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Research Article

Acetylcholine esterase inhibition activity of *gloriosa superba* and molecular docking study of its constituents against bacterial proteins

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ABSTRACT

Plant materials are invaluable sources in treatment of various diseases and research on certain plants has opened the way to development of various therapeutic agents. In the present study, the chloroform extract from the flowers of *Gloriosa superba* which belongs to the Colchicaceae family was subjected to column chromatography and it led to the isolation of myristyl alcohol which was identified by spectral methods. Eventhough *G. superba* exhibited a large number of biological activities, the acetylcholinesterase inhibition activity was not yet explored. The chloroform extract was studied for the acetylcholine esterase inhibition activity which showed an IC₅₀ value of 14µg/ml. This indicates that the chloroform extract of *Gloriosa superba* exhibits a strong AChE inhibition activity. Two compounds very often isolated from this genus colchicine and glorisine were subjected to molecular docking studies against the bacterial proteins 1UAG, 2X5O, 3UDI and 3TYE. It exhibited very good scores involving conventional H-bonding, alkyl, pi-alkyl and various other interactions.

Keywords: *Gloriosa superba*; Colchicaceae; acetylcholine esterase; colchicine; glorisine; docking.

INTRODUCTION

Gloriosa superba belongs to the family Colchicaceae is a perennial tuberous climbing herb found in Southern Africa, India, Srilanka, Malayasia and Burma. It is also planted outdoors in the Southern United states. In India, it is found in Rajasthan, Maharashtra, Karnataka, Kerala, Tamilnadu, Goa and other few states. It is a branching climber that grows to about 5 m. It is the state flower of Tamil Nadu in India and is also the national flower of Zimbabwe. In Tamil Nadu, *Gloriosa* cultivation is promoted by government subsidy schemes and several hundred acres are grown as a cash crop. All parts of the plant contain high content of colchicine, which is a medicinal alkaloid, and the seeds are used to extract colchicines. (Angunawela et al., 1971, Gooneratne et al., 1966). *G. superba* is a good abortifacient and causing expulsion of fetus from the womb. Roots possess purgative, cholagogue, anthelmintic, bitter, acrid, astringent and germicidal properties. Paste is an antidote of snakebite and extract of plant also possess Central Nervous System (CNS) depressant properties. (John et al., 2009, Suryavanshi et al., 2012). The tuberous root of *G. superba* boiled with sesamum oil is applied twice a day

on the joints, affected with arthritis reduces pain. (Singh 1993). It is also used in wounds, skin related problems, fever, piles, inflammation, uterine contractions, blood disorders, general body toner and poisoning. (Haron et al., 2008). Based on the above mentioned comments, it is not surprising that the pharmacological benefits of *G. superba* have been attracting great interest.

Accidental poisoning and suicidal misuse of tubers are well known in areas where the plant grows. In addition to colchicine, the plant also contains other compounds such as 3-desmethyl colchicine, beta-lumicolchicine, *N*-formyl-desacetyl colchicine, 2-desmethyl colchicine, chelidonic acid, and salicylic acid. The flowers of *G. superba* have also been pharmacologically documented to possess anticancerous activity but so far the phytoconstituents present in the flowers was not explored. (Kaliyaperumal Ashokkumar 2015).

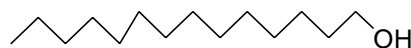


Figure 1: Structure of compound 1

Alzheimer's Disease (AD) is the main cause of dementia in our ageing society. Traditionally it was thought that it is an untreatable degenerative condition, but recent advances in drug therapy have challenged this view. Acetylcholinesterase inhibitors (AChEI) are used clinically to counteract Alzheimer's disease. Treatment is known to improve symptoms by enhancing cholinergic functions and increasing the amount of acetylcholine present in cholinergic synapses. Numerous plant ex-

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tracts have been investigated for their potential to treat cognitive disorders and neurodegenerative diseases. In the present work *G. superba* extract was tested for this activity for the first time.

The phytochemicals from tubers of *G. superba* have with antimicrobial activity of showed a higher activity against the gram positive and negative bacteria. (Haroon et al., 2011, Hemaiswarya et al., 2009, Kamna et al., 2012, Senthil kumar 2013, Suryavanshi et al., 2012). Colchicine is the major compound isolated from the seed and rhizome of this plant (Sarin et al., 1974) and other important compound is gloriosine. (Angunawela et al., 1971, Gooneratne et al., 1966). It was reported for its antibacterial activity also. The inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acids synthesis and antimetabolites (John et al., 2009) are the mechanisms followed by the antimicrobial agents.

MATERIAL AND METHODS

Plant material

Gloriosa superba belongs to Colchicaceae family was collected from the Dindigul district, Tamil Nadu, during the month of January 2017. It was identified by the Botanical Survey of India, Coimbatore. A voucher specimen was stored in the department.

