Role of nitric oxide-scavenging activity of *Karanjin* and *Pongapin* in the treatment of Psoriasis

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Abstract

In the present study, *Karanjin* and *Pongapin*, two important furanoflavone, constituents of *Pongamia pinnata* were studied in the management of Psoriasis. Presently, we have experimentally studied the free radical quenching property of *Karanjin* and *Pongapin*. A modified method was used to estimate the scavenging effect of the *Karanjin* (the highest activity of 95.60%) and *Pongapin* (68.05%) compared to the ascorbic acid as standard (11.60%) against nitric oxide. Furthermore, Molecular docking studies were performed using CLC drug discovery workbench software version 3.0 of the studied flavones (*Karanjin* and *Pongapin*) with the receptors responsible for psoriasis (*viz.* IL-17A, IL-17F, IL-23, ROR γ t, and TLR-7). Docking scores of *Karanjin* and *Pongapin* with different studied receptors were found to be comparable to that of Methotrexate, a known drug for treating Psoriasis. Docking results suggest that *Karanjin* and *Pongapin* might also help in controlling the disease. Overall, our results indicate that flavones (*Karanjin* and *Pongapin*) could be a natural and better alternative in curing psoriasis without any side effects.

Keywords Psoriasis · Nitric oxide · Methotrexate · Flavones

Introduction

Psoriasis is an autoimmune inflammatory disease characterized by abnormal growth of an epidermal layer of the skin. This is related to increased production of epidermal keratinocytes and incomplete epidermal differentiation. Current research on psoriasis demonstrates that nitric oxide is the signaling molecule for keratinocyte growth and plays a pivotal role in this disease (Daniela et al. 1998). Recent studies have shown that, in psoriatic plaques, there is an

increased concentration of nitric oxide (NO). Scavengers of nitric oxide compete with oxygen leading to a reduced production of nitrite. Serum level of nitrite–nitrate is also seen to increase in psoriasis patients (Tekin et al. 2006). The concentration of nitrite in aqueous solution has been assayed spectrophotometrically using the Griess reagent with which nitrite reacts to give a stable product. The compounds which can scavenge or trap nitric oxide radical can be helpful in the treatment of *Psoriasis vulgaris*. Hence, the study of flavones (Online resource 1) which could act as a potential NO inhibitor has been presented in this paper. The furanoflavone (*Karanjin* and *Pongapin*) isolated from *Pongamia pinnata*, could be a breakthrough in the management of Psoriasis. In contrast, methotrexate, a well-known drug used in the treatment of Psoriasis has exhibited NO inhibiting activity (Murrell et al. 1996). Presently, we have studied the nitric oxide quenching property of the furanoflavones—*Karanjin* and *Pongapin*—using biochemical and bioinformatics approach. Docking study of these two compounds with receptors responsible for psoriasis—ROR γ t (Retinoid-related orphan receptor) (Huh and Littman 2012), IL (Interleukin)-23, 17A, 17F (Wasilewska et al. 2016), and TLR (Toll-like receptor)-7 (Gilliet et al. 2004) have also shown encouraging results determined by interaction (docking)

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scores. Recent research has shown the pivotal role of IL-23 in psoriasis which was previously only considered to be a pro-inflammatory cytokine. Not only this, IL-23 is solely responsible for the production of IL-17A, which had been identified to play the key role in autoimmunity (Langrish et al. 2005). TLR has shown to play a pivotal role in autoimmune disorders including Psoriasis (Di Cesare et al. 2009; Mahgoub et al. 2014). It has also been noted that long-term use of Methotrexate can be carcinogenic, whereas the use of flavones has no side effects. Though Methotrexate decreases the NO levels in psoriasis and it is used quite effectively in the treatment of psoriasis, but the exact mechanism of the action is still unknown (Tekin et al. 2006). Thus, flavones can cure psoriasis by the simple pathway of scavenging radicals with safer and effective mechanism of action.

