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In Vitro Antioxidant, Antibacterial Activity and Phytochemical Studies of *Primula Denticulata* – An Important Medicinal Plant of Kashmir Himalaya

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Abstract
Objective: To evaluate *in vitro* antioxidant, antibacterial activity and phytochemical analysis of ethanolic extract of leaves of *Primula denticulata*- an important medicinal herb of Kashmir Himalaya.
Methods: Phytochemical study was performed by using various standard phytochemical methods. The antioxidant capacity of the extract was assessed by employing different *in vitro* assays such as DPPH free radical scavenging assay, Hydrogen peroxide scavenging activity and Lipid per oxidation assay. Calf thymus DNA was monitored by TBARS formation. The results were compared with standard antioxidant (α-tocopherol). Antibacterial activity of the extract was determined by agar well diffusion method.
Results: DPPH free radical scavenging assay, hydrogen peroxide scavenging activity revealed plant extract to be active radical scavenger. *P.denticulata* extract dose dependently inhibited the MDA formation or lipid per oxidation and as such might intercept the free radical chain of oxidation. The leaf extract also prevents calf thymus DNA from oxidative damage induced by hydroxyl radical generated by $FeSO_4$ and H_2O_2 in Fenton reaction using agrose gel electrophoresis. The ethanolic extract showed anti-microbial activity which was visible as the zones of inhibition formed in the different cultures of Gram positive and Gram negative bacteria.
Conclusion: These results suggest that the ethanolic extract of *P.denticulata* plays an important role in the intonation of oxidative stress. As this analysis is a groundwork study, a detailed study is needed which will provide more information on the active principles responsible for their pharmacological properties and may also lead to the development of novel drugs which may act as possible antioxidants for biological systems susceptible to free radical-mediated reactions.
Keywords: *Primula denticulata*, antioxidant activity, antibacterial activity, phytochemicals

1. Introduction

Oxidative stress is an imbalance between reactive oxygen species (ROS) production and the intracellular capacity for removing ROS. It leads to excessive damage of all bio molecules like DNA, RNA, lipids, proteins etc [1] which can further initiate the development of many diseases such as ageing, atherosclerosis, lipofuscinosis, oxygen toxicity, cancer and liver injury [2, 3]. Antioxidants are compounds which inhibit oxidation, or free radicals induced oxidative damage and therefore are potential quenchers of free radicals or reactive oxygen species. Recent investigations have also revealed plant products as potential antioxidants against various diseases, induced by free radicals due to presence of these phytochemicals [4]. These phytochemicals in plants also react with other organisms in the environment, inhibiting bacterial or fungal growth hence responsible for the antimicrobial activity of plants [5]. These substances are considered as basis for developing new antimicrobial drugs as they inhibit pathogens and have little toxicity to host cells.

Primula denticulata Sm., locally known as Doker-neej (Kashmiri) and Drumstick Primula (English), of family Primulaceae, grows wild in Kashmir Himalaya and bear remarkable medicinal importance [6, 7]. The chemical profiling of *Primula denticulata* (Indian anti-snake venom plant) showed presence of Primetin-19 (5, 8-dihydoxyflavone) which posses strong sensitizing properties and is a powerful contact allergen [8]. Pharmacological studies indicate that the extracts of genus *Primula* are rich in saponins and phenolic glycosides [9]. *Primula veris* is an effective ABTS (2-azino-bis3-ethylbenzthiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical and superoxide anion radical scavenger, and has total reducing power and metal chelating activities on ferrous ions activities [10]. Investigation

conducted by Heshmatollah[11] on *Primula heterochroma* to determine amount of protective effect of polyphenolic flavonoid extracts showed good results. *Primula macrophylla* possess antifungal activity which is due to presence of flavone compound i.e. 2-phenylchromone [12]. The biological effects of epicuticular substances in farinose exudates accumulated on inflorescence shafts and calyces on human acute myeloid leukemia cells (HL-60) were analyzed and the results revealed that crude material of *Primula denticulata* possessed little antioxidative capacity but strong cytostatic properties [13]. However, there is not enough information regarding the antimicrobial and antioxidant activities of ethanolic extract of *Primula denticulata* leaves. The intention of the present study was mainly to comprehend the phytochemical screening, antioxidant and antimicrobial activities of ethanolic extract of *Primula denticulata* leaves as part of the exploration of new and novel bio- active compounds.

