PHYTOTOXICITY, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF FOUR SELECTED MEDICINAL FLOWERS

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***Abstract*:** Natural products derived from plant sources have gained significant attention in recent years due to their potential pharmacological activities. *Althea officinalis, Hyssopus officinalis, Nymphaea alba,* and *Sphaeranthus indicus* are widely recognized for their traditional medicinal properties and have been used for centuries in various folk medicine systems. For this purpose, the biological activities of ethanolic extract of *A. officinalis, H. officinalis, N. alba,* and *S. indicus* flowers was carried out. The phytotoxic activity revealed the highest FI_{50} value (2007.4 µg/ml) for *S. indicus* while the lowest FI_{50} value (360.0 µg/ml) had shown by *H. officinalis.* Among all the flowers the highest antibacterial activity was shown by the *H. officinalis* (24.33±2.05 mm) against *P. aueroginosa* at 9 mg/ml while, all the selected followers *A. officinalis, H. officinalis, N. alba,* and *S. indicus* did not show any significant activity against Bacillus subtilis in 3 mg/ml. The antifungal activity exhibited a maximum zone of inhibition (29 mm) was shown by *S. indicus* against *Aspergillus niger* while the lowest zone of inhibition (5 mm) was shown by *N. alba* against *A. niger*. The highest percentage of radical scavenging activity (83.25) were reported by *H. officinalis* while the lowest % RSA (39.74) showed by *S. indicus* followed by *N. alba* (50.56%). Similarly, the highest IC_{50} values (891.09 µg/ml) were reported by *H. officinalis* while the lowest IC_{50} values (0.91 µg/ml) showed by *H. officinalis.* The present study concluded the effective use of flowers of plants might be the presence of active secondary metabolites against various bacterial, fungal diseases and for the isolation of herbicidal compounds.

Index-term: Antimicrobial Activities, Medicinal Flowers, Phytotoxic Activity, Antioxidant Activity.

I. INTRODUCTION

Human beings had attached to plants throughout the ages for their simple necessities as being for purpose of protection, foodstuffs, fertilizers, and medicines. For this purpose, plants are study under the discipline of pharmacognosy. Pharmacognosy is the study of biological, taxonomical, and morphological aspects of crude drugs, their isolation, evaluation of secondary metabolites, and finding new drugs from natural resources (Clark, 1996). Pharmacognostic and biological studies are preferred to carry out on medicinal plants. Medicinal plants show a primary role in health care systems in large proportions of the world's population and mainly herbal remedies have a continuous history of long use in developing countries (Dar et al., 2017, Rehman et al., 2020). Among medicinal plant parts there is potential in flowers used in traditional medicines and prescribe by the

practitioners. *A. officinalis* flowers act as an expectorant, diuretic, demulcent, emollient, rheumatism, constipation, and intermittent fever (Rizvi and Ali, 2016). It contains mucilage polysaccharides, sterols, quercitin, and volatile oils ((Soleimany et al., 2016, ur Rehman et al., 2019). Flowers extract of *H. officinalis* contained volatile oil, diosmin, limonene, eugenol, and myristicin, which are responsible for muscle relaxant, anthelmintic, antibacterial, and antifungal activities (Dehghanzadeh et al., 2012, Wali et al., 2019). *N. alba* possessed analgesic, antioxidant, anticancer, and astringent properties (Rehman, 2019). Due to active constituents like nymphalin, gallic acid, and cardiac glycoside (Selvakumari et al., 2016). The aqueous extract of *S. indicus* flowers had insecticidal, piscicidal, larvicidal, and biocidal activities due to the presence of sphaeranthanolide and eudesmenolides such as frullanolide (Reddy et al., 2015).

Phytochemistry is an important foundation for the validation of numerous therapeutic industries, identification of crude drugs, and have a positive anthropological action (Mukherjee et al., 2015). Phytotoxicity has become a significant implement to pinpoint the plants with bioactive compounds for the use in the development of natural herbicides that are more explicit and cause less environmental compensations (Imatomi et al., 2015). Over the previous years, wellbeing assistances are at risk because of the use of many common and less effective antibiotics against various diseases (Bhalodia and Shukla, 2011). Innate antibacterial constituents have the welfare of developed constancy, less spinoff, and toxic effects. We need to prepare inherent or developed resistance antifungal medicines over the existing antifungal drugs for the treatment of infectious diseases, which is inevitable (Pinto et al., 2017). Reactive oxygen species (ROS) or oxidants are oxygen containing free and non-free radical molecules. Commonly defined ROS are superoxide anion (O2•−), hydroxyl radical (HO•), peroxyl (RO2•) etc. These ROS species are produced as inevitable byproduct of aerobic metabolism like cellular respiration, photosynthesis etc. and must be controlled through antioxidant activity by exposing to different biological metabolites (Nanda, 2019). The present study has been conceived to inspect the role of flowers of *A. officinalis*, *H. officinalis*, *N. alba*, and *S. indicus* through phytotoxicity, antibacterial and antifungal activities. The reason beyond the selection of the flowers is due to the frequent used in traditional medicine, unavailability of keen observation on various activities as a whole on the store-dried form from the market and to confirm that whether the flowers have the medicinal abilities or not. The current study as aimed to investigate the herbicidal potential of selected flowers and to evaluate its antimicrobial perspectives and free radical scavenging activity.

