Antimicrobial activities and phylogenetic study of *Erythrina senegalensis* DC (Fabaceae) seed lectin

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Abstract

Erythrina senegalensis (Fabaceae) have been traditionally used in the treatment of microbial ailments, and the specific agent mediating its efficacy has been investigated in several studies. In this study, the antimicrobial activity of purified *E. senegalensis* lectin (ESL) was analyzed. The phylogenetic relationship of the gene encoding lectin with other legume lectins was also established to investigate their evolutionary relationship via comparative genomics. Antimicrobial activity of ESL against selected pathogenic bacteria and fungi isolates was evaluated by the agar well diffusion method, using fluconazole (1 mg/ml) and streptomycin (1 mg/ml) as positive controls for fungi and bacteria sensitivity, respectively. Potent antimicrobial activity of ESL against *Erwinia carotovora*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Aspergillus niger*, *Penicillium camemberti*, and *Scopulariopsis brevicaulis* was observed, with inhibition zones ranging from 18 to 24 mm. Minimum inhibitory concentrations of ESL ranged between 50 and 400 μg/ml. Primer-directed polymerase chain reaction of *E. senegalensis* genomic DNA detected a 465-bp lectin gene with an open reading frame encoding a 134-amino acid polypeptide. The obtained nucleotide sequence of the ESL gene shared high sequence homology: 100, 100, and 98.18% with *Erythrina crista-galli*, *Erythrina corallodendron*, and *Erythrina variegata* lectin genes, respectively, suggesting that the divergence of *Erythrina* lectins might follow species evolution. This study concluded that ESL could be used to develop lectin-based antimicrobials, which could find applications in the agricultural and health sectors.

Key words: antimicrobial, *Erythrina senegalensis*, lectin, phylogenetic analysis, polymerase chain reaction

Introduction

Plants contain a huge amount of bioactive compounds that are being used worldwide for various purposes, especially in ethnomedicine. These compounds range from a myriad of secondary and primary metabolites such as flavonoids, alkaloids, polyphenols, and bioactive proteins, which can be used in targeted therapy and treatment of diverse microbial diseases (Van Holle and Van Damme, 2018; Górniak et al., 2019; Othman et al., 2019; Bilanda et al., 2020; Gupta et al., 2020; Taiwo et al., 2020; Mammari et al., 2021; Sun et al., 2021). An increase in microbial resistance and detrimental environmental consequences associated with the use of chemical antimicrobial agents has also contributed to the search for natural plant-based alternatives, especially those with protein origin (Vandenborre et al., 2011; Singh et al., 2019).

Lectins are unique proteins or glycoproteins, having at least one domain devoid of catalytic activity and capable of binding specific glycan moieties reversibly (Alen'kina et al., 2014; Nubi et al., 2021). These proteins are heterogeneous in structures and proficient in agglutinating or precipitating cells and carbohydrate-relate structures via specific interactions (Singh et al., 2016;

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Adedoyin et al., 2021). Lectins mediate a wide range of biological and biochemical processes in many organisms, such as nitrogen fixation, cellular development, mitogenesis, symbiosis, immunomodulation, and defense (Smýkal et al., 2015; Osman and Konozy, 2017; Rambo et al., 2019; Van Holle and Van Damme, 2019; Fonseca et al., 2022a).

Belonging to the legume family (Fabaceae) and characterized by peculiar red flower coloration, the genus *Erythrina* is widely distributed across the tropical and subtropical regions of the world. Members of this genus comprise more than one hundred species, which are rich in many bioactive molecules. However, lectins have only been detected in a few members of *Erythrina* (Focho et al., 2009; Doughari, 2010; Otimenyin and Uzochukwu, 2010; Atsamo et al., 2013).

Erythrina senegalensis is generally used for ethnomedicinal purposes in Africa, especially in the traditional treatment of ailments such as arterial hypertension, gastrointestinal disorders, leprosy, diabetes, and hemorrhoids (Doughari et al., 2010). *Erythrina* spp. possess anxiolytic and anticonvulsant properties, especially in the central nervous system (Focho et al., 2009). These pharmacological activities of *E. senegalensis* are attributable to bioactive molecules inherent in them (Atsamo et al., 2013; Nembo et al., 2015; Nordström R, Malmsten et al., 2017; Armas et al., 2019; Van Holle and Van Damme, 2019).

Evolutionary relationships and functional diversity of genes and proteins are usually analyzed using phylogenetic analysis (Song et al., 2018). Exploring the similarities between sequences by mapping the existing data of characterized proteins belonging to a particular phylogenetic family tree can provide predictive insights into the functions and biochemical properties of members belonging to the family (Murphy et al., 2011). In the present study, the antimicrobial activity of purified lectin from *E. senegalensis* seeds was investigated, and phylogenetic analysis of the gene encoding lectin with other legume lectins was carried out to identify their evolutionary and functional relationship.

Materials and methods

Chemicals and reagents

Glutaraldehyde, bovine serum albumin (BSA), sugars (glucose, lactose, maltose, mannose, sorbose, galactose, *N*-acetylglucosamine, and mannitol), acrylamide, ammonium persulfate, *N,N,N',N'*-tetramethylethylenediamine (TEMED), sodium dodecyl sulfate, agarose, and coomassie brilliant blue R250 were purchased from the Sigma Chemical Company, St Louis, Missouri, USA. Sepharose 4B and Sephadex G-150 were bought from Pharmacia Fine Chemicals, Uppsala, Sweden. Nutrient agar and nutrient broth were from Lab M Ltd, Lancashire, United Kingdom. Gel-loading dye (BioLabs®, B7025S), ethidium bromide, deoxyribonucleoside triphosphates (dNTPs), Taq polymerase, DNA master mix, 100-bp DNA ladder (BioLabs®, NO551S), and ZYMO DNA Extraction Kit were purchased from South Africa (Inqaba Biotech, Hatfield Pretoria). All other reagents and chemicals used in this study were of analytical grade.

Selected microbial species

Erwinia carotovora, *Pseudomonas aeruginosa*, *Lactobacillus acidophilus*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Aspergillus niger*, *Fusarium* species, *Penicillium camemberti*, *Scopulariopsis brevicaulis*, *Trichophyton interdigitale*, and *Trichoderma* species were obtained from the culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Collection and identification of E. senegalensis seeds

The *E. senegalensis* plant was collected from a regrowth forest located $7°31'9''$N $4°31'36''$E within the Obafemi Awolowo University, Ile-Ife, Nigeria. Seeds were identified at the IFE HERBARIUM, Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria, where the plant was deposited, with the voucher specimen number IFE-17749.

