

Original Article

Antibacterial and antifungal activity of methanolic extracts of *Salix alba* L. against various disease causing pathogens

Atividade antibacteriana e antifúngica de extratos metanólicos de *Salix alba* L. contra vários patógenos que causam doenças

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Abstract

The present study was aimed to manifest the antibacterial and antifungal activity of methanolic extracts of *Salix alba* L. against seven Gram-positive and Gram-negative bacterial pathogens e.g. *Streptococcus pyogenes*, *Staphylococcus aureus* (1), *S. aureus* (2), *Shigella sonnei*, *Escherichia coli* (1), *E. coli* (2) and *Neisseria gonorrhoeae* and three fungal isolates from the air such as *Aspergillus terreus*, *A. ornatus*, and *Rhizopus stolonifer*. Two different serotypes of *S. aureus* and *E. coli* were used. The agar well-diffusion method results showed the dose-dependent response of plant extracts against bacterial and fungal strains while some organisms were found resistant e.g. *E. coli* (1), *S. sonnei*, *A. terreus* and *R. stolonifer*. The highest antibacterial activity was recorded at 17.000±1.732 mm from 100 mg/mL of leaves methanolic extracts against *S. pyogenes* while the activity of most of the pathogens decreased after 24 h of incubation. The highest antifungal activity was reported at 11.833±1.0 mm against *A. ornatus* at 50 mg/mL after 48 h of the incubation period. These experimental findings endorse the use of *S. alba* in ethnopharmacological formulations and suggest the use of methanolic extracts of the said plant to develop drugs to control the proliferation of resistant disease causing pathogenic microbes.

Keywords: antimicrobial, plant extract, *Salix alba*, MIC, MBC, Willow.

Resumo

O presente estudo teve como objetivo manifestar a atividade antibacteriana e antifúngica de extratos metanólicos de *Salix alba* L. contra sete patógenos bacterianos Gram-positivos e Gram-negativos. *Streptococcus pyogenes*, *Staphylococcus aureus* (1), *S. aureus* (2), *Shigella sonnei*, *Escherichia coli* (1), *E. coli* (2) e *Neisseria gonorrhoeae* e três isolados de fungos do ar, como *Aspergillus terreus*, *A. ornatus*, e *Rhizopus stolonifer*. Dois sorotipos diferentes de *S. aureus* e *E. coli* foram usados. Os resultados do método de difusão em ágar mostraram a resposta dependente da dose de extratos de plantas contra cepas de bactérias e fungos, enquanto alguns organismos foram considerados resistentes, e.g. *E. coli* (1), *S. sonnei*, *A. terreus* e *R. stolonifer*. A maior atividade antibacteriana foi registrada em 17.000 ± 1.732 de 100 mg/mL de extratos metanólicos de folhas contra *S. pyogenes*, enquanto a atividade da maioria dos patógenos diminuiu após 24 h de incubação. A maior atividade antifúngica foi relatada em 11,833 ± 1,0 contra *A. ornatus* a 50 mg/mL após 48 h do período de incubação. Esses achados experimentais endossam o uso de *S. alba* em formulações etnofarmacológicas e sugerem o uso de extratos metanólicos da referida planta para o desenvolvimento de fármacos que controlem a proliferação de doenças resistentes que causam micróbios patogênicos.

Palavras-chave: antimicrobiano, extrato vegetal, *salix alba*, microfone, MBC, salgueiro.

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1. Introduction

The plants represent themselves as a treasury of natural therapeutic compounds and are pronounced as 'Medicinal Plants' that are well renowned for having anti-infectious properties and are used for the treatment of almost all kinds of ailments like skin, respiratory, reproductive, digestive disorders, chickenpox, dysentery, fever, hysteria, malaria, abdominal pain, mania, infantum, tonsillitis, and vomiting. Infectious diseases are chronic and are a major cause of premature death throughout the world (Shinwari, 2010). The use of plant parts and extracts of plants for the treatment of various disorders dates back to human history. The highly effective drugs that are being sold today are derived from the plants' or the plants' products (Fouche et al., 2008).

The use of Willow as a herbal remedy was cited 6,000 years ago in Mesopotamia. Sumerian, Babylonian, Egyptian, Assyrian, Chinese, Greek, and Roman civilizations used Willow for relieving pain and inflammation. Hippocrates recommended the chewing of Salix stem bark to relieve pain and inflammation. Brew of Willow is also evident for relieving excruciating pregnancy pains. An English clergyman Edward stone used Willow as a remedy to cure backache, toothache, headache, and menstrual cramps. *S. alba* is commonly known as Willow, White Willow, Silver Willow, and Golden Willow. It belongs to the genus *Salix* and the family *Salicaceae*. It is a tall tree with yellow, grey or red-brown branches, juvenile leaves are yellowish-green with acute apex and silky texture. The plant is habituated in Europe, the Mediterranean region, Western, South and Central Asia. The plant is widely naturalized in Pakistan and grows in nutrient-rich soil (Adnan et al., 2015; Fortini et al., 2016; Alamgeer et al., 2018). Fewer phytochemical studies reported the presence of glycosides, tannins, flavonoids and aromatic aldehydes (Gligorić et al., 2019; Marinho et al., 2021). Salicin has identified from *S. alba* by a French scientist H. Leroux in 1829. Salicylic acid is a glycoside in nature and is found to be responsible for all pain-relieving actions of *S. alba* (Sulima and Przyborowski, 2019).

