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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 202017(3):2085-2093.

OPEN ACCESS

Antimicrobial Activity, Phytochemical Profiling, Antioxidant Potential and Total phenol of Medicinal Plant of *Juglin regia* L.

Sher Hayat¹, Imran Khan¹, Amir Hasan khan^{1*}, Ali Hazrat², Qaisar ali¹, Ateeur Rahman¹, Saira Naz¹, Ahmad Hassan khan¹, Zubir Shah¹, Khaleeq Ahmad¹ and Ashfaq Ahmad³,

¹Department of Botany Shaheed Benazir Bhutto University, Sheringal, Dir upper, **Pakistan**

²Department of Botany, University of Malakand, KP. **Pakistan**

³Department of Forestry Shaheed Benazir Bhutto University, Sheringal, Dir upper, **Pakistan**

*Correspondence: amirhasankust@gmail.com Received 19-05-2020, Revised: 23-07-2020, Accepted: 30-08-2020 e-Published: 10-09-2020

The current research was carried out to examine the phytochemical testing and antimicrobial properties of *Juglin regia* L leaves. The methanol extract was exposed to qualitative phytochemical analysis utilizing standard processes. The of tannins, glycosides, saponins, Steroid, Flavonoid, Phenol and terpenoids. The antimicrobial properties assessed through disc diffusion method. Data shown that *Staph aureus* was inhibited through methanol (8mm), n-hexane (7mm) Distilled Water (6mm) Ethanol (1mm), The inhibition zones towards *Venturiainaequilis* fungi were varied from 1.9mm to 5.4mm. Largest zone was seen for methanol 5.8mm (32%) used by n-hexane 3.8mm (65%) and distilled water 3.4mm (58%) while minimal zone of inhibition had been recorded in Methanol extract 1.9mm (32%). The detected phytochemicals were saponins, terpenoid, flavonoids, phenol and tannin while steroid and glycoside were observed absent in phytochemical screening. The anti-oxidant found at various amount 60µg/ml (61.45±0.11) at 60 80µg/ml (65.23±0.10) as well as at 100µg/ml (71.78±0.12) correspondingly. Total phenolic content of aqueous soluble low fraction regarding the crude extract was (2.07%) of dry extract. The outcome of the investigation proven the existence of antioxygenic compounds in leaves, extracts revealed capacity as a natural antioxidant to that restrict the lipid per oxidation in food items.

Keywords: Medicinal plant, Antimicrobial activity, Phytochemical screening, Total phenol, antioxidant activity

INTRODUCTION

Juglans regia L. is a very important plant actually the nut highly famous and mostly utilized. In Pakistan, this species is highly distributed through the entire country especially in newly merged district of Khyber Pakhtunkhwa. Not just dried fruit are utilized but additionally green walnuts, kernel, leaves, shells and bark have already been utilized in pharmaceutical industry (Stampar et al., 2006). In current years an enhancing propensity usage of natural substances

rather than the artificial types has been noticed. While the artificial materials and items are more complicated compared to natural compound, quite a long time for them to in order for them to complete his natural time periods and come back to nature; therefore creating lots of environmental contamination. Similarly using the rise in the buying cost of raw materials, the issue of price advantages for chemical generation is now extra substantial. Natural antioxidants that because phenolic substance, utilized as natural

antioxidants are getting benefit, because of the importance for human health, reducing the possibility of chronic ailment through decrease in suppression of macromolecular oxidation and oxidative stress (Silva et al., 2004; Pulido et al., 2000). Their usage as protecting food ingredients (Zupko et al., 2001) had maximizing attention. As well as antioxidant activity, many studies exhibited the antimicrobial activity of phenols and/or phenolic extracts (Pereira et al., 2006, 2007a,b; Proestos et al., 2005; Puupponen-Pimiä et al., 2001; Rauha et al., 2000; Sousa et al., 2006; Zhu et al., 2004), creation these an excellent substitute for antibiotics and preservative chemicals. There was a prolonged curiosity about utilizing natural antimicrobial substances, while the user stress on the food industry supplement, in order to prevent preservative chemicals as a result of enhancing immunity to antibiotics (Oliveira et al., 2007; Cowan, 1999). *Juglin regia* L green shell is a by-product associated with *Juglin regia* L production, having limited utilization. Therefore, utilizing husk like a source of phytochemicals will increase the significance of the walnut yield, along with offer usage for a by-product that is produced in a huge amount. Various studies revealed the possibility antioxidant of *Juglin regia* L yield, particularly fruits (Espinet et al., 2000; Pereira et al., 2008) but also leaves (Pereira et al., 2007b) as well as liqueurs produced through green fruits (Stampar et al., 2006). Various studies reported the antimicrobial activity of *Juglin regia* L products, especially of bark (Alkhwajah, 1997), leaves (Pereira et al., 2007b), fruits (Pereira et al., 2008) therefore the particular compound juglone (Clark et al., 1990) although till this time around the knowledge regarding *Juglin regia* L green husks is almost found.