2. Material and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, catechin, linolenic acid, were purchased from Sigma Aldrich. Trichloroacetic acid (TCA), thiobarbituric acid (TBA), ferric chloride, hydrogen peroxide (H_2O_2) was obtained from Merck. All the other reagents used were of analytical grade.

2.2 Plant material collection and identification

Healthy and disease free plants of *Primula denticulata* were collected in May- June 2011 from Gulmarg region of Kashmir Himalaya (latitude 34°3'27"N; longitude 74°23'9"E), at an altitude of 2500 m. The healthy plant species were randomly collected by hand-picking and latter identified at the Centre of Plant Taxonomy and Biodiversity, University of Kashmir by Dr. Anzar A. Khuroo. A specimen was deposited under voucher number KASH-1743 (Kashmir University Herbaria) for future reference.

2.3 Preparation of extract

Fresh and healthy leaves of *Primula denticulata* were cleaned with double distilled water, dried under shade (25± 2°C) for 5-6 days. The dried plant material (1000g) was chopped and ground to fine powder using an electric grinder. The plant powder was packed in Soxhelt apparatus and extracted with ethanol at 60-65°C for 2-3 days. The extract was filtered through Whatmann filter paper No.1. The pellet was discarded and the supernatant was collected and concentrated under reduced pressure at required temperature using a rotavapor. The percentage yield was found to be 21.2% w/w. The extract was dried and stored in screw cap glass vials at 4^{o}C for experimental analysis.

2.4 Phytochemical screening

The leaf extract of *Primula denticulata* was screened for the presence of major bioactive constituents like alkaloids, phenolics, flavonoids, tannins, cardiac glycosides, terpenes, anthraquinone glycosides saponins, steroids, and carbohydrates using standard qualitative phytochemical methods [14, 15].

2.5 Determination of total phenolics and flavonoids

The total phenolics in leaf extract of *Primula denticulata* was determined by Folin–Ciocalteu method [16] with slight modifications. To 0.2 ml of plant extract (1mg/ml) was added to 2.5 ml of 10% diluted Folin–Ciocalteu reagent and 2 ml 2.5% aqueous Na_2CO_3. The reaction mixture was at room temperature with slight shaking. The absorbance of the blue colour solution was measured at 765 nm on UV–visible spectrophotometer [Shimadzu UVPC-1650 (Japan)]. Gallic acid (50 mg %) was used as standard. The absorbance of solution was compared with gallic acid calibration curve. The aluminium chloride colorimetric method [17] was used to determine the total flavonoid content of leaf extract. This method is based on the formation of a complex flavonoid–aluminium, having the absorbance maximum at 430 nm. 0.5 ml of plant extract (1mg/ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 15 minutes, the absorbance of the reaction mixture was measured at 430 nm on UV–visible spectrophotometer [Shimadzu UVPC-1650 (Japan)]. Total flavonoid content was expressed as catechin equivalents (mg CE/g dry weight) through the calibration curve with catechin as standard.

2.6 Antioxidant activity assays

2.6.1 DPPH free radical scavenging assay

The DPPH assay was performed by using the method of Braca [18]. Various concentrations of plant extracts (100-1000 µg/ml) were added to 1ml of the 20mg% methanol solution of DPPH, and the mixture was vortexed vigorously. The tubes were then incubated at room temperature for 30 minutes in dark, and the absorbance was taken at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. α-tocopherol was taken as positive control. The percentage inhibition was calculated by using the formula.

$$\text{Percent inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance of the control and As is absorbance in the presence of plant extracts and known standards.

2.6.2 Hydroxyl radical scavenging assay

Deoxyribose assay was used to evaluate the hydroxyl radical scavenging potential of *Primula denticulata* leaf extract [19]. The hydroxyl radicals generated in Fenton reaction attack deoxyribose to form products that, upon heating with thiobarbituric acid at low pH, yield a pink chromogen (thiobarbituric acid reactive species). The reaction mixture containing 25mM deoxyribose, 10mM ferric chloride, 100mM ascorbic acid, 2.8 mM H_2O_2 in 10mM KH_2PO_4 (pH 7.4) with or without plant extract at various concentrations (20-120 µg/ml). The reaction mixture was incubated at 37^0C for 1h. Then 1 ml of 1% (w/v) thiobarbituric acid and 1 ml of 3% (w/v) trichloroacetic acid were added and heated at 100^0C for 20 min. The TBARS was measured spectrophotometrically at 532 nm. The results were expressed as percentage inhibition of deoxyribose oxidation, as determined by the following formula.

