Research Article

Antioxidant Activity of Six Selected Medicinal Plants, Pakistan

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Abstract | Presently, there is universal interest to discover new and safe antioxidant compounds from natural sources, to reduce oxidative injury to the cells and overcome oxidative deterioration of foods affecting human life. The study was designed to assess the antioxidant capability of six selected medically principal plants; *Hyssopus officinalis, Origanum vulgare, Thymus vulgaris, Glycyrrhiza glabra, Cordia latifolia* and *Zizyphus jujuba*. Antioxidant activity was determined by total phenolic content determination, Pyrogallol method, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical scavenging assay, and free-radical-scavenging assay. Results revealed that *O. vulgare* and *T. vulgaris* possess high free radical scavenging capability and significant antioxidant activity. On the other hand, *H. officinalis* plant extracts exhibited low free radical scavenging ability and antioxidant potential. Obtained knowledge via present study have been extended further for isolation of potential bioactive components with natural and inherent antimicrobial and antioxidant properties.

Received | March 31, 2021; **Accepted** | January 19, 2022; **Published** | June 20, 2022
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Citation | Liaqat, I., Bibi, U., Arshad, M. and Arshad, N., 2022. Antioxidant activity of six selected medicinal plants, Pakistan. *Journal of Innovative Sciences,* 8(1): 13-19.
DOI | https://dx.doi.org/10.17582/journal.jis/2022/8.1.13.19
Keywords | Antioxidant activity, *Origanum vulgare*, *Thymus vulgaris*, *Zizyphus jujuba*, DPPH assay

1. Introduction

Among 6,500 medically important plant species reported in Asia, about 6000 species of include flowering plants, among which, 124 species are important from medical point of view (Ahmed *et al*., 2014). In past, different studies have published significantly important information about medicinal plants existing in Pakistan (Liaqat, 2017; Liaqat *et al*., 2016; 2017; Rashid *et al*., 2019). However, limited information is available from recent studies (Umair *et al*., 2017).

Medicinal plants are important owing to their ability of chemical compounds and various plant active components were isolated in early 19th century. For example, analgesic medicines (codeine, morphine) are obtained from opium; digitoxin from *Digitalis purpurea* and *D. lanata* and cocaine from *Erythroxylum coca* (Menger *et al*., 2013). The drug discovery process is complex. Only a plant ecologist or ethnobotanist/ ethnopharmacologist can collect and identify their desired medicinal plant(s) easily.

One of the main factor contributing to the various ailments both acute and chronic such as arthritis, cardiovascular and neurodegenerative diseases is oxidative stress. Despite the massive antioxidant enzymes and molecules, sometimes these agents may not be sufficient to detox system from the oxidative damage caused by physiological, environmental and other pathological conditions, which enhance generation of free radicals. These free radicals consisting of superoxide, peroxyl radical and hydroxyl radical usually cause lipid pathological conditions, which enhance generation of free radicals. These free radicals consisting of superoxide, peroxyl radical and hydroxyl radical usually cause lipid peroxidation, protein degradation and DNA oxidation thus leading to various chronic diseases as mentioned above (Jayathilake *et al*., 2016). Antioxidant compounds protect body from serious diseases by terminating the attack of free radicals. Almost every organism has enzymes (catalase, super-oxide dismutase) and compounds (flavonoids, and compounds, flavonoids, phenolic acids, polyphenols, ascorbic acid, tocopherol and glutathione). However, any pathological or physiological condition may trigger imbalance and necessitates the help from external supplementation to maintain the oxidative balance in body. Associated toxicity with synthetic compounds for example, butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) recommend usage of natural plant based antioxidants. Medicinal plants possess various antioxidants including terpenoids, limonene, flavonoids, curcumin, coumarins, gallic acid, carotenoids, tannins, eugenol, β sitosterol *etc*. which have the potential to protect against damage caused by reactive oxygen species (Jawaid *et al*., 2014).

