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EVALUATION OF PHENOLIC COMPOUNDS IN SOME LOCALLY AVAILABLE FLOWERING PLANTS

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ABSTRACT

Phenolic compounds in plants are responsible for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. Plants synthesize a greater array of secondary compounds which having numerous medicinal properties that can be beneficial for human health and play a key role in health protection all over the world. The present study reveals that phenolic compounds are present in flower petals and to some extent in leaves. These are also related to antimicrobial and anti-oxidant activities of plant due to their high tendency to chelate metals. Phenolic possess hydroxyl and carboxyl groups, able to bind metals. Maximum phenolic contents were present in Dhalia flowers (468.61mg/100g) and minimum was found in Gainda leaves (5.34 mg/100g). Highest level of flavonoids was present in Jasmin flowers (58.97mg/100g) and mostly leaves did not possess flavonoid. Phenolic contents may be related to antibacterial and antioxidant activities as Dhalia Flower has maximum phenolic compounds, it exhibit maximum antibacterial activity and DPPH Scavenging activates (80 % and 16.65% respectively), others having less phenol did not show antimicrobial activities. Maximum antifungal activity was found in Morning glory flower (19 %) which may be related with Flavonoids contents which is maximum in Morning glory flowers.

KEYWORDS: phenolic compounds; antioxidant activities; flower; leaves; flavonoids and antimicrobial activities.

INTRODUCTION

Over the past few decades, the plant kingdom has been a valuable source of natural products for curing a variety of human diseases. This is why significance of plants having medicinal importance owing to their several beneficial effects on human health has been understood and hence, they are now playing a key role in health protection all over the world.[1, 2] More than two thousand plant species having medicinal value have been recognized in the conventional Asian mode of medication[3] since, they produce a wide range of bioactive secondary metabolites such as tannins, steroids and phenolic compounds etc., present in essential oils with potential antimicrobial and antioxidant properties.[4-6] Consequent upon these properties, extracts of these plants also find application as food preservatives.[7] These extracts are added to drugs and food items to inhibit the growth of harmful microorganisms responsible for chronic as well as infectious diseases like cancer, cardiovascular disease, neurological disorders, atherosclerosis, inflammatory injuries, ageing etc.[1, 8] caused by the development of reactive oxygen species (ROS) such as peroxides, hydroxyl radicals and superoxide anions generated in the body through metabolism process. These reactive species lead to oxidative stress and hence, biomolecules such as proteins, lipids, carbohydrates and nucleic acids are destroyed.[9]

Various synthetic antioxidants such as *butylated hydroxytoluene*, *butylated hydroxyanisole* and *propyl gallate* etc. are believed to cause negative impact on human health and also, drug resistance of some microorganisms against these chemical oxidants has been increased. Therefore, natural antimicrobials with lesser side effects seem to be the most appropriate alternative solution to these concerns.[10-12] Above 30,000 antimicrobial compounds isolated from plants have been discovered so far. Among variety of these antimicrobial and antioxidant compounds, flavonoids (polyphenols) and other phenolic compounds such as phenolic acids, stilbenes, tannins, lignans, and lignin, having manifold health impacts are known to inhibit the growth of cancer and heart diseases.[6-7] These compounds are present in both edible as well as non-edible plants and are commonly found in leaves, flowering tissues in addition to woody parts like stems and barks. In the past few years, researchers have reported the presence of appreciable quantities of various phytochemicals, like carbohydrates, proteins, phytosterols, flavonoids, glycosides, tannins and other phenolic compounds in plants including *Gliricidia sepium, Toona ciliate, C. formosana, M. candidum, Callistemon viminalis* and *Lantana camara,* that can be successfully isolated for medicinal applications due to their potential antioxidant and antimicrobial activities against tested

microorganisms.[13-16]

