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A STUDY ON THE PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITIES OF TWO COMMON WEEDS

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ABSTRACT

The principal objective of this study was to analyze the phytochemical and antioxidant activities of two common weeds. Invasion of weeds pose an enormous threat to our ecosystem. If we are able to mobilize the use of such weed by establishing its medicinal use we can steer the management process into utilization instead of weed control. The qualitative phytochemical prescreening was performed by color method. The total polyphenolic content of the extracts were determined by using Folin-Ciocalteau reagent and the total flavonoid content was determined according to colorimetric method. The antioxidant activity was assessed by using DPPH radical scavenging method. The phytochemical screening revealed the presence of carbohydrates, tannins, glycosides, flavonoids and phenols in *Lantana camara*, and presence of carbohydrates, alkaloids, saponin, tannin, glycosides, flavonoid and phenol in *Erigeron bellidioides.* The antioxidant activity test for *L. camara* revealed that the IC 50 value of Methanol, Ethyl acetate and Hexane extract were 13.3583 mcg/ml, 83.786 mcg/ml and >100 mcg/ml respectively where as for *E. bellidioides* the IC 50 value of Hexane, Ethyl acetate, Methanol extract were >100 mcg/ml, 99.52 mcg/ml and 26.331 mcg/ml respectively. The findings of this study revealed that the methanolic extracts of *L.camara* and *E. bellidioides* have the highest antioxidant activity.

KEYWORDS: antioxidant, *L.camara*, *E.bellidioides*, polyphenolic compounds, flavonoid, DPPH radical.

INTRODUCTION

Ever since the ancient times, nature has been a foundation for medical agents and a remarkable amount of modern drugs have been derived by the virtue of it; many of such derivations were based on the uses of the agents in traditional medicine.[1] Natural products play a significant role in drug development in the pharmaceutical industry with nearly half of all modern clinical drugs arising from natural origin.[2]

Oxidative stress can be defined as the inequality between the antioxidants and pro-oxidants with the latter being higher, potentially leading to damage in biological targets.[3] Oxidation, apart from being a natural cellular metabolic process is a crucial process in the formation of free radicals commonly known as reactive oxygen species (ROS) or reactive nitrogen species (RNS), such as Superoxide, Hydroxyl, Hydrogen peroxide, Peroxyl radical, Ozone, Nitric oxide, Peroxynitrite, Peroxynitrous acid, Nitrogen dioxide free radicals. The so produced free radicals are capable of damaging cellular membranes, proteins, fats and nucleic acid. There are important processes such as antioxidant defense mechanism endowed by nature upon every cell for protective purposes. The failure of such mechanisms to oppose the free radicals plays an important role in many chronic and degenerative diseases such as heart diseases, cancer, diabetes mellitus, asthma, neurodegenerative diseases, Parkinson's, Alzheimer's etc. and ageing.[4]

Antioxidants have established itself to be of remarkable significance by possessing the ability to defend the body from damage due to free radical induced oxidative stress.[5] Antioxidants work by neutralizing free radicals by interfering with the oxidation process by oxygen scavenging activity, chelating activity and catalytic activity. Antioxidant compounds such as phenolic acids, polyphenols and flavonoids trap free radicals to reduce oxidative stress and its potential harm of degenerative diseases.[6]

L. camara is an important medicinal plant with several medicinal uses in traditional medication system. It has been used to cure many health problems in different parts of the World. Leaves are used to treat cuts, rheumatisms, ulcers, catarrhal infection, tetanus, rheumatism, malaria, cancer, chicken pox, asthma, ulcer, swelling, eczema, tumor, high blood pressure, and bilious fever, ataxy of abdominal viscera, sores, measles, fevers, cold and high blood pressure.[7] In Nepal, it has established itself to be an invasive and nuisance weed.

