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RESEARCH ARTICLE

VALORIZATION OF PRUNUS NEPALENSIS PLANT PARTS: EXTRACTION AND EVALUATION OF IN VITRO ANTIOXIDATIVE POTENTIAL AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

Prunus nepalensis (L.), locally known as Sohiong, is an important indigenous plant in Meghalaya, India. The plant has been underutilized besides having high nutritive value. The present investigation was carried out with the objective to valorize different parts of *P. nepalensis* as natural sources of antioxidants and antibacterial agents for food and pharmaceutical industry. Fruit, leaf, stem and seed extracts of *P. nepalensis* were prepared in aqueous and organic solvents (methanol, ethanol and acetone) and analyzed for phytochemical screening and in vitro antioxidant activity. The antibacterial activity of the extracts was performed using agar well diffusion method. Results confirm the presence of alkaloids, flavonoids, tannins and others phytochemicals in different extracts obtained. Ethanolic leaf extract revealed highest TPC (803.43 ± 7.76 mg GAE/ 100g DM). Methanolic leaf and stem extracts exhibited the highest radical scavenging activity (16.565 ± 0.334 and 16.679 ± 0.371 mg AEAC/g DM, respectively) whereas aqueous extract of fruit exhibited highest FRAP value (21.593±0.914 mmol AEAC/ 100g DM). Agar well diffusion test revealed good antibacterial activity of the extracts. Our findings suggest that *P. nepalensis* have great potential therapeutic values that need to be fully exploited.

INTRODUCTION

The free radicals generated under normal metabolic processes pose beneficial as well as deleterious effects in our body. An increased generation of free radicals induces an imbalance between the oxidants and antioxidants therefore causing oxidative damage to the cellular components. Natural antioxidants present in herbs, fruits and vegetables have radical scavenging ability and reduce oxidation reactions thereby strengthening our defense system. For decades, synthetic antioxidants were widely, used however lately the mutagenic and toxic effects associated with their excess consumption have brought about restrictions and limitations in their applications. On the contrary, the natural antioxidants confer fewer side effects. Further, microorganisms can cause food spoilage and infectious diseases in humans. In fact, over the years, pathogenic microorganisms have contributed to growing concerns over new disease outbreaks worldwide. Off late the focus of research has shifted to the plant bioactive compounds having the dual effect rendering antioxidant and antimicrobial activity thus having therapeutic importance and preventing

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food spoilage and improving its shelf life (Ganie *et al*., 2011; Tajkarimi *et al*., 2010).

In recent years, underutilized and aboriginal species widespread to specific locations having potent pharmacological activities are gaining attention. The north eastern region (NER) of India is the hub of plant biodiversity; however the fruits and vegetables grown are consumed and traded in the local vicinity (Rai *et al*., 2005). Studies have reported that these underutilized crops contribute more in terms of nutritive value as compared to widely consumed commercial foods (Agrahar-Murugkar and Subbulakshmi, 2005; Seal, 2011). *Prunus nepalensis* (Sohiong) belonging to the *Rosaceae* family is an indigenous plant in Meghalaya. The plant grows to 15-20 m height and bears deep purple coloured fruit that are round in shape and has a smooth surface. Primarily the utilization of the plant, as fresh fruits or processing at a very small scale for production of squash, ready to serve beverage, jam, fruit preserve and wine (Patel *et al*., 2008) is limited only to its native place. Information pertaining to its composition and antioxidant and antimicrobial properties may offer significant support to improve its commercialization and utilization of the plant. Thorough review of ethno-botanical, ethno-pharmacological and modern scientific validation data revealed that no work has been done on the selected plant. The present study aims to evaluate the *in vitro* antioxidative potential and antibacterial activity of different parts of *P. nepalensis* (fruit, leaf, stem and seed).

MATERIALS AND METHODS

Chemicals

Chemicals used in the study were of analytical grade and procured from credible concerns viz. Sigma, Merck, BDH and Qualigens.

