Anti inflammatory evaluation of Andrographis paniculata leaf extract formulation using combination of in silico and in vitro methods

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Abstract
The investigation on medicinal plants have been gaining importance around the world especially in Asian nations due to the presence of wide range of bioactive phytochemicals. The presence of diverse bioactive compounds makes medicinal plants more demandable for curing several diseases, such as inflammatory diseases, and diabetes. *Andrographis paniculata* is one such plant found very commonly throughout the country and reported to have a massive traditional properties. The methanolic extract yielded eight phytochemical compounds as identified by GC-MS. This study addresses the *in vitro* and *in silico* study of the plant leaf extract for a potential anti inflammatory effect. The plant showed adequate anti inflammatory effect through in silico and in vitro models. The compounds identified by GC-MS are put through molecular docking studies and virtual toxicity studies. Out of all the compounds, Apigenin (-8.8 kcal/mol), and Andrographiside (-8.4 kcal/mol), have the better binding energy as compared to the standard diclofenac (-8.1 kcal/mol) indicating that the extract can find its usage as anti inflammatory drug. Further the claim was confirmed by *in vitro* study using BSA method. As compared to standard diclofenac (IC50: 0.47 mg/ml) the IC50 value of *Andrographis paniculata* extract was found to be only 0.284 mg/ml indicating a significant increase in inhibition at low concentration of the extract. A set of herbal cream was prepared using the extracts using 0, 10, 20, 30 mg/ml concentration. All formulations were found to have similar evaluative values with formulation F3 standing out, The formulations remained stable even after 6 months of storage. Thus the leaf extract of *Andrographis paniculata* can find huge potential in developing a new drug strategy against inflammation.

Keywords: *Andrographis paniculata.* I*n vitro* anti-inflammatory assay, *In silico* docking study, Phytochemicals

1. Introduction

Natural remedies have been effectively used in treatment of various disease over many centuries around the world because of their ease in availability and fewer adverse effects. Although there are quite a few researches on synthetic drugs and some of them are available in market for treatment of various disorders but most customers are not satisfied by them owing to severe side effects in most of them. This has lead to people gravitating towards natural products. Even though the onset of action is comparatively low in natural products but they are known for curing illness from root level [1]. One of the underrated yet chronic disease is inflammatory diseases. Chronic inflammation is a long-term condition where your body's immune system is constantly working overtime, even when there's no immediate threat. This can lead to damage in tissues and organs. While the exact causes vary, factors like unhealthy lifestyle, infections, and autoimmune diseases can contribute. Symptoms might include pain, fatigue, and digestive issues. If left untreated, chronic inflammation can increase the risk of serious conditions like heart disease, diabetes, and cancer [2].

So, in order to control this massive threat like inflammatory conditions some effective herbal medicine may be required. In order to combat this, a very well known traditional medicinal plant, that is, *Andrographis paniculata* better known as kalmegh, belonging to the family of acanthaceae. It is characterized by its tall, slender stems and small, white flowers. The plant is particularly valued for its medicinal properties, primarily due to the presence of diterpenoid compounds known as andrographolides. These compounds have been shown to possess antiviral, and hepatoprotective activities, making *Andrographis paniculata* a popular herb in traditional medicine [3]. In this study, the anti inflammatory property of *Andrographis paniculata* has been explored in a topical cream formulation and it evaluation.

2. Materials and Methods

2.1 Materials

The authenticated fresh leaves of *Andrographis paniculata* were collected from West Bengal Medicinal Plant Board, Kalyani, West Bengal, India. The solvents of AR grade were procured from Oxford Lab Fine Chem LLP. Other chemicals and reagent were obtained from SRL labs. All water were of deionized grade

2.2 Extraction and chemical tests of plant material

The plant materials were dried and extracted in methanol and ethyl acetate using ultrasonic extraction 20 kHz (Labman LMU3CD). The presence of phytochemicals were detected by chemical tests of alkaloids, glycosides, resins, tanins, gums, proteins, volatile oil and fixed oil. Based on the results, it was observed that methanolic extracts had presence of more primary and secondary metabolites as compared to the ethyl acetate extract, so it was chosen for further experimentation [4].

2.3 In silico anti-inflammatory study

The extract was subjected to GC-MS (Perkin Elmer Claurus 600) and eight prominent terpenoid compounds Apigenin, Andrographolide, Neoandrographolide, Isoandrographolide, Dehydroandrographolide, Andrograpanin, Deoxyandrographolide and Andrographiside. The structure were drawn in Chem Draw Professional 15.0 Three-dimensional structures of the ligands were created in Open Babel and saved in SDF format for further preparation and

molecular docking analysis. The protein data bank provided the crystallographic structures of the structure of Vioxx bound to human COX-2 (PDB ID: 5KIR)[5]. The protein was prepared using the BIOVIA Discovery Studio 2021 Client application. The docking was performed using PyRx having Auto dock Vina tool. A grid dimension of 69.02 Å x 69.58 Å x 50.40 Å was chosen for the experiment. The intermolecular interactions between the phytochemicals and the residues of the COX2 protein were identified and visualized using the Discovery Studio 2021 Client software[6]. Toxicity prediction of the compounds were performed using https://tox.charite.de/protox3/ [7].