Microorganisms are responsible to cause different ailments and antibiotics gain resistance against them. The different microorganisms were selected to explore the broad-spectrum antibiotic potential of the *Salix* plant. *S. aureus* is a major cause of pneumonia, respiratory tract infections, cardiovascular disorders, and surgical site infections. The *S. aureus* is also a causative agent of nosocomial bacteremia. It is widely isolated from hospital-acquired infections. The infections caused by *S. aureus* are difficult to treat because of the emergence of resistance against penicillin and other low-spectrum antibiotics (Lowy, 1998). Another bacterium *S. pyogenes* is very virulent and causes various acute and chronic illnesses. The *S. pyogenes* causes pharyngitis and necrotizing fasciitis and it is the prevailing cause of death in both developing and developed countries like the USA and UK (Lamagni et al., 2008). Shigellosis is a gastrointestinal infection that is very severe in children of less than 5 years and is caused by pathogenic *S. sonnei*. Ampicillin is used as a drug to eradicate this infection but due to the selective pressure bacterium is becoming resistant which involves the development of new antibacterial drugs (Kapperud et al., 1995). The *N. gonorrhoeae* is a causative agent of gonorrhoea which is a

Sexually Transmitted Disease (STD). Sexual contact with an infected person results in the transfer of disease and is habituated in the human genital tract and very unsustainable outside the human body (Lycke et al., 1980). Another well-renowned bacterium that is responsible for causing diseases is *E. coli*. It causes gastrointestinal infections in both infants and adults (Harrington et al., 2006). These microorganisms have specialized strategies to resist antibiotics which involve enzymatic inhibition of antimicrobial drugs, change in the target site and active efflux of drugs from the bacterial cell which help the microorganisms to develop resistance against antibiotics. The prevailing antimicrobial drug resistance is a challenge for the modern world to develop antibiotics to control the growing population of resistant pathogens (Javed et al., 2020; Silva et al., 2021).

Herein we report the use of the methanolic extracts of *S. alba* leaves and bark for the treatment of various bacterial and fungal pathogens. The dose and the time-dependent response were reported to evaluate the potential of extracts to curb the population of disease-causing pathogens. A complete pictorial layout of the study is represented in Scheme 1.

2. Materials and Methods

2.1. Collection of the plant samples and extract preparation

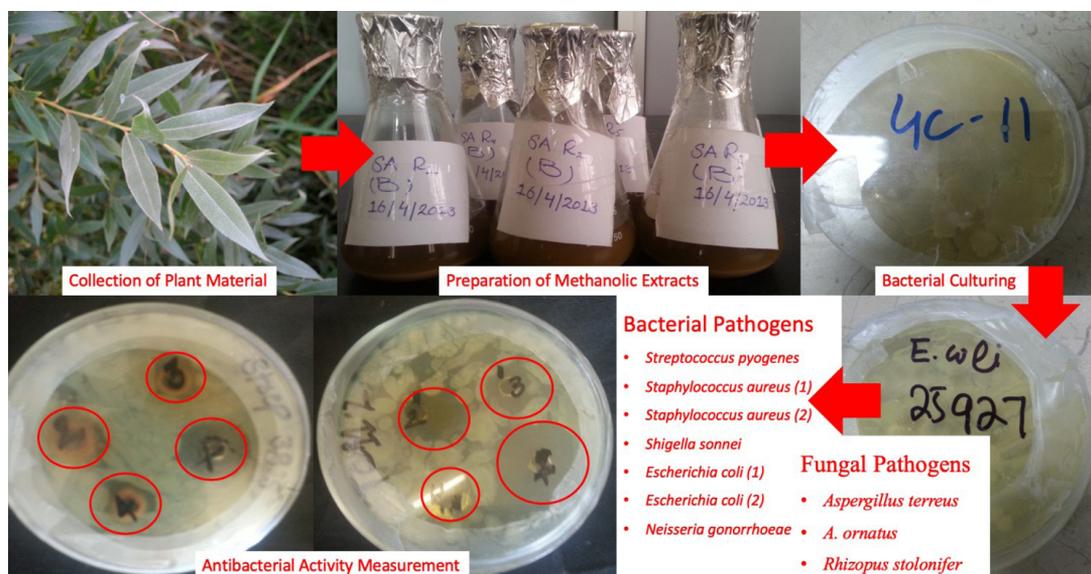
The plant samples of *S. alba* L. were collected from the Bajwat Wildlife Sanctuary, Sialkot, Pakistan (32° 40' 00.0" N, 75° 40' 00.0" E). The leaves were separated and the stem bark was peeled off from the stem. After drying, the plant parts were ground and stored in an airtight container in dark at 4 °C until extraction. The 1 to 10 ratio of the air-dried powdered plant material and methanol was used respectively to prepare organic plant extract (Dafallah Bilal and Hossain, 2019).

2.2. Adjustment of the McFarland standard

The McFarland standards were used to adjust the number of bacterial and fungal cells in a liquid suspension. The turbidity was adjusted at 0.5 McFarland (approximately 1.5×10^8 cells) by measuring the light wavelength at 600 nm (Jalalvand et al., 2019).

2.3. Antibacterial susceptibility testing of the crude plant drug

The antibacterial activity of methanolic plant extracts (stem bark and leaves) was evaluated by using the agar well diffusion assay. Luria-Bertani (LB) agar medium was poured in autoclaved Petri plates under sterilized conditions. The bacterial inoculum was spread for the uniform distribution of the seedling. The wells of 6 mm diameter were bored which were used for the loading of the plant extracts. The gentamicin was used as positive control while dimethyl sulfoxide (DMSO) was considered as a negative control. Four wells were pricked in each Petri plate. After loading the wells, plates were wrapped with paraffin tape and placed in an incubator at 37 °C for 24 h in an inverted position. The zone of inhibition around wells



Scheme 1. Schematic representation of the work.

was measured by a ruler and expressed in millimeters (mm). The zone of inhibition was measured again after 48 h to determine the efficacy of the crude plant drugs over time (Javed et al., 2020). The Activity Index (AI) and Percentage Activity (PA) were determined by using the following Equations 1 and 2:

$$\text{Activity Index} = \frac{\text{Zone of inhibition}}{\text{Control}} \quad (1)$$

$$\text{Percentage Activity} = \text{Activity Index} \times 100 \quad (2)$$

2.4. Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)

The serial dilution method was implied to determine the MIC and to prepare the different concentrations (75 mg/mL, 37.5 mg/mL, 18.75 mg/mL, 9.37 mg/mL, 4.68 mg/mL, 2.34 mg/mL and 1.17 mg/mL) of the plant extracts in LB broth. The bacterial inoculum was used to seed the LB broth containing certain plant extract doses and then incubated at 37 °C for 24 h. The control tubes were prepared without the bacterial inoculation while the positive control was prepared with gentamicin. After 24 h of incubation, the MIC was recorded by comparing the visual turbidity of the seeded tubes with control tubes. At the end of the incubation period, a loop full of bacterial culture was placed on the LB solid medium and incubated for 24 h at 37 °C to document the MBC (Bhuyan et al., 2017).