Methods

Receptors and ligands

Protein 3D structures (PDB format) of studied receptors IL-17F (PDB, 1JPY), IL-17A (PDB, 5HI3), IL23 (PDB, 3QWR), ROR- γ t (PDB, 4NIE), and TLR7 (5GMF) were downloaded from Protein Databank (PDB) (<http://www.rcsb.org>). Chemical three-dimensional (3D) conformer of the ligands in SDF format was downloaded from PubChem database (Bolton et al. 2008). Flavones: Karanjin (PubChem CID, 100633) Pongapin (PubChem CID, 3083586) and drug: Methotrexate (PubChem CID, 126941).

Receptor-ligand docking

CLC drug discovery workbench software version 3.0 was used to perform the receptor-ligand docking studies. Downloaded 3D structure of receptors (protein) and ligands (flavones and antitumor drug) were imported into CLC drug discovery workbench software version 3.0. As per the user manual, protein and ligand optimization was performed, then potential-binding pockets within the protein structure were searched and targeted molecular docking between receptor and ligand was conducted. CLC drug discovery visualization tool was used to study the details of the interaction between protein (receptor) and ligand.

Isolation of Karanjin and Pongapin from *P. pinnata*

Air-dried crushed root bark of *P. pinnata* was extracted with methanol, concentrated, and then fractionated using *n*-hexane and ethyl acetate. The *n*-hexane concentrate was subjected to column chromatography using a silica gel column (60–120) mesh. The column was eluted with *n*-hexane and subsequently with *n*-hexane-ethylacetate mixtures as

solvents with increasing polarity. The fraction obtained from *n*-hexane-ethylacetate (9:1) afforded Karanjin and Pongapin (98.5% purity). All these compounds were characterized from spectral analysis and compared with the literature data. The NMR spectral analysis (300 MHz ^1H NMR, ^{13}C NMR, and ^{135}C DEPT) of Karanjin and Pongapin was carried out to identify the correct compounds and also to check the purity of the two compounds (Online resource 3).

Determination of the nitric oxide quenching activity

Nitric oxide was estimated according to the Green et al., (1982) method. The nitric oxide-scavenging activity was determined according to the modified method of Marcocci et al. (1994). High concentration of nitrite has to accumulate for being detected with Greiss reagent. To produce a constant flow of nitric oxide over a period of hours, a chemical source of nitric oxide was used, viz., sodium nitroprusside, which spontaneously produces nitric oxide when dissolved in aqueous solution at physiological pH (7.37). Sodium nitroprusside solution was freshly prepared by taking 10 mM sodium nitroprusside and 20 mM phosphate buffer (pH 7.4) previously bubbled with argon. Greiss reagent was prepared by adding solution A to solution B. Solution A was prepared by taking 2% w/v sulfanilamide in distilled water and then added to 4% w/v of phosphoric acid. Solution B contained 0.2% w/v naphthyl ethylene diamide dihydrochloride in distilled water. 0.5 mL (1 mg/mL) ethanolic extract of the compounds was taken and treated with 0.5 mL of sodium nitroprusside solution and incubated at 25 °C for 150 min. To prepare the control solution, 0.5 mL of the sample solutions were added to 20 mM phosphate buffer (pH 7.4) and incubated at 25 °C for 150 min. No sodium nitroprusside was added. On completion of the incubation, 1 mL of Greiss reagent was added to each sample solution and nitric oxide level was estimated spectrophotometrically at 542 nm. The absorbance of the sample solutions was recorded against the absorbance of the control samples. Percentage of inhibition of nitric oxide of the compounds was estimated from the difference of absorbance of control and test samples:

$$\frac{\text{control absorbance} - \text{test sample absorbance}}{\text{control absorbance}} \times 100$$

= % of nitric oxide inhibition.

