WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 13, 654-666. Research Article ISSN 2277– 7105

# COMPARATIVE *IN VITRO* ANTIOXIDANT ACTIVITY OF EXTRACTS OF AERIAL PARTS OF *GINKGO BILOBA* L. FROM KUMAUN HIMALAYA

**Lalit M. Tewari[1], Brij M. Upreti[1], Geeta Tewari[2]*, Manoj K. Singh[3] and Tapan Nailwal[3]**

[1]1Department of Botany, D. S. B. Campus, Kumaun University, Nainital, Uttarakhand, India.
[2]Department of Chemistry, D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India.
[3]Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital, Uttarakhand. India.

Article Received on
25 August 2017,
Revised on 15 Sep. 2017,
Accepted on 06 Oct. 2017
DOI: 10.20959/wjpr201713-9839

***Corresponding Author**
**Geeta Tewari**
Department of Chemistry,
D.S.B. Campus, Kumaun
University, Nainital,
Uttarakhand, India.

## ABSTRACT

The present study was carried out to evaluate the antioxidant activity of leaf and twig methanol, hexane and DCM extracts of *Ginkgo biloba* L., a valuable rare exotic medicinal plant. This was achieved by radical scavenging, metal chelating, FRAP assay and ABTS radical scavenging activity determination. Total phenolic and flavonoid contents were also evaluated. Among the extractants, methanol gave the best results for phenolic content (285±0.12 mg gallic acid equivalent (GAE)/g dry weight)) and FRAP (224±0.20 mg gallic acid equivalent (GAE)/g dry weight) assay especially, in case of leaf extract. For flavonoids content, DCM extract (278.64±0.77 AAE /100 g dry weight) showed the best result with leaf extract. Total phenolic, flavonoid contents and FRAP activity were estimated in the highest amount from leaf extracts as compared of the twig extracts. For DPPH activity, hexane leaf extract and DCM twig extract showed maximum activity with, however, for ABTS activity, hexane leaf and hexane twig and for metal chelating, DCM leaf and methanol twig extracts showed maximum $EC_{50}$ in comparison to the standards. FRAP and DPPH antioxidant activity showed significant positive relationships with total phenolic content while flavonoid was significantly correlated with FRAP antioxidant activity. Flavonoid content showed significant negative correlation with the antioxidant activity performed by metal chelating method.

**KEYWORDS:** Antioxidant activity, total phenolic content, FRAP, flavenoids, *Ginkgo biloba* L.

## INTRODUCTION

Plant species of the Himalayan region medicine has been known for a long time. The importance of medicinal plants is being reported as a source of natural antioxidant and functional foods.[1] Antioxidant property is considered to be the best among various functional foods because it controls and reduces the oxidation processes in the body[2] by protecting against reactive oxygen species (ROS).[3] Now–a–days secondary metabolites present in medicinal plants have been receiving a great attention mainly due to their role in preventing diseases that arises due to oxidative stress. As a result of stress, the body system releases reactive oxygen species (ROS) such as singlet oxygen and various radicals involved in a number of disorders including cardiovascular malfunctions, tissue injury, DNA damage and tumour promotion. Free radicals are cause of many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney and liver disease, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders and aging. Several studies suggested that antioxidants could be helpful in preventing accumulation of these reactive oxygen species radicals and beneficial for their treatment.[4,5,6] Because of their natural origin, the plant based antioxidants are of greater beneficial effects in comparison to the synthetic ones. Excessive ROS content can have deleterious effects on protein, lipid, RNA and DNA since they are present in a very small quantity and highly reactive.[7] However, during environmental stress time and cell dysfunction, ROS level can increase dramatically. In order to prevent the ROS-induced oxidative damage, there is an antioxidant defence mechanism in human beings that includes metal chelating, free radical-scavenging activities, ABTS, FRAP and total phenol, flavonoid content to neutralize these free radicals immediate after they are *Ginkgo biloba* L. (Ginkgoaceae) known as median hair tree with remarkable order of great antiquity, is represented by a sole survivor regarded as a "living fossil". It came into existence during the Permian and achieved worldwide distribution and luxuriance during the Triassic and Jurassic periods of Mesozoic age. *Ginkgo* has various uses in traditional medicine and has been used for a numerous types of health complaints and diseases. *Ginkgo biloba* L. is medicinally important due to the presence of several groups of compounds, such as terpenoids, flavonoids, glycosides, polyphenols, organic acids and amino acids.[8,9] (Boonkaew and Camper, 2005; Beek and Montoro, 2009).

