



## Antioxidant Capacity and Total Phenolic Content of *Swertia chirayita* (Roxb. ex Fleming) H. Karst. in Uttarakhand

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Accepted on: 16-09-2013; Finalized on: 31-10-2013.

### ABSTRACT

The antioxidant capacity and Total phenolic content in the whole plant of *Swertia chirayita* (Roxb. ex Fleming) H. Karst. were investigated in different solvents. The antioxidant capacity was evaluated by studying Free radical scavenging capacity (DPPH assay) and Total phenolic content was by Folin Ciocalteu reagent method. Butylated hydroxy toluene was used for antioxidant capacity and gallic acid for phenolic content as a standard. The EC<sub>50</sub> value of Methanol extract was 27.70 µg/ml, compared to 17.75 µg/ml, for BHT and the results clearly indicate that methanolic extract of the whole plant of *Swertia chirayita* is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

**Keywords:** Antioxidant capacity, DPPH, Phenolic content, *Swertia chirayita*.

### INTRODUCTION

India is much known for its variety of agro-climatic diversity as for its rich heritage of traditional systems of medicines. The Indian System of Medicine (ISM) comprises of Ayurveda, Siddha, Unani and Amchi system of medicine along with the other folk medicinal practices and age old home remedies. These all systems have placed India as leading nation, in natural medicinal therapeutic field, based on the knowledge of the use of medicinal plants. India possesses a rich treasure of biodiversity which has been used for health care for more than four thousand years.

Several Indian medicinal plants are known for their beneficial therapeutic effects which also might have antioxidant properties.<sup>1</sup> Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA.<sup>2</sup> It is well known that free radicals are the major cause of various chronic and degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer.

Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging.<sup>3,4</sup> That is why, antioxidants along with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases where oxidants or free radicals are implicated<sup>5</sup> Phenolic compounds with antioxidant activity, which are widely distributed in many fruits, vegetables, and tea are

believed to account mainly for the antioxidant capacity of many plants.<sup>6-8</sup>

Plants contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids (including carotenoids) and some other endogenous metabolites, which are rich in antioxidant activity.<sup>9,10</sup> The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.<sup>11</sup> Flavonoids, phenolic acids, and phenolic diterpenes<sup>12,13</sup> lignans are the examples of phenolic components with antioxidant properties. *Swertia chirayita* (Roxb. ex Fleming) H. Karst (Family Gentianaceae,) is one of the important traditional medicinal plant, an erect annual or perennial herb found in Himalaya and Meghalaya at an altitude of 1200-1300 meters.<sup>14</sup> The entire plant is used in medicine, however the root is mentioned to be the most powerful part. It have been reported to have anti-inflammatory<sup>15</sup>, anti-viral,<sup>16</sup> antihelmintic,<sup>17</sup> anticarcinogenic<sup>18</sup>, hepatoprotective<sup>19</sup> hypoglycemic<sup>20</sup> and wound<sup>21</sup> healing activity as well as antibacterial activity.

The main objectives of this study were to determine the antioxidant capacity and total phenolic contents of *Swertia chirayita* in different solvents. The inter-relationship between phenolic content and antioxidant activity have been also carried out.

### MATERIALS AND METHODS

#### Chemicals

All solvents used are of analytical grade, DPPH [2,2-diphenyl-1-picrylhydrazyl], butylated hydroxytoluene (BHT), Gallic acid, and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich Co.(USA). All other



chemicals and solvents were of analytical grades and obtained from Merck (Germany) companies. All solutions, including freshly prepared doubled distilled water.

### Collection of plant

The plant *Swertia chirayita* were collected in September from the Kauntalani Nursery, which is located at N-30° 45'123" Latitude and E- 077° 53'00 and is 2580 m.amsl. [Fortrex 201 GPS]. Kauntalani Nursery belongs to the Chakrata Forest Division, District Dehra Dun, Uttarakhand.

### Identification of plant

The plant was first identified at the field using standard keys and descriptions. All the plants were packed in the Kraft paper and herbarium sheets were prepared. Its botanical identity was further confirmed and authenticated by the plant taxonomist of Department of Botany, Forest research institute, Dehradun. The accession number 16430 has been assigned to the specimen submitted to FRI herbarium for identification and is identified as *Swertia chirayita* (Roxb.Ex Flem)Karst.

### Extraction

The whole plant were dried under shade and powdered in a mechanical grinder. The powdered material (200gms) was extracted successively in Petroleum Ether, Chloroform, Acetone and Methanol by using Soxhlet apparatus for 18 h. The extracts were concentrated in vacuum evaporator and kept in vacuum desiccators for complete removal of solvent and weighed.