Percent inhibition = [(A-B)/A] ×100

Where A was the malonaldehyde produced by Fenton reaction treated alone (without extract) and B was the malonaldehyde produced in the presence of plant extract. Butylated hydroxytoluene and α- tocopherol were taken as the positive control.

2.6.3 Lipid peroxidation (LPO)

LPO was performed according to the method of Wright [20]. The reaction mixture in a total volume of 2.0ml, contained 1ml of linoleic acid, 0.2ml ferric nitrate (20mM), 0.2ml of ascorbic acid (200mM) and 0.2ml of H_2O_2 (30mM) and different concentrations of plant extracts (50-250µg/ml). This was followed by incubation at 37^oC in a water bath for 1hr. The reaction was stopped by the addition of 1.0ml TCA (10% w/v), following which 1.0ml of TBA (1%w/v) was added and all the tubes were placed in a boiling water bath for 20min. The tubes were then centrifuged at 5000rpm for 10min. The amount of malonaldehyde formed in each of the samples was assessed by measuring the optical density of the supernatant at 535nm against a reagent blank.

2.6.4 Prevention of oxidative DNA damage

The Prevention of oxidative damage to calf thymus DNA by leaf extract of *Primula denticulata* was performed as previously described by Ghanta [21]. Calf thymus DNA (0.37µg) with and without plant extract (10, 30, 50, 80 and 100µg) was incubated with 20 mM ferric nitrate 30 mM H_2O_2 in 20.0 mM phosphate buffer saline (pH 7.4) in a final reaction mixture volume of 20µl for 1h at 37°C. Hydroxyl radical generated by Fenton reaction were used to induce oxidative damage to DNA. The reaction was terminated by the addition of loading buffer bromophenol blue (0.25%) and glycerol (30%) and the mixture was subjected to gel electrophoresis in 0.7% agarose/TAE buffer run at 100V. DNA was visualized and photographed by gel doc.

2.6.5 Antibacterial activity assay

Antibacterial activity of *Primula denticulata* was determined by agar well diffusion method [22]. Each microorganisms were grown overnight at 37°C in Mueller-Hinton Broth (Himedia). 100µl of standardized inoculum (0.5 Mac-Farland) of each strain was inoculated on molten Mueller-Hinton agar, homogenized and poured into sterile 90mm petri dishes and were allowed to solidify under laminar air flow. Standard cork borer of 5mm diameter was used to made wells, into which extracts were added. Kenamycin (30µg/disc) was used as positive control and ethanol alone as negative control. Every petri dish was sealed with laboratory film to avoid evaporation. The plates were then incubated at 37±1°C for 24hr. Finally zone of inhibition was measured to the nearest size in mm with the help of standard scale. The experiments were carried out in triplicates and results were calculated as mean ± SD.

2.6.7 Statistical analysis

All of the experiments were done in triplicate. The data were recorded as means ± standard deviations and were analysed with SPSS software.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical screening of *Primula denticulata* leaf extract showed that the plant is rich in various active ingredients (secondary plant metabolites). The result of the phytochemical screening revealed strong to moderate presence of alkaloids, phenolics, flavonoids, tannins, cardiac glycosides, terpenes, saponins, steroids, and carbohydrates (Table-1). Using quantitative assays, total phenolic content and total flovonoids content was found to be 15.93 ± 5.43 (mg GAE/g dry extract) and 7.78 ± 2.99 (mg catechin/g dry extract) respectively.

