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## Investigation of *Salsola imbricata* extracts against human pathogenic bacteria and fungi and *in vitro* antioxidant activities

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Antimicrobial potential of *Salsola imbricata* Forssk. was carried out through zone of inhibition and MIC assays while *in vitro* antioxidant potential was explored via different assays, i.e. total phenolic contents, total flavonoids content, DPPH assay, ABTS assay and metal chelating. It is accomplished through results that both parts had good to satisfactory antimicrobial and antioxidant activities. The maximum zone of inhibition showed by methanol extract of bark against *Bacillus subtilis* (40±1.5mm) and maximum antifungal potential exhibited by chloroform extract of bark against *Aspergillus niger* (27±1.5mm). Aqueous extract of selected plant didn't show any activity against test organisms. The significant MIC value showed by fruit extract against *Pseudomonas aeruginosa* was 0.017±0.01 at 0.6mg/ml. Aqueous extracts exhibited good potential in all assays except in ABTS and metal chelating. Bark extract of methanol showed maximum potential in ABTS assay whereas chloroform bark extract exhibited maximum % bound iron in metal chelating.

Keywords: Salsola imbricata Forssk, antibacterial, antifungal, antioxidant, Zone of inhibition, MIC

### INTRODUCTION

Many countries have been practiced traditional medicine by a significant proportion of the population for many centuries. Pharmacological active compounds which are extracted from medicinal plants have increased worldwide (Magbool et al. 2019). In Central Asia, infectious diseases are cured by medicinal plants and medicinal plants produced bioactive compounds which have potential to act against multi resistant bacteria (Ajaib et al. 2018; Singh et al. 2011). The World Health Organization (WHO) is encouraging and facilitating the countries for effective herbal health programs. Some new drugs are still unexplored which is extracted from different plants (Ajaib et al. 2020). Hence, last decade as witnessed for human disease management which practiced through new biomolecules (Singh et al. 2011). Oxygen is most important molecule in our environment for the survival of living beings but sometime it is toxic due to uncoupling of electrons. As a result of uncoupling reactive oxygen species (ROS) formed which includes singlet oxygen [O], hydroxyl radicals (HO), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hypochlorous acid (HCIO). These species are very damaging because they cause lipid peroxidation in cell membrane and antioxidant prevents this lipid peroxidation by scavenging of ROS (Ajaib et al. 2017; Nishanthini et al. 2012).

Synthetic antioxidants are being limited due to their carcinogenicity effect and in replacement natural antioxidants were used. Antioxidant phytochemicals were found in medicinal plants which play a crucial role in treatment of diseases related to humans. The scrutiny of some plants were carried out and findings claimed that they have antioxidant and radical scavenging potential (Nishanthini et al. 2012; Siddiqui et al. 2017).

The selected plant *Salsola imbricata* Forssk. belongs to Chenopodiaceae locally used for skin diseases and for intestinal worms (Ajaib et al. 2019). Family Chenopodiaceae comprised of 35 genera and 106 species in Pakistan. These genera are largely distributed in desert, semidesert and along sea-shores (Munir et al. 2014).

Salsola imbricata Forssk. is a small shrub having height 0.3-1.2m. Stems and lower leaves heavily covered with ascending, short and straight hairs (Fig.1). Leaves succulent, broadly ovate to circular in outline and crushed leaves having strong fishy smell and taste (Ajaib et al. 2019). It is used for producing alkali and eaten by camels only. It is distributed in Africa, the Arabian Peninsula, S Iran, Pakistan (Baluchistan), S and E Afghanistan, NW India (Freitag et al.2001).



Figure 1: Salsola imbricata Forssk. in natural habitat.

### MATERIALS AND METHODS

### Plant material:

Selected plant *Salsola imbricata* Forssk. was collected from Bahawalpur District, Pakistan. The plant material was identified with the voucher No. SAH. 2945 and deposited in Sultan Ahmed

herbarium (SAH), Department of Botany, GC University, Lahore.

### Test organisms:

The bacterial test organisms chosen for examination *i.e.*, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* were collected from the Department of PMI, Lahore.

Fungal strains *Aspergillus niger* and *Aspergillus oryzae* selected for the exploration of antifungal potential were collected from the Institute of Industrial Biotechnology GC University, Lahore.

