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RESEARCH ARTICLE

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Effect of topical marshmallow (*Althaea officinalis*) on atopic dermatitis in children: A pilot double-blind active-controlled clinical trial of an *in-silico*-analyzed phytomedicine

Vahedeh Naseri[1] | Zahra Chavoshzadeh[1,2] | Azadeh Mizani[3] | Babak Daneshfard[3] | Farzaneh Ghaffari[4] | Mahdi Abbas-Mohammadi[5] | Latif Gachkar[6,7] | Mohammad Kamalinejad[8] | Razieh Jafari Hajati[3] | Zahra Bahaeddin[3] | Soghrat Faghihzadeh[9] | Mohsen Naseri[3,10]

[1]Pediatrics Infectious Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2]Department of Allergy and Clinical Immunology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3]Traditional Medicine Clinical Trial Research Center, Shahed University, Tehran, Iran

[4]School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[5]Department of Organic Chemistry, Faculty of Chemistry, University of Kashan, Kashan, Iran

[6]Infectious Disease and Tropical Medicine Reseaerch Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[7]Infectious Diseases Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[8]School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[9]Deptartment of Biostatistics, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

[10]Hikmat, Islamic and Traditional Medicine Department, The Academy of Medical Sciences, Tehran, Iran

Correspondence
Mohsen Naseri, Traditional Medicine Clinical Trial Research Center, Shahed University, No. 1471, North Kargar Ave., Enghelab Sq., Tehran 1417953836, Iran.
Email: naseri@shahed.ac.ir

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Atopic dermatitis (AD) is a chronic relapsing eczematous skin disease, which primarily affects infants and young children. Due to the side effects of commonly used drugs for its treatment, the development of safer therapeutic strategies is needed. There are many reports on the topical use of marshmallow (*Althaea officinalis*) for a range of skin diseases in Persian medicine. The main aim of the present investigation was evaluating the efficacy of marshmallow in children with mild-to-moderate atopic dermatitis. Another aim of the study was screening the anti-allergic and anti-inflammatory potential of phytocomponents against target proteins, including TNF-alpha, IL6, and PDEs A, B, and D enzymes with PDB IDs: 2AZ5, 1P9M, 3I8V, 4KP6, and 1Y2K, respectively, along with their respective standard ligands using computational docking analysis. A pilot clinical trial was designed to investigate the safety and efficacy of *Althaea officinalis* in children with AD. The diagnosis of AD was made according to the criteria of Hanifin and Rajka. Children between 3 months and 12 years old were participated in this trial and randomly allocated into two parallel intervention and control groups. The intervention group used *Althaea officinalis* 1% ointment while the positive control group used Hydrocortisone 1% ointment twice a day for a week and after that, three times per week for a period of 3 weeks. The severity of AD was

Abbreviations: AD, atopic dermatitis; CAM, complementary and alternative medicine; IL, interleukin; IQR, interquartile range; OPLS, optimized potentials for the liquid simulations; PDB, Protein Data Bank; PDEs, phosphodiesterases; PM, Persian medicine; RMSD, root mean square deviation; ROS, reactive oxygen species; SCORAD, SCORing atopic dermatitis; SPSS, statistical package for social sciences; TNF-alpha, tumor necrosis factor-alpha; USP, United States Pharmacopeia.

measured using the SCORAD score at the end of each assessment visits. A total number of 22 patients completed the study. A significant decrease of the SCORAD score was observed in both groups. At the end of the study, this score change, which indicates the improvement of the patients was significantly higher in the intervention group in comparison to the baseline (p-value = .015) and week 1 (p-value = .018). In the docking analysis of the study, 33 phytochemical compounds were identified, which were docked into the active site of IL6, TNF-alpha, and human PDE4 isoenzymes. Affinity toward the selected enzymes was significantly higher in glycosylated compounds. The results of this pilot study showed that the efficacy of *Althaea officinalis* 1% ointment in a decrease of disease severity is more than Hydrocortisone 1% in children with AD. However, further studies are needed to confirm this finding. Moreover, the docking analysis revealed that the inhibitory activity of compounds with free hydroxyl groups such as glycosylated compounds was better than others, probably due to the hydrogen bond interaction of hydroxyl groups of the ligands with the enzymes.

KEYWORDS

Althaea officinalis, atopic dermatitis, marshmallow, molecular docking, Persian medicine, SCORAD index

1 | INTRODUCTION

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases with high morbidity. AD, also known as atopic eczema, is characterized by pruritic, chronic itching, dry skin, and eczematous lesions with a relapsing nature (Saini & Pansare, 2019). Clinical symptoms of AD are often associated with psychosocial complications, which have a negative impact on the quality of life of patients and their families (Simpson et al., 2018). It affects between 0.3 and 20% of children around the world (Charman, Chambers, & Williams, 2003). The prevalence of AD has risen in recent years together with an increased prevalence of asthma. It has also imposed a major burden on health care systems (Humbert et al., 2019).

