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ORIGINAL ARTICLE

In vitro assessment of antioxidant potential and determination of polyphenolic compounds of *Hedera nepalensis* K. Koch

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Abstract Recently, the interest in discovering natural antioxidants has increased tremendously for their application to control onset of multiple diseases. The present study was designed to determine polyphenolic compounds and antioxidant potential of *Hedera nepalensis*. Crude extract of *H. nepalensis* and its fractions (*n*-hexane, ethyl acetate and aqueous) was prepared by solvent–solvent extraction. Total flavonoid and phenolic contents were determined by colorimetric methods using quercetin and gallic acid as standard. Further quantitative analysis of phenolic and flavonoid compounds was carried out by using high performance liquid chromatography coupled with diode array detector (HPLC-DAD). Ethyl acetate fraction showed the highest amount of total flavonoid (2.4 ± 0.164 mg QE/100 mg) and phenolic contents (12.90 ± 0.15 mg GAE/100 mg). Using HPLC-DAD, catechin was identified in aqueous fraction and caffeic acid was identified in ethyl

Abbreviations: *H. nepalensis*, *Hedera nepalensis*; HPLC-DAD, high performance liquid chromatography with diode detector; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DMSO, dimethylsulfoxide; HNC, crude extract of *H. nepalensis*; HNN, *n*-hexane fraction of *H. nepalensis*; HNE, ethyl acetate fraction of *H. nepalensis*; HNA, aqueous fraction of *H. nepalensis*; GAE, gallic acid equivalents; QE, quercetin equivalents

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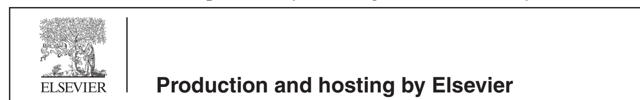

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acetate fraction of *H. nepalensis*. The antioxidant capacity of *H. nepalensis* was evaluated by measuring the scavenging potential of hydrogen peroxide (H_2O_2), total antioxidant capacity and reducing power. The extract/fractions showed significant ($P < 0.05$) hydrogen peroxide scavenging potential with their IC_{50} values ranging from 31.19 to 200 μg/ml. Among crude extract and all the fractions of *H. nepalensis*, ethyl acetate fraction showed the highest total antioxidant activity by phosphomolybdenum method followed by *n*-hexane, crude extract and aqueous fraction. Moreover, ethyl acetate fraction has shown the highest reducing power followed by aqueous fraction, *n*-hexane fraction and crude extract. The present study provides evidence that ethyl acetate fraction of *H. nepalensis* has significant antioxidant potential which correlates well with its high phenolic and flavonoid contents. To the best of our knowledge catechin and caffeic acid have been reported for the first time in *H. nepalensis*.

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1. Introduction

Free radicals are highly reactive oxygen species that are produced as a result of respiration and metabolism at cellular level. If the effects of these radicals are not mitigated, they may cause damaging effect to macromolecules of cell like lipids, nucleic acids, proteins and carbohydrates (Kulbacka et al., 2009). Furthermore oxidative stress imposed by excessive accumulation of these free radicals may cause a broad range of diseases like inflammation, stroke, heart disease, diabetes mellitus, cancer, Parkinson' disease and Alzheimer's disease (Mensor et al., 2001; Parejo et al., 2002; Hou et al., 2003; Orhan et al., 2003; Tepe et al., 2005; Ozgen et al., 2006). Antioxidants are the compounds capable of preventing or reducing harmful effects of these free radicals. The exogenous antioxidants mainly consist of synthetic and natural antioxidants. However, there have been increasing safety concerns over synthetic antioxidants. For example, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), the two well-known synthetic antioxidants, have been restricted for their DNA damaging and other toxic effects (Sasaki et al., 2002). As an alternative, natural resources got substantial attention as a source of biologically active antioxidants (Dillard and German, 2000).

Antioxidant constituents of the plants act as a radical scavengers and help in converting the free radicals to less reactive species. Most naturally occurring antioxidants of plants are flavonoids, vitamins, phenols, carotenoids and dietary glutathione (Larson, 1988). Manach et al. (1998) have reported that plant derived antioxidants can quench singlet oxygen, scavenge free radicals, inhibit enzymes and decompose peroxides. Therefore flavonoids, tannins, carotenoids and other constituents of plants have attracted great attention of the world as potential antioxidants (Record et al., 2001). These antioxidants can be used for the prevention of a wide range of diseases including cancer. Therefore the worldwide research is now focusing on natural antioxidants.

