Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(1): 371–373

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

Isolation of Morin from *Turbinaria ornata* and *In vitro* Analysis of Monoamine oxidase B Inhibition of Morin

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Received: 30 Dec 2020 | Revised accepted: 12 Feb 2021 | Published online: 19 Feb 2021

ABSTRACT

Flavonols are the main dietary groups of flavonoids which are having various medicinal values. In the present study identification of the flavonol morin in the brown seaweed *Turbinaria ornata* was done. In-silico analysis of morin as drug and inhibitor of MAO-B inhibitor was done and inhibition efficiency was elucidated. Among the two flavonols we isolated morin as yellow amorphous powder and confirmed with ^{1}H NMR study. Purified morin was analyzed for its enzyme inhibition activities, which elucidates that at a concentration of 100 µM morin exhibited 80% inhibition and on dialysis that morin exhibited reversible inhibition due to the dissociable nature of enzyme-inhibitor complexes.

***Key words*:** Morin, Enzyme inhibition, Flavonols, *Turbinaria ornata*

Seaweeds are a rich source of bioactive phytochemicals like as phloroglucinol, terpenoids, phenolics, glycolipids, sterols and fucoidans. The extracts and isolated bioactive molecules of seaweeds are reported to have bioactivities like antibacterial, antioxidant, wound healing, anti-inflammatory, anti-diabetic, hypolipidemic and neuro-rescue activities [1]. Polyphenolic compounds including flavonoids are generally referred as safe and non-toxic with anti-oxidation property. Numerous investigations reported that dietary intake of plant phenolics renders longevity, decreases the onset of chronic metabolic diseases like neurodegenerative diseases, cancer, diabetes, obesity etc. Phenolic compounds are rich in seaweeds. ROS production in seaweeds is due to various forms of environmental stresses. Seaweeds generally have higher antioxidant activity due to a higher content of ascorbic acid, reduced glutathione, phenols and flavonoids [2]. *Turbinaria ornata* is a edible brown seaweed, which grows in tropical ocean waters mostly habitats include exposed rocky intertidal area, tide pools, intertidal beaches, reef flats and deeper water [3]. *Turbinaria ornata* is edible and having significant role in food industry, since alginate isolated from it have many important roles in food industry, and also it is used in making pickles, omelets, salads, dried and crisped powder is useful in cooking for seasoning in the pacific islands [4]. Flavonols such as kaempferol, morin, quercetin and myricetin are the main dietary groups of flavonoids which are having various medicinal values [5]. The present study dealt with the isolation and characterization of the morin, a flavonol from the brown seaweed *Turbinaria ornata* and the inhibitory effect morin on monoamine oxidase B.

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MATERIALS AND METHODS

Plant material

The marine brown alga *Turbinaria ornata* was collected from the Mandapam coast of Rameshwaram sea waters (Longitude 78º 8'E, Latitude 9º 17' N). The algal material was identified and authenticated by Dr. B. Andrews, Project Scientist, National Biodiversity of India, Chennai, Tamil Nadu and a voucher specimen was maintained for future reference. The dried seaweed (50 g) was grounded in electrical mixer and extracted using 250 ml of various solvents such as Acetone, ethanol and methanol for 24 hours in Soxhlet apparatus. Each filtrate was concentrated to dryness and was lyophilized into fine powder.

Thin layer chromatography

TLC study of methanolic extract was carried out in the light of previous study [6]. Methanolic extract was dissolved in minimum amount of methanol and spotted on the silica plates. The plates were developed using mobile phase, ethyl acetate: formic acid: acetic acid: water (10: 1.1:1.1:2.7, v/v/v/v). After development, the plates were observed.

Drug likeliness evaluation

As per the Lipinski rule of five drug likeliness of the isolated morin from *Turbinria ornata* was studied as 1. Molecular mass less than 500Da, 2. Less than 5 hydrogen bond donors, 3. Less than 10 hydrogen bond acceptors and 4. High lipophilicity (expressed as Log P less than 5) and also it was confirmed whether morin satisfies all the rules based on the 3D structure using the Lipinski drug filter [7].