Glycrrhiza glabra (Fabaceae) is a herb of commercial importance *viz*., glabridin, thus attracting various pharmaceutical and food sectors (Esmaeili *et al*., 2019). There is a wide distribution of Cordia (Family Boraginaceae), a genus contains 300 species or more, in Africa, Asia and America. Owing to the various ethnobotanical and ethnopharmacological aspects, scientific studies of this species have intensified (Oza and Kulkarni, 2017). Siddiqui *et al*. (2006) reported for the very first time two new abietane diterpenes from *Cordia latifolia*, thus signifying its pharmacological importance. The medicinal plant *Hyssopus officinalis* (family Lamiaceae), is novel medicinal plant present in Asia. Existing literature has shown important phytochemical contents and biological activities (Fathiazad *et al*., 2011). *Orignaum vulgare*, a popular perennial shrub and flavoring herb, is valued for several biological properties. Its antibacterial and antioxidant activities as well as cytotoxicity associated to its active components such as carvacrol and thymol components are well studied in literature (Coccimiglio *et al*., 2016). *Thymus vulgaris,* an aromatic plant is equally important due to its antioxidant as well as antimicrobial properties owing to existing phenolic compounds (El-Guendouz *et al*., 2019). *Zizyphus jujuba Mill*., is also a great crop in Asia. Various bioactive components found in the leaves of this plant confer it important physiological and pharmacological properties. This plant flavonoid possesses anti-aging, anticancer, antidiabetic, cardio protective and neuroprotective effects (Zhang *et al*., 2019).

There is not much data on the antioxidant potential of medicinal plants although found in Pakistan (Liaqat, 2017; Liaqat *et al*., 2016, 2017). Though biological screening of *in vitro* activities (anti-inflammatory, anti-cancer and antioxidant activities are well known. With above background, the current study has been aimed to analyse the antioxidant potential of six Pakistani medicinal plants.

2. Materials and Methods

2.1 Plants collection and extraction

First, the mature leaves of *G. glabra*, *C. latifolia*, *H. officinalis*, *O. vulgare*, *T. vulgaris* and *Z. jujube* were collected and dehydrated below 40°C in a chamber. After dehydration, powder was prepared by using mechanical grinder and finally stored in an air-tight container. Methanol was used for extracting the plants dried powder material. Semisolid mass obtained after the complete removal of solvent under reduced pressure. It was lyophilized and dissolved in methyl alcohol for the current study.

Various methods including total phenolic content determination, DPPH free-radical and OH radical scavenging activities and Pyrogallol method determination were used for determining the antioxidant potential of six selected medicinal plants.

2.2 Assay for total phenolic content (TPC)

Following spectrophotometric method, total phenolic content was determined. Three sets of test tubes were taken, washed and incubated at 150°C. 0.1 mL sample was blend with Folin–Ciocalteu reagent. 01 mL quantity of Na_2CO_3 solution (7%) was mixed after

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five minutes followed by adding autoclaved distilled water and mixing, completely. After this, the solution was placed in incubator in the absence of light for 90 minutes at 25°C and then the optical density (OD_{750}) was noted. TPC was measured by drawing calibration curve, prepared using gallic acid.

2.3 Diphenyl-1 Picrylhydrazyl (DPPH) free radical scavenging assay

This assay was used to determine the electron or hydrogen atom donating potential of pure compounds. In brief, methanolic stock solution of DPPH from 20 ˚C was used to prepare the working solution at OD_{517} to get final absorbance of 0.98±0.03. At different concentrations of 10 – 500 µg/mL, the 3 mL solution aliquot was blended with 100 µL of the sample. Mixture was shaken completely and incubated in the absence of light at normal temperature for 15 minutes. After this the absorbance recorded at 517 nm. Measurement of scavenging activity was determined as below:

Scavenging effect (%) = [(control absorbance - sample absorbance)/(control absorbance)] × 100

