Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 22 | No. 2 | pp. 1023-1028 | 2023

Original Research Paper

https://doi.org/10.46488/NEPT.2023.v22i02.049

Open Access Journal

Molecular Docking Analysis of *Embelia ribes* for Selected Constituents as *Spodoptera frugiperda* (Fall Armyworm) Beta Glycosidase and Caspase-1 Inhibitors

Srinivasan Kumaraswamy*, Vasantha-Srinivasan Prabhakaran and Radhakrishnan Narayanaswamy***†**

*Department of Biochemistry, St. Peter's Institute of Higher Education and Research (SPIHER), Avadi, Chennai-600 054, Tamilnadu, India

**Department of Bioinformatics, Saveetha School of Engineering, SIMATS (Deemed to be University), Thandalam, Chennai-602 105, Tamilnadu, India

***Department of Biochemistry, Saveetha Medical College and Hospital, SIMATS (Deemed to be University), Thandalam, Chennai-602 105, Tamilnadu, India

†Corresponding author: Radhakrishnan Narayanaswamy; kishnanbio07@gmail.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 25-10-2022
Revised: 08-12-2022
Accepted: 24-12-2022

Key Words:
Molecular docking
Spodoptera frugiperda
Embelia ribes
Embelin
Vilangin

ABSTRACT

Insect pest control is one of the major issues facing the agriculture sector because of the need for new agrochemicals and biocontrol agents that are environmentally friendly, economically affordable, and safe for human health. *Spodoptera frugiperda* (fall armyworm) is one of the insect pests that causes huge damage to various crops around the globe due to its generalist nature. In the present study, three selected *Embelia ribes* Burm F (Myrsinaceae) constituents, which include embelin, 5-O-methylembelin, and vilangin; one semi-synthetic compound (potassium embelate); three synthetic compounds, namely coenzyme Q_{10}, dopaquinone, and idebenone; and two reference compounds (azadirachtin and amitraz) were assessed on the docking behavior of *S. frugiperda* (beta glycosidase and caspase-1. The docking studies showed that coenzyme Q_{10} exhibited the highest binding energies (-130.61 and -434.56 kcal. mol^{-1}) for the target enzymes *S. frugiperda* (beta glycosidase and caspase-1, respectively). Thus, the present investigation provides new knowledge in understanding *Embelia ribes* Burm F (Myrsinaceae) constituents as possible inhibitors against *S. frugiperda* (beta glycosidase and caspase-1) enzyme activities. Furthermore, the present work can help to develop new insecticides and pesticides against *S. frugiperda* and other related insect pests.

INTRODUCTION

The long-term usage of synthetic fertilizers and pesticides contaminates soil and surface water, and they are also reported to cause ill effects in animals (fish) and humans (Patel & Gajar 2001). Moreover, the use of synthetic pesticides leads to environmental issues like bioaccumulation and biomagnification, which cause huge losses to our biological wealth and resources. Similarly, overuse of chemical fertilizers (like nitrogenous, phosphoric, and potassium) has negatively affected the soil texture and altered the pH value of the soil, causing an imbalance in nutrient absorption for crops, fruits, plants, and vegetables (Dhar 2020). The increasing awareness about the ill effects of synthetic fertilizers and pesticides has forced us to look for alternative ways (organic farming) and technologies (like traditional hydroponics and aquaponics). Moreover, there is a huge rise in demand for the use of plant-based (botanical) pesticides, especially for crop protection (Nas 2004). Now, worldwide agricultural scientists are investigating and developing botanical pesticides (using indigenous plant materials) to protect against insect infestations (Pavunraj et al. 2011). Moreover, the use of indigenously available plants or herbs in the control and management of pests is a traditional, adopted, and practiced agricultural technology in many countries, including India (Roy et al. 2005). The advantage of using botanical pesticides is that they are non-selective toxins or poisons that are active against a wide range of agricultural pests (Pavunraj et al. 2011). Several phytochemicals have been reported to be potent alternatives to chemical pesticides (Leatemia & Isman 2004). The mechanism of action of phytochemicals has been reported by

ORCID details of the authors:

Vasantha-Srinivasan Prabhakaran: https://orcid.org/0000-0003-3415-3315
Radhakrishnan Narayanaswamy: https://orcid.org/0000-0001-5259-761X

many researchers. For instance, the antifeedant (Raja et al. 2005), larvicidal (Kabaru & Gichia 2001), ovicidal (Pavunraj et al. 2006), repellent (Paredes-Sanchez et al. 2021), etc. are the few reported mechanisms of action employed by the phytochemicals for the control and management of pests. In the present study, we focus on *Spodoptera frugiperda* (Fall Armyworm), which is one of the major pests and has the following crops like cotton, maize, millet, rice, sorghum, soybean, and sugarcane (Deshmukh et al. 2021).

