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Quantitative phytochemical profile, cholinesterase enzyme inhibition assay, antibacterial and antioxidant activities of *Sarcococca saligna*

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Abstract: Aqueous methanol extracts of *Sarcococca saligna* leaves and roots were used in this work to explore its phytochemical contents, antioxidant, enzyme inhibition, and antibacterial activities. Total phenolic contents were found to be in higher concentrations then total flavonoids contents in aqueous methanolic extracts of leaves. Antioxidant activity was performed using DPPH radical scavenging assay. In our findings both leaves and roots extracts were found to show substantial antioxidant potential. Aqueous methanolic extracts of both the leaves and roots gave significant inhibition against butyryl cholinesterase whereas against acetyl cholinesterase extracts of roots gave significant inhibition. The results were compared with the standard drug Eserine. The aqueous methanolic extract of leaves, roots and crude saponins isolated from leaf extracts gave moderate to significant antibacterial activity against the tested bacterial strains using agar disc diffusion method. According to the conclusions, *S. saligna* possesses significant antioxidant, enzyme inhibition, and antibacterial activities. Hence it is assumed that *S. saligna* has the potential to be used in the discovery and development of new bioactive compounds.

Keywords: Acetyl cholinesterase, butyryl cholinesterase, *Sarcococca saligna*, antibacterial activity, phytochemical analysis.

INTRODUCTION

The main focus of phytochemistry research is on the characterization and bioactivities of plants especially their bioactive metabolites, rather than on the synthesis of new molecules (Saleem *et al*., 2020). Therapeutic constituents, directly or indirectly from plants or parts of plants have been in use since ancient ages for different ailments (Errayes *et al*., 2020). According to the literature, the plant's therapeutic effect may be due to secondary metabolites' combined effect. Since the past decade, natural phyto therapeutics is increasingly in demand by local people and local practitioners. Their popularity is due to their low price and fewer side effects than allopathic medicines (Bhatti *et al*., 2022). As medicinal plants are in use since ages for both nutritive and medical purposes, there is still a need to be investigated scientifically for the claimed activity and examine the group of chemicals responsible for such action (Niculet *et al*., 2020). In Pakistan, almost 75% of the population uses traditional medicine system for their primary health care. The number of registered herbal practitioners in Pakistan is 50,000 (Shinwari *et al*., 2009). In this study aqueous methanolic extracts from *S. saligna* leaves, and roots were screened against Acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) enzymes because of its extensive use in traditional medicine. Two structurally and functionally very similar, yet distinct enzymes form the family of cholinesterases (ChEs). To date, cholinesterase inhibitors have been shown to be the most successful treatments for Alzheimer's disease, owing to their capacity to alter both the cognitive and functional aspects of the disease. Alzheimer's disease is being treated using cholinesterase inhibitors, which are considered the standard treatment. Furthermore, there is a requirement for the development of safe and effective cholinesterase inhibitors with a favorable pharmacological profile that would efficiently cross the blood-brain barrier as well as disappear from the body with minimum or no side-effects (Mushtaq *et al*., 2014). This hypothesis was developed because *Sarcococca saligna* (Buxaceae) has tremendous profile of therapeutic uses also on the basis of some previously published evidences of anticholinesterase compounds from its leaves (Gilani *et al*., 2005). In this study both the significance of leaves and roots were focused keeping in view its ethnopharmacology. Traditionally *S. saligna* has been used in the treatment of

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bacterial infections, G.I.T disorders, fever, malaria, ulcer, rheumatoid arthritis and exhibit potent immunosuppressant and anti-diabetic actions (Rehman *et al*., 2018). Therefore, it was subjected to preliminary study against AChE and BChE enzymes with the hope that this specie will help in providing evidence for the presence of potential novel anticholinesterase drug molecule. The extracts were also subjected for qualitative and quantitative phytochemical composition as well as antioxidant and antibacterial activities.

MATERIALS AND METHODS

Plant material collection and extraction

Sarcococca saligna was collected from Nathiya Galli hills, identified by Prof. Dr. Manzur Hussain, chairman Botany department Hazara University. Both leaves and roots were washed thoroughly using tap water, shade dried, ground to a fine powder, and stored in tight air vessels. The aqueous methanol (20:80) extracts were prepared from both leaves and roots of *S. saligna* using triple maceration method. Crude extracts were concentrated at 40°C using rotary evaporator (GUU R-1001) (Edeoga *et al*., 2005).

