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PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAVES OF *Elsholtzia ciliata* (Thunb.) Hyl. (LAMIACEAE)

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Abstract

The present study aimed to determine the antioxidant potential and phytochemical screening of *Elsholtzia caliata* leaves. Five different assays i.e Ferric reducing antioxidant power (FRAP), DPPH free radical scavenging activity, Metal chelating activity (MCA), Total phenolic contents (TPC) and ABTS were used to find out the antioxidant potential. For this purpose the plant extract was prepared and then fractionated by using n-hexane, butanol, chloroform, ethyl acetate and aqueous extracts. The presence of different phytochemicals like Alkaloid, flavonoid, saponins, tannins, terpenoids and cardiac glycosides were tested by different qualitative assays using different solvents. Total phenolic content (TPC) ranged from 50.91-11.69 mg/ml of standard gallic acid and highest value shown by n-hexane. Percentage bound iron in metal chelating activity ranged from 96.29-74.07%. Highest value was shown by n-hexane. In FRAP assay the highest value was shown by chloroform and lowest by aqueous extract. In DPPH free radical scavenging activity, the values varied from 99.33-46.17 mg/ml. In ABTS the highest value was shown by butanol and lowest by ethyl acetate extracts. In the present work, it was found that *Elsholtzia caliata* exhibited higher antioxidant activity with high amount of phenolic contents. Therefore, it can be concluded that the phenolic compounds are mainly contributing to the antioxidant potential of selected plant species.

Keywords: Antioxidant, Phytochemical, *Elsholtzia caliata*, Pharmacolog

Introduction

The medicinal plants are effective for healing and for protection of human diseases like cancer, diabetes and cardiovascular disorders (Modak *et al*., 2007; Tiwari *et al*., 2013) because of the presence of secondary metabolites (Wadood *et al*., 2013). There are number of plants which have shown useful antioxidant potential by their constituents. Carotenoids, tocotrienols, flavonoids, cinnamic acids, folic acid, benzoic acids, ascorbic acid, tocopherols, etc., are some therapeutic agents produced by the plant for their survival in environment. Betacarotene, alpha tocopherol and ascorbic acid are the mostly used antioxidants to cure diseases (Bharti *et al*., 2012). Antioxidant agents found in different parts of the plants that protect the cells of human body from the side effects caused by reactive oxygen species (ROS). ROS such as hydrogen peroxide that play main function in the proliferation of many diseases like cancer.

Antioxidants found in plant materials inhibit the action of free radicals found in humans and protect the body from many lethal diseases leading to death (Oluwajuyitan *et al*., 2021). There is spreading a trend in the whole world for finding the unexploited resources of medicinal plants. Reactive oxygen species (ROS) is a way of the threat in foods lowering the self-stability and in living organisms causing serious disorders and diseases. Present research is focused on identifying naturally occurring antioxidants present in plants (Conti *et al.*, 2016).

Elsholtzia ciliata belongs to family Lamiaceae. It is considered to be one of the richest family from medicinal point of view. Lamiaceae species have been studied to have a broad range of biological activity, and a wide range of phytochemicals (Rehan *et al.*, 2014). *Elsholtzia ciliata* spreads mainly by small seeds. These seeds have a very high percentage of germination. They are most likely spread mechanically. The phytochemical evaluation indicates that it is full of alkaloids, flavonoids, tannins, and oligosaccharides (Ijeh *et al*., 2004). In many parts of the world it has been used in past as a culinary and medicinal herb to cure diseases. However, the use of this herb for such objectives was not yet fully in focus except with few traditional methods. The herb acts mainly on the digestive and nervous systems, stomach cramps and indigestion. The leaves can be harvested in the whole growing season and then used in fresh or dried form. Therefore, in the present study, we evaluated the phytochemical constituents of extracts of this plant, and determined their antioxidant.

Materials and Methods

Plant collection and preparation of extract: Leaves of *Elsholtzia caliata* collected from Swat by Prof. Dr. Anjum Perveen and dried at room temperature and grounded to powdered form. Standard maceration techniques were employed for obtaining different extracts in solvents of different polarity i-e. n-hexane, chloroform, ethyl acetate, butanol and water.

Phytochemical screening: The phytochemical screening of extracts were performed using standard procedures by following Ayoola *et al.* (2008).

Determination of antioxidant activity: The parameter considered to verify the antioxidant activity included FRAP after Benzie & Strain (1996); DPPH according to Lee *et al*. (1998); MCA after Dinis *et al.* (1994); ABTS was performed by method of Miller *et al.* (1993); TPC according to Makkar *et al*. (1993).

