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In-Silico Exploration of Antiasthmatic Properties of Flavonoids Isolated from Lanneacoromandelica (Houtt.) Merr

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ABSTRACT
Lanneacoromandelica (Houtt.) Merr. (LC) is an important Indian medicinal plant used in folklore medicine to treat various diseases.Current study adopted chromatographic and analytical techniques for isolating phytochemicals and to recognise protein-ligand interactions, assessantiasthmatic potential against specific molecular targets.The L. coromandelicaleaf extract was subjected to column chromatography using silica gel with gradient mobile phases and two isolated compounds.The structures were established using detailed spectral analyses, such as mass spectrometry, FT-IR, ^{1}H NMR, and ^{13}C NMR.Molecular docking analysis was performed for these isolated molecules and their interactions with targets, such as 1IJZ Human IL (13), 3NOS (nitric oxide synthasised by human endothelial cells), 3LB6(IL-13 in complex with IL-13R alpha2), 1N26 (Human Interleukin-6 Receptor alpha), and 2AZ5 (TNF-alpha) which are important in the pathophysiology of asthma.Phytochemical examination detected the presence of diverse phytochemicals, and isolation through column chromatography led to the separation of Lupeol and Isoquercitrin in pure form.Molecular docking analysis revealed potential interactions between these ligands and the selected target molecules suggesting their involvement in the antiasthmatic properties of L. coromandelica.One flavonoid and One triterpenoid were isolated from the leaves of L. coromandelicaand in silico docking studies revealed good binding interactions with the targets involved in asthma, that guide further in-vitro and in-vivo studies.L. coromandelica

Keywords: Lanneacoromandelica (Houtt.) Merr., Isolation, Antiasthmatic, In-silico, Molecular docking

1. INTRODUCTION

Lanneacoromandelica (Houtt.) Merr. is a tall, deciduous Indian ash tree that belongs to the Anacardiaceae family.It can grow to a height of 10–20 m with unique leaves, which are estipulate and alternately arranged.The tree also has yellow-green unisexual flowers and drupe fruits with a hard covering and compressed seeds.L. coromandelicahas been mentioned in various texts and cultures, stating its effectiveness against heart problems, diarrhoea, ulcers, inflammation, infections, and hepatitis. Recent literature revealed the presence offlavonoids, tannins,steroids and glycosides with a broad pharmacological profile [1-5].

Asthma is a chronic complex condition characterised by inflammation and airway narrowing, leading to breathing difficulties.Various proteins and cytokines are involved in asthma pathophysiology.IL-13, produced mainly by Th2 immune cells, plays a crucial role in Asthma by driving mucus overproduction, airway sensitivity, and structural changes in the airways.Similarly, IL-6, another cytokine, is elevated during asthma attacks and contributes to the overall inflammatory response.TNF-α, a potent inflammatory mediator, has been linked to severe forms of Asthma by inducing inflammation and airway changes [6-9].

Nitric oxide (NO) produced from endothelial nitric oxide synthase (eNOS) facilitates to keep the airways open, excessive amounts indicate airway inflammation and can further aggravate asthma symptoms.Together, these targets highlight the intricate network of molecules that contributes to the complexity and severity of Asthma [10].

Flavonoids have also been shown to exert anti-inflammatory effects.They achieved this by targeting various mechanisms associated with inflammation and progression.Recent studies have shown that flavonoids can regulate NF-κB and its inhibitory molecule IkB, indicating their potential role in controlling cell activation during inflammation.Flavonoids also affect other transcription factors that regulate the activity of CD_4^+ T helper 2 (Th2) cells.These cells are essential for specific inflammatory responses, particularly those associated with allergies and Asthma [11-12].

The current study aimed to isolate the major phytochemicals from the ethanol extract of L. coromandelicaleaves using column chromatography, followed by structure elucidation and in-silico docking studies on targets involved in the pathophysiology of Asthma.

2. METHODOLOGY

2.1 Phytochemical evaluation

The ethanolic extracts of L. coromandelicaleaves included a variety of phytochemicals, which were qualitatively identified through the application of conventional procedures for phytochemical screening[13].

2.2. Total flavonoid content

The Dowd colorimetric method using $AlCl_3$ was adapted to determine the total flavonoid content in the ethanol extract of the L. coromandelica[14].