In our study walnut (*Juglin regia* L) collected from Tehsil utman khel District Bajaur, were examined, regarding their antimicrobial activities. Antioxidant, Antioxidant potential, and total phenols content

MATERIALS AND METHODS

Plants collection and Extraction

Juglin regia L plant were shade dried and ground into a fine powder. From each plant 50g of powder were soaked in 200ml of different solvents (methanol, ethanol, distilled water and n-hexane) and powder of the *Juglin regia* L plant was stored at room temperature for two weeks and was vigorously shaken each day. The impregnated plant material was first filtered through plain filter

and whattmann filter paper # 41. By rotary evaporator under vacuum, solvents were completely vaporized to obtain the crude extracts. The filtrates acquired were kept in a refrigerator (Chaudhary et al., 2011).

Antibacterial activity

The antibacterial potential of *juglin regia* L plant was determined by a modified agar well diffusion assay reported by Khan et al., (2011).

Requirements/ Materials

Plant samples, nutrient agar and broth, bacterial strain cultures, saline solution bacterial slants, sterile cork borer, disc (for diffusion), micropipette, Petri plates, organic solvent (DMSO), incubator, spirit lamp, Filter paper.

Preparation of samples

Samples were prepared from *Juglin regia* L plant using methanol, ethanol, n-hexane and distilled water.

Preparation of media for bacteria

1. Media was prepared by adding 0.13g of nutrient broth to 10ml of dist. water, adjusting the pH to 7.0 & autoclaved.

2. Nutrient agar (NA) medium was organized through dissolving 23gm/10ml nutrient agar in dist. water, adjusting the pH to 7.0 and autoclaved.

Bacterial strains used

A gram positive strain called *Staphylococcus aureus* was used in the study. The bacteria were preserved at 4°C on nutrient agar medium.

Antifungal assay

The antifungal activity of *Juglin regia* L plant was determined by agar well diffusion assay stated by Khan et al., (2011) with some modifications.

Microorganism used:

Venturia inaequalis, an ascomycetes fungi was used in the study

Assay procedure

Media was prepared by taking 6.5 g Sabouraud agar and dissolve in 100 ml of dist. water. The pH was adjusted to 5.6. Methanol is used as a negative control. 15 ml of each medium was poured into a P. dish (9cm), allowed to solidify. Subsequently, a 7-day-old well-tested fungal culture was obtained with a pre-sterilized cork drilling machine and placed upside down in

the center of the plate. Petri dishes were placed for 7 days in the dark at 25 ± 2 ° C in incubator. Extension diameter (mm) was measured at 24-hour intervals for seven days. The assay was performed in triplicate.

The rate of fungal inhibition was found out by the following formula:

$$\% \text{ inhibition of fungi growth} = \frac{\text{Linear growth in test sample} \times 100}{\text{Linear growth in control}}$$

Phytochemical assay

Phytochemical process is carried out after plant extract preparation by using the detailed protocol is as follows:

Test for saponins

A total of 3ml from extract was added to 5ml of dist. water in a test tube and shaken dynamically thus will result in a stable foam, a +v sings for saponins.

Test for steroid

To 5 ml of extract, 2 ml of chloroform and conc. H₂SO₄ was added. Red color production in chloroform inferior layer indicates steroids presence.

Test for Glycosides

3ml of extract was mixed with 2ml of chloroform, then 2ml of conc. H₂SO₄ was added and gently shaken. A reddish brown colour was obtained which shows the presence of steroidal ring.

Test for Terpenoids

A total of 5 ml extract was mixed in 2ml of chloroform and dissolve till dryness. Then 2ml of conc. H₂SO₄ was included and heat up for approximately two minutes. A grayish colour appeared which indicate the existence of terpenoids.