Table 1: Phytochemicals Assessment

Tests	n-hexane	Ethyl acetate	Chloroform	Butanol	Aqueous
Flavonoids	+	+	+	+	_
Phytosterols	_	+	+	+	+
Cardiac Glycosides	_	+	+	+	+
Tannins	_	_	+	+	+
Anthraquinones	+	_	+	_	+

Results

In present study phytochemical analysis and antioxidant activity were carried out according to standard procedures. Their results are described below:

1. Phytochemical screening: Phytochemical analysis involved just qualitative identification of taken plant constituents. The results of phytochemical analysis of leaves of *Elsholtzia caliata* revealed the presence of Flavonoids, Terpenoids, Saponins, Anthraquinones, Cardiac glycosides and reducing sugars. Tannins are also present. The results of phytochemical screening are shown in **Table-2.**

2. Antioxidant Activity of extracts: The following standard procedures were used to find the antioxidant activity of the plant.

1. DPPH free radical scavenging activity: The highest value was indicated by n-Hexane and lowest by aqueous and the percentage scavenging activity of each extract was calculated as shown in **Fig. 1**.

Ferric Reducing Antioxidant Power (FRAP) Assay: FRAP assay was performed and the results were compared with standard ferric chloride curve (**Fig. 2)**. Results showed that Chloroform extract showed highest value while the aqueous extract showed the lowest value of FRAP assay in **Fig. 3.**

3. Total Phenolic Content (TPC): TPC was performed and absorbance of the solvents was noted at 725nm, which was then compared with standard Gallic acid curve (**Fig. 4**). The Results showed that n- Hexane with the highest value and aqueous with lowest value of the Total Phenolic Contents. The results are formulated in **Fig. 5.**

4. Metal chelating activity: Percentage bound iron was calculated by subtracting the value of control absorbance from sample absorbance. The results showed that the maximum percentage bound iron of ferrozine-ferrous complex formation was shown by Chloroform extract which was 98.76% whereas Butanol extract showed lowest value 74.07% as shown in **Fig. 6.**

5. ABTS Assay*:* ABTS assay was performed and absorbance of different extracts was noted at 734nm. Percentage inhibition was calculated. The results showed maximum value of percentage inhibition of the Butanol extract and minimum of Ethyl acetate as shown in **Fig. 7.**

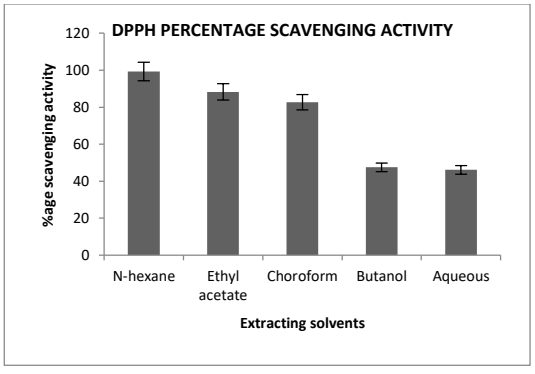

Fig 1: %age scavenging activity of different extracts of *E. caliata*

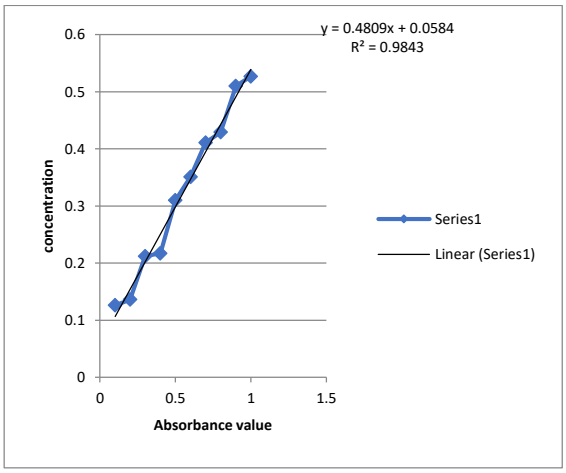

Fig. 2: Standard curve of Ferric chloride

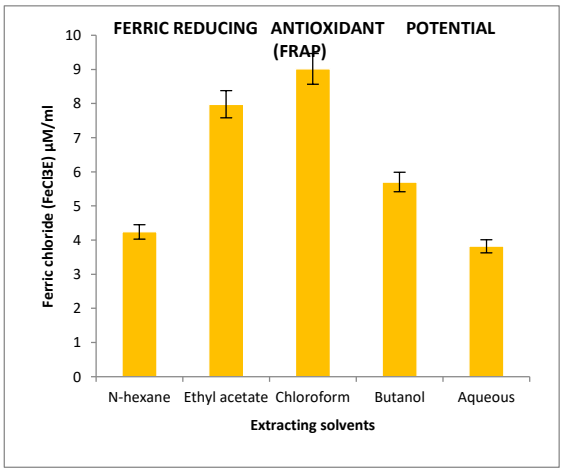

Fig 3: Graphical representation of Ferric reducing antioxidant power (FRAP) assay of Different extracting solvents of *E. caliata*

