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Development and application of magnolol derivative in cosmetics

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Introduction:

At present, different types of preservatives have been used in food, medicine and cosmetics, and most of them are chemical preservatives. There are dozens of commonly used preservatives, such as acid preservatives (benzoic acid), ester preservatives (paraben esters) and etc[1]. However, it is well known that most of the commercial chemical preservatives have adverse effects on the human body, such as causing skin allergy[2]. Therefore, natural preservatives which are mild and safe to human are in urgent need of various industries.

Magnolia officinalis is the dried bark, root bark and branch of magnolia officinalis, which is an important Chinese herbal medicine.Magnolol (MG) has excellent antioxidant，bacteriostatic properties and therefore it has often been used in cream-based cosmetics. However, the water solubility of MG is very poor,which greatly limits the application of MG in water-based cosmetics. The aim of this study was to increase the water solubility of MG by chemical modification and expand the application of MG in cosmetics.

Methods that have been reported about the modification of MG mainly focused on allyl or phenolic hydroxyl group to improve antiviral and anti-tumor efficacies[3-4]. In contrast, in this study, the allyl and phenolic hydroxyl groups of MG are expected to be preserved, while several biological activities[5], including antibacterial activity, antifungal activity, antimalarial activity, anti-inflammatory activity and etc are rendered through the formation of Mannich bases. In this study, we selected MG as the lead compound, and a kind of 3,3'- (2,2'-diethanlamine)-methyl magnolol (MGDEA) was designed and synthesized to evaluate its water solubility, safety, antibacterial and anti-inflammatory effects.

Materials & Methods:

1.Synthesis of MGDEA

Magnolol has two active α hydrogens in its molecular structure, which can undergo Mannich reaction with secondary amines and aldehydes. Magnolol derivatives were synthesized by reacting magnolol with diethanolamine and formaldehyde.

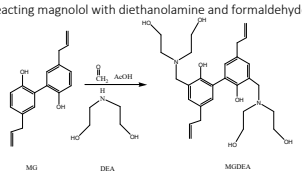


Figure1. Mannich reaction for the synthesis of MEGEA

2.Cells tests

Cell experiments were conducted to evaluate the safety of MG and MGDEA on HaCaT cells. Different concentrations （0--200ppm） of MGDEA and MG were set to confirm the safety dose to HaCaT cells by CCK-8 assay.

3.Minimal inhibitory concentration MIC test and Preservative efficacy test

Referring to the requirements of the "Technical Specification for Safety of Cosmetics" (2015 edition), the MIC of magnolol derivative to 5 kinds of microorganisms commonly found in cosmetics was determined by half-dilution method. Based on this MIC value, we selected the appropriate dosage (2-3times of MIC value) of MGDEA for the spray formulation (without any preservatives), and then carried out a 28-day Preservative Efficacy Testing (USP 51，EP 7.0).

4.Anti-inflammatory activity test

The anti-inflammatory effect of MGDEA on the expression of IL-6 in HaCaT cells was detected by Western blotting. The principle of this method is to combine protein with specific antibody to detect its expression.

5.Antioxidant test

The DPPH test is less time-consuming with high reproducibility. 1,1-diphenyl-2-hydrazine (DPPH) can bind to free radicals. Its alcohol solution is dark purple and has strong absorption at 520nm. When free radicals are present in the sample, the DPPH alcohol solution would turn into yellow and the decrease of its absorbance could be quantitatively analyzed by ELIASA.

Results & Discussion:

1.Synthesis of MGDEA

The structure of MGDEA was determined by ^{1}HNMR (Fig.2),the number of hydrogen atoms of the MGDEA are consistent with 3,3'- (2,2'-diethanlamine)-methyl magnolol . The water solubility of MGDEA was more than 10%.

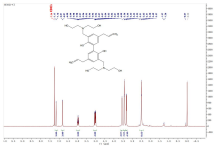


Figure2. ^{1}HNMR-CDCl$_3$ spectra of MGDEA

Table 1. Aqueous solubility

Dissolution quality	MG	MGDEA
Solvent aqueous（100g）	--	＞10g

2.Cells tests

As shown in Figure 3, MGDEA (IC_{50}=150.24ppm) showed lower toxicities toward HaCaT cells than MG (IC_{50} =18.01ppm). Based on the above results, the cytotoxicity of magnolol was greatly reduced after modification.

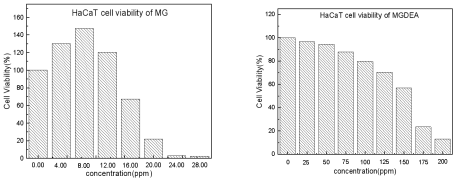


Figure 3.Safety evaluation of MG and MGDEA

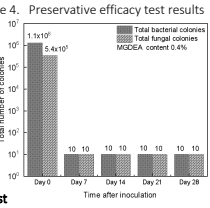
3.Minimal inhibitory concentration MIC test and Preservative efficacy test

The MIC against *Pseudomonas Aeruginosa* and *Escherichia coli* reached 0.08% and 0.063%, respectively, indicating that the antibacterial activity of MGDEA was greatly improved.Only 0.4% of MGDEA is required in water-based spray to pass the 28-day Preservative Efficacy test.

Table 2. The MIC test results of MGDEA

Bacteria \ %	MGDEA	10%MG (Ethanol solution)	Methyl paraben	Phenoxyethanol
Escherichia coli	0.063	1.33	0.156	0.25
Staphylococcus aureus	0.032	0.0053	0.078	0.125
Pseudomonas aeruginosa	0.08	1.33	1.25	1
Candida albicans	0.08	0.0063	0.626	0.25
Aspergillus niger	0.125	0.0063	0.626	0.5

Figure 4. Preservative efficacy test results



4.Anti-inflammatory activity test and Antioxidant test

The IL-6 expression in MGDEA group(70ppm) is significantly lower than that in the MG group(70ppm). The DPPH clearance rate of MGDEA （1000ppm） reached to 86.09%.

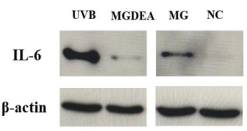


Figure 5. IL-6 inflammatory factor protein expression

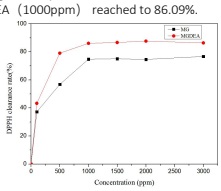


Figure 6. DPPH test results

Conclusions:

- ✓ The 1HNMR and LC-MS results showed that magnolol derivatives(3,3'- (2,2'-diethanlamine)-methyl magnolol) were successfully synthesized .
- ✓ The **water solubility** of MGDEA was **more than 10%** and the **cytotoxicity** of MGDEA **decreased approximately 8 times**, compared with MG . The **free radical scavenging ability** of MGDEA was **15% improved**.
- ✓ MGDEA exhibits a powerful anti-microbial activity against 5 kinds of microorganism commonly found in cosmetics and the anti-bacterial activity of MGDEA was better than phenoxyethanol and methyl paraben. **Only 0.4% dosage** of MGDEA was required in water-based spray to **pass the 28-day Preservative efficacy testing**.

In conclusion, MGDEA has better aqueous solubility and is less toxic to skin cells than MG. MGDEA has broad anti-bacterial spectrum and anti-inflammation effect, making it a promising new active ingredient and powerful natural preservative for high-end skin care products.

Acknowledgements:

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