Phenolic compounds are known to behave as reducing agents, hydrogen donators, and singlet oxygen quenchers because of redox properties responsible for their antioxidant activity.[7] These compounds work as better antimicrobials in combination with each other as compared to individual compounds.[8] Phenolic extracts of various parts of *Asparagus acutifolius, Bryonia dioica, Cytisus multiflorus, Sambucus nigra, Rosa micrantha, Filipendula ulmaria, Castanea sativa* and *Cistus ladanifer* containing ellagic, caffeic, and gallic acids, quercetin, kaempferol, and rutin have been reported to show considerable antibacterial effects against gram-positive and gram-negative bacteria i.e., *K. pneumoniae*, *S. epidermidis,* and *S. aureus*, at lower concentrations**.**[6] Xiaoyong et al. evaluated the polyphenol compositions, antioxidant and antimicrobial properties of blueberry-leaf extracts; the results proved that the blueberry leaves possess significant antioxidant and antimicrobial potential due to the occurrence of polyphenols.[17]

Studies have shown that aqueous and organic extracts of aerial parts of various flowering plants have potential antioxidant and antimicrobial activity against pathogens, compatible with that of commercial antibiotics and can be employed effectively in the development of new antimicrobial drugs.[18-25] Several studies reveal that flowers possess a wide range of secondary metabolites of medicinal value having antibacterial, antifungal and antioxidant activities; anthocyanins, flavanones and flavonols are found in petals, responsible for floral pigmentation. Moreover, biosynthesis of fragrant volatiles, including terpenoid and phenylpropanoid/benzenoid compounds, indols, thiols and aliphatic esters, takes place in the petal tissues.[26] Therefore, aqueous and organic extracts of flowers of plants belonging to various families exhibiting strong antioxidant and antibacterial activities have been subjected to screening of phytochemicals that can be isolated for use as an alternative source of antimicrobial agent against the human pathogens.[27-31]

Our country is also endowed with a wide range of plants of medicinal importance. Among these, *Elaeagnus umbellate* a wild shrub from Elaeagnaceae family, is splendidly present in Himalayan regions of Pakistan. *Silybum marianum*, a flowering plant of medicinal importance, is richly found in Khyber Pukhtoonkhwa and Punjab areas of Pakistan. The data revealed the occurrence of flavonoids, phenols and tannins in both blue and white flowering plants of *S. marianum*. Furthermore, activity of the plant against gram positive bacteria has been confirmed**.**[32]

According to an estimate, quite low percentage of all the flowering plants present on Earth has been recognized by the scientists for their medicinal use.[16] It is therefore worthwhile to explore more natural sources and establish their potential use as a substitute for synthetic antibiotics. Hence, the present study is aimed at the evaluation of various winter season flowering plants grown in Lahore. Twenty extracts prepared from ten locally available plants belonging to different genera were subjected to determination of total flavonoids and total phenols as well as antioxidant, antibacterial and antifungal activities.

MATERIAL AND METHODS

Plant materials

Samples of locally available flowering plants were collected in winter season. The plants materials were ground after drying at room temperature and fine powder was used for all activities. Phenol, Flavonoid and all activities were measured as colorimetric methods. Analyses were taken at UV-Visible Spectrophotometer Analytikajena Specord 200.

Determination of total flavonoids

Total flavonoids were estimated using the method as described by Ordonez and his coworker.[33] 0.5ml of 2% $AlCl_3$ ethanol solution was added to 0.5ml of ethanolic extract and was filtered after keeping for one hour at room temperature; absorbance of the filtrate was noted at 420 nm and the total flavonoid contents of petals and leaves were calculated as quercetin equivalent 2, 4, 6, 8 µg/mL had absorbance 0.1922, 0.3892, 0.5977, 0.7854 from the calibration curve.

Determination of total phenols

Total phenols were determined by using Folin Ciocalteu reagent.[34] A dilute extract of each plant extract (0.5 ml of 1:10 g ml-1) or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml of 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetric at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L-1 solutions of Gallic acid in methanol: water (1:1, v/v). Total phenol values are expressed in terms of gallic acid equivalent from calibration curve related to Beer Lambert Law for absorbance. 0.5, 1.0, 1.5, 3.0 ug/mL of Gallic acid gave absorbance 0.0432, 0.05213, 0.0611, 0.0741 which is a common reference compound.