Extraction procedure

Fresh flowers of *Gloriosa superba* (250 g) were collected, shade-dried and ground into coarse powder and extracted with 350 ml chloroform by cold percolation method (72 hrs) and repeated for three times to yield the extract. The solvent was distilled out and concentrated to yield the extract.

Isolation of compounds

Thin layer chromatography

The chloroform extract was examined by thin layer chromatography using petroleum ether: ethyl acetate (8:2) solvent system. It showed the presence of at least 5 compounds with R_f values 0.96, 0.85, 0.72, 0.56 and 0.40. The extract was subjected to column chromatography.

Column chromatography

A column was set up using TLC silica gel about 20 g and petroleum ether. Silica gel slurry was prepared using 100% petroleum ether and poured into the column and allowed to settle in the column slowly. Then the crude extract was introduced into the column. The column was eluted with petroleum ether, ethyl acetate in the order of increasing polarity. Fractions of 5 ml was collected and monitored by thin layer chromatography. Fraction number 3 (KS-1) and fraction number 6 (KS-2) were obtained as single spots in TLC.

Acetylcholine esterase inhibition activity

Acetylthiocholine iodide (ATCI), 5, 5"-thiobis-2-nitrobenzoic acid (DTNB), Acetylcholine esterase enzyme was purchased from sigma Aldrich. Acetylcholine esterase activity (Shahwar et al., 2012) was carried out for the chloroform extract of the flowers of *Gloriosa superba*. Spectrophotometric assay was used to determine the inhibitory potential of the compounds against acetylcholine esterase enzyme isolated from red blood cells. Acetyl thiocholine iodide was used as a substrate. 2.81ml of phosphate buffer of pH 8 was taken in each test tube. The test sample solutions of different concentrations of 2µg, 4µg, 6µg, 8µg, 10µg were added and 30µl of enzyme were added. The mixture was allowed standing for 10min. The coloring reagent DTNB (dithiobisnitro benzoic acid) was added which produces the yellow anion of 5-thio-2-nitro benzoic acid and then substrate 30µl followed by incubation for 20 min. The absorbance was measured at 412nm. The percentage inhibition in enzyme activity can be calculated as follows:

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

Molecular Docking

Molecular docking studies have been being carried out for colchicine and gloriosine reported from *G. superba*. Four different bacterial proteins are used for docking studies, namely 1UAG, 2X5O, 3UDI involved in cell wall synthesis and 3TYE which is involved in the synthesis of dihydrofolic acid. The softwares used are Chemdraw, Pyrx, Chimera, and Discovery.

Preparation of the protein

The bacterial proteins were downloaded from Protein Data Bank with PDB id: 1UAG, 2X5O, 3UDI and 3TYE.

Structure of the ligands

The 2D structures of the ligands colchicine and gloriosine were drawn using Chemdraw 8.0

RESULT AND DISCUSSION

Compound 1

The ¹H-NMR spectra of the compound showed a triplet at δ 0.81 for three protons suggesting the presence of a methyl group adjacent to a methylene group. Further the strong singlet at δ 1.20 for sixteen protons indicates the presence of a long chain methylene protons in the compound. The spectra also exhibited a signal at δ 4.06 for two protons indicating the presence of a methylene group under an oxygen function. The signal at δ 1.40 and 1.92 indicates the presence of two other methylene groups which are α and β methylene groups to the -CH₂OH group. The absence of the signal in the unsaturated region of the spectrum indicates that the compound is a saturated compound myristyl alcohol (Figure 1). The above facts suggest that the compound is a long chain saturated alcohol.

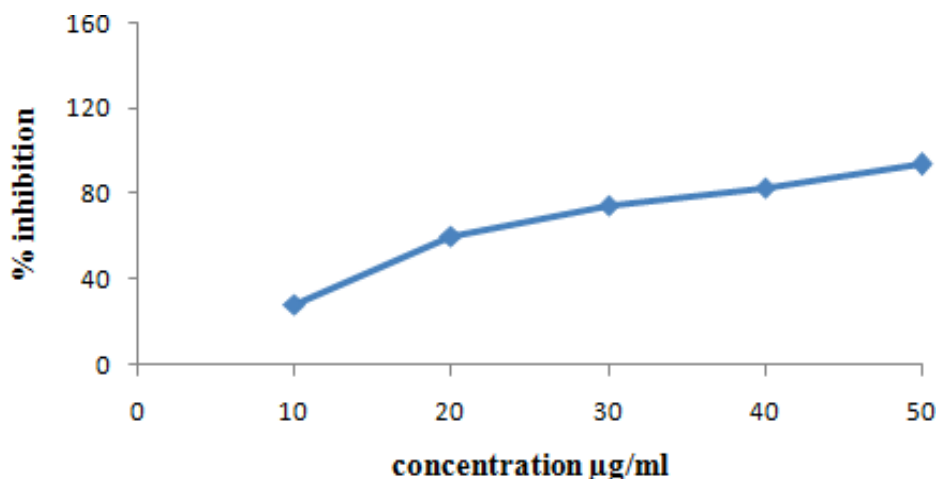


Figure 2: Acetylcholinesterase inhibition activity of the chloroform extract of flowers from *G. Superb*