2.4 Hydroxyl radical scavenging assay

Extracted solution (400 µL) of every sample (0.2, 0.5, 0.8, and 1.2 mg/mL) was blended with 200 µL of O-phenanthroline (7.5 mmole/L), Phosphate buffer (1 mL), ferrous sulphate (7.5 m mole/L) and 0.1% Hydrogen peroxide. Dilution was made to 5mL with H_2O and incubated at normal temperature for half an hour. At 510 nm, absorbance was measured. 400 µL of water was added instead of sample for control preparation. The scavenging performance of scavangers on OH radical was calculated by using the given below equation:

Scavenging activity (%) = (Absorbance of sample - Absorbance of control / Absorbance of control) × 100

2.5 Pyrogallol method determination

3 mL of tris buffer was mixed with 1mL sample and incubated of 25 ˚C for 25 minutes in water bath. 20 µL of pyrogallol (45 milli mole) preheated at 25 ˚C was added and measured spectrophotometrically at 325 nm. Termination of reaction was done by adding 20 µL of 8 mM HCl. Immediately, the absorbance was noted again after passing 5 minutes. The scavanging activity of superoxide anion was measured using the following equation.

Scavenging affect (%) = AS – AC/AC×100

2.6 Statistical analyses

Data presentation was in form of mean and standard error of means (SEM). Data was analyzed statistically by using one way analysis of variance (one-way ANOVA) at $p < 0.05$. Duncan's Multiple Range (DMR) test was used for sorting out the differences in mean with the help of windows SAS 9.1.

3. Results and Discussion

Current study showed high antioxidant potential of *O. vulgare* and *T. vulgaris* are found in Pakistan and different parts of the world. *Oregano* and *Thymus* EO chemical nature depends upon the environmental conditions, geographical place, methods of extraction and other variables (Diniz do Nascimento *et al*., 2020). Chemotype (sabinyl/ cymyl source full of sesquiterpenes) and sensory factors such as sweet and floral varies depending upon geographical regions. Węglarz *et al*. (2020) reported different concentrations of rosamarinic acid, a compound rich for antioxidant potential during plant growth development, developing at blooming to the starting of seed growth in common and Greek oregano species.

3.1 Total phenolic contents (TPC)

TPC were highest (72.38 ± 1.73) in *Z. jujuba* and lowest in *H. officinalis* (9.563 ± 0.197) compared to other plants. Concentration of phenolic content was decreased in the following order; *Z. jujuba* > *O. vulgare* > *G. glabra* > *C. latifolia* > *T. vulgaris* > *H. officinalis*. The value of phenolic content was 41.11 in *O. vulgare*, 33.36 in *G. glabra*, 21.66 in *C. latifolia*, 9.563 in *T. vulgaris* (Figure 1). As a standard, gallic acid was.

3.2 DPPH free radical scavenging activity

The DPPH is known to abstract the fluctuating H and the potential to scavenge the DPPH radical is associated to the blockage of peroxidation of lipid. Expression of this activity was showed after 30 minutes of reaction. Thriple time the measurements were noted and their scavenging affects were determined according to the percentage of scavenged DPPH. Radical scavenging activities of DPPH varied from 12.82 % to 106.06 %. *O. vulgare* has the highest antioxidant capacity 106.06% of DPPH inhibition), followed by *T. vulgaris* extract (100.69%), *G. glabra* (91.45 %) and *Z. jujuba* (43.30%). *C. latifolia* extract showed the lowest antioxidant capacity (12.82 %) (Figure 2).