Traditionally medicinal plants are used for prevention as well as for the treatment of several diseases. Among the properties behind these virtues, the antioxidant activity holds a place of first order (Rahman, 2008). *Hedera nepalensis* (Araliaceae) is a well-known medicinal plant distributed in west Asia, Japan, Afghanistan and the Himalayas (Nasir, 1975). Traditionally, *H. nepalensis* is used for the cure of diabetes (Gilani et al., 2007) and cancer (Hamayun et al., 2006). Its antitumor activity has been recently reported by Kanwal et al. (2011). Phytochemical analysis showed the presence of flavonoids, steroids, tannins, terpenoids and cardiac glycosides in *H. nepalensis* (Kanwal et al., 2011). Considering the medicinal activity of *H. nepalensis* based on traditional information, the present study was undertaken to analyze its antioxidant potential and to identify selected antioxidant compounds by using HPLC coupled with DAD.

2. Materials and methods

2.1. Equipment

The chromatographic system used consisted of Agilent Chem station Rev. B.02-01-SR1(260), Agilent 1200 series binary gradient pump coupled with diode array detector (DAD; Agilent technologies, Germany), Discovery-C18 analytical column (4.6 × 250 mm, 5 μm particle size, Supelco, USA). UV/VIS-DAD spectrophotometer (8354 Agilent Technologies, Germany), rotary evaporator (Buchi, Switzerland), and incubator IC83 (Yomato, Japan).

2.2. Reagents and chemicals

All the solvents *i.e.* methanol, acetonitrile, acetic acid, dimethylsulfoxide (DMSO) and water were of HPLC grade and were purchased from Sigma (Sigma–Aldrich, Germany). All the reference compounds were purchased from Sigma–Aldrich *i.e.* rutin, quercetin, gallic acid, caffeic acid, catechin, kaempferol and myricetin.

2.3. Plant material

The aerial parts of *H. nepalensis* were collected from Nathia gali Murree road, District Rawalpindi, Punjab, Pakistan in September 2010. The plant species was identified by Professor Dr. Rizwana Aleem Qureshi (Taxonomist), Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan and a voucher specimen numbering HMP-461 was deposited in Herbarium of Medicinal Plants of Pakistan, Quaid-i-Azam University, Islamabad 45320, Pakistan.

2.4. Preparation of crude extract and fractions

Plant material (1 kg dry weight) was macerated in methanol and chloroform mixture (4 L, 1:1) for 7 days at room

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temperature, filtered and concentrated in rotary evaporator at 45°C under low pressure to get crude extract. The crude extract (60 g) was then suspended in 500 mL distill water. The water suspension was partitioned three times (3 × 500 ml) with *n*-hexane to get *n*-hexane fraction. The residual aqueous suspension was then partitioned with ethyl acetate (3 × 500 mL) to get ethyl acetate fraction and aqueous fraction. All the organic solvent layers and remaining aqueous layer were concentrated by using rotary evaporator to get *n*-hexane fraction (HNN, 18.4 g), ethyl acetate fraction (HNE, 17.0 g) and aqueous fraction (HNA, 23.0 g) respectively.

2.5. Determination of flavonoid content

For total flavonoid content determination, aluminum chloride colorimetric method was used (Chang et al., 2002). The crude extract and its fractions (0.5 ml of 1.0 mg/ml methanol) were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1.0 M potassium acetate and 2.8 ml of distilled water. The resulting mixture was kept at room temperature for 30 min. Then absorbance of the reaction mixture was measured at 415 nm using UV/VIS-DAD spectrophotometer. The calibration curve was drawn by using quercetin as standard at final concentrations 0.0–8.0 μg/ml.

2.6. Determination of phenolic content

The total phenolic content was determined according to Velioglu et al. (1998) by using Folin–Ciocalteu reagent. The crude extract and its fractions were prepared at a concentration of 1 mg/ml and 200 μl of each solution was transferred into a test tube and 1.5 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) was added and mixed. The resulting mixture was kept at room temperature for 5 min and then 1.5 ml of 6% (w/v) sodium carbonate was added and mixed gently. The resulting reaction mixture was kept at 25 °C for 90 min and the absorbance was measured at 725 nm by using UV/VIS-DAD spectrophotometer. The calibration curve (0.0–25 μg/ml) was plotted by using gallic acid. The total phenolic content was expressed as gallic acid equivalents (GAE).

2.7. HPLC-DAD analysis

2.7.1. Preparation of standard solution for HPLC analysis

Stock solutions of rutin, kaempferol, myricetin, gallic acid, catechins, caffeic acid and quercetin were prepared in methanol, at concentration of 1 mg/ml and then further diluted with methanol to get 10, 20, 50, 100, 150 and 200 μg/ml for the preparation of standard calibration curve. All the solutions were filtered through 0.2 μm sartolon polyamide membrane filter (Sartorius).