### Method

#### Free radical scavenging capacity (DPPH assay)

Antioxidant capacity was evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay according to the procedure described by Blios et al.<sup>22</sup> A DPPH stock solution was first prepared and stored at 20°C. 2ml of a volume of 25 to 200 µl of the sample was then poured into an optical glass cuvette and the stock solution was added to a final volume of 4ml. After 30 min incubation at 28 to 30°C in dark, the absorbance at 517 nm using spectrophotometer (Thermofisher Scientific UV-2700) was measured against a blank of pure of pure methanol and DPPH(2ml each). Than compared with synthetic antioxidant butylated hydroxy toluene (BHT), this was used as standard. All the determinations were performed in triplicate. The antioxidant capacity was expressed as Ec50 value, which represents the concentration of the sample in cuvette necessary to decrease the initial DPPH concentration by 50%.

The Ec50 value was calculated using the following formula:

$$\% \text{ DPPH radical - scavenging} = \left[ \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \right]$$

EC50 of the extract was determined by using SPSS software.

### Determination of the total phenolic content

Total phenol contents were determined by Folin Ciocalteu reagent method Singleton and Rossi<sup>23</sup> using gallic acid as a standard phenolic curve. Appropriate dilutions of the samples (.25ml) were oxidized with Folin-Ciocalteu reagent for five minutes at room temperature followed by addition of 7.5% Sodium carbonate solution. The absorbance of the resulting blue color was measured at 740nm on UV-VIS spectrophotometer (Thermofisher Scientific UV-2700) after heating at 50°C for 5.0 minutes in a water bath. Quantification was done on the basis of the standard curves of gallic acid prepared from 1ml of each of the 200,150,100 and 50 ppm gallic acid solutions assaying in the manner similar to that used for the extracts. The results were expressed as % gallic acid equivalents (GAE).

### RESULTS AND DISCUSSION

All the four extracts obtained by using extraction of the whole plant of *Swertia chirayita* with solvents namely Petroleum ether (PE), Chloroform (CHL), Acetone (ACE) and Methanol (MET) were screened for their antioxidant capacity using DPPH free radical scavenging assay.

DPPH is a stable free radical which accepts electrons and becomes a stable diamagnetic molecule. The purple color or violet color of the DPPH solution changes to yellow as diamagnetic molecule form and the absorbance at 517nm wavelength maximum decreases. The decrease in absorbance of DPPH caused by antioxidant is due to the reaction between the antioxidant molecule and its radical which results in the scavenging of the radical by H-donation.

Data shown in the table 1, reveals that the extract MET had with DPPH radical scavenging activity from 51% to 90% at a concentration of 17µg/ml. MET scavenging more than 90% of the DPPH radical compared to the BHT control. The BHT control was arrayed at 100 µg/ml, corresponding to the maximum allowable concentration for BHT additions to food stuffs.<sup>24</sup> Values between 25-175 µg/ml were used to determine the inhibition concentration (EC<sub>50</sub>), which is the amount of antioxidant required to deplete the initial DPPH by 50%. The EC<sub>50</sub> value of MET was 27.70 µg/ml, compared to 17.75 µg/ml, for BHT.

DPPH free radical scavenging capacities of the plant extractive are attributed to their phenolic constituents. A large number of studies have been reported<sup>25,26</sup> wherein the free radical scavenging capacity of the plant positively correlated with their phenolic constituents. Thus the extracts MET conferring free radical scavenging capacity was also examined for its TPC<sub>s</sub> in MET extract as determined by Folin Ciocalteu reagent method were found to be 0.24% GAE. It was observed that the phenolic contents of MET extracts are associated with an increased radical scavenging capacity. This effect may be due to the

electron donating ability of the phenolic compounds present in the MET.

**Table 1:** Free radical scavenging capacity of methanol extract

Concentration of extracts (µg/ml)	Antioxidant activity (%)
	Methanol Extract
25	51.5086
50	66.81034
75	66.81034
100	64.65514
125	85.16228
150	85.16228
175	90.4173
200	90.4173
EC <sub>50</sub>	27.704

**Table 2:** Results of total phenolic content in methanolic extract

Solvent Used	Total Phenol content (%)	EC <sub>50</sub> (%)
Methanol	0.234	27.704

## CONCLUSION

This study confirms the in vitro antioxidant capacity of methanolic extracts of *Swertia chirayita*, with results comparable to those of the standard compounds such as BHT gallic acid and can therefore be proposed as new potential sources of natural additives for the pharmaceutical industries. The data clearly indicated that the methanolic extracts of *Swertia chirayita* showed good antioxidant activity among all three solvent extract. However, the components responsible for the antioxidant activities of the extracts were not identified and further work should be conducted to isolate and identify these bioactive compounds.