Table 1: Qualitative phytochemical screening of *primula denticulata* leaf extract

Phytoconstituents	Test	Result
Alkaloids	Wagner's test	+ +
Phenolics	phenol test	+ +
Tannins	Ferric chloride test	+ +
Cardiac glycosides	Keller-Killani test	+ +
Terpenes	Salkwaski's test	+
Flavonoids	Shinoda's test	+ +
Saponins	Frothing test	+

(++) = strong presence, (+) = moderate presence

3.2 *In-Vitro* Antioxidant Activity

3.2.1 DPPH radical scavenging activity

DPPH radical scavenging potential of *Primula denticulata* leaf extract at different concentrations investigated in the present study was determined together with standard antioxidant (α-tocopherol) at the same concentrations (Fig. 1). *Primula denticulata* leaf extracts (ethanolic and methanolic extracts) showed significant scavenging effect on DPPH free radical in concentration dependent manner. When compared with standard antioxidants used in the experiment, the extract showed relatively lower DPPH free radical scavenging potential.

3.2.2 Hydrogen peroxide scavenging activity

Hydroxyl radical ($HO^{\bullet}$) is an extremely reactive species and is capable of damaging almost every molecule found in living cells. In the present study, hydroxyl radical scavenging ability was estimated by generating hydroxyl radicals using ascorbic acid–iron-H_2O_2 (Fenton reaction). Antioxidant efficiency of *Primula denticulata* leaf extract was determined as the ability to scavenge the free radicals generated. The extract exhibited a concentration dependent scavenging of hydroxyl radicals which was comparable to the reference standard (α-tocopherol) at the same doses respectively (Fig. 2).

3.2.3 Effect of ethanolic extract of *p. Denticulata* on lipid per oxidation

This procedure contained Fe++/ascorbic acid as oxidizing agent to initiate lipid per oxidation in Linoleic acid. The damages were assessed by the estimation of formation of the MDA, the product of lipid per oxidation which forms a pink chromagen with TBA whose absorbance is measured at 535nm. *P. denticulata* extract dose dependently inhibited the MDA formation or lipid per oxidation. The amount of MDA formed in the presence of known antioxidants and test compounds are shown in fig 3.

3.2.4 Antioxidant activity against oxidative damage to DNA

In the presence of ethanolic extract, the extent of DNA damage is reduced and the protective effects observed were dose dependent (Fig. 4). Antioxidant effect of ethanol leaf extract was investigated using a free radical induced DNA break system *in vitro*. As revealed in fig1 there was no significant difference in the DNA (normal control, lane 1) and DNA treated with H_2O_2 alone (lane 8). Conversely, in the presence of all the three reagents together (Ferric nitrate, Ascorbic acid and H_2O_2, an extensive damage was found (lane 2). In the presence of ethanol extract, the extent of DNA damage is reduced and the protective effects observed were quantity dependent (lane 3-7). The results confirmed that the extract was active scavenger of hydroxyl radicals as DNA damage caused by Fenton's reagent was delayed by the addition of ethanolic extract of *P. denticulata.*

Fig 1: Graphic representation showing the percentage inhibition of DPPH radicals by extracts of *P. denticulata*