### Methodology adopted:

The selected plant, *Salsola imbricata* Forssk. firstly, spread into its parts such as bark and fruit and then dried under shade at room temperature for 20-30 days. Ultimately, dried plant material was crushed and subjected to maceration. The static-state maceration carried out in petroleum ether, methanol, chloroform and distilled water and concentrated extract obtained, stored at 20°C.

The percentage (%) yield of crude extracts was calculating by using the formula which is given below:

Percentage(%) Extraction yi

 $= \frac{\text{Wt. of plant extract}}{\text{Wt. of initial plant sample}}$ 

× 100

Antimicrobial potential of *Salsola imbricata* Forssk. was calculated by considering by zone of inhibition produced as a result of agar well diffusion method whereas MIC was calculated by broth dilution assay following Murray et al. (1999) with slight altering this technique. For the estimation *in vitro* antioxidant potential of *S. imbricata* the determination of total flavonoids contents assay carried following Dewanto et al. (2002). Estimation of Total Phenolic Contents (TPC) following Makkar et al. (1993). DPPH radical scavenging activity following Ajaib et al (2013). Metal Chelating activity following Dinis et al. (1994) and ABTS<sup>+</sup> Assay following Miller et al (1993).

### Statistical analysis of assessment:

All parameters were conceded out in the form of triplicates and outcomes achieved were analyzed statistically to conclude the considerable value of analysis.

### **RESULTS AND DISCUSSION**

The technique which is followed in this research was maceration of plants because its cost is low and it presents high percentage extraction yield. The procedure used for extraction or components was an important factor because it considers different parameters i.e. commercial viability, extraction yield and its quality (Ajaib et al. 2014b). Aqueous extract of both parts had exhibited highest percentage (%) extraction yield, fruit exhibited 11.18% extraction yield (Fig.2). The minimum yield exhibited by methanol bark extract of S. imbricata Forssk. i.e. 5.115%. Ajaib et al. (2014b) had been shown methanol % extraction yield in the range of 4.86-6.81% during the investigation of antimicrobial potential of Clerodendrum splendens.

The percentage extraction yield varies; it is because of two reasons. Firstly, the % extraction yield increases progressively with the increase in the polarity of solvents, i.e. the % extraction yield have direct relation to the index of solvents' polarity. Secondly, bioactive compounds in the plant material were soluble in their characteristic solvents and the distribution of the components was uneven in the various parts of the plant (Ajaib et al. 2014c).

For the assessment of antimicrobial activity of Salsola imbricata Forssk., four bacterial strains were selected i.e. Staphylococcus aureus, Bacillus subtilis (gram positive) and Escherichia coli, Pseudomonas aeruoginosa (gram negative). These bacteria have one same characteristic which is all were pathogenic. Moreover, for antifungal potential Aspergillus oryzae and Aspergillus niger were chosen.

To come across the receptiveness of the test organisms (microbial strains), standard discs were exploited (Table 1, 2). According to these standards, the specimens were categorized as susceptible, intermediate and resistant susceptible.

The extract of bark had exhibited maximum potential than fruit of chloroform extract. Methanolic extract of bark and fruit possess antibacterial potential that closely related i.e.  $13\pm1.5$  and  $14\pm1$ . Petroleum ether extract of both parts had same potential i.e.  $12\pm1$ . Aqueous extract of both parts fail to show any activity (Table 3 & Fig. 3). The extract of bark had exhibited maximum potential against *S. aureus* as compared to the bark of the with the petroleum ether extract. The methanolic extract of bark exhibited more potential than fruit. Similarly, chloroform bark extract proved to be more potential

as compared to fruit. The minimum potency was exhibited by aqueous extract of both parts. Petroleum ether bark showed highest potential. Aqueous extract of both parts fail to show any activity (Table 4 & Fig.4).

Bark extract of petroleum ether exhibited more potential as compared to fruit extract. Similarly, methanol and chloroform extract of bark have more potential as compared to fruit extract of methanol and chloroform. Methanol bark had exhibited maximum potential i.e. 31±1.5. The minimum potency was exhibited by aqueous extract of both parts. All extracts of bark proved to be more potent than fruit against *P. aeruginosa*. Aqueous extract of both parts fail to show any activity (Table 5 & Fig. 5).

Bark extract of methanol had exhibited maximum potential as compared to fruit extract. Likewise, petroleum ether and chloroform bark extract had more potential as compared to fruit. The maximum potential possesses by bark extract which was macerated in methanol as reported by Ajaib et al. (2016) during evaluation of biological activities of *Chenopodium ambrosioides*. Minimum potential exhibited by aqueous extracts. The bark extracts proved to be more potent than leaf against *B. subtilis* (Table 6 & Fig. 6).