*Ginkgo biloba* L. has been traditionally used to treat cardiovascular and neurological diseases and the leaf extract is used in the formulations of cosmetics and skin care products. There are certain reports on the essential oil composition[10,11,12,13] extract composition[14,15] antimicrobial activities[16,17,18] and conservation strategies[19] of *G. biloba*. *Ginkgo biloba* L. has been previously reported to have strong antioxidant activities due to the presence of glycosides that scavenge free radicals.[20,21,22,23] These antioxidants may help in protecting cellular damages due to oxidative stress and also lower the risk of chronic diseases[24] by regular intake of antioxidants.[25] Examination of biological activity of separate plant parts allows a significant contribution to medicinal plant study. Therefore, in the present work, an attempt has been made to evaluate the antioxidant activity of various extracts of leaf and twig of *Ginkgo biloba* L. from Kumaun Himalaya.

## MATERIALS AND METHODS

### Collection and extraction of plant material

*Ginkgo biloba* L. plant parts were collected from the D.S.B. Campus Nainital Uttarakhand. The plants parts viz. leaves and twig were separated and allowed to shade dried. The dried parts were powdered in a blender to obtain a coarse powder. Ten gm of leaf (L) and twig (T) sample were taken and mixed with 100 mL of Methanol (ML &MT), Hexane (HL & HT) and Dichloromethane (DL & DT), kept as air tight for 48 hr, and were shaken frequently for uniform mixing of powdered samples distribution. The solutions were filtered and extracts of sample were stored for further analysis.

### Determination of total phenolic contents

Total phenolic content (TPC) in the leaf and twig extracts of *Ginkgo biloba* L. was determined by Folin–Ciocalateu's calorimetric method.[28] One mL of each plant extract (1.0 mg/mL) was mixed with Folin–Ciocalteu's reagent (1.0 mL) and allowed to stand for reaction for 5 minutes. This mixture was then neutralized by 7% sodium carbonate (10 mL) and kept in dark at room temperature for 90 min at 23$^{\circ}$C. The absorbance of resultant blue color was measured at 750 nm using UV–VIS spectrophotometer and quantification of the total phenolic content was done on the basis of standard curve of gallic acid prepared in 80% (v/v) methanol as mg of gallic acid equivalents (GAE) per gram of dried sample (mg GAE/g dried extract).

**Determination of total flavonoid contents**

Aluminium chloride calorimetric method was used for flavonoids determination.[29] 0.3 mL of each extract was mixed with 3.4 mL of 30% methanol, 0.15mL of $NaNO_2$ (0.5M) and 0.15mL of 10% aluminium chloride. After 5 min, one mL of NaOH was added, the solution was well mixed and measure the absorbance against reagent blank at 506 nm. The standard curve for flavonoids was made using quercetine standard solution (0 -100 µg/mL) under the same procedure and the total flavonoids were presented as mg of quercetine equivalent per g of dry extract.

***Evaluation of FRAP***

Ferric reducing antioxidant power (FRAP) assay is a technique which determines the total antioxidant power as a function of the reducing capability. The FRAP assay was first given by Benzie and Strain (1996)[30] and later on by Faria et al. (2005)[31] with some modifications. A mixture of 300 mM acetate buffer (pH 3.6) (i.e. 3.1 g of sodium acetate and 16 mL glacial acetic per liter); 10 mM TPTZ (2, 4, 6-tri-2-pyridyl-1, 3, 5-triazin) in 40 mM HCl (1mL) and 20 mM ferric chloride (1 mL) was made to prepare the FRAP reagents. This reagent mixture was pre warmed at 37ºC for 8 minutes. Absorbance was taken at 593 nm UV-VIS spectrophotometer a blank sample was prepared by ascorbic acid and results were expressed in mg ascorbic acid equivalent (AAE) per g dry extract.

**ABTS radical scavenging activity**

Total antioxidant activity was measured by the ABTS (2, 2'- Azinobis 3-ethylbenzothiazoline- 6-sulphonic acid) radical scavenging method.[32] ABTS (7.0 mM) and potassium per sulfate (2.45 mM) was added in to an amber coloured bottle and kept for 16–18 h in the dark at room temperature. The ABTS solution was diluted with 80% (v/v) ethanol till an absorbance of 0.70 ± 0.05 at 734 nm was obtained. ABTS cation solution (3.90 mL) was added to 0.1 mL of supernatant extract and mixed thoroughly and allowed to stand for six minutes in dark at 23ºC temperature. The absorbance was recorded at 734 nm on the UV-VIS spectrophotometer (U-2001, Hitachi, Japan). The extract samples were diluted 80% (v/v) in different solvent to obtain the concentration. Standard curve was prepared by using a range of concentration of ascorbic acid for quantification of antioxidant potential and the activity of each sample was expressed as $EC_{50}$ (the concentration required for 50% reduction of ABTS.The percentage of inhibition was calculated by the formula:

$$Scavenging\ effect = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \times 100$$