Chemicals and drugs

In this study, the chemical and solvents used were of analytical grade and were acquired from Sigma Chemical Company (St. Louis, MO).

Extraction of crude saponins

Leaves of *S. saligna* were selected for the extraction of crude saponins on the basis of qualitative phytochemical test results; schematic diagram is given in figure 1. Crude extracts of leaves were prepared using ratio of distilled water and methanol in 20:80 proportion, followed by maceration for 7 days. The extracts were filtered and concentrated in a vacuum rotary evaporator. The concentrated extracts were redissolved in methanol followed by treatment with n-butanol (already saturated with distilled water). The mixture is shaken in a separating funnel, allowed it to stand till the two layers got separated. The lower layer of n-butanol was taken, it was concentrated and redissolved in methanol. It was then treated with precooled diethyl ether drop wise, light yellowish color precipitates were collected and stored for further analysis (Rehman *et al*., 2018).

Phytochemical analysis

The aqueous methanolic extracts of both the leaves and roots were screened separately for the existence of flavonoids, saponins, alkaloids, glycosides, cumarins, tannins and carbohydrates. The results are given in table 1.

Phenolic contents quantification

Total phenolic content in the samples investigated were quantified by mixing 1 ml of aqueous methanolic extracts of leaves with 5mL of 10times diluted Folin ciocalteu reagent as well as 4mL of 7.5% Na_2CO_3. The combination was let to remain at room temp for 90minutes and the absorption was monitored at 760nm. The results were reported in milligrams (mg) of gallic acid equivalents per 100grammes of fresh body weight. (Mg GAE/100g, FW) (Ayele *et al*., 2022). Same procedure was repeated for roots as well.

Flavonoid contents quantification

Total flavonoid contents (TFC) were determined using the conventional colorimetric technique, with a few modifications to account for differences in flavonoid concentrations across samples. In a falcon tube, 5mL of aqueous methanolic extracts of leaves was combined with 0.3mL of 5 percent $NaNO_3$ for 5 minutes to get a 5 percent $NaNO_3$ solution. After that, 0.3mL of a 10 percent $AlCl_3$ solution was added to complete the reaction. After 5 minutes, 2mL NaOH was added to the liquid to stop the reaction. The mixture was diluted further with distilled water until it reached a final concentration of 10mL. In this experiment, the absorbance was measured at 510nm, and the findings were computed in mg of Quercetin equivalent per 100grammes of fresh weight (mg RtE/100g, FW) (Kumaresan *et al*., 2019).

Acetylcholinesterase and Butyrylcholinesterase inhibition assay

Ellman method was followed (Čolović *et al*., 2013) with slight modifications for anti-ACHE and anti BChE assay. 100µl of the assay mixture's total volume contains 60µl of sodium phosphate (Na_2HPO_4) buffer having pH 7.7 conc. of 50mM, 10µl of test sample having conc. Of 0.5mM was poured in well-1, 10µl of the enzyme having conc of 0.005 units was also added to it, mixed well and reading was taken at a wavelength of 405 nm then incubated for 10 min at 37ºC. With the addition of 10µl substrate having conc. of 0.5 mM acetylthiocholine iodide for AChE and butyryl thiocholine chloride for BChE, the reaction was initiated. Next, 10µl of DTNB with conc of 0.5mM was also added. It was incubated for 15 minutes, and once again, its absorption was recorded at 405nm using 96 well plate readers (Synergy HT Bio Tek, USA) and percentage inhibition, as well as an IC_{50}, were calculated using the following formula (Čolović *et al*., 2013):

$$\text{Inhibition (\%)} = \text{Control-Test / Control} \times 100$$

Antibacterial screening of Sarcococca saligna

Agar disc-diffusion method was used for the screening (Rehman *et al*., 2018). Bacterial strains used in the study were gram positive including *Staphylococcus aureus* (ATCC 14028), *Methicillin-resistant Staphylococcus aureus* (MRSA) (ATTC 33592) and gram negative *Shigella dysenteriae* (ATCC 13313), *Chlamydia trachomatis* (ATCC VR-573), *Neisseria gonorrhoeae* (ATCC 49226). The dried methanol extracts from *S.*

saligna have been dissolved in DMSO at a final concentration of up to 30 mg/ml. Each pre sterilized petri plates was added with 30 ml of agar media. Around 10µl test microbes were seeded into each agar plate. Each 6 mm (diameter) disc was saturated at a concentration of 30 mg/ml with 10µl of the plant sample solution (300µg/disc) and put on the Petri plate on the inoculated medium. Standard antibiotic drug Ciprofloxacin (30µl) were used as positive control. At 37°C, the plates were incubated for 24 hours. A clear zone of bacterial growth inhibition was seen surrounding all of the experimental and control drugs after 24hours of incubation at 37°C.

