"ESTIMATION OF RADICAL SCAVENGING POTENTIAL OF SELECTED MEDICINAL PLANTS OF LAHORE, PAKISTAN"

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ABSTRACT: In the current research, *in vitro* antiradical potential of some medicinal trees of Lahore, Pakistan was investigated. Various fruits and pods of 25 medicinal plants (*Prosopis juliflora*, *Carissa carandas*, *Ceiba speciosa*, *Heterophragma adenophyllum Cestrum diurnum Jacaranda mimosifolia*, *Diospyros malabarica*, *Terminalia bellerica*, *Ficus lyrata*, *Diospyros peregrine*, *Cinnamomum verum*, *Erysimum cheiri*, *Buchanania lanzan*, *Withania somnifera*, *Fagonia arabica*, *Berberis lycium*, *Strychnos potatorum*, *Matthiola incana*, *Ziziphora tenuior*, *Centaurea behen*, *Rosa indica*, *Punica granatum*, *Lodoicea maldivica*, *Cassia absus*, *Celastrus paniculatus*) were extracted by ethanol. Different concentrations of fruit extracts (1, 0.5, 0.25, 0.125 mg/mL) were prepared to evaluate *in vitro* antioxidant activity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method was performed and compared with standards like Vitamin E, BHT (Butylated hydroxytoluene) and ascorbic acid. Many plant extracts exhibited 80% of DPPH radical scavenging activity. *F. Arabica, S. potatorum, M. incana, P. granatum, C. behen, W. somnifera*, *T. bellerica*, and *H. adenophyllum* showed significant anti-radical potential (98.4±0.52, 96.40±0.52, 96.42±0.51, 94.58±0.52, 94.47±0.50, 94.65±0.56, 94.55±0.50, 91.42±0.51% inhibition, respectively) at 1 mg/mL equivalent to standards *i.e.* Vitamin E, BHT and ascorbic acid inhibition (88.25±0.2, 78.1±0.3 and 40.51±0.17%), respectively. Moreover, the dose dependent activity was observed as decline in the percentage inhibition at lower extracts concentration. The 50% inhibitory concentration (IC50) was also determined for the extracts that varied from 0.014± 0.001 (*W. somnifera* and *J. mimosifolia*) to 2.69±0.001 (*B.lanzan*) mg/mL depending on the sample extracts. The natural antioxidants in plants may be used in foods and natural products as a substitute to synthetic antioxidants, which have side effects.

Keywords: Antiradical potential, *In vitro* Natural antioxidant, Medicinal plants, IC50, DPPH.

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INTRODUCTION

Reactive oxygen species (ROS) often known as free radicals are a cause of more than 100 ailments including diabetes, malignancy, carcinomas, CNS disorders, atherosclerosis, liver carcinoma and nephrotoxicity (Zengnin *et al*., 2014). ROS are different types of enacted oxygen, including superoxide, hydroxyl radicals ($OH^{\bullet}$), peroxide (H_2O_2) and singlet oxygen (O) affecting directly to tissues and contributes to the vascular failure (Zagouri *et al*., 2012). The utilization of medicinal plants everywhere throughout the world originates before the introduction of anti-infection agents and other synthetic drugs. Bioactive compounds from herbal origin have been appeared as a viable source of chemotherapy without harming living tissues (Yadav *et al*., 2014). Nowadays, some vegetables and flavors are being assessed for their medicinal properties in the cure of chronic ailments like cancer and Alzheimer (Ekor, 2013).

Clinical as well as experimental studies have proved that ROS has a vital role in the etiology of cancers and carcinomas (Varunraj *et al*., 2011). All living organisms including humans have antioxidant defense system protecting them from ROS damage. But sometimes natural antioxidant defense systems become insufficient and the use of dietary intake of antioxidant components becomes crucial (Ravikumar *et al*., 2014). It is recommended that fruits, vegetables and plants are the main source of natural antioxidants. Natural antioxidants can scavenge free-radicals; acting as scavenging agents and oxygen quenchers. Herbal foods being a major source of natural antioxidants have the capacity to neutralize the toxic ROS (Lekha *et al*., 2010).