Molecular docking study

A molecular docking study was carried out to elucidate insilico efficiency of morin in monoamine oxidase B. Human monoamine oxidase B was used as a drug target protein and the 3D structure of the target was downloaded from PDB (1GOS and Chain A). The ligand, morin was selected for docking against the target protein and the structure was retrieved from pubchem database (Table 1). The active site amino acid sequence of Ser15, Glu34, Arg42, Tyr60, Cys172, Val235, Tyr345, Tyr398, Thr426 and Gly434 in monoamine oxidase B was taken for docking study [8]. The software Auto Dock 4.2 was used for molecular docking of hit compounds to explore the binding conformation and hydrogen bond interactions. Auto Dock generated 10 possible binding conformations, i.e.,10 runs for each docking by using Genetic Algorithm (GALS) searches. A default protocol was applied, with population size of 150 randomly placed individuals, a maximum number of 2.5 × 105 energy evaluations, and a maximum number of 2.7 × 104 generations, gene mutation rate of 0.02 and cross over rate of 0.8 were used. The docking result of hit compounds was evaluated using binding energy, inhibition constant and hydrogen bond interactions. All visualization of docking results was analyzed using PyMOL [8].

Chromatographic extraction and isolation of Morin

The methanolic extract of *Turbinaria ornata* was extracted with petroleum at a temperature of 50°C and then extracted with benzene to remove non flavonoid materials. The flavonoid fraction was separated in a silica gel column (75 µm, 6.0cm × 40cm) and eluted with methanol of increasing polarities with water as 25 to 100% of methanol. The volume of 4 fractions (I, II, III, and IV) was 50 ml each. Appreciable antioxidant activity was found *in vitro* in fraction III and IV. The most active III fraction obtained (14.11mg) was again subjected to silica gel column chromatography (Silica gel 60, 230-400 mesh, 1.0 cm i.d. x 20 cm) and eluted with n-hexane:Ethy acetate, EtOAc, acetone and methanol [9-10].

In vitro Monoamine oxidase B inhibition of morin

The MAO-B enzyme (0.05 mg/mL protein) was incubated with the test compound morin 25.0 µM, 50.0 µM, 75.0 µM, 100.0 µM and standard inhibitor Deprenyl 50.0 µM in a total reaction mixture volume of 1 mL, containing 100 mM potassium phosphate buffer (pH 7.4). After 20 min incubation at 37°C, the reaction was stopped by chilling the tubes on the ice bath. All the samples were dialyzed against potassium phosphate buffer (25 mM; pH 7.4) at 4°C for 14 h (three buffer changes). Control enzyme (without inhibitor) was also run through the same procedure and activity of the enzyme was determined before and after the dialysis [11].

RESULTS AND DISCUSSION

TLC analysis

Based upon the preliminary phytochemical studies, Methanolic extract was chosen for TLC studies. The extracts showed distinct spots on the TLC plate spots were identified with the help of various visualizing agents and the results indicated that the leaves contained an appreciable amount of bioactive compounds. The spots of purple and yellow color elucidate the presence of phenols and flavonoids (Fig 1). Our results are very much similar to the results of previous study [11].

Fig 1 TLC analysis of *Turbinaria ornata*

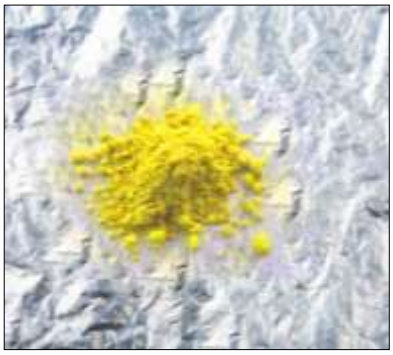

Fig 2 Yellow amorphous powder of morin

Extraction and characterization of morin

As per the procedures of [9-10] with slight modifications the isolation was done to get the desired bioactive compound. 5.7mg of compound 1 was yielded from n-hexane: EtOAc (3:7) fraction which is yellow crystals. 4.87mg of compound 2 was yielded from methanol: Chloroform (0.5:9.5) fraction as yellow amorphous powder (Fig 2). ^{1}H-NMR-400 MHz, analysis of two compounds elucidated that the compound 1 was myricetin and compound 2 chemical shifts recorded δ values confirmed the compound 2 as morin [9].