Purification and hemagglutinating activity of E. senegalensis lectin (ESL)

Purification and hemagglutinating activity analysis of ESL were conducted as described in our previous study (Kuku et al., 2012). Purification involves a combination of ammonium sulfate precipitation, gel filtration on a Sephadex G-150 column, and affinity chromatography on a lactose-sepharose 4B column. In brief, *E. senegalensis* seeds (50 g) were pulverized and defatted using petroleum ether. The obtained flour was air-dried at room temperature and extracted with 10 mM phosphate buffered saline (PBS), pH 7.2 (1 : 10 w/v), at $4°C$ for 24 h. The resulting mixture was centrifuged (6000 rpm,

30 min), and the supernatant was collected as a crude lectin extract. Hemagglutinating activity of lectin was determined in a 96-well microtiter plate. The crude lectin aliquot (100 μl) was serially diluted with PBS (100 μl), and 50 μl of erythrocyte suspension (4% v/v) was added to each well. The resulting mixture was left undisturbed for 60 min at room temperature. Hemagglutinating activity was expressed as units taken to be the reciprocal of the highest dilution that shows visible agglutination. Specific activity was expressed as units per mg protein. In the purification stage, the crude lectin extract was subjected to 70% ammonium sulfate precipitation and left overnight (4°C). The resulting precipitate was collected by centrifugation (6000 rpm, 30 min). The recovered precipitate was resuspended in PBS, pH 7.2, and dialyzed against PBS and distilled water for 48 h. An aliquot (4 ml, approximately 28 mg protein) of ammonium sulfate dialysate was loaded on the Sephadex G-150 column (2.5 × 40 cm) equilibrated with PBS. Fractions (4 ml) were collected at a flow rate of 30 ml/h. Elution was monitored at 280 nm, and the hemagglutinating activity of the fractions was assayed. Active peak fractions were pooled and dialyzed for 48 h. Then, an aliquot (2 ml, approximately 12 mg protein) of the pooled Sephadex G-150 active fraction was layered on the Lactose-Sepharose 4B column (1.5 × 10 cm) equilibrated with PBS. Unadsorbed protein was washed with the equilibration buffer, and the adsorbed protein was eluted with the same buffer containing 0.2 M lactose. Fractions (1 ml) were collected at a flow rate of 20 ml/h. Elution was monitored at 280 nm, and the hemagglutinating activity of the fractions was assayed. Active peak fractions were pooled, dialyzed exhaustively (against PBS and distilled water), lyophilized, and kept frozen at −20°C. The protein concentration of ESL was determined at each purification stage following the method of Lowry et al. (1951). Purification was repeated several times to obtain pure ESL in sufficient quantities for antimicrobial study. The purity and molecular weight of ESL were determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on a 12.5% acrylamide gel using low-molecular-weight protein markers (Nubi et al., 2021).

Analysis of antimicrobial activity of ESL

Agar well diffusion method was used to analyze the antimicrobial activity of ESL against selected pathogenic microorganisms (Cheesbrough, 2006). In brief, fungi were cultivated on potato dextrose broth at 25°C for 48–72 h, whereas bacteria were cultivated on nutrient broth at 37°C for 24 h. The microbial cell density of both cultures was standardized (10^8 CFU/ml of 0.5 McFarland standards) at 600 nm. A sterile swab stick moistened with the inoculum was spread on potato dextrose agar (PDA) and Mueller Hinton agar (MHA) for fungi and bacteria, respectively. Then, 6-mm-diameter wells were bored equidistant from each other using a sterile cock borer into the respective agar media and filled with an equal volume (50 μl) of ESL (0.1–0.4 mg/ml). Fluconazole (1 mg/ml) and streptomycin (1 mg/ml) were used as the positive control for fungi and bacteria sensitivity, respectively, whereas PBS buffer was used as the negative control. Plates were incubated at 25°C for 48–72 h for fungi and 37°C for bacteria. Zones of inhibition were measured in millimeters.

Minimum inhibitory concentration (MIC) of ESL

MIC analysis of ESL was carried out following the method of Cheesbrough (2006). In brief, a serial dilution of ESL was prepared. Aliquots (2 ml) of the different concentrations were added to 18 ml of presterilized malt extract agar and Sabouraud dextrose agar (SDA) for bacteria and fungi at 40°C to achieve a final concentration regimen of 0.05 and 10 mg/ml, respectively. Respective media were poured into sterile petri plates and allowed to set. The surface of the media was dried under laminar flow before streaking with 18-h-old bacterial and fungal cultures. The plates were incubated at 37°C for 24 h and 25°C for up to 72 h for bacteria and fungi, respectively, and then, they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Isolation of genomic DNA (gDNA) and polymerase chain reaction (PCR) amplification of ESL gene

E. senegalensis gDNA was extracted using the ZYMO Plant DNA Mini Kit (ZYMO Research, California, USA). The purity of the isolated gDNA was evaluated by measuring the absorbance quotient at 260 nm/280 nm, and the concentration was determined using a Nanodrop spectrophotometer (Beckman Coulter). For amplification, an aliquot of 30 ng/μl gDNA was prepared via PCR. Gene-specific primers were designed corresponding to the conserved regions of the nucleotide sequences of

Erythrina lectins (accession numbers: FJ8396683.2; AY158072.1; X52782.1) available on the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/nuccore) and then synthesized (Inqaba Biotech, South Africa). PCR amplification was performed in a 25 μl total reaction mixture containing template gDNA (30 ng), 10 μM each of primers (forward: 5′–TGGGGACGTAACAAAAGGAG–3′; reverse: 5′–CTGGCTTGGACTTTGTTGGT–3′), dNTPs (10 mM), MgCl$_2$ (25 mM), Taq polymerase (5 Units/μl; Promega), buffers (5×), and deionized water. The PCR amplification consisted of initial denaturation at 94 °C for 5 min, followed by 36 denaturation cycles of 30 s at 94 °C, 30 s annealing at 55 °C, and 90 s extension at 56 °C in a 96-well Thermal Cycler (Eppendorf Master Cycler Nexus Gradient 230), and a final 4-min extension at 72 °C.

Confirmation of PCR products on agarose gel

Amplified products (amplicons) were separated on agarose gel (1% w/v) and stained using ethidium bromide (0.7 μg/ml). Electrophoresis of the PCR amplicons was carried out using a 100-bp DNA ladder (Promega) at 60–90 V for 3 h. The gels were visualized, and the results were monitored using an ultraviolet (UV) transilluminator gel documentation system (Bio Olympics). The amplified product was recovered and purified using a QIA quick gel extraction kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Sequencing of the amplified gene

The purified PCR products were sequenced with an Applied Biosystems (ABI) 3500XL automated sequencer using both forward and reverse primers. The cleaned products were injected into the Applied Biosystems ABI 3500XL Genetic Analyser with a 50-cm array, using POP7.

Sequence alignment and phylogenetic analysis

A homology search of the ESL nucleotide sequence in the GenBank database using the Basic Local Alignment Search Tool (BLAST search) was also conducted. Sequence alignment with other plant lectins was analyzed using ClustalW. Nucleotide sequence alignment results were used for phylogenetic analysis using the MEGA7.0 software (Molecular Evolutionary Genetics Analysis, version 7.0) program to analyze the evolutionary relationships (Kumar et al., 2016). Integrity of the phylogenetic tree was estimated using 1000 bootstrap replicates.

Statistical analysis

Data were expressed as mean ± standard error. One-way analysis of variance was used to determine the significance of the findings, and P-values < 0.05 were considered statistically significant.

Results and discussion

Antimicrobial study

Legumes are rich in bioactive proteins and peptides, including lectins with diverse biological functions, such as playing key defense roles against invading microbial pathogens (Mani-López et al., 2021; Fonseca et al., 2022b). In the present study, homogeneous ESL was obtained via the combination of ammonium sulfate precipitation and column chromatography techniques as reported in our previous study (Kuku et al., 2012). *E. senegalensis* lectin has an estimated subunit molecular weight of 35 kDa as confirmed by SDS-PAGE (Fig. 1). Konozy et al. (2003, 2012) revealed that the molecular weights of *E. lysistemon* and *E. speciosa* lectins were 30 and 26.7 kDa, respectively, after SDS-PAGE analysis, which is consistent with the findings of the present study. Similarly, lectins with an estimated molecular weight range of 26–30 kDa were also detected in *Erythrina crista-galli*, *Erythrina americana*, *Erythrina flabelliformis*, *Erythrina lysistemon*, *Erythrina rubrinerva*, and *Erythrina vespertilio* (Ortega et al., 1990; Bonneil et al., 2004). The range of molecular weight is not surprising as *Erythrina* lectins are made up of one-chain protomer with a molecular weight of 27–35 kDa that forms a dimer (Osman and Konozy, 2017).

