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In vitro anthelmintic activity of *Macaranga denticulata* and *in silico* molecular docking analysis of its isolated compounds with tubulin

Mohammad Shah Hafez Kabir[1], Arkajyoti Paul[2], Mohuya Mojumdar[2], Abul Hasanat[1,3], Md. Mohaiminul Islam[3], Afia Nabila[4], Md. Jakiul Islam[3], Md. Rabiul Hossain[4], Nishan Chakrabarty[1], Mohammad Nazmul Islam[1*]

[1]Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh
[2]Department of Pharmacy, BGC Trust University Bangladesh, Chittagong-4000, Bangladesh
[3]Department of Pharmacy, State University of Bangladesh (SUB), Dhaka- 1205, Bangladesh
[4]Department of Pharmacy, University of Science & Technology Chittagong, Chittagong-4202, Bangladesh

***Corresponding author e-mail:** sayeadiiuc@gmail.com

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ABSTRACT

The aim of the present study was to evaluate anthelmintic activity on *Tubifex tubifex* worm of methanol extract of *Macaranga denticulata* (Muell. Arg.) leaves and *in silico* molecular docking used for six phytoconstituents namely 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol, stigmasterol isolated from *M. denticulata*, to identify whether these compounds interact with tubulin. *M. denticulata* leaves extract exhibited strong anthelmintic activity *in vitro*. Where it paralyzed (10.22±0.69min) and produced death (19.43±0.80 min) of the *Tubifex tubifex* at 10 mg/ml dose and the standard, Levamisole (3.3±0.38min and 6.5±0.76min) at 1 mg/ml. A wide range of docking score found during molecular docking by Schrodinger. 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol, stigmasterol showed the docking score -5.733, -3.951, -7.584, -6.115, -8.307-, and 8.021 respectively. Among all the compounds, β-sitosterol showed highest docking score. So, β-sitosterol is the best compounds as anthelmintic drug, as it possessed higher value in Molecular docking. Methanol extract of *M. Denticulata* leaves showed well anthelmintic activity. Further *in vivo* investigation need to identify that isolated compounds from *M. denticulata* have anthelmintic activity.

Key Words: *Macaranga denticulata*, anthelmintic activity, Levamisole

INTRODUCTION

Tubulin is a known anticancer and anthelmintic drug target. The investigation of tubulin inhibitors could lead to the development of new anthelminthic drugs. Inhibitors bind selectively to β-Tubulin of nematodes, cestodes and fluke, a protein subunit of microtubule and thereby disrupting microtubule structure and function [1-3]. Microtubules are highly dynamic, ubiquitous cellular organelles serving a variety of vital functions including mitosis, motility and transport, in all eukaryotes. Many of these structures exist in a dynamic equilibrium in which assembly and disassembly of the soluble subunits are balanced. In such systems, the drug–tubulin interaction results in a shift of this equilibrium with a net loss of microtubules and accumulation of free tubulin. In view of the crucial roles, that microtubules play in many cellular processes, their drug-induced destruction eventually

leads to the death of the organism [3]. Some anthelmintic drugs act rapidly and selectively on neuromuscular transmission of nematodes. Levamisole, pyrantel and morantel are agonists at nicotinic acetylcholine receptors of nematode muscle and cause spastic paralysis. Dichlorvos and haloxon are organophosphorus cholinesterase antagonists [4]. Diethylcarbamazine blocks host, and possibly parasite, enzymes involved in arachidonic acid metabolism, and enhances the innate, nonspecific immune system. Some drugs are known to affect the fatty acid oxidation pathway in mammals, caused a reduction in oxygen consumption rates in C. elegans and genome-wide gene expression profiles provided an additional confirmation of its mode of action [5], antiallergenic, antibacterial, antihistamic antiemetic and antimigraine agents. It is used as an anthelmintic for humans and farm animals against intestinal roundworms and pinworms infection; administered orally. Because of its broad spectrum usage it is used as a standard drug in our study and there are various research articles available which support our study.

In silico molecular docking technique play an important role in the drug design and discovery to predict the conformations of each ligand molecule at the active site, hence the molecular docking study was carried out to predict the β-Tubulin inhibitory activity and results are reported. Even though Benzyl derived compounds are known to have antiparasitic effect, it is now banned in many countries. Piperzine has broad spectrum activities like anthelmintic.