Fruit extract of methanol exhibited maximum potential as compared to bark. Similarly, fruit extract showed more potential as compared to bark macerated in petroleum ether. Contrary to the methanol and petroleum ether extract, bark and fruit of chloroform extract somewhat same potential i.e.  $10\pm0.5$ . and minimum potential exhibited by aqueous extracts as similar finding also reported by Ajaib et al. (2015a) during antimicrobial evaluation of *Casearia tomentosa*. The fruit extract showed good activity against *A. oryzae*. Aqueous extract of both parts failed to show any activity (Table 7 & Fig. 7).

Bark extract of methanol exhibited maximum potential as compared to fruit. Similarly, bark extract showed more potential as compared to macerated petroleum fruit in ether. Correspondingly, to the methanol and petroleum ether extract, bark of chloroform extract proved to be more potent fruit. Minimum potential exhibited by aqueous extracts. The highest potential depicted by bark of chloroform. The bark extract showed good activity against A. nigerand proved to be more potent than fruit extracts. Aqueous extract of both parts failed to show any activity (Table 8 & Fig. 8).

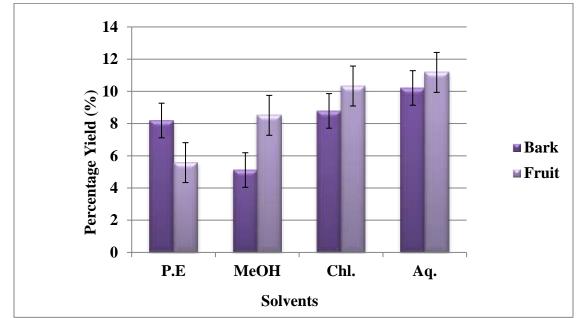


Figure 2: Graphical representation of (%) extraction yield of different parts of Salsola imbricata Forssk.

Table 1: Zone of Inhibition (mm) produced by the bacterial strains against standard antibiotic
discs

Antibiotic	Conc.	Zone of inhibition (mm)			
standard disc	(µg)	S.aureus	E.coli	P.aeruginosa	B. subtilis
Azithromycin	15	16 ±2.5	15±1.0	10±0.6	22±0.6
Piperacillin	15	-	30±1.2	20±0.4	-
Amoxicillin	20	12±1.2	25±1.0	0±0	10±0.2
Ampicillin	10	8±1.7	9±2.0	-	-
Ceftazidime	30	8±0.6	20±1.0	0±0	-
Cefotaxime	10	35±2.5	13±1.7	0±0	8±0.2
Amikacin	30	18±0.8	-	21±1.0	-
Final respon	se	Resistant- Susceptible	Intermediate- Susceptible	Susceptible	Resistant- susceptible

## Table 2: Zone of Inhibition (mm) of fungal strains against the standard antifungal discs

Antifungal standard disa	Conc. (µg/ml)	Zone of inhibition (mm)	
Antifungal standard disc		A.niger	A.oryzae
Nystatin	100	24±2.0	32±1.5
Tezole	100	22±0.5	69±2.9
Fungivin	100	15±3.4	42±1.7
Griseofluvin	100	22±0.5	27±0.5
Final response		Intermediate	Intermediate

# Table 3: Zone of inhibition produced by extracts of different parts of Salsola imbricata Forssk.against E. coli.

Diant nanta	Zone of inhibition (mm)			
Plant parts	Petroleum Ether	Methanol	Chloroform	Aqueous
Bark	12±1	13±1.5	23±1	0±0
Fruit	12±1	14±1	22±2.08	0±0
LSD	2.267	2.92	3.7	0

# Table 4: Zone of inhibition produced by extracts of different parts of Salsola imbricata Forssk. against S. aureus.

Plant parts	Zone of inhibition (mm)				
Fiant parts	Petroleum Ether Methanol Chloroform Aqueous				
Bark	30±1.5	26±2	23±1.5	0±0	
Fruit	17±1.5	21±1	11±0.5	0±0	

# Table 5: Zone of inhibition produced by extracts of different parts of Salsola imbricata Forssk. against P. aeruginosa.