II. MATERIALS AND METHODS

Plant collection, preservation, and extract preparations

This study complied with relevant institutional, national, and international guidelines and legislation of Pakistan. The dried flowers of *A. officinalis* L, *H. officinalis* L, *N. alba* L, and *S. indicus* L. were purchased from the local Qissa Khwani Bazar in Peshawar, and no specific permits were required to collect the plant materials. The Plants were authenticated through literature and confirmed by Dr. Ghulam Jellani (Taxonomist) and Prof. Dr. Ghulam Dastagir (Medicinal Plant Expert) Department of Botany, University of Peshawar. Using an electric grinder, the flowers had reduced to a powder. Before the extraction, the powder from each flower was stored in its own individual, airtight flask. In a 500 ml conical flask, 50 g of powder from each flower had combined with 250 ml of ethanol to make the extract. After 15 days (Kept for extra days in order to get bulk of extracts), extracts had prepared by filtering the mixture through Whatman filter paper No. 42 into a new flask and placing it in a water bath at 60 ^{0}C to evaporate the solvents. The resulting extracts were stored in the refrigerator at 4 degrees

Celsius for future research (Sharma, 2013). Initial phytotoxic, antibacterial and antifungal activities were all performed using the ethanolic extract.

Phytotoxic activity:

Lemna minor used as a test organism for determining the phytotoxicity of floral ethanolic extracts. Before adding 1000 ml of distilled water and adjusting the pH with dissolved KOH tablets to a range of 5.5-6.0, we produced the e-medium by first adding different salts concentrations. For 15 minutes at 121 degrees Celsius, the e-medium had placed in an autoclave. The stock solutions were prepared for each sample by mixing 15mg extract with 1.5ml DMSO (No activity of DMSO). The volume of 10µl, 100µl and 1000µl were poured in disinfected Petri plates with micropipette. Petri plates had left out at room temperature for a whole night to allow the solvents to evaporate. The next day, ten Lemna minor plants, each with three fully expanded fronds, had placed in Petri dishes with E-medium (20 ml) and put in a growth cabinet. Number of fronds had counted and compared to the norm after a week's growth. A percentage of inhibition (FI50) was determined using Biostat's 2006 software using the following formula.

$$\text{Inhibition } \% \ = 100 \ \frac{\text{Fronds figure in the sample test}}{\text{Fronds figure in control}} \text{ X } 100$$

Antibacterial activity

The well diffusion method had used to test the ethanolic extract for antibacterial activity against several strains of bacteria obtained from the COBAM, UOP laboratories: *Bacillus subtilis* (Ehrenberg), *Salmonella typhi* (Lignieres), *Pseudomonas aureginosa* (Migula), and *Staphylococcus aureus* (Rosenbach). We autoclaved nutrient agar at 121 degrees Celsius for 15 minutes. After cooling the molten nutritional agar to 40 degrees Celsius, 20 to 30 milliliters had placed into each of the Petri plates. After the medium in the Petri dishes had set, we smeared it over the facet with a cotton pad and drilled four wells in it with a sterile metal borer of 6 mm diameter to hold the bacterial culture. The stock solutions were prepared by using 3, 6, and 9 mg/ml of DMSO (1%). A volume of 100 µl was used in each well from each stock solution. A positive control of ampicillin (25 µl) against each bacterial strain was used while DMSO (1%) was kept as a negative control. The plates had kept in a 37 C incubator for 24 hours. Millimeter readings had taken of the inhibition zone surrounding each well (Bhattarai and Basukala, 2016).

Antifungal activity

The ethanolic extract was tested for its antifungal properties using the test-tube dilution technique against four distinct strains of fungus obtained from the COBAM, UOP laboratories: *Aspergillus niger, A. terrus, A. solani, and Alternaria alternata*. Fungus straining cultures that were a week old were stored in the fridge in test tubes. Plant extract (12, 24, and 36 mg/ml of 1% DMSO) was dissolved to make the stock solutions. After being autoclaved at 121 degrees Celsius for 15 minutes, cooled to 40 degrees Celsius, and then transferred to test tubes, the Sabouraud dextrose agar (SDA) growth material was solid. Each test tube that containing SDA in slanting condition followed by fungal culture on top of the medium and then a volume of 67µl through micropipette from the stock solutions were applied. Voriconazole (10 µl) and 1% DMSO (as a negative control) had provided in separate test tubes. After a week incubation at 37 degrees Celsius, the diameters of the inhibitory zones throughout the test tubes were measured (Leite et al., 2014).

Antioxidant activity

When testing for antioxidant activity, a DDPH photometric experiment had performed using 2mg DPPH in 100 ml of ethanol. The stock solutions were prepared by dissolving 10 mg extract in 1ml of ethanol, making the

final volume of 20ml stock solution having 20mg extract. A number of different concentrations (2, 3, 4, and 5 ml) from the stock solution were taken and treated with 2 ml (0.3Mm) of DPPH solution. The mixture of stock solution and DPPH were kept in the dark for 30 minutes. The volume of 50, 100, 150, and 200 µl were checked for absorbance values at 517 nm after the dark period, and the antioxidant activity had converted to a percentage using the following formula

$$\text{Antioxidant \%} = \frac{\textbf{Abs Control} - \textbf{Abs Sample}}{\textbf{Abs Control}} \textbf{ X 100}$$

Negative control DPPH had taken in the same concentration as a stock solution while Ascorbic acid had used as a positive control (standard solution). The IC50 values had calculated by linear regression of plots where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of antioxidant activity from three separate tests (Nanda, 2019).

Statistical Analysis

The statistical analysis (TWO-WAYS ANOVA) was performed by using GraphPad Prism 8. 0. 3. The FI_{50} and IC_{50} values were find out through the software Biostat 2006 and Excel 2016 respectively.