2.7.2. Preparation of sample solution for HPLC analysis

The samples for HPLC analysis were prepared at concentration of 10 mg/ml in methanol. The samples were dissolved in methanol *via* sonication and were filtered through 0.2 μm Sartolon Polyamide membrane filter. All the samples were freshly prepared and used for an analysis immediately or stored at 4 °C if not tested for more than 1 h.

2.7.3. Chromatographic conditions

Chromatographic analysis was carried out by using HPLC-DAD attached with Discovery C-18 analytical column. The method followed was as described by Zu et al. (2006) with a slight modification according to system suitability. Briefly, mobile phase A was methanol–acetonitrile–water–acetic acid (10:5:85:1) and mobile phase B was methanol–acetonitrile–acetic acid (60:40:1). A gradient of time 0–20 min for 0–50% B, 20–25 min for 50–100% B and then isocratic 100% B till 30 min was used. Flow rate was 1 ml/min and injection volume was 20 μl. Rutin and gallic acid were analyzed at 257 nm, catechin at 279 nm, caffeic acid at 325 nm and quercetin, myricetin, kaempferol were analyzed at 368 nm. Different wavelengths were selected according to the method described by Zu et al. (2006). Every time column was reconditioned for 10 min before the next analysis.

2.8. Antioxidant studies

2.8.1. Hydrogen peroxide scavenging activity

The ability of the plant extract to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of 2 mM H_2O_2 was prepared in 50 mM phosphate buffer (pH 7.4). An aliquot of 100 μl of the sample was transferred to an eppendorf tube to have final concentration in a reaction mixture of 200, 66.6, 22.2 and 7.4 μg/ml and the volume was made up to 400 μl with 50 mM phosphate buffer (pH 7.4). After addition of 600 μl of H_2O_2 solution, tubes were vortexed and absorbance of H_2O_2 at 230 nm was determined after 10 min, against a blank. Ascorbic acid was used as positive control. The percentage H_2O_2 scavenging ability of samples (extract/fraction) was than calculated by using the following equation:

$$\text{Hydrogen peroxide scavenging activity} = [1 - As/Ac] \times 100.$$

As = Absorbance of sample
Ac = Absorbance of control
Experiments were performed in triplicate.

2.8.2. Total antioxidant capacity (Phosphomolybdenum method)

Total antioxidant capacity was determined by the phosphomolybdenum method (Prieto et al., 1999). An aliquot of 0.1 ml of the sample was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammoniummolybdate). After an incubation of 90 min at 95 °C, samples were cooled to room temperature and absorbance of the mixture was measured at 695 nm. In the case of blank 0.1 ml of DMSO was used in place of the sample. The antioxidant capacity of each sample was expressed as ascorbic acid equivalent. Experiments were performed in triplicate.

2.8.3. Reducing power

The reducing power of *H. nepalensis* was determined according to the method described by Oyaizu (1986). The reducing power of the sample was determined by using 200 μl aliquot of extract prepared in DMSO mixed with 500 μl of 2 M phosphate buffer at pH 6.6 and 500 μl of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50 °C for 20 min. A volume of 500 μl of 10% trichloroacetic acid was added to the mixture,

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which was then centrifuged at 3000 rpm for 10 min. The upper layer of the mixture (500 μl) was mixed with an equal volume of distilled water and 100 μl of 0.1% ferric chloride ($FeCl_3$). Absorbance was measured at 700 nm. Blank was prepared by adding 200 μl of DMSO instead of the extract. The reducing power of each sample was expressed as ascorbic acid equivalent. Experiments were performed in triplicate.

3. Results

3.1. Total flavonoid content

Total flavonoid content was calculated as quercetin equivalents (QE) as shown in Fig. 1. Total flavonoid contents of the crude extract of *H. nepalensis* and its fractions varied noticeably. This study showed the highest flavonoid content in HNE (2.5 mg QE/100 mg sample), followed by HNC (2.3 mg QE/100 mg sample), HNA (0.57 mg QE/100 mg sample) and HNN (0.001 mg QE/100 mg sample).