M. denticulata Muell. Arg. (Euphorbiaceae) is a small to medium-sized, evergreen tree and is a common pioneer species in moist open areas and secondary forests [6]. In the mountains of Northern Thailand, *M. denticulata* is used as a fallow enriching species by Karen hill tribe farmers [7]. In folk medicine, traditional healers use fresh or dried leaves of some Macaranga species to treat swellings, cuts, sores, boils and bruises [8]. A phytochemical review of literatures indicates the genus Macaranga to be a rich source of the Isoprenylated, geranylated and farnesylated flavonoids and stilbenes. Furthermore, more classes of secondary metabolites like terpenes, tannins, coumarins and other types of compounds are known to be isolated from different species of the genus Macaranga. Flavonoids and stilbenes are regarded as the major constituents and are most likely responsible for most of the activities found in the plants of this genus. It is experimentally validated that *M. denticulata* Possess thrombolytic and Cytotoxicity [9].

The aim of the present study to identify the anthelmintic activity of methanol extract of *Macaranga denticulata* and also we have described molecular docking analysis of 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol, stigmasterol, which were isolated from *M. denticulata* [10] with tubulin (PDB: 1SA0).

METHOD AND MATERIAL

Plant collection

The leaves of *M. denticulata* were collected from the Chittagong city area in front of Chittagong Medical college hostel gate of Bangladesh in October, 2014 then identified by Dr. Sheikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh. Voucher specimens, collection id: CTG 121, for *M. denticulata* kept in the Department of Pharmacy, International Islamic University Chittagong, Chawkbazar, Chittagong-4203, Bangladesh for further reference.

Extracts preparation

The collected plant was washed thoroughly with water and air dried for a week at 35 to 40 °C and pulverized in electric grinder. The obtained powder was successively added to methanol with vigorous shaking at 55 to 60 °C temperature. The extracts were made to dry by using rotary evaporator under reduced pressure. The extract was preserved at 4^0 C for further use.

Experimental Worms

Experimental worms collected from local aquarium shop. Then these are authenticated by local zoologist.

Chemicals and Reagents

Loperamide (Square Pharmaceuticals Ltd., Bangladesh), castor oil (WELL's Heath Care, Spain), normal saline solution (0.9% NaCl) and charcoal meal (10% activated charcoal in 5% gum acacia), Albendazole were used for antihelmintic activity tests. All other reagents were of analytical grade.

Preparation of test doses

The extracts were suspended in the vehicle. Various strengths were prepared from a stock solution 40 mg/ml. the solutions were prepared freshly solutions were administered orally.

In-vitro Anthelmintic Assay

The anthelmintic activity of methanol extract of *M. denticulata* was carried out as per the procedure of Ajaiyeoba et al. 2001 [11, 12] with some minor modifications. The aquarium worm *T. tubifex* were used in the present study because it has anatomical similarity and belongs to the same group of intestinal

worm i.e. annelid [13, 14]. The worms were collected from the local market of Chittagong, average size of worms 2-2.5 cm. in length were used for the study. The standard drug Levamisole (1 mg/ml) and three different concentrations of methanol extract of *M. denticulata* (2.5, 5 and 10 mg/ml) in double distilled water [15, 16] were prepared freshly and used for the study of anthelmintic activity. One group was composed of water and it was considered as controlled group. The anthelmintic activity was determine at two different stage 'time of paralysis' and 'time of death' of the worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors [17]. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased [18].

Statistical Analysis

The results are expressed as mean ± standard error of the mean (SEM). Data were analyzed using one way factorial ANOVA tests using SPSS Data Editor for Windows, Version 16.0 (SPSS Inc., USA) followed by Dennett's tests on each group except control for anthelmintic. The results obtained were compared with the negative control group for antidiarrheal activity and $P < 0.05$, $P < 0.01$ and $P < 0.001$ was considered to be statistically significant in Dennett's tests. Statistical program GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) was used for graphical presentation.

***In silico* analysis**

Molecular docking analysis of isolated compounds from *M. denticulata*

Preparation of protein structure

The 3D coordinates of crystal structure of tubulin (PDB: 1SA0) was downloaded from the RCSB protein data bank (http://www.rcsb.org/pdb) set up at Brookhaven National Laboratory in 1971. It is a worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids. Water molecules were removed from the protein 1SA0 before the instigation of molecular docking. The protein structure was corrected by the utilization of alternate conformations and valence monitor options as some crystallographic disorders as well as some unfilled valance atoms were present in the protein file. The resultant protein file was subjected to energy minimization by applying Chemistry at HARvard Macromolecular Mechanics (CHARMm) force fields. CHARm is a program which provides a large suite of computational tools that encompass numerous conformational and path sampling methods, free energy estimates, molecular minimization, dynamics, and analysis techniques, and mode l- building capabilities (http://www.charmm.org/). After the energy minimization the protein fie was subjected to define and edit binding site option available on tools panel to explore the plausible binding site within the protein (1SA0).