The most common treatment of moderate to severe AD is topical corticosteroids, emollients, oral antihistamines, antibiotic agents, immunosuppressive agents, or non-pharmacologic therapies. Oral corticosteroids are well tolerated and improve the lesions. The most common side effects of topical steroids are hypo-pigmentation, skin atrophy, adrenal suppression, cataracts, glaucoma, growth retardation, secondary infections, and acne. Although antihistamines can treat pruritus, they can affect children's ability to learn. On the other hand, topical cyclosporine as an alternative treatment of AD has showed little success due to the insufficient penetration of the drug through the skin. Accordingly, these medications do not ensure successful treatment in all patient with AD (Kim et al., 2011; Ruzicka et al., 1997).

Today, complementary and alternative medicine (CAM) includes various medical systems with a significant increase of popularity among physicians and patients in the last decades (Daneshfard, Sanaye, & Nimrouzi, 2019). Available resource, integration with conventional medicine, lower costs, fewer side effects, cultural or autochthon accommodation, and recommendation of easy methods for patients have complied patient's tendency to be involved in these kind of treatments (Abolhassani, Naseri, & Mahmoudzadeh, 2012). Persian medicine (PM) is one of the CAM medical systems composed of all knowledge and practices used in diagnosis, prevention, and elimination of diseases in ancient Persia till now, which has passed down from generation to generation (Ghaffari, Naseri, Movahhed, & Zargaran, 2015; Goshtasebi, Mazari, Behboudi Gandevani, & Naseri, 2015; Naseri et al., 2016). As a holistic school of medicine, PM is based on the theory of humoral medicine and temperaments considering both physical and non-physical aspects of a human being (Atarzadeh, Daneshfard, Dastgheib, Jaladat, & Amin, 2016; Nimrouzi, Daneshfard, Tafazoli, & Akrami, 2019). It has a great potential for solving some of the medical problems, as demonstrated in some recent researches in these fields (Ahmadi et al., 2010; Khodaei et al., 2017; Nozad, Naseri, Safari, Al Ahadi, & Ghaffari, 2016; Qaraaty et al., 2014). According to the most famous PM textbooks, marshmallow (*Althaea officinalis* L.) is a good candidate for the treatment of AD (Avicenna, 2005; Shirazi, 2009).

Marshmallow (*Althaea officinalis*) is a medicinal plant native to Asia, Europe, and the United States of America. It is a rich source of bioactive constituents possessing different biological activities such as antimicrobial, anti-inflammatory, immune-modulatory, demulcent, soothing, and antitussive effects (Shah et al., 2011). In addition, marshmallow is a safe medicinal herb. There are several reports on its topical use for a variety of diseases (Shah et al., 2011). A large number of secondary metabolites such as pectin, starch, di-saccharide sucrose, mucilage, flavonoids (Hypolaetin-8-glucoside, isoquercitrin, kaempferol,

caffeic, pcoumaric acid), coumarins, scopoletin, phytosterols, tannins, asparagine, and many amino acids have been found in *Althaea officinalis* (Gudej & Bieganowska, 1990; Sendker et al., 2017).

In this pilot clinical trial study, we evaluated the effectiveness of topical *Althaea officinalis* in children with mild-to-moderate AD. In addition, its chemical constituents were screened against three isoforms of human PDE4, TNF-alpha, and interleukin 6 (IL6) receptors to evaluate their interaction with the standard ligands.

2 | MATERIALS AND METHODS

2.1 | Ethics and trial registration

The study protocol was in accordance with the Declaration of Helsinki (1989 revision). It was approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences with number: 91/132. The trial protocol was also registered in the Iranian Registry of Clinical Trials database (ID: IRCT2017020831205N2). Moreover, all of the parents of the enrolled children signed the written informed consent before participation in the trial.

2.2 | Study design and participants

This research was conducted at the Pediatrics infectious disease research center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, from 2012 to 2013. It was designed as a randomized, double-blind, active-controlled, and single-center trial following the Consolidated Standards of Reporting Trials (CONSORT) guideline to investigate the efficacy of *Althaea officinalis* in the treatment of children with AD. The parallel groups had a 1:1 allocation ratio. Study design and outcomes remained unchanged until the end of the study.