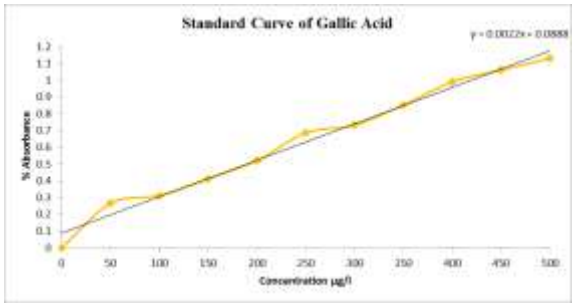

Fig. 4: Standard curve of Gallic acid

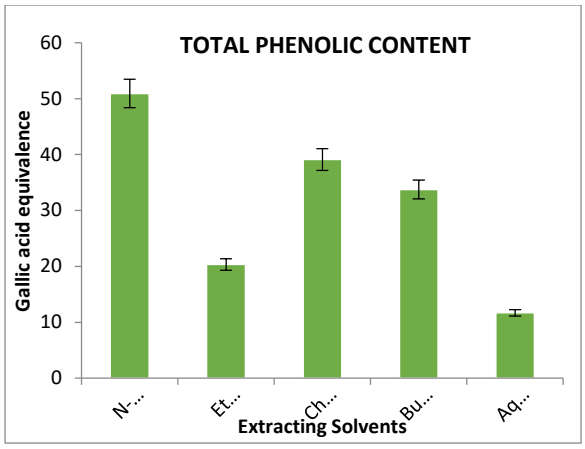

Fig 5: Graphical representation of total phenolic content present in different extractingSolvents of of *E. caliata* in mg/ml of Gallic acid

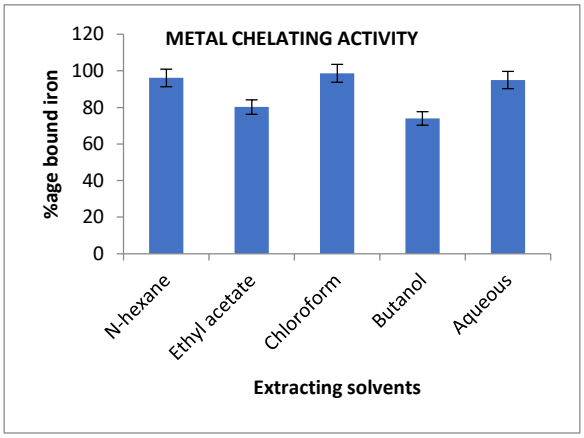

Fig 6: Graphical representation of Metal chelating activity of different extracting solvents of *E. caliata*

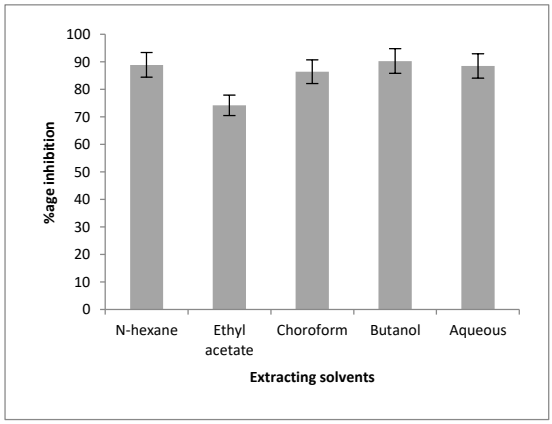

Fig: 7 Graphical representation of ABTS of different extracts of *E. caliata*

Discussion

Medicinal qualities of plants have been taken as an important step to discover medicines in the past few years. Plants form a diverse group of secondary metabolites with antioxidant activity. Antioxidants block the action of free radicals in living organisms which have been found in the proliferation of many diseases, infections and in the aging process. A vital role is being played by free radicals in processing the different biological processes which are vital for the body. They have their role in cell-signaling mechanism taking place in living organisms. This indicate that free radicals are also required but at the same time lethal for the body in case of excess amount. Hence there are number of mechanisms in order to hinder free radical to cause damage. The damage was repaired with the help of many enzymes found freely or attached in body like superoxide, reductase, catalase, and peroxidase. In addition to this, the antioxidants play a key role in these defense systems which are mostly vitamin C, vitamin E, vitamin A and polyphenols (Okwu & Josiah, 2001). In the present study, the chemical composition and antioxidant activity of *Elsholtzia caliata leaves* extract were calculated. The presence of flavonoids, saponins alkaloids, tannins and cardiac glycosides were detected as exact amount was not confirmed because it was a qualitative experiment. In some extracts they were in large amount and in some extracts the polyphenols were in small amount. DPPH radical free scavenging activity, (TPC) total phenolic content, (MCA)metal chelating activity, ABTS and reducing power (FRAP) of different extracts were 9.83 %, 50.91 mg/g gallic acid equivalent, 96.29%, 90.31% and 9.0 respectively. These are the highest values shown by some of the extracts. Results of the work had shown that the phytochemicals were responsible for medicinal effects of this plant. Aqueous, n- hexane, chlorofom and methanol extracts of leaves were analysed qualitatively for tannin, saponin and alkaloids.

Conclusion

Results of the work had shown that the phytochemicals are responsible for medicinal effects of this plant. Aqueous, n- hexane, chlorofom and methanol extracts of leaves were analysed qualitatively for tannin, saponin and alkaloids.

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