Preparation of ligand

The structures of compounds 3-acetylaleuritolic acid, oleanolic acid, macarangin, scopoletin, β-sitosterol, stigmasterol were drawn using ChemBioDraw software. ChemBioDraw™ is software from PerkinElmer for development of chemical structures of bioactive compounds. The prepared ligand was then subjected to add the hydrogen bonds and the energy has been minimized using CHARm force field.

Docking analysis

To find out the accurate binding model for the active site of tubulin, molecular docking analysis was performed using ligand fit of GLIDE software from Schrodinger (http:/ /www.schrodinger.com /) . Molecular docking analysis was performed using crystal structure of tubulin (PDB: 1SA0). The structure of crystal structure of tubulin (PDB: 1SA0) were obtained from Protein Data Bank (http://www.rcsb.org). The mechanism of ligand position is based on the fitting points. Fitting points are incorporated into the hydrogen bonding groups on the ligand and the proteins. The ligand fit module [19] from GLIDE software was utilized to execute the molecular docking analysis, based on shape-based searching and Monte Carlo methods. At the time of docking, variable trials Monte Carlo conformation was applied where the number of steps depends on the n umber of rotatable bonds present in the compounds/ligands. By default the torsion number is 2, the maximum minimizations steps is 300 and maximum successive failure is 110. During the docking process the top ten conformations were engendered for each of the compound after the minimization of the energy [20].

RESULTS AND DISCUSSIONS

Anthelmintic activity

Consequences of study were recorded as shown in Table 1 as in the form of time required to get following attacks of paralysis and at the end time required for complete death of parasite. From the above study it was seen that the methanolic extract showed dose dependent anthelmintic activity as compared to a standard drug levamisole. Methanol

extract *M. denticulata* show Time taken for paralysis (10.22±0.69 min, 15.36±0.78 min, 20.17±1.26 min) and Time taken for Death (19.43±0.80 min, 31.73±1.29 min, 43.28±1.58 min) at dose 10, 5, and 2.5 respectively where compared to a standard drug Levamisole (1 mg/ml) showed Time taken for paralysis (3.3±0.38 min) Time taken for Death (6.5±0.76 min).

***In silico* analysis**

Molecular docking analysis

In this study, the binding mode of tubulin was investigated by doing computational analysis, glide docking. Both glide standard (SP) and extra precision (XP) mode had been introduced, where extra precision mode used for cross validation purpose. The results of docking analysis were described in Table 2 and the docking figure showed in Figure 1. Among all the compounds, β-Sitosterol showed well docking score, glide emodel and glide energy.

