STUDY ON PHYTOCHEMICAL AND ANTIOXIDATIVE POTENTIAL OF LEAF EXTRACT OF STINGING NETTLE, *URTICA DIOICA* L IN UTTARAKHAND, INDIA

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***Abstract*:**

In present day scenario, free radicals are produced naturally in human body linked to diseases such as cancer and cardiovascular diseases. Antioxidant agents can act against free radicals either by retarding their formation (preventive antioxidants) or by in-activating in reaction medium (chain breaking antioxidants). Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. Stinging nettle**,** (*Urtica dioica* L), also called common nettle**,** weedy perennial plant of the nettle family (Urticaceae), and known for its stinging leaves. The present study comprised of collection of plant *Urtica dioica* L from different locations of Uttarakhand followed by ethanol extraction for phytochemical analysis. Estimation of total phenol and flavonoid contents with the emphasis for determination of antioxidant activities of these plants. The percentage yield of crude extract in *Urtica* sample ranges from 58 - 64%. Phytochemical studies showed that alkaloids, flavonoids, carbohydrates, proteins, tannins, saponins, terpenoids, steroids, glycosides were present in all the samples of *Urtica dioica* while anthocyanin and cardiac glycosides were absent in all the samples. The phenolic concentration of samples ranges from 0.44±0.03 to 0.64±0.02. The flavonoid content of the samples of *Urtica dioica* L varies from 0.83±0.23 to 0.68±0.009. The study was also conducted with reference to the reducing power activity which was highest in sample 3 which is of Pauri. The antioxidant activities of three samples were calculated by DPPH scavenging activity. The IC_{50} value of samples ranged from 227.191-389.066 µg/ml. Thus, the plant *Urtica dioica* L. can be used as a food supplement and herbal antioxidants which can reduce the effect of free radical and maintaining the health.

***Index terms*: *Urtica dioica* L, antioxidative potential, phenols, flavonoids**

1. INTRODUCTION

The world is presently over-dependent on a few plant species for a study of value chain and processing. Diversification of production and consumption habits to include a broader range of plant species, particularly those currently identified as under-utilized, could significantly contribute to improve health and nutrition, livelihoods and ecological sustainability. Wild plants have played a significant role in supplementing staple foods by supplying trace elements, vitamins, and minerals in order to obtain a balanced diet, and they may do so again in the future. Several epidemiological studies suggest that a high intake of foods rich in natural antioxidants reduces the risk of some cancers, heart, and degenerative diseases.

In present day scenario, although free radicals are produced naturally in the body, the modern lifestyle can surely accelerate their production in human body. Lifestyle factors like exposure to toxic chemicals, air pollution, smoking, consumption of alcohol and unhealthy fried or junk foods not only triggers the free radical production but are also linked to diseases such as cancer and cardiovascular diseases. Free radicals are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals (Bagchi and Puri,1998; Kumpulainen and Salonen,1999; Pourmorad *et al*., 2006; Singh and Aswal, 2018) and free radicals can cause oxidative stress, a process that can trigger cell damage. Antioxidant agents can act against free radicals either by retarding their formation (preventive antioxidants) or by in-activating in reaction medium (chain breaking antioxidants) (Singh and Aswal, 2018). Antioxidants are broadly divided into by two groups on the basis of their occurrence i.e., naturally or synthetic. The natural compounds would seem to have better antioxidant activity than the currently used synthetic antioxidants, making them a particularly attractive ingredient for commercial foods (Brewer, 2011; Lobo *et al.*, 2010).

Stinging nettle**,** (*Urtica dioica*), also called common nettle**,** weedy perennial plant of the nettle family (Urticaceae), and known for its stinging leaves. Stinging nettle is distributed nearly worldwide but is especially common in Europe, North America, North Africa, and parts of Asia. In India, it grows naturally in Kashmir, Uttarakhand, West Bengal, Arunachal Pradesh, Tamil Nadu and Sikkim (Kumar *et al.*, 2017*).* In Uttarakhand, it is found in almost all the areas. The compounds present in plant belong to the classes of lignan, secolignan, norlignan, flavonoid, alkaloid, sesquiterpenoid, triterpenoid, sterol, and sphingolipid (Abdeltawab *et al.*, 2012; Wang *et al*., 2011). The stinging trichomes are believed to contain a mixture including formic acid, acetyl choline, histamine and serotonin. The plant is common in herbal medicine, and young leaves can be cooked and eaten as a nutritious potherb. *Urtica dioica* can be used in osteoarthritis, benign prostatic hypertrophy, allergic rhinitis and asthma, bleeding, diabetes