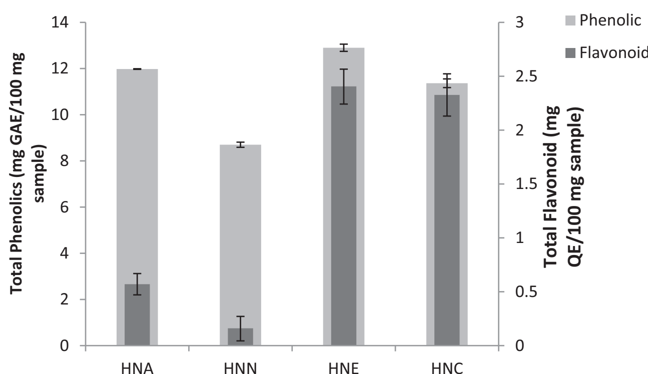

Figure 1 Total phenolic and flavonoid content (mg/100 mg of extract/fraction) of *H. nepalensis* crude extract and its fractions. Data are expressed as mean ± S.D.

3.2. Total phenolic content

Total phenolic contents of all the extract/fractions were calculated in terms of gallic acid equivalents (GAE) as shown in Fig. 1. The present study showed that total phenolic contents in the subjected plant as: HNE (12.9 mg GAE/100 mg sample) > HNA (12 mg GAE/100 mg sample) > HNC (11.4 mg GAE/100 mg sample) > HNN (8.7 mg GAE/100 mg sample).

3.3. HPLC analysis

Qualitative analysis of the extract and fractions was carried out by using reverse phase HPLC and their chromatographic profile was compared with the retention times and absorption spectrum of reference standards (rutin, kaempferol, myricetin, gallic acid, catechins, caffeic acid and quercetin). From the HPLC profile it was observed that the crude extract contains catechin (126 μg/g) and caffeic acid (20.30 μg/g) (Table. 1), while among fractions, HNE contains caffeic acid (42 μg/g) (Fig. 2) and aqueous fraction contains catechin (192.50 μg/g) (Table. 1).

3.4. H_2O_2 scavenging activity

The percentage scavenging of H_2O_2 of the crude extract and its three fractions is shown in Fig. 3a. Among all fractions, ethyl

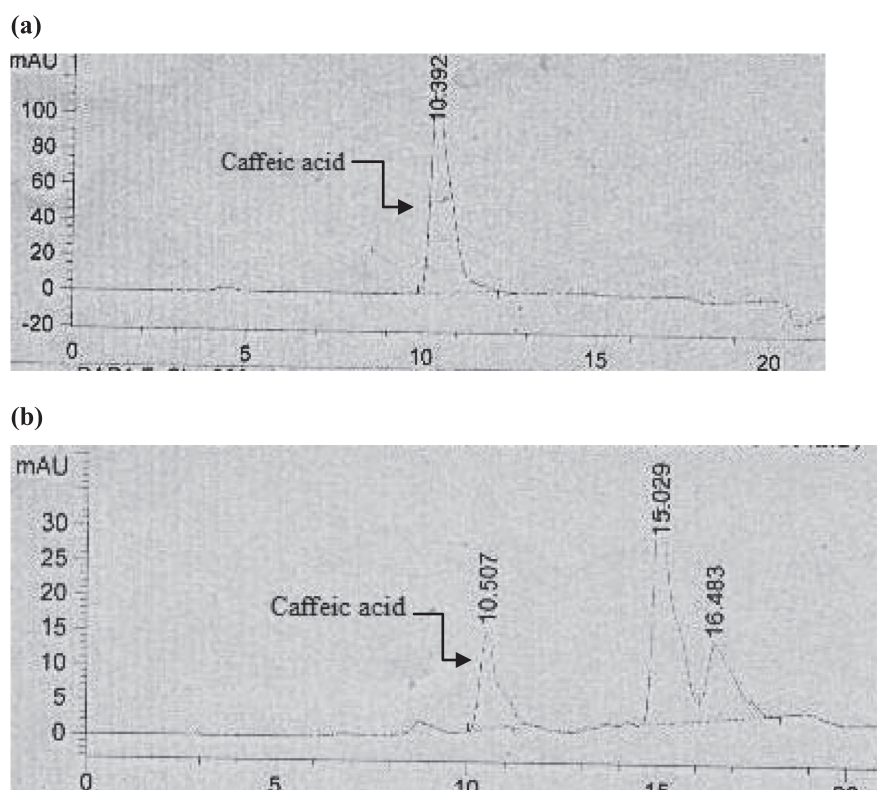

Figure 2 HPLC profile of (a) standard (caffeic acid) (b) ethyl acetate fraction of *H. nepalensis*.