Volunteers referring to the Immunologic and Allergic clinic of Mofid children hospital and met Hanifin's and Rajka's criteria (Hanifin & Rajka, 1980) were enrolled and randomly allocated to two parallel groups: intervention and positive control groups. Participants were evaluated for AD before and after applying the ointment. In a standardized interview, parents were asked for AD symptoms, medication, and physicians' diagnosis. Furthermore, a detailed questionnaire was filled out by the participants asking about name, age, sex, address, phone number, duration of the disease, former medical history of diseases such as asthma, allergic rhinitis or AD, and history of atopy in the family.

2.3 | Inclusion and exclusion criteria

The inclusion criteria were children aged 3 months to 12 years old who have been recently diagnosed as AD with a SCORAD score (Kunz et al., 1997) of 25–50. Non-entrance criteria were: usage of topical drugs in the last 4 weeks prior to study entry, history of acute local infections, history of contact dermatitis to administrated drugs, and history of immunodeficiency diseases.

Exclusion criteria were considered: skin sensitivity during the treatment period, lack of cooperation, irregular drug consumption, and failure to follow the treatment protocol.

2.4 | Drug preparation

Marshmallow with the scientific name of *Althaea officinalis* (Malvaceae family) is a species in the genus Althaea (www.theplantlist.org). Its flowers were bought from Dineh Medicine Distribution Co, Tehran, Iran. The identification of the plant material was confirmed by plant taxonomy books. Voucher specimens of *Althaea officinalis* L. (No. SBMU-8064) were deposited in the Herbarium Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Quality control tests were done according to British Pharmacopeia (British Pharmacopoeia Commission, 2013).

To prepare the ethanolic extract, the maceration method was applied for the extraction of 100 g marshmallow dried flowers, using 1,000 ml of 96° ethanol as solvent. The yield obtained by maceration contained 15 g of dried marshmallow extract. The 1% ointment was manufactured according to Physicians' Desk Reference (Sanborn, 2007) using a petrolatum base in the School of Pharmacy, Shahid Beheshti University of Medical Sciences. The microbial tests were conducted with acceptable results based on the United States Pharmacopeia 34 (USP34) reference values.

2.5 | Intervention

During the study period, all of the children were asked to take a bath before ointment usage in addition to receiving common care and special food dietary prior to intervention. Children in each group applied either of *Althaea officinalis* 1% or Hydrocortisone 1% topical ointments in the involved areas as intervention and control groups, respectively. They were trained to use 1.5 cc of the ointments on their involved body parts.

The patients were instructed to use their prescribed medication for 7 days twice a day and after that, three times per week for a period of 3 weeks.

2.6 | Outcome measure

Children were visited three times for clinical examination by an allergy specialist physician prior to intervention, 1 week and 4 weeks after the beginning of the intervention.

The outcome measure of the study was the SCORAD score. This standardized valid scoring index has been developed by the European Task Force for AD (Kunz et al., 1997). Both objective signs (including intensity and extent) and subjective symptoms (including sleep loss and pruritus) are assessed by this questionnaire. Considering the

SCORAD index total score, AD is classified into mild (<25), moderate (25–50), and severe (≥50) categories (Pucci et al., 2005). The severity of AD was measured by the physician using the SCORAD index at the end of each assessment visit.

2.7 | Randomization and blinding

Children were assigned to the groups using a simple randomization method. Each participant was given a randomization number applying a single sequence of random assignments. Both *Althaea officinalis* 1% and Hydrocortisone 1% ointments were manufactured, labeled, and packed by Sina Daroo Corporation, Tehran, Iran. The packages of ointments were identical in shape, color, and fragrance. All of the physicians, researchers, and statisticians were kept blind to the allocation of the patients.

2.8 | Safety

All adverse events were evaluated at each visit asking the parents if their children experienced any complication. Over the treatment period, we evaluated safety outcomes, including any serious adverse events.

2.9 | Molecular docking analysis

The molecular docking study was carried out using the Glide application in the Schrodinger package (Schrodinger, LLC) (Singh & Muthusamy, 2013). The TNF-alpha, IL6, and PDEs A, B, and D enzymes structure file (PDB IDs: 2AZ5, 1P9M, 3I8V, 4KP6, and 1Y2K, respectively) were retrieved from the Protein Data Bank (PDB) and then edited using protein preparation on Maestro 10.6, where all water molecules and co-crystallized ligands were removed. Partial atomic charges were assigned according to the optimized potentials for the liquid simulations (OPLS3) force field. The grid box of the enzymes was centered at particular residues of the proteins and was generated with the Grid generation application. The ligands of *Althaea officinalis* were obtained from the Dictionary of Natural Products as well as some published reports in the literature. The 2D structures of the desired compounds were drawn on ChemDraw Professional 15.0 and 3D structures generated using the Chem3D suite. The structures were saved in SDF format and prepared for docking. The Ligand Preparation module (Lig-Prep) in Maestro was used to prepare the ligands in this study. The drawn ligands were geometry optimized using the OPLS3 force field. They were subjected to impact minimization with a cut off Root Mean Square Deviation (RMSD) of 0.3 Å. Docking analyses were carried out using Glide-5.5. Flexible ligand docking was accomplished for all of the compounds. Rolipram, ibuprofen, celecoxib, diclofenac, and cetirizine were used as the positive controls.