(Baumgardner, 2016), gout, excessive menstrual bleeding, acne treatment, etc. Additionally, stinging nettle has been used as a source of bast fibers for textiles and is sometimes used in cosmetics (Petruzzello, 2017). Most of the studies on *Urtica dioica* were concentrated on its general study that whether it is beneficial or harmful (Baumgardner, 2016), its Pharmacognostical and phytochemical study (Kumar *et al*, 2017, Joshi *et al.*,2014) of *Urtica dioica* (Asgarpanah *et al.*, 2012). Therefore in the present study *U. dioica* was used for determination of phytochemical constituents and antioxidative potential under different locations of Uttarakhand, India as it is a weedy plant, can be cultivated easily and do not need very much monitoring.

2. MATERIAL AND METHODOLOGY

2.1. Location of the experiment and climatic conditions

The present investigation was carried out at biotechnology laboratory in department of Botany, Sri Guru Ram Rai College of Basic and Applied Sciences, Sri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand. The Doon Valley is situated between two of the India's mightiest rivers-The Ganges on the east and The Yamuna on the west. Dehradun is a picturesque city with the mild climate. It is as the capital of Uttarakhand, and is located between the latitude $29^0$55' and $38^0$31'N and longitude $77^0$35' and $78^0$20' E, covering an area of 2002.4 sq. Km with an elevation of 2000 m above the sea level.

The climate of Dehradun is generally temperate, although it varies from tropical to severely cold, depending upon the seasons and the altitude of the area. The nearby hilly regions often get snowfall during winter but the temperature of Dehradun does not go below 0^0C. The weather is considered to be good during winter in the hilly regions but it is often hot in valley. The agriculture is good here due to the fertile alluvial soil and the adequate water drainage and rainfall.

2.2. Collection of plant samples

The aerial parts of plant were collected from three different locations of Uttarakhand namely Dehradun, Rudraprayag and Pauri district. The plant samples were dried in shade at 25 to 35^0 C for 10-15 days then crushed to coarse powder using grinder. The dried plant material was stored in paper bags.

2.3. Extraction of crude extract

About 50 gm accurately weighed dry plant samples were soaked with 70% ethanol for 48 hours at room temperature. The content were refluxed for 2 hours at temperature not exceeding 60 ^{0}C, cooled and filtered. Filtrates were evaporated on the water bath till they were finally reduced to dryness to get dry extracts. The extract was then transferred to previously weigh airtight containers. Percentage yield of the crude extracts were calculated with the formula:

Percentage yield (%) =weight of extract/weight of powdered drug×100

2.4. Phytochemical analysis

The ethanol extract of *Urtica dioica* were subjected to primarily phytochemical investigation. The various tests performed were conducted for qualitative estimation of alkaloids, proteins, carbohydrate, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, anthraquinines, coumarins, anthocyanin, cardiac glycoside. Besides the qualitative analysis of these phytoconstituents, plant extract was also analyzed for quantification of phenol and flavanoids and reducing power and DPPH radical scavenging assay.

2.4.1. Determination of total phenolic content

The total phenolic content was determined by using Folin-Ciocalteu reagent according to the method described by Singleton and Rossi (1945). First of all the dilutions of Gallic acid were prepared (ranges from 20µl to 200µl) from the stock solution of Gallic acid (2 mg/10 ml).

0.1 ml (100µl) of sample and 50 µl of 2 N Folin-Ciocalteu reagent were added to a 5 ml volumetric flask. The solutions were mixed and stand for 5 min at room temperature. Next, 0.3ml (300µl) of 20% sodium carbonate solution was added and kept aside for 15 min. Finally, 5 ml of distilled water was added. The blue colour was measured against the reagent blank at 725nm using UV-spectrophotometer. The total phenolic content of the sample was determined by comparison with the optical density values of different concentrations of the standard phenolic compound Gallic acid. Each sample was analysed in duplicates and a calibration curve of Gallic acid was constructed by plotting absorbance versus concentration. The total phenol content was expressed as gram of Gallic acid equivalents (GAE) per 100 gm extract.