*Embelia ribe*s Burm. f., a dense shrub of the family Myrsinaceae, has been reported to possess numerous medicinal uses. *E. ribes* has been known to possess phytoconstituents, namely, christembine, daucosterol, 5-*O*-methylembelin, embelinol, embeliol, embeliaribyl ester, embelin, embolic acid, rapanone, sitosterol, and vilangin, etc. (Mhaskar et al. 2011). *Brassica juncea* and *E. ribe*s have been reported to safeguard the seeds from several fungal diseases and pests. In addition, treatment with these plant extracts also stimulates disease resistance after the germination of the seeds (Dhar 2020). Moreover, embelin (a bioactive constituent of *E. ribes*) has been reported to be effective against stored grain pests (Dhar 2020). Thus, the background mentioned above prompts us to carry out the present work on three selected constituents of *E. ribes,* namely, embelin, 5-O-methylembelin, and vilangin; one semi-synthetic compound (potassium embelate); three synthetic compounds, namely coenzyme Q10, dopaquinone, and idebenone; and two reference compounds (azadirachtin and amitraz), which were assessed on the docking behavior of *S. frugiperda* (beta glycosidase and caspase-1) by using the PatchDock method.

MATERIALS AND METHODS

Ligand Preparation

Chemical structures of three ligands (constituents of *E. ribes*) namely 1) embelin (CID no: 3218); 2) 5-*O*-methylembelin (CID no:171489); 3) vilangin (CID no:417182); one semi-synthetic compound 4) potassium embelate (CID no: 23677950); three synthetic compounds namely, 5) coenzyme Q10 (CID no: 5281915); 6) dopaquinone (CID no: 439316); 7) idebenone (CID no: 3686); two reference compounds namely, 8) azadirachtin (CID no: 5281303); and 9) amitraz (CID no: 36324) were downloaded from PubMed database. These selected ligands were prepared according to the earlier report (Mohan et al. 2022). These prepared structures were used for further study (PatchDock).

Identification and Preparation of Target Protein

The three-dimensional (3D) structure of the *Spodoptera frugiperda* beta glycosidase (PDB ID: 5CG0 with a resolution of 2.09 Å) and caspase-1 (PDB ID: 1M72 with a resolution of 2.30 Å) were obtained from Protein Data Bank (PDB). A chain of both proteins was processed individually by removing other chains, ligands, and crystallographically observed water (H_2O) molecules (i.e., water without hydrogen bonds) by using UCSF Chimera software.

Molecular Docking Study

A docking study was performed for the selected (three *E. ribes* constituents; one semi-synthetic; three synthetic, and two reference compounds) ligands using the PatchDock online server. Finally, the binding site analysis was carried out for the best-docked pose using the PyMOL software (Mohan et al. 2022).

RESULTS AND DISCUSSION

Spodoptera frugiperda has gained much attention worldwide due to its outbursts in Africa (since 2016) and Asia (since mid-year 2018). In 2018, *S. frugiperda* has been reported for the first time in India, and that too in South India (Shivamogga and Davanagere districts of Karnataka State) on the maize crop (Deshmukh et al. 2021). *S. frugiperda* has been reported to cause primarily huge damage to maize crops, followed by other host crops like millets, vegetables, and sorghum (Day et al. 2018).

In general, beta glycosidases have been involved in hydrolyzing glycosides to release carbohydrates (Marana et al. 2000). *Spodoptera frugiperda* beta glycosidase (Sfβgly) is a secreted digestive enzyme present in midgut epithelial cells. *Spodoptera frugiperda* beta glycosidase (Sfβgly) has been previously reported in relation to its i) catalysis, ii) substrate recognition, and iii) thermal stability (Otsuka et al. 2020). Thus, in the present study, *Spodoptera frugiperda* beta glycosidase (Sfβgly) was selected as one of the target enzymes.