Plant parts	Zone of inhibition (mm)				
	Petroleum Ether Methanol Chloroform Aqueous				
Bark	28±1.5	31±1.5	19±1.5	0±0	
Fruit	20±1.5	17±1.5	16±1.5	0±0	
LSD	3.46	3.46	3.46	0	

# Table 6: Zone of inhibition produced by extracts of different parts of Salsola imbricata Forssk. against *B. subtilis*.

Plant parts	Zone of inhibition (mm)					
Fiant parts	Petroleum Ether	Methanol	Chloroform	Aqueous		
Bark	30±1	40±1.5	18±1.5	0±0		
Fruit	17±1.5	17±2.08	10±0.5	0±0		
LSD	2.92	4.13	2.61	0		

## Table 7: Zone of inhibition produced by extracts of different parts of Salsola imbricata Forssk. against *A. oryzae.*

Diant parts	Zone of inhibition (mm)			
Plant parts	Petroleum Ether	Methanol	Chloroform	Aqueous
Bark	10±0.5	12±0.5	10±0.5	0±0
Fruit	11±1.5	13±1.5	10±0.5	0±0
LSD	2.61	2.61	1.3	0

# Table 8: Zone of inhibition produced by extracts of different parts of Salsola imbricata Forssk. against A. niger.

Plant parts	2	Zone of inhibition (mm)		
Plant parts	Petroleum Ether	Methanol	Chloroform	Aqueous
Bark	12±1	20±1	27±1.5	0±0
Fruit	11±1	12±1.1	10±0.5	0±0
LSD	2.267	2.43	2.61	0

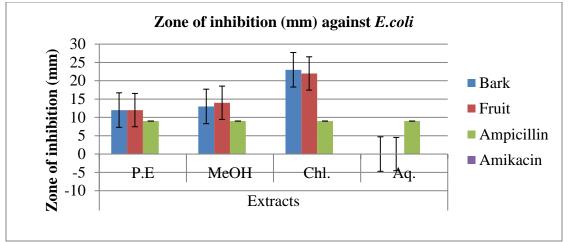


Figure 3: Graphical representation of Zone of inhibition produced by different parts of *S. imbricata* Forssk. against *E. coli.* 

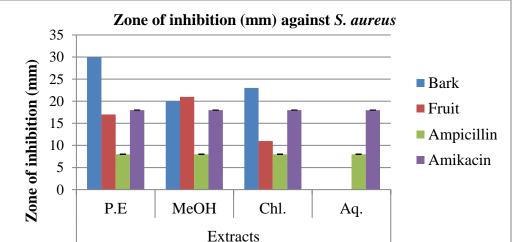
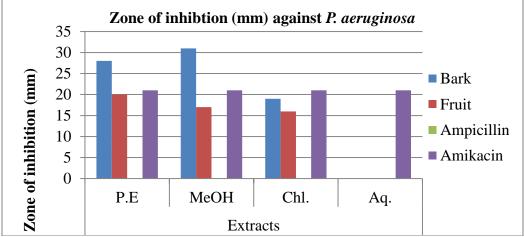
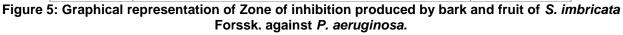


Figure 4: Graphical representation of Zone of Inhibition produced by bark and fruit of *S. imbricata* Forssk. against *S. aureus*.





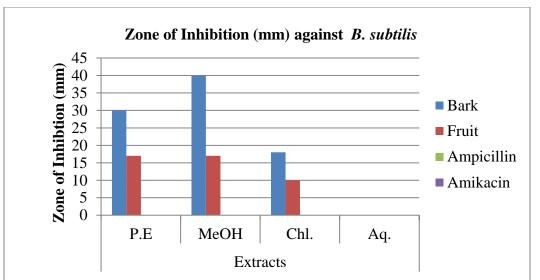


Figure 6: Graphical representation of Zone of inhibition produced by bark and fruit of *S. imbricata* Forssk. against *B. subtilis.* 

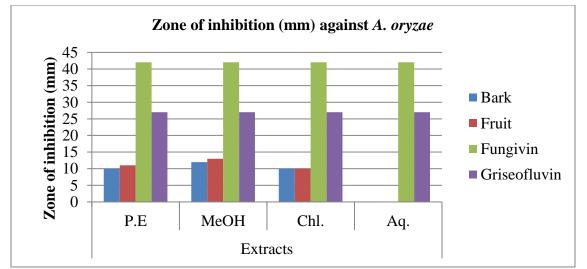


Figure 7: Graphical representation of Zone of inhibition produced by bark and fruit of *S. imbricata* Forssk. against *A. oryzae* 