2.4.2. Determination of total flavonoid content

The total flavonoid content was determined by aluminium chloride ($AlCl_3$) method using quercitin as a standard. First of all the dilutions of quercitin were prepared (ranges from 20µl to 200µl) from the stock solution (2mg/10 ml water). The 0.25 ml (250µl) extract was mixed with 1.25ml (1250µl) of double distilled water which was followed by addition of 75 µl of 5% $NaNO_2$ (5gm in 100 ml of water). This mixture was incubated for 5 min at room temperature and then 0.15 ml (150 µl) of 10% $AlCl_3$ (10 gm in 100 ml water) was added. The reaction mixture was treated with 0.5 ml (500µl) of 1mM NaOH. After a incubation of 6 min at room temperature finally the reaction mixture was diluted with 5 ml of double distilled water followed by the incubation of 20 min at room temperature. The absorbance was measured at 510nm. The flavonoid content was calculated from a quercitin standard curve. The total flavonoid content was expressed in milligrams of quercitin equivalents (QE) per gram of samples.

2.4.3. Determination of reducing power activity:

The reducing power of sample was determined by the Oyaizu (1986) method with some modifications. Reducing power activity is based on the reduction of ferric cyanide (Fe^{3+}) in stoichiometric excess relative to the amount of antioxidants (Benzie and Strain, 1996). Firstly, dilutions of different concentrations were prepared (250µl, 500µl, 750µl and 1000µl).

50µl sample with different concentrations were mixed with 0.5 ml (500µl) of 0.2 M sodium phosphate buffer (pH.6) and 0.5 ml (500µl) of 1% potassium ferric cyanide and incubated at 50^0C for 20 min. After incubation, 2 ml of 10% of trichloroacetic acid was added to the mixture, followed by centrifugation at 30000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2 ml of

deionised water and 0.5 ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700nm. Ascorbic acid was used as references.

2.4.4. DPPH Radical scavenging assay

The scavenging activity was measured using 2,2-diphenyl- 1-picryl hydrazyl (DPPH). The extracts were redissolved in 70% ethanol. The 5ml assay mixture contained 3.98ml methanol, 20µl extract and 1ml DPPH (0.15mM in methanol). After incubation at room temperature for 30 min, the decrease in absorbance was measured at 517nm using a spectro- photometer. Ascorbic acid was used as references. The IC50 value indicated the concentration of tested sample required to reduce the free radical concentration by 50%. The experiment was performed in duplicate.

DPPH scavenging activity (%) $=A_0-A_1/A_0\times100$

2.5. Statistical Analysis:

Results were expressed as mean ± standard deviation using MS- excel.

3. RESULT AND DISCUSSION:

Urtica dioica is an annual plant which possesses wide range of biological effects and being used in folk medicines since the ancient times. Despite the numerous studies concerning the biological activities of this plant, there are only several studies regarding the chemical composition of stinging nettle leaves (Kumar *et al*, 2017, Joshi *et al*.,2014, Petruzzello, 2017). Deeper chemical investigation revealed the presence of different class of compounds in leaves. The present study was conducted to study the phytochemical, phenol, flavonoid and antioxidant properties of *Urtica dioica*. The findings of present study were as follows:

3.1. Yield and Phytochemical estimation of crude extract:

Research studies suggest it's possible that various phytochemicals may help protect from cancer or possible slow down the growth of cancer, reduce inflammation and help regulate hormones. Phytochemicals are often extracted from plants, processed and sold as dietary supplements. They're generally considered to be safe, but there's not much regulation regarding their dosages or even effectiveness, so it's important to speak with the healthcare provider before taking these supplements, especially if we have any health conditions (Singh and Aswal, 2018). Mojab *et al.* (2003) had done phytochemical screening of some species of Iranian plants which includes *Urtica dioica* and found the presence of alkaloids, tannins, saponins, flavonoids. On the other hand, Moses *et al.* (2013) demonstrated the preliminary phytochemical screening of eight selected medicinal herbs which also shows the presence of saponins, tannins, steroids, terpenoids and flavonoids.

Dark green appearance of extraction is obtained by simple filtration method and there is no colour difference among the three samples. The percentage yield of crude extract ranged from 58-64%. The highest yield was 64% of sample 3 (Pauri) and lowest was of sample 1 (Dehradun) (Table -3.1).

Table no. 3. 1. Yield of crude extracts from ethanol solvent of different samples of *Urtica dioica* L

S.N.	Samples	Quantity of seed material(gm)	Appearance	Weight of extract(gm)	Percentage yield
1.	Sample1(Dehradun)	50	Dark green	0.58	58
2.	Sample2(Rudraprayag)	50	Dark green	0.60	64
3.	Sample3(Pauri)	50	Dark green	0.64	60

Phytochemical analysis showed the presence of proteins, amino acids, carbohydrates, flavonoids, tannins, phenol compounds and alkaloids compounds. Anthocyanin and cardiac glycosides were not present in all the samples (table 3.2).