DPPH scavenging assay

The antioxidant activity of *Sarcococca saligna* leaves and roots extracts was measured by free radical scavenging ability against 1, 1-diphenyl-2-picrylhydrazyl stable radicals (DPPH). The assay was performed as per the protocol given by (Anjum *et al*., 2019). Aqueous methanolic extracts (10 µl, 20 µl and 30µl were added an equal volume in ethanolic solution of DPPH (0.1mM), incubated after thirty minutes at room temperature. Using UV spectrophotometer its absorbance was calculated at 517nm. The test was repeated thrice. standard control used was Butylated Hydroxytoluene (BHT). Following formula was used for calculation of percentage inhibition free radical by DPPH;

$I (\%) = 100 \times (A_{blank} \_\_ A_{sample} / A_{blank})$

A_{blank} is the absorbance of the control reaction mixture exclusive of the test samples, and A_{sample} is the absorbance of the test compounds (Oguz *et al*., 2020).

STATISTICAL ANALYSIS

Experiment results will be expressed in mean ± SD. The data were analyzed by Student's t-test using statistical package for social sciences (SPSS) version.19, IBM Inc. (USA).

RESULTS

Phytochemical analysis

Qualitative phytochemical screening of aqueous methanolic extract of *Sarcococca saligna* leaves and roots confirmed presence of flavonoids, alkaloids, tannins, saponins and glycosides whereas cumarins and carbohydrates were negative as described in table 1.

Quantitative phytochemical analysis justified a significant concentration of total flavonoids at 3 different concentrations of aqueous methanolic extract of leaves of *Sarcococca saligna* were found to be 1.0222 at 500µg/ml, 0.9345 at 300µg/ml, 0.6201 at 100µg/ml. Total phenolic contents were measured to be 1.3330 at 500µg/ml, 1.0590 at 300µg/ml, and 0.8999 at 100µg/ml. Results are described in table 2.

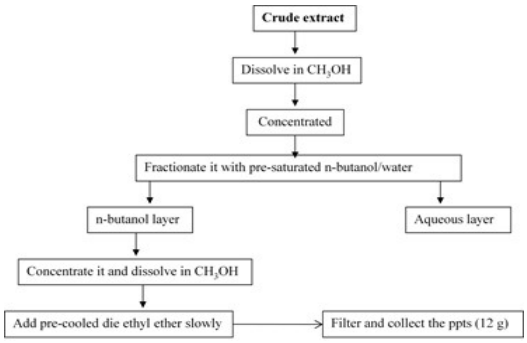

Fig. 1: Scheme for the extraction of crude saponins from *Sarcococca saligna*

Enzyme inhibition assay

EZ–Fit enzyme kinetic software (Perrella Scientific Inc. Amherst, USA) was used to record IC_{50} values of serial dilutions of the samples in different concentrations, starting from 0.5mM to 0.25 mM, 0.125 mM, 0.0625 mM, 0.03125 mM, 0.015625mM. IC_{50} values were calculated only for those samples which inhibition rate was 50% or above. All the values are mean of 3 self-determining experiments which are discussed in Table 3. Aqueous methanolic extracts of *Sarcococca saligna* leaves and roots and crude saponin of leaves were tested against Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), at 0.5 mg/ml dose. The aqueous methanolic extracts of leaves gave 89.26±0.26 %

Inhibition and IC_{50} of 9.71±0.11µg/ml against BChE, the same extracts were considered inactive against AChE (because its inhibition was less than 50%). The percent inhibition of aqueous methanolic extracts of roots was 56.34±0.26% with IC_{50} of 141.9±0.15µg/ml against AChE. Inhibition was 91.34±0.12% against BChE and 4.51±0.09µg/ml IC_{50}. The crude saponin of leaves gave inhibition less than 50% against AChE (i.e., 24.33±0.21%), whereas its activity against BChE was 57.39±0.24% inhibition and an IC_{50} of 161.26±0.16 µg/ml. Results of the tested extracts samples were compared with the results of standard drug (Eserine) whose inhibition was 82.82±1.09 % with an IC_{50} of 0.85±0.0001µg/ml against AChE and 91.29±0.001µg/ml with IC50 of 0.04±0.09µg/ml against BChE.