Caspases have been involved in the carrying out of the apoptosis process, and the apoptotic pathway is highly conserved in the metazoans. Thus, caspases serve as a fascinating target for medical treatment. *Spodoptera frugiperda* caspase-1 (Sf-caspase 1) is the chief effector caspase of *S. frugiperda* (Forsyth et al. 2004). Therefore, in the present study, *Spodoptera frugiperda* caspase-1 (Sf-caspase 1) was selected as the second target enzyme.

Table 1 shows the chemical nature and botanical source of nine selected ligands. Out of nine ligands, seven ligands belong to the quinone (55.6%). Similarly, alpha amino acid (11.1%), tertiary amino compound (11.1%), and triterpenoid (11.1%) are three other classes of selected ligands.

Table 1: Nature of chemical class and source of nine selected ligands.

S.No.	Ligand name	Nature of chemical class	Source
1.	Coenzyme Q_{10}	Quinone	*Arachis hypogaea*, *Brassica oleracea*, *Brassica rapa*, *Citrus clementina*, *Daucus carota*, *Glycine max*, *Malus domestica*, *Petroselinum crispum*, *Pisum sativum*, *Solanum lycopersicum and Solanum tuberosum*
2.	Dopaquinone	Alpha amino acid	Present in the human skin and feces
3.	Embelin	Quinone	*Embelia ribes, Ardisia humilis, Connarus ritchiei, Embelia barbeyana*, *Embelia kilimandscharica*, *Embelia robusta*, *Embelia tsjersium-cottam*, *Myrsine africana*, *Myrsine capitellata*, *Myrsine semiserrata* and *Rapanea umbellate*
4.	Idebenone	Quinone	Synthetic one
5.	5-*O*-Methylembelin	Quinone	*Embelia ribes*
6.	Potassium embelate	Quinone	Synthetic one
7.	Vilangin	Quinone	*Embelia ribes*
8.	Azadirachtin	Triterpenoid	*Azadirachta indica*
9.	Amitraz	Tertiary amino compound	Synthetic one

Table 2: The interaction energy analysis of nine selected ligands with the *Spodoptera frugiperda* beta glycosidase using the PatchDock method

S.No.	Ligand names	-ACE* (-kcal.mol^{-1})	Interaction of amino acid residue	Bond distance (Å)
1.	Coenzyme Q 10	130.61	Arg189 Asn249 Glu271	2.9 2.8 2.8
2.	Dopaquinone	+271.02	Gln39 Ser247 Asn249 Tyr331 Glu451 Trp452	3.5 2.9 2.9 3.3 2.4 3.1
3.	Embelin	+40.45	His142 Glu187 Glu451 Trp452	3.6 2.5 2.9 3.2
4.	Idebenone	34.07	Gln39 His142 Arg267 Glu271	2.1 2.6 3.0 3.5
5.	5-*O*-Methylembelin	+52.22	Glu187 Glu399	1.6, 2.3 and 2.5 2.4
6.	Potassium embelate	+22.46	His142 Glu187	3.3 1.8 and 3.3
7.	Vilangin	83.22	No interactions	-
8.	Azadirachtin	+5.95	Arg189	3.2 and 3.4
9.	Amitraz	+53.98	No interactions	-

Note: ACE*- Atomic contact energy

The docking studies revealed that coenzyme Q_{10} exhibited the highest (ACE) atomic contact energy (-130.61 kcal.mol^{-1}) with the *Spodoptera frugiperda* beta glycosidase enzyme. In contrast, idebenone showed the lowest binding energy (-34.07 kcal.mol^{-1}) with the target enzyme *Spodoptera frugiperda* beta glycosidase (Table 2).