Medicinal property of a plant to control certain disease is attributed to the antioxidant potential of its constitutive compounds such as phenols, terpenoids and flavonoids that work as chemo-preventive and anticancer by affecting metabolic enzymatic reactions taking part in the initiation of carcinogenic compounds and cellular arrest (Malik *et al*., 2012).

D. malabarica (Desr.) Kostel is highly medicinal with antimicrobial, antioxidant and blood purifying compounds (Ravikumar *et al*., 2014). *F. arabica L.* is considered as anticancer and neuroprotective, *B. lanzan Spreng* has antispasmodic, antioxidant and antimicrobial properties (Mashwani *et al*., 2013). *R. indica L.* contains cardio-tonic, diuretic, expectorant and antiviral properties (Zengin *et al*., 2014). *F. lyrata L.* contains antioxidant, antimicrobial and anticancer properties (Bidarigh *et al*., 2011). *H. adenophyllum Wall.* is used for various ailments including cancer and epilepsy (Rahmatullah *et al*., 2010). *C. paniculatus Wild* is used as anticancer and insomnia treatment (Lekha *et al*., 2010). *S. potatorum L.f.* has been used for the treatment of gonorrhea, leukeorrhea and other eye diseases (Yadav *et al*., 2014). *C. behen L.* has been used in the treatment of cancer and neurological disorders. *D. peregrina f. javanica Kosterm* have antimicrobial, antioxidant and anticancer potential (Malik *et al*., 2015). *C. carandas L.* is rich in iron, vitamin C and used in the treatment of anemia (Shafeeq *et al*., 2014). *C. verum J.* have strong antibacterial, antipyretic, antitumor, anticancer and neuroprotective compounds (Hamidpour *et al*., 2015).

MATERIALS AND METHODS

Fresh fruits, seeds and bark of the selected plants were collected from Lahore. The plants were identified and deposited in the herbarium of Lahore College for Women University, Lahore. i.e *P. juliflora* (Sw.) DC. (LCWU-15-128), *C. carandas* L. (LCWU-15-129), *C. speciosa* A.St.Hil. Ravenna (LCWU-15-130), *H. adenophyllum* Wall. (LCWU-15-131), *C. diurnum* L. (LCWU-15-132), *J. mimosifolia* D. (LCWU-15-133), *D. malabarica* (Desr.) Kostel. (LCWU-15-134), *T. bellerica* Roxb. (LCWU-15-135), *F. lyrata* L. (LCWU-15-117), *D. peregrina* f. *Javanica* Kosterm. (LCWU-15-136), *C. verum* J. (LCWU-15-137), *E. cheiri* L. (LCWU-15-138), *B. lanzan Spreng*. (LCWU-15-139), *W. somnifera* L. (LCWU-15-89), *F. arabica* L. (LCWU-15-140), *B. lycium* Royle. (LCWU-15-141), *S. potatorum* L.f. (LCWU-15-142), *M. incana* (L.) W.T.Aiton. (LCWU-15-143), *Z. tenuior* L. (LCWU-15-144), *C. behen* L. (LCWU-15-145), *R. indica* L. (LCWU-15-122), *P. granatum* L. (LCWU-15-146), *L. maldivica* (Pers.ex H. Wendl.) (LCWU-15-147), *C. absus* L. (LCWU-15-148), *C. paniculatus* Willd. (LCWU-15-149). The fruits, seeds and bark after washing were shade dried at room temperature i.e 20°C-30°C. The dried plants were pulverized into fine powder and stored for the future use. The powdered material was extracted with ethanol by maceration for seven days. The resulting crude extracts after filtration were concentrated by using a rotary evaporator following (Malik *et al*., 2015).