2.3. Isolation of phytochemicals using column chromatography

The present work involved the chromatographic investigation of L. coromandelica, wherein a silica gel column (60-120 mesh) was employed to isolate the major phytochemicals from the ethanolic leaf extract.A normal phase column chromatography technique was adopted, and a series of elutions with n-hexane and an ethyl acetate blend were performed to transition gradually from non-polar to polar compounds.The progress of chromatographic separation was assessed using thin-layer chromatography (TLC), employing a mobile solvent system consisting of ethyl acetate, n-hexane and Methanol in a volumetric ratio of 7:2.5:0.5.An anisaldehyde-sulfuric acid reagent was sprayed to facilitate spot visualisation [15].

Fractions with similar TLC chromatograms were combined, and two fractions with different retention factor (Rf) values were chosen for further purification.The first fraction was obtained as residue.It was then purified by re-chromatography, employing a silica gel column with a mesh size of 100-200.This process brings about generation of LC-1.Similarly, second fraction was obtained, resulting in the pure form of LC-2. LC-1 and LC-2 were characterised by infrared spectra (IR), proton and carbon nuclear magnetic resonance spectral analysis ^{1}H NMR, ^{13}C NMR and mass spectrometry.

2.4. Molecular-docking analysis

Lupeol and Isoquercitinwere docked into active site using cDOCKER procedure, which relies on the "CHARMM force field applied in the receptor-ligand binding protocol" of version 3.5 of "Discovery Studio software" to dock with the binding sites of 1IJZ (Human IL-13), 3NOS (human endothelial nitric oxide synthase), 3LB6(IL-13 in complex with IL-13R alpha2), 1N26 (Human Interleukin-6 Receptor alpha), and 2AZ5 (TNF-alpha).The molecular ligands exhibited adequate flexibility as far as they easily traverse the active site, hence facilitating the analysis of binding orientations, modes of interaction, and correlation accompanied by existing literature. A greater -cDOCKER interaction energy indicates a more significant and favourable interaction of the protein with ligand. This formed basis for the scoring function[16-18].

3. RESULTS

3.1. Preliminary phytochemical screening

The L. coromandelicawas subjected to a preliminary phytochemical examination using standard methods. The analysis showed existence of various components illustrated in the Table 1.

Table 1. Phytochemical evaluation of L. coromandelica

Phytochemicals	L.coromandelica
Alkaloids	+
Glycosides	+
Flavonoids	++
Terpenoids	++
Steroids	+
Tannins	++
Proteins	++
Carbohydrates	++
Amino acids	+
Saponins	+

+ Present,- Absent

3.2. Total flavonoid content

The quantification of flavonoids using the $AlCl_3$ colorimetric method resulted in a high total flavonoid content of 73.7 ± 0.67 mg QE/g in the L. coromandelicaethanolic extract, which is significant, reflecting the robust

potential of the plant as a source of antioxidants.The high correlation coefficient (R^2 = 0.9956) of the calibration curve supported the accuracy and reliability of this method for flavonoid estimation.

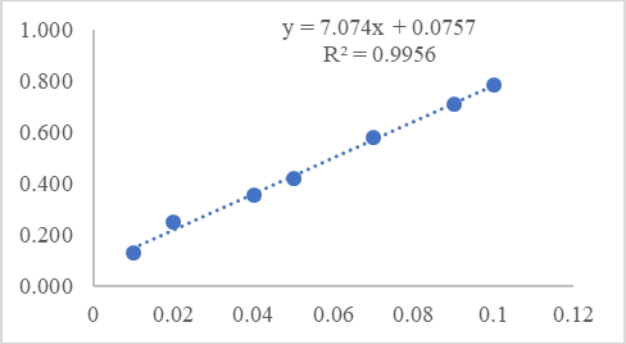

Figure 1. Standard curve of quercetin

3.3. Isolation of Phytochemicals

Phytochemical investigation of the L. coromandelicaleaf ethanolic extract revealed the presence of an important class of secondary metabolites such as alkaloids, glycosides, polyphenolics, terpenes, and terpenoids.The study attempted to isolate the phytochemicals from the leaf extract, and gradient elution with n-hexane and ethyl acetate led to the separation of two compounds, namely LC-1 and LC-2.Structural elucidation using advanced analytical techniques confirmed the presence of Lupeol and Isoquercitrin.