**Determination of metal chelating activity**

Hundred microlitter of 1 mM $FeCl_2$ was added to 1 mL of each extract and 3.7mL of distilled water. The reaction was initiated by the addition of 0.2 mL of 5mM ferrozine solution. The mixture was vigorously shaken and left to stand for 2 minutes at room temperature. The absorbance of the solution was measured at 562 nm.[33] The standard curve was prepared by using a range of concentrations of ascorbic acid for quantification of antioxidant potential of each extract. The activity of each extract was expressed as $EC_{50}$ (the concentration required for 50% reduction of metal chelating). Sodium salt of EDTA was used as positive control as the percent activity was determined using the formula:

$$Scavenging\ effect = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \times 100$$

**DPPH scavenging activity**

The method for evaluating DPPH activity given by Brand-William, Cuvelier and Berset (1995)[34] is based on scavenging of 1, 1-diphenyl-2-picryl-hydrazyl radical (DPPH). 400µM DPPH was dissolved in pure 80% ethanol. DPPH cation (DPPH) (3.0 mL) was mixed with extract (1 mL) and kept in dark at room temperature for 20 minutes. Reduction in the absorbance at 520 nm was recorded by UV-VIS spectrophotometer. The control was prepared without any sample and standard curve was prepared by using a range of concentrations of ascorbic acid for quantification of antioxidant potential. The activity of each sample was expressed as $EC_{50}$ (the concentration required for 50% reduction of DPPH). The scavenging activity was calculated by using the following equation:

$$Scavenging\ effect = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \times 100$$

**Statistical analysis**

All the data of antioxidant activity assay were conducted in three replicates and the data for each activity was presented as the mean value ± standard deviation (SD). Significant difference among means was tested by one way ANOVA (Duncan's test) using SPSS 16.0 and correlation between phenolic content, flavonoids content and the antioxidant activity was performed by Pearson's correlation test using SPSS 16.0. Significance level of $p \leq 0.05$ was considered as statistically significant level.

## RESULTS AND DISCUSSION

The present work was performed to evaluate the reducing or protecting power of natural antioxidants present in the *Ginkgo biloba* L. leaves and twig extracts.

### Total phenol content (TPC)

Phenolic compounds are the important class of secondary metabolites that are synthesized by the plants in response to biotic and abiotic stresses. The total phenolic content was observed to be the highest in metanolic extract (285±0.12 - 255±0.51 mg GAE/g dry extract) followed by DCM (240±0.73 -210±0.22 mg GAE/g dry extract) and hexane extract (165±0.30 - 105±0.50 mg GAE/g dry extract) for both leaf and twig (Table1). The leaf extracts had greater phenolic content as compared to twig extract. There are some previous reports on the antioxidative properties of some medicinal plants. The total phenolic content of methanolic rhizome extract of *Hedychium spicatum* Buch. Ham. ex D. Don from Uttarakhand was 4.692±0.025 mg gallic acid equivalent/g dry[25] while methanolic twig extract of *Ocimum tenuiflorum* from Pollachi, Tamilnadu showed 3.66 mg GAE/g dry weight phenol content and 2.84 mg GAE/g dry weight. Previous studies on the methanolic leaf extracts of *Ginkgo biloba* L. showed the total phenolic content of 75.30±0.69 mg/g dry weight from Egypt.[26]

### Flavonoid content

Flavonoids are the important components of human and animal diet because of their common presence in the plants. The irregular consumption of plants for secondary metabolites has serious positive and negative consequences to health as they have different biological activities. Total concentration of flavonoids in the DCM leaf extract was 278.64±0.77 mg QE/g dry weight while in the DCM twig extract was 263.52±0.81 mg QE/g dry weight. Methanol leaf extract showed 257.04±0.36 mg QE/g of flavonoid while methenolic twig extract showed 241.92±0.35 QE/g dry weight. Hexane leaf and twig extracts showed 155.52±0.37 and 142.56±0.39 QE/g of flavonoids content respectively (Table 1). DCM leaf and twig extracts were observed to contain the highest flavonoids concentration. Flavonoids content estimated by a previous study in methanolic leaf extract of *Ginkgo biloba* L. was 84.59±1.43 mg QE /g from Egypt.[26]