Drug likeliness evaluation

The Lipinski rule of five for morin isolated from *Turbinaria ornata* was analyzed using Lipinski drug filter (Table 1). These results showed that morin satisfied Lipinski rule of five and can be recommended to be used as a drug. Morin with molecular mass, 302 have 5 hydrogen bond donor and 7 hydrogen bond acceptor. The Log P value was 2.010900 and the molar refractivity was 74.050476 which showed the drug like property of morin. These results showed that morin fulfilled the lipinski rule of five for using them as possible drugs in treating diseases.

Table 1 Drug likeliness evaluation of morin

Compound	Mass	Hydrogen bond donor	Hydrogen bond acceptor	LOGP	Molar Refractivity
Morin	302	5	7	2.010900	74.050476

Molecular Docking study

The molecular docking results (Fig 3) clearly elucidates that morin have the appreciable properties of drug and have better binding interactions with Monoamine oxidase B. The binding energies of the protein-ligand interactions also confirm that the morin got fit into the active pockets of receptor tightly and can inhibit the binding of dopamine in the active site. Morin may be a potential drug candidate that inhibits the monoamine oxidase B with appreciable binding energy of -9.20987 kcal/mol. Our results are very much similar to the previous investigation [12].

Inhibition and binding properties of the inhibitor morin

The inhibition and binding properties of the inhibitor morin was investigated by the enzyme-inhibitor complex dissociation by dialysis. Different concentrations of the morin and standard inhibitor deprenyl (25.0 µM, 50.0 µM, 75.0 µM, 100.0 µM and standard inhibitor Deprenyl 50.0 µM) were

allowed to interact with the enzyme (MAO- B) for 20 min and the resulting enzyme-inhibitor complex mixtures were dialyzed overnight against buffer solutions. The enzyme activities were analyzed before and after the dialysis. The recombinant MAO-B lost about 20% of the enzyme activity during overnight dialysis. Incubation of morin (100 μM) with MAO-B produced almost 80 % inhibition of the enzyme activity respectively compared to control and drug standard, which was almost completely recovered after dialysis (Fig 4). These findings suggested that morin exhibited reversible inhibition due to the dissociable nature of enzyme-inhibitor complexes [13].

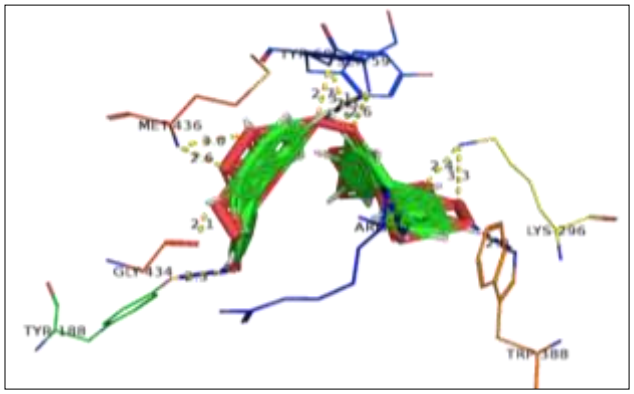

Fig 3 Molecular docking results of human monoamine oxidase B. with morin visualized in PyMOL

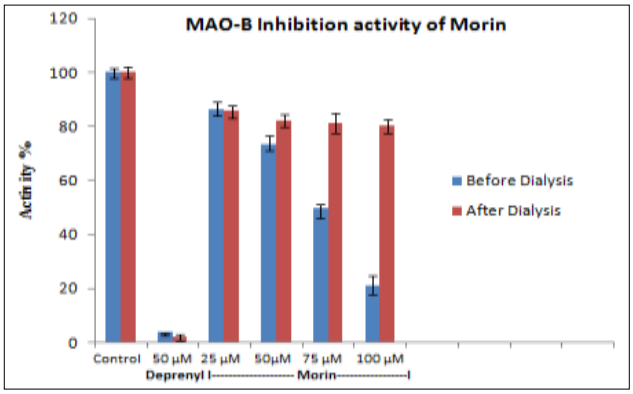

Fig 4 Morin enzyme inhibition and enzyme-inhibitor complex dissociation by dialysis