Test for Flavonoids

5 ml of extract had been included to 2ml of 2% NaOH solution which resulted in yellow colour. Then a small number of drops of dilute acid were put into make the solution colour less which is a positive notice for the existence of flavonoids.

Test for Phenols and Tannins

From extract a total of 0.5 ml was combined 2ml of water and 2 to 3 drops of FeCl₃ were added. The appearance of blackish color indicate the existence of tannins and phenols.

Total phenolic assay

The magnitude of total phenol was identified through the use of Folin-Ciocalteu assay reported by (Oliveira et al., 2008). Plant sample extract 0.1g were added with 60 to 80ml of distilled water. Now it is heated and boiled for 30 minutes in a sand bath and filtered. Final volume was up to 100ml with distilled water. From the above extract 0.1ml added to test tube (screw cap) plus 3.9ml dist water is added and agitated. Add 1ml of Folin-Ciocalteu reagent and then added 5ml of 20% of sodium carbonate and shake vigorously. Keep it at room temperature for 20 minutes. After that O.D of sample will be observed at 720nm wave length. Experiments were performed in triplicate.

Antioxidant assay

Antioxidant assay was performed by scavenging effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Oliveira et al., 2008). A total 0.25 ml DPPH 0.8 mg in MeOH was included with test tubes to accurately weigh the weighted amounts of the extracts break up in 3.75 ml MeOH, accordingly to an extraction level of concentration ranging from 0.01 and 0.2 mg / ml. Subsequently preparing, the samples were kept at room temperature for half an hour in the dark. Absorbance at 517 nm was calculated utilizing a UV / Vis V-530 spectrophotometer and examined having a non-extracted control. A blank for every sample was ready utilizing methanol rather than of the DPPH solution. Ascorbic acid had been utilized as the reference compound. Antioxidant activity was shown as a percentage of DPPH radical inhibition and was measured by comparison:

$$\% \text{ scavenging DPPH free radical} = 100 \times \frac{(1-AS)}{AD}$$

These values are described the same as the inhibitory concentration of the extract necessary to reduce the first DPPH radical concentration by 50% and shown in mg/ml.

STATISTICAL ANALYSIS

Standard deviation was applied on the data and recorded in mean values then converted into percent. The Formula of calculation in percent is as follows:

$$\text{Percent inhibition} = \frac{\text{Zone of inhibition of extract (mm)} \times 100}{\text{Zone of inhibition of standard}}$$

RESULTS

Anti-bacterial activity of selected plants extracts

The current study also carried out assess the

antimicrobial activity of crude extracts (Distilled Water, ethanol, methanol, and n-hexane) of *Juglin regia* L against *Staphylococcus aureus* bacteria strains and one fungal strain. The *Juglin regia* L leaves were utilizing the agar disc diffusion method by calculating the diameter of growth inhibition zones with 50 µl of distilled water, ethanol, methanol and n-hexane. Antibacterial activity was carried performed against gram positive *Staphylococcus aureus* bacteria. In *Juglin regia* L different zone of inhibition were observed. Zones of inhibition were varied from 1mm to 8mm in *Juglin regia* L plant extracts. Largest zone was found with Methanol extract 8mm, used by n-hexane 7mm, after which Distilled water 6mm. But minimal zone of inhibition associated with Ethanol extract 1mm against *Staphylococcus aureus* (Table. 1). Analysis regarding the data show that methanol, n-hexane and Distilled Water extracted samples have a significant inhibitory impact against *Staphylococcus aureus* as evaluated to the ethanol extracted samples. Methanol and n-hexane extracts had been more efficient against *Staphylococcus aureus* at each the amounts level as compared towards the ethanol extracts, 8.3 mm (59% Z.I) by methanol while 7.2 mm (51% Z.I) by n-hexane and 6mm (43%) Distal water and 1 mm (7%) by ethanol by ethanol at the concentration of 1 and 2mg disc-1.(Table 2). Highest zone of inhibition percentage (59%) was found at methanol extract while the lowest zone of inhibition percentage (7%) observed in ethanol extract (Fig 1)

Anti-fungal activity

The inhibitory effect of various extract (viz distilled water, methanol, ethanol and n-hexane) of *Juglin regia* L, were examined against fungal strains. In *Juglin regia* L different zone of inhibition were noticed. The inhibition zones were varied from 1.9mm to 5.4mm in *Juglin regia* L plant

extracts.(Table 3) Various zone of inhibition percentage was seen that is for methanol 3.8mm (56%) used by n-hexane 3.5mm (51%) and then distilled water 3.4mm (50%) and similarly the observed zone of inhibition for ethanol was 1.9mm (28%) against *Venturiainaequilis* fungi (Table 4). Highest zone of inhibition percentage (56%) was found at methanol extract while the lowest zone of inhibition percentage (28%) observed in ethanol extract (Fig 2)

Phytochemical study of *Juglin regia* L.