Determination of Anti-radical potential of extracts: Quantitative measurement of radical quenching potential was performed according to the method of Erasto *et al*., (2004). The commercial known antioxidants, butylated hydroxytoluene (BHT), Vitamin E and ascorbic acid were used as positive control for comparison. *In vitro* antioxidant activity of extracts was recorded by measuring the decrease in absorbance of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. Equal volume (2.0 mL) of ethanolic extracts / positive control (1mg/mL, 0.5mg/mL, 0.25mg/mL and 0.125mg/mL) and DPPH, contained in test tubes were incubated at 30°C for 15 minutes. Solution containing (DPPH and methanol) was used as blank. Absorbance was measured at 517 nm by UV-Vis spectrophotometer. The actual absorbance of extracts was calculated by subtracting the absorbance of ethanolic extracts, from absorbance of corresponding DPPH extracts. Decline in absorbance directed the antioxidant potential of extracts. Free radical scavenging action of extracts was estimated by the following formula. Percentage Inhibition of DPPH = [(Ablank – Asample)/Ablank] × 100

Determination of IC_{50} values: The IC_{50} value (mg/mL) is the actual effective plant concentration by which DPPH radicals were scavenged almost 50% of the original value. These values were calculated by interpolation by using GRAPHPAD PRISM software.

Statistical analysis: The tests were performed in triplicate and the results were interpreted as mean ± standard deviation. The IC50 was determined by GRAPHPAD PRISM software for extracts and standards.

RESULTS AND DISCUSSION

The antiradical activity of extracts was compared with the quenching potential of standard antioxidants i.e. Vitamin E, BHT and ascorbic acid (Figure-1). All the medicinal plants selected had remarkable free radical scavenging activity. During *in vitro* experimentation, all extracts presented the scavenging activity as *P. juliflora* (82.2±1.05), *C. carandas* (84.38±0.53), *C. speciosa* (82.34±0.56), *H. adenophyllum* (91.42±0.51), *C. diurnum* (91.42±0.51), *J. mimosifolia* (92.57±0.51), *D. malabarica* (88.54±0.50), *T. bellerica* (94.55±0.50), *F. lyrata* (94.59±0.52), *D. peregrina* (94.42±0.51), *C. verum* (68.51±1.33), *E. cheiri* (94.47±0.50), *B. lanzan* (22.42±0.51), *W. somnifera* (94.65±0.56), *F. arabica* (98.4±0.52), *B. lycium* (91.56±0.51), *S. potatorum* (96.40±0.52), *M. incana* (96.42±0.51), *Z. tenuior* (92.66±0.57), *C. behen* (94.47±0.50), *R. indica* (92.47±0.50), *P. granatum* (94.58±0.52), *L. maldivica* (91.41±0.52), *C. absus* (82.62±0.54), *C. paniculatus* (40.51±0.50) at 1 mg/mL concentration. The result showed a direct comparison of the percentage DPPH inhibition of medicinal plant

extracts with standard antioxidants, Vitamin E (88.25±0.2), BHT (78.18±0.3) and ascorbic acid (40.51±0.17). Ethanolic extracts of *F. arabia, S. potatorum, M. incana, P. granatum, C. behen, W. somnifera, T. bellerica* and *H. adenophyllum* showed substantial anti-radical potential (98.4±0.52, 96.40±0.52, 96.42±0.51, 94.58±0.52, 94.47±0.50, 94.65±0.56, 94.55±0.50, 91.42±0.51 at 1mg/mL equivalent to standards Vitamin E, BHT and ascorbic acid (88.25±0.2, 78.1±0.3 and 40.51±0.17). Moreover, the decreased inhibition of DPPH was observed with the deceased extracts concentration *i.e.* (0.5, 0.25, 0.125 mg/mL) as explained in Figure-1**.** All remaining plant extracts were found to be active against DPPH and demonstrated a wide range of DPPH scavenging activity. The result of our previous study revealed that these selected medicinal plants had higher terpenoid contents that may be the major contributing factors for the highest antioxidant potential of these medicinal plants (Malik *et al*., 2017).