In Nepal, *Erigeron bellidioides* has its identity as a problematic and nuisance weed. It is known to significantly reduce crop yields. The most intensive research on this herb is in its infancy. It has been reported to be toxic to cattle and goats as per the locals. It is mainly used as bug repellant; people use it in bedstraw to keep bugs out of mattresses. It is indigenously used as a blood purifier in the north western Himalayan part of India.[8]

Nepal is especially vulnerable to the establishment of invasive foreign species owing to its wide range of biodiversity and environmental conditions.[9] The invasion of weeds is creating challenges in our biodiversity, causing more harm than good in terms of resource availability, ecosystem, economy and health of the inhabitants as well as posing as an expensive and insidious environmental problem.[10,11] Various studies on weed control have recommended that the substitution of chemical methods to control such environmental atrocity by any other means physical or biological is not as effective. If we are able to mobilize the use of such weed by establishing its medicinal use we can steer the management process into utilization instead of weed control. There are various management practices implemented in order to control the infestation and invasion, but using our resources and manpower to research and develop any basis that can help us to use this plant is truly necessary. The very basis of our being is the power of intellect the humans have over the other living creatures, so instead of just destroying something that has been disturbing us we can invent and research better ways to manage such invasive plants by establishing scientific evidence of its medicinal properties. This does not only elevate the status of the plant but also creates alternatives from which we can derive useful substances.

METHODS AND METHODOLOGY

Plant material: The aerial parts of the plant, *L. camara* was collected from Kathmandu, whereas the aerial parts of plant *E. bellidioides* was collected from Shankenjung VDC - 4, Illam, Nepal and was duly identified at the National herbarium center and plant laboratory, Godawari, Lalitpur, Ministry of Forest and Soil Conservation, Government of Nepal.

Chemicals and apparatus: Hexane, ethyl acetate, methanol, quercetin dihydrate, gallic acid, ascorbic acid, diphenyl-1-picrylhydrazyl reagent (Hi-Media), potassium acetate of E merck were purchased from the local supplier. UV- spectrophotometer (SHIMADZU), soxhlet apparatus (Borosil) and rotary vacuum evaporator (ATICO India) were used.

Plant processing: The plant material was cut into pieces and shade dried at room temperature. Dried sample was crushed into powder by electric grinder and was subject to extraction by using solvent in the following order of polarity- hexane, ethyl acetate and methanol. The so obtained liquid extracts were then subjected to evaporation using a rotary evaporator under vacuum at 37±5ºC until the solid mass was obtained. The extracts were then weighed and kept in glass sample tube and stored in refrigerator at 4°C for further analysis.

Phytochemical Prescreening: The qualitative phytochemical prescreening was performed in order to identify the various groups of chemical constituents such as alkaloids, saponins, glycosides, tannins, terpenoids, flavonoids, carbohydrates and proteins. Different extracts of *L.camara* and *E.bellidioides* was tested for the presence of chemicals by their color reactions with different reagents.[12]

Total polyphenolic content determination: The total polyphenolic content of the extracts were determined by using Folin-Ciocalteau reagent.[13] Gallic acid was used for constructing the standard curve (10 to 80µg/ml) as shown in figure 1. The total polyphenolic compound concentration in the extracts was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of the extract.

Total flavonoid content determination: The total flavonoid content was determined according to colorimetric method.[14] Quercetin was used for constructing the standard curve (10, 20, 30, 40 and 50µg/ml) as shown in figure 2. The total flavonoid content concentration in the extracts was expressed as milligrams of quercetin equivalent per gram (QE/gm) of dry weight of extracts.

Antioxidant activity by DPPH Scavenging: Reference samples of ascorbic acid and sample solutions of plant extract were prepared in methanol at different concentrations (100, 80, 60, 40, 20 and 5 µg/ml). 0.01 mM solution of DPPH was prepared in methanol and 4 ml of this solution was added to 1ml of each concentration of sample plant extracts and ascorbic acid solutions. The mixture was kept in dark for 30 minutes. Similarly, as control, 4ml of 0.1mM DPPH was mixed with 1ml of methanol. Thirty minutes later absorbance was measured at 517 nm. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$percentage\ scavenging = \frac{Ao - AT}{Ao} \times 100$$

Where, A_o = absorbance of DPPH solution and absorbance of test or reference sample.