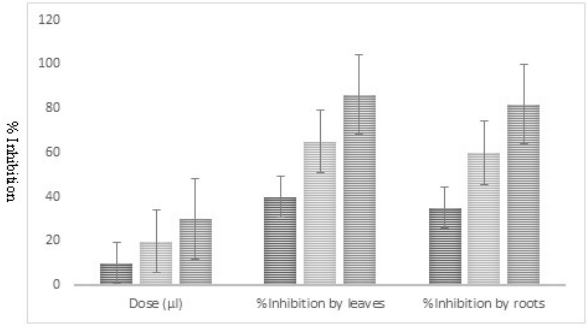

Fig. 2: Antioxidant activity of *Sarcococca saligna* leaves and roots.

Table 1: Phytochemical analysis of leaves and roots of *Sarcococca saligna*

S.No.	Test	Reagent	Observations	Leaves	Roots
1	Alkaloids	Dragendorff Mayer Wagner	Reddish-brown ppts. Yellowish ppts. Reddish-brown ppts	+++ +++ +++	+++ +++ +++
2	Flavonoids	Shinoda test Alkaline reagent test	Red or pink color Yellow color which discolors on addition of dilute HCl	++ ++	+++ +++
3	Saponins	Froth test Lead acetate test $[Pb(C_2H_3O_2)_2]$	Persistent froth White ppt's	+++ +++	++ ++
4	Glycosides	Na-Nitroprusside test	Pink color	+	+
5	Tannins	1% gelatin with 10% NaCl	White precipitates	+++	+++
6	Coumarins	NaOH test	Florescence	-	-
7	Carbohydrates	Molisch's reagent	Purple ring formation	-	-

+ = presence of phytochemicals and - = absence of phytochemicals. +++ = high concentration, ++ = moderate concentration

Tale 2: Total flavonoids and phenolic contents of aqueous methanolic extracts of leaves

Samples	Dose		
	500µg/ml	300µg/ml	100µg/ml
Total Flavonoids	1.0222	0.9345	0.6201
Total Phenolics	1.3330	1.0590	0.8999

Table 3: Percentage inhibition and IC_{50} of *S. saligna* leaves and roots against AChE, and BChE

S. No.	Sample	Acetylcholinestrase (AChE)		Butrylcholinestarase (BChE)	
		Inhibition	IC_{50}	Inhibition	IC_{50}
1	Aqueous methanolic ext. of leaves	**	-	89.26±0.26	9.71±0.1
2	Aqueous methanolic ext. of roots	56.34±0.26	141.9±0.15	91.34±0.12	4.51±0.09
3	Cr. Saponin from leaves	**	-	57.39±0.24	161.26±0.16
4	Eserine (Standard)	82.82±1.09	0.85±0.0001	91.29±0.001	0.04±0.09

The values are mean of ± SD of triplicate values. (** = Inhibition was less than 50)

Table 4: Antibacterial screening of *S. saligna*

S.No.	Sample	ZOI in mm against tested Bacterial strains				
		Shigella dysenteriae	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*	MRSA	*S. aureus*
1	Aqueous methanolic extracts of leaves	24±0.56	18±0.37	19±0.58	16±0.24	20±0.27
2	Aqueous methanolic extracts of roots	14±0.67	12±0.57	16±0.54	18±0.44	22±0.5
3	CSp from leaves	09±0.43	14±0.40	13±0.50	12±0.60	16±0.60
4	Ciprofloxacin	30±0.25	28±0.32	28±0.51	28±0.57	26±0.36
5	DMSO	09±0.22	09±0.29	09±0.30	09±0.40	09±0.20

The values are mean of ± SD of triplicate values.

Antioxidant assay

Free radical scavenging activity was also carried out against *S. saligna* extracts and the percentage inhibition for leaves and roots extracts were recorded as 86.3% with IC_{50} 8.5±0.9µg/ml and 86% with IC_{50} 7.0±0.5µg/ml respectively using DPPH scavenging assay method, results are expressed in fig. 2.

Antibacterial assay

Aqueous methanolic extracts of leaves and roots as well as crude saponins (CSp) isolated from leaves were tested against gram positive bacterial strains including *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), and gram negative strains including *Shigella dysenteriae, Neisseria*

gonorrhoeae (gonococci) and *Chlamydia trachomatis*. Results are described in Table 4. All the results were recorded by measuring zone of inhibition (ZOI) in mm.