The phytochemical screening reflect that various phytochemicals were found in methanolic extract of *Juglin regia*L (leaves). Such as saponins, terpenoid, flavonoids, phenol and tannin although steroid and glycoside were found missing. Methanolic extracts showed the existence of greater amounts of phenolic compounds as compared to ethanolic one. (Table 5).

Total Phenol Contents of *Juglin regia* L.

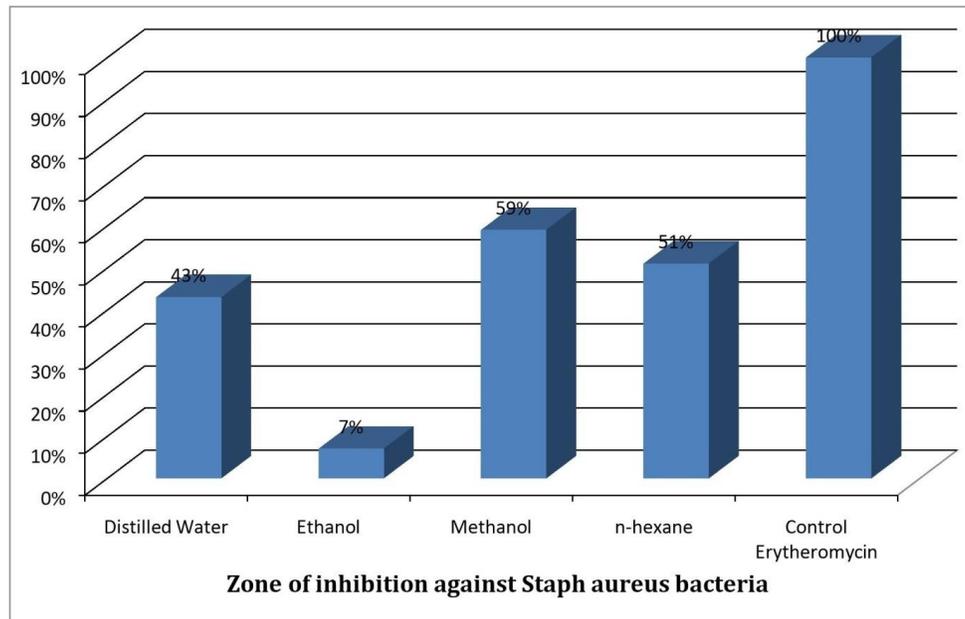
The total phenolic levels of the aqueous extracts was confirmed by using Folin Ciocalteu chemical agent. In the assay, gallic acid was utilized a standard compound and total quantity of phenolic contents were indicated like mg/g gallic acid equivalent utilizing the standard curve equation: $y = 0.0012x - 0.0018$, in which y is absorbance at 720 nm and x is total phenolic content in *Juglin regia* L, extracts indicated in mg/gm. The results showed that *Juglans regia* L possessed the greatest source of phenolic contents (2.07%) (Table 6). The maximum content of phenol compounds in *juglan regia* L revealed that these substances contribute to anti-oxidant activity. It could be perceived as many promising plant species for natural herbal provision of antioxidants with a high active value for the manufacture of medicines.

Table: 1. Zone of inhibition of *Juglin regia* L plants extracts against *Staphylococcus aureus* bacteria.

Name of the Organism	Plants extract	Zone of Inhibition in (mm)	Mean± Standard Deviation	Chi –Square
<i>Staphylococcus aureus</i>	Distilled Water	6	6.0±0.15	0.5
	Ethanol	1	1±0.17	
	Methanol	8.3	8.3±0.18	
	n-hexane	7.2	7.2±0.20	
	Control Erytheromycin	14	14±0.13	

Table2: Percentage inhibition of *Juglin regia* L. plants extracts against *Staphylococcus aureus* bacteria.