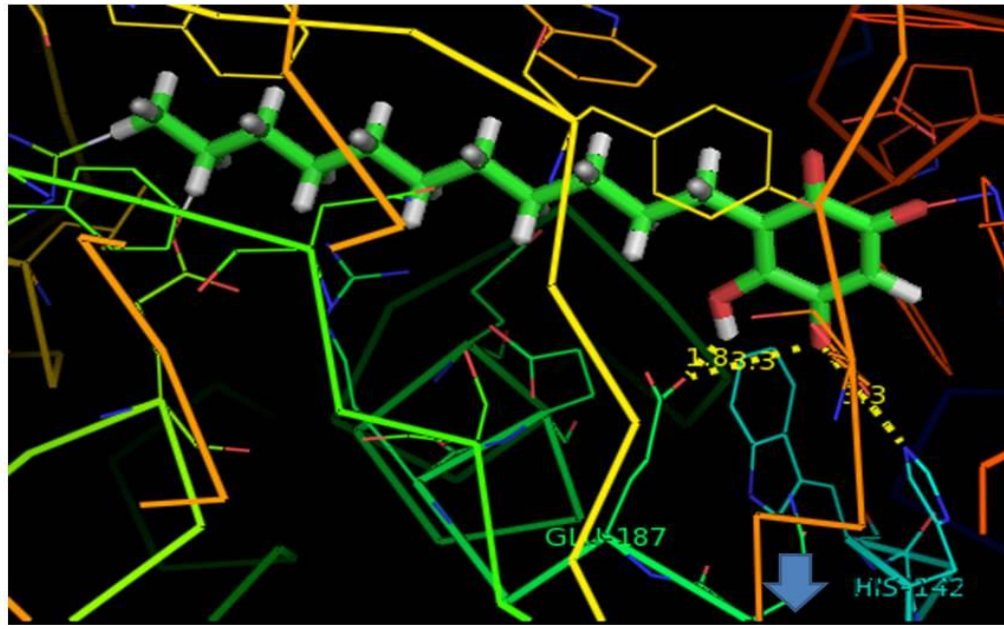
In the present study, six ligands namely, dopaquinone (+271.02 kcal.mol^{-1}); amitraz (+53.98 kcal.mol^{-1}); 5-*O*-Methylembelin (+52.22 kcal.mol^{-1}); embelin (+40.45 kcal.mol^{-1}); potassium embelate (+22.46 kcal.mol^{-1}) and azadirachtin (+5.95 kcal.mol^{-1}) have shown poor (ACE) atomic contact energy values (Table 2).

Three ligands such as embelin, 5-*O*-Methylembelin, and potassium embelate were shown to interact with the Glu 187 amino acid residue of the *Spodoptera frugiperda* beta glycosidase enzyme (as shown in Table 2). Similarly,

Tamaki et al. (2016) reported that the mutation in Glu 187 amino acid position results in the worst effect on *Spodoptera frugiperda* beta glycosidase hydrolytic activity. In the present study, three ligands such as embelin, idebenone, and potassium embelate (Fig. 1) are shown to interact with the His 142 amino acid residue of the *Spodoptera frugiperda* beta glycosidase enzyme. Similarly, Tamaki et al. (2016) reported that the His 142 amino acid residue is present in the glycone binding site of the *Spodoptera frugiperda* beta glycosidase enzyme.

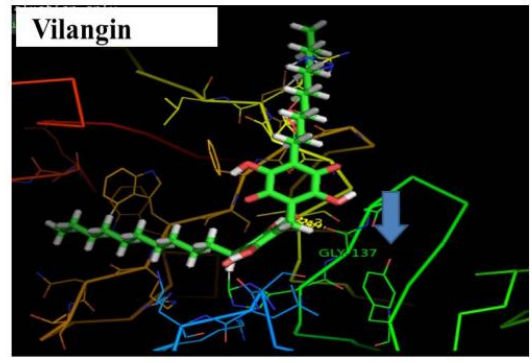
The docking studies showed that azadirachtin exhibited the highest (ACE) atomic contact energy (-504.85 kcal. mol^{-1}) with the *Spodoptera frugiperda* caspase-1 enzyme. In contrast, vilangin showed the lowest binding energy (-155.16 $kcal.mol^{-1}$) with the target enzyme *Spodoptera frugiperda* caspase-1 (Table 3).

The binding energy results showed the following order: azadirachtin (-504.85 $kcal.mol^{-1}$), < coenzyme Q_{10} (-434.56 $kcal.mol^{-1}$), < idebenone (-292.08 $kcal.mol^{-1}$), < 5-O-Methylembelin (-258.03 $kcal.mol^{-1}$), < potassium embelate (-246.62 $kcal.mol^{-1}$), < amitraz (-236.96 $kcal.mol^{-1}$), < embelin (-210.73 $kcal.mol^{-1}$), < vilangin (-155.16 $kcal.mol^{-1}$).