2.10 | Statistical analysis

The statistical analysis was applied by the statistical package for social sciences (SPSS) (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, version 22.0. Armonk, New York: IBM Corp.). Groups' baseline data contrasted by Fisher's exact test and Wilcoxon rank-sum test. For each group, we used the Friedman test to study the trend of repeated measures of SCORAD AD severity score during the one-month period as a proxy of effectiveness. In addition, the Wilcoxon rank-sum test employed to compare the SCORAD score decrease (△) between two groups during different time spans. Data are presented as Median and Interquartile range (IQR). Differences were considered significant by the $p < .05$. It should also be mentioned that per-protocol analysis was applied in this study.

3 | RESULTS

3.1 | Study flow

Of the 30 individuals enrolled in the study, 22 children completed the trial (Figure 1). All of the dropouts in both groups were due to the inappropriate consumption of the drug. The intervention group consisted of four (36.5%) females and seven (63.6%) males with a median age of six (IQR of 4–9 and range of 4–24) and the positive control group consisted of one (9.1%) female and 10 (90.9%) males with a median age of five (IQR of 4–6 and range of 3–14). Groups were not different regarding gender and age (p-value of 0.311 and 0.302, respectively). All of these participants well obeyed the instruction of study while no side effect was reported by them. Moreover, there were no co-interventions such as using supplements during the study.

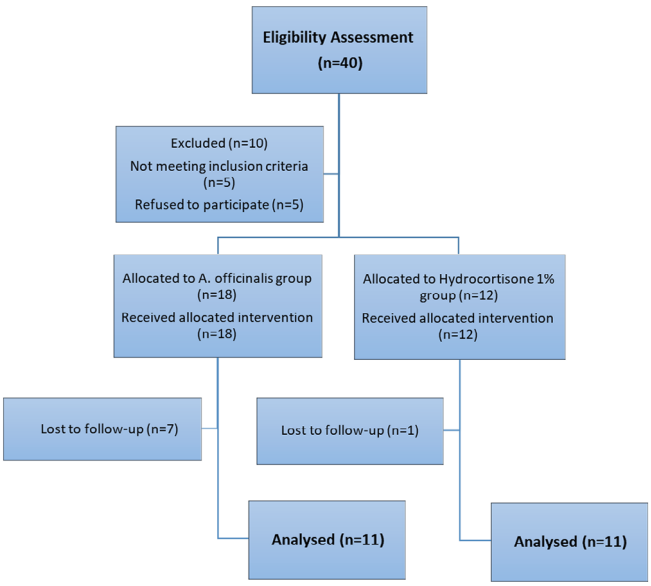

FIGURE 1 CONSORT diagram of the study flow [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Comparing SCORAD atopic dermatitis severity score at baseline and follow-up (end of week 1 and 4) between intervention (*A. officinalis*) and control (Hydrocortisone 1%) groups

	Positive control group	Intervention group	*p*-value[a]
Baseline	25 [25–35][b]	30 [25–35]	1
Week 1	10 [5–20]	10 [0–30]	.687
Week 4	10 [0–30]	0 [0–10]	.076
Trend analysis[c]	<0.0001	<0.0001	-

[a]Wilcoxon rank-sum test.
[b]Median [interquartile range (IQR)].
[c]Friedman test.

TABLE 2 Comparing decrease in SCORAD atopic dermatitis severity score between intervention (*A. officinalis*) and control (Hydrocortisone 1%) groups

	Positive control group	Intervention group	*p*-value[a]
△ week 1 – Baseline	−15 [−25 to −15][b]	−15 [−20 to −15]	0.973
△ week 4 – Week 1	0 [−5 to −15]	−5 [−10 to −0]	0.018
△ week 4 – Baseline	−15 [−25 to −5]	−25 [−30 to −20]	0.015

[a]Wilcoxon rank-sum test.
[b]Median [interquartile range (IQR)].

3.2 | Clinical trial outcome

According to Table 1, trend analysis showed the decrease of SCORAD score in both groups while this score was significantly lesser (*p*-value = .076) in the intervention group in week 4. Moreover, the score change of the intervention group was significantly higher at the end of the study comparing to the baseline (*p*-value = .015) and week 1 (*p*-value = .018) (Table 2).