The percentage scavenging was then plotted against concentration and regression equation was obtained from which IC50 (micromolar concentration required to inhibit DPPH radical formation by 50%) values were calculated for each plant extract.

Statistical analysis: All the quantitative tests were conducted in triplicate. The data was presented in the form of mean ± SD (standard deviation). The results were analysed statistically by the help of Microsoft excel 2007.

RESULTS

The preliminary phytochemical screening of *L. camara* showed the presence carbohydrates and flavonoids and phenols in all extracts. Alkaloids were present only in hexane and ethyl acetate extract. Glycosides, tannins and terpenoids were found only in the methanol extract. However, saponins and protein were absent in all three extracts. *E. bellidioides* showed the presence of alkaloids in Hexane and Methanol extract whereas absent in Ethyl acetate extract. Saponin was present in Ethyl acetate and Methanol extract. Carbohydrates were found to present in all extracts. Tannin was present in Hexane and Methanol and absent in Ethyl acetate extract. Protein was absent in all the extracts. Glycosides were found in Hexane and Methanol extract. Finally, Phenols and Flavonoid were found in all extracts as observed quantitatively for both plants. The results obtained are presented in Table 1.

Table 1. Phytochemical screening of *L. camara* and *E. bellidioides*.

S. No.	phytoconstituents	*L. camara* extracts			*E. bellidioides* extracts		
		Hexane	Ethyl acetate	methanol	Hexane	Ethyl acetate	Methanol
1.	Alkaloids	+	+	+	+	-	+
2.	Saponins	-	-	-	-	+	+
3.	Glycosides	-	-	+	+	-	+
4.	Tannins	-	-	+	+	-	+
5.	Carbohydrates	+	+	+	+	+	+
6.	Flavonoids	+	+	+	+	+	+
7.	Phenols	+	+	+	+	+	+
8.	Proteins	-	-	-	-	-	+
9.	Terpenoids	-	-	+	-	-	+
Note: presence '+'; Absence '-'							

The value of total phenolic content of *L. camara* extracts ranged from 12.89 mg GAE/g in hexane, 67 mg GAE/g in ethyl acetate and 81.047 mg GAE/g in methanol. The total phenolic

contents of *E. bellidioides* extracts were found to be 4.6 mg GAE/g for hexane, 21.6 mg GAE/g for ethyl acetate and 48.4 mg GAE/g for methanol.

The value of total flavonoid content of *L. camara* ranged from 24.51 mg QE/g in hexane, 41.48 mg QE/g ethyl acetate and 20.83 mg QE/g in methanol. Whereas, the total flavonoid content for *E. bellidioides* were found to be 28.727 mg QE/g for hexane extract, 21.909 mg QE/g for ethyl acetate extract and 30 mg QE/g for methanol extract.