### Total FRAP

It has been observed that the transition metal ions contribute to the oxidative damage in neurodegenerative disorder like Alzheimer's and parkinsons disease and hence, determination of the iron (II) chelating activity of plant extract is of great significance. Total FRAP content

of leaf extract varied from 80±0.65 to 224±0.20 mg ascorbic acid equivalents (AAE)/g dry extract (Table 1). The FRAP content was found to be the highest for methanolic extract (224±0.20 AAE/g dry extract for leaf and 241.92±0.35 mg AAE/g dry extract for twig) and the lowest for hexane extracts (80±0.65 AAE/g dry extract for leaf and 64±0.38 AAE/g dry extract for twig ). For DCM and hexane extracts, it was higher for leaf extract whereas with methenol extract, the FRAP content was higher for twig as compare to leaf.

**DPPH scavenging activity**

In the present study, free radical scavenging activity of *Ginkgo biloba* L. extracts of leaf and twig were evaluated. DPPH radical scavenging ability is helpful in evaluating the antioxidant potential of medicinal plants. The standard (ascorbic acid) $EC_{50}$ value of 45. 277 µg/ mL. It was observed that methanol and hexane leaf extracts showed better scavenging activity as compared to DCM leaf extract while vice versa was true for twig extracts (Figure1). The order of DPPH scavenging activity for leaf extract was HL>ML>DL and for twig extract was following the order : DT>HT>MT. Table 2 showed the scavenging effect of leaf extracts (HL,ML,DL) on DPPH radical and was observed in the following order: HL (46.483µg/mL) >ML (46.516µg/mL)>DL (47.961µg/mL) while twig extracts were DT (46.123 µg/mL)>MT (47.872 µg/mL)>HT ( 49.391 µg/mL) in order. Hexane leaf and DCM twig extract showed the highest activity. DPPH free radical scavenging activity % of inhibition was 71% for leaf extract and 11% for twig extract. Methanolic extracts of *G. biloba* had a noticeable effect on scavenging free radical (75.86%) at a concentration of 150 µg/mL.[27]

**Metal chelating property**

Metal chelating standard showed 44.510 µg/mL $EC_{50}$ value while leaf and twig extracts had activity in following order: DL (48.941 µg/mL) >ML ( 49.012 µg/mL)>HL ( 49.333 µg/mL) for leaf extracts while twig extracts had the following order: MT (46.567 µg/mL)>HT (46.760 µg/mL)>DT ( 48.843 µg/mL). DCM leaf and methanol twig extracts showed the highest chelating activity as shown in Figure 2. Methanolic twig extract showed maximum metal chelating activity among all extracts. The percentages of metal scavenging capacity at 200 µg/ ml of methanolic extract of *Ginkgo biloba* L. found to be 32.2%[27] *ABTS activity.*

ABTS standard showed 45.433 µg/mL $EC_{50}$ value (Figure 3) while leaf and twig extracts had ABTS activity in following order: HL (48.932 µg/mL) >ML (49.284 µg/mL) >DL (50.267 µg/mL) for leaf extracts and HT> (47.967µg/mL) >MT (48.180µg/mL) >DT (48.264µg/mL) for twig extracts. DCM leaf and methanol twig extracts showed the highest activity.

**Correlation study**

Correlation matrix revealed significant positive relationship of total phenolic contents with FRAP and DPPH antioxidant activity while flavonoid with FRAP antioxidant activity. Flavonoid content showed significant negative correlation with the antioxidant activity performed by metal chelating method (Table 3).

**Table 1: Total phenolic, flavonoid and FRAP content of the leaf and twig extracts and extract yield.**

| Antioxident assays | Leaf extract | | | Twig extract | | |
|---|---|---|---|---|---|---|
| | Hexane | Methanol | DCM | Hexane | Methanol | DCM |
| Phenolic content (mg gallic acid equivalent (GAE)/g dry weight) | 165.00±0.30 [b] | 285.00±0.12 [f] | 240.00±0.73 [d] | 105.00±0.50 [a] | 255.00±0.51 [e] | 210.00±0.22 [c] |
| Frap | 80.00±0.65 [b] | 224.00±0.20 [f] | 144.00±0.30 [d] | 64.00±0.38 [a] | 192.00±0.32 [e] | 112.00±0.33 [c] |
| Flvinoid AAE /100 g dry weight | 155.52±0.37 [b] | 257.04±0.36 [d] | 278.64±0.77 [f] | 142.56±0.39 [a] | 241.92±0.35 [c] | 263.52±0.81 [e] |
| Extract yield %(w/w) | 0.77±0.21 [b] | 5.64±0.23 [f] | 0.59±0.04 [a] | 1.50±0.15 [d] | 4.50±0.25 [e] | 1.00±0.05 [c] |