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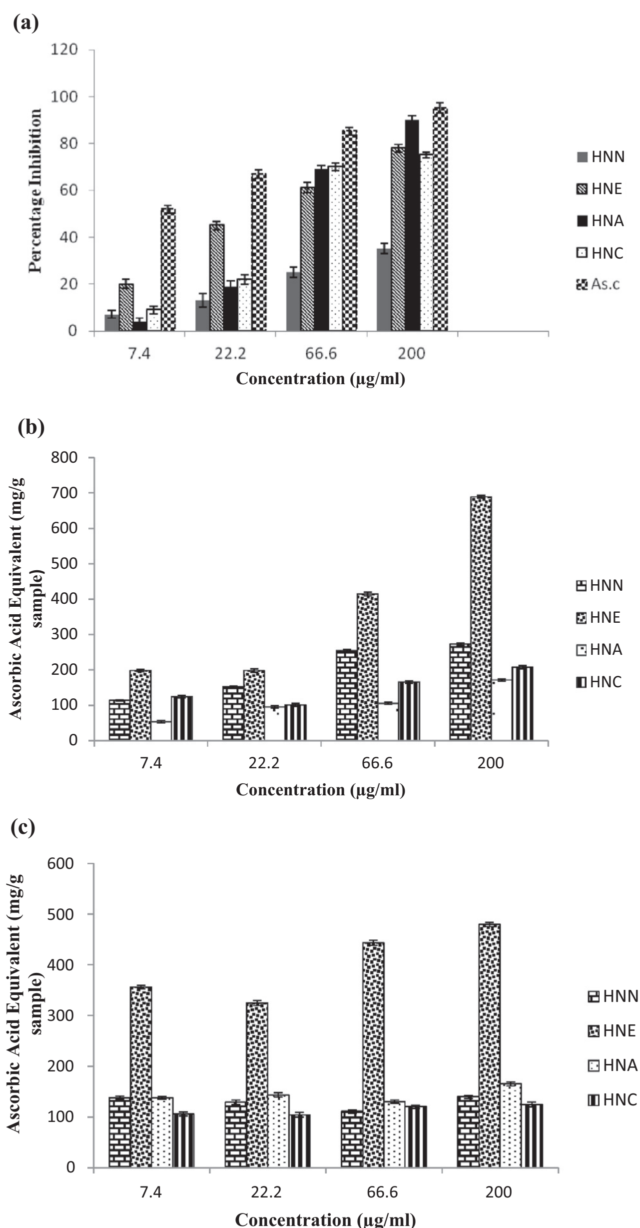

Figure 3 (a) Percentage scavenging of hydrogen peroxide (b) Total antioxidant capacity and (c) Reducing power of crude extract of *H. nepalensis* and its fractions. Data are expressed as mean ± S.D. ($P < 0.05$).

acetate fraction showed lowest IC_{50} value *i.e.* 31.19 μg/ml followed by crude extract, aqueous fraction and *n*-hexane fraction 37.87, 43.16 and 200 μg/ml respectively (Table. 2). The extract/fractions of *H. nepalensis* have shown an increase in percentage scavenging with an increase in concentration (Fig. 3a).

3.5. Total antioxidant capacity

Total antioxidant capacity of the crude extract and its fractions was determined by the phosphomolybdenum method and expressed as equivalents of ascorbic acid (mg/g of extract) as shown in Fig. 3b and was found to decrease in the order, such that ethyl acetate fraction (HNE) > *n*-hexane fraction (HNN) > crude extract (HNC) > aqueous fraction (HNA). All the samples exhibited an increase in antioxidant capacity with an increase in concentration.

3.6. Reducing power assay

HNE showed the highest reducing power with 479.37 mg ascorbic acid equivalents/g sample measured at 200 μg/ml of extract concentration followed by 165.35, 139.64 and 124.70 mg ascorbic acid equivalents/g sample measured at 200 μg/ml of sample concentration for HNA, HNN and HNC respectively (Fig. 3c).

4. Discussion

In the present study, we have opted to determine the antioxidant capacity of *H. nepalensis* and reported for the first time identification of catechin and caffeic acid in the subject plant. Our study indicated that ethyl acetate fraction contains significant amounts of phenolic compounds having good antioxidant potential and their beneficial effects on human nutrition and health are considerable.

Phenolic compounds are strong candidates exhibiting free radical scavenging potential (Qian et al., 2008) and their hydroxyl groups confer scavenging potential (Yildirim et al., 2000). Further Kessler et al. (2003) has reported that the mechanism of action of flavonoids is through scavenging or chelation. Sahreen et al. (2010) observed a positive correlation between plant phenolics and their antioxidant character. Moreover, Shon et al. (2004) investigated a positive correlation in antioxidant and antimutagenic potential of phenolics. The presence of high concentration of phenolic contents in HNE makes it a valuable discovery for its possible use as source of natural antioxidants.

Springfield et al. (2005) has mentioned that HPLC is the best way for chemical profiling of plant extract, therefore simple, rapid, reproducible and specific RP-HPLC fingerprinting was also established in the current research work for

Table 1 Quantitative analysis of polyphenols in the crude extract of *H. nepalensis* and its fractions.