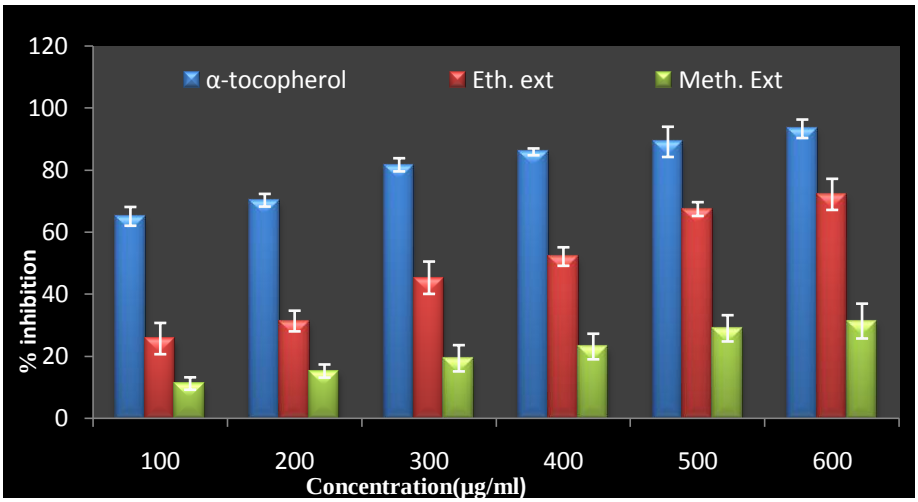


Fig. 2: Graph showing Hydrogen peroxide scavenging activity of ethanolic extract

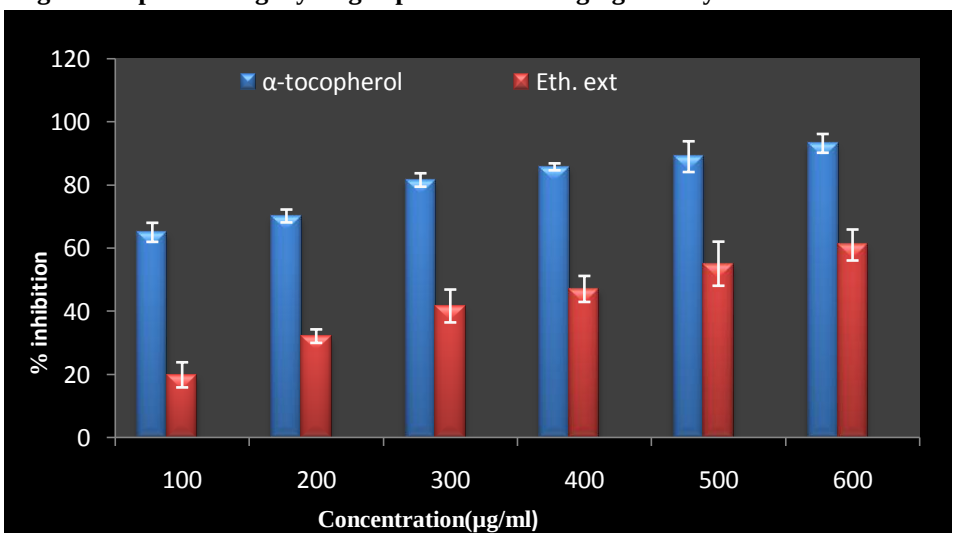


Fig. 3: Graphic representation of the effect of ethanolic and methanolic extracts on lipid per oxidation

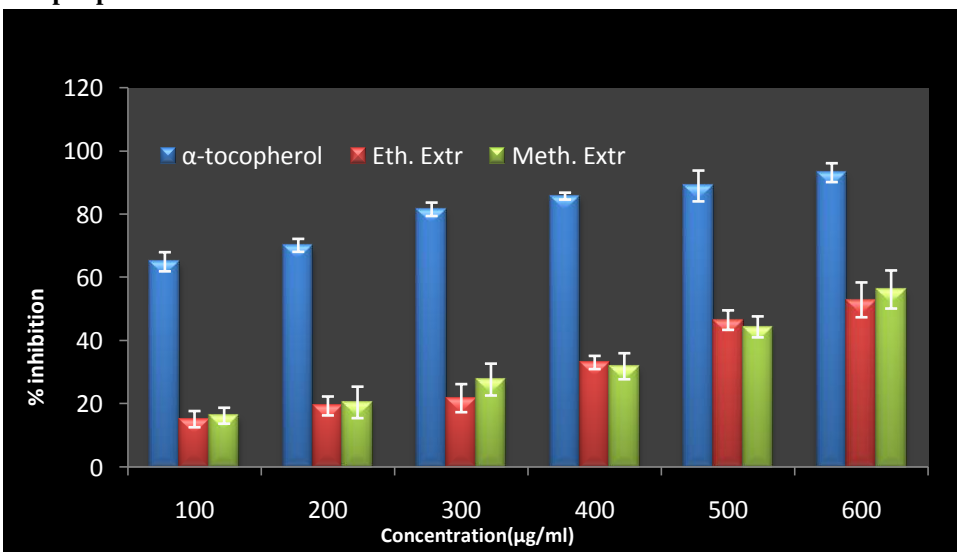
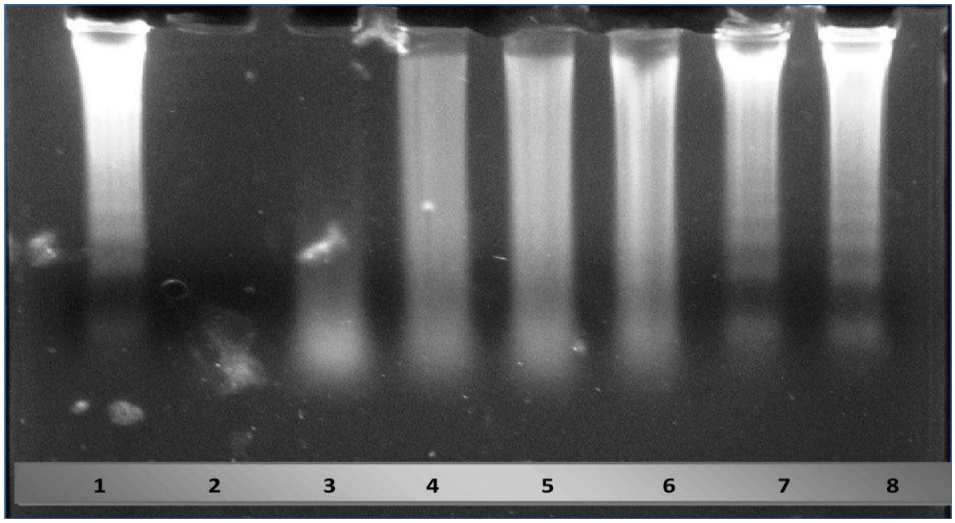