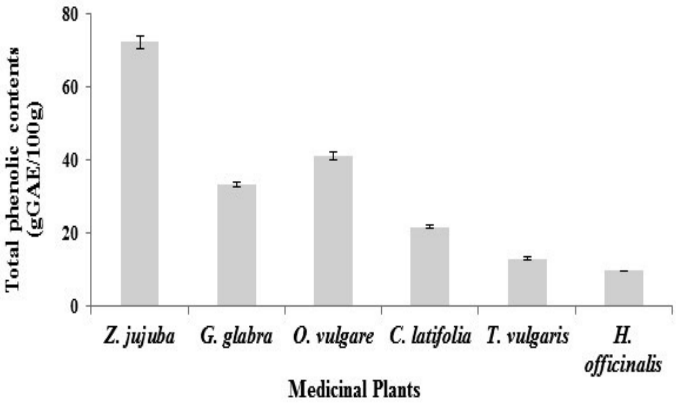

Figure 1: Determination of total phenolic contents (mg GAE/g dry wt.) in six medicinal plants *in vitro*. Data represent mean ± SD, using one way analysis of variance (one way ANOVA), data was statistically analyzed. Differences in means were sorted out using Duncans Multiple Range (DMR) test with the help of SAS 9.1 for windows. The P< 0.05 was considered statistically significant.

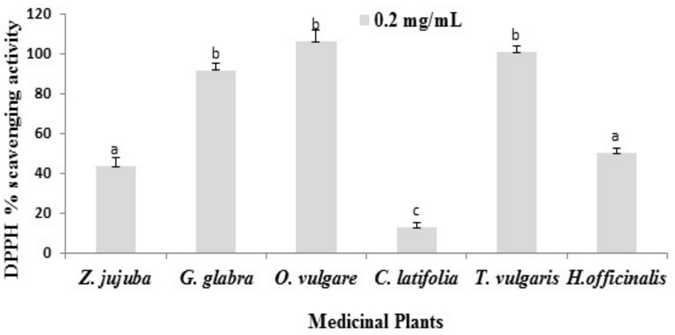

Figure 2: *In vitro* antioxidant activity of six medical plans by DPPH assay. Data represent mean ± SD, using one way analysis of variance (one way ANOVA), data was statistically analyzed. Differences in means were sorted out using Duncans Multiple Range (DMR) test with the help of SAS 9.1 for windows. The P< 0.05 was considered statistically significant.

3.3 OH radical scavenging assay

The scavenging effect of various plant fractions was concentration dependent. *T. vulgaris* displayed strong hydroxyl radical scavenging activity (100.69%), followed by *O. vulgare* (100.05%), *G. glabra* (98.65%), *Z. jujuba* (76.85%), *H. officinalis* (79.16%), *C. latifolia* (21.68%) at 0.2 mg/mL. The percentage scavenging effect of all plant extracts except *H. officinalis* at different concentration (0.5 mg/mL, 0.8 and 1.2 mg/mL) were 100%. *T. vulgaris* and *O. vulgare* extracts showed highest hydroxyl radical scavenging activity 100.69 % and 100.05%, respectively at 0.2 mg/mL. While *C. latifolia* showed lowest hydroxyl radical scavenging activity (Figure 3). The percentage scavenging effect of *H. officinalis* extracts at 0.5, 0.8 mg/ mL was 26.4% and 13.6%, respectively (Data not shown).

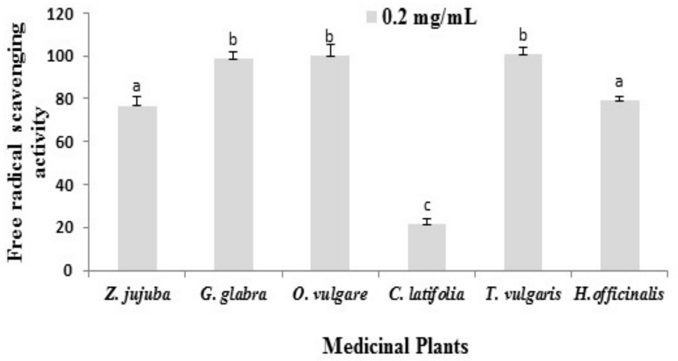

Figure 3: Hydroxyl radical scavenging activity. Data represent mean ± SD, using one way analysis of variance (one way ANOVA), data was statistically analyzed. Differences in means were sorted out using Duncans Multiple Range (DMR) test with the help of SAS 9.1 for windows. The P< 0.05 was considerd statistically significant.