In vitro antibacterial assay showed that ESL significantly ($P < 0.05$) inhibits the growth of *E. carotovora*, *K. pneumonia*, *P. aeruginosa*, and *S. aureus* at 0.4 mg/ml, with inhibition zones ranging from 18 to 24 mm (Fig. 2). *E. carotovora* and *S. aureus* were most susceptible to the protein, with inhibition zones of 20 ± 0.33 and 24 ± ± 0.33 mm, respectively (Table 1). However, *L. acidophilus* was resistant to lectin at this concentration, which suggests that ESL might be devoid of binding affinity to the glycomoieties expressed on the membrane of this organism. The MIC of ESL against susceptible bacteria ranged from 0.05 to 0.2 mg/ml (Table 1).
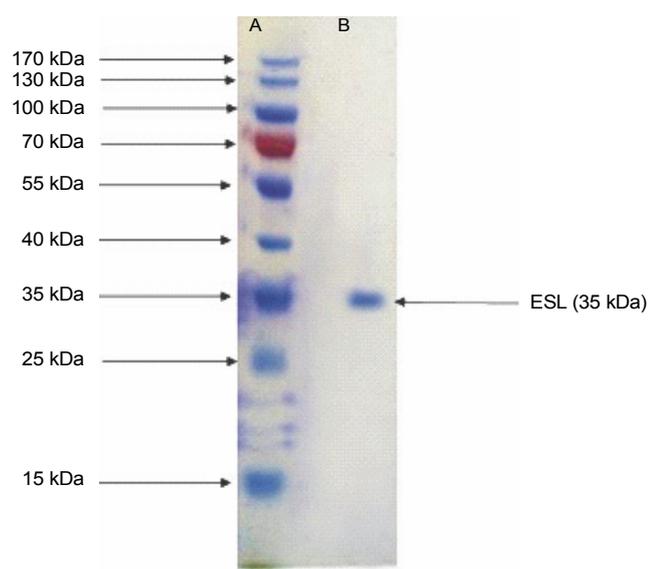
Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electro-
phoresis electrophoretogram of *Erythrina senegalensis* lectin
and standard protein markers (15–170 kDa)

Lectins have high specificity, enabling them to iden-
tify unique sugar structures (Adedoyin et al., 2021). This
unique property allows them to mediate specific micro-
bial binding, innate immune response, and cell-to-cell
signaling, coupled with host–pathogen interactions (Pa-
iva et al., 2010; Procópio et al., 2017). While eliciting
antibacterial activities, plant lectins may cause pore
formation, followed by interactions with definite bac-
terial cell wall components. Potential targets include
lipopolysaccharides, peptidoglycans, teichoic and tei-
churonic acids, as well as muramic- and muramyl-asso-
ciated structures. These interactions attract extensive
interest due to arrays of exposed microbial glycomoie-
ties that are potential lectin-binding sites (Gomes et al.,
2013; Singh et al., 2016). In addition, lectins can form
aggregates with microbial glycoconjugates, which might
block bacteria binding sites, thus preventing host
infection (Charungchitrak et al., 2011). Usually associa-
ted with their carbohydrate recognition domains (CRDs),
antibacterial mechanisms may also include restricting
microbial invasion, growth, migration, and competitive
inhibition of microbial proteins from specific receptors
(Paiva et al., 2010; Breitenbach Barroso Coelho et al.,
2018; Monika et al., 2020).

Antibacterial activity exhibited by ESL in the present
study was quite impressive as legume lectins were toxic
to bacterial pathogens. This is attributable to inter-

actions involving inherent aromatic amino acids and
hydrogen bonding between hydroxyl oxygen of sugars
and lectin CRD (Dias et al., 2015; El-Araby et al., 2020;
Santana et al., 2022). Santana et al. (2022) showed that
lectin isolated from the tropical legume *Cratylia argentea*
elicited potent toxicity toward *Listeria monocytogenes*
by reducing its systemic loads. El-Araby et al. (2020)
showed that purified lentil and pea lectins inhibited the
growth of *S. aureus* and *P. aeruginosa*, with inhibition
zones of 35 and 33.4 mm, respectively, as against 20 and
22 nm manifested by ESL in the present study. As
reported by Krishnaveni et al. (2022), lectins from the
legumes *Cicer arietinum* Black (CiarBL) and *Prunus
dulcis* nut raw (PruDuNRL) showed potent antibacterial
activity at 100 mg/ml against *Streptococcus oralis*,
Streptococcus pyogens, *Propionibacterium acnes*, and *S.
aureus*. Similarly, lectins from other legumes such as
Canavalia ensiformis, *Trigonella foenumgraecum*, *Ara-
chis hypogaea*, *Cajanus cajan*, *Pisum sativum*, and *Pha-
seolus vulgaris* inhibited *Streptococcus mutans*-induced
biofilm formation, supporting the antibacterial proper-
ties of these quintessential bioactive proteins (Islam
et al., 2012; Lagarda-Diaz et al., 2017).

ESL antibacterial activity reported in the present
study compared favorably with the results of specific clas-
sical antimicrobials including streptomycin, kasugamycin,
chloramphenicol, and oxytetracycline, against pathogenic
bacteria: *S. aureus*, *Enterococcus faecalis*, *Escherichia
coli*, *K. pneumonia*, *Erwinia amylovora*, *P. aeruginosa*,
Proteus vulgaris, *Hafnia alvei*, *Serratia marcescens*, *S. au-
reus*, and *Micrococcus luteus* (Ulagesan and Kim 2018;
Al-esnawy et al., 2021; Slack et al., 2021).

Findings of the present study showed that ESL signi-
ficantly ($P < 0.05$) inhibited the hyphal growth of *A. ni-
ger*, *P. camemberti*, and *S. brevicaulis* at 0.4 mg/ml, with
inhibition zones ranging between 18 and 19 mm (Fig. 3).
The MIC obtained for the susceptible fungi ranged be-
tween 0.2 and 0.4 mg/ml. The hyphal growth of *S. bre-
vicaulis* was most inhibited by ESL with an inhibition
zone of 19 ± 0.76 mm (Fig. 3). However, *T. interdigitale*
and *Trichoderma* spp. were resistant to lectin at this
concentration (Table 2). This is not surprising as legume
lectins are reported to be moderate antifungals (Ynalvez
et al., 2021). The growth inhibitory activities of lectins
vary between fungal species; however, their mechanism
of action is poorly understood (Yan et al., 2005; Araújo
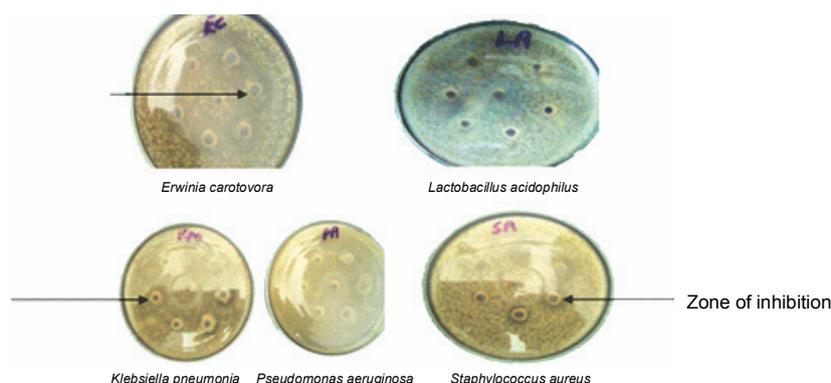
Fig. 2. Plates showing the antibacterial sensitivity of *Erwinia senegalensis* lectin against *Erwinia carotovora* (EC), *Pseudomonas aeruginosa* (PA), *Lactobacillus acidophilus* (LA), *Klebsiella pneumonia* (Kpn), and *Staphylococcus aureus* (SA)