Fig. 4: Photograph showing the agrose gel electrophoretic analysis of Fenton- mediated DNA oxidation and protective effect of the ethanolic extract on oxidative damage to calf thymus DNA



Lane 1: Native calf thymus DNA;
Lane 2: DNA+ 20mM Ferric Nitrate + 100mM Ascorbic Acid + 30mM H_2O_2.;
Lane 3: DNA+ 20mM Ferric Nitrate + 100mM Ascorbic Acid + 30mM H_2O_2 + 10µg of ethanolic extract;
Lane 4: DNA+ 20mM Ferric Nitrate + 100mM Ascorbic Acid + 30mM H_2O_2 +30µg of ethanolic extract;
Lane 5: DNA+ 20mM Ferric Nitrate + 100mM Ascorbic Acid +30mM H_2O_2+ 50µg of ethanolic extract;
Lane 6: DNA+ 20mM Ferric Nitrate + 100mM Ascorbic Acid +30mM H_2O_2+ 80µg of ethanolic extract;
Lane 7: DNA + 80µg of ethanolic extract;
Lane 8: DNA + 30mM H_2O_2

3.3 Anti-microbial activity of *primula denticulata* ethanolic extracts

All the extracts of *Primula denticulata* (methanol, ethanol and aqueous) were tested for antimicrobial activity, however, only the ethanolic extract showed anti-microbial activity which was visible as the zones of inhibition formed in the different cultures of Gram positive and Gram negative bacteria as well as in case of fungal cultures. Five bacterial strains *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Salmonella typhi* and one fungal strains *Aspergillus niger* were tested for their susceptibility to *Primula denticulata* extract. The maximum activity was seen for *Escherichia coli* with inhibition zone diameter of 23.53 ± 3.71mm followed by *Klebsiella pneumoniae* with inhibition zone diameter of 20.74 ± 3.33mm. In case of antifungal activity, the ethanolic extract was found to be effective against the growth of *Aspergillus niger* with maximum inhibition zone diameter of 12.00 ± 2.60 mm as is depicted in Table 2.

Table 2: Anti-bacterial activity of 10% (w/v) ethanol extract of *Primula denticulata* against different bacterial cultures as represented by the diameter of zone of inhibition

S. No.	Test Bacteria	Volume of ethanolic extract used (µl)	Diameter of zone of inhibition (mm)	Diameter of zone of inhibition(mm) *Chloramphenicol disc	Blank
1.	*Escherichia coli*	50	15.66 ± 1.98	25.66 ± 4.04	0
		90	23.53 ± 3.71		
2.	*Staphylococcus aureus*	50	8.43 ± 1.09	30.66 ± 4.04	0
		90	15.67 ± 3.88		
3.	*Klebsiella pneumoniae*	50	11.44 ± 2.53	32.00 ± 5.00	0
		90	20.74 ± 3.33		
4.	*Salmonella typhi*	50	8.90 ± 2.76	17.33 ± 1.15	0
		90	14.05 ± 4.50		