Name of the Organism	Plants Extract	Percentage (%)
Staphylococcus aureus	Distilled Water	43%
	Ethanol	7%
	Methanol	59%
	n-hexane	51%
	Control Erytheromycin	100%

Fig 1. Inhibition zone of *Juglin regia* L. extracts against *Staphylococcus aureus* bacteria.**Table3: Zone of inhibition in (mm) against fungal strain *Venturia inaequilis*.**

Name of the Organism	Plant Extract	Zone of Inhibition in (mm)	Mean± Standard Deviation	Chi -Square
Venturia inaequilis	Distilled Water	3.4	3.4±0.152	0.5
	Ethanol	1.9	1.9±0.115	
	Methanol	3.8	3.8±0.208	
	n-hexane	3.5	3.5±0.152	
	Control (methanol)	6.8	6.8±0.152	

Table 4: Percentage inhibition of *Juglin regia* L plants extracts against fungal strain *Venturia inaequilis*.

Name of the Organism	PLANT EXTRACTS	Percentage (%)
Venturiainaequilis	Distilled Water	50%
	Ethanol	28%
	Methanol	56%
	n-hexane	51%
	Methanol (-ve control)	100%

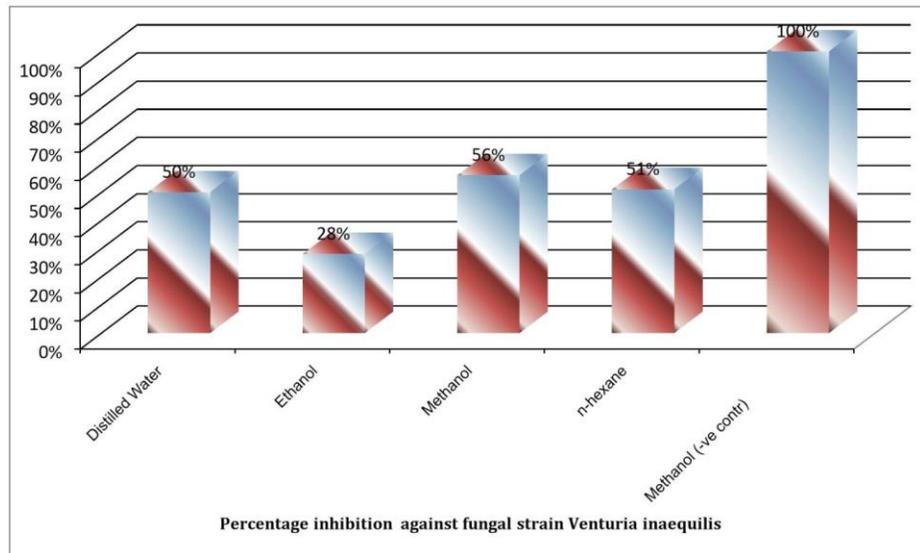


Fig 2. Zone of inhibition of *Juglin regia* L extracts against fungal strain *Venturia inaequilis*.

Table 5: Phytochemical screening of *Juglinregia*L

S.No	PHYTOCHEMICAL COMPOUND	Methanol extract
1	Saponin	+
2	Steroid	—
3	Glycoside	—
4	Terpenoid	+
5	Flavonoid	+
6	Phenol and Tannin	+

Table 6: Total phenolic analysis of *Juglinregia*L plants extracts by Folin-Ciocalteu assay

Treatment	Sample weight (g)	O.D	Blank	O.D Blank	X= y + 0.0018 / 0.0012	Total phenol (ppm)	%age
<i>Juglin regia</i> L. 1	0.11	0.16	0.113	0.047	40.62	20333.3	2.01±1.24
<i>Juglin regia</i> L. 2	0.11	0.161	0.113	0.048	41.5	20750	2.07±1.11

Table 7: Antioxidant potential of aqueous plant extracts of *Juglinregia*L plant.

Concentration	Anti-oxidant activity (%)	
	Mean	Mean± Standard Deviation
60 µg	61	61.45±0.11
80 µg	65	65.23±0.10
100 µg	72	71.78±0.12

Antioxidant activity:

The antioxidant potential of *Juglin regia* L. was found out utilizing aqueous extracts of the *Juglin regia* L plant by scavenging effect assay. Various amount (60, 80 and 100 µg/ml) of the extracts were utilized in the assay as shown in (Table 7). At 60µg/ml the aqueous fraction of *Juglansregia* L showed the highest radical scavenging activity (61.45±0.11) At 80 and 100µg/ml, a maximum anti-oxidant activity (65.23±0.10 & 71.78±0.12) for the aqueous extracts of *Juglin regia* L. Usually a concentration dependant activity was documented for targeted extract. The powerful property that is antioxidant of extracts because of the feature of having hydroxyl groups within the phenolic substances.