Note: ↓ Down arrow mark represents the Histidine (His) amino acid at position 142 of the *Spodoptera frugiperda* beta glycosidase enzyme interacting with the ligand (potassium embelate). The dotted lines represent the hydrogen bonds.

Fig. 1: Interaction of potassium embelate with the target enzyme *Spodoptera frugiperda* beta glycosidase.



Note: ↓ Down arrow mark represents the Glycine (Gly) amino acid at position 137 of the *Spodoptera frugiperda* caspase-1 enzyme interacting with the ligand (vilangin). The dotted lines represent the hydrogen bonds.

Fig. 2: An interaction of potassium embelate with the target enzyme *Spodoptera frugiperda* caspase-1.

Table 3: The interaction energy analysis of nine selected ligands with the *Spodoptera frugiperda* caspase-1 using the PatchDock method.

S.No.	Ligand names	-ACE* (-kcal.mol^{-1})	Interaction of amino acid residue	Bond distance (Å)
1.	Coenzyme Q 10	434.56	No interactions	-
2.	Dopaquinone	187.98	Arg40 Tyr202 Ile204	3.5 1.6 and 3.3 2.5
3.	Embelin	210.73	Arg203	2.3. 2.4 and 3.2
4.	Idebenone	292.08	Ile204	3.1 and 3.4
5.	5-O-Methylembelin	258.03	No interactions	-
6.	Potassium embelate	246.62	Arg203 Ile204	3.2 3.1, 3.1 and 3.4
7.	Vilangin	155.16	Gly137	2.6 and 3.3
8.	Azadirachtin	504.85	Arg203	2.5
9.	Amitraz	236.96	No interactions	-

Note; -ACE*- Atomic contact energy

In the present study, three ligands such as embelin, potassium embelate, and azadirachtin were shown to interact with the Arg 203 amino acid residue of the *Spodoptera frugiperda* caspase-1 enzyme. Interestingly one ligand (vilangin) was shown to interact with the Gly 137 amino acid residue (Fig. 2) of the *Spodoptera frugiperda* caspase-1 enzyme. This result is on par with the previous report (Forsyth et al. 2004 and Wang et al. 2021).

Secondly, metabolites from *Azadirachta indica* have been known to possess antifeedant and repellent activity against *Spodoptera frugiperda* (Paredes-Sanchez et al. 2021). *Embelia ribes* has been reported as one of the plants used in crop disease management practices in ancient medieval and premodern India (Nene 2003). Similarly, *E. ribes* leaf extracts have shown to possess growth inhibition and feeding deterrence on *Spodoptera litura* (Noosidum & Chandrapatya 2015). To the best of our knowledge, no docking reports are available for the *S. frugiperda* enzymes (beta glycosidase and caspase-1) till date.

CONCLUSION

In the present study, all the nine selected ligands have been shown to dock with both the target enzymes. To our understanding, we are the first to describe the docking behavior of *S. frugiperda* enzymes (beta glycosidase and caspase-1) with that of nine selected ligands even though *E. ribes* crude extract has been known to possess antifeedant activity. These results provide new insight into understanding their inhibitory activities and pave way for further *in vitro* investigation of these 9 selected ligands as possible inhibitors against both the *Spodoptera frugiperda* enzymes (beta glycosidase and caspase-1).

REFERENCES

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R., Godwin, J. and Gomez, J. 2017. Fall armyworm: impacts and implications for Africa. Outlooks Pest Manag., 28(5): 196-201.

Deshmukh, S.S., Prasanna, B.M., Kalleshwaraswamy, C.M., Jaba, J. and Choudhary, B. 2021. Fall armyworm (*Spodoptera frugiperda)*. In: Polyphagous Pests of Crops, Springer, Singapore., pp. 349-372.

Dhar, A. 2020. Plant protection practices by using botanicals for sustainable agriculture. Plant Arch., 20(2): 3741-3746.

Forsyth, C.M., Lemongello, D., LaCount, D.J., Friesen, P.D. and Fisher, A.J. 2004. Crystal structure of an invertebrate caspase. J. Biol. Chem., 279(8): 7001-7008.

Kabaru, J.M. and Gichia, L. 2001. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. African Journal of Science and Technology, 2(2).

Leatemia, J.A. and Isman, M.B. 2004. Insecticidal activity of crude seed extracts of *Annona* spp., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae. Phytoparasitica, 32(1): 30-37.