Samples	Polyphenols (μg/g dry weight)						
	Rutin	Kaempferol	Myricetin	Gallic acid	Catechins	Caffeic acid	Quercetin
HNN	–	–	–	–	–	–	–
HNE	–	–	–	–	–	42	–
HNA	–	–	–	–	192.5	–	–
HNC	–	–	–	–	126	20.30	–

HNN: *Hedera nepalensis n*-hexane fraction, HNE: *Hedera nepalensis* ethyl acetate fraction, HNA: *Hedera nepalensis* aqueous fraction, HNC: *Hedera nepalensis* crude extract.

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Table 2 IC_{50} (μg/ml) values of crude extract of *H. nepalensis* and its fractions.

Samples	H_2O_2 scavenging activity
HNN	> 200
HNE	31.19
HNA	43.16
HNC	37.87
As.c	6.64

HNN: *Hedera nepalensis n*-hexane fraction, HNE: *Hedera nepalensis* ethyl acetate fraction, HNA: *Hedera nepalensis* aqueous fraction, HNC: *Hedera nepalensis* crude extract, As.c: ascorbic acid.

quantification of seven major polyphenols (rutin, kaempferol, myricetin, gallic acid, catechins, caffeic acid and quercetin) in the *H. nepalensis*. Standards were selected on the basis of their reported medicinal properties for instance; caffeic acid reduces the acute immune and inflammatory response (Huang et al., 1998) and catechin is the class of flavonoids with potent antioxidant and cancer chemopreventive properties (Weyant et al., 2001). Out of seven compounds, catechin and caffeic acid were identified for the first time (to best of our knowledge) in *H. nepalensis*. These results are in agreement with previous report (Inayatullah et al., 2012) where rutin and chlorogenic acid were identified in the crude extract of *H. nepalensis*. However, between catechin and caffeic acid, caffeic acid is more antioxidant than catechin (Chen and Ahn, 1998) It has also been illustrated that these compounds are strong antioxidants, in which caffeic acid is considered to be the highest antioxidant due to the dihydroxylation of 3, 4- position on the phenolic ring of caffeic acid (Kim and Lee, 2004). So it can be deduced from the above mentioned results, that due to the presence of caffeic acid out of seven standards, HNE might have shown significant antioxidant potential. Antioxidant capacity of compounds is related to its ability of protecting a biological system against the potentially harmful effects of reactive oxygen and nitrogen species (karadag et al., 2009). Yildirim et al. (2000) has reported that the antioxidant character is attributed to the different mechanisms such as prevention of chain initiation, reducing capacity, radical scavenging and decomposition of peroxides. Therefore, the use of a variety of methods for the determination of antioxidant capacity of medicinally important plants is suggested (Salazar et al., 2008). Hence in the current research work different antioxidant assays were performed to check the antioxidant potential of the plant because antioxidant activity occurs by different mechanisms, which means employing a method depending on one mechanism may not reflect the true antioxidant capacity (karadag et al., 2009).

Hydrogen peroxide itself is not very reactive because of its weak oxidizing and reducing capabilities but it can inactivate few enzymes by oxidizing essential thiol (–SH) groups and it also can cross the cell membrane rapidly and once inside the cell, in the presence of metal ions it is converted into highly toxic hydroxyl radical which may originate many of its toxic effects (Lee et al., 2004). Therefore it is biologically important for cells to scavenge hydrogen peroxide that gets entered into the cell. Hence *in vitro* testing for H_2O_2 scavenging activity of phytoextracts is one of the valuable tools to discover new natural antioxidants to control diseases induced by H_2O_2 and its products. From the result, it appeared that HNE of the plant has shown the highest H_2O_2 scavenging ability. It is therefore reasonable to conclude that highest phenolic compounds in the ethyl acetate fraction are a good electron donor; they may increase H_2O_2 scavenging potential by reducing it to water (Khan et al., 2012a; Ruch et al., 1984).

Literature is scarce about total antioxidant activity of *H. nepalensis* by the phosphomolybdenum method. In the presence of antioxidant compounds, Mo (VI) is reduced to Mo (V) and forms a green colored complex of phosphomolybdenum (V) that gives absorbance at 695 nm. The method is based upon the spectrophotometric quantitation of total antioxidant capacity and employs cost-effective reagents (Prieto et al., 1999). The phosphomolybdenum method is quantitative since the antioxidant capacity is expressed as the number of equivalents of ascorbic acid. The significant antioxidant capacity of ethyl acetate fraction can be attributed to the elevated amounts of polyphenols (Khan et al., 2012b). This is in accordance with previous reports of Sharififar et al. (2004) in which they suggested that flavonoids and polyphenols contribute significantly to the total antioxidant potential of medicinal plants.