Table 1. Sensitivity pattern and minimum inhibitory concentration exhibited by *Erythrina senegalensis* lectin against bacteria isolates

Bacteria isolates	Zone of inhibition [mm]	Streptomycin [mm] [mg/ml]	MIC [mg/ml]
Erwinia carotovora	20 ± 0.33	19 ± 0.12	0.05
Pseudomonas aeruginosa	18 ± 0.58	22 ± 0.41	0.2
Lactobacillus acidophilus	0.0	ND	ND
Klebsiella pneumonia	19 ± 0.67	18 ± 0.53	0.2
Staphylococcus areus	24 ± 0.33	20 ± 0.38	0.1

Data were expressed as mean ± standard error (n = 3); ND – not detected
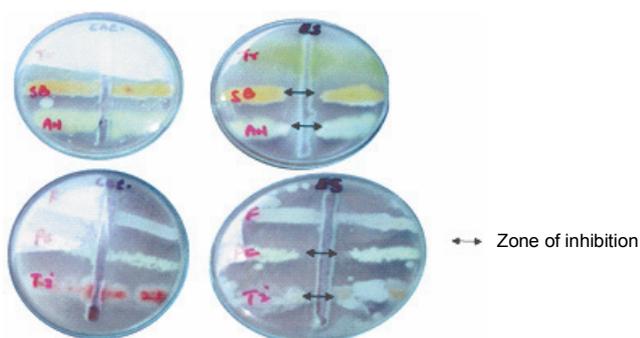
Fig. 3. Plate showing the antifungal sensitivity of *Erythrina senegalensis* lectin against *Aspergillus niger* (AN), *Fusarium species* (F), *Penicillium camemberti* (PC), *Scopulariopsis brevicaulis* (SB), *Trichophyton interdigitale* (Ti), and *Trichoderma* species (Tr)

et al., 2010; Lam and Ng, 2011; Del Rio et al., 2020). This is because plant lectins cannot suppress fungal growth directly since they do not penetrate the cell wall or cell membrane to reach the cytoplasm (Lagarda-Diaz et al., 2017). Despite this, indirect responses induced by lectin's attachment to key carbohydrate structures on the fungal surface have been observed in fungal survival (Wong et al., 2010). These structural targets include fungal cell wall chitin components. Specific interactions might result in the inhibition of cell wall synthesis, dissolution of the fungal cell wall, or cell metastasis, resulting in cell death (Lam and Ng, 2011; Breitenbach Barroso Coelho et al., 2018). Similarly, they can also associate with fungal hyphae, suppressing growth because of poor nutrient absorption and spore germination or predisposing the target organism to a certain stress condition by inducing detrimental morphological changes that ultimately lead to cell death (Van Parijs et al., 1991; El-Araby et al., 2020).

Consistent with the present findings, Sitohy et al. (2007) and Devi et al. (2013) reported that at 100 mg/ml lectins isolated from *P. sativum* and *Pongamia glabra* inhibited the growth of *Candida albicans*, *Aspergillus flavus*, *Trichoderma viride*, and *Fusarium oxysporum*. Yan et al. (2005) showed that a lactose-specific lectin from the legume *Astragalus mongholicus* also inhibited the growth of *Botrytis cincerea, F. oxysporum, Colleto-*

Table 2. The sensitivity pattern and minimum inhibitory concentration exhibited
by *Erythrina senegalensis* lectin against fungal isolates

Fungal isolates	Zone of inhibition [mm]	Fluconazole [mm] [mg/ml]	MIC [mg/ml]
Aspergillus niger	18 ± 0.88	20 ± 0.88	0.4
Fusarium sp.	0.0	ND	ND
Penicillium camemberti	18 ± 0.70	22 ± 0.50	0.2
Scopulariopsis brevicaulis	19 ± 0.76	18 ± 1.41	0.2
Trichophyton interdigitale	0.0	ND	ND
Trichoderma sp.	0.0	ND	ND

Data were expressed as mean $\pm$ standard error ($n = 3$); ND – not detected

trichum spp., and Drechslera turcica at a concentration range of 100–200 μg. Plant lectins have also been reported to cause lethal hypersensitivity reactions by triggering specific cytokines while mediating their antifungal activity (Kanzaki et al., 2008). Similar to ESL, polymyxin, a peptide-based antifungal, has been reported to inhibit the growth of *C. albicans*, *Cryptococcus neoformans*, and *Fusarium* spp. (Hsu et al., 2017).

Corresponding bioactivities of ESL have also been reported in lactose-binding lectins from *Chondrilla caribensis* and *Bufo arenarum* (Sánchez Riera et al., 2003; Marques et al., 2018). This suggests that the antibacterial and antifungal activities of ESL might be related to its sugar specificity, as legume lectins are emerging as promising molecules for designing novel therapeutics (Barbosa et al., 2021).

Sequence and phylogenetic analysis

Many plant families contain homologous proteins, such as lectins, that have a common biological function but different specificity toward substrates (Kalinina et al., 2004). This is observed in the complexity of lectin gene organization as variations exist between species. Available data on legume lectin structures suggest that they are closely related proteins (Rougé and Risler, 1990). Furthermore, their homology also indicates that they are well conserved during evolution (Sparvoli et al., 2001; Lioi et al., 2003). To investigate the evolutionary relationship of ESL with other plant lectins, a comparative analysis of the ESL gene was carried out with plant lectin families in a phylogenetic framework. gDNA of *E. senegalensis* seed was extracted. The gene encoding ESL was isolated using specific primers and analyzed
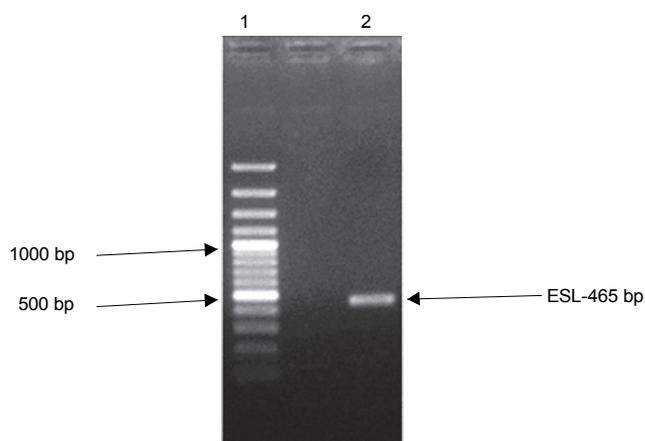
Fig. 4. Agarose gel electrophoresis of the polymerase chain reaction amplification product of *Erythrina senegalensis* lectin gene. Standard 100-bp DNA ladder (Lane 1) and amplified ESL-465 (Lane 2)

using the BLAST. One set of primers detected the lectin-coding gene of *E. senegalensis* (ESL) via direct PCR analysis. Agarose gel electrophoresis of the PCR products revealed a distinct amplicon fragment (Fig. 4). The lectin gene after Sangers sequencing showed a 465-bp consensus sequence designated as ESL-465.