Table 1: Molecular docking studies of colchicines and gloriosine against bacterial proteins 1UAG, 2X5O, 3UDI and 3TYE

Ligands	Docking details	1UAG	2X5O	3UDI	3TYE
Colchicine	Binding score	-7.8	-7.5	-6.2	-7.4
	Conventional H-bond	ASN:211 ASN:331	ASN:211	-	GLY:70 ALA:190
	Alkyl and pi-alkyl	-		ILE:148 PRO:184	PRO:69 PHE:71
	Others	THR:270 ASP:214 LEU:177	THR:270 LEU:177	-	PHE:71
Gloriosine	Binding score	-7.9	-7.5	-7.1	-7.6
	Conventional H-bond	ASN:268 LEU:299 PHE:303 VAL:305	ASN:138 LYS:319	GLU:301 VAL:391	ARG:234,68,254
	Alkyl and pi-alkyl	PHE:303	ALA:414 HIS:183	LYS:393	LYS:220 PRO:69 PHE:189
	Others	GLY:265 GLY:298	ASP:346 GLY:73	GLN:419	-

Complementing the above data the ^{13}C -NMR spectra exhibited signals at δ 14.19 for a methyl carbon, the signals at δ 21.03, 29.35, 29.69, 31.92 and 38.17 belongs to the long chain methylene carbon atoms. The signal at δ 60.36 suggests the presence of a $-\text{CH}_2\text{OH}$ carbon.

Acetylcholinesterase (AChE) Inhibition activity

Eventhough *G. superba* exhibited a large number of biological activities, the acetylcholinesterase (AChE) inhibition activity was not yet explored. In the present work we tested the acetylcholinesterase (AChE) inhibition activity of the chloroform extract of *G. superba*. The results are shown in the Figureure and the IC_{50} value for the acetylcholinesterase (AChE) inhibition activity is found to be 14 $\mu\text{g}/\text{ml}$ (Figure 2).

Molecular docking

Considering the current increase of antibiotic

resistance, the requirement of novel compounds to treatinfections with lower side effects becomes important. In this regard, the alkaloids from *Gloriosa superba* were tried as antimicrobial compounds although their mechanisms of action are not known. Herein, we intended to extend the knowledge on possible interactions between these compounds colchinine and gloriosine and target proteins that would allow understanding and describing the mechanism of action.

The compound colchinine showed docking scores of -7.8, -7.5, -6.2 and -7.4 K cal/mole with the 1UAG, 2X5O, 3UDI and 3TYE proteins respectively. It forms H-Bonds with the protein with ASN; 211, ASN;331 of 1UDI, ASN:211 of 2X5O and GLY:70, ALA:190 of 3TYE. Further it showed many alkyl and pi-alkyl interactions and pi-pi T stacked interactions with the bacterial proteins (Figure 3).