3.3.1. Compound-1 (LC-1)

The ^{1}H NMR spectrum revealed angular methyl proton signals at δ 0.76 (s), 0.79 (s), 0.83 (s), 0.94 (s), 0.96 (s), 1.03 (s), and 1.67 (s), corresponding to (C24), (C28), (C25), (C27), (C23), (C26), and (C30) correspondingly indicating the presence of seven methyl groups in the compound. A distinct doublet observed at δ of 3.2 ppm was designated at 3-H. Additionally, two olefinic proton signals at δ 4.57 and 4.69 ppm corresponded to an exocyclic double bond.

The ^{13}C NMR spectra analysis revealed 30 distinct carbon signals, comprising seven methyl, 6 quaternary carbons eleven methylene and six methane, characteristic of a lupane-type triterpenoid skeleton. A signal at δ 78.996 ppm was attributed to the carbon bonded to a hydroxymoeity located at C-3 position. The exocyclic double bond carbons appeared at δ 150.966 ppm (quaternary carbon, C-20) and δ 109.312 ppm (methylene carbon, C-29). Furthermore, a mass spectrum with an mass to charge ratio of 409 confirmed a prominent peak signal of lupeol (molecular weight 426.7).

Figure 2. Structure of Lupeol

3.3.2. Compound-2 (LC-2)

The compound melting point (M.P.) was observed at range from 240-244°C. The ^{1}H NMR spectrum displayed distinct signals, including δ 6.14 1H, d (C-2), 6.32 1H, d (C-3), 6.96 1H, d (C-2'), 6.72 1H, d (C-5'), 7.01 1H, d (C-6'), and 5.6 1H, d (C-1''). The broad peaks at range of δ 3.32–3.76 ppm relate to the hydroxyl groups in the sugar moiety, further corroborated by the carbon NMR spectrum. The peaks in the δ 6 ppm region reflect the aromatic ring arrangement in the compound.

The ^{13}C NMR spectrum revealed 21 carbon signals, with no indications of free methyl or methoxy groups. The δ values were recorded as follows: 157.86-C(2), 135.41-C(3), 177.12-C(4), 160.64-C(5), 99.31-C(6), 164.35-C(7), 94.45 -C(8), 158.34-C(9), 104.68 (C(10), 121.42-C(1'), 115.87-C(2'), 145.24-C(3'), 149.13-C(4'), 116.21-C(5'), 121.48-C(6'), 101.47-C(1''), 73.47-C(2''), 75.21-C(3''), 70.26-C(4''), 76.44-C(5''), and 62.17-C(6'').

The quaternary carbon atom at C-9, which is connected to oxygen, along with carbon with hydroxy moiety, exhibited greater chemical shifts in the ^{13}C NMR chart. The mass spectral data with mass to charge ratio of 464 validated the distinct fragment peak, consistent with isoquercitin (molecular weight 464.38).

Figure 3. Isoquercitin chemical structure

3.4. Computational docking analysis

The outcomes of docking interpretation were illustrated in Table 2.Lupeol and Isoquercitin could fit comfortably in the binding sites of the examined targets (Figure 4 and 5).