The creation of a multiple-sequence alignment maximizes the chance of positional homology between nucleotides or amino acids by establishing gaps in the evolutionary comparisons of primary sequence data (Talavera and Castresana 2007). BLAST analysis of the ESL-465 nucleotide sequence against nucleotide sequences of plant lectins available in the NCBI database resulted in a significant alignment at 100% nucleotide identity with *Erythrina variegata* lectin (accession number: FJ839683.2), 100% nucleotide identity with *E. cris-*
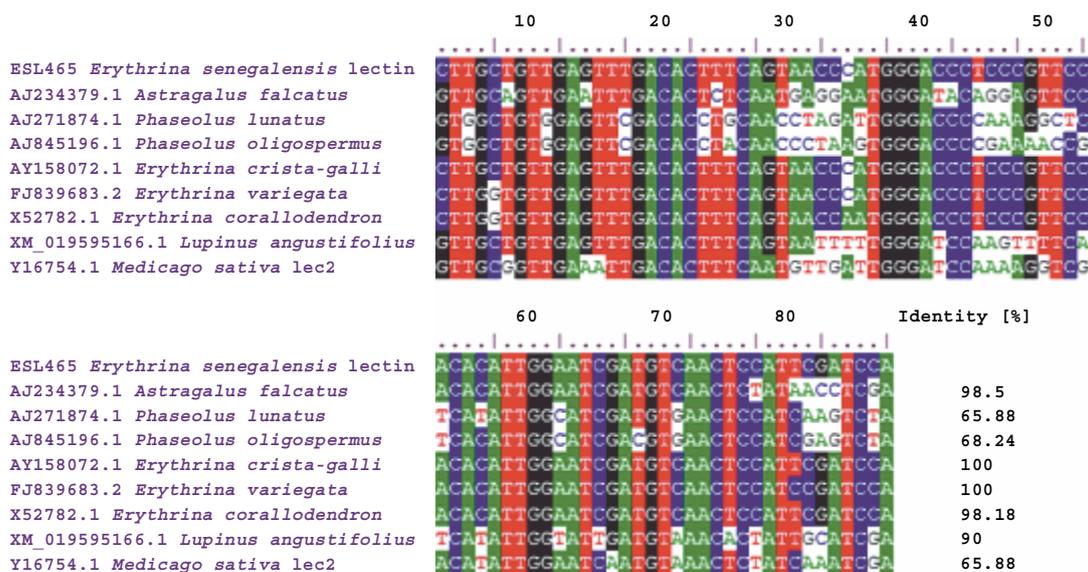
Fig. 5. Multiple-sequence alignment of *Erythrina senegalensis* lectin nucleotide sequence with related plant lectin genes

Table 3. Translated amino acid sequence of ESL465 gene

ESL00A-465	
Translated amino acid sequence (134 amino acids)	WGRNKRRVWGFTTHQDSKWHAGLGLNGPNSIYTCAHLGDHRHRQLNEILLFHTTLF HTTLHTPTPRWFSILYGTNKQVQQSPSQLKVMDTSEYSTTQNRITHTKHLLLSLTLSV THGTLPRFHTLESMSTPFDP
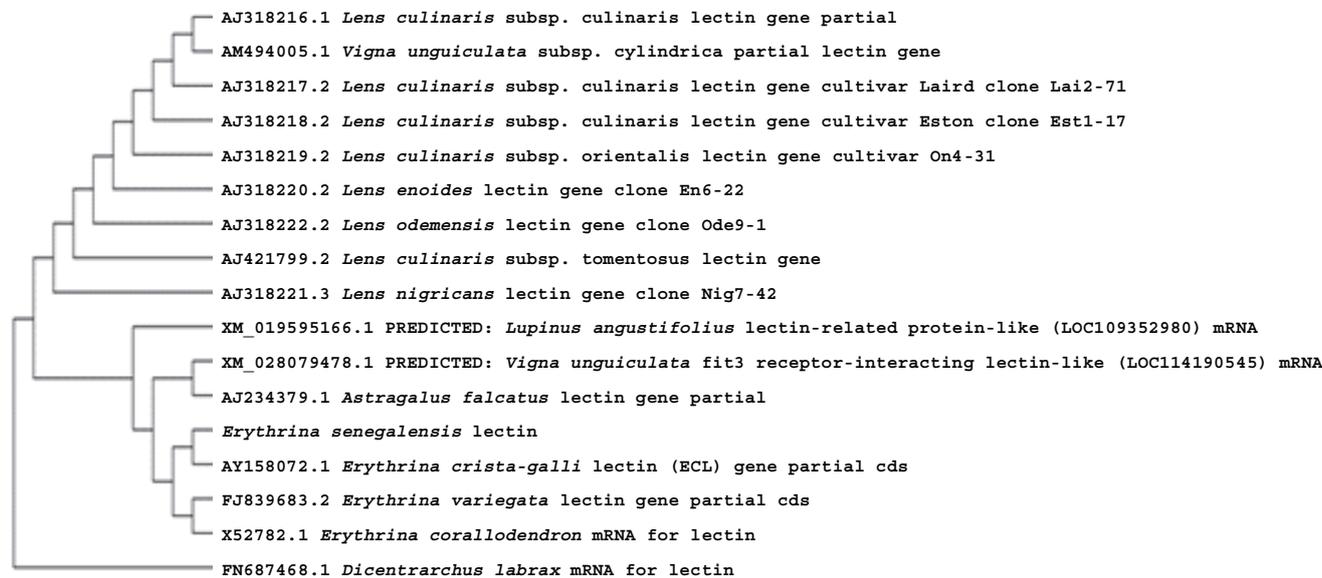
Fig. 6. Maximum likelihood phylogenetic tree of ESL-465 and other legume lectin genes; phylogenetic analysis was performed using the MEGA 7.0 program, and a consensus tree was constructed using the neighbor-joining method with 1000 replicate bootstrap values

ta-galli lectin (accession number: AY158072.1), and 98.18% nucleotide identity with *Erythrina corallodendron* lectin (accession number: X52782.1) (Fig. 5). This suggests that the lectin gene might be conserved within the *Erythrina* genus. Analysis of sequence variations or similarities provides insights into their structural divergence and evolution (Williams and Lovell, 2009). Findings of the present study also showed a profound nucleotide alignment at 98.5, 68.24, and 65.88% nucleotide identity with *Astragalus falcatus*, *Phaseolus oligospermus*, and *Phaseolus lunatus* lectin genes, respectively. Although information regarding the bioactivity of lectins from *A. falcatus*, *P. lunatus*, *P. oligospermus*, *E. cristagalli*, *E. variegata*, and *E. corallodendron* is scarce in the literature, the high sequence homology reported for these proteins with ESL might suggest their potential antimicrobial activities. The translation of the ESL-465 nucleotide sequence to the amino acid sequence resulted in a sequence of 134 amino acids (Table 3), which is within the amino acid composition range of legume lectins (Osman and Konozy, 2017).

Recently, functional and structural analysis of individual proteins is becoming more practical via phylogenetic analysis (Song et al., 2018). To elucidate the gene structure and construction of the gene family, nucleotide and/or amino acid sequences can be used. This makes the knowledge of protein sequences in predicting evolutionary relationships acceptable, suggesting that proteins of the same family might, to a greater extent, exhibit similar biological activities (Dey and Meyer, 2015; Xie et al., 2015; Li et al., 2016; He, et al., 2017). By multiple-sequence alignment using ClustalW, the evolutionary relationship between the *E. senegalensis* gene (ESL-465) and other plant lectins was revealed (Fig. 6). The analysis revealed that ESL-465 formed a cluster with other lectin genes from different leguminous families. This is not surprising as legume lectins are uniquely closely related homologous proteins, which are well conserved during evolution (Lioi et al., 2006; Lagarda-Diaz et al., 2017).

Conclusion

Several studies reported that lectins from the *Leguminosae* family have unique bioactivities, including toxicity toward pathogenic bacteria and fungi. In this study, *E. senegalensis* seed lectin elicited impressive antimicrobial activity against *E. carotovora*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *A. niger*, *P. camemberti*, and *S. brevicaulis*. The phylogenetic analysis of the ESL gene also showed its evolutionary relationship with other plant lectins (mostly legumes), suggesting the potential antimicrobial activities of these lectins. However, further research on the antimicrobial and structural properties of these lectins is needed to confirm these findings. Furthermore, lectin can serve as a potential target for developing practical applications in agriculture, health, and even pharmaceutical research.