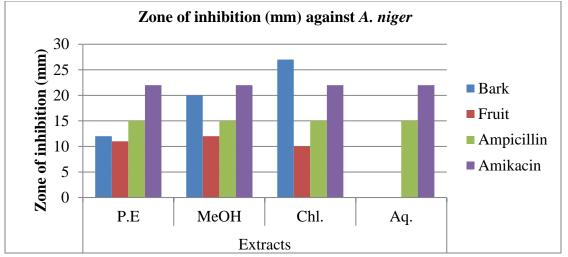


Figure 8: Graphical representation of Zone of inhibition (mm) produced by bark and fruit of *S. imbricata* Forssk. against *A. niger* 

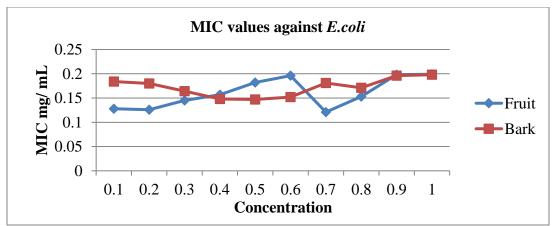
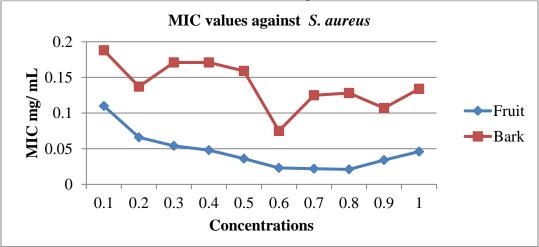


Figure 9: Graphical representation of MIC values (mg/mL) exhibiting potential of the bark and fruit of S. imbricata Forssk. against E. coli.





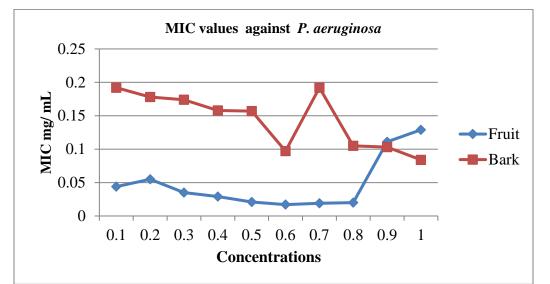


Figure 11: Graphical representation of MIC values (mg/mL) exhibiting potential of the bark and fruit of *S. imbricata* Forssk. against *P. aeruginosa.* 

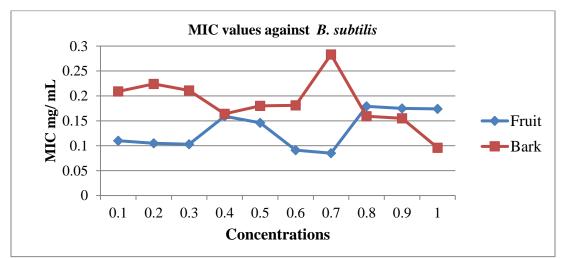


Figure 12: Graphical representation of MIC values (mg/mL) exhibiting potential of the bark and fruit of *S. imbricata* Forssk. against *B. subtilis* 

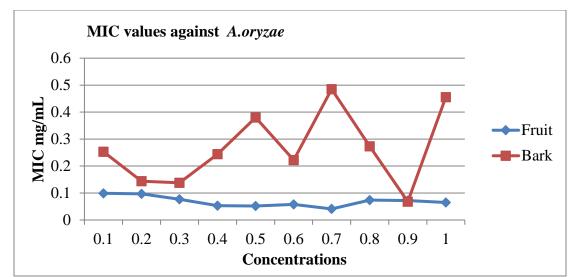


Figure 13: Graphical representation of MIC values (mg/mL) exhibiting antifungal potential of the bark and fruit of *S. imbricata* Forssk. against *A. oryzae.* 