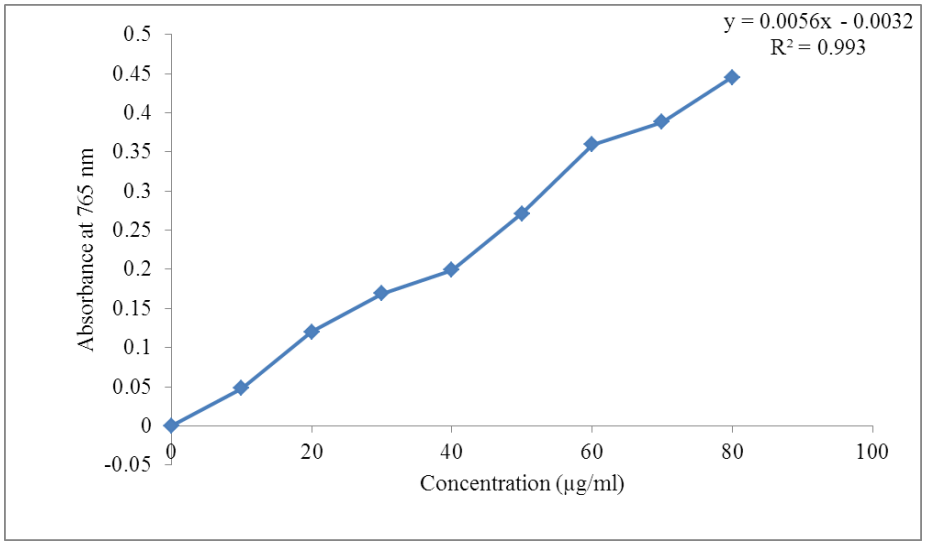

Figure 1: Calibration curve of Gallic acid

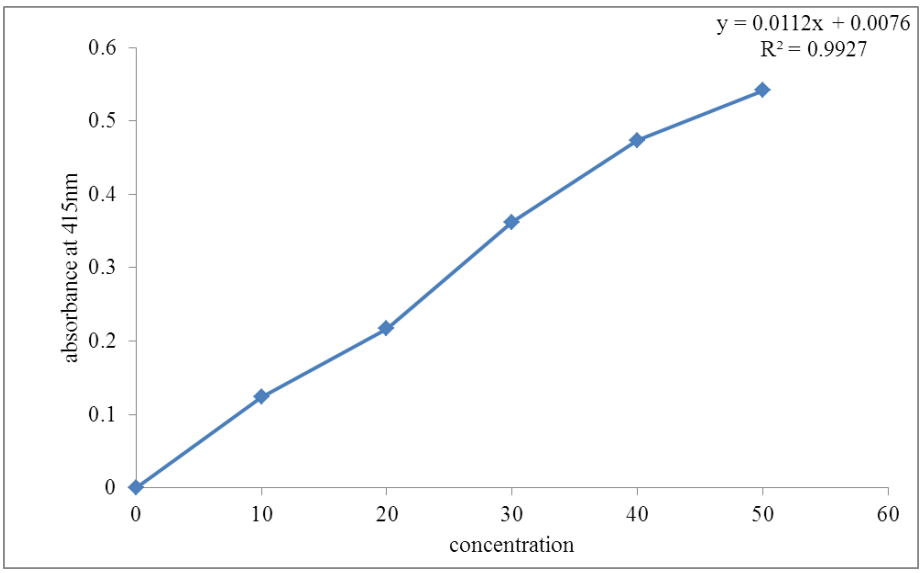

Figure 2. Calibration curve of quercetin.

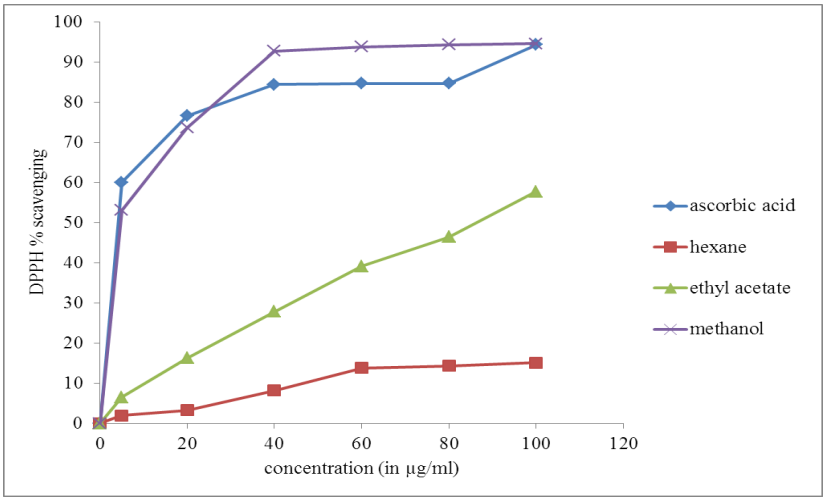

Figure 3. DPPH radical scavenging activity of L. camara.