III. RESULTS

Phytotoxic Activity

The phytotoxic activity of *A. officinalis*, *H. officinalis*, *N. alba*, and *S. indicus* flowers was carried out against Lemna minor. Individually the highest value of inhibition of A. officialis against Lemna minor was 47.77±1.57% at 1000 µg/L followed by 44.44±1.57% at 100 µg/L and 36.68±2.70% in 10 µg/L (Table 1 and Fig. 1). The *H. officinalis* had 48.89±4.16% inhibition at 1000 µg/L, 45.55±4.16% at 100 µg/L and 6.68±2.70 at 10 µg/L. N. alba showed 51.11±1.57% inhibition at 1000 µg/L, 46.66±2.72% at 100 µg/L and 40.00±2.72% at 10 µg/L. The S. indicus exhibited 42.22±5.67%, 36.68±2.70% and 20.00±5.44% inhibition at 1000 µg/L, 100 µg/L and 10 µg/L respectively. Comparatively, among all flowers the highest (51.11±1.57%) phytotoxicity was shown by *N. alba* at 1000 µg/L while the lowest (20.00±5.44%) was shown by S. indicus at 1000 µg/L. The highest FI50 value (2006.13±3.78 µg/ml) had shown by *S. indicus* while the lowest FI50 (359.93±1.96 µg/ml) value was shown by *H. officinalis* (Table 1). The Two-ways ANOVA of Tukey recommended test with alpha level 0.05 showed that *N. alba* had very significant (***) results having P-value 0.0002 in multiple comparison with control at each concentration (Fig. 1). The overall results concluded that phytotoxicity of these flowers are dose dependent as the concentration increases, more inhibition will be occurring.

Table 1. Phytotoxic activity of the ethanol extract of selected medicinal flowers.

Samples	Dosage (µg/L)	Fronds figure in test	Fronds figure in contr	Inhibition %	Fl50 (µg/ml)
A. officinalis	10	19.00±0.82	30.00±0	36.68±2.70	1402.03±4.19
	100	16.67±0.47	30.00±0	44.44±1.57	
	1000	16.67±0.47	30.00±0	47.77±1.57	
H. officinalis	10	19.00±0.82	30.00±0	36.68±2.70	359.93±1.96
	100	16.33±1.25	30.00±0	45.55±4.16	
	1000	15.33±1.25	30.00±0	48.89±4.16	
N. alba	10	18.00±0.82	30.00±0	40.00±2.72	600.2±2.14
	100	16.00±0.82	30.00±0	46.66±2.72	
	1000	14.67±0.47	30.00±0	51.11±1.57	
S. indicus	10	24.00±1.63	30.00±0	20.00±5.44	2006.13±3.78

	100	19.00±0.82	30.00±0	36.68±2.70
	1000	17.33±1.70	30.00±0	42.22±5.67

Keywords; FI_{50} (Frond Inhibition)

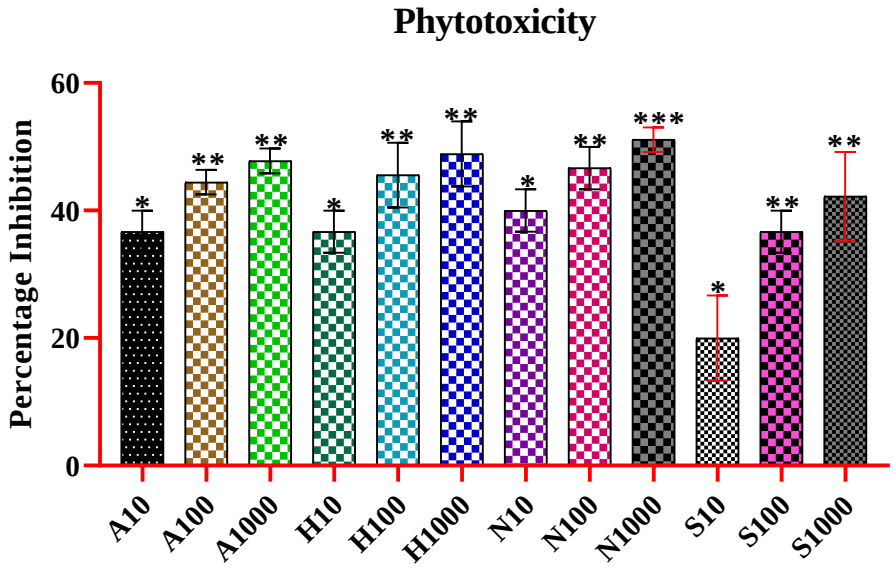

Fig. 1. 10, 100, 1000 showing the concentrations with first alphabetic letter of each test plant. The Tukey recommended Two ways ANOVA with alpha level (0.05) showing different significance level with (*) significant, (**) intermediate significance and (***) very significant data.

Antibacterial Activity

The antibacterial activity of *A. officinalis*, *H. officinalis*, *N. alba,* and *S. indicus* flowers was carried out against *Pseudomonas aueroginosa*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus.* The results revealed that *A. officinalis* the highest ZOI (23.00±0.82 mm) against *P. aueroginosa* at 9 mg/ml while the lowest ZOI (11.33±1.25 mm) against *S. typhii* at 3 mg/ml. The *H. officinalis* had the highest ZOI (24.33±2.05 mm) against *P. aueroginosa* at 9 mg/ml while the lowest ZOI (10.33±0.47 mm) against *B. subtilis* at 3 mg/ml. The *N. alba* had the highest ZOI (21.67±1.25 mm) against *S. aeurusi* at 9 mg/ml while the lowest ZOI (11.00±0.82 mm) against *S. aeurusi* at 3 mg/ml. The *S. indicus* had the highest ZOI (20.33±1.25 mm) against *S. aeurusi* at 9 mg/ml. While, it had the lowest ZOI (11.00±0.82 mm) against *S. aeurusi* 3 mg/ml. Comparatively, among all the flowers the highest antibacterial activity was shown by the *H. officinalis* (24.33±2.05 mm) against *P. aueroginosa* at 9 mg/ml while, all the selected followers *A. officinalis*, *H. officinalis*, *N. alba,* and *S. indicus* did not show any significant activity against *Bacillus subtilis* in 3 mg/ml (Table 2). The Two-ways (Mixed Model) ANOVA of Tukey recommended test were performed with alpha level 0.05 which exhibited the $P < 0.0001$ for *H. officinalis* against *P. aueroginosa*. The most significant (****) results were recorded with multiple comparison of all plants and standard during statistical analysis (Fig. 2).