Results and discussion

Natural flavones are safer and better alternative than synthetic drugs in the treatment or prevention of Psoriasis. Two furano-flavones (Karanjin and Pongapin) isolated in abundance from *P. pinnata* were selected for the present study, keeping it furanoflavonoid specific study. In addition, the antipsoriatic activity of Karanjin has been reported (Divakara et al. 2013;

Alex et al. 2017). This study will help to focus on the mechanism of its antipsoriatic activity. Hence, present work deals with the experimental study of NO-scavenging activities of two furanoflavones (Karanjin and Pongapin). Molecular docking was performed to study the interaction between the selected ligands (Karanjin, Pongapin, and Methotrexate) and receptors related to psoriasis (IL-17F, IL-17A, IL23, ROR- γ t, and TLR7). CLC Drug Discovery Workbench Software was used to achieve the accurate predictions. PLANTSPLP score (Korb et al. 2009) is used to predict the docking score in the Drug Discovery Workbench. PLANTSPLP score keeps an excellent balance between evaluation time and score accuracy, during protein and ligand interaction. Best interaction between protein and ligand with least binding energy is given as result. Ligands with low-binding score (higher binding efficiency) with interacting receptor could be more effective to act as a novel drug for treating psoriasis. Table 1 suggests that the binding energies of Karanjn and Pongapin are comparable within and to the known psoriasis drug Methotrexate, with all the studied receptors (Online resource 2). Thus, this study suggests that Karanjn and Pongapin could play a vital role in preventing psoriasis.

The NMR spectral analysis (300 MHz ^1H NMR, ^{13}C NMR, and $^{135}^\circ$ DEPT) of Karanjn and Pongapin was done to identify the correct compounds and to check the purity of two compounds which is 98.5% (Katekhaye et al. 2012; Pavanaram and Ramachandra 1956) (Online resource 3). The concentration of nitrites and nitrates are parallel to NO levels, and thus, measuring the concentration of nitrites can give an idea about the amount of NO present. The concentration of nitrite in aqueous solution has been assayed spectrophotometrically using the Griess reagent with which nitrite reacts to give a stable product at 542 nm. The absorbance of the sample solutions was recorded against the absorbance of the control samples. Percentage of inhibition of nitric oxide of the compounds was estimated from the difference of absorbance of control and test samples. Ascorbic acid was used as a standard. Karanjn showed the highest activity of 95.60% and Pongapin 68.05% compared to the standard; ascorbic acid of 11.60% percentage inhibition of NO (Fig. 1).

Fig. 1 Nitric oxide-scavenging activity of flavones (Karanjin and Pongapin) from *Pongamia pinnata* with ascorbic acid as a control

Overall, results suggest that the furanoflavones (Karanjin and Pongapin) could emerge as a natural, safe alternative in preventing the progression of psoriasis by controlling the NO level. We have experimentally demonstrated that the NO-scavenging activity of Karanjn (95.60%) and Pongapin (68.05%) is higher than control. Thus, Karanjn and Pongapin should be studied in further detail as it could emerge as a promising natural drug for controlling autoimmune disease like psoriasis. For a drug to be effective in preventing the progression of psoriasis, it should be able to interact and have good binding efficiency with the psoriasis-related receptors. Molecular docking results further suggests that the binding efficiency of the studied flavones (Karanjin and Pongapin) with receptors responsible for psoriasis, viz., IL-17A, IL-17F, IL-23, ROR γ t, and TLR-7, is good and comparable to the binding scores of methotrexate, a drug for treating psoriasis. Binding scores of the flavones (Karanjin and Pongapin) with receptors and NO-scavenging property suggest that the studied furanoflavones could be promising plant-derived drugs in controlling psoriasis effectively.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Table 1 Molecular docking (binding) score of studied ligands with psoriasis-related receptors

Receptors	Karanjin	Pongapin	Methotrexate
IL17-A	− 49.15	− 53.30	− 59.76
IL17-F	− 26.93	− 28.15	− 40.89
IL23	− 40.05	− 42.42	− 49.75
ROF	− 52.95	− 51.62	− 59.33
TLR7	− 35.58	− 32.50	− 45.98

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