DISCUSSION

The results revealed that various phytochemicals that as saponins, terpenoid, flavonoids, phenol and glycoside were found in the leaves of (Qadir et al., 2014) Who reported that methanolic plant extracts were observed to reveal the existence of large concentration of flavonoids, saponins alkaloids, and phenolic compounds however he did not report glycoside. It is documented that such chemical constituents are important for the antimicrobial property against some individual pathogens and thus might recommend the utilization indigenously for the treating different diseases (Wright and Linda, 2011; Uddin et al., 2011). The antimicrobial properties of multiple extracts of *Juglin regia* L was examined against *Staphylococcus aureus* bacteria plus one fungus *Venturia inaequilis*. Results showed that each extracts of *Juglin regia* L were most effective against *Staphylococcus aureus* other than ethanol extract which reveal less response comparatively methanol. It was additionally observed that crude methanolic extract of *Juglin regia* L show activity that is good compared to ethanol. Methanol extract inhibited the growth of *Staphylococcus aureus*. (Cruz-Vega et al 2008). Who stated that methanol and n-

hexane plant extracts were found to indicate development of inhibition against bacterial strains 100 and 125 mg/ml, correspondingly? In the current study *Juglin regia* L exhibit maximal antifungal activity in n-hexane plant extract. Ethanol extract indicated medium antifungal activity but minimal inhibition zone was seen in methanol extract against the fungal strains (Pereira et al, 2008).. Who stated that *Juglin regia* L fruits, leaves and bark aqueous and solvents extract showed antifungal activity against extended range of fungi and the same investigations was carried out by (Oliveira et al 2008). His work reflects that all most walnut species exhibit antifungal activity against *Cryptococcus neoformans* and *Candida albicans* petroleum ether extract. High zones of inhibition had been seen with cv. Lara extracts (MIC of 1 mg/mL). But in his result aqueous plant extract of green husks were observed with no antifungal activity against *Cryptococcus neoformans* and *Candida albicans* (Oliveira et al. 2008). In the present study it was reported that *juglanregia* L have high content of phenol compounds . The same studies were carried out on *Juglin regia* L, by Akin, et al., (2013); Oliveira et al., (2008) who documented that such plants are a potential source of phenolic substances and therefore justifying our results effects. Numerous work result that antioxidant activity 26 of phenolic substances contained in plants have redox characteristics that allow it to work as reducing agents and hydrogen donators (Mustafa et al., 2010). The outcome have been in conformity to the results published by Oliveira et al., (2008); Rahman et al., 2016) where a concentration dependant anti-oxidant activity were recorded for *Juglin regia* L.

CONCLUSION

The phytochemical profiling revealed the existence of glycosides, tannins, alkaloids, reducing sugar, phlobatanins, terpenoids, steroids, saponins, , flavonoids. While glycosides and steroid did not observed in methanolic

extract of *Juglin regia* L. Methanol extract samples indicate excellent activity against *Venturiainaequilis* and *Staphylococcus aureus*.

CONFLICT OF INTEREST

The authors declared that current study was carry out in the absence of all conflict of interest.

Funding sources.

This research study was sponsored through Prime minister Reimbursement scheme, Pakistan.

ACKNOWLEDGEMENT

This research work was sponsored by Prime minister Reimbursement scheme, Pakistan which is highly acknowledged. The authors extend their high appreciation and acknowledgment to the NIFA staff by giving moral support to the authors.

AUTHOR CONTRIBUTIONS

All author contributed in the paper here. Sher Hayat designed and carry out the experiments AHK wrote the manuscript. Ahmad Hasan, Saira Naz, Qaisar ali, Ateeq ur rahman, Zubir shah performed Data analysis, Ashfaq Ahmad and Khaleeq Ahmad helped in plant collection identification dehydration and powder preparation. Imran Khan and Ali Hazrat provide technical suggestions on the draft and identified the language and grammatical mistakes. IK supervised all the stages. All authors read and approved the final version.

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