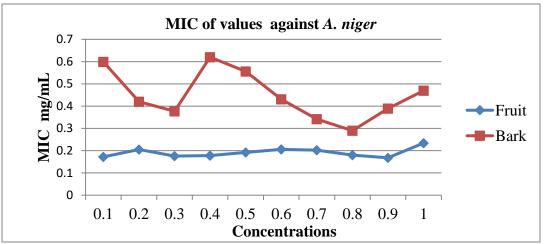
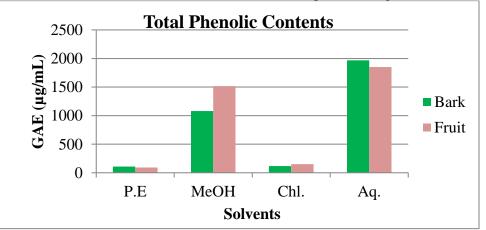


Figure 14: Graphical representation of MIC values (mg/mL) exhibiting antifungal potential of the bark and fruit of *S. imbricata* Forssk. against *A. niger.* 





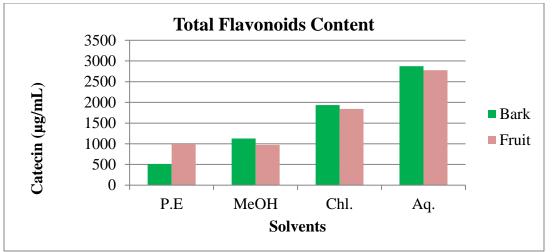


Figure 16: Graphical representation of Total Flavonoids Content (TFC) of different parts of *S. imbricata* Forssk

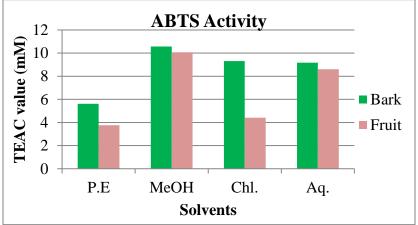


Figure 17: Graphical representation of ABTS Activity of different parts of S. imbricata Forssk.

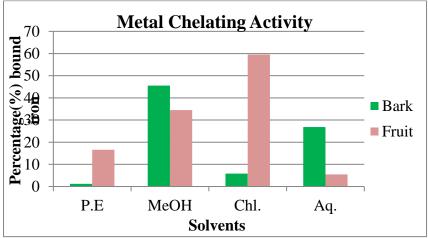


Figure 18: Graphical representation of the Metal Chelating Activity of different parts of *S. imbricata* Forssk.

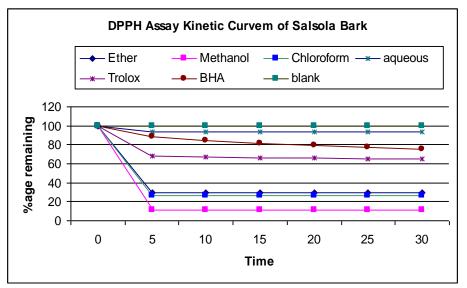


Figure 19: Graphical representation of DPPH kinetic curve of bark extracts of S. imbricata Forssk.

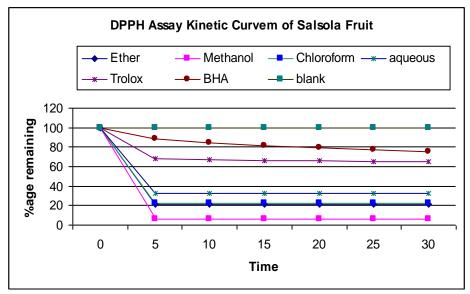


Figure 20: Graphical representation of DPPH kinetic curve of fruit extracts of S. imbricata Forssk.

The potential of *Salsola imbricata* Forssk. against bacterial strains was *B.subtilis> S. aureus> P. aeruginosa> E.coli.* The potential of *S. imbricata* Forssk. against fungal strains was *A. niger>A. oryzae.* Plant exhibited good to satisfactory antifungal potential except water extracts. They didn't show any activity against fungal and bacterial strains (Ajaib et al., 2014c).

Broth agar dilution method was used to carry out minimum inhibitory concentration (MIC). 10 dilutions were prepared according to thistechnique against all bacterial strains and minimum concentration attained which restrict the growth of bacteria at particular concentration.

Bark possesses 0.075±0.01 at 0.6mg/mL concentration against *S. aureus* which was minimum amongst all bacterial strains. Fruit possess 0.017±0.01 at 0.6mg/mL concentration against *P. aeruginosa*. Bark and fruit extracts seems to be more effective against *S. aureus* and *P. aeruginosa* respectively amongst all bacterial strains.