Collection of Plant Material and Preparation of Extracts

Ripened fruits, matured leaves and stem of *Prunus nepalensis* (L.) were collected in September-October, 2014 from Meghalaya (Laitryngew). The edible part of fruits was separated and finely chopped before subjecting to freeze drying. The stones were broken to retrieve the seeds. The leaves, seeds and stem were shade dried. The dried samples were kept below 4^{o}C until further use. All samples were pulverized to powder prior to extraction using electric blender and passed through 0.5mm sieve.

Dried powdered samples were subjected to extraction using distilled water, methanol, ethanol and acetone. The extracts were prepared by steeping dried powder with suitable volume of extractant (1:10) in covered flasks. The solutions were placed in a shaker incubator to facilitate thorough mixing at ambient temperature for 24 hours so that soluble compounds attain equilibrium in the solvents. The extracts were then filtered and centrifuged. The solvents were removed under reduced pressure at 50^{o}C using rotary evaporator and were stored at -20^{o}C until further analysis.

Preliminary Phytochemical Screening

The extracts were subjected to qualitative tests for phytochemical screening of alkaloids, amino acid, carbohydrate, flavonoids, glycoside, phenols, phlobatanin, protein, saponin, tannin and terpenoids using standard protocols (Evans and Trease, 1989; Harborne, 1973; Sofowara, 1993).

Determination of Total Phenol Content

Total phenol content (TPC) of extracts was analyzed using Folin Ciocalteu (FC) method by Singleton and Rossi (1965). Briefly, 1ml of sample was mixed with 70ml of distilled water to which 5ml of FC reagent (diluted 1:10 with distilled water) was added. The mixture was allowed to stand for 2 minutes followed by adding 15ml of Na_2CO_3 (200g/ L). The volume was made up to 100ml with distilled water, mixed and incubated for 2 hours at room temperature. The absorbance was measured at 765 nm. Gallic acid was used as standard and the TPC was expressed in terms of mg gallic acid equivalent per 100 g dry matter (mg GAE/100 g DM).

Determination of In Vitro Antioxidant Activity

Antioxidant activity (AA) was measured by DPPH (2, 2-diphenyl-1-picrylhydraxyl) and FRAP (Ferric reducing antioxidant power) assays.

For DPPH assay, the method was adapted from Brand-Williams *et al.* (1995) with slight modifications. An aliquot of the extract solution was diluted 1:4 times prior to estimation, to obtain reading within the linear range of the spectrophotometer. Briefly, 0.1ml of the diluted sample was treated with 3.9ml of 0.1mM methanolic DPPH solution and allowed to stand for 30 minutes in the dark at 37 ^{o}C. The absorbance was measured at 517 nm. Percent inhibition of the DPPH radical by the sample was calculated using the following equation:

$$\% \text{ Inhibition} = \left(\frac{A_b - A_s}{A_b} \right) \times 100$$

where, A_s is absorbance of sample extract and A_b is absorbance of blank. Ascorbic acid was used as standard and results was expressed in terms of mg ascorbic acid equivalent antioxidant capacity per gram dry matter (mg AEAC/g DM). All measurements were performed in triplicate.

The FRAP assay was performed according to the method given by Benzie and Strain (1996) with minor modifications. Sample extract was diluted 1:100 times with distilled water to obtain reading within the linear range of the spectrophotometer at 593 nm. Briefly, 0.1ml of the diluted sample was added to 3ml of FRAP reagent consisting of acetate buffer (300mM, pH 3.6), TPTZ (0.031mg in 10ml 40mM HCl) and ferric chloride (20mM) in the ratio of 10:1:1. After 4 minutes, the absorbance was recorded at 593nm. The FRAP values were derived from standard curve using ascorbic acid as standard and results were expressed as mmol ascorbic acid equivalent antioxidant capacity per 100 gram dry matter (mmol AEAC/ 100 g DM).