## REFERENCES

- Amudha P, Shanthy P, Antioxidant activity of some rare medicinal plants Journal of Pharmacy Research, 4(3), 2011, 698-699.
- Iuliano L, Luigi I, Colavita AR, Leo R, Pratico D, Violi F, Oxygen free radicals and platelet activation, Free Radical Biology & Medicine, 22, 1997, 999-1006.
- Scalbert A, Manach C, Remesy C, Morand C, Dietary polyphenols and the prevention of diseases, Critical Reviews in Food Science and Nutrition, 45, 2005, 287-306.
- Halliwell B, Gutteridge, J.-M.-C. 1999, "Free Radicals in Biology and Medicine", 3rd edition, Oxford University Press, Oxford, U.K.
- Soares JR, Dinis TCP, Cunha AP, Almeida LM, Antioxidant activities of some extracts of *Thymus zygis*, Free Rad. Res, 26, 1997, 469-478.
- Kaur C, Kapoor HC, Antioxidant activity and total phenolic content of some Asian vegetables, Int J Food Sci Tech, 37(2), 2002, 153-162.
- Vinson JA, Hao Y, Su X, Zubik L, Phenol antioxidant quantity and quality in foods: vegetables, J Agric Food Chem, 46, 1998, 3630-3634.
- Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL, Lipophilic and hydrophilic antioxidant capacities of common foods in the United States, J Agric Food Chem, 52, 2004, 4026-4037.
- Zheng W, Wang SY, Antioxidant activity and phenolic compounds in selected herbs, J Agr Food Chem, 49, 2000, 5165-5170.
- Cai YZ, Sun M, Corke H, Antioxidant activity of betalains from plants of the Amaranthaceae, J Agr Food Chem, 51, 2003, 2288-2294.
- Osawa T, Novel natural antioxidants for utilization in food and biological systems, In I.Uritani, V. V. Garcia, & E. M. Mendeoza (Eds.), Postharvest biochemistry of plant food-materials in the tropics, pp. 241-251, 1994, Tokyo, Japan: Japan Scientific Societies Press.
- Pietta PG, Flavonoids in medicinal plants, In C. A. Rice- Evans, & L. Packer (Eds.), Flavonoids in health and disease, New York: Dekker, 1998, pp. 61-110,
- Shahidi F, Janitha PK, Wanasundara PD, Phenolic antioxidants, Critical Reviews of Food Science & Nutrition, 32(1), 1992, 67-103.
- Kirtikar KR, Basu BD, Indian Medicinal Plants, 2<sup>nd</sup> Ed., vol. III, Allahabad, 2006, 1664-1666.
- Islam CN, Banerjee MK, Das PC, Preliminary studies on the anti-inflammatory effects of *Swertia chirata* in albino rats, Indian J. Pharmacol, 27, 1995, 37-39.
- Verma H, Patil PR, Kolhapure RM, et al, Antiviral activity of the Indian medicinal plant extract *Swertia chirata* against herpes simplex viruses: a study in vitro and molecular approach, Ind J Med Microbiol, 26, 2008, 322-326.
- Iqbal Z, Lateef M, Khan MN, et al, Antihelminthic activity of *Swertia chirata* against gastrointestinal nematodes of sheep, Fitoterapia, 77, 2006, 463-465.
- Saha P, Mandal S, Das A, Das PC, Das S, Evaluation of anti-carcinogenic activity of *Swertia chirata* Buch. Ham, an Indian medicinal plant on DMBA-induced mouse skin carcinogenesis model, Phytothe. Res., 18, 2004, 373-378.
- Mukherjee S, Sur A, Maiti BR, Hepatoprotective effect of *Swertia chirata* on rat, Indian Journal of Experimental Biology, 35(4), 1997, 384-388.
- Bajpai MB, Asthana RK, Sharma NK, et al, Hypoglycemic effect of swerchirin from the hexane fraction of *Swertia chirayita*, Planta Med, 57, 1991, 102-104.
- Saxena AM, et al., "Mode of action of three structurally different hypoglycemic agents: a comparative study", Indian J Exp Biol, 34, 1996, 351-355.
- Blois MS, Zhao XY, Antioxidant determinations by the use of a stable free radical, Nature, 181, 1958, 1199-1200.
- Singleton VL, Joseph AR, Jr, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, Am. J. Enol. Vitic, 16:3, 1965, 144-158.
- Ribeiro SMR, Barbwa LC,A, Oweiro JH, Knodler M, Schieber A, Phenolic compounds and antioxidant capacity of Brazilian Mango (*Mangifera indica* L.), Food chem., 110, 2008, 620-626.
- Yang JH, Lin HC, Mau JL, Antioxidant properties of several commercial mushrooms, Food Chem, 77, 2002, 229-235.
- Kaur C, Kapoor HC, Antioxidant activity and phenolic content of some Asian vegetables, International Journal of Food Science and Technology, 37, 2002, 153-161.

Source of Support: Nil, Conflict of Interest: None.