The 50% inhibitory concentration (IC_{50}) was also determined for all the extracts (Figure-2) that varied from 0.014± 0.001 (*W. somnifera* and *J. mimosifolia*) to 2.69±0.001 (*B. lanzan*) mg/mL depending on the sample extracts. Figure-2 represents the overall comparison of IC_{50} of all extracts i.e. *P. juliflora* (0.045±0.005), *C. carandas* (0.057±0.001), *C. speciosa* (0.181±0.001), *H. adenophyllum* (0.083±0.001), *C. diurnum* (0.275±0.001), *J. mimosifolia* (0.014±0.001), *D. malabarica* (0.359±0.001), *T. bellerica* (0.357±0.001), *F. lyrata* (0.062±0.001), *D. peregrina* (0.53±0.001), *C. verum* (0.802±0.001), *E. cheiri* (0.148±0.50), *B. lanzan* (2.696±0.001), *W. somnifera* (0.015±0.001), *F. arabica* (0.149±0.001), *B. lycium* (0.804±0.001), *S. potatorum* (0.057±0.001), *M. incana* (0.189±0.001), *Z. tenuior* (0.577±0.001), *C. behen* (0.624±0.001), *R. indica* (0.06±0.001), *P. granatum* (0.271±0.001), *L. maldivica* (0.157±0.001), *C. absus* (0.684±0.001), *C. paniculatus* (2.19±0.001).

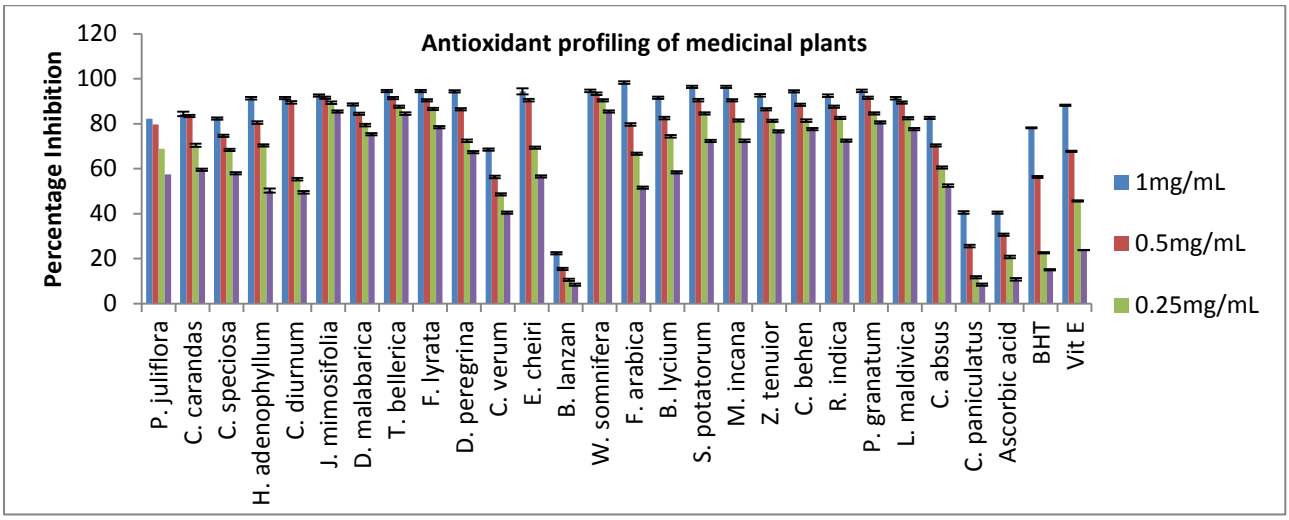

Figure-1. **Percentage inhibition of DPPH by ethanolic medicinal plant extracts.**