**Table 2: $EC_{50}$ values (µg/mL) of different antioxidants activities.**

| Plant part | DPPH (standard 45.28 µg/mL) | | | Metal (standard 44.52 µg/mL) | | | ABTS (standard 45.43 µg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Hexane | Methanol | DCM | Hexane | Methanol | DCM | Hexane | Methanol | DCM |
| Leaf | 46.48 | 46.52 | 47.96 | 49.333 | 49.01 | 48.94 | 48.93 | 49.28 | 50.27 |
| Twig | 49.39 | 47.87 | 46.12 | 46.76 | 46.57 | 48.84 | 47.97 | 48.18 | 48.26 |

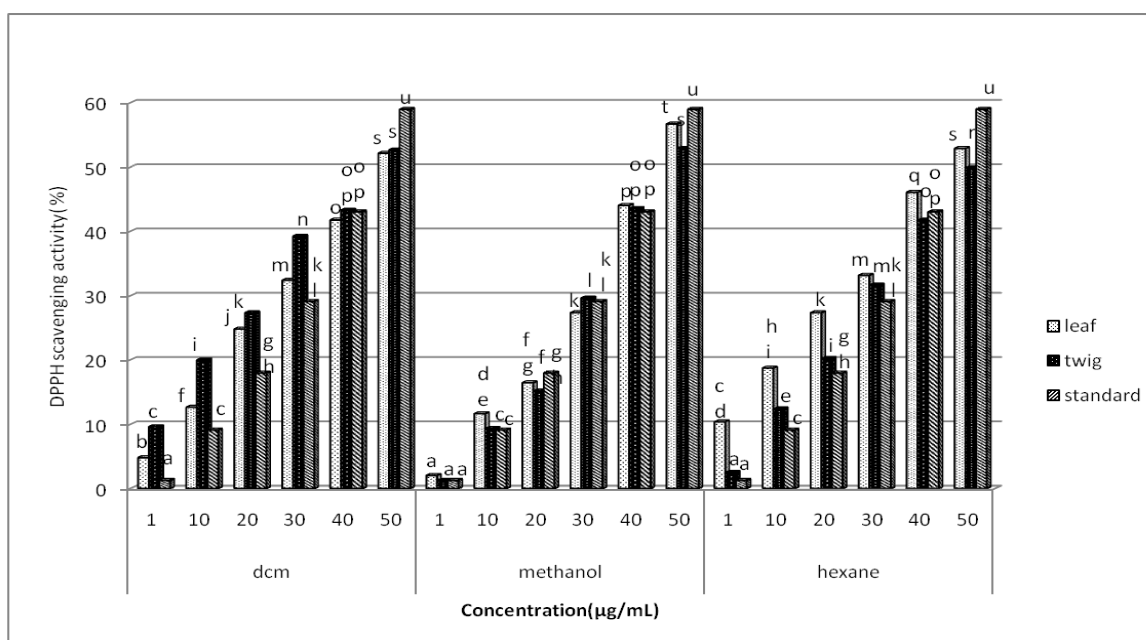


**Figure 1: Comparative DPPH scavenging activity in *Ginkgo biloba* L. leaf and twig extracts with standard**

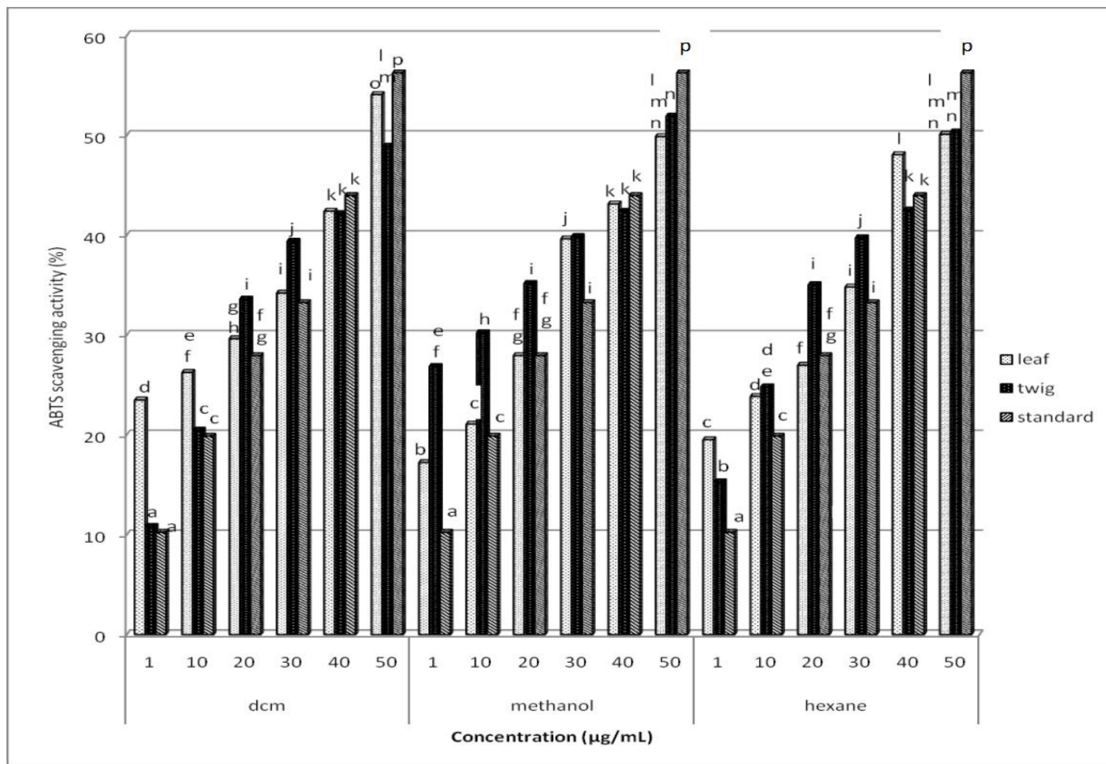


**Figure 2: Comparative ABTS radical scavenging activity in *Ginkgo biloba* leaf and twig extracts with standard**

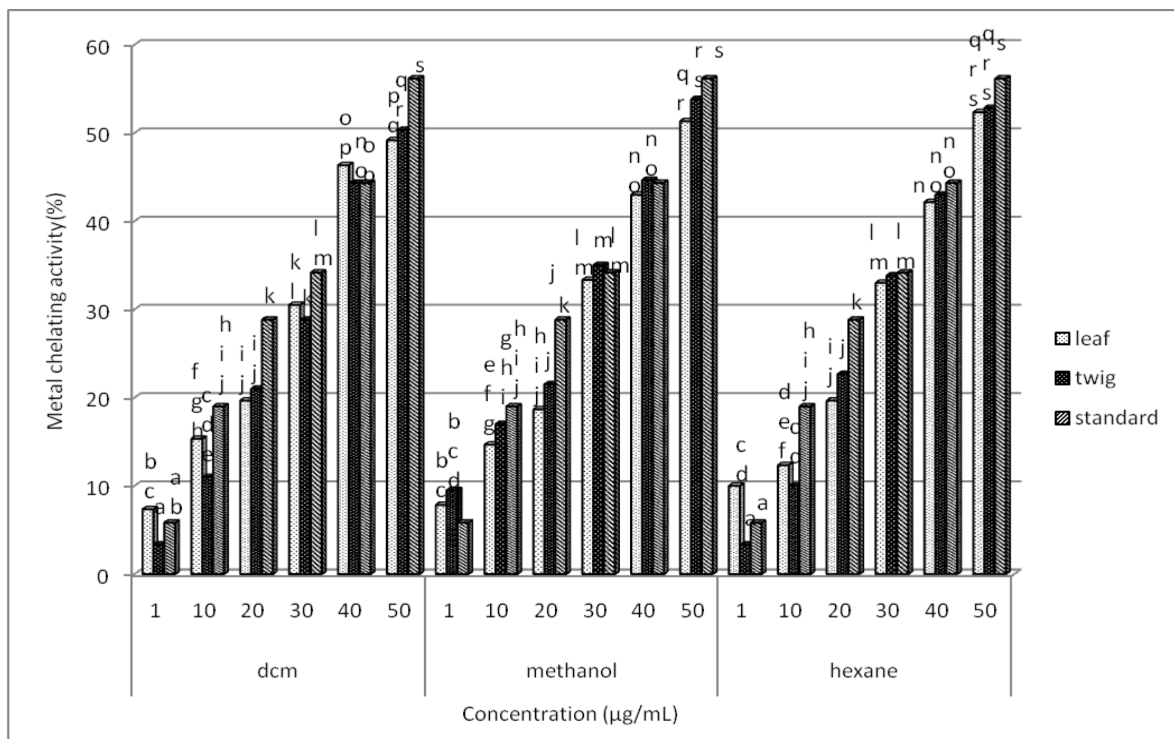


**Figure 3: Comparative metal chelating ability of *Ginkgo biloba* L. leaf and twig extracts with standard**

**Table 3: Correlation between total phenolic and flavonoid contents and antioxidant activity in *Ginkgo biloba* L.**

| | DPPH | ABTS | Metal | Frap | Phenol | flavonoid |
|---|---|---|---|---|---|---|
| DPPH | 1 | -0.145 | -0.161 | 0.200 | 0.675** | 0.420 |
| ABTS | | 1 | -0.099 | 0.332 | 0.226 | 0.289 |
| Metal | | | 1 | 0.731** | -0.229 | -0.502* |
| Frap | | | | 1 | 0.591** | 0.894** |
| Phenol | | | | | 1 | 0.857** |
| Flavonoid | | | | | | 1 |

## CONCLUSION

In the present study, analysis of antioxidant activity (DPPH, ABTS, Metal chelating and FRAP) revealed that *Ginkgo biloba* L. methanolic leaf and twig extracts had the best FRAP activity. Leaf extracts showed better antioxidant activities as compared to twig extracts.