*The concentration of the Chloramphenicol used was 50µg/disc

4. Discussion

DPPH is a purple stable free radical at room temperature with characteristic absorbance at 517 nm. The nitrogen free radical of DPPH is easily quenched by an antioxidant to yellow coloured complex (1,1-diphenyl-2-picrylhydrazine). The decolourization of purple colour is stoichiometric depending on the number of electrons gained [23]. *Primula denticulata* leaf extracts showed significant scavenging effect on DPPH free radical in concentration dependent manner. When compared with standard antioxidants used in the experiment, the extract showed relatively lower DPPH free radical

scavenging potential. This DPPH radical scavenging activity of *Primula denticulata* leaf extracts as such might prevent reactive radical species from damaging biomolecules such as DNA, protein, polyunsatured fatty acids (PUFA) and sugars in susceptible biological and food systems. Hydroxyl radical ($HO^{\bullet}$) is an extremely reactive species formed in biological systems and attacks nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis and mutagenesis. It initiates the lipid peroxidation process by abstracting hydrogen atoms from polyunsaturated fatty acids from membrane lipids. It is capable of damaging almost every molecule found in living cells [24]. *Primula denticulata* leaf extract depicted the ability to scavenge the free radicals generated and the extract exhibited a concentration dependent scavenging of hydroxyl radicals which was comparable to the reference standard (α-tocopherol) at the same doses respectively. It is known that free radical cause auto-oxidation of unsaturated lipids in food [25]. On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of lipid [26]. Lipid peroxidation assay contained Fe++/ascorbic acid as oxidizing agent to initiate lipid per oxidation in Linoleic acid. The damages were assessed by the estimation of formation of the MDA, the product of lipid per oxidation which forms a pink chromagen with TBA whose absorbance is measured at 535nm. *P.denticulata* extract dose dependently inhibited the MDA formation or lipid per oxidation and as such might intercept the free radical chain of oxidation.

DNA is susceptible to oxidative damage and hydroxyl radicals oxidize guanosine or thymine to 8-hydroxyl-2-deoxyguanosine and thymine glycol which change DNA and lead to mutagenesis and carcinogenesis [27]. *Primula denticulata* leaf extract prevents calf thymus DNA from oxidative damage induced by hydroxyl radical generated by $FeSO_4$ and H_2O_2 in Fenton reaction using agrose gel electrophoresis. The protective effect of *Primula denticulata* leaf extract on calf thymus DNA is shown in the Fig 1. Hydroxyl radicals generated were found to induce DNA strand breaks and causes complete DNA damage (Lane-2). *Primula denticulata* extract at 10–100 µg/ml concentration offered concentration dependent protection to DNA damage induced by hydroxyl radicals (Lane 3-7). Catechin (10 µg/ml) was used as standard (Lane-8). Thus, the results indicate that *Primula denticulata* leaf extract prevents DNA damage against oxidative stress. The hydroxyl radical quenching ability of polyphenolic compounds of *Primula denticulata* could be responsible for the protection against oxidative damage to DNA.

Our investigation thus is in congruence with the argument that traditional medicines remain a worthless source in the potential discovery of natural product pharmaceuticals. The present study also revealed that extract of *P. denticulata* contains various secondary metabolites. These phytochemicals might serve as a major source for pharmaceutical products, so the plant species can hold an immense potential to serve as therapy for various chronic diseases. The crude extract of the species have also promising antioxidant and antimicrobial activity which provide a scientific substantiation for the traditional use of this plant and further investigations are needed to obtain novel antioxidant and antimicrobial drugs.

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References

[1] Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R. Chemical composition and *in vitro* antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. *Leben Wiss Technol*. 2007; 40: 239–248.

[2] Iyer D, Devi PU. Radioprotective activity of *Murraya koenigii* L. On cellular antioxidants in swiss albino mice. *J Pharmaceut Res*. 2009; 2: 495-501.

[3] Smerq J, Sharma M. Possible mechanism of *Murraya Koenigi* and *Cinnamomum tamala* in Swiss albino mice with reference to antioxidant activity. *Int J Pharmaceut Sci Drug Res*.2011; 3: 260- 264.