Determination of Antibacterial Activity

Three pathogenic bacteria (*Bacillus cereus*, *Bacillus subtilis* and *Escherichia coli*) were selected for antibacterial assay. The antibacterial activities of the different extracts were evaluated by the agar well diffusion method using nutrient agar plates as described by Sen and Batra (2012). Agar plates were swabbed with 24 hours old - broth culture of respective bacteria using sterile cotton swabs. The plates were divided into four quarters and wells of 10mm were made using sterile cork borer. About 100 µL of extracts of given concentration were added into the wells. The plates were incubated at 37^{o}C for 24 hours. The diameter of inhibition zone formed was measured and recorded.

Statistical Analysis

Analyses of Variance (ANOVA) were conducted by using IBM SPSS Statistics Version 20.0 and Duncan tests were performed to test the significant differences between treatments ($p \leq 0.05$).

RESULTS

Preliminary Phytochemical Screening

The different extracts of *P. nepalensis* (fruit, leaf, stem and seed) were prepared using different organic solvents (methanol, ethanol, and acetone) and water. The *P. nepalensis* extracts revealed the presence of phenolic compounds, alkaloids, glycosides, tannin, flavonoids and other phytochemicals, summarized in Table 1.

Table 1 Preliminary phytochemical screening of various extracts of *P. nepalensis*.

Solvent	Fruit				Leaf				Stem				Seed			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Alkaloids	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Amino acid	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-
Carbohydrate	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phlobatanin	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-
Protein	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+
Saponin	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

*A= methanol extract; B= ethanol extract; C= acetone extract; D= aqueous extract; + = present; - =absent.

Total Phenol Content

The total phenol content of different extracts of *P. nepalensis* plant was quantified spectrophotometrically based on the reduction of FC reagent. The TPC of the extracts of different parts of *P. nepalensis* are presented in Figure 1. Overall, leaves, stem and fruits of *P. nepalensis* revealed to possess optimal phenolic content in an order: ethanolic leaf extract (803.4 ± 7.76 mg GAE/ 100g DM) > methanolic fruit extract (798.0 ± 10.62 mg GAE/ 100g DM) > aqueous leaf extract (794.9 ± 9.78 mg GAE/ 100g DM) > aqueous stem extract (790.1 ±10.11 mg GAE/ 100g DM). On the other hand, seed extracts exhibited least TPC ranging from 50.5 ± 1.50 to 357.4 ± 4.22 mg/100g DM. Furthermore, it was observed that aqueous and methanol extracts had the highest TPC closely followed by ethanol extracts whereas acetone extracts showed minimal TPC.

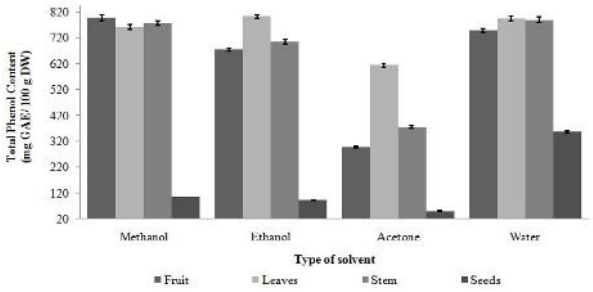

Figure 1 Total phenol content (TPC) of methanol, ethanol, acetone and water extracts of fruit, leaves, stem and seeds of *Prunus nepalensis* (L.)

(a) Bars represent mean value ± SD (n=3)