Disclosure of conflict of interest
None

References

Adedoyin I.O., Adewole T.S., Agunbiade T.O., Adewoyin F.B., Kuku A. (2021) *A puried lectin with larvicidal activity from a woodland mushroom, Agaricus semotus Fr.* Acta Biol. Szeg. 65(1): 65–73. https://doi.org/10.14232/abs.2021.1.65-73

Alen'kina S.A., Bogatyrev V.A., Matora L.Y., Sokolova M.K., Chernyshova M.P., Trutneva K.A., Nikitina V.E. (2014) *Signal effects of the lectin from the associative nitrogen-fixing bacterium Azospirillum brasilense Sp7 in bacterial-plant root interactions.* P. Soil. 381(1–2): 337–349. https://doi.org/10.1007/s11104-014-2125-6

Al-esnawy A.A., Ereiba K.T., Bakr A.M., Abdraboh A.S. (2021) *Characterization and antibacterial activity of Streptomycin sulfate loaded bioglass/chitosan beads for bone tissue engineering.* J. Mol. Struct. 1227: 129715. https://doi.org/10.1016/j.molstruc.2020.129715

Araújo F.J.H., Vasconcelos I.M., Martins-Miranda A.S., Gondim D.M., Oliveira J.T. (2010) *A ConA–like lectin from Dioclea guianensis benth. Has antifungal activity against Colletotrichum gloeosporioides, unlike its homologues, ConM and ConA.* J. Agric. Food Chem. 58: 4090–4096. https://doi.org/10.1021/jf903254b

Armas F., Pacor S., Ferrari E., Guida F., Pertinhez T.A., Romani A.A., Scocchi M., Benincasa M. (2019) *Design, antimicrobial activity and mechanism of action of Arg-rich ultra-short cationic lipopeptides.* PloS One 14(2): e0212447. https://doi.org/10.1371/journal.pone.0212447

Atsamo A., Néné-Bi S., Kouakou K., Fofié K., Nyadjeu P., Watcho P., Datté J., Kamanyi A., Nguelefack T. (2013) *Cardiovascular and antioxidant effects of the methanol extract from the stem bark of Erythrina senegalensis DC (Fabaceae).* J. Physiol. Pharmacol. Adv. 3(4): 110–120. https://doi.org/10.5455/jppa.20130430124448

Barbosa P.P.D.S., Araújo F.N.D., Almeida J.M.D., Gadelha T.S. (2021) *Leguminosae lectins as biological tools in medical research: a review.* Braz. Arc. Biol. Technol. 64. https://doi.org/10.1590/1678-4324-2021200170

Bilanda D.C., Dzeufiet P.D., Fouda Y.B., Ngapout R.F., Tcheutchoua Y., Owona P.E., Wouamba S.C., Tatchou L.T., Dimo T., Kamtchouing P. (2020) *Antihypertensive and antidiabetic activities of Erythrina senegalensis DC (Fabaceae) stem bark aqueous extract on diabetic hypertensive rats.* J. Ethnopharm. 10: (246)112200. https://doi.org/10.1016/j.jep.2019.112200

Bonneil E., Young N.M., Lis H., Sharon N., Thibault P. (2004) *Probing genetic variation and glycoform distribution in lectins of the Erythrina genus by mass spectrometry.* Arch. Biochem. Biophys. 426(2): 241–249. https://doi.org/10.1016/j.abb.2004.01.006

Breitenbach Barroso Coelho L.C., Marcelino dos Santos Silva P., Felix de Oliveira W., de Moura M.C., Viana Pontual E., Soares Gomes F., Guedes Paiva P.M., Napoleão T.H., dos Santos Correia M.T. (2018) *Lectins as antimicrobial agents.* J. App. Micro. 125(5): 1238–1252. https://doi.org/10.1111/jam.14055

Charungchitrak S., Petsom A., Sangvanich P., Karnchanatat A. (2011) *Antifungal and antibacterial activities of lectin from the seeds of Archidendron jiringa Nielsen.* Food Chem. 126(3): 1025–1032. https://doi.org/10.1016/j.foodchem.2010.11.114

Cheesbrough M. (2006) *District laboratory practice in tropical countries.* Part 2. Cambridge University Press. https://doi.org/10.1017/cbo9780511543470

Del Rio M., de la Canal L., Regente M. (2020) *Plant antifungal lectins: mechanism of action and targets on human pathogenic fungi.* Curr. Pro. Pep. Sci. 21(3): 284–294. https://doi.org/10.2174/1389203720666190906164448

Devi S.S., Dhanalakshmi J., Selvi S. (2013) *The antibacterial and antifungal activity of lectin from seeds of Pongamiaglabra.* Inter. J. Curr. Biotechnol. 1: 10–14.

Dey G., Meyer T. (2015) Phylogenetic profiling for probing the modular architecture of the human genome. Cell Syst. 1(2): 106–115. https://doi.org/10.1016/j.cels.2015.08.006

Dias R.D.O., Machado L.D.S., Migliolo L., Franco O.L. (2015) *Insights into animal and plant lectins with antimicrobial activities.* Molecules 20(1): 519–541. https://doi.org/10.3390/molecules20010519

Doughari J.H. (2010) *Evaluation of antimicrobial potentials of stem bark extracts of Erythrina senegalensis DC.* Afr. J. Microbiol. Res. 4(17): 1836–1841.

El-Araby M.M., El-Shatoury E.H., Soliman M.M., Shaaban H.F. (2020) *Characterization and antimicrobial activity of lectins purified from three Egyptian leguminous seeds.* AMB Express. 10(1): 1–14. https://doi.org/10.1186/s13568-020-01024-4

Focho D.A., Ndam W.T., Fonge B.A. (2009) *Medicinal plants of Aguambu-Bamumbu in the Lebialem highlands, southwest province of Cameroon.* A. J. Pharm. Pharmacol. 3(1): 1–13.

Fonseca V.J.A., Braga A.L., de Almeida R.S., da Silva T.G., da Silva J.C.P., de Lima L.F., Dos Santos M.H.C., dos Santos Silva R.R., Teixeira C.S., Coutinho H.D.M., et al. (2022a) *Lectins ConA and ConM extracted from Canavalia ensiformis (L.) DC and Canavalia rosea (Sw.) DC inhibit planktonic Candida albicans and Candida tropicalis.* Arch. Microbiol. 204(6): 1–11. https://doi.org/10.1007/s00203-022-02959-x

Fonseca V.J.A., Braga A.L., Ribeiro Filho J., Teixeira C.S., da Hora G.C. Morais-Braga M.F.B. (2022b) *A review on the antimicrobial properties of lectins.* Int. J. Biol. Macromol. 195: 163–178. https://doi.org/10.1016/j.ijbiomac.2021.11.209

Gomes F.S., Procópio T.F., Napoleão T.H., Coelho L.C., Paiva P.M. (2013) *Antimicrobial lectin from S chinus terebinthifolius leaf.* J. Appl. Micro. 114(3): 672–679. https://doi.org/10.1111/jam.12086

Górniak I., Bartoszewski R., Króliczewski J. (2019) *Comprehensive review of antimicrobial activities of plant flavonoids.* Phytochem. Rev. 18(1): 241–272. https://doi.org/10.1007/s11101-018-9591-z

Gupta B., Sadaria D., Warrier V.U., Kirtonia A., Kant R., Awasthi A., Baligar P., Pal J.K., Yuba E., Sethi G., et al. (2020) *Plant lectins and their usage in preparing targeted nanovaccines for cancer immunotherapy.* [in:] *Seminars in cancer biology.* Pt. 14. Academic Press. https://doi.org/10.1016/j.semcancer.2020.02.005