2.5. Antifungal susceptibility testing of the crude plant drug

The antifungal activities of different concentrations (75 mg/mL, 50 mg/mL, 25 mg/mL) of plant extracts were evaluated by applying the agar well diffusion assay.

The potato dextrose agar medium was poured into sterilized Petri plates under a germ-free environment of the Laminar air flow hood. The media was allowed to stabilize for 5-10 min at room temperature. The Petri plates were inoculated by decanting the spore suspension in the mid of the media and spread with the help of a bent rod. Each Petri plate was dug with four wells of 6 mm diameter. The wells were pricked with the help of a cork borer at a certain distance of 25.4 mm (1 inch) from the edges of the plate and were further filled with 100 µl of plant extracts. 5-10 minutes were given to the plant extracts to diffuse freely in the agar. The paraffin tape was used to seal the plates and incubated in an inverted position at 28 °C for 48 h. The antifungal activity was measured in millimeters (mm) with the help of a calibrated transparent scale (Mativandlela et al., 2006).

2.6. Statistical analysis

The experiments were performed in triplicate and the results were expressed in mean and standard deviation. All results were analyzed statistically to determine the significance and insignificance values with the help of MSTAT C® software in the form of the multifactorial Analysis of Variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). $P < 0.05$ was used to indicate the significant difference.

3. Results

3.1. Antibacterial activity of the methanolic extracts of *S. alba*

3.1.1. Antibacterial activity of the methanolic extracts of the leaves at 75 mg/mL after 24 & 48 h of incubation

The antibacterial activity of the leaves and the stem bark extracts of *S. alba* was assessed at 75 mg/mL

(Table 1) and 100 mg/mL after two-time intervals i.e. 24 and 48 h. It was observed that the susceptibility of all bacterial strains to the said extracts differed significantly ($P < 0.05$). The highest inhibitory activity was observed against *S. aureus* (1), followed by the *N. gonorrhoeae* and *E. coli* (2) with a zone of inhibitions at 13.333 ± 1.155 , 12.667 ± 0.577 and 10.667 ± 1.528 mm respectively. These extracts could not inhibit the growth of *E. coli* (1) and *S. sonnei*. The comparison with the standard antibiotic revealed that the highest activity in terms of activity index (AI) and percent activity (PA), was observed against the *N. gonorrhoeae* (AI = 1.15 and PA = 115) followed by *S. aureus* (1) (AI = 0.95 and PA = 95). The susceptibility of the crude plant drug remained stable with a very meager decrease in activity against used pathogens even after incubation of 48 hours (Figure 1 & Figure 2).

3.1.2. Antibacterial activity of the methanolic extracts of the stem bark at 75 mg/mL after 24 & 48 h of incubation

The antibacterial activity of the stem bark extracts at 75 mg/mL after 24 and 48 h is represented in Table 2.

The highest activity was observed against *E. coli* (2), followed by *S. aureus* (1), *S. pyogenes* and *N. gonorrhoeae* with a zone of inhibitions at 10.333 ± 0.577 , 9.667 ± 0.577 , 9.333 ± 1.155 and 9.333 ± 1.155 mm respectively. There was no growth inhibition of *E. coli* (1) and *S. sonnei* as reported by methanolic leaves extract. The efficacy of the crude methanolic extract decreased after 48 h of incubation except there was an increase in inhibition of *S. aureus* (2) (Figure 1 & Figure 2).

3.1.3. Antibacterial activity of the methanolic extracts of the leaves at 100 mg/mL after 24 & 48 h of incubation

The antibacterial activity of the *S. alba* leaves extracts at 100 mg/mL was observed after 24 and 48 h of incubation and is represented in Table 3. An increase in the concentration of the crude plant drug resulted in increasing the inhibition zone. The highest activity was observed against *S. pyogenes*, followed by *S. aureus* (1) and *N. gonorrhoeae* with a zone of inhibitions at 17.000 ± 1.732 , 15.333 ± 1.155 and 15.000 ± 1.732 mm respectively. These extracts have not inhibited the growth of *E. coli* (1) and

Table 1. Antibacterial activity of *S. alba* leaves extracts at 75 mg/mL.

Bacterial Strains	Incubation Period							
	24 Hours				48 Hours			
	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)
<i>S. aureus</i> (1)	$13.333 \pm 1.155a$	14	0.95	95	$11.667 \pm 0.577a$	14	0.83	83
<i>S. pyogenes</i>	$9.000 \pm 1.000c$	19	0.47	47	$9.000 \pm 1.000bc$	20	0.45	45
<i>N. gonorrhoeae</i>	$12.667 \pm 0.577a$	11	1.15	115	$11.000 \pm 1.000a$	15	0.73	73
<i>S. aureus</i> (2)	$8.667 \pm 1.155c$	10	0.87	87	$10.333 \pm 2.082ab$	12	0.86	86
<i>E. coli</i> (1)	$0.000 \pm 0.000d$	16	0	0	$0.000 \pm 0.000d$	9	0	0
<i>E. coli</i> (2)	$10.667 \pm 1.528b$	15	0.71	71	$8.000 \pm 0.000c$	11	0.73	73
<i>S. sonnei</i>	$0.000 \pm 0.000d$	13	0	0	$0.000 \pm 0.000d$	13	0	0

Different letters show a significant difference ($p < 0.05$). Small alphabets represent the results of DMRT. All samples in three replicates \pm Standard Deviation.

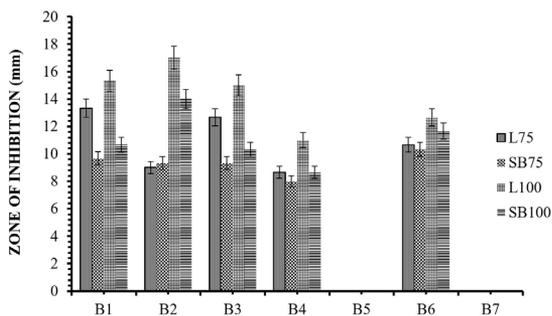


Figure 1. Comparison of zones of inhibition of leaves and stem bark extracts of *S. alba* after 24 h of incubation. L=*S. alba* leaves. SB=*S. alba* stem bark. 75 and 100 represents concentration in mg/mL. B1=*S. aureus* (1), B2=*S. pyogenes*, B3=*N. gonorrhoeae*, B4=*S. aureus* (2), B5=*E. coli* (1), B6=*E. coli* (2), B7=*S. sonnei*.