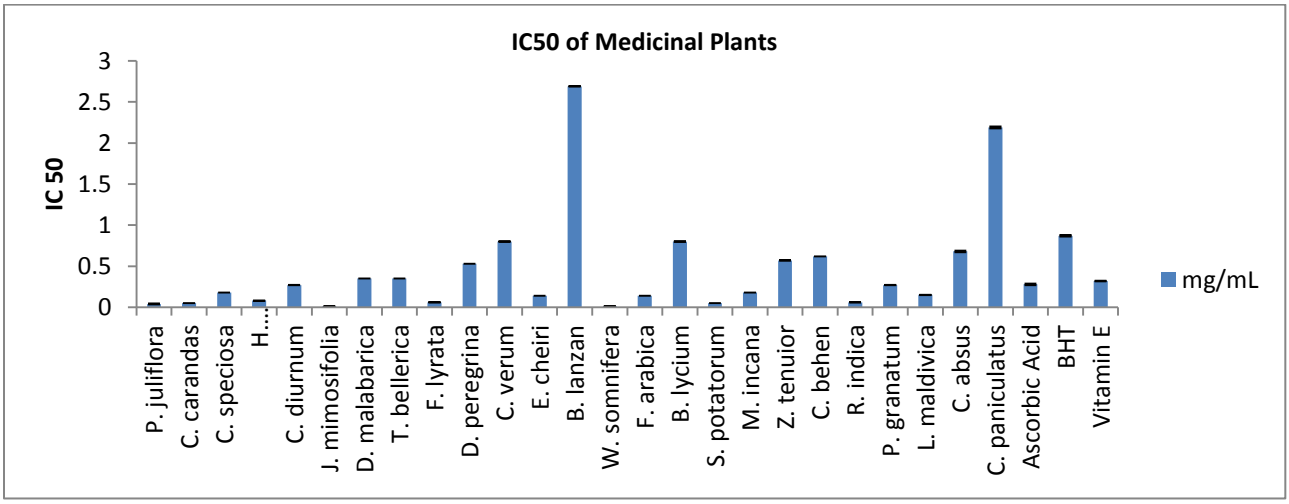

Figure-2: IC_{50} of ethanolic extracts of selected medicinal plants of region Lahore, Pakistan

In this study, ethanol extracts from 25 trees were evaluated for the antioxidant activity. The antiradical potential of the extracts was calculated by their ability to scavenge DPPH radical. Percentage DPPH inhibition was a common method to determine the antioxidant potential of bioactive compounds present in plants (Malik *et al.,* 2012). Results also revealed that all the extract tested had higher scavenging activity showing the presence of biologically active compounds. The presented results are also comparable with the findings of Shafeeq *et al*., 2014 and Hamidpour *et al*., 2015, who measured antioxidant potentials of leaves and fruits of medicinal plants against reducing oxidative stress. Antioxidant property may be due to the higher level of bioactive compounds reacting differently with free radicals (Yadav *et al*., 2014). Secondary metabolites such as tannins, tarpenoid, alkaloids, vitamins and flavonoids/anthocyanins sometimes act on free radicals synergistically or individually based on their structure and biological properties reported by Loizzo *et al*., 2010 and Varunraj *et al*., 2011).

The 50% inhibitory concentration (IC50) was also determined for the extracts that varied from 0.014± 0.001 (*W. somnifera* and *J. mimosifolia*) to 2.69±0.001 (*B. lanzan*) mg/mL depending on the sample extracts (Fig 2). The antiradical activity of the extracts showed their ability to give hydrogen or electron atom (Zengin *et al*., 2014). Free radical causes damage to the human body at cellular and tissue level and result into dangerous diseases like cancer and AIDS. These medicinal plants can be therapeutically useful in tissue injuries caused be free radicals. All the plant extracts tested quenched DPPH radical but, in different manners indicating the difference in reactivity of bioactive compounds with DPPH radical (Loizzo *et al*., 2010, Yadav *et al*., 2014).

Conclusion: The developing countries, particularly Pakistan, a big figure of population rely on folk medicine to treat chronic diseases. This experimental work will be helpful for the search of new bioactive compounds for human welfare.

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