Table 2. Antibacterial activity of some selected medicinal flowers.

Flowers	Conc. (mg/ml)	Dose (µL)	Inhibition Zone (mm)			
			B. subtilis	*P. aueroginosa*	*S. typhi*	*S. aeurus*
Ciprofloxacin	2	100	28.67±0.47	29.67±1.25	32.00±0.82	30.33±1.25
A. officinalis	3	100	0	12.33±1.25	11.33±1.25	12.33±0.47
	6		13.67±1.25	14.33±1.25	14.67±0.47	16.00±0.82
	9		17.67±0.47	23.00±0.82	20.00±0.82	20.33±0.47
H. officinalis	3	100	0	13.33±0.47	10.67±1.89	16.00±0.82
	6		10.33±0.47	15.33±0.47	20.00±0.82	16.33±0.47
	9		12.00±1.41	24.33±2.05	21.33±2.05	20.33±1.25
N. alba	3	100	0	15.00±0.82	16.33±0.47	11.00±0.82
	6		14.00±0.82	17.00±0.82	19.33±0.47	16.67±0.47
	9		18.67±0.47	19.67±0.47	20.67±0.47	21.67±1.25
S. indicus	3	100	0	11.67±0.47	11.33±1.25	11.00±0.82
	6		13.67±0.47	15.00±2.16	17.00±3.56	15.67±1.25
	9		18.33±0.47	20.33±1.25	19.67±1.25	20.00±1.63

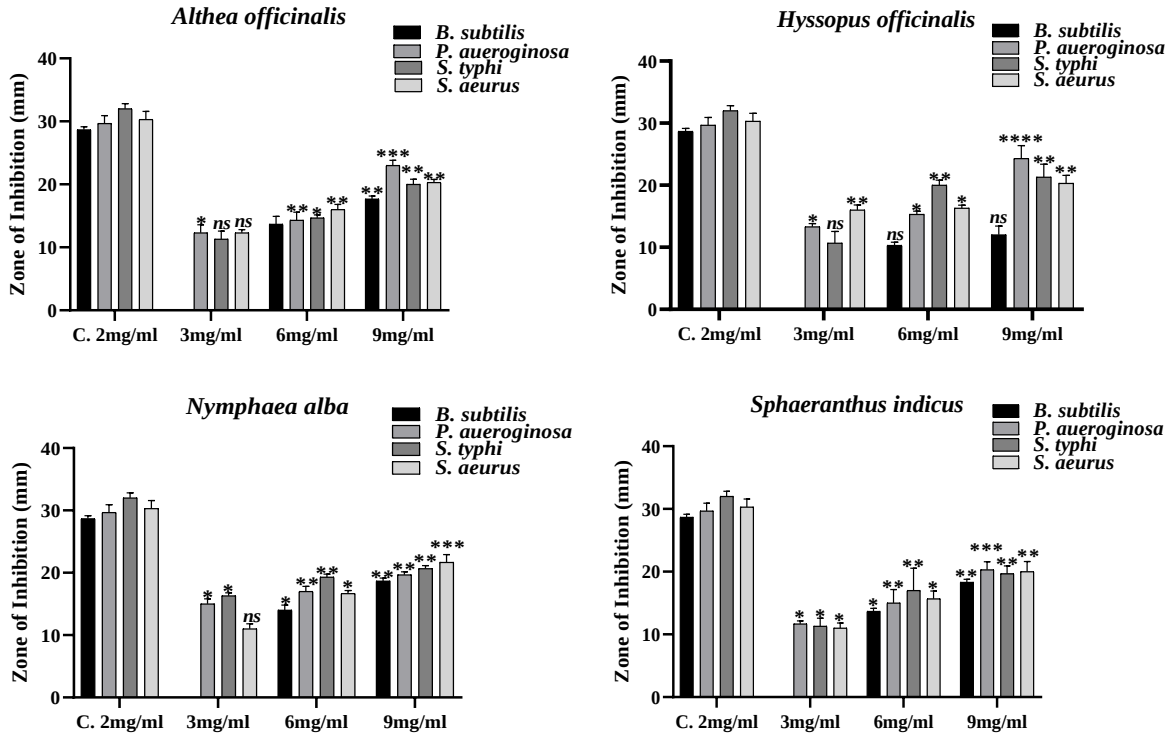

Fig. 2. The Two ways ANOVA of antibacterial activity with alpha level (0.05) showing different significance level with (*) significant, (**) intermediate significance and (***) very significant, most significant (****) and not significant (ns) results.

Antifungal Activity

The antifungal activity of *A. officinalis*, *H. officinalis*, *N. alba*, and *S. indicus* flowers were carried out against *A. alterneta*, *A. niger*, *A. terrus*, and *A. solani*. The results showed that *A. officinalis* had the highest inhibition zone (21.67±2.05 mm) in 36 mg/ml against *A. niger* while the lowest (11.67±0.94 mm) in 12 mg/ml against *A. terrus*. The *H. officinalis* had the highest antifungal activity (22.00±1.63 mm) in 36 mg/ml against *A. solani* while the lowest (10.33±0.47 mm) at 24 mg/ml against *A. alternata*. The *N. alba* had the highest (22.00±1.63 mm) in 36 mg/ml against *A. solanii* while the lowest (11.67±1.89 mm) in 12 mg/ml against *A. niger*. The *S. indicus* had the highest (21.33±0.94 mm) at 36 ml/mg against *A. solanii* while the lowest (12.33±0.47 mm) in 12 mg/ml against *A. alterneta*. Comparatively, among all the flowers extract was shown the highest antifungal activity (22.00±1.63 mm) by *H. officinalis* and *N. alba* against *A. solanii* in 36 mg/ml. While, *A. officinalis* in 12 mg/ml against *A. alternata*, *H. officinalis* in 12 mg/ml against *A. alternata* and *A. niger*, and S. indicus in 12 mg/ml against *A. terrus* did not show any significant activity (Table 3). The Two-ways (Mixed Model) ANOVA of Tukey recommended test were performed with alpha level 0.05 which exhibited the $P < 0.0001$ for both *H. officinalis* and *N. alba* against *A. solanii*. The most significant (****) results were recorded with multiple comparison of all plants and standard during statistical analysis (Fig. 3).