CONCLUSIONS

The inhibitory effect of the compound morin isolated from *Turbinaria ornata* on human monoamine oxidase was investigated. Morin satisfied the drug likeness evaluation, and morin was proved to be good inhibitor of MAO-B in in-silico molecular docking and also morin exhibited reversible inhibition on the enzyme monoamine oxidase B. The results of our study revealed that morin may be a drug lead as selective MAO- B inhibitor in treating various neurological disorders.

Acknowledgements

The authors acknowledging the support of Dr. D. Vijayraja, Head, Experimental Neuroscience Laboratory, Rev. Jacob Memorial Christian College, Ambilikkai, Tamilnadu, India and Dr. V. Manikandan, Post-doctoral Research Fellow, University of Texas Health Science Centre, San Antonio.

LITERATURE CITED

1. Chakraborty K, Joseph D. 2016. Antioxidant potential and phenolic compounds of brown seaweeds *Turbinaria conoides* and *Turbinaria ornata* (class: Phaeophyceae). *Journal of Aquatic Food Product Technology* 25(8): 1249-1265.
2. Farasat M, Khavari-Nejad RA, Nabavi SM, Namjooyan F. 2014. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical Research* 13(1): 163.
3. Zubia M, Stiger-Pouvreau V, Mattio L, Payri CE, Stewart HL. 2020. A comprehensive review of the brown macroalgal genus Turbinaria JV Lamouroux (Fucales, Sargassaceae). *Journal of Applied Phycology* 32(5): 2743-2760.
4. Novaczek I. 2001. A guide to the common edible and medicinal sea plants of the Pacific Islands. *Secretariat of the Pacific Community, University of the South Pacific*. pp 33.
5. Karak P. 2019. Biological activities of flavonoids: An overview. *International Journal of Pharmaceutical Sciences and Research* 10(4): 1567-1574.
6. Muthukrishnan S, Sivakkumar T. 2018. Physicochemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of *Schleichera oleosa* (Lour.) Oken. *Indian Journal of Pharmaceutical Sciences* 80(3): 525-32.
7. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 23(1/3): 3-25.
8. Monteiro AF, Viana JD, Nayarisseri A, Zondegoumba EN, Mendonça Junior FJ, Scotti MT, Scotti L. 2018. Computational studies applied to flavonoids against alzheimer's and parkinson's diseases. *Oxidative Medicine and Cellular Longevity* 30: 2018.
9. Dhanraj V, Karuppaiah J, Balakrishnan R, Elangovan N. 2018. Myricetin attenuates neurodegeneration and cognitive impairment in Parkinsonism. *Frontiers in bioscience* (Elite edition). 1: 10: 481-494.
10. Suganya T, Fumio I, Siriporn O. 2007. Antioxidants active principles isolated from Psidium guajava grown in Thailand. *Sci. Pharm*. 75: 179-193. doi:10.3797/scipharm.2007
11. Engelbrecht I, Petzer JP, Petzer A. 2019. Evaluation of selected natural compounds as dual inhibitors of catechol-O-methyltransferase and monoamine oxidase. *Central Nervous System Agents in Medicinal Chemistry* (*Formerly Current Medicinal Chemistry-Central Nervous System Agents*) 19(2): 133-145.
12. Girija K, Hemalatha A, Saranya C, Parthiban C, Anantharaman P. 2013. Extraction and isolation of phlorotannins from brown seaweed *Turbinaria ornata* (Turner) J. Agardh and its antioxidant activity. *International Journal of Bioassays* 2: 1185-9.
13. Larit F, Elokely KM, Chaurasiya ND, Benyahia S, Nael MA, León F, Abu-Darwish MS, Efferth T, Wang YH, Belouahem-Abed D, Benayache S. 2018. Inhibition of human monoamine oxidase A and B by flavonoids isolated from two Algerian medicinal plants. *Phytomedicine* 40: 27-36.