The fact that the phenolic compounds are good electron donors, they exhibit reducing power and have ability to reduce ferric ion to ferrous ion by donating electron (Shon et al., 2004) may explain a current interest in the applicability of the reducing power assay in determining the antioxidant capacity of plant extracts. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In the present research work, the reducing potential of the extracts indicates the reducing power of *H. nepalensis* as a potential source of antioxidants. Moreover our study reveals that the HNE has high phenolic contents and antioxidant potential due to high electron donors' attribute (Shahidi et al., 1992). This study, therefore, suggests that the best reducing power of HNE and HNA might be due to the presence of its phenolic contents which is in agreement with the report of Li et al. (2009).

5. Conclusion

It is concluded that among all tested samples, ethyl acetate fraction (HNE) contained the highest amount of flavonoids and phenolic contents and exhibited significant antioxidant potentials. Among other polyphenols, to the best of our knowledge catechin and caffeic acid have been reported for the first time in *H. nepalensis*.

These results pave the way for the possible development of natural additives to replace synthetic ones. Therefore, further investigations for the isolation of active constituents and their pharmacological evaluation are required.

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References

Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10, 178–182.

Chen, X., Ahn, D.U., 1998. Antioxidant activities of six natural phenolics against lipid oxidation induced by Fe2+ or ultraviolet light. JAOCS 75, 12.

Dillard, C.J., German, J.B., 2000. Phytochemicals: nutraceuticals and human health. J. Sci. Food Agric. 80, 1744–1756.

Gilani, S.A., Qureshi, R.A., Gilania, S.J., 2007. Indigenous uses of some important ethnobotanical herbs of Ayubia national park, Abbottabad, Pakistan.

Hamayun, M., Khan, S.A., Sohn, E.Y., Lee, I.J., 2006. Folk medicinal knowledge and conservation status of some economically valued medicinal plants of district Swat, Pakistan. Lyonia 11 (2), 101–113.

Hou, W.C., Lin, R.D., Cheng, K.T., Hung, Y.T., Cho, C.H., Chen, C.H., Hwang, S.Y., Lee, M.H., 2003. Free radical scavenging activity of Taiwanese native plants. Phytomedicine 10, 170–175.

Huang, M.T., Smart, R.C., Wong, C.Q., Conney, A.H., 1998. Inhibitory effect of curcumin, chlorogenic acid, caffeic acid and ferulic acid on tumor promotion in mouse skin by 12-O tetradecanoylphorbol-13-acetate. Cancer Res. 48, 5941.

Inayatullah, S., Prenzler, P.D., Obied, H.K., Rehman, Ata.-ur., Mirza, B., 2012. Bioprospecting traditional Pakistani medicinal plants for potent antioxidants. Food Chem. 132, 222–229.

Kanwal, S., Ullah, N., Ihsan-ul-Haq, Afzal, I., Mirza, B., 2011. Antioxidant, antitumor activities and phytochemical investigation of *Hedera nepalensis* K. Koch, an important medicinal plant from Pakistan. Pak. J. Bot. 43, 85–89.

Karadag, A., Ozcelik, B., Saner, S., 2009. Review of methods to determine antioxidant capacities. Food Anal. Methods 2, 41–60.

Kessler, M., Ubeaud, G., Jung, L., 2003. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacol. 55, 131–142.

Khan, R.A., Khan, M.R., Sahreen, S., Ahmed, M., 2012a. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. Chem. Cent. J. 6, 12.

Khan, R.A., Khan, M.R., Sahreen, S., Ahmed, M., 2012b. Assessment of flavonoids contents and in vitro antioxidant activity of *Launaea procumbens*. Chem. Cent. J. 6, 43.

Kim, D.O., Lee, C.Y., 2004. Comprehensive study on vitamin C equivalent antioxidant capacity of various polyphenolics in scavenging a free radical and its structural relationship. Crit. Rev. Food Sci. Nutr. 44, 253–273.

Kulbacka, J., Saczko, J., Chwilkowska, A., 2009. Oxidative stress in cells damage processes. Pol Merkur Lekarski 27, 44–47.

Larson, R.A., 1988. The antioxidants in higher plants. Phytochemistry 27, 969–978.

Lee, M.C., Shoji, H., Miyazaki, H., Yoshino, F., Hori, N., Toyoda, M., Ikeda, Y., Anzai, K., Ikota, N., Ozawa, T., 2004. Assessment of oxidative stress in the spontaneously hypertensive rat brain using electron spin resonance (ESR) imaging and in vivo L-Band ESR. Hypertens. Res. 27, 485–492.