Determination of antioxidant activity (DPPH Assay)

The DPPH scavenging activity was determined according to Brand-Williams *et al.* using 1,1-diphenyl-2-picryl-hydrazil (DPPH) reagent.[35] A Stock solution of DPPH, [DiPhenyl 2-picryl hydrazyl] 0.1 m mole was prepared by dissolving 3.94 mg in 100 mL of methanol: water (50:50) . Pre weighed 1mg/mL of petals and leaves were taken in test tubes, stock solution (3 mL) was added in such a way to achieve a concentration of 250 ug/mL of reagent DPPH. Then the samples were shaken vigorously and kept in dark for 0.5 hours. The absorption of samples was measured on a spectrophotometer, Perkin Elmer UV-Visible spectrophotometer at 517 nm. All tests were run in triplicate and reported on average quantity.[36] Rutin was used as standard controls and %inhibition was calculated using the formula: (10mg/mL Rutin gave 86.65% DPPH activity). (Blank-sample)/blank x100=% inhibition.

Antimicrobial Activity

Determination of antibacterial activity

Weighed amount of petals and leaves was taken in methanol solution. The test solutions were autoclaved and an inocculum of *E. coli* (20ul) prepared in saline solution was added to each solution. Initial absorbance of each test solution was noted at 600nm[37-39] and were placed in shaking incubator at 37°C. After 24 hours other readings were noted at 600nm and %age inhibition of petals and leaves from clearance of turbidity produced by *E. Coli* after 24 hours.

Determination of antifungal activity

Aspergillus niger was selected for the antifungal activity at concentration of 1mg/5mL in nutrient broth. The solutions of petals and leaves in methanol were prepared 1mg/mL in test tubes, then added 4mL nutrient broth in each test tube with concentration of 200ug/mL. After autoclaving of test tubes having test solution, an inocculum of A*spergillus niger* (20ul) prepared on slant of agar was added to each test tube. Take initial absorbance at 550nm, then keep test tube in shaking incubator at 26±2°C. After 72 hours readings at 550nm[39] were taken and noted the % inhibition flowers and leaves from the absorbance of blank after 72 hour at 550nm. Clearance of turbidity showed the antifungal activity of petals and leaves.

RESULTS AND DISCUSSION

In the present study winter season flowering plants were collected from different areas of Lahore city and their petals and leaves were analyzed separately for phenolic compounds i.e., phenols and polyphenols (Flavonoids) and analysis showed that they are responsible for their antibacterial, antifungal and antioxidant activities of plants.

The result in Table-1 showed that maximum phenolic contents were present in Dhalia flowers (468.61mg/100g) and minimum was found in Gainda leaves (5.34mg/100g). Maximum flavonoids were present in Morning glory flowers (70.69mg/100g) shown in figure-1 and mostly leaves did not possess flavonoid.

Due to phenolic compounds plants possess antibacterial and antifungal activities accordingly. It was observed interestingly that amount of phenols in petal and leaves may be corresponds to antibacterial and antioxidant activities and amount of Flavonoids related to antifungal activities as revealed in two tables. Dhalia Flower has maximum phenolic compounds, it exhibit maximum antibacterial activity (80%) and antioxidant activity (16.65%) as in Table-2 and others having less phenolic contents showed less antibacterial and antioxidant activities. Maximum antifungal activity was found in Morning glory flowers (19 %) may be it is related to Flavonoid contents as Morning glory flowers have maximum Flavonoids.

Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolic compounds possess hydroxyl and carboxyl groups, able to bind particularly iron and copper.[40] The roots of many plants exposed to heavy metals exude high levels of total phenol. They may inactivate iron ions by chelating and additionally suppressing the superoxide-driven Fenton reaction, which is believed to be the most important source of ROS.[41]

Table-1: Estimation of phenols, flavonoids (polyphenol) of winter flowering plants

Plant	Parts	Colour	Total Phenol mg/100g	Flavonoids mg/100g
Dhalia	Flowers	Yellow	468.61	35.30
	Leaves	Light green	8.64	ND
Sunflower	Flowers	Yellow	135.27	6.58
	Leaves	Light green	63.38	ND
Winter seasonal	Flowers	Pink/purple	226.38	13.44
	Leaves	Green	37.67	ND
Gainda	Flowers	Dark yellow/orange	94.20	5.65
	Leaves	Green	5.34	ND
Knair	Flowers	Pink	123.80	33.05
	Leaves	Green	73.81	9.86
Hibiscus	Flowers	Red	55.33	14.12
	Leaves	Green	4.212	ND
Rose	Flowers	Red	267.16	12.17
	Leaves	Dark green	72.17	ND
Jasmin	Flowers	Off white	376.99	58.98
	Leaves	Green	53.42	ND
Morning glory	Flowers	Purple	138.72	**70.69**