Table no. 3. 2. Phytochemical analysis of different samples of *Urtica dioica* L

S.N.	Constituents	Sample1 (Dehradun)	Sample2 (Rudraprayag)	Sample3 (Pauri)
1.	Alkaloids	+	+	+
2.	Proteins	+	+	+
3.	Carbohydrates	+	+	+
4.	Flavonoids	+	+	+
5.	Tannins	+	+	+
6.	Saponins	+	+	+
7.	Terpenoids	+	+	+
8.	Steroids	+	+	+
9.	Glycosides	+	+	+
10.	Coumarins	+	+	+
11.	Anthocyanin	-	-	-

12.	Cardiac glycoside	-	-	-

*(+) and (-) signs indicates presence and absence of the compound, respectively.

3.2. Total phenol and flavonoid concentration of different samples of *Urtica dioica* L

Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992). Due to their ability to act as antioxidant agents, there is a growing interest to use those components in traditional medicine or treatment of different diseases (Fattahi *et al.*, 2014*).* Phenolic compounds are plant secondary metabolites possessing aromatic ring with one or more hydroxyl groups from the aromatic amino acids phenylalanine produced via the phenylpropanoid pathway (Tura, 2002). The two major classes of phenolic compounds include flavonoids and phenolic acids. The presence of phenolic compounds enables plants to act as reducing agents, hydrogen donators and singlet oxygen quenchers. The total phenol content of the sample *Urtica dioica* varies from 0.44±0.03 to 0.64±0.015. The highest phenol content was found in sample 3 (Pauri) while the lowest was found in sample 1 (Dehradun). Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo* (Geetha *et al*.,2003) The flavonoid content of the samples of *Urtica dioica* varies from 0.83±0.23 to 0.68±0.009 and the highest was from sample 2 (Rudraprayag) and lowest if of sample 3 (Pauri). Total flavonoid concentration of different samples were expressed as regression equation of the calibration curve as shown in figure no. 3.1 and 3.2. Total phenol and flavonoid concentration of different *Urtica* samples were expressed as regression equation of the calibration curve was shown in figures below

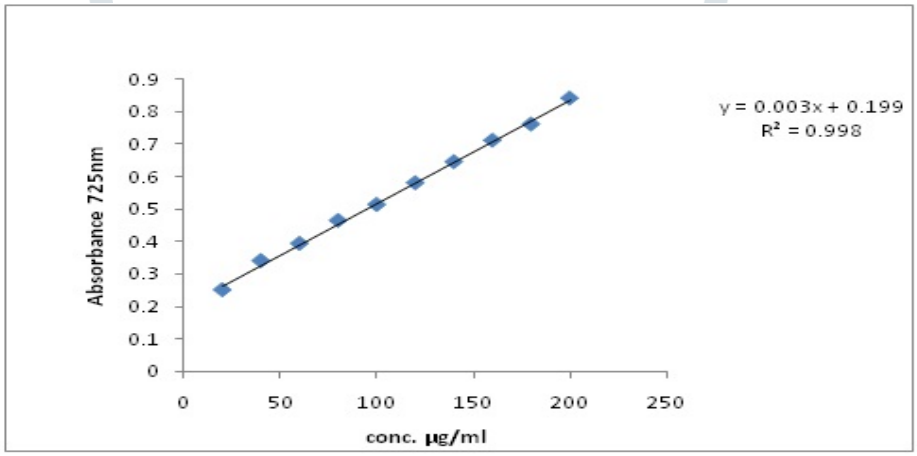

Figure no. 3.1. Standard curve represent concentration of Gallic acid (µg/ml) against absorbance

Table no 3. 3. Total phenol and flavonoid concentration of different *Urtica dioica* L samples

S.N.	Plant samples	Total phenol concentration (Mean ±S.E.)	Total flavonoid concentration (Mean ±SE)
1.	Sample1(Dehradun)	0.44±0.03	0.69±0.003
2.	Sample2(Rudraprayag)	0.46±0.015	0.83±0.23
3.	Sample3(Pauri)	0.64±0.02	0.68±0.009