Marana, S.R., Terra, W.R. and Ferreira, C. 2000. Purification and properties of a β-glycosidase purified from midgut cells of Spodoptera frugiperda (Lepidoptera) larvae. Insect Biochemistry and Molecular Biology, 30(12): 1139-1146.

Mhaskar, M., Joshi, S., Chavan, B., Joglekar, A., Barve, N. and Patwardhan, A. 2011. Status of *Embelia ribes* Burm f.(Vidanga), an important medicinal species of commerce from northern Western Ghats of India. Curr. Sci., 100(4): 547-552.

Mohan, S., Prabhakaran, V.S. and Narayanaswamy, R. 2022. *In silico* analysis of *Cissus rotundifolia* constituents as human neutrophil elastase (HNE), matrix metalloproteinases (MMP 2 and MMP 9), and tyrosinase inhibitors. Appl. Biochem. Biotechnol., 194(1): 232-245.

Nas, M.N. 2004. *In vitro* studies on some natural beverages as botanical pesticides against *Erwinia amylovora* and *Curtobacterium flaccumfaciensis* subsp. *poinsettiae*. Turk. J. Agric. For., 28(1): 57-61.

Nene, Y.L. 2003. Crop disease management practices in ancient medieval and pre-modern India. Asian Agri-Hist., 7(3): 185-201.

Noosidum, A. and Chandrapatya, A. 2015. Growth inhibition and feeding deterrence from leaf extracts of *Embelia ribes* Burm. f. on *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Khon Kaen Agr. J., 43(4): 773-780.

Otsuka, F.A., Chagas, R.S., Almeida, V.M. and Marana, S.R. 2020. Homodimerization of a glycoside hydrolase family GH1 β-γlucosidase suggests distinct activity of enzyme different states. Protein Sci., 29(9): 1879-1889.

Paredes-Sánchez, F.A., Rivera, G., Bocanegra-García, V., Martínez-Padrón, H.Y., Berrones-Morales, M., Niño-García, N. and Herrera-Mayorga, V. 2021. Advances in control strategies against *Spodoptera frugiperda*. A review. Molecules, 26(18): 5587.

Patel, M.B. and Gajjar, S.N. 2001. Bioefficacy of various plant leaf extracts against *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera). Indian J. Appl. Entomol., 15(2): 32-34.

Pavunraj, M., Muthu, C., Ignacimuthu, S., Janarthanan, S., Duraipandiyan, V., Raja, N. and Vimalraj, S. 2011. Antifeedant activity of a novel 6-(4, 7-hydroxy-heptyl) quinone® from the leaves of the milkweed *Pergularia daemia* on the cotton bollworm *Helicoverpa armigera* (Hub.) and the tobacco armyworm *Spodoptera litura* (Fab.). Phytoparasitica, 39(2): 145-150.

Pavunraj, M., Subramanian, K., Muthu, C., Seenivasan, S.P., Duraipandiyan, V., Packiam, S.M. and Ignacimuthu, S. 2006. Bioefficacy of *Excoecaria agallocha* (L.) leaf extract against the armyworm *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). Entomon., 31(1): 37.

Raja, N., Jeyasankar, A., Venkatesan, S.J. and Ignacimuthu, S. 2005. Efficacy of *Hyptis suaveolens* against lepidopteran pests. Curr. Sci., 88(2): 220-222.

Roy, B., Amin, R., Uddin, M.N., Islam, A.T.M.S., Islam, M.J. and Halder, B.C. 2005. Leaf extracts of Shiyalmutra (*Blumea lacera* Dc.) as botanical pesticides against lesser grain borer and rice weevil. J. Biol. Sci., 5(2): 201-204.

Tamaki, F.K., Souza, D.P., Souza, V.P., Ikegami, C.M., Farah, C.S. and Marana, S.R. 2016. Using the amino acid network to modulate the hydrolytic activity of β-glycosidases. PLoS One, 11(12): e0167978.

Wang, Y., Goodman, C.L., Ringbauer Jr, J., Li, Y. and Stanley, D. 2021. Prostaglandin A2 induces apoptosis in three cell lines derived from the fall armyworm, *Spodoptera frugiperda*. Arch. Insect Biochem. Physiol., 108(3): e21844.