He L., Li Y., He R.L., Yau S.S. (2017) *A novel alignment-free vector method to cluster protein sequences.* J. Theo. Biol. 427: 41–52. https://doi.org/10.1016/j.jtbi.2017.06.002

Hsu L.H., Wang H.F., Sun P.L., Hu F.R., Chen Y.L. (2017) *The antibiotic polymyxin B exhibits novel antifungal activity against Fusarium species.* Int. J. Ant. Agents. 49(6): 740–748. https://doi.org/10.1016/j.ijantimicag.2017.01.029

Islam B., Khan A.U. (2012) *Lectins: to combat infections.* [in:] *Protein Purification.* Ed. Ahmad R. InTech. Rijeka (Croatia): 167–188. https://doi.org/10.5772/30212

Kalinina O.V., Novichkov P.S., Mironov A.A., Gelfand M.S., Rakhmaninova A.B. (2004) *SDPpred: a tool for prediction of amino acid residues that determine differences in functional specificity of homologous proteins.* Nucl. Acids Res. 32(suppl. 2): W424–W428. https://doi.org/10.1093/nar/gkh391

Kanzaki H., Saitoh H., Takahashi Y., Berberich T., Ito A., Kamoun S., Terauchi R. (2008) *NbLRK1, a lectin-like receptor kinase protein of Nicotiana benthamiana, interacts with Phytophthora infestans INF1 elicitin and mediates INF1-induced cell death.* Planta 228(6): 977–987. https://doi.org/10.1007/s00425-008-0797-y

Konozy E.H. (2012) *Characterization of a D-Galactose-binding lectin from seeds of erythrina lysistemon.* Turkish J. Biochem. 1: 7–18.

Konozy E.H., Bernardes E.S., Rosa C., Faca V., Greene L.J., Ward R.J. (2003) *Isolation, purification, and physicochemical characterization of a D-galactose-binding lectin from seeds of Erythrina speciosa.* Arch. Bioch. Biophys. 410(2): 222–229. https://doi.org/10.1016/S0003-9861(02)00695-1

Krishnaveni M., Kavipriya M., Jayasudha J.B. (2022) *Extraction and characterization of lectin from Cicerarietinum black and Prunusdulcis nut raw, its anti-inflammatory, anti-*

bacterial activity against oral pathogens. Mat. Today: Pro. 51: 1706–1713 https://doi.org/10.1016/j.matpr.2020.10. 567

Kuku A., Odekanyin O.O., Okonji R.E. (2012) *Physicochemical properties of a lactose specific lectin from the seeds of Erythrina senegalensis DC.* Ife J. Sci. 14(1): 143–153.

Kumar S., Stecher G., Tamura K. (2016) *MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger data-sets.* Mol. Biol. Evol. 33(7): 1870–1874. https://doi.org/ 10.1093/molbev/msw054

Lagarda-Diaz I., Guzman-Partida A.M., Vazquez-Moreno L. (2017) *Legume lectins: proteins with diverse applications.* Int. J. Mol. Sci. 18(6): 1242. https://doi.org/10.3390/ijms 18061242

Lam S.K., Ng T.B. (2011) *Lectins: production and practical applications.* Appl. Micro. Biotech. 89: 45–55. https:// doi.org/10.1007/s00253-010-2892-9

Li Y., Tian K., Yin C., He R.L., Yau S.S. (2016) *Virus classi-fication in 60-dimensional protein space.* Mol. Phyl. Evol. 99: 53–62. https://doi.org/10.1016/j.ympev.2016.03.009

Lioi L., Galasso I., Santantonio M., Lanave C., Bollini R., Spar-voli F. (2006) *Lectin gene sequences and species relation-ships among cultivated legumes.* G. Res. Crop Evol. 53(8): 1615–1623. https://doi.org/10.1007/s10722-005-8719-3

Lioi L., Sparvoli F., Galasso I., Lanave C., Bollini R. (2003) *Lectin-related resistance factors against bruchids evolved through a number of duplication events.* Theor. Appl. Gen. 107(5): 814–822. https://doi.org/10.1007/s00122-003-1343-8

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951) *Protein measurement with the Folin phenol reagent.* J. Biol. Chem. 193(1): 265–275. https://doi.org/10.1016/ s0021-9258(19)52451-6

Mammari N., Krier Y., Albert Q., Devocelle M., Varbanov M. (2021) *Plant-derived antimicrobial peptides as potential antiviral agents in systemic viral infections.* Pharmaceuti-cals 14(8): 774. https://doi.org/10.3390/ph14 080774

Mani-López E., Palou E., López-Malo A. (2021) *Legume pro-teins, peptides, water extracts, and crude protein extracts as antifungals for food applications.* Trends Food Sci. Technol. 112: 16–24. https://doi.org/10.1016/j.tifs.2021. 03.035

Marques D.N., de Almeida A.S., de Oliveira Sousa A.R., Pe-reira R., Andrade A.L., Chaves R.P., Carneiro R.F., de Vas-concelos M.A., do Nascimento-Neto L.G., Pinheiro U., et al. (2018) *Antibacterial activity of a new lectin isolated from the marine sponge Chondrilla caribensis.* Int. J. Biol. Macromol. 109: 1292–1301. https://doi.org/10.1016/ j.ijbiomac.2017.11.140

Monika D., Sharma S., Kumar N. (2020) *Use of plant-derived antimicrobials as an alternative to antibiotics.* J. Pharm. Phytochem. 9(2): 1524–1532. https://doi.org/10.22271/ phyto.2020.v9.i2y.11069

Murphy C., Powlowski J., Wu M., Butler G., Tsang A. (2011) *Curation of characterized glycoside hydrolases of fungal origin.* Database 2011: bar020. https://doi.org/10.1093/ database/bar020

Nembo E.N., Atsamo A.D., Nguelefack T.B., Kamanyi A., He-scheler J., Nguemo F. (2015) *In vitro chronotropic effects of Erythrina senegalensis DC (Fabaceae) aqueous extract on mouse heart slice and pluripotent stem cell-derived cardiomyocytes.* J. Ethnopharm. 165: 163–172. https:// doi.org/10.1016/j.jep.2015.02.002

Nordström R., Malmsten M. (2017) *Delivery systems for antimicrobial peptides.* Adv. Coll. Int. Sci. 242: 17–34. https://doi.org/10.1016/j.cis.2017.01.005

Nubi T., Adewole T.S., Agunbiade T.O., Osukoya O.A., Kuku A. (2021) *Purification and erythrocyte-membrane per-turbing activity of a ketose-specific lectin from Moringa oleifera seeds.* Biotech. Rep. 31: e00650. https://doi.org/ 10.1016/j.btre.2021.e00650

Ortega M., Sanchez C., Chacon E., Rendon J.L., Estrada R., Masso F., Montaño L.F. Zenteno E., (1990) *Purification and characterization of a lectin from Erythrina americana by affinity chromatography.* Plant Sci. 72(1): 133–140. https://doi.org/10.1016/0168-9452(90)90195-T

Osman M.E., Konozy E.H. (2017) *Insight into Erythrina lec-tins: properties, structure and proposed physiological significance.* Open Bio. Comp. J. 5: 57–71. https://doi.org/ 10.2174/1874847301705010057

Othman L., Sleiman A., Abdel-Massih R.M. (2019) *Antimicro-bial activity of polyphenols and alkaloids in middle eastern plants.* Fr. Micro. 10: 911. https://doi.org/10.3389/fmicb. 2019.00911

Otimenyin S.O., Uzochukwu D.C. (2010) *Spasmolytic and anti-diarrhea effects of the bark of Erythrina senegalensis and root of Kigelia africana.* Asian J. Pharmaceut. Clin. Res. 3(4): 11–14..