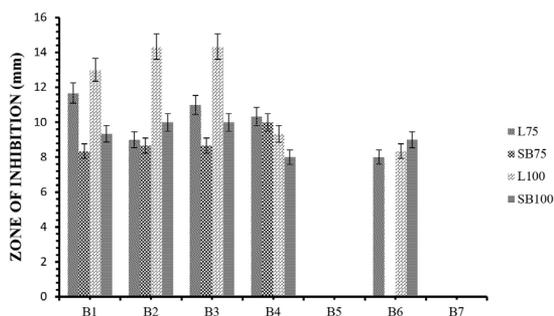


Figure 2. Comparison of zones of inhibition of leaves and stem bark extracts of *S. alba* after 48 h of incubation. L=*S. alba* leaves. SB=*S. alba* stem bark. 75 and 100 represents concentration in mg/mL. B1=*S. aureus* (1), B2=*S. pyogenes*, B3=*N. gonorrhoeae*, B4=*S. aureus* (2), B5=*E. coli* (1), B6=*E. coli* (2), B7=*S. sonnei*.

Table 2. Antibacterial activity of *S. alba* bark extracts at 75 mg/mL.

Bacterial Strains	Incubation Period							
	24Hours				48Hours			
	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)
<i>S. aureus</i> (1)	9.667±0.577a	17	0.57	57	8.333±0.577b	14	0.60	60
<i>S. pyogenes</i>	9.333±1.155a	24	0.39	39	8.667±0.577b	16	0.54	54
<i>N. gonorrhoeae</i>	9.333±1.155a	18	0.52	52	8.667±1.155b	22	0.39	39
<i>S. aureus</i> (2)	8.000±0.000b	16	0.50	50	10.000±1.000a	15	0.67	67
<i>E. coli</i> (1)	0.000±0.000c	12	0	0	0.000±0.000c	10	0	0
<i>E. coli</i> (2)	10.333±0.577a	11	0.94	94	0.000±0.000c	8	0	0
<i>S. sonnei</i>	0.000±0.000c	11	0	0	0.000±0.000c	11	0	0

Different letters show a significant difference ($p < 0.05$). Small alphabets represent the results of DMRT. All samples in three replicates \pm Standard Deviation.

Table 3. Antibacterial activity of *S. alba* leaves extracts at 100 mg/mL.

Bacterial Strains	Incubation Period							
	24Hours				48Hours			
	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)
<i>S. aureus</i> (1)	15.333±1.155ab	11	1.39	139	13.000±1.732a	12	1.08	108
<i>S. pyogenes</i>	17.000±1.732a	14	1.21	121	14.333±2.517a	14	1.02	102
<i>N. gonorrhoeae</i>	15.000±1.732ab	16	0.94	94	14.333±1.528a	15	0.96	96
<i>S. aureus</i> (2)	11.000±1.732c	17	0.65	65	9.333±1.528b	13	0.71	71
<i>E. coli</i> (1)	0.000±0.000d	16	0	0	0.000±0.000c	13	0	0
<i>E. coli</i> (2)	12.667±2.309bc	15	0.85	85	8.333±0.577b	12	0.69	69
<i>S. sonnei</i>	0.000±0.000d	13	0	0	0.000±0.000c	11	0	0

Different letters show significant differences ($p < 0.05$). Small alphabets represent the results of DMRT. All samples in three replicates \pm Standard Deviation.

S. sonnei. The highest activity in terms of AI and PA was observed against *S. aureus* (1) (AI = 1.39 and PA = 139) followed by *S. pyogenes* (AI = 1.21 and PA = 121) and *N. gonorrhoeae* (AI = 0.94 and PA = 94) (Figure 1 & Figure 2).

3.1.4. Antibacterial activity of the methanolic extracts of the stem bark at 100 mg/mL after 24 & 48 h of incubation

The antibacterial activity of the *S. alba* stem bark extracts was assessed at 100 mg/mL after a time interval of 24 hours and is given in Table 4. The highest activity in terms of the zone of inhibition (14.000±0.000 mm) was observed against *S. pyogenes*, followed by that of *E. coli* (2) (11.667±1.155 mm) while there was a gradual decrease in inhibition activity after 48 h of incubation. These extracts have not inhibited the growth of *E. coli* (1) and *S. sonnei*. The highest activity in terms of AI and PA was observed against *E. coli* (2) (AI = 1.06 and PA = 106) followed by *S. aureus* (1) (AI = 0.63 and PA = 63) and *S. pyogenes* (AI = 0.58 and PA = 58) (Figure 1 & Figure 2).

3.1.5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC)

The lowest MIC value (0.585 mg/mL) was recorded against *S. aureus* (2), followed by *E. coli* (2) (2.34 mg/mL), *S. pyogenes* (4.68 mg/mL), *E. coli* (1) (9.37 mg/mL), *S. aureus* (1) (18.75 mg/mL) and *N. gonorrhoeae* (18.75 mg/mL) when crude methanolic leaves extract was used. While in the case of the stem bark extracts, the lowest value (1.17 mg/mL) of MIC was recorded against *S. aureus* (2), followed by *S. pyogenes* (9.37 mg/mL), *E. coli* (1) (18.75 mg/mL) and *S. aureus* (1), *N. gonorrhoeae* and *E. coli* (2) (each with 150 mg/mL) as shown in Table 5.

The lowest MBC value (1.17 mg/mL) was recorded against *S. aureus* (2), followed by *E. coli* (2) (4.68 mg/mL), *S. pyogenes* (9.36 mg/mL), *E. coli* (1) (18.74 mg/mL) and *N. gonorrhoeae* and *S. aureus* (1) (each with 37.5 mg/mL) by using leaves extract. Almost the same was the case with the stem bark extract except that the values for MBC were very high against *S. aureus* (1), *N. gonorrhoeae* and *E. coli* (2) (each with a value of 150 mg/mL) (Table 5).