The ZOI of tested samples were compared with standard drug Ciprofloxacin as positive control. The maximum ZOI was demonstrated by standard drug i.e., 26±0.00mm against *S. aureus*, 30 ± 0.00 mm against *S. dysenteriae*, 28±0.00 mm each against *N. gonorrhoeae*, *C. trachomatis* and MRSA. ZOI below 10mm was considered as inactive when compared to 9.0±0.00 ZOI of negative control DMSO. The ZOI shown by aqueous methanolic extracts of leaves against *S. dysenteriae* observed was 24±0.56 mm whereas aqueous methanolic extracts of roots and CSp has shown 14±0.67 mm and 9±0.43mm respectively. Keeping in view the traditional use of *S. saligna* in sexual transmitted diseases its extracts were significantly evaluated against *N. gonorrhoeae and C. trachomatis*. Aqueous methanolic extracts of leaves were significantly active, its ZOI was calculated as 18±0.37mm against *N. gonorrhoeae* and 19±0.58mm against *C. trachomatis* whereas aqueous methanolic extracts of roots shown 12±0.57mm ZOI against *N. gonorrhoea* and 16±0.57mm against *C. trachomatis*. Similarly, CSp isolated from leaves of *S. saligna* gave moderately active results with ZOI of 14±0.4mm against *N. gonorrhoeae* and 13±0.5mm against *C. trachomatis*. MRSA is common disease-causing pathogenic microbe, in this study aqueous methanolic ext. of leaves gave ZOI i.e., 16±0.24mm against MRSA while results shown by aqueous methanolic ext. of roots and CSp were recorded to be 18±0.44mm and 12±0.06 respectively. *S. aureus* is commonly found on the surface of human skin and cause different types of skin infections. *S. saligna* is traditionally used against skin disorders by local people. Keeping in view the traditional uses of this plant species, it was investigated against *S. aureus* and according to the results, aqueous methanolic ext. of leaves and roots gave 20±0.3mm and 22±0.5mm ZOI whereas CSp isolated from leaves had shown the ZOI of 16±0.6mm.

DISCUSSION

Aqueous methanolic extracts of leaves and roots of *S. saligna* were investigated against Acetylcholinesterase and butyrylcholinesterase enzymes. Anticholinesterase molecules are known to inhibit acetylcholine hydrolysis, thus producing a cholinergic action (Ozdemir *et al*., 2019). AChE and BChE possess the potential to decrease acetylcholine level, resulting in blocking neurotransmission hence responsible for slowing down the motor activity (Apatzidou *et al*., 2018). However, cholinesterase inhibitors (ChEI), most notably of AChE, are the most successful method to date for managing the cognitive symptoms of Alzheimer's disease (AD). They have shown significant therapeutic efficacy in improving both cognitive performance and quality of life in these individuals (Elufioye *et al*., 2010). In the light of findings of this study, it has been justified that aqueous methanolic extracts of both leaves and roots as well as CSp exhibit inhibitory potential against both AChE and BChE in a dose dependent manner and they could be considered for further molecular level research which can be helpful in drug discovery for the treatment of AD. The current study revealed that aqueous methanolic extract roots has enzyme inhibitory activity more than the leaves extract and crude saponins. However this inhibitory effect of roots extract is most significant in case of AChE. As the According to the literature *Sarcococca saligna* has been reported certain pregnane type steroidal alkaloids which exhibit both acetyl cholinesterase and butyrylcholinesterase inhibitory potential. These including known alkaloids axillarine C, sarcorine, N3-demethylsaracodine, saligcinnamide, axillarine F, salignenamide A, axillaridine A, vaganine A, sarsalignone, sarsalignenone, salignenamide C and salignenamide D. According to a published study, these compounds are significantly active against both enzymes in a dose-dependent manner with the reported IC_{50} values ranging from 5.21–227.92µM against acetylcholinesterase and 2.18–38.36µM against butyrylcholinesterase (Khalid *et al*., 2002). These compounds have been isolated from the leaves of this specie. In this study we have targeted the inhibitory potential of both leaves and roots. Previous studies has reported about justified other species of medicinal plant's inhibitory potential against AChE and BChE e.g., well-known medicinal plants such as *Huperzia serrata*, *Panax ginseng*, *Gingko biloba,* have been identified to be effective in inhibition of AChE and BChE enzymes which are considered to be related to the mechanism of memory dysfunction (Orhan *et al*., 2004). *Bombax bromoposenze, Dioscorea dumetorum, Garcinia kola* showed significant inhibitory activity (>83%) towards BChE. The leaves of *Crinum jagus*, stembark of *Peltophorum pterocarpum* and *Pycnanthus angolensis*, root bark and stem bark of *Spondias mombin* all gave significant activity against AChE. There are also some evidances from literature which showed certain plants have inhibitory potential against both AChE and BChE like *Spondias mombin* and *Pycnanthus angolensis* (Tettevi *et al*., 2022) gave high inhibition against both AChE and BuChE (Elufioye *et al*., 2010: Diniso *et al*., 2022).