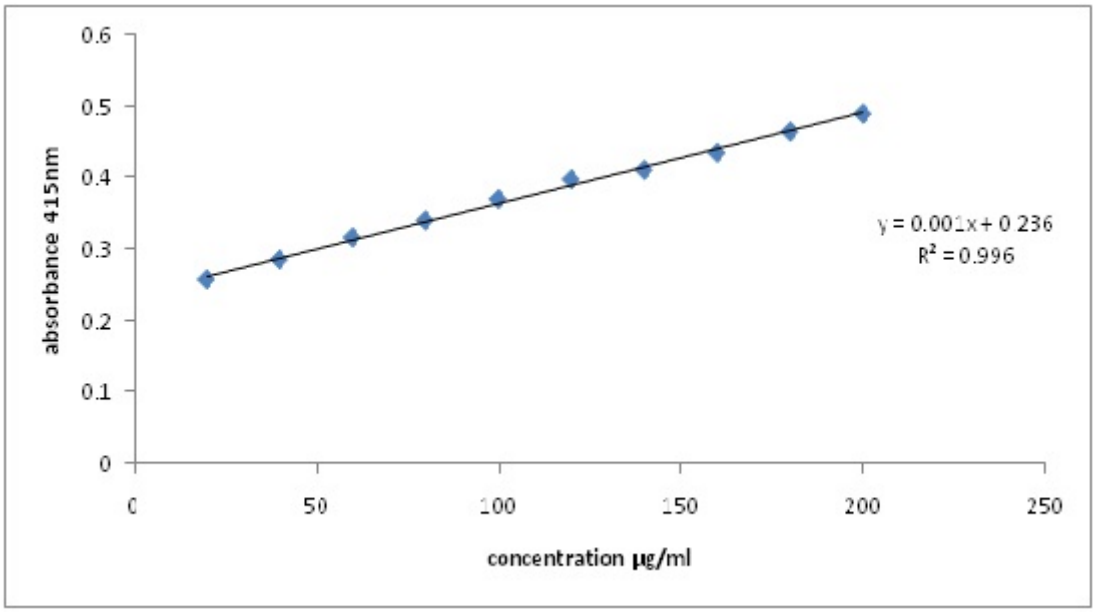

Figure no.3.2.Standard curve represents concentration of Quercitin (µg/ml) against absorbance.

3.3. Antioxidant activity

In the present study, Reducing power activity and DPPH radical scavenging assay was used for the determination of antioxidative potential of the plant extract.

3.3.1. Reducing power activity

The reducing power of three samples of *Urtica* were determined from distinct colour changes at 700nm, depending on the reducing power of the sample concentration. The high absorbance of the reaction mixture indicates high reducing power. The study showed that the highest reducing power activity was shown by sample 3 (Pauri). The reducing power assay shows that the least value out of three samples is of sample 2 (Rudraprayg) (figure no3.3 and table no.3.4).

Table no.3.4. Reducing power activity analysis of different samples of *Urtica*

S.N.	Samples	Concentration (µg/ml)	Absorbance (700nm)
1.	Sample1(Dehradun)	250	0.114
		500	0.215
		750	0.229
		1000	0.364
2.	Sample2 (Rudraprayag)	250	0.181
		500	0.199
		750	0.212
		1000	0.229
3.	Sample3 (Pauri)	250	0.189
		500	0.134
		750	0.243
		1000	0.469
4.	Standard (ascorbic acid)	250	0.036
		500	0.062
		750	0.073
		1000	0.187