## ACKNOWLEDGMENT

The authors are grateful to UGC, New Delhi, IERP, GBPIHED, Kosi, Katarmal and Head, Department of Botany and Head, Department of Chemistry, D. S. B. Campus, Kumaun University, Nainital for providing necessary facilities.

## REFERENCES

1. Vaidya ADB, Devasagayam TPA (Current status of herbal drugs in India: an overview). Journal of Clinical Biochemistry and Nutrition, 2007; 41: 1–11.
2. Krishnaih D, Sarbatly R, Bono A, (Phytochemical antioxidants for health and medicine-a move towards nature). Biotechnology and Molecular Biology Reviews, 2007; 2: 97–104.
3. Lan S, Yin J, Charles D, Zhou K, Moore J, Yu L, (Total phenolic content, chelating capacities and radical scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf). Food chemistry, 2007; 100: 990–997.
4. Spadiene A, Savickiene N, Skesters A, Silova A, Rodovicius H, (The effects of *Ginkgo biloba* L. and *Camellia sinensis* L. extracts on oxidative stress in patients with type 2 diabetes). African Journal of Pharmacy and Pharmacology, 2012; 6(44): 3080-3085.
5. Milan S, Niciforovic N, Topuzovic M, Solujic S, (Total phenolic content, flavonoid concentrations and antioxidant activity, of the whole plant and plant parts extracts from *Teucrium montanum* L. var. *montanum*, f. *supinum* (L.) Reichenb). Biotechnology & Biotechnology, 2011; 25(1): 2222-2227.

6. Rawat S, Bhatt I, Rawal R, (Total phenolic compounds and antioxident potential of *Hedychium spicatum* Buch. Ham. ex D. Don in west Himalaya, India). Journal of Food Composition and Analysis, 2011; 24: 574 -579.
7. Lu JM, Lin PH, Yao Q, Chen C, (Chemical and molecular mechanisms of antioxidants: Experimental approaches and model sytwigs). Journal of Cellular and Molecular Medicine, 2010; 14(4): 840-860.
