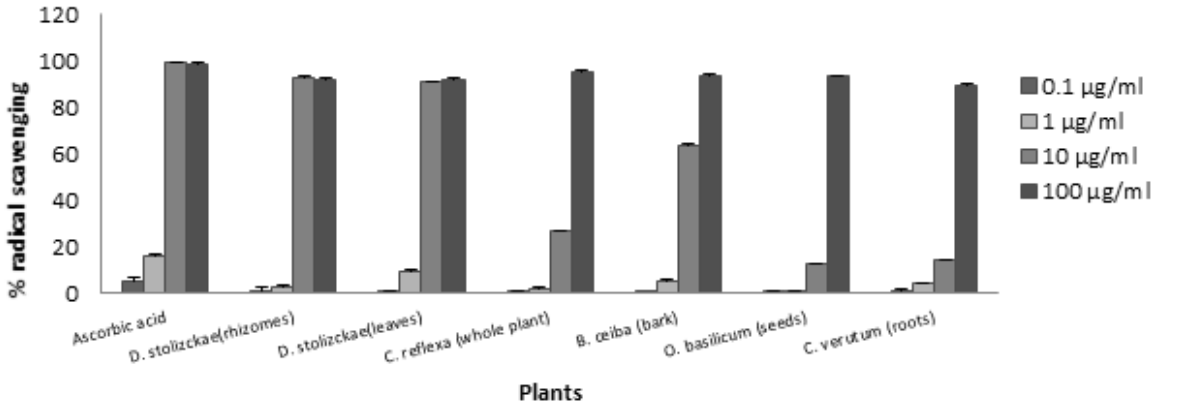

Fig. 2: Graphical representation of percentage scavenging of DPPH free radicals by extracts/ ascorbic acid at 517 nm.

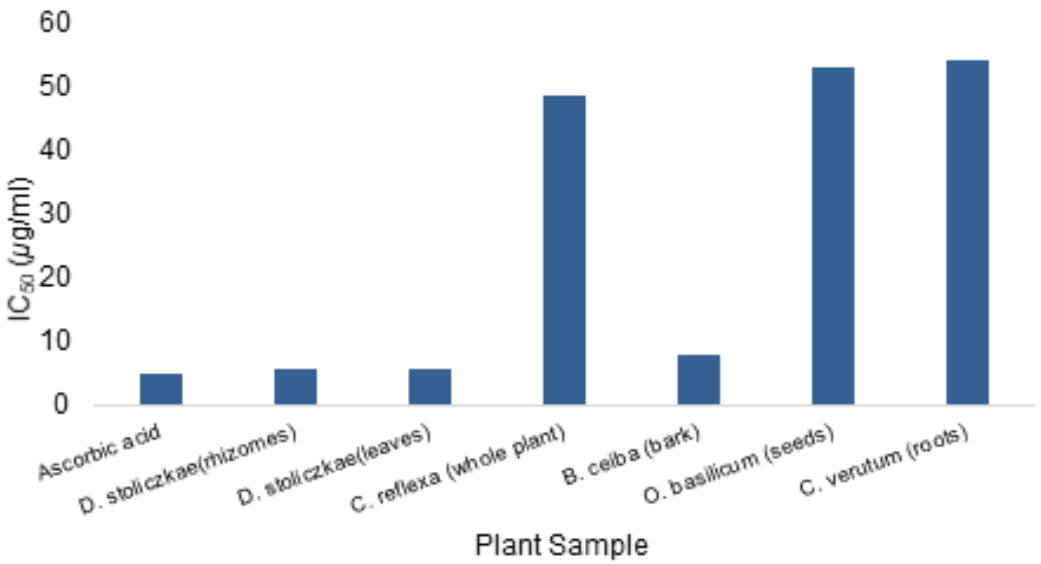

Fig. 3: Graphical representation of IC_{50} (µg/mL) values of plant extracts for free radicals

3.2 Antibacterial activity determination

Each plant crude extracts (50 mg/mL) were measured and compared with the zone of inhibitions produced by the (1 mg/mL) standard antibiotics ofloxacin and cefpodoxime. Among the six sample extracts the rhizomes and leaves of *D. stoliczkae* and bark of *B. Ceiba* showed better antibacterial activity against all test organisms while other extracts showed no zone of inhibition. The inhibition zone diameter is given in the table

Table 1: Antibacterial activity

SN	Plant sample/Conc.	Diameter of inhibition zone (mm) *		
		Gram -ve bacteria		Gram +ve bacteria
		E. coli	*P. vulgaris*	*S. aureus*
		50 mg/mL	50 mg/mL	50mg/mL
1	*D. stoliczkae* (rhizomes)	27	32	10
2	*D. stoliczkae* (leaves)	29	33	10
3	*B. ceiba* (bark)	30	30	11
4	Ofloxacin (1mg/mL)	42	47	38
5	Cefpodoxime (1mg/mL)	33	32	18
6	10% DMSO (1mg/mL)	0	0	0

*Diameter of inhibition zones includes the diameter of well (6 mm)

The standard drugs ofloxacin and cefpodoxime showed higher zone of inhibition as compared to sample extracts.

3.3 Determination of total phenol

The extract that displayed the higher concentration of total phenols were that of *D. stoliczkae* (rhizomes), *D. stoliczkae* (leaves) and *B. ceiba* (bark) i.e. 380 ± 3.81, 375.69 ± 0.49 and 254.71 ± 2.43 mg GAE/g dry extract wt. respectively while other extracts showed lower phenol content.