3. Antifungal activity of some selected medicinal flowers.

Flowers	Conc. (mg/ml)	Dose (µL)	Inhibition Zone (mm)			
			A. alternata	***A. niger***	***A. terrus***	***A. solanii***
Voriconazole	2	67.5	29.33±0.47	30.33±1.70	32.00±0.82	31.00±0.82
A. officinalis	12	67.5	0.00	12.67±1.25	11.67±0.94	12.67±0.47
	24		15.00±0.82	14.67±1.25	17.00±1.63	16.00±0.82
	36		18.00±0.82	21.67±2.05	19.67±0.47	19.67±1.25
H. officinalis	12	67.5	0.00	0.00	11.00±0.82	11.33±0.94
	24		10.33±0.47	12.67±1.25	16.67±0.47	17.33±0.94
	36		13.67±1.89	20.67±1.25	21.33±2.05	22.00±1.63
N. alba	12	67.5	12.33±0.47	11.67±1.89	12.00±0.82	13.33±2.05
	24		13.33±1.70	15.33±0.47	16.00±1.63	16.67±1.25
	36		17.67±0.47	20.67±1.25	21.33±0.47	22.00±1.63
S. indicus	12	67.5	12.33±0.47	13.67±0.94	0.00	13.33±2.05
	24		14.00±2.45	15.67±0.94	13.67±1.70	16.67±3.09
	36		17.67±0.47	20.67±1.25	19.00±1.63	21.33±0.94

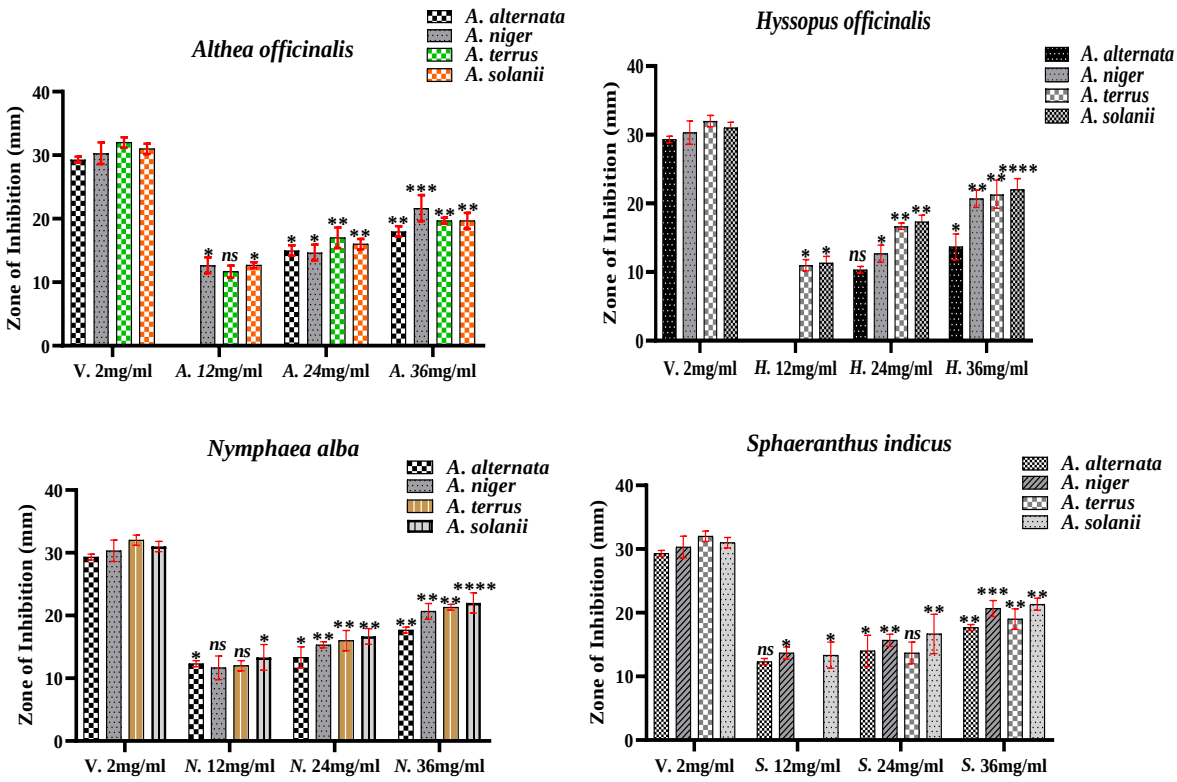

Fig. 3. The Two ways ANOVA of antifungal activity with alpha level (0.05) showing different significance level with (*) significant, (**) intermediate significance and (***) very significant, most significant (****) and not significant (ns) results.

Antioxidant Activity

The antioxidant activity of *A. officinalis*, *H. officinalis*, *N. alba*, and *S. indicus* flowers has been recognized through significant studies by using different concentrations (Table 4). The highest percentage of radical scavenging activity (83.25) were reported by *H. officinalis* followed by *N. alba* (79.88%), *S. indicus* (77.92%), and *A. officinalis* (73.86%) at the concentration of 200 µl. While, the lowest % RSA (39.74) showed by *S. indicus* followed by N. alba (50.56%), *A. officinalis* (54.42%) and *H. officinalis* (58.39%) at 50 µl concentration (Fig. 4, 5 and 6). Comparatively, the most significant antioxidant activity was reported by *H. officinalis* (83.25%) compared with ascorbic acid (95.05%) (Fig. 4). The highest IC_{50} values (891.09 µg/ml) were reported by *H. officinalis* followed by *N. alba* (838.75 µg/ml), *S. indicus* (680.03 µg/ml), and *A. officinalis* (110.79 µg/ml) at the concentration of 200 µl. While, the lowest IC_{50} values (0.91 µg/ml) showed by *H. officinalis* followed by *N. alba* (27.94 µg/ml), *A. officinalis* (30.31 µg/ml) and *S. indicus* (92.95 µg/ml) at 50 µl concentration. Comparatively, surprisingly superior significance IC50 values showed by *H. officinalis* compared with ascorbic acid (44.09 µg/ml) (Fig. 5 and 6).