8. Boonkaew T and N Camper, (Biological activities of *Ginkgo* extracts). Phytomedicine, 2005; 12: 318-323.
9. Beek TAV, Montoro P, (Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals). Journal of Chromatographia A, 2009; 1216: 2002-2032.
10. Yonghong Z, Jingmian, W, Desheng L and Yinhua Z, (Chemical studies on volatile constituents of *Ginkgo biloba* L. leaves) Natural Product Research and Development, 1999; 11(2): 62-66.
11. Zhang WC, Bang, SZ, Hong, TW and Qing Y, (Chemical constituents of essential oil from the leaves of *Ginkgo biloba).* Journal of Tropical and Subtropical Botany, 2008; (4): 329-332.
12. Hongliang LU, Chienese Tobacco Science, 2011; 32: 66.
13. Tewari G, Mohan B, Kishor K, Tewari LM and Nailwal TK, (Volatile Constituents of *Ginko biloba* L. leaves from Kumaun: a source of (E)- neroliodol and phytol.) Journal of Indian Chemical Society, 2015; 92: 1583-1586.
14. Chi J, Xu L, Ma C and Liu A, (Chemical constituents of the leaves of *Ginkgo biloba).* Zhongguo Zhong Yao Za Zhi, 1997; 22(2): 106-107.
15. Beek, TA van, (Chemical analysis of *Ginkgo biloba* leaves and extracts). Journal of Chromatography A, 2002; 967: 21–55.
16. Sati SC and Joshi, S, (Antibacterial activities of *Ginkgo biloba* L. leaf extracts). Scientific World Journal, 2011; 11: 2237–2242.
17. Sati P, Pandey, A and Palni, LMS, (Antimicrobial potential of leaf extracts of *Ginkgo biloba* L., growing in Uttarakhand, India). National Academy Science Letters, 2012; 35(3): 201-206.
18. Tewari G, Mohan B, Tewari L, Kishor K, Nailwal TK and Singh M, (Comparative phytochemical composition and antimicrobial potential of leaf and twig extracts of *Ginkgo biloba* L. from India), G- Journal of Environmental Science and Technology, 2015; 2(6): 97-100.