In Vitro Antioxidant Activity of Extracts

DPPH free radical scavenging activities and ferric reducing antioxidant potential (FRAP) of *P. nepalensis* extracts are shown in Figure 2. All extracts exhibited scavenging ability, however, methanolic extracts of fruit, leaf and stem (11.7 ± 0.30, 16.6 ± 0.33 and 16.7 ± 0.37 mg AEAC/g DM, respectively) showed higher radical inhibitory activity as compared to ethanol (5.4 ± 0.11, 16.5 ± 0.49 and 12.9 ± 0.29 mg AEAC/g DM, respectively), acetone (1.7 ± 0.03, 7.9 ± 0.36 and 2.9 ± 0.52 mg AEAC/g DM, respectively) and aqueous (9.18 ± 0.39, 14.9 ± 0.33 and 16.2 ± 0.68 mg AEAC/g DM, respectively) extracts. On the other hand, the radical scavenging capacity of seed extracts was more pronounced in ethanol extract (12.9 ± 0.29 mg AEAC/g DM). Further, the aqueous extracts of fruit (21.6 ± 0.91 mmol AEAC/100g DM) and stem (14.4 ± 0.30 mmol AEAC/100g DM) demonstrated significantly higher reducing power followed by methanolic extracts for fruit and stem (14.2 ± 0.36 and 12.7 ± 0.28 mmol AEAC/100g DM, respectively). However, the methanolic leaf extract (16.8 ± 0.34 mmol AEAC/ 100g DM) exhibited higher ferric reducing capacity than aqueous (14.4 ± 0.29 mmol AEAC/ 100g DM) and ethanol (9.6 ± 0.41 mmol AEAC/ 100g DM) extracts. Overall, reducing power of seeds was less indicating least antioxidative potential.

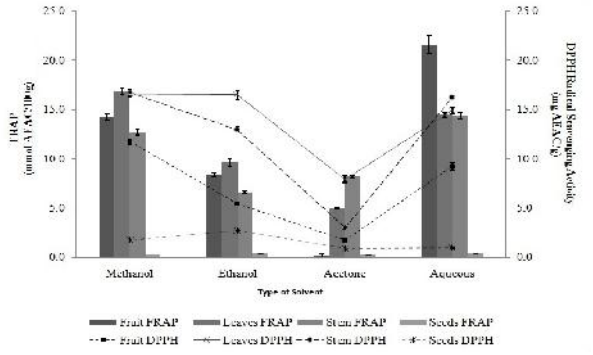

Figure 2 *In vitro* antioxidant activity (FRAP and DPPH) of methanol, ethanol, acetone and water extracts of fruit, leaves, stem and seeds of *Prunus nepalensis* (L.)

(a) Bars represent mean value ± SD (n=3)

Antibacterial Activity of Extracts

The antibacterial activities of *P. nepalensis* extracts are presented in Table 2. Both gram positive (*Bacillus cereus* and *Bacillus subtilis*) and gram negative bacteria (*Escherichia coli*)

tested were found to be sensitive to the different extracts. *B. cereus* was observed to be least affected when compared to *B. subtilis* and *E. coli* with inhibition zone ranging (12 to 18) mm, (12 to 28) mm and (14 to 23) mm, respectively.

Table 2 Antibacterial activity of various extracts of *Prunus nepalensis*

Type of Solvent	Inhibition zone (mm)			
	Fruit	Leaf	Stem	Seed
		Bacillus cereus		
Methanol	16 ± 0.46	18 ± 0.44	12 ± 0.26	12 ± 0.00
Ethanol	14 ± 0.26	16 ± 0.35	14 ± 0.26	14 ± 0.20
Acetone	14 ± 0.43	16 ± 0.20	12 ± 0.50	12 ± 0.40
Water	15 ± 0.61	12 ± 0.35	12± 0.00	-
		Bacillus subtilis		
Methanol	28 ± 0.72	14 ± 0.26	14 ± 0.35	16 ± 0.10
Ethanol	22 ± 0.46	15 ± 0.10	16 ± 0.35	18 ± 0.40
Acetone	20 ± 0.44	14 ± 0.60	14 ± 0.26	19 ± 0.53
Water	23 ± 0.87	12 ± 0.10	12 ± 0.20	-
		Escherchia coli		
Methanol	21 ± 0.53	18 ± 0.36	15 ± 0.35	17 ±0.87
Ethanol	19 ± 0.36	20 ± 0.44	15 ± 0.35	18 ±0.40
Acetone	18 ± 0.35	15 ± 0.61	16 ± 0.50	17 ±0.50
Water	23 ± 0.50	14 ± 0.06	15 ± 0.36	-

Values are mean ± SD (n=3). Diameter of inhibition zone includes diameter of well.