3.3 | Docking analysis

Medicinal plants are rich sources of different bioactive constituents that can be used for the inhibition of various enzymes. So far, several phytochemical studies have been performed on *Althaea officinalis* to isolate and characterize its bioactive constituents. The literature review revealed that a number of coumarin, flavonoid, alkaloid, and some other polyphenolic derivatives have been reported from this plant (Gudej & Bieganowska, 1990; Kumar, Sudhakar, Kapil, & Snigdha, 2016; Rani, Khan, & Ali, 2010; Sendker et al., 2017). Thirty-three phytochemical constituents were found from *Althaea officinalis*, including glycine betaine (**1**), p-coumaric acid (**2**), caffeic acid (**3**), lauric acid (**4**), altheacalamene (**5**), N-(*E*)-cinnamoyl-L-aspartate (**6**), kaempferol (**7**), N-(*E*)-coumaroyl-L-tyrosine (**8**), N-(*E*)-coumaroyl-L-dopa (**9**), N-(*E*)-caffeoyl-L-tyrosine (**10**), N-(*E*)-caffeoyl-L-dopa (**11**), 4-stearylcatechol (**12**), altheahexacosanyl lactone (**13**), Beta-sitosterol (**14**), lanosterol (**15**), dihydrokaempferol 4′-O-glucoside (**16**), Hypolaetin-8-glucoside (**17**), isoquercitrin (**18**), haploperoside A (**19**), altheaecoumaryl glucoside (**20**), 8-O-(2-O-Sulfo-Beta-D-glucopyranoside)-hypolaetin 4′-methyl ether (**21**), hypolaetin-8-O-ß- D -(2″-O-sulfo)glucopyranoside (**22**), 4′-O-methylisoscutellarein-8-O-ß-D-(3″-O-sulfo)-glucuronopyranoside (**23**), theograndin II (**24**), 4′-O-methylhypolaetin-8-O-ß-D-(2″-O-sulfo)glucopyranoside (**25**), 3′-O-sulfate, 8-O-Beta-D-glucopyranoside-hypolaetin 4′-methyl ether (**26**), 4′-O-methylhypolaetin-8-O-ß-D-(3″-O-sulfo)glucurono-pyranoside (**27**), tiliroside (**28**), hypolaetin-8-O-[Beta-D-glucopyranosyl-(1 6)-Beta-D-glucopyranoside] (**29**), hypolaetin-8-O-ß-D-glucopyranosyl-(1″4″)-ß-D-glucuronopyranoside (**30**), althaeaoctatetracontenoic acid (**31**), 5,13-dihydroxynonacosanyl gadoleate (**32**), and β-D-glucopyranuronosyl-(1 → 3)-α-D-galactopyranuronosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 4)-[β-D-glucopyranuronosyl-(1 → 3)]-α-D-galactopyranuronosyl-(1 → 2)-L-rhamnopyranose (**33**).

All identified compounds were docked into the active site of TNF-alpha, IL6, and human PDE4 isoenzymes by employing the Glide module (Schrodinger suite). The output of all ligands is shown in Table 3 given by docking score in kcal/mol, indicating that glycosylated compounds presented a statistically significant higher affinity toward the selected enzymes. It could probably be due to the hydrogen bond interaction of hydroxyl groups in the glycosyl part of the ligands with enzymes. The docking score of an oligosaccharide (Compound **33**) was highest against enzymes 3I8V, 4KP6, 1Y2K, and 1P9M with the values of −13.796, −14.401, −14.375, and −11.713 kcal/mol, respectively; however, it was inactive against 2AZ5 enzyme. More investigation of the results of binding energies indicated that hypolaetin-8-O-[Beta-D-glucopyranosyl-(1 6)-Beta-D-glucopyranoside] (**29**), tiliroside (**28**), and 4′-O-methylhypolaetin-8-O-ß-D-(3″-O-sulfo)glucurono-pyranoside (**27**) were the active compounds against PDE A enzyme (PDB ID: 3I8V) with docking score values of -11.373, -10.125, and -9.877 kcal/mol, respectively. In addition, two flavonoids tiliroside (**28**) and 4′-O-methylhypolaetin-8-O-ß-D-(3″-O-sulfo)glucurono-pyranoside (**27**) as well as the glycosylated coumarin altheaecoumaryl glucoside (**20**) possessed high docking scores on PDE B (PDB IDs: 4KP6) enzyme activity with the values of −12.536, −11.768, and −11.601 kcal/mol, respectively. The affinity of glycosylated flavonoids hypolaetin-8-O-[Beta-D-glucopyranosyl-(1 6)-Beta-D-glucopyranoside] (**29**), hypolaetin-8-O-ß-D-glucopyranosyl-(1″4″)-ß-D-glucuronopyranoside (**30**), and 4′-O-methylhypolaetin-8-O-ß-D-(3″-O-sulfo)glucurono-pyranoside (**27**) was also high to the PDE D enzyme (PDB ID: 1Y2K) based on the binding energies of -10.293, -8.675, and -8.527 kcal/mol, respectively. Compounds **30**, hypolaetin-