	Leaves	Dark green	57.80	ND
Cana	Flowers	Red	204.59	0.83
	Leaves	Dark green	32.81	ND

Table-2: Estimation of antimicrobial, antioxidant activities of winter flowering plants

Plant	Parts	Antibacterial activity against E. coli (1+4 nutrient broth) inhibition (%)	Antifungal activity against Aspergillus niger (1+4 nutrient broth) inhibition (%)	DPPH anti-scavenging activity (%)
Dhalia	Flowers	80.0	10	16.6
	Leaves	04.0	ND	01.91
Sunflower	Flowers	30.0	6.0	06.93
	Leaves	12.0	3.0	03.73
Winter seasonal	Flowers	68.0	8.0	13.02
	Leaves	9.0	ND	1.17
Gainda	Flowers	28.0	6.0	8.05
	Leaves	ND	ND	1.81
Knair	Flowers	33.0	10.0	4.97
	Leaves	24.0	7.0	1.26
Hibiscus	Flowers	09.0	ND	6.41
	Leaves	ND	ND	2.77
Rose	Flowers	64.0	16.0	8.35
	Leaves	15.0	6.0	1.21
Jasmin	Flowers	76.0	12.0	11.46
	Leaves	16.0	4.0	1.33
Morning glory	Flowers	23.0	19.0	1.74
	Leaves	11.0	3.0	0.46
Cana	Flowers	56.0	8.0	8.35
	Leaves	12.0	ND	3.52
Rutin(10mg/mL)		---	---	84.09 ±0.375

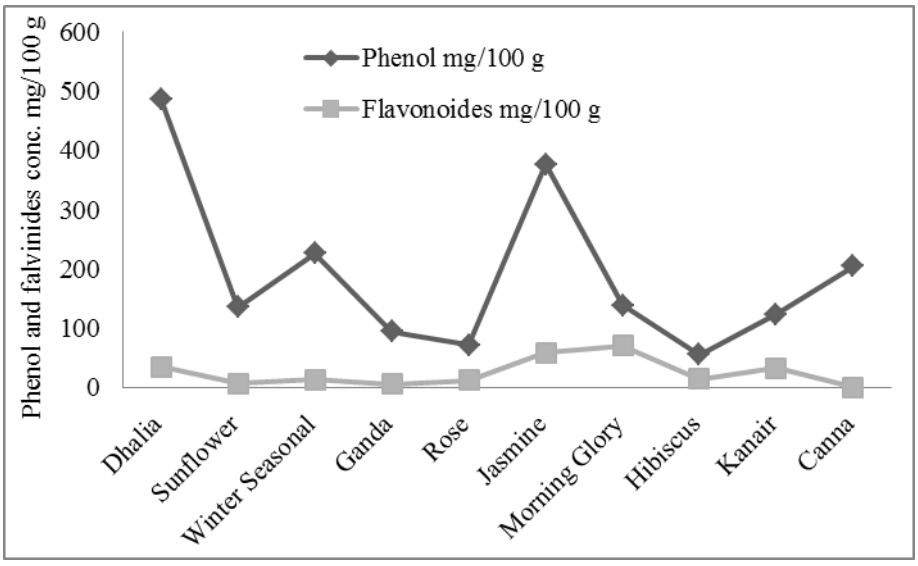

Figure 1: Phenol and flavonoids concentration (mg/100 g) in plants flower

CONCLUSIONS

Following conclusion were drawn from studies

- Phenols are responsible for microbial and antioxidant activities in flowering plants to improve their defensive system as the data revealed that higher the amount of phenols, higher these activities.
- Phenols impart color to petals but petals without color also possess phenols as in the case of jasmine flowers.
- Amount of phenol and flavonoids (polyphenols) are irrespective to each other as maximum phenol was found in Dhalia flower and maximum flavonoid was found in morning glory flowers.
- Phenol contents may be responsible for antibacterial activity and DPPH Scavenging activities and Flavonoids responsible for antifungal activities as in case of Dhalia and Morning glory flowers.

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