The antioxidant test for *E. bellidioides* revealed that methanol extract has highest antioxidant property, ethyl acetate extract presented moderate antioxidant activity whereas, and hexane extract has shown the least antioxidant property. The sequence of antioxidant activity of *E. bellidioides* followed the order of methanol (IC50, 26.331µg/ml) > ethyl acetate (IC50, 99.52 µg/ml) > hexane (IC50, >100 µg/ml).

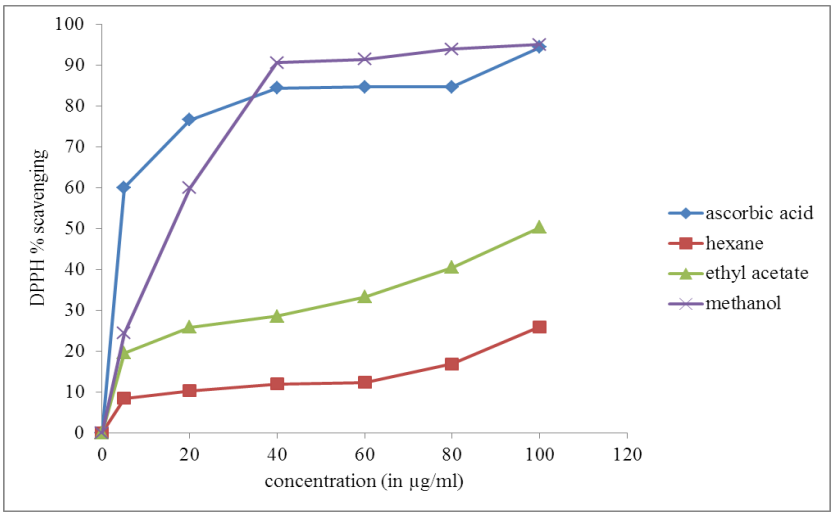

Figure 4: DPPH radical scavenging activity of *E.bellidioides*

Table-2: The TPC and TFC values of *L.camara* and *E. bellidioides*

	Lantana camara			**Erigeron bellidioides**		
	Hexane	**Ethyl acetate**	**Methanol**	**Hexane**	**Ethyl acetate**	**Methanol**
Total phenolic content	12.89 mg GAE/g	67 mg GAE/g	81.047 mg GAE/g	4.6 mg GAE/g	21.6 mg GAE/g	48.4 mg GAE/g
Total flavonoid content	24.51 mg QE/g	41.48 mg in QE/g	20.83 mg QE/g	28.727 mg QE/g	21.909 mg QE/g	30 mg QE/g

The plant *L. camara* presented antioxidant activity, which was highest for methanolic extract and least for hexane extracts. The sequence of antioxidant activity of *L. camara* followed the order of methanol (IC50, 13.35 µg/ml) > ethyl acetate (IC50, 83.786 µg/ml) > hexane (IC50, >100 µg/ml).