Table 4. Antioxidant activity of *A. officinalis*, *H. officinalis*, *N. alba* and *S. indicus* flowers.

	A. officinalis			*H. officinalis*			Ascorbic Acid			DPPH Abs
	Abs	**%RSA**	**IC50 values**	**Abs**	**%RSA**	**IC50 values**	**Abs**	**%RSA**	**IC50 values**	
50	0.93	54.42	30.31	0.85	58.39	0.91	0.99	51.54	44.097	2.043
100	0.88	56.58	38.95	0.69	66.17	304.3	0.72	64.75	245.54	2.043
150	0.65	67.84	74.87	0.53	74	607.76	0.52	74.3	466.99	2.043
200	0.53	73.86	110.79	0.34	83.25	891.09	0.21	89.72	648.44	2.043
	N. alba			***S. indicus***			**Ascorbic Acid**			**DPPH Abs**
		%RSA	**IC50 values**	**Abs**	**%RSA**	**IC50 values**	**Abs**	**%RSA**	**IC50 values**	
50	1.01	50.56	27.94	1.23	39.74	92.95	0.99	51.54	44.097	2.043
100	0.64	68.42	298.21	1	50.95	288.64	0.72	64.75	245.54	2.043
150	0.55	72.98	568.48	0.73	64.17	484.34	0.53	74.3	466.99	2.043
200	0.41	79.88	838.75	0.45	77.92	680.03	0.21	89.72	648.44	2.043

s: **Conc**. (concentration), **Abs** (Absorbance).

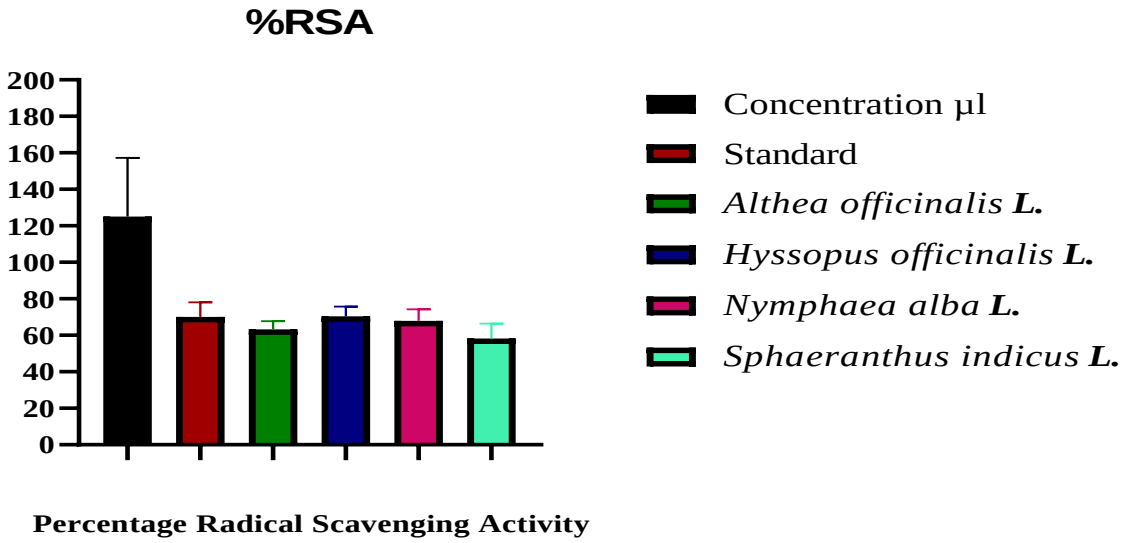

Fig. 4. Percentage radical scavenging activity of selected medicinal flowers.

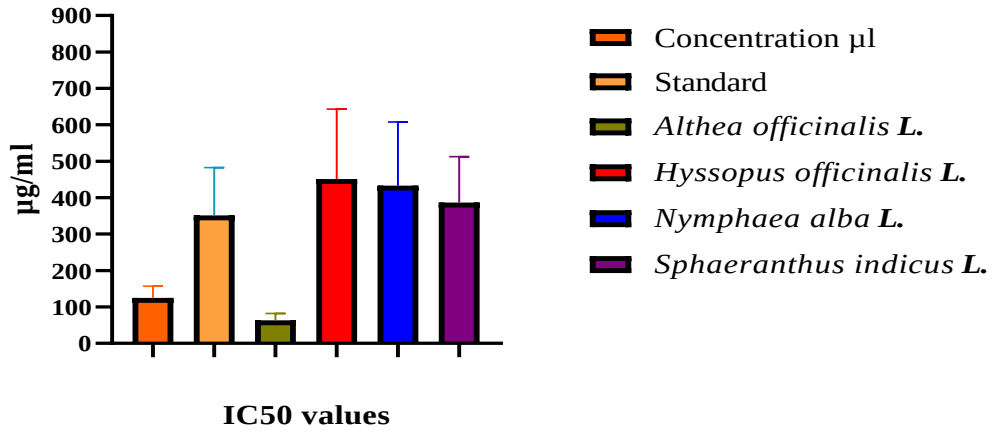

Fig. 5. Inhibitory Concentration (IC 50) values of selected medicinal flowers.