TABLE 3 Docking analysis of the chemical constituents of *Althaea officinalis* against TNF-alpha, IL6 Interleukin, and PDE4s

	Docking score (Kj/Mol)				
Compound	**3I8V[a]**	**4KP6[b]**	**1Y2K[c]**	**2AZ5[d]**	**1P9M[e]**
AO-1	−4.111	−3.459	−1.917	−1.938	−2.474
AO-2	−3.504	−5.493	−3.592	−4.803	−4.557
AO-3	−4.541	−6.478	−4.967	−5.904	−5.865
AO-4	−1.27	−3.621	−1.092	−2.282	−1.868
AO-5	−3.294	−6.715	−2.276	−5.334	−2.988
AO-6	−5.64	−9.973	−5.025	−6.512	−6.041
AO-7	−4.995	−6.557	−4.485	−5.96	−5.773
AO-8	−6.488	−7.155	−3.739	−6.3	−6.267
AO-9	−6.456	−9.175	−4.769	−7.497	−7.331
AO-10	−7.678	−7.122	−5.659	−7.392	−5.495
AO-11	−7.552	−10.43	−4.756	−8.513	−5.44
AO-12	−3.692	−5.352	−4.073	−8.15	−5.71
AO-13	−2.551	−7.528	−2.876	−4.765	−3.747
AO-14	0.587	0.597	−2.14	−5.249	−2.249
AO-15	−1.228	−6.568	−2.013	−4.967	−2.687
AO-16	−8.692	−8.539	−5.861	−8.934	−8.329
AO-17	−8.214	−11.099	−6.877	−6.113	−10.014
AO-18	−7.966	−10.781	−5.312	−9.307	−7.426
AO-19	−6.686	−10.528	−6.073	−9.412	−8.621
AO-20	−3.02	−11.601	−5.552	−7.16	−7.089
AO-21	−6.473	−9.492	−4.788	−8.635	−7.375
AO-22	−9.015	−9.708	−4.975		−8.31
AO-23	−8.928	−9.633	−7.97	−6.465	−8.519
AO-24	−9.707	−10.401	−8.518	−10.735	−9.638
AO-25	−8.377	−8.705	−7.158	−6.924	−9.823
AO-26	−9.729	−9.977	−6.755	−9.75	−9.525
AO-27	−9.877	−11.768	−8.527	−10.524	−9.347
AO-28	−10.125	−12.536	−7.751	−7.196	−9.79
AO-29	−11.373	−10.942	−10.293	−11.088	−9.92
AO-30	−9.566	−10.217	−8.675	**−11.987**	−10.277
AO-31				−8.355	
AO-32	−3.961	−0.078		−8.755	
AO-33	**−13.796**	**−14.401**	**−14.375**		**−11.713**
Rolipram-R	−3.675	−6.107	−2.858	−5.705	−2.726
Rolipram-S	−3.714	−7.16	−2.194	−5.507	−3.216
Celecoxib				−5.09	−3.472
Cetirizine				−7.973	−4.737
Diclofenac				−5.676	−4.649
Ibuprofen				−5.393	−4.375

Note: The most active compounds against each enzyme are highlighted in bold.
[a]PDB codes of PDEs A.
[b]PDB codes of PDEs B.
[c]PDB codes of PDEs D.
[d]PDB code of TNF-alpha.
[e]PDB code of IL6.