3.4 Pyrogallol method determination

Pyrogallol method was used for the determination of superoxide. Different fractions of scavenging effect of superoxide radicals of was compared with the alike doses/amount of pyrogallol (126.1 g/L). The superoxide scavenging activities at 0.2 mg/mL concentration were in the following order; *O. vulgare* > *T. vulgaris* >*G. glabra* > *Z. jujuba* > *C. latifolia* > *H. officinalis*. *O. vulgare* plant showed the highest antioxidant potential while *Z. jujuba* plant showed the lowest antioxidant potential at 0.2 % concentration (Figure 4).

Natural products capability of electron donation is studied by 2, 2 – DPPH radical solution bleaching of purple coloration. This procedure is based on addition of antioxidants for scavenging the DPPH that decolorize the DPPH solution. The color change degree is proportional/related to the potency and concentration of antioxidants (Kedare and Singh, 2011). In the current study, among all the plants studied *O. vulgare*, *G. glabra*, *H. officinalis*, *C. latifolia* showed higher inhibition percentage. While *G. glabra* and *Z. jujuba* showed lower inhibition percentage. Similar findings were made by Sylvie *et al*. (2014), who studied the antioxidant and antiradical properties of methanolic extracts *in vitro* using medicinally important plants. Authors reported that extracts of *Acalypha racemosa*, *Garcinia lucida* and *Hymenocardia lyrata* showed antioxidant properties. Roots of *H. lyrata* exhibited the highest antioxidant activity while its bark was rich in total phenol content.

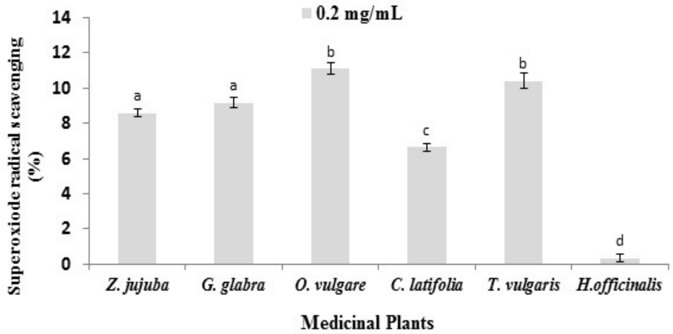

Figure 4: Superoxide radical scavenging activity. Data represent mean ± SD, using one way analysis of variance (one way ANOVA), data was statistically analyzed. Differences in means were sorted out using Duncans Multiple Range (DMR) test with the help of SAS 9.1 for windows. The P< 0.05 was considerd statistically significant.

Both solvent and polarity is important for determining the phenol content. Besides, various intrinsic and extrinsic factors such as extracting solvent, storage and handling also affect it. The plants are a treasure of wide variety of several antioxidants and possibly the compounds having antioxidant potential differ and all can't be measured with exact accuracy. Todate various methods have developed to study the antioxidant potential of various plant components. Current study uses the extracts of *Z. jujuba, O. vulgare, G. glabra* and *C. latifolia* showed high phenolic content. *T. vulgaris* and *H. officinalis* showed lower phenolic content. Recently, Johari and Khong (2019) evaluated for antioxidant potential and TPC of *Pereskia bleo* leaves. Authors observed that methanolic extract possess higher total phenolic contents and antioxidant activity compared to other extracts (hexane, chloroform), thus indicating methanolic extract ha higher potential for scavenging free radicals when compared with other plants.