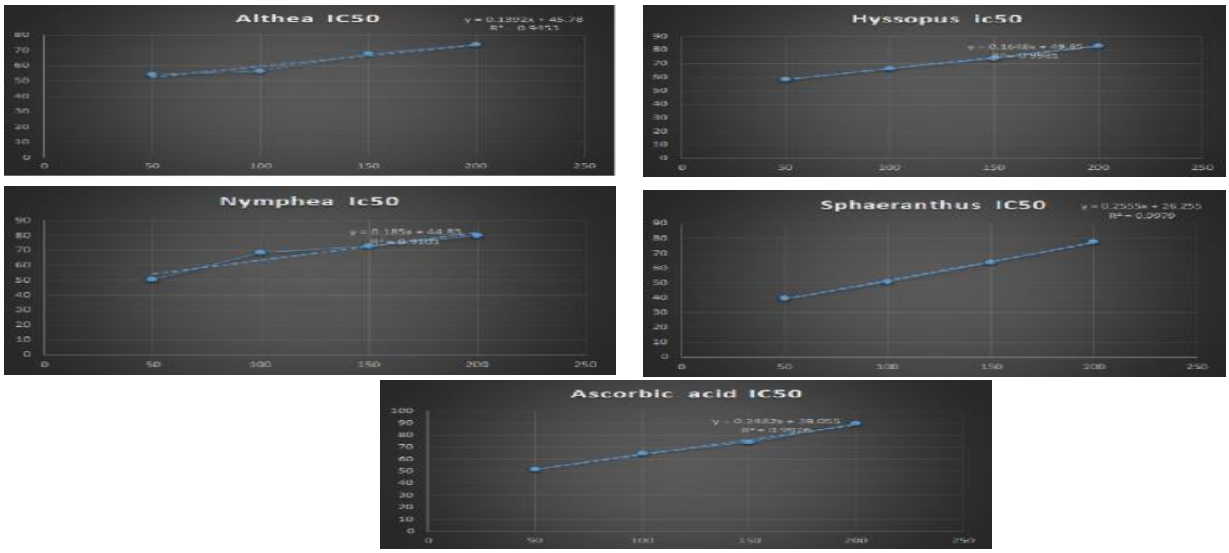

Fig. 6. Inhibitory Concentration (IC 50) values of selected medicinal flowers.

IV. DISCUSSION

In the present study, we sought to determine phytotoxic activity, antibacterial activity, antifungal activity and antioxidant activity of the selected medicinal flowers.

Phytotoxic Activity

Phytotoxicity refers to the toxic effects of a substance on plants, and it has long been a topic of interest among scientists, agriculturists, and environmental health professionals. The study of phytotoxicity provides important information on the effects of environmental pollutants and other chemicals on plant growth and survival. Numerous studies have investigated the phytotoxic effects of various chemicals and have identified a wide range of toxic mechanisms, including oxidative stress, cellular damage, and altered gene expression. Numerous studies have explored the effects of phytotoxins on various plant species, including crop plants and wild plants, as well as the potential for bioremediation of contaminated soils (Al-Shammari et al., 2018, Gharieb et al., 2019). The

findings from these studies have important implications for the protection of crops and the preservation of plant biodiversity, as well as for the assessment of the ecological risk of pollutants and other chemicals. The results of the phytotoxic activity of *A. officinalis*, *H. officinalis*, *N. alba*, and *S. indicus* flowers against *Lemna minor* show some similarities and differences compared to other studies in literature. Similar to our results, previous studies have also reported *N. alba* to have a high level of phytotoxicity. For example, a study by Loponen et al. (2011) found *N. alba* to have a toxic effect on the growth of the duckweed *Lemna gibba*. This result is also consistent with other studies that have reported the toxic effects of *N. alba* on various aquatic plants (Cudalbeanu et al., 2019, Loponen et al., 2011). However, the results of our study differ from a study by Al-Ani et al. (2016) which found that *H. officinalis* had no significant phytotoxicity against the aquatic plant *Lemna minor*. This difference may be due to the fact that Al-Ani et al. (2016) used a different concentration of *H. officinalis* extract in their experiment, which may have affected the results. Our results also showed that *S. indicus* had the lowest level of phytotoxicity compared to the other flowers, with an FI50 value of 2006.13 ± 3.78 µg/ml. This result is in contrast to a study by Al-Ani et al. (2016) which found that *S. indicus* had a moderate level of phytotoxicity against *Lemna minor*. This difference may be due to the different extraction methods used in the two studies, as well as the different concentrations of *S. indicus* extract used. In conclusion, our results suggest that the phytotoxicity of these flowers is dose dependent and that the concentration of extract affects the level of phytotoxicity. Further studies using different extraction methods and concentrations of the extracts may provide additional insight into the phytotoxicity of these flowers. The present results had strongly supported by the findings of Chandrasiri et al. (2015) on *Austroeupatorium inulifolium* flowers, who reported similar phytotoxic activity. The phytochemical screening indicated the presence of various phytochemicals including phenols that might be responsible for the phytotoxic effect. The outcomes supported Soltys et al. (2013) described that the water-soluble allelochemical present in various plants helping in reducing of growth of weeds, which resulted from highly chemically reactive compounds of phenols.