Dilutions were also made against fungal strains. Bark possess 0.068±0.02 at 0.9mg/mL concentration against *A. oryzae* which was more efficient than *A. niger.* Fruit exhibited 0.041±0.01

against *A. oryzae* at 0.7mg/mL concentration. Bark and fruit proved to be more competent against *A. oryzae* than *A. niger* (Fig. 13, 14).

Five assays TPC, TFC, ABTS, Metal chelating activity and DPPH were used to determination of antioxidant activity. Plants provide antioxidant constituents which documents as free radicals (Ajaib et al. 2015b. Trend has been altered considerably as there is increased consideration to explore natural assets having antioxidant potential and can alternate synthetic antioxidants (Ajaib et al. 2014a).

Aqueous extract of bark of *S. imbricata* Forssk. had exhibited maximum potential than aqueous extract of fruit. Petroleum ether bark extract had more potential as compared to fruit extract. Fruit had exhibited more potential than bark macerated in methanol. Correspondingly, fruit of chloroform extract showed more Gallic acid equivalent ( $\mu$ g/mL) than of bark. Highest Gallic acid equivalent ( $\mu$ g/mL) values exhibited by bark aqueous extract i.e. 1969.25±0.8 $\mu$ g/mL. The minimum GAE potential showed by fruit extract of petroleum ether as compared to others. The values showed presence of total phenolic contents in both parts of plant (Fig. 15).

Maximum potential exhibited by bark extract as compared to fruit macerated in distilled water. Highest amount of flavonoid contents was present in aqueous bark extract i.e.2876.18 $\pm$ 2.4µg/mL. Following aqueous extracts, methanol and chloroform bark extract had more Catecin (µg/mL) value than fruit extract. However, there is contradiction between petroleum ether and other bark extracts as petroleum ether bark extract proved to be less potent than fruit extract. Petroleum ether bark had exhibited minimum Catecin (µg/mL) value. The maximum and minimum peak exhibited by aqueous bark extract and petroleum ether bark extract respectively (Fig. 16).

Methanol bark extract exhibited maximum TEAC value (mM) as compared to fruit extract. Similarly, bark extract of petroleum ether, chloroform and aqueous had exhibited more potential than fruit extract. The maximum TEAC value exhibited by methanol extract of bark i.e. 10.57±1.5mM and 3.76±0.2mM minimum TEAC value which was exhibited by petroleum ether fruit extract. The highest and lowest peak depicted by methanolic extract of bark and petroleum ether fruit extract correspondingly (Fig. 17).

Chloroform fruit extract of fruit had exhibited maximum percentage (%) bound iron as compared to bark extract. Following aqueous extract, petroleum ether fruit exhibited more percentage (%) bound iron value than of bark extract. Contrary to other extracts, methanol and aqueous bark proved to be more efficient than fruit. The maximum percentage (%) bound iron exhibited by chloroform fruit i.e. 59.5±0.9% and minimum percentage (%) bound iron showed by bark petroleum ether extract i.e. 1.2±0.3% (Table 4.33). The highest peak and lowest peak depicted by chloroform fruit extract and petroleum ether bark extract respectively (Fig. 18).

Bark extract had exhibited maximum % DPPH (remaining) value than of fruit macerated with distilled water. Following the aqueous bark extract, petroleum ether, methanol and chloroform extract of bark had more % DPPH (remaining) as compared to fruit. The highest % DPPH (remaining) showed by aqueous bark extract i.e. 93.6% and minimum value exhibited by fruit methanol extract i.e. 6.33%. Hence, bark extracts of *Salsola imbricata* Forssk. proved to be more efficient as compared to fruit (Fig. 19, 20).

### CONCLUSION

The appraisal of antibacterial activity showed that both parts of chosen plant have good to satisfactory activity against bacteria. Aqueous extracts exhibited minimum potential against bacterial and fungal strains. The plant also exhibited antifungal potential but plant had less antifungal potential as compared to antibacterial. Plant also depicted good antioxidant activity for five assays i.e. TPC, TFC, DPPH, ABTS assay and Metal chelating. All extracts showed significant antioxidant potential. From above results it is concluded that respective plant contains bioactive compounds which is functional against peroxidative damage in living systems to cure cancer, aging and chronic diseases.

### CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

### AUTHOR CONTRIBUTIONS

MA designed and SFperformleed the experiments and both also wrote the manuscript. MTZ, MI, FS, SI and KHB reviewed the manuscript. All authors read and approved the final version.

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