[4] Hou WC, Lin RD, Cheng KT, Hung, YT, Cho CH, Chen CI, Hwang SY, Lee MH. Free radical scavenging activity of Taiwanese native plants. *Phytomedicine*, 2003, 10: 170-175.

[5] Bruneton J. Pharmacognosy, Phytochemistry of Medicinal Plants. Lavoiser publishers France, 1995: pp. 265–380.

[6] Kaul MK. Medicinal plants of Kashmir and Ladakh, Temperate and Cold Arid Himalaya, India. Indus Publishing co. New Delhi, 1997.

[7] Kala CP, Dhyani PP, Sajwan B. Developing the medicinal sector in northern India challenges and opportunities. *Journal of Ethnobiology and ethenomedicine*, 2006, 32: 11.

[8] Harborne JB. Primulaceae Hirsutin and Gossypetin in Dionysia. *Phytochemishtry* 1971; 10: 472.

[9] Gamze, B., Bulbull., Ozmen, A., Halil, H. and Ozge. 2008. Antimitotic and antibacterial effects of the *Primula veris* flower extracts. *Caryologia.* 61: 88-91.

[10] Demr, N., Nadaroglu, H. and Demr, Y. The antioxidant and radical scavenging activities of cowslip (*Primula veris).* Phytopharmacology and Therapeutic Values. 2009; 157- 170.

[11] Heshmatollah, A., Mahboobeh, Z., Seyed, F. N., Alireza, N and Seyed, M. N. Assessing the protective effect of *Primula heterochroma* extracts against Sodium fluoride-Induced hemolysis in rat erythrocytes research report. *Fluoride*. 2011; 44: 238–242.

[12] Saqib, N., Alam, F. and Ahmad, M. Antimicrobial and cytotoxicity activities of the medicinal plant *Primula macrophylla*. *J Enzyme Inhib Med Chem* 2009; 24: 697-701.

[13] Gangwar K, Deepali K, Gangwar RS. Ethnomedicinal plant diversity in Kumaun Himalaya of Uttarakhand, India. *Natural Sciences* 2010, 8(5): 66-78.

[14] Harborne, J. B. Phytochemical methods. Chapman and Hall, London; 1973.

[15] Trease GE, Evans IC. Text book of pharmacognosy. 12th ed, Oxford, Alden Press, 1983, 343-383.

[16] Slinkard K, Singleton VL.Total phenol analyses: automation and comparison with manual methods. *Am. J. Enol. Viticult*, 1977; 28: 49–55.

[17] Mcdonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 2001; 73: 73–84.

[18] Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. *Journal of Natural Products* 2001; 64: 892–895.

[19] Halliwell B, Guttridge JMC, Aruoma OI. *Analytical biochemistry*, 1987; 165: 215-219.

[20] Wright JR, Colby HD, Miles PR. Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. *Achieves of Biochemistry and Biophysics*, 1981, 206: 296–304.

[21] Ghanta S, Banerjee A, Poddar A, Chattopadhyay S. Oxidative DNA damage preventive activity and antioxidant potential of *Stevia rebaudiana* (Bertoni) Bertoni, a natural sweetener. *Journal of Agricultural Food Chemistry*, 2007; 55: 10962–10967.

[22] Perez C, Pauli M, Bazerque P. An antibiotic assay by the well agar method. *Acta Biologiae Medicinae Experimentalis* 1990; 15: 113–115.

[23] Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of *Thymus zygi*. *Free Radical Research* 1997; 26:469–478.

[24] Manian R, Anusuya N, Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis*. *Food Chemistry*, 2008; 107:1000–1007.

[25] Kaur G, Tirkey N, Bharrhan S, Chanana V, Rishi P, Chopra K. Inhibition of oxidative Stress and cytokine activity by curcuminin amelioration of endotoxin-induced Experimental hepatotoxicity in rodents. *Clinical and Experimental Immunology*, 2006; 145: 313–321.

[26] Jain A, Soni M, Deb A, Jain SP, Rout, Gupta VB. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* roxb. leaves. *Journal of Ethnopharmacology*, 2008; 115:61–66.

[27] Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America, 1993; 90: 7915–7922.