Table-3: Antioxidant activity presented by *L. camara* and *E.bellidioides*

	Concentration(µg/ml)	Percentage scavenging; Mean ± sd		
		Hexane extract	Ethyl acetate	Methanol
L.camara	5	1.88 ± 0.43	6.46 ± 0.98	53.09 ± 0.14
	20	3.23 ± 0.64	16.07 ± 2.29	73.58 ± 0.68
	40	8.08 ± 0.26	27.66 ± 3.05	92.72 ± 0.88
	60	13.71 ± 0.28	39.06 ± 2.09	93.8 ± 0.28
	80	14.28 ± 0.33	46.36 ± 0.7	93.99 ± 0.27
	100	15.09 ± 0.17	57.67 ± 1.91	94.6 ± 0.58
IC50		>100 µg/ml	83.78 µg/ml	13.35 µg/ml
E. bellidioides	5	8.44 ± 0.33	19.58 ± 0.458	24.34 ± 0.67
	20	10.33 ± 0.45	25.87 ± 0.79	59.92 ± 0.25
	40	11.94 ± 0.77	28.507 ± 0.73	90.56 ± 0.30
	60	12.30 ± 1.10	33.24 ± 0.70	91.37 ± 1.0
	80	16.89 ± 0.55	40.43 ± 0.44	93.89 ± 0.70
	100	25.96 ± 0.77	50.31 ± 0.77	94.96 ± 0.67
IC50		>100 µg/ml	99.52 µg/ml	26.331 µg/ml

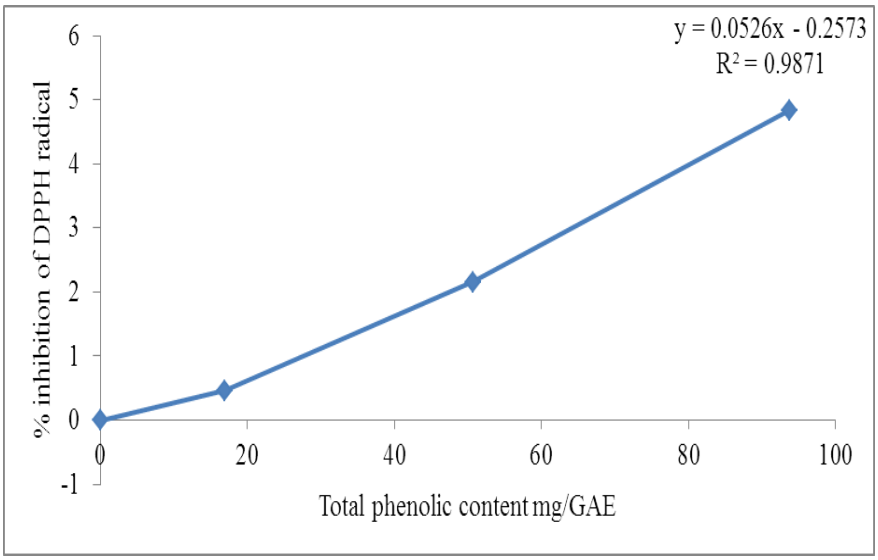

Figure 5.Linear correlation between antioxidant activity and total phenolic content of *E. bellidioides*

Table-4: Antioxidant activity of ascorbic acid (Standard)

Standard	Concentration (µg/ml)	Percentage scavenging	IC $_{50}$ value
Ascorbic acid	5	53.09	**< 5µg/ml**
	20	73.58	
	40	84.36	
	60	84.63	
	80	84.63	
	100	94.33	

A high correlation was observed between total phenolic content and antioxidant activity for both the plants. However the correlation between flavonoids and antioxidants were very low.