Paiva P.M., Gomes F.S., Napoleão T.H., Sá R.A., Correia M.T., Coelho L.C. (2010) *Antimicrobial activity of secon-dary metabolites and lectins from plants.* Curr. Res. Tech-nol. Edu. Topics Appl. Microbiol. Micro. Biotech. 1(2): 396–406. https://doi.org/10.1016/j.jbiotec.2010.09.661

Procópio T.F., Moura M.C., Albuquerque L.P., Gomes F.S., Santos N.D., Coelho L.C., Pontual E.V., Paiva P.M., Napo-leão T.H. (2017) *Antibacterial lectins: action mechanisms, defensive roles and biotechnological potential. Antibac-terials: synthesis, properties and biological activities.* Nova Sci Pub. New York: 69–90.

Rambo D.F., Biegelmeyer R., Toson N.S., Dresch R.R., Mo-reno P.R., Henriques A.T. (2019) *The genus Erythrina L.: a review on its alkaloids, preclinical, and clinical studies.* Phyto. Res. 33(5): 1258–1276. https://doi.org/10.1002/ ptr.6321

Rougé P., Risler J.L. (1990) *Evidence for internal sequence homologies in Leguminosae lectins: phylogenetic implica-tions.* Biochem. Sys. Eco. 18(1): 29–37. https://doi.org/10. 1016/0305-1978(90)90029-f

Sánchez Riera A., Daud A., Gallo A., Genta S., Aybar M., Sán-chez S. (2003) *Antibacterial activity of lactose-binding lec-tins from Bufo arenarum skin.* Biocell 27(1): 37–46. https://doi.org/10.32604/biocell.2003.27.037

Santana L.N., Tavares L.S., Dorvigny B.M., Souza F.D.A.L., de Albuquerque Paiva B.H., Evêncio-Neto J., Hounkonnou S.G.C., Silva A.F.B., Ramos M.V. Lima-Filho J.V. (2022) *Anti-infective activity of Cratylia argentea lectin (CFL) against experimental infection with virulent Listeria monocytogenes in Swiss mice.* Phytomedicine 94: 153839. https://doi.org/10.1016/j.phymed.2021.153839

Singh R.S., Walia A.K., Kanwar J.R. (2016) *Protozoa lectins and their role in host – pathogen interactions.* Biotechnol. Adv. 34(5): 1018–1029. https://doi.org/10.1016/j.biotechadv.2016.06.002

Singh R.S., Walia A.K., Kennedy J.F. (2019) *Structural aspects and biomedical applications of microfungal lectins.* Int. J. Biol. Macromol. 134: 1097–1107. https://doi.org/10.1016/j.ijbiomac.2019.05.093

Sitohy M., Doheim M. Badr H. (2007) *Isolation and characterization of a lectin with antifungal activity from Egyptian Pisum sativum seeds.* Food Chem. 104(3): 971–979. https://doi.org/10.1016/j.foodchem.2007.01.026

Slack S.M., Walters K.J., Outwater C.A., Sundin G.W. (2021) *Effect of kasugamycin, oxytetracycline, and streptomycin on in-orchard population dynamics of Erwinia amylovora on apple flower stigmas.* Plant Dis. 105(6): 1843–1850. https://doi.org/10.1094/pdis-07-20-1469-re

Smýkal P., Coyne C.J., Ambrose M.J., Maxted N., Schaefer H., Blair M.W., Berger J., Greene S.L, Nelson M.N., Besharat N., et al. (2015) *Legume crops phylogeny and genetic diversity for science and breeding.* Crit. Rev. Plant Sci. 34(1–3): 43–104. https://doi.org/10.1080/07352689.2014.897904

Song L., Wu S., Tsang A. (2018) *Phylogenetic analysis of protein family. Fungal gen.* Humana Press. New York: 267–275. https://doi.org/10.1007/978-1-4939-7804-5_21

Sparvoli F., Lanave C., Santucci A., Bollini R. Lioi L. (2001) *Lectin and lectin-related proteins in Lima bean (Phaseolus lunatus L.) seeds: biochemical and evolutionary studies.* Plant Mol. Biol. 45(5): 587–597. https://doi.org/10.1023/a:1010647310311

Sun S., Huang S., Shi Y., Shao Y., Qiu J., Sedjoah R.C.A.A., Yan Z., Ding L., Zou D., Xin Z. (2021) *Extraction, isolation, characterization and antimicrobial activities of non-extractable polyphenols from pomegranate peel.* Food Chem. 351: 129232. https://doi.org/10.1016/j.foodchem.2021.129232

Taiwo A.S., Adenike K. Aderonke O. (2020) *Efficacy of a natural coagulant protein from Moringa oleifera (Lam) seeds in treatment of Opa reservoir water, Ile-Ife, Nigeria.* Heliyon 6(1): e03335. https://doi.org/10.1016/j.heliyon.2020.e03335

Talavera G., Castresana J. (2007) *Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments.* System. Biol. 56(4): 564–577. https://doi.org/10.1080/10635150701472164

Ulagesan S., Kim H.J. (2018) *Antibacterial and antifungal activities of proteins extracted from seven different snails.* Appl. Sci. 8(8): 1362. https://doi.org/10.3390/app8081362

Van Holle S., Van Damme E.J. (2018) *Signaling through plant lectins: modulation of plant immunity and beyond.* Biochem. Soc. Trans. 46(2): 217–233. https://doi.org/10.1042/bst20170371

Van Holle S., Van Damme E.J. (2019) *Messages from the past: new insights in plant lectin evolution.* Front. Plant Sci. 10: 36. https://doi.org/10.3389/fpls.2019.00036

Van Parijs J., Broekaert W.F., Goldstein I.J., Peumans W.J. (1991) *Hevein: an antifungal protein from rubber-tree (Hevea brasiliensis) latex.* Planta 183: 258–264. https://doi.org/10.1007/bf00197797

Vandenborre G., Smagghe G., Van Damme E.J. (2011) *Plant lectins as defense proteins against phytophagous insects.* Phytochemistry 72(13): 1538–1550. https://doi.org/10.1016/j.phytochem.2011.02.024

Williams S.G., Lovell S.C. (2009) *The effect of sequence evolution on protein structural divergence.* Mol. Biol. Evol. 26(5): 1055–1065. https://doi.org/10.1093/molbev/msp020

Wong J.H., Ng T.B., Cheung R.C., Ye X.J., Wang H.X., Lam S.K., Lin P., Chan Y.S., Fang E.F., Ngai P.H., et al. (2010) *Proteins with antifungal properties and other medicinal applications from plants and mushrooms.* Appl. Micro. Biotech. 87(4): 1221–1235. https://doi.org/10.1007/s00253-010-2690-4

Xie X.H., Yu Z.G., Han G.S., Yang W.F., Anh V. (2015) *Whole-proteome based phylogenetic tree construction with inter-amino-acid distances and the conditional geometric distribution profiles.* Mol. Phylogen. Evol. 8: 37–45. https://doi.org/10.1016/j.ympev.2015.04.008

Yan Q., Jiang Z., Yang S., Deng W., Han L., (2005) *A novel homodimeric lectin from Astragalus mongholicus with antifungal activity.* Arch. Biochem. Biol. 442(1): 72–81. https://doi.org/10.1016/j.abb.2005.07.019

Ynalvez R.A., Gonzalez K.M., Ynalvez M.A., Escalante J.F. (2021) *The characterization and determination of the antimicrobial, and anti-HIV reverse transcriptase activities of leguminosae (Arachis hypogaea, Dolichos biflorus, Erythrina crista-galli, and Glycine max) lectins.* Amer. Int. J. Biol. 9(1): 1–12. https://doi.org/10.15640/aijb.v9n1a1