Li, H., Wang, X., Li, Y., Li, P., Wang, H., 2009. Polyphenolic compounds and antioxidant properties of selected China wines. Food Chem. 112, 454–460.

Manach, C., Morand, C., Crespy, V., Demigne, C., Texier, O., Regerat, F., Remesy, C., 1998. Quercitin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett. 426, 331–336.

Mensor, L.L., Menezes, F.S., Leitao, G.G., Reis, A.S., Dos Santos, T.C., Coube, C.S., Leitao, S.G., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15, 127–130.

Nasir, E., 1975. Flora of Pakistan. In: Stewart herbarium, Gordon College, Rawalpindi and Department of Botany, University of Karachi, Karachi.

Orhan, I., Aydin, A., Colkesen, A., Sener, B., Isimer, A.I., 2003. Free radical scavenging activities of some edible fruit seeds. Pharm. Biol. 41, 163–165.

Oyaizu, M., 1986. Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakkaishi 35, 771–775.

Ozgen, U., Mavi, A., Terzi, Z., Yildirim, A., Coskun, M., Houghton, P.J., 2006. Antioxidant properties of some medicinal Lamiaceae species. Pharm. Biol. 44, 107–112.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J., Codina, C., 2002. Comparison between the radical scavenging activities and antioxidant activity of six distilled and nondistilled mediterranean herbs and aromatic plants. J. Agric. Food Chem. 50, 6882–6890.

Prieto, P., Pineda, M., Aguliar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. Ann. Biochem. 269, 337–341.

Qian, Z.J., Jung, W.K., Byun, H.G., Kim, S.K., 2008. Protective effect of an antioxidative peptide purified from gastrointestinal digests of oyster, *Crassostrea gigas* against free radical induced DNA damage. Bioresour. Technol. 99, 3365–3371.

Rahman, I., 2008. Antioxidant therapeutic advances in COPD. Ther. Adv. Respir. Dis. 2, 351–374.

Record, I.R., Dreosti, I.E., McInerney, J.K., 2001. Changes in plasma antioxidant status following consumption of diets high or low in fruit and vegetables or following dietary supplementation with an antioxidant mixture. Br. J. Nutr. 85, 459–464.

Ruch, R.J., Chung, S.U., Klaunig, J.E., 1984. Spin trapping of superoxide and hydroxyl radicals. Methods Enzymol. 105, 198–209.

Ruch, R.J., Cheng, S.J., Klaunig, J.E., 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen 10, 1003–1008.

Sahreen, S., Khan, M.R., Khan, R.A., 2010. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chem. 122, 1205–1211.

Salazar, R., Pozos, M.E., Cordero, P., Pérez, J., Salinas, M.C., Waksman, N., 2008. Determination of the antioxidant activity of plants from Northeast Mexico. Pharm. Biol. 46, 166–170.

Sasaki, Y.F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., et al, 2002. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat. Res. 519, 103–119.

Shahidi, F., Janitha, P.K., Wanasundara, P.D., 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 32, 67–103.

Sharififar, F., Dehghn-Nudeh, G., Mirtajaldini, M., 2004. Major flavonoids with antioxidant activity from *Teucrium polium* L. Food Chem. 112, 885–888.

Shon, M.Y., Choi, S.D., Kahng, G.G., Nam, S.H., Sung, N.J., 2004. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. Food Chem. Toxicol. 42, 659–666.

Springfield, E.P., Eagles, P.K.F., Scot, G., 2005. Quality assessment of South African herbal medicines by means of HPLC fingerprinting. J. Ethnopharmacol. 101, 75–83.

Tepe, B., Sokmen, M., Akpulat, H.A., Sokmen, A., 2005. *In vitro* antioxidant activities of the methanol extracts of four *Helichrysum* species from Turkey. Food Chem. 90, 685–689.

Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46, 4113–4117.

Weyant, M.J., Carothers, A.M., Dannenberg, A.J., Bertagnolli, M.M., 2001. Catechin inhibits intestinal tumor formation and suppresses focal adhesion kinase activation in the Min/1 Mouse1. Cancer Res. 61, 118–125.

Yildirim, A., Mavi, A., Oktay, M., Kara, A.A., Algur, O.F., Bilaloglu, V., 2000. Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argenta DesfEx DC*), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. J. Agric. Food Chem. 48, 503.

Zu, Y., Li, C., Fu, Y., Zhao, C., 2006. Simultaneous determination of catechin, rutin, quercetin, kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. J. Pharm. Biomed. Anal. 41, 714–719.