19. Tewari LM, Upreti BM, Bohra N, Bharti M, Pandey N, Chopra N, Tewari G and Nailwal T, Conservation of *Ginkgo biloba* L. Through in- vitro Techniques and its Molecular Characterization, "Microbiological and pharmacological aspects of Biodiversity". (Eds) Pankaj Sharma, Neha Gautam Sharma and Pankaj Sharma, 2017; ISBN 978-93-5056-878-1: 22-36.
20. Kudolo G.B, Delaney D, Blodgett J, (Short-term oral ingestion of *Ginkgo biloba* extract (EGb 761) reduces malondialdehyde levels in washed platelets of type 2 diabetic subjects). Diabetes Research and Clinical Practice, 2005; 68: 29-38.
21. Yeh YC, Liu TJ, Wang LC, Lee HW, Ting CT, Lee WL, Hung CJ, Wang KY, Lai HC, (A standardized extract of *Ginkgo biloba* suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes). British Journal of Pharmacology, 2009; 156(1): 48–61.
22. Sati P, Pandey A, Rawat S, Rani A, (Phytochemical and antioxidant in leaf extracts of *Ginkgo biloba* with reference to location, seasonal variation and solvent system). Journal of Pharmacy Research, 2013; 7(9): 804-809.
23. Atoui A.K, Mansouri A, Boskou G, Kefalas P, (Tea and herbal infusions: their antioxidant activity and phenolic profile). Food Chemistry, 2005; 89: 27–36.
24. Alasalvar C, Al-Farsi M, Quantick PC, Shahidi F, Wiktorowicz R, (Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots). Food Chemistry, 2005; 89: 69–76.
25. Rawat S, Bhatt I, Rawal R, (Total phenolic compounds and antioxidant potential of *Hedychium spicatum* Buch.Ham. ex D. Don in west Himalaya, India). Journal of Food Composition and Analysis, 2011; 24: 574 -579.
26. Hossam S, Mona el-beltagi, Badawi H, (Comparison of antioxidant and antimicrobial properties or *Ginkgo biloba* and Rosemary (*Rosmarinus officinalis* L.) from Egypt). Notulae Botanicae Horti Agrobotanici, 2013; 41(1): 126-135.
27. EL-Beltagi HS and Badawi M H, (Comparison of Antioxidant and Antimicrobial Properties for *Ginkgo biloba* and Rosemary (*Rosmarinus officinalis* L.) from Egypt). Notulae Botanicae Horti Agrobotanici, 2013; 41(1): 126-135.
28. Kim DO, Jeong SW, Lee CY, (Antioxident capacity of phenolic phytochemicals from various cultivars of plums). Food Chemistry, 2003; 81: 321-326.

29. Park YS, Jung ST, Kang SG, Heo B.K, Arancibia-Avila P, Toledo F, Drzewiecki J, Namiensik J, Gorinstein S, (Antioxident and protein in ethylene – treated kiwi fruits). Food Chemistry, 2008; 107: 640–648.
30. Benzie IFF, Strain JJ, (The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': the FRAP assay). Analytical Biochemistry, 1996; 239: 70–76.
31. Faria A, Oliveira J, Neves P, Gamerio P, Santos-Buelga C, Freitas De V, Mateus N, (Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts). Journal of Agricultural and Food Chemistry, 2005; 53: 6896–6902.
32. Cai Y, Luo Q, Sun M, Corke H, (Antioxidant activity and phenolic compounds in 112 traditional Chinese medicinal plants associated with anticancer). Life Sciences, 2004; 75: 2157–2184.
33. Dinis TCP, Madeira VMC, Almeida LM, (Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers). Archives of Biochemistry and Biophysics, 1994; 315: 161-169.
34. Brand-William WM, Cuvelier E, Berset C, (Use of a free radical method to evaluate Antioxidant activity). Lebensmittel Wissenschaft and Technologie, 1995; 28: 25–30.