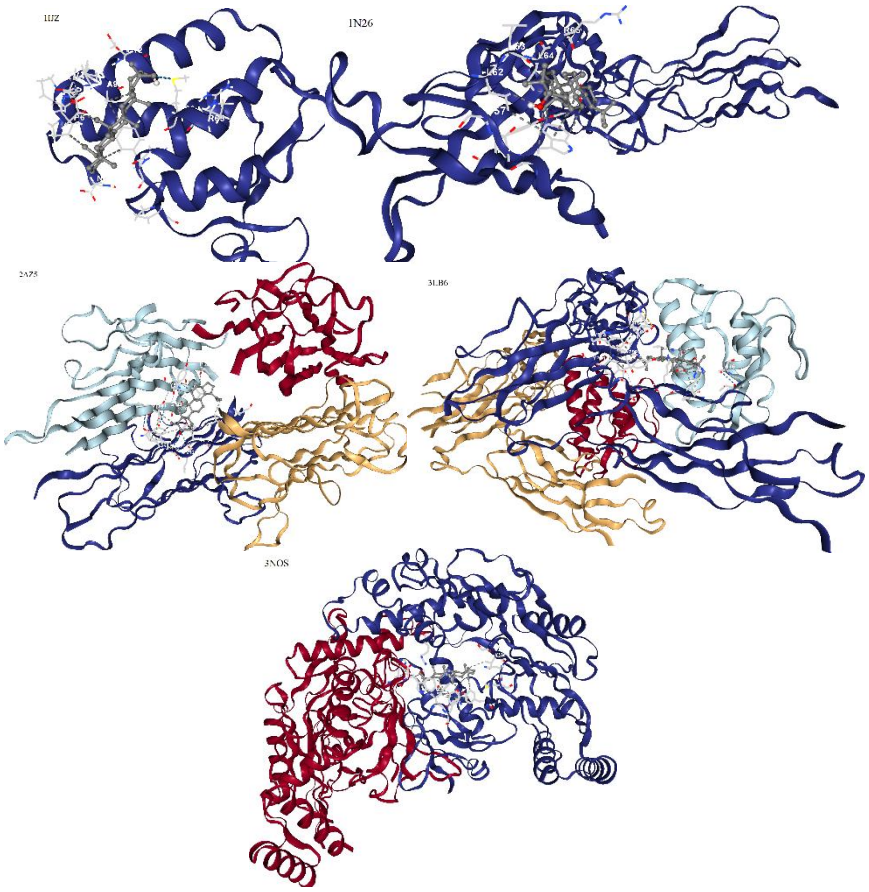

Figure 4. Binding score of Lupeol against different targets

For Human IL(13) (PDB ID: 1IJZ), Lupeol binds with a slightly superior affinity at -7.2 Kcal/mol compared to Isoquercitrin(-6.7 Kcal/mol). Conversely, with Human endothelial nitric oxide synthase (PDB ID: 3NOS), Isoquercitrin outperformed lupeol, displaying stronger attraction at -8.9 Kcal/mol versus Lupeol (-8.5 Kcal/mol).Both compounds exhibit comparable binding strengths when interacting with IL-13 (PDB ID: 3LB6), with Lupeol at -7.9 Kcal/mol and Isoquercitrin closely following at -7.8 Kcal/mol. A similar pattern is seen with Human Interleukin-6 (PDB ID: 1N26), where Lupeol again shows a higher affinity (-7.8 Kcal/mol) relative to Isoquercitrin (-6.9 Kcal/mol). Notably, in the case of TNF-α(PDB ID: 2AZ5), Lupeol demonstrates a significantly stronger interaction, marked at -9.9 Kcal/mol, as opposed to Isoquercitrin(-7.9 Kcal/mol), suggesting a pronounced difference in binding potential between the two compounds for this particular protein.

Table 2. Binding affinityscore of isolated compounds against various targets

S. No.	PDB	Lupeol	Isoquercitin
		Binding energy (Kcal/mol)	
1.	1IJZ Human IL (13)	-7.2	-6.7
2.	3NOS	-8.5	-8.9
3.	3LB6-IL-13	-7.9	-7.8
4.	1N26	-7.8	-6.9
5.	2AZ5 (TNF-α)	-9.9	-7.9

In-silico docking studies of these isolated compounds against the targets 1IJZ Human IL (13), 3NOS, 3LB6 (IL-13), 1N26 (Human Interleukin-6), and 2AZ5 (TNF-α) were performed. From the results, it is evident that Lupeol appears to have a broad spectrum of binding affinities across the various protein targets listed and also demonstrates consistent binding energies between -7.2 to -7.9 Kcal/mol for other proteins, hinting at its potential broad-spectrum activity across these targets. In contrast, Isoquercitrin showed varied binding affinities across protein targets. Its strongest binding occurs with human endothelial nitric oxide synthase (PDB ID: 3NOS) at -8.9 Kcal/mol. However, for most of the targets, except nitric oxide synthase, Isoquercitrin possesses a slightly weaker or comparable binding affinity to lupeol. These results align with the in-silico and in-vivo results of studies conducted by Rathinavelet al., and a study conducted by Saleem et al. reported the inflammationmodulating attributes of lupeol [19-20].The Vasconcelos group study supported the antiasthmatic potential of lupeol in mice [21].