Table 4. Antibacterial activity of *S. alba* bark extracts at 100 mg/mL.

Bacterial Strains	Incubation Period							
	24Hours				48Hours			
	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)	Zone of Inhibition (mm)	Gentamicin (Control)	Activity Index (AI)	Percentage Activity (PA)
<i>S.aureus</i> (1)	10.667±0.577bc	17	0.63	63	9.333±0.577a	11	0.85	85
<i>S. pyogenes</i>	14.000±0.000a	24	0.58	58	10.000±1.000a	14	0.71	71
<i>N. gonorrhoeae</i>	10.333±1.155c	18	0.57	57	10.000±1.000a	16	0.63	63
<i>S. aureus</i> (2)	8.667±0.577d	16	0.54	54	8.000±0.000b	17	0.47	47
<i>E. coli</i> (1)	0.000±0.000e	12	0	0	0.000±0.000c	16	0	0
<i>E. coli</i> (2)	11.667±1.155b	11	1.06	106	9.000±1.000ab	15	0.6	60
<i>S. sonnei</i>	0.000±0.000e	11	0	0	0.000±0.000c	13	0	0

Different letters show a significant difference (p<0.05). Small alphabets represent the results of DMRT. All samples in three replicates ± Standard Deviation.

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *S. alba* extracts.

Bacterial Strains	Leaves		Bark	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>S. aureus</i> (1)	18.75	37.5	150	N.O
<i>S. pyogenes</i>	4.68	9.37	9.37	18.75
<i>N. gonorrhoeae</i>	18.75	37.5	150	N.O
<i>S. aureus</i> (2)	0.585	1.17	1.17	2.34
<i>E. coli</i> (1)	N.O	N.O	N.O	N.O
<i>E. coli</i> (2)	2.34	4.68	150	N.O
<i>S. sonnei</i>	N.O	N.O	N.O	N.O

N.O = Not Observed.

The overall picture reflected that MIC and MBC of leaves extract against all selected bacterial strains were lower than that of bark extract (Figure 3).

3.2. Antifungal activity of the methanolic extracts of *Salix alba*

The antifungal activity of the *S. alba* leaves and bark extracts were recorded at regular intervals of 48 h and 96 h to explore the efficacy and potency of the crude drug (Table 6). The analysis of variance revealed highly significant results (P<0.05). The plant extracts were prepared in doses of 25 mg/mL, 50 mg/mL and 75 mg/mL to determine the lowest effective applied concentration of a drug. Both leaves and the stem bark extracts of the plant were found not effective against *A. terreus* and *R. stolonifer* on all applied concentrations while *A. ornatus* was found potent against all experimental concentrations with a diameter (11.833±1.041 mm) at 50 mg/mL of leaves methanolic extract. At 25 mg/mL the inhibition zone diameter (11.5±0.500 mm) was greater than the concentration of 75 mg/mL (9.667±1.155 mm). The bark extract of the plant was also found impotent against both *A. terreus* and *R. stolonifer*. *A. ornatus* expressed activity

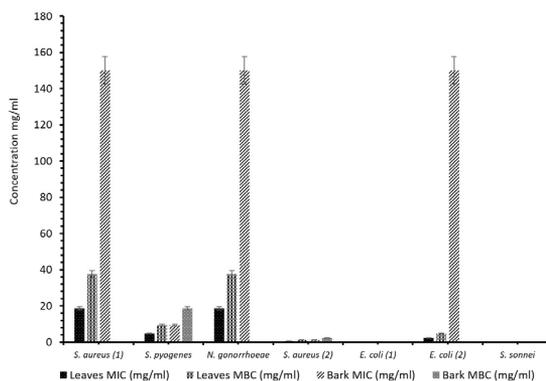


Figure 3. Comparison of MIC and MBC of leaves and stem bark extracts against selected bacterial strains.

on 25 mg/mL, 50 mg/mL and 75 mg/mL as 12.5±0.500, 10.333±1.528 and 12.667±2.082 mm respectively. The efficacy of the antifungal crude drug was measured at an interval of 96 hours and the inhibition zone diameter has decreased against the *A. ornatus*.

Table 6. Antifungal activity of *S. alba* extracts.

Concentration (mg/mL)	Zones of Inhibition (mm)											
	Leaves						Bark					
	25 mg/mL		50 mg/mL		75 mg/mL		25 mg/mL		50 mg/mL		75 mg/mL	
	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
<i>A. oryzae</i>	11.5±0.5	10.66±0.5	11.833±1.0	10.33±0.5	9.667±1.1	11.5±0.5	12.5±0.50	11.5±0.5	10.33±1.5	9±1	12.667±2.0	11.33±1.5
<i>A. terreus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>R. stolonifer</i>	0	0	0	0	0	0	0	0	0	0	0	0

All samples in three replicate ± Standard Deviation.

4. Discussion

Antibiotics act as a powerful therapeutic agent and play a very important role to combat microbial infections. Due to the emergence of resistance against antibiotics, diseases that were considered treated and managed earlier are now appearing more severe than ever before. The emergence of potentially active microbial strains, having multiple drug resistance is a new hot talk of the present time (Dafallah Bilal and Hossain, 2019; EL Moussaoui et al., 2019). The leaves and the stem bark methanolic extracts of *S. alba* were used for the determination of antibacterial and antifungal activity. Figure 1 represents the antibacterial activity of *S. alba* leaves and stems bark methanolic extracts and it was manifested that the zone of inhibition increased with increasing the dose of the drug from 75 mg/mL to 100 mg/mL. Another parameter was also evaluated in this study which involves the measurement of the efficacy of the antibacterial drug after 48 h of time interval (Figure 2). The *S. alba* leaves crude drug was also found active after an incubation period of 48 h which showed the effectiveness of the antimicrobial drug against applied concentration. The highest inhibition zone was measured at 11.667 ± 0.577 mm against *S. aureus* (1) by using a 75 mg/mL dose which was initially measured as 13.333 ± 1.155 mm (Table 1). A decrease in the inhibition zone shows the reduction of the drug effectiveness and increase in bacterial growth which could be a result of increased selective pressure and degradation of the drug by a pathogen. Another reason can be the loss of effectiveness of plant phytochemicals against pathogens. The bacterial strains *E. coli* (1) and *S. sonnei* formally found ineffective at 24 h of incubation, were also found undefeated after an interval of 48 h which shows the ineffectiveness of the methanolic extract against these strains at all applied concentrations.