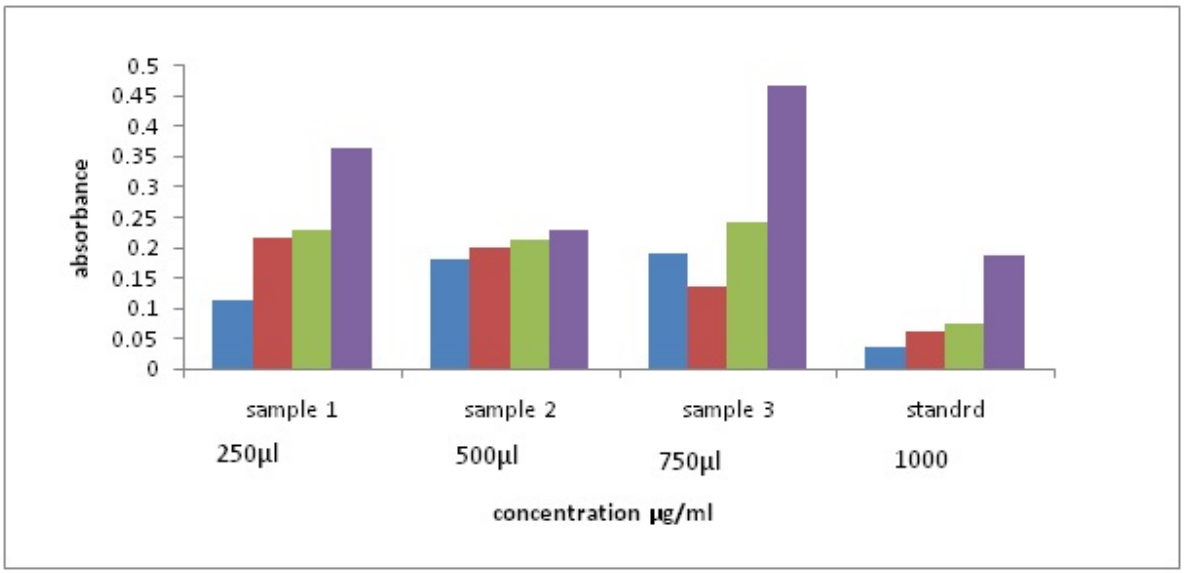

Figure no 3.3. Reducing power comparison of different samples

3.3.2. Result of DPPH radical scavenging assay

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al*., 2002).

DPPH scavenging activity of different samples of *Urtica* was compared with standard (ascorbic acid) by evaluating efficiencies, known as IC_{50}. The IC_{50} is the concentration of an antioxidant at which 50% inhibition of free radical activity is observed. The lower the IC_{50}, the greater the overall effectiveness of the antioxidant in sample. The IC_{50} µg/ml ranged from 227.191 to 389.066 µg/ml. The IC_{50} values and % inhibition of different samples shown in following figures 3.4 and tables3.5:

Table no.3.5. DPHH assay (IC_{50} values) of different samples of *Urtica dioica* along with the standard (Ascorbic acid)

S. N.	Plant Sample	DPHH assay - IC_{50}(µg/ml)
1.	Sample 1(Dehradun)	350.75
2.	Sample 2 (Rudraprayag)	253.28
3.	Sample 3(Pauri)	389.066
4.	Sample 4 (standard-ascorbic acid)	227.191

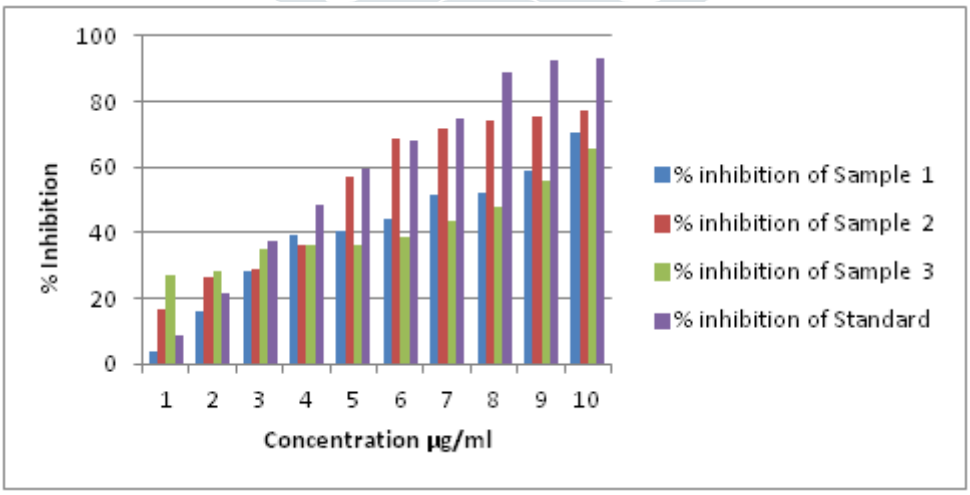

Figure no. 3.4. % inhibition of different samples of *Urtica dioica* L with standard (Ascorbic acid)

CONCLUSIONS

The present studies revealed that the plant stinging nettle, *Urtica dioica* L contains biologically active compounds such as phenols, flavonoids used in reducing the free radical formation accelerated due to modern livelihood factors. Thus, the plant

Urtica dioica L can be used as a food supplement and herbal antioxidants which can reduce the effect of free radical and maintaining the health besides using this plant for the production of eco-fibers, fodder etc.

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