Due to increasing antibiotic resistance in the world, scientists are approaching new sources for antibiotic agents. Natural medicinal plants provide us good source for this purpose because phytochemicals present in medicinal plants are known to exhibit various biological activities. These phytochemicals can provide us in discovery of new antibiotic drugs which can be helpful to overcome antibiotic resistance. Diverse approaches have been used over the last few decades to reveal novel antibacterial compounds from traditional medicines. *S. saligna* has historically been used to treat a variety of

conditions, particularly skin conditions, gonorrhoea, and syphilis (Moghaddam *et al*., 2010). Therefore in the present study *S. saligna* was investigated against pathogenic bacterial strains such as *Shigella dysenteriae*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, MRSA, *S. aureus* in order to justify its traditional uses in GIT, skin and sexual disorder as reported by (Jaine *et al*., 2021). The findings of this study has proved its significant against the tested bacterial strains when ZOI were compared with standard antibiotic drug Ciprofloxacin. This plant is a valuable source of steroidal alkaloids; multiple antibacterial steroidal alkaloids, including saligcinnamide, Na-methyl epipachysamine-D, as well as epipachysamine D, have been isolated from its roots and stems. These molecules have been reported to show significant activity against the bacterial strains such as *Klebsiella pneumoniae*, *Streptococcus areus*, *Streptococcus pyrogenus*, *Pseudomonas aeruginosa*, *Salmonella typhii*, and *Shigella boydii* (Atta-ur-Rahman *et al*., 1998). *Sarcococca hokoriana* is another specie of Buxaceae family, it has been known to contain five - pregnene type steroidal alkaloids, including two new hookerianamides J and K and eight previously identified molecules, including hookerianamides H and I, epipachysamine E, N-methypachysamine A, 2, 3-dehydrosarsalignone, sarcovagine C and vagenine A. Holaphylline a compound from *S. saligna* has been reported with antibacterial, antifungal, phytotoxic and insecticidal activity against *S. aureus* (79%), *B. subtilus* (72%), and *P. aeruginosa* (69%), respectively (Naeem *et al*., 2019). Besides these active isolated moieties, the n-hexane, crude extracts, Ethyl acetate, $CHCl_3$ and aqueous fraction of arial parts of *S. saligna*, have confirmed significant antibacterial potential according to earlier published research (Anwar *et al*., 2019). From the results it has been clearly evident that aqueous methanolic extract of the leaves has substantial antibacterial potential against all the bacterial under study which was found greater than the plant root extract as well as the isolated saponins.

Through the DPPH assay it was revealed that *S. saligna* exhibit significant antioxidant potential. This activity may be due to the presence of phenolic and other phytochemicals compounds in both roots and leaves which were confirmed through qualitative screening method. The results of this study is also in agreement with the results of other species of same genus i.e. *Sarcococca hookoriana* leaf extracts and *S. saligna* fruits extracts being evaluated for antioxidant activity (Ahmad *et al*., 2015: Baral *et al*., 2022). The present study revealed that both root and leaves extracts have same magnitude of antioxidant potential. Any compound which possesses antioxidant activity will ultimately has cytotoxic potential (Barma *et al.,* 2022). Further studies can be conducted to evaluate the this plant's cytotoxic activity.

CONCLUSION

A comprehensive report on the phytochemical profile and pharmacological activities of *S. saligna* were explored in this study. Results obtained from our study indicate that leaves and roots of *S. saligna* possess significant inhibitory potential against AChE, and BChE enzymes. It also gave significant antioxidant and moderate to strongly significant antibacterial activity against the tested pathogenic bacterial strains. The study findings provide a new paradigm for the use of the *S. saligna* leaves and roots as a natural source of biologically active molecules, including as antioxidants, enzyme inhibitors and antibacterial agents (Barma *et al*., 2021).

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