DISCUSSION

The presence of bioactive compounds such as phenol, flavonoids and tannins in different extracts of *P. nepalensis* probably contributes to high scavenging capacity of the plant. Free radical mediated oxidative stress has been one of the prime factors in the pathogenesis of degenerative diseases. Several studies have demonstrated the bioactive components viz. alkaloids have anti-inflammatory (Souto *et al*., 2011), antimalarial (Dua *et al*., 2013), antibacterial, antispasmodic, analgesic (Harborne, 1973), cytotoxicity and other pharmacological properties (Thite *et al*., 2013); flavonoids and tannins contribute to antioxidant, antimicrobial and anticancer activity (Benevente-Gracia *et al*., 1997; Giovanelli *et al*., 2000); saponins have antifungal and cardioprotective properties; glycosides are used in treatment of congestive heart failure and cardiac arrhythmia (Bhandary *et al*., 2012); Terpenoids have hepaticidal, anti-ulcer, antimalarial, anticarcinogenic, antimicrobial and diuretic activities; phlobatanins have diuretic properties (Daniel *et al*., 2015). The underutilized parts of *P. nepalensis* can thus be used for the treatment of various ailments associated with pathogenic microbes and free radical mediated chronic diseases.

The variation in total phenol content (TPC) in different extracts revealed that extraction of compounds is greatly influenced by the type of solvent and the part of the plant which is probably due to difference in solubility index of the bioactive compounds in various solvents (Neenah *et al*., 2011; Shobowale *et al*., 2013; Sultana *et al*., 2009). Our results are in agreement with the previous studies showing that water, methanol and ethanol (in decreasing order of polarity) have higher concentration of phenol compounds as compared to acetone (less polar) from the different parts of *P. nepalensis*.

The antioxidant effect may be accounted to different mechanisms such as free radical scavenging as well as reducing properties, decomposition of peroxides or prevention of chain initiation (Gulcin *et al*., 2002). Moreover, due to chemical complexity of extracts from different parts of plant and diversity of plant phenols, it is desireable to perform different methods to determine the antioxidant activity (Dai and Mumper, 2010). DPPH scavenging test, to assess their free radical scavenging capacity and FRAP assay for estimating total antioxidant power was used in parallel for estimating the total antioxidant activity of plant extracts. The present study demonstrated that the different extracts of *P. nepalensis* were able to reduce DPPH concentration and showed a decreasing trend in absorbance at 517 nm. FRAP assay is a robust, sensitive, fast and precise analytical method to assess antioxidant property of different compounds (Benzie and Strain, 1996; Benzie and Strain, 1999). FRAP assay suggest that the bioactive compounds in *P. nepalensis* are likely to contribute significantly toward the antioxidant effect observed. Furthermore, DPPH and FRAP assay of extracts clearly indicate the influence the type of solvent and part of plant used for analysis. The extracts prepared in high polarity solvents, viz. water, methanol and ethanol, considerably showed higher antioxidant activity in contrast to the ones prepared using less polar solvents like acetone. Earlier studies conducted on *Medicago sativa* (Caunii *et al*., 2012) and *Polygonum minus* (Hassim *et al*., 2015) have shown similar results reporting antioxidant activity of extracts to strongly depend on the type of solvents used.

The results also indicate the efficacy of the extracts against the pathogenic bacteria, viz., *Bacillus cereus, Bacillus subtilis and Escherichia coli*. The antibacterial activity was subjective to the type of solvent and part of the plant. *B. subtilis* and *E. coli* were more sensitive to various fruit extracts where as *B. cereus* was more sensitive to stem extracts. The antibacterial activity may be attributed to the presence of phytochemicals such as flavonoids, phenolics and tannins which have also been reported in previous studies (Al-daihan and Bhat, 2012; Cowan, 1999).

CONCLUSION

Prunus nepalensis (L.) exhibits high antioxidative effect and optimal antibacterial activity. The study affirms high antioxidative potential of aqueous, methanol and ethanol extracts of different parts of *P. nepalensis*. Further investigation is required to isolate, characterize the bioactive compounds and elucidate their mode of action in vitro and in vivo studies.

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