Antibacterial Activity

Antibacterial activity has been a topic of great interest in the scientific community due to the increasing threat of antibiotic resistance. The World Health Organization (WHO) reports that bacteria are becoming increasingly resistant to antibiotics, making it harder to treat common infections (Talebi Bezmin Abadi et al., 2019). This has led to an urgent need to find new and alternative ways to combat bacterial infections. In recent years, research has focused on the discovery of natural compounds with antibacterial properties as a potential solution to the problem of antibiotic resistance (Ahmad et al., 2019, Hsueh et al., 2015). Several studies had conducted to evaluate the antibacterial activity of different plant extracts and essential oils. These studies have shown promising results, and it had believed that natural compounds might play a crucial role in the development of new antibacterial agents. Innate antibacterial constituents have the welfare of developed constancy, less spinoff, and toxic effects. We need to prepare inherent or developed resistance antibacterial medicines over the existing antibiotics for the treatment of infectious diseases, which is inevitable (Pinto et al., 2017). The Present results agree with other studies such as Ruban and Gajalakshmi (2012) on Hibiscus rosa-sinensis flowers, who reported a similar inhibition zone against *Salmonella* spp. The results of the study indicate that *H. officinalis* showed the highest antibacterial activity against P. aueroginosa at 9 mg/ml, with a zone of inhibition of 24.33±2.05 mm. On the other hand, none of the other selected flowers, A. officinalis, *H. officinalis*, *N. alba,* and *S. indicus,* showed significant activity against *Bacillus subtilis* at 3 mg/ml. These results are in line with previous research work on

the antibacterial properties of medicinal plants. For example, a study by Khursheed et al. (2017) showed that *H. officinalis* had antibacterial activity against several pathogenic bacteria, including *P. aeruginosa.* The results of this study also align with the findings of a study by Chatterjee et al. (2018) that reported the antibacterial activity of *H. officinalis* against *P. aeruginosa* and other pathogenic bacteria. However, the results differ from previous research on the antibacterial activity of other medicinal plants. For example, a study by Al-Lafi and Al-Lafi (2017) reported that *N. alba* had strong antibacterial activity against *Bacillus subtilis*, while the results of the current study showed no significant activity against this bacterium. This difference in results could be due to variations in extraction methods, sample preparation, and the concentration of the extracts used. The phytochemical screening indicated that the presence of various phytochemicals including (terpenes, phytosterols, saponins, and steroidal glycosides) that might be responsible for antibacterial activity. The result supported inferences of Cimanga et al. (2018), who reported that similar phytochemical compounds exhibited a combined intensity of antibacterial activity. In conclusion, the results of this study suggest that *H. officinalis* has strong antibacterial activity against *P. aeruginosa*, while the other selected flowers showed no significant activity against *Bacillus subtilis*. These results are consistent with previous research on the antibacterial properties of *H. officinalis*, but they differ from previous studies on the antibacterial activity of other medicinal plants. Overall, these results highlight the importance of investigating the antibacterial properties of different plant species, as well as the importance of considering the specific concentrations used in these studies.

Antifungal Activity

The antifungal activity of various plant extracts had extensively studied due to their potential as natural sources of antifungal agents. The ability of plant extracts to inhibit fungal growth had attributed to the presence of various secondary metabolites, such as flavonoids and terpenoids (Khan et al., 2018). In recent years, there has been a growing interest in the use of natural compounds as alternatives to synthetic antifungal agents, due to concerns over the development of drug resistance and toxic side effects associated with synthetic drugs (Pereira et al., 2019). This has led to numerous studies investigating the antifungal properties of plant extracts and identifying the active compounds responsible for their antifungal activity. To our knowledge, the antifungal effect these medicinal flowers extract from market had not explored yet in previous studies. From result is clear that these flowers extracts have an antifungal effect. The results agree with the findings of Rizwan et al. (2016) on *Malva Sylvestris* flower and Pushpavathi et al. (2017) on *Kigelia africana* flower. The phytochemical analysis indicated the occurrence of various phytochemicals including (tannins and hydrolysable tannins) that exhibited antifungal activities against several microorganisms (Dam et al., 2019).

Antioxidant Activity

The role of antioxidants in maintaining human health and preventing chronic diseases has gained significant attention in recent years. Antioxidants are compounds that protect the body from oxidative stress, a process that can lead to cellular damage and contribute to the development of various diseases. Plant extracts (including leaves, flowers, and stems) are a rich source of antioxidants, and a growing body of research had focused on identifying and evaluating the antioxidant properties of different plant species (Kim et al., 2018). To the best of our knowledge, yet there is no previous study has been investigated the antioxidant potential of these market available medicinal flower's extract. These ROS species are produced as inevitable byproduct of aerobic metabolism like cellular respiration, photosynthesis etc. and must be controlled through antioxidant activity by exposing to different biological metabolites. The present agrees with the finding of Nanda (2019) that reported several

medicinal edible flowers having significant antioxidant activities. Antioxidant effects had brought by the presence of phytochemicals in Plant extracts like phenolics, flavonoids, alkaloids, saponins, steroids, glycosides, tannins, protein and amino acids (Armoskaite et al., 2011). Plant-based phytochemicals antioxidant have been used in herbal medicine against various diseases like cardiovascular, cancer, aging and diabetes diseases (Paul et al., 2016).

V. CONCLUSION

The present study concluded that the presence of several secondary metabolites might exhibited significant phytotoxic, antibacterial, antifungal activities and antioxidant activities. The phytotoxic activity confirmed that the flower must be further processed for the preparation and isolation of herbicides. Additionally, the antimicrobial activities revealed that the usage of dried flowers from the market are therapeutically rich against bacterial and fungal strains and should be processed for antibiotic in future. Furthermore, the antioxidant results proved that the flowers have the abilities to overcome the effect of ROS and should be scrutinized for antioxidant compounds. Moreover, the flowers also used traditionally for medicinal purposes, which confirmed through this study that these flowers have still their therapeutic values and safe to use.

Declarations

Ethics approval and consent to participate

"*This study complied with relevant institutional, national, and international guidelines and legislation of Pakistan. The dried flowers of A. officinalis L, H. officinalis L, N. alba L, and S. indicus L. were gathered from Qissa Khwani Bazar in Peshawar, and and no specific permits were required to collect the plant materials*".

Consent for publication

Not applicable.

Competing interests

The authors have declared that no competing interests exist.

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Availability of data and materials

The data used to support the findings of this study are included within the article.

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