3.4 Determination of flavonoids content

The extract that displayed the higher concentration of flavonoids were that of *D. stoliczkae* (rhizomes), *D. stoliczkae* (leaves) and *B. ceiba* (bark) i.e. 401.1 ± 2.80, 372.1 ± 5.70 and 307.6 ± 2.10 mg QE/g dry extract wt. respectively while other extracts showed lower flavonoids content.

4. Discussion

The present study was performed to identify the hepatoprotective plants from Western region of Nepal, around Kaski district and evaluate their biological importance scientifically. For this, ethnomedicinal survey was carried out on plants that have been used by local people for hepatic ailments such as jaundice and hepatitis. On basis of the survey, some plants were selected for their biological activities that have been less studied scientifically. Most commonly used was found to be *C. reflexa,* about 85% of the respondents were aware about the plant. People also mostly used the fruits such as *C. papaya*, *S. officinarum* and *C. pepo* and found useful for efficient cure of liver diseases. The survey was useful as it also revealed those medicinal plants that were widely available around our locality but were less studied scientifically for liver ailments such as *D. stoliczkae, C. verutum, O. basilicum, B. Ceiba*, etc. Herbal remedies for liver disorders have been widely used and has been popularized world over by leading pharmaceuticals and the most widely used medicines for liver ailments are based on herbal remedies (Singh *et al*. 2012).

From the data available from the survey, six plants were selected for determining their antioxidant and antimicrobial activities. Antioxidant activity was determined by well-established method, DPPH assay and further evaluation of total phenol and flavonoid content. DPPH forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in determination of radical scavenging activity of natural products as well as synthetic compounds (Bhuiyan et al 2019). Plant phenols act as primary antioxidants or free radical terminators while flavonoid as one of the most important natural phenols, possesses radical scavenging

properties. The presence of a certain structure and particularly hydroxyl position in the molecule determine their antioxidant properties; in general, these properties depend on the ability to donate hydrogen or electron to a free radical (Luis *et al*. 2009). Liver is a major organ attacked by reactive oxidative species or free radicals and presence of antioxidants can play role to scavenge such reactive species and protect liver from injuries and diseases (Sanchez-Valle *et al*. 2012). The plants containing polyphenols and flavonoids can be useful for their role in such degenerative diseases. Among the studied plant extracts, the rhizomes and leaves extracts of *D. stoliczkae* and bark extracts of *B. ceiba* showed radical scavenging activity and IC_{50} value close to that of standard. Also these plant extracts showed the higher phenol and flavonoid content than other plant extracts.

Among the studied plant extracts, the rhizomes and leaf extracts of *D. stoliczkae*, as well as the bark extracts of *B. ceiba*, exhibited excellent radical scavenging and antibacterial activities. This was attributed to these specific plant extracts showcasing higher phenol and flavonoid content compared to other plant extracts.

From this survey, a total of 41 plant species, including *D. stoliczkae* and *B. ceiba*, were identified as being beneficial in the treatment of hepatic disorders

These in vitro assays indicate that these plant extracts could be significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. In a research conducted by Gandhare and colleagues in 2010, an antioxidant assay was performed using the bark extract of *B. ceiba*. The findings of the study demonstrated the antioxidant capabilities of the plant. It was suggested that phenols and flavonoids present in the extract could be accountable for this activity. This conclusion was supported by a subsequent study conducted by Wanjari *et al*. in 2016. The present study also proved the antioxidant potential and higher phenol and flavonoid content of the bark of *B. ceiba*. Previous study of *D. stoliczkae* for its antioxidant activity by Parajuli *et al*. 2012, also revealed the better radical scavenging activity and higher flavonoid and phenol content of the rhizomes extract of the plant (Parajuli *et al*. 2012). The present study proved not only the rhizomes extract of the plant *D. stoliczkae*, but also the leaves also show better antioxidant activity. This can be useful for further biological activity study. The present study also determined the antibacterial activity of the selected plant extracts. Despite the widespread use of broad spectrum antibiotics, bacterial infection is responsible for up to a quarter of the deaths of patients with liver disease. The plant with antibacterial activities can be useful for protection of liver from bacterial infections (Wyke 1987; Semwal *et al*. 2021). The above study showed the better antibacterial effect of the extracts of *D. stoliczkae* and *B. ceiba* as well. The result, therefore, can be useful for further analysis of hepatoprotective activities of these plants. A study by Ravi *et al*., 2010, showed the hepatoprotective activity of *B. ceiba* Linn against drug-induced toxicity in experimental rats (Ravi *et al*. 2010). Currently, there hasn't been any experimental research conducted on the plant *D. stoliczkae.* However, based on the findings presented earlier and by comparing them with results from other plants, it's evident that the rhizomes and leaf extracts of *D. stoliczkae* exhibit superior in vitro activities. This suggests potential benefits for further investigation into its biological properties through in vivo studies, particularly to confirm its potential hepatoprotective effects.

5. Conclusion

The above study was useful to find out the medicinal plants that can be used for hepatoprotection from the survey and biological assays as well. The study concluded the better biological activity of the plant *D. stoliczkae.* The plant can be further taken for evaluation of hepatoprotective and other pharmacological activities evaluation that can be useful for newer drugs development in modern medicine for treatment of various ailments.

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