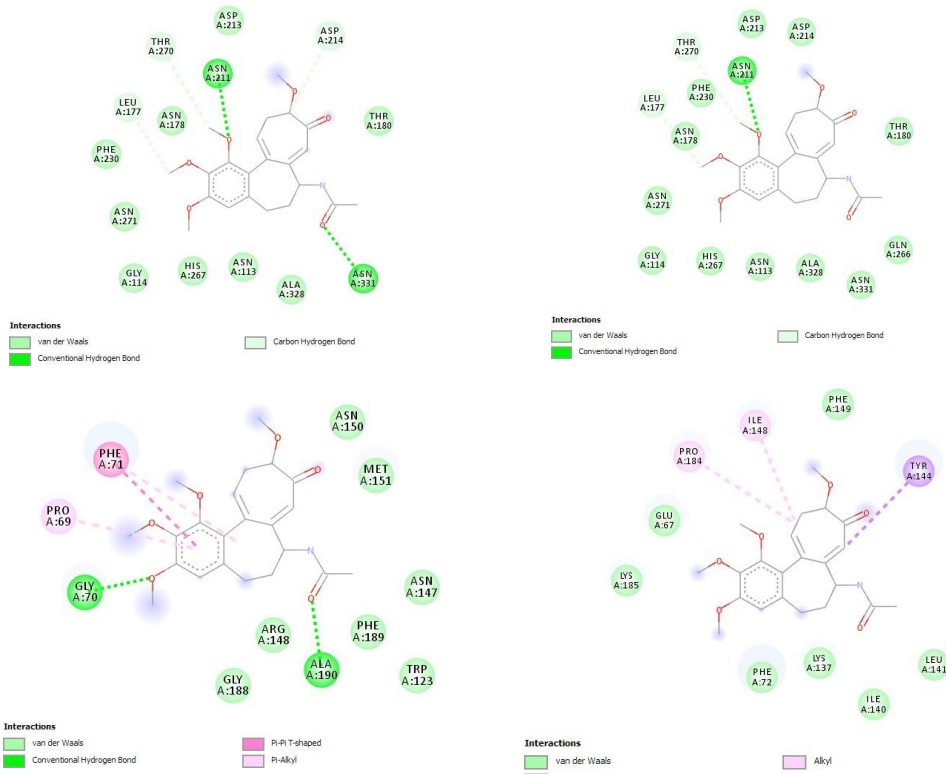


Figure 3: Docking images of the compound colchicine with the bacterial proteins a. 1UAG, b. 2X50, c. 3UDI and d. 3TYE

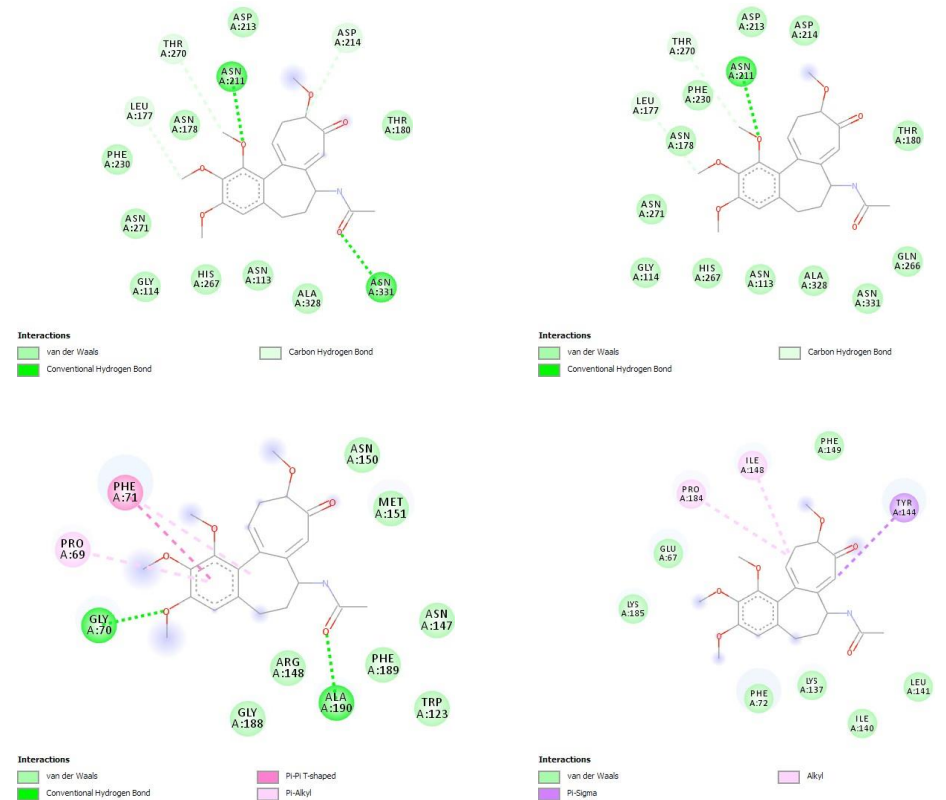


Figure 4: Docking images of the compound gloriosine with the bacterial proteins a. 1UAG, b. 2X50, c. 3UDI and d. 3TYE

The compound gloriosine showed docking scores of -7.9, -7.5, -7.1 and -7.6 with the proteins 1UAG, 2X5O, 3UDI and 3TYE. It forms hydrogen bonds with ASN:268, LEU:299 and PHE:303 of 1UAG, ASN:138 and LYS:319 of 2X5O, GLU:301, VAL:391 of 3UDI protein and with ARG:234, ARG:68 and ARG:254 of 3TYE (Figure 4).

CONCLUSION

The chloroform extract from the flowers of *G. superba* subjected to column chromatography which led to the isolation of compound **1** and was identified by spectral methods. The chloroform extract was studied for the acetylcholine esterase inhibition activity which showed an IC₅₀ value of 10µg/ml. Two compounds very often isolated from this genus colchicine and gloriosine were subjected to docking studies against the bacterial proteins 1UAG, 2X5O, 3UDI and 3TYE in order to study the mechanism of action. It exhibited very good scores involving conventional H-bonding and alkyl-pialkyl interactions.

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