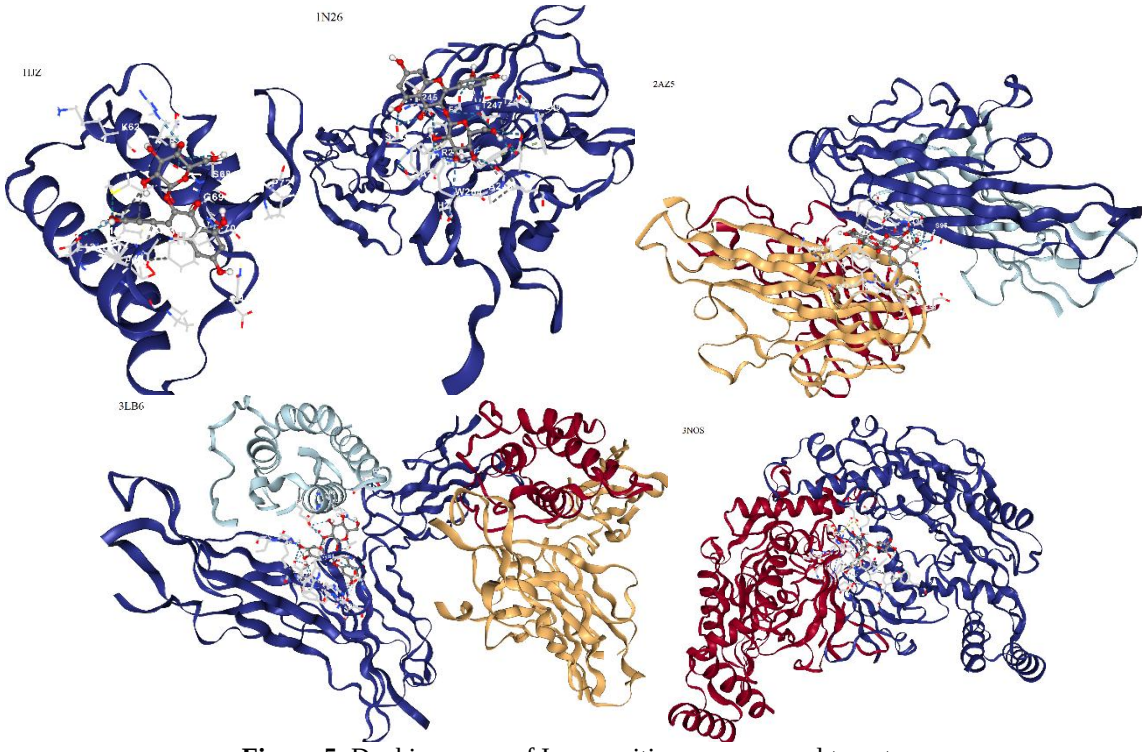

Figure 5. Docking score of Isoquercitin across several targets

Our findings align with those of Chen and Rogerio, who have also demonstrated the antiasthmatic potential of Isoquercitin in allergic asthma models [22-23].

The antiasthmatic potential of these compounds was investigated through in silico docking studies targeting various human proteins related to inflammation and Asthma, such as IL-13, endothelial nitric oxide synthase, Interleukin-6, and tumour necrosis factor-α. The docking studies revealed that Lupeol demonstrated broad-spectrum binding affinities with consistent values ranging from -7.2 to -7.9 Kcal/mol across the various protein targets. Isoquercitin showed variable binding affinities, with notably strong binding to the Human endothelial nitric oxide synthase (3NOS) at -8.9 Kcal/mol.

Lupeol and Isoquercitin, sepatated from the ethanol extract of L. coromandelicaleaves, exhibit distinct binding profiles against several protein targets implicated in inflammatory processes. The consistent binding affinity of lupeol suggests that it may have broad-spectrum potential across multiple inflammatory markers. In contrast, the binding affinities of Isoquercitrin were more variable, with a particular affinity for endothelial nitric oxide synthase, which could indicate a more targeted effect on this enzyme.

The comparable binding affinities of both ligands to IL-13 protein targets suggest possible interactions between both compounds and IL-13-mediated pathways. Moreover, the superior affinity of lupeol for TNF-α and Interleukin-6 points to its potentially greater efficacy in modulating inflammatory cytokines. These results indicate that Lupeol and Isoquercitrin could be promising molecules for further research into their antiasthmatic properties and potential therapeutic applications in addressing asthma-related complications.

4. CONCLUSION

The current study deals with the phytochemical analysis of the L. coromandelicaleaf ethanolic extract, which has uncovered a diverse range of secondary metabolites.Two specific compounds, Lupeol and Isoquercitrin, were isolated using gradient elution and identified using advanced analytical methods.

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Conflict of interest

none to declare.

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