Free radical (hydroxyl radical) scavenging activity of *T. vulgaris* extracts might be due to the presence of high molecular weight phenolics such as catechin, and rutin derivatives. Though, thymol was reported to be the major constituent in various investigated applications of *T. vulgaris* regardless of extraction technique of geographic origin. The variable levels of thymol were recorded between 18.11% and 55.44% (Diniz do Nascimento *et al*., 2020). *Thymus* sp. possess biological properties that are directly affected by chemical constituents, determined by variations in temperature, humidity, harvesting and post-harvest factors, alongwith vegetative stage (Diniz do Nascimento *et al*., 2020). The *Z. jujuba* extracts exhibits remarkable lower hydroxyl radical scavenging capacity rendering, their utilization in different ailments associated with oxidative stress. Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms and food. It is rapidly decomposed in to oxygen and water and this may produce hydroxyl radical that can initiate lipid peroxidation and cause DNA damage. Reduced form of molecular oxygen superoxide anion produced by gaining single electron. This is an initial free radical produced from electron transport system of mitochondria. Four different chain reaction used for generating energy by mitochondria and cause reduction of oxygen to water. Escaping electrons from chain reaction react directly with oxygen and produce superoxide anion. It plays crucial role in other reactive oxygen species formation, including hydroxyl radical hydrogen peroxide, or singlet oxygen in systems of living organisms (Lee *et al*., 2004).

In the present study, it was observed that *O. vulgare* and *T. vulgaris* have higher superoxide scavenging activities. The EO of *Oregano* and *Thymus* has chemical nature, determined by the environmental factors, geographical origins, extraction methods and other variable factors (Diniz do Nascimento *et al*., 2020). For example, using different methods, such as DPPH and reducing power, it was noted that reducing power and scavenging radicals potential of O. vulgare vulgare was higher significantly compared to *O. vulgare hirtum*. It was concluded that carvacrol and thymol are powerful reductants. Regarding environmental effect, shade drying EO of *O. vulgare* (31.48%) with carvacrol (45%) exhibited comparatively high DPPH radical scavenging ability compared to oven drying method (26.19%) (Ozdemir *et al*., 2018). It was observed that different methods including FRAP and DPPH assays showed variable antioxidant potential of EO from *O. vulgare* in different geographical origins (Diniz do Nascimento *et al*., 2020). Similarly, Aprotosoaie *et al*. (2019) also used FRAP and DPPH assays to evaluate EO from thyme and observed that the antioxidant activity was significantly influenced under lab conditions. It was concluded by authors that 55.44% thymol resulted in high antioxidant potential. Lagouri *et al*. (2011), reported that the methanolic extracts of *O. vulgare* and *T. vulgaris* against DPPH radical were more potent compared to the hexane extracts and *O. vulgare* showed stronger activity compared to thyme. *G. glabra*, *C. latifolia* and *H. officinalis* have intermediate superoxide scavenging activities, while *Z. jujuba* plant showed lowest scavenging activity.

The synthetic replacement with natural antioxidants may have some advantages. In the current study, analysis of free radical scavenging activity, DPPH free-Radical test and total phenolic contents showed that mainly the *T. vulgaris* and *O. vulgare* have good antioxidant potential and high free radical scavenging activity. While extracts of *H. officinalis* plant showed low antioxidant activity and capacity of free radical scavenging. Research on medicinal plants will discover new therapeutics approaches with precise policy and appropriate guidelines regarding use of the traditional herbs ensuring scientific evidence, safety and understanding.

Novelty Statement

This work reported for the first time that mong six medicinal plants six medicinal plants; *G. glabra, C. latifolia, H. officinalis, O. vulgare, T. vulgaris* and *Z. jujube*, mainly the *T. vulgaris* and *O. vulgare* have good antioxidant potential and high free radical scavenging activity. While extracts of *H. officinalis* plant showed low antioxidant potential and free radical scavenging capacity.

Author's Contribution

Najma Arshad is the supervisor and planned the work. Umaima Carried out the experiments. Iram Liaqat wrote the manuscript. Iram Liaqat and Dr. Najma analysed the data. Iram Liaqat, Muhammad Arshad and Najma Arshad supervised the complete study, guided in preparing manuscript. All authors approved the manuscript.

Conflict of interests

The authors have declared no conflict of interest.

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