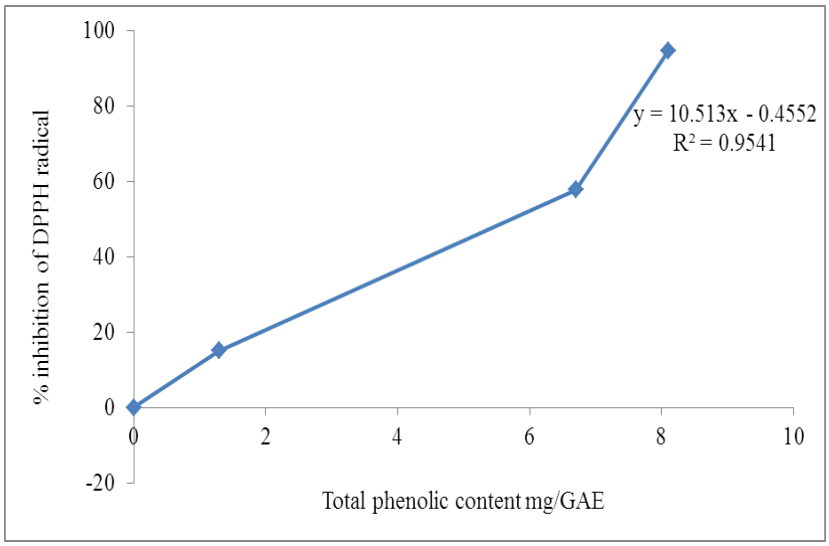

Figure 5.Linear correlation between antioxidant activity and total phenolic content of *L. camara*

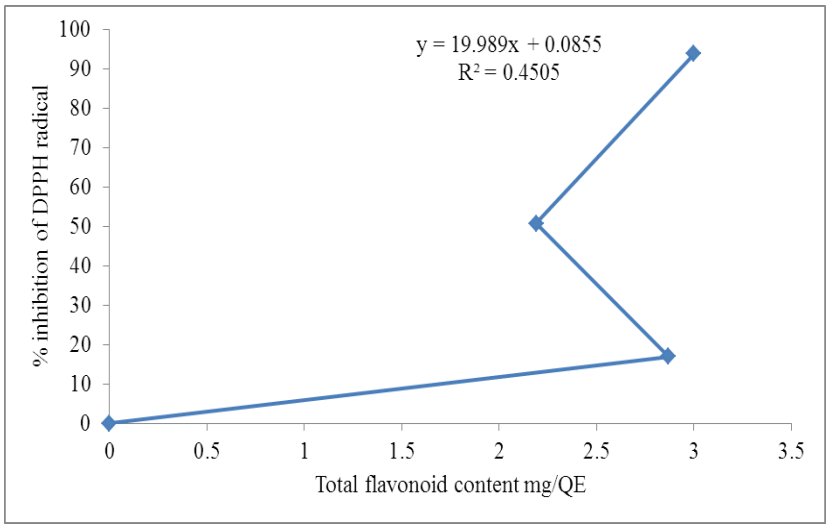

Figure 5.Linear correlation between antioxidant activity and total flavonoid content of *E. bellidioides*

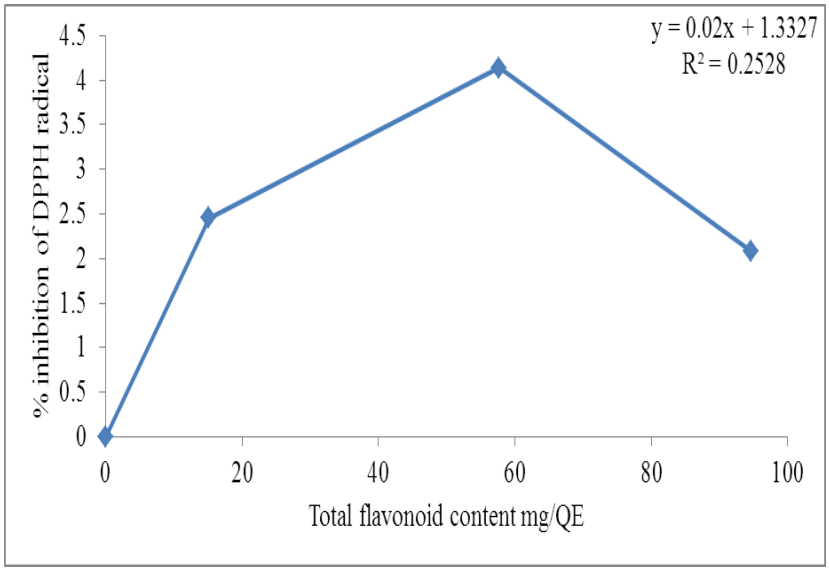

Figure 5.Linear correlation between antioxidant activity and total phenolic content of *L. camara*