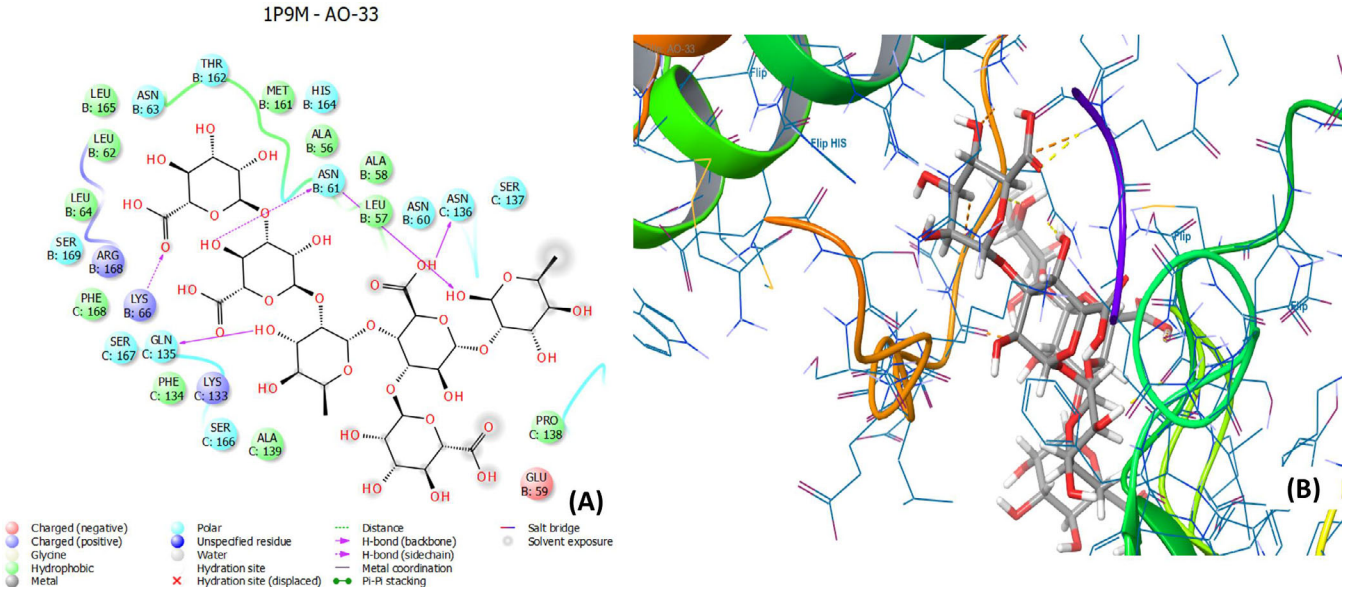

FIGURE 2 The view of 2D structure (A) and 3D structure (B) of docked ligand **AO-33** with IL6 [Colour figure can be viewed at wileyonlinelibrary.com]

8-glucoside (**17**) and **29** were also found to have a high activity against IL6 (PDB ID: 1P9M) with the scores of −10.277, −10.014, and −9.92 kcal/mol, respectively. A docking score value of −11.987 kcal/mol was also recorded for the glycosylated flavonoid hypolaetin-8-O-ß-D-glucopyranosyl-(1″ 4″)-ß-D-glucuronopyranoside (**30**) as the best inhibitor against 2AZ5 enzyme, followed by three other flavonoids, including hypolaetin-8-O-[Beta-D-glucopyranosyl-(1 6)-Beta-D-glucopyranoside] (**29**), theograndin II (**24**), and 4′-O-methyl hypolaetin-8-O-ß-D-(3″-O-sulfo) glucurono-pyranoside (**27**) with docking values of −11.088, −10.735, and −10.524 kcal/mol, respectively.

4 | DISCUSSION

The results of this study indicated a significant beneficial effect of a topical formulation of *Althaea officinalis* 1% on the SCORAD index in children with AD. Although both groups improved regarding their total SCORAD score, this improvement was significantly higher in the intervention (topical marshmallow) group.

For a long time, plants have played a significant role in the treatment of many diseases, especially in Asia (Golshani, Daneshfard, Mosleh, & Salehi, 2015). Herbal extracts have various functions in nature, including anti-aging, photoprotection, antioxidant, moisturizing, astringent, anti-irritant, and antimicrobial effects. In addition, there are several herbal medicines, which have been evaluated for their possible effects on AD. A mixture of honey, beeswax, and olive oil (Al-Waili, 2003), Evening primrose oil/Borage oil (Lee & Bielory, 2010), licorice extract of *Glycyrrhiza glabra* (Saeedi, Morteza-Semnani, & Ghoreishi, 2003), Korean herbal formulation Hwangryunhaedoktang (Kim et al., 2011), and two herbal compounds, Zemaphyte and Kamillosan (Latchman, Whittle, Rustin, Atherton, & Brostoff, 1994; Patzelt-Wenczler & Ponce-Pöschl, 2000 are some of the topical herbal medicines that have been shown to have beneficial effects on AD.

Althaea officinalis from the Malvaceae family has widely used as an herbal medicine for the treatment of the irritation of oral cavity, pharyngeal mucosa, dry cough, mild gastritis, skin burns, or insect bites, as well as catarrh of the mouth and throat, gastrointestinal and urinary tract complains, inflammation, and ulcers possessing immunomodulatory and anti-microbial effects. Many compounds, including pectins, mucilage, flavonoids, vanillicacid, coumarins, scopoletin, phytosterols, tannins, asparagines, amino acids, and di-saccharide saccharose have been extracted from different parts of *Althaea officinalis* (Al-Snafi, 2013).

There are several possible mechanisms of action, which are thought to be related to the clinical effectiveness of this herbal ointment. Mucilaginous and bio-adhesive effects of the polysaccharide fraction from aqueous extract of marshmallow roots leading to the formation of polysaccharide layers on inflamed or destructed epithelial tissue. According to Deters et al, this situation leads to better protection and rehydration of the tissue and an improved shielding of the mucosal barrier against physical or microbial agents (Deters et al., 2010).