It was observed that an increase in the concentration of the antimicrobial drug was directly proportional to the inhibition of the bacterial colonies. Increasing the concentration of the crude drug dose results in increasing the effectiveness and concentration of phytochemicals that accelerate the process of inhibition of bacterial growth (Mogashoa et al., 2019). To accomplish this task, the concentration of *S. alba* crude plant methanolic extract was prepared as 100 mg/mL (Table 3). Hence, increased concentration in the case of *E. coli* (1) and *S. sonnei* had not reported any positive effects for growth inhibition which shows the complete impotency of the experimental crude drug against referenced pathogens. The plant secondary metabolites are characteristically attributed to plant families which are formed by diverse metabolic pathways in different plant parts. Different plant parts have different functional attributes. The secondary products formed in different parts have different physiological roles and have different activities against microbes (Putra et al., 2016). Our results are in favor of some previously published work that reported the use of *Mentha longifolia* (Hafedh et al., 2010), *M. piperita* (Desam et al., 2017), *Pelargonium sidoides* (Mativandlela et al., 2006), *Platymiscium gracile* (Cuellar et al., 2018), *Allium saralicum* (Jalalvand et al., 2019), *Eucalyptus* (Bhuyan et al., 2017), different South African

(Shai et al., 2008) and Norwegian (Burčová et al., 2018) plants for the determination of the antibacterial activities.

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial drug that can impede microbial growth and the Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration which can kill the microbial population (Desam et al., 2017; Kutluk et al., 2018; Silva et al., 2019). Figure 3 is representing the MIC and MBC comparison of the leaves and bark methanolic extracts. A comparison revealed the more efficient activity of *S. alba* leaves methanolic extracts at a lower dose which depends on the more effective action of the phytochemicals found in the plant leaves. Another reason is the presence of more diverse and dynamic metabolic pathways for the synthesis of secondary metabolites that operates in the leaves. These results are in favor of some previously published scientific literature (Mativandlela et al., 2006; Putra et al., 2016; Dias et al., 2018; Sati et al., 2018).

Figure 4a and b represents the antifungal activity of the plant methanolic extracts. The antifungal susceptibility results of the *S. alba* showed the ineffectiveness of the crude drug against *A. terreus* and *R. stolonifera* on all applied concentrations while *A. ornatius* was found vulnerable to both extracts prepared from different plant parts, on all applied concentrations. Increasing the incubation period did not increase the effectiveness of the plant drug against

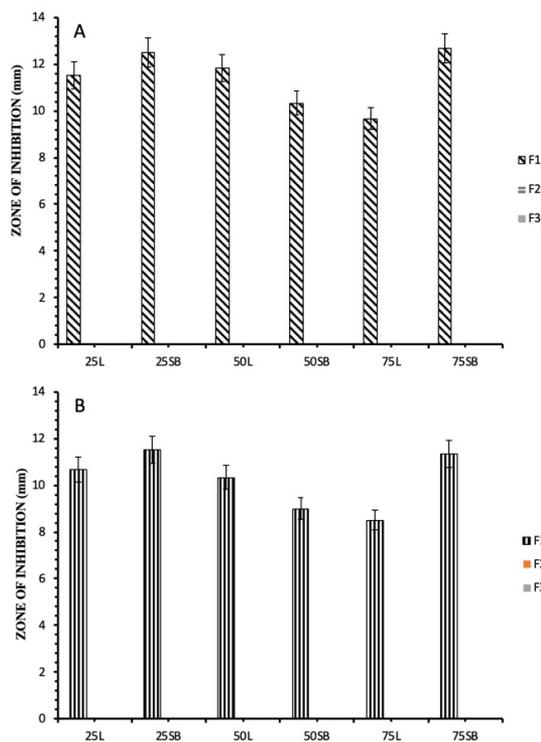


Figure 4. Antifungal activity of *S. alba* extracts after 48 h and 96 h of incubation. L= *S. alba* leaves. SB= *S. alba* stem bark. 25, 50 and 75 represents concentration in mg/mL. F1= *A. ornatius*, F2= *A. terreus*, F3= *R. stolonifera*.

the aforementioned strains which shows the ineffectiveness of the drug and the resistance of the pathogens. These findings suggest the broad-spectrum antibacterial activities of *S. alba* methanolic extracts while narrow antifungal activities were reported.

5. Conclusion

Herein we reported the use of methanolic extracts of *Salix alba* prepared from the leaves and bark powder as an effective antibacterial and antifungal agent to curb the growth of various bacterial and fungal strains. Dose and the time responsive activities were measured and increasing the concentration resulted in increasing the inhibition zone diameter while there was a gradual decrease in activity with an increasing incubation period. The experimental evidence suggested that the *Escherichia coli* (1), *Shigella sonnei*, *Aspergillus terreus*, and *Rhizopus stolonifer* are not inhibited by both leaves and stem bark methanolic extracts while the growth of *Streptococcus pyogenes*, *Staphylococcus aureus* (1), *Staphylococcus aureus* (2), *Shigella sonnei*, *Escherichia coli* (2) and *Neisseria gonorrhoeae*, *A. ornatus* were suppressed by both leaves and bark methanolic extracts. These findings endorse the use of methanolic preparations of *S. alba* to treat various bacterial and fungal pathogens.

Conflict of interest

The authors declare no conflict of interest.