Table-5: Correlation of Total Phenolic and Flavonoid Content with antioxidant activity

Correlation coefficient	Antioxidant activity	
	Lantana camara	***Erigeron bellidioides***
Total Phenolic Content	0.9541	0.9871
Total Flavonoid Content	0.2525	0.4505

DISCUSSION

The present study was performed in order to study the invasive plants posing as problematic weed in Nepal; *L. camara* and *E. bellidioides*. Although *L. camara* was traditionally used as a medicinal plant it has garnered a reputation for being an invasive and problematic weed. *E. bellidioides*, a rather poisonous weed has not been studied for its possible medicinal activities. Locally used as bug repellant, in diarrhea, cholera and hemorrhage, however its further research is still in its infancy. *L. camara*, on the other hand is an invasive weed but has a wide variety of traditional uses all over the world. It possesses various bioactive molecules elucidating their biological activity which also explains its traditional uses. According to a review article, there are various useful constituents in this plant such as mono- & sesquiterpenes, triterpenes, iridoid glycosides and phenyl ethanoid glycosides which explains the pertinent biological activity.[15] In our current study, we observed the presence of carbohydrates and flavonoids and phenols in all extracts of *L. camara*. Alkaloids were present only in hexane and ethyl acetate extract. Glycosides, tannins and terpenoids were found only in the methanol extract. *E. bellidioides* indicated the presence of alkaloids in Hexane and Methanol extract. Saponin was present in Ethyl acetate and Methanol.

Carbohydrates were found to be present in all extracts. Tannins were present in Hexane and Methanol and glycosides were found in Hexane and Methanol. Phenols and flavonoids were present in all three extracts.

Antioxidants have emerged as one of the most intriguing compounds with tremendous prospects and potential, gaining them immense popularity over the years. Antioxidants provide us a new resource for developing compounds and help us in preventing degenerative diseases caused by free radicals in our body. It possesses an ability to neutralize free radicals produced in our body due to oxidative stress. In this study, the methanolic extracts of both the plants exhibited good antioxidant activity which was quite similar to the standard (ascorbic acid). According to a study performed with methanolic extracts of different parts of *L. camara* assessed for antioxidant activity, the leaves revealed the best antioxidant properties having IC50 value at 16.02 µg/ml and fruits revealed a very poor antioxidant properties having IC50 value at 90.11µg/ml.[5] Another study revealed the leaves extract of *L. camara* presented antioxidant activity having IC50 value 16.13±0.35 µg/ml. HPLC was used to identify the phenolic compounds such as, gallic acid, chlorogenic acid, caffeic acid, quercentin and rutin. L. camara contained large amount of caffeic acid (14.69 mg/g dry sample). A good correlation between the antioxidant activities and the phenolic compounds content was also observed in this study.[16] Derived from phenylalanine in plants, phenols play a vital role in plant defense by protecting against pathogens and herbivore predators, as well as cell growth and cell division.[17] In our study, we observed a high correlation between total phenolic content and antioxidant activity for both the plants. In a previous study conducted in different parts of *L. camara*, the leaf extract presented with highest phenolic content (245.5±3.54 mg GAE/g) which was attributed to the antioxidant activity.[5] It can be reasonably concluded that the antioxidant activity may be attributed to the active ingredient of the plants.

CONCLUSION

The findings of this study revealed that the methanolic extracts of *L.camara* and *E. bellidioides* has the highest antioxidant activity and total phenolic content; the ethyl acetate extract has the highest flavonoid content among other extracts. In conclusion, the methanolic extracts of both plants showed DPPH radical scavenging activities quite similar to ascorbic acid, which was used as a standard. A very high correlation was observed between the total phenolic content and the antioxidant activity. Hence we can conclude that this correlation

may be attributed to the high antioxidant activity. Both the plants may serve as a promising natural source of antioxidants and are suggested for further investigation.

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