On the other hand, Tannin can improve cutaneous wound healing in rats, which probably results from powerful angiogenesis and antibacterial activity (Li et al., 2011). Pectins, another component of marshmallow provides moist and enough oxygen in damaged skin and promote angiogenesis, epithelization, and bacterial killing in the damaged site (Boateng, Matthews, Stevens, & Eccleston, 2008).

Flavonoids as polyphenolic antioxidants found in *Althaea officinalis* can elimination the reactive oxygen species (ROS) resulted from oxidative stress in damaged tissue, which in turn leads to wound

healing and minimizing the damage (Hemmati, 2013). According to some researches, mucilage, as the main polysaccharide component of marshmallow, can improve epithelization and phenolic acid can decrease the duration of wound healing with its anti-inflammatory effect (Hemmati, 2013; Hemmati, Houshmand, Ghorbanzadeh, Nemati, & Behmanesh, 2014).

Phosphodiesterases and cytokines such as TNF alpha and IL-6 have an important role in almost all incidences of inflammation and its associated processes. The central role of PDE4A, B, and D have also been known in inflammatory diseases through hydrolysis of cyclic adenosine monophosphate in inflammatory and immune cells. It is well established that inhibition of the activity of related enzymes can be considered a therapeutic strategy in patients with AD disease.

In silico studies reveal the affinity, activity, and binding orientation of ligands to the target proteins. In drug design, the stability of the interaction between a drug and a receptor is crucial to identify the molecular and macromolecular interactions. This information is vital for the dosage determination of drug so that medical treatment is in accordance with what is expected. Herein, docking analysis was performed to establish the putative binding model of the chemical constituents of *Althaea officinalis* to the active site of selected enzymes.

Our findings showed that some of the chemicals of *Althaea officinalis* have a high ability to bond to the enzymes and block their activity. Based on the obtained results, the inhibitory activity of compounds with free hydroxyl groups was characterized to be better than others. Specially, five derivatives of a flavone (hypolaetin), including compounds **24** and **27**–**30** as well as a disaccharide (**33**) indicated the highest inhibitory activity against enzymes. As shown in Table 3, the binding energy of these ligands is lower than standard ligands, indicating a greater affinity to enzymes and so, resulting in strong interaction between ligands and enzymes, which causes the high therapeutic effects of ligands. Accordingly, the chemical synthesis or purification of naturally occurring hypolaetin derivatives and their biological evaluation by in vitro and in vivo models could be considered in future studies.

Visualization of the interaction between ligands and enzymes revealed that the high affinity of compounds with free hydroxyl groups could be concluded from two major interactions, including the H-bond interaction of the hydroxyl groups and hydrophobic π-π stacking of the aromatic rings of ligands with the active site of enzymes. In the best-docked result, oligosaccharide **33** showed a strong H-bond interaction with ASN-61B (two interactions), LYS-66B, GLN-135C, and ASN-136C (Figure 2).

Recent advances in the discovery of the AD pathogenesis noted several treatment strategies. Based on the molecular docking analysis, an oligosaccharide and various glycosylated derivatives of hypolaetin indicated the strongest interaction with enzymes with low binding energies compared with other ligands and controls. These compounds may possess a significant inhibitory effect on inflammatory mediators, and therefore, could be used as drug candidates in AD treatment in further studies.

Considering the lack of rigorous regulations, the need for manufacturing the nutraceutical with proved efficacy, safety, and quality of a marketed product is less strongly enforced in the pharmaceutical sector. Therefore, many available products might be ineffective (Williamson, Liu, & Izzo, 2020). Accordingly, much more efforts should be put on the production of standardized nutraceuticals.

Although this study evaluated a well-analyzed herbal product with significant findings, it had some limitations. It was a pilot study in a small sample of patients with short term follow-up. Moreover, although a standard questionnaire was used for the assessment of the patients, this study lacks objective outcome measures to investigate the mechanisms of action. Such points should be considered in future studies.

5 | CONCLUSION

Results of this pilot active-controlled trial revealed that topical use of *Althaea officinalis* ointment has a higher efficacy in children with AD in comparison to topical Hydrocortisone 1%. Considering the side effects related to available drugs and their low efficacy, it appears that *Althaea officinalis* 1% ointment could be an appropriate remedy for children suffering from AD. However, further clinical investigations are needed to more rigorously prove its efficacy.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

ORCID

Babak Daneshfard https://orcid.org/0000-0001-6729-9113
Mohsen Naseri https://orcid.org/0000-0001-9239-768X

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