References

- ADNAN, M., BIBI, R., AZIZULLAH, A., ANDALEEB, R., MUSSARAT, S., TARIQ, A., ULLAH, R., ELSALAM, N.M., KHAN, A.L. and BEGUM, S., 2015. Ethnomedicinal plants used against common digestive problems. *African Journal of Traditional, Complementary, and Alternative Medicines*, vol. 12, no. 5, pp. 99–117. <http://dx.doi.org/10.4314/ajtcam.v12i5.15>.
- ALAMGEER, UTTRA, A.M., AHSAN, H., HASAN, U.H. and CHAUDHARY, M.A., 2018. Traditional medicines of plant origin used for the treatment of inflammatory disorders in Pakistan: a review. *Journal of Traditional Chinese Medicine*, vol. 38, no. 4, pp. 636–656. [http://dx.doi.org/10.1016/S0254-6272\(18\)30897-5](http://dx.doi.org/10.1016/S0254-6272(18)30897-5). PMID:32186090.
- BHUYAN, D.J., VUONG, Q.V., CHALMERS, A.C., VAN ALTENA, I.A., BOWYER, M.C. and SCARLETT, C.J., 2017. Phytochemical, antibacterial and antifungal properties of an aqueous extract of *Eucalyptus microcorys* leaves. *South African Journal of Botany*, vol. 112, pp. 180–185. <http://dx.doi.org/10.1016/j.sajb.2017.05.030>.
- BURČOVÁ, Z., KREPS, F., GREIFOVÁ, M., JABLONSKÝ, M., HÁZ, A., SCHMIDT, Š. and ŠURINA, I., 2018. Antibacterial and antifungal activity of phytosterols and methyl dehydroabietate of Norway spruce bark extracts. *Journal of Biotechnology*, vol. 282, pp. 18–24. <http://dx.doi.org/10.1016/j.jbiotec.2018.06.340>. PMID:29940188.
- CUELLAR, J.E., MARTÍNEZ, J., ROJANO, B., GIL, J.H., and DURANGO, D., 2018. Chemical composition and antioxidant and antibacterial activity of *Platymiscium gracile* Benth.: a species threatened by extinction. *Journal of King Saud University - Science*, vol. 32, no. 1, pp. 702–708. <https://doi.org/10.1016/j.jksus.2018.11.006>.
- DAFALLAH BILAL, M.A. and HOSSAIN, M.A., 2019. Antibacterial activity of different crude extracts of *Suaeda maritima* used traditionally for the treatment of hepatitis. *Biocatalysis and Agricultural Biotechnology*, vol. 22, pp. 101383. <http://dx.doi.org/10.1016/j.bcab.2019.101383>.
- DESAM, N.R., AL-RAJAB, A.J., SHARMA, M., MYLABATHULA, M.M., GOWKANAPALLI, R.R., and ALBRATTY, M., 2017. Chemical constituents, in vitro antibacterial and antifungal activity of *Mentha × Piperita* L. (peppermint) essential oils. *Journal of King Saud University – Science*, vol. 31, no. 4, pp. 528–533. <https://doi.org/10.1016/j.jksus.2017.07.013>.
- DIAS, I.J., TRAJANO, E.R.I.S., CASTRO, R.D., FERREIRA, G.L.S., MEDEIROS, H.C.M. and GOMES, D.Q.C., 2018. Antifungal activity of linalool in cases of *Candida* spp. isolated from individuals with oral candidiasis. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 78, no. 2, pp. 368–374. <http://dx.doi.org/10.1590/1519-6984.171054>. PMID:28977047.
- EL MOUSSAOUI, A., JAWHARI, F.Z., ALMEHDI, A.M., ELMSELLEM, H., FIKRI BENBRAHIM, K., BOUSTA, D. and BARI, A., 2019. Antibacterial, antifungal and antioxidant activity of total polyphenols of *Withania frutescens* L. *Bioorganic Chemistry*, vol. 93, pp. 103337. <http://dx.doi.org/10.1016/j.bioorg.2019.103337>. PMID:31627061.
- FORTINI, P., DI MARZIO, P., GUARRERA, P.M. and IORIZZI, M., 2016. Ethnobotanical study on the medicinal plants in the Mainarde Mountains (central-southern Apennine, Italy). *Journal of Ethnopharmacology*, vol. 184, pp. 208–218. <http://dx.doi.org/10.1016/j.jep.2016.03.010>. PMID:26969402.
- FOUCHE, G., CRAGG, G.M., PILLAY, P., KOLESNIKOVA, N., MAHARAJ, V.J. and SENABE, J., 2008. In vitro anticancer screening of South African plants. *Journal of Ethnopharmacology*, vol. 119, no. 3, pp. 455–461. <http://dx.doi.org/10.1016/j.jep.2008.07.005>. PMID:18678239.
- GLIGORIĆ, E., IGIĆ, R., SUVAJDŽIĆ, L. and GRUJIĆ-LETIĆ, N., 2019. Species of the genus *Salix* L.: Biochemical screening and molecular docking approach to potential acetylcholinesterase inhibitors. *Applied Sciences (Switzerland)*, vol. 9, no. 9, pp. 1842. <https://doi.org/10.3390/app9091842>.
- HAFEDH, H., FETHI, B.A., MEJDI, S., EMIRA, N. and AMINA, B., 2010. Effect of *Mentha longifolia* L. ssp. *longifolia* essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. *African Journal of Microbiological Research*, vol. 4, no. 11, pp. 1122–1127.
- HARRINGTON, S.M., DUDLEY, E.G. and NATARO, J.P., 2006. Pathogenesis of enteroaggregative *Escherichia coli* infection. *FEMS Microbiology Letters*, vol. 254, no. 1, pp. 12–18. <http://dx.doi.org/10.1111/j.1574-6968.2005.00005.x>. PMID:16451173.
- JALALVAND, A.R., ZHALEH, M., GOORANI, S., ZANGENEH, M.M., SEYDI, N., ZANGENEH, A. and MORADI, R., 2019. Chemical characterization and antioxidant, cytotoxic, antibacterial, and antifungal properties of ethanolic extract of *Allium Saralicum* R.M. Fritsch leaves rich in linolenic acid, methyl ester. *Journal of Photochemistry and Photobiology. B, Biology*, vol. 192, pp. 103–112. <http://dx.doi.org/10.1016/j.jphotobiol.2019.01.017>. PMID:30731424.
- JAVED, B., NAWAZ, K. and MUNAZIR, M., 2020. Phytochemical analysis and antibacterial activity of tannins extracted from *Salix alba* L. Against different Gram-Positive and Gram-Negative bacterial strains. *Iranian Journal of Science and Technology, Transaction A: Science*, vol. 44, pp. 1303–1314.
- KAPPERUD, G., RORVIK, L.M., HASSELTVEDT, V., HOIBY, E.A., IVERSEN, B.G., STAVELAND, K., JOHNSEN, G., LEITAO, J., HERIKSTAD, H., ANDERSSON, Y., LANGE LAND, G., GONDROSEN, B. and LASSEN, J., 1995. Outbreak of *Shigella sonnei* infection traced to imported