CONCLUSION

From the study it was found that, *M. denticulata* could be great source of new anthelmintic drug. Both *in vitro* and *in silico* models support that it has potential anthelmintic activity and all the isolated compounds from *M. denticulata* could be anthelmintic drug. Among all the compounds, β-Sitosterol showed highest docking score. So, β-Sitosterol is the best compounds for anthelmintic activity, as it possessed higher value in Molecular docking. Further *in vivo* investigation need to identify that isolated compounds from *M. denticulata* have anthelmintic activity.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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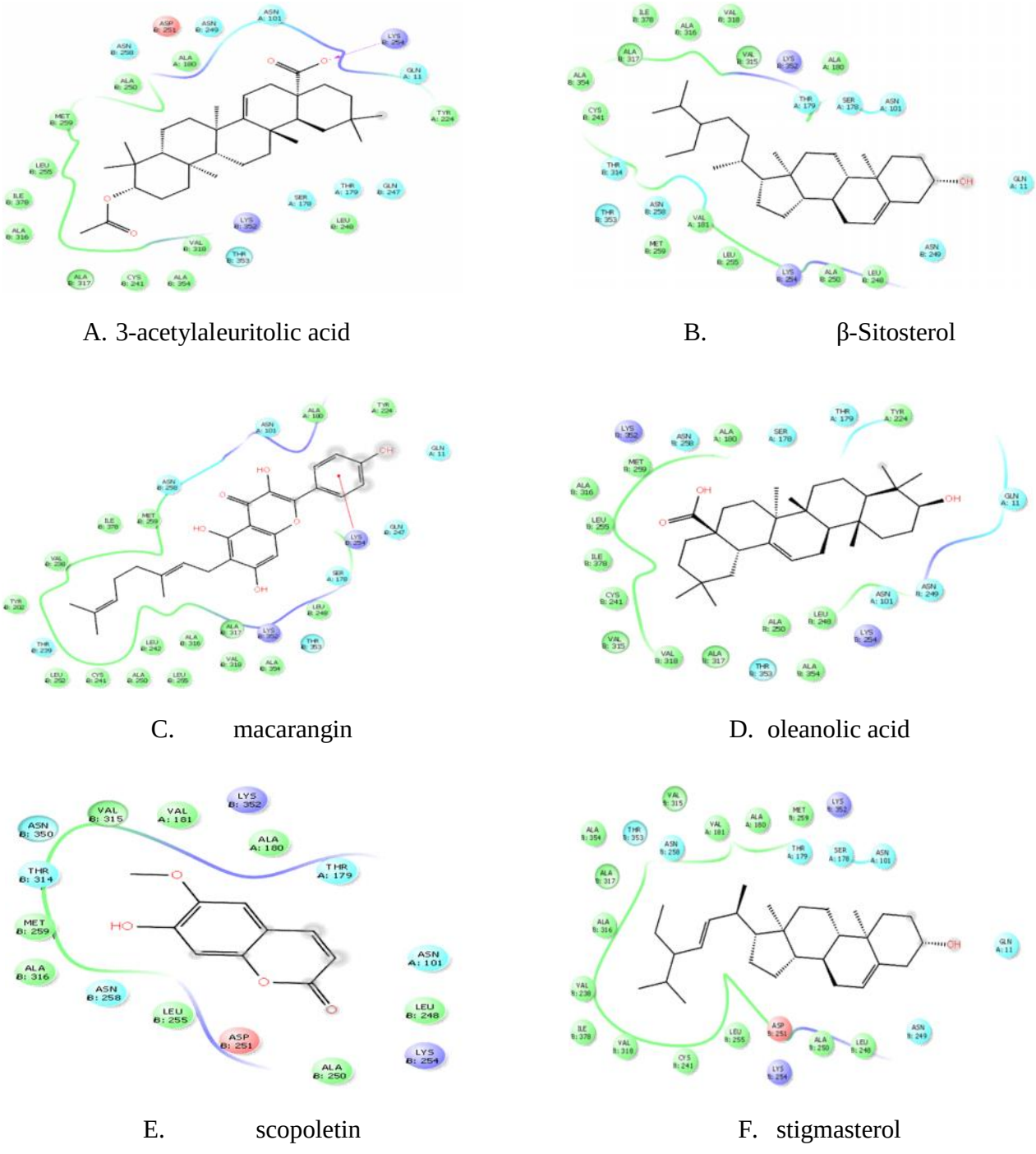
Table 1: Anthelmintic activity of methanol extract of *M. denticulata*.

Treatment	Time taken for paralysis (min)	Time taken for Death (min)
Control(Water)	0	0.00
Levamisole (1 mg/ml)	3.3±0.38	6.5±0.76
M. denticulata (10 mg/ml)	10.22±0.69[b]	19.43±0.80[b]
M. denticulata (5 mg/ml)	15.36±0.78[b]	31.73±1.29[b]
M. denticulata (2.5 mg/ml)	20.17±1.26[b]	43.28±1.58[b]

Values are mean ± SEM, (n = 3); [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$, Dennett's test as compared to positive control (Levamisole, 1 mg/ml). Statistical representation of the effective paralysis and dead time by *M. denticulata* methanol extract, positive anthelmintic control (Levamisole, 1 mg/ml) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis by using SPSS for windows, version 16.0.

Table 2: Docking results with 3-acetylaleuritolic acid, β-Sitosterol, macarangin, oleanolic acid, scopoletin and stigmasterol in the tubulin (PDB: 1SA0).

Compound name	Docking score	Glide emodel	Glide energy
3-acetylaleuritolic acid	-5.733	-54.699	-38.909
β-Sitosterol	-8.307	-58.732	-45.953
macarangin	-7.584	-75.188	-53.495
oleanolic acid	-3.951	-59.881	-45.178
scopoletin	-6.115	-40.903	-29.391
stigmasterol	-8.021	-58.652	-43.527



A. 3-acetylaleuritolic acid B. β-Sitosterol

C. macarangin D. oleanolic acid

E. scopoletin F. stigmasterol

Figure 1: Molecular docking analysis of A. 3-acetylaleuritolic acid, B. β-Sitosterol, C. macarangin, D. oleanolic acid, E. scopoletin and F. stigmasterol with tubulin (PDB: 1SA0) receptor complex obtained from Glide docking.

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