- iceberg lettuce. *Journal of Clinical Microbiology*, vol. 33, no. 3, pp. 609-614. <http://dx.doi.org/10.1128/jcm.33.3.609-614.1995>. PMID:7751364.
- KUTLUK, I., ASLAN, M., ORHAN, I.E. and ÖZÇELİK, B., 2018. Antibacterial, antifungal and antiviral bioactivities of selected *Helichrysum* species. *South African Journal of Botany*, vol. 119, pp. 252-257. <http://dx.doi.org/10.1016/j.sajb.2018.09.009>.
- LAMAGNI, T.L., NEAL, S., KESHISHIAN, C., ALHADDAD, N., GEORGE, R., DUCKWORTH, G., VUOPIO-VARKILA, J. and EFSTRATIOU, A., 2008. Severe streptococcus pyogenes infections, United Kingdom, 2003-2004. *Emerging Infectious Diseases*, vol. 14, no. 2, pp. 202-209. <http://dx.doi.org/10.3201/eid1402.070888>. PMID:18258111.
- LOWY, F.D., 1998. Staphylococcus aureus infections. *The New England Journal of Medicine*, vol. 339, no. 8, pp. 520-532. <http://dx.doi.org/10.1056/NEJM199808203390806>. PMID:9709046.
- LYCKE, E., LÖWHAGEN, G.B., HALLHAGEN, G., JOHANNISSON, G. and RAMSTEDT, K., 1980. The risk of transmission of genital Chlamydia trachomatis infection is less than that of genital Neisseria gonorrhoeae infection. *Sexually Transmitted Diseases*, vol. 7, no. 1, pp. 6-10. <http://dx.doi.org/10.1097/00007435-198001000-00002>. PMID:6771879.
- MARINHO, T.A., OLIVEIRA, M.G., MENEZES-FILHO, A.C.P., CASTRO, C.F.S., OLIVEIRA, I.M.M., BORGES, L.L., MELO-REIS, P.R. and SILVA-JR, N.J., 2021. Phytochemical characterization, and antioxidant and antibacterial activities of the hydroethanolic extract of *Anadenanthera peregrina* stem bark. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e234476. <http://dx.doi.org/10.1590/1519-6984.234476>. PMID:33681898.
- MATIVANDLELA, S.P.N., LALL, N. and MEYER, J.J.M., 2006. Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. *South African Journal of Botany*, vol. 72, no. 2, pp. 232-237. <http://dx.doi.org/10.1016/j.sajb.2005.08.002>.
- MOGASHOA, M.M., MASOKO, P. and ELOFF, J.N., 2019. Different Combretum molle (Combretaceae) leaf extracts contain several different antifungal and antibacterial compounds. *South African Journal of Botany*, vol. 126, pp. 322-327. <http://dx.doi.org/10.1016/j.sajb.2019.06.035>.
- PUTRA, M.Y., HADI, T.A. and MURNIASIH, T., 2016. In vitro antibacterial and antifungal activities of twelve sponges collected from the Anambas Islands, Indonesia. *Asian Pacific Journal of Tropical Disease*, vol. 6, no. 9, pp. 732-735. [http://dx.doi.org/10.1016/S2222-1808\(16\)61119-2](http://dx.doi.org/10.1016/S2222-1808(16)61119-2).
- SATI, P., DHYANI, P., BHATT, I.D. and PANDEY, A., 2018. Ginkgo biloba flavonoid glycosides in antimicrobial perspective with reference to extraction method. *Journal of Traditional and Complementary Medicine*, vol. 9, no. 1, pp. 15-23. <http://dx.doi.org/10.1016/j.jtcme.2017.10.003>. PMID:30671362.
- SHAI, L.J., MCGAW, L.J., MASOKO, P. and ELOFF, J.N., 2008. Antifungal and antibacterial activity of seven traditionally used South African plant species active against *Candida albicans*. *South African Journal of Botany*, vol. 74, no. 4, pp. 677-684. <http://dx.doi.org/10.1016/j.sajb.2008.04.003>.
- SHINWARI, Z.K., 2010. Medicinal plants research in Pakistan. *Journal of Medicinal Plants Research*, vol. 4, no. 3, pp. 161-176.
- SILVA, F.R.G., MATIAS, T.M.S., SOUZA, L.I.O., MATOS-ROCHA, T.J., FONSECA, S.A., MOUSINHO, K.C. and SANTOS, A.F., 2019. Phytochemical screening and in vitro antibacterial, antifungal, antioxidant and antitumor activities of the red propolis Alagoas. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 79, no. 3, pp. 452-459. <http://dx.doi.org/10.1590/1519-6984.182959>. PMID:30379200.
- SILVA, K.B., PINHEIRO, C.T.S., SOARES, C.R.M., SOUZA, M.A., MATOS-ROCHA, T.J., FONSECA, S.A., PAVÃO, J.M.S.J., COSTA, J.G., PIRES, L.L.S. and SANTOS, A.F., 2021. Phytochemical characterization, antioxidant potential and antimicrobial activity of *Averrhoa carambola* L. (Oxalidaceae) against multiresistant pathogens. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 3, pp. 509-515. <http://dx.doi.org/10.1590/1519-6984.220259>. PMID:32876163.
- SULIMA, P. and PRZYBOROWSKI, J.A., 2019. Purple willow (*Salix purpurea* L.) and its potential uses for the treatment of arthritis and rheumatism. *Bioactive food as dietary interventions for arthritis and related inflammatory diseases*. USA: Elsevier.