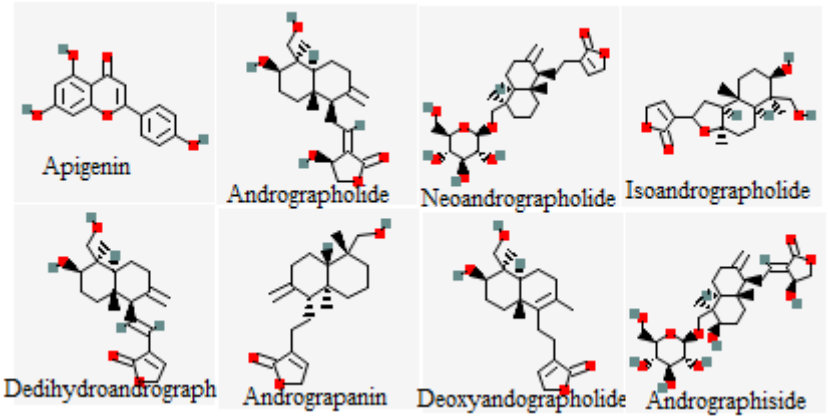

Fig 1. Ligands from *A. paniculata*

2.4 In vitro anti inflammatory study

Standard diclofenac was used as reference drug. 0.2g of powdered drug was added in 20 ml of deionized water. Serial dilution between the range of 0.1mg/ml-1mg/ml was performed for *A. paniculata* leaf extract. All samples contained 5.0 ml of total volume. Reaction mixtures were prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of BSA. Then 2 ml of *A. paniculata* extract from each different concentration were mixed with reaction mixtures. A similar procedure was used for reference drug and they were used as positive controls for this study. In addition, distilled water was used as negative control. The reaction mixtures containing extracts and drug. were incubated in a water bath at 37°C ± 2°C for 30 min, and later, it was heated at 70°C at which the reaction mixture was maintained for 15 min. Then, the reaction mixture was allowed to cool down at room temperature for 15 min. Absorbance of reaction mixture before and after denaturation was measured for each concentration (0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, and 0.4 mg/ml) at 680 nm using an UV spectrophotometer (Shimadzu 1900i) [8]. The inhibition is calculated by following formula.

$$\%Inhibition = \left(\frac{Absorbance of control - Absorbance of test}{Absorbance of control}\right) \times 100.$$

2.5 Formulation of herbal cream

In a 100 ml beaker oil phases bees wax(4 gm) and liquid paraffin (7 ml) were mixed and heated at 70^{o}C. In another 100ml beaker borax (2 gm) and water (q.s) mixed together and also heated at 70^{o}C. The process was followed by bees wax and liquid paraffin mixture added slowly into borax and water mixture with continuous stirring and maintain the heat.

After that *A. paniculata* extract were added in different concentration (0, 10, 20, 30 mg/ml) in the mixture to get F1, F2, F3 and F4 and clove oil (q.s) is added for essence [9].

2.6 Evaluation of herbal cream

The creams were evaluated based on their physical attributes like color, odor, state, consistency, pH, spreadability, washability, non-irritancy and stability.

2.7 Statistical analysis

The values are expressed as the mean ± standard error of the mean. The result is also expressed as an IC50 value. The IC50 value was calculated using logarithmic regression analysis.

3. Results and discussion

PyRx docking was utilized to ascertain the binding affinities and significant interactions between *A. paniculata* -derived phytochemical ligands and crystallographic structures of the structure of Vioxx bound to human COX-2 (PDB ID: 5KIR). The binding affinities of the acquired ligands and the standard NSAID drug Diclofenac. Table 2 shows the binding affinity obtained from the protein bound ligands and the standard drug Flurouracil [10] . The binding affinity of the *A. paniculata* ranged from -7.0 to -8.8 kcal/mol. The molecular interactions between the most active ligands and the active site of the COX II protein were visualized using the Discovery Studio 2021 Client program (Fig. 1). These samples showed the expected interactions with the protein active-region amino acids, indicating strong antagonistic characteristics against the Vioxx bound to human COX-2. For the protein coded 5KIR, Apigenin had the highest binding affinity of -8.8 kcal/mol followed by Andrographiside (-8.4 kcal/mol). As compared to the standard drug Diclofenac (-8.1 kcal/mol), the binding affinities of two natural compounds (Apigenin, and Andrographiside) derived from *A. paniculata* were found to be higher indicating their future usage in inhibition of inflammation. The same set of ligands were studied for their toxicity using ProTox II software showed Apigenin, Andrographiside II had a predicted class 4 to class 5 toxicity with higher LD50 ranging between 1400-4000 mg/kg values indicating their safe usage in human [11].

Table :1 Docking results of *A. paniculata* ligands

Ligands	Binding Affinity (cΔG in kcal/mol)
	3EQM
Andrographolide	-7.2
Neoandrographolide	-7.2
Isoandrographolide	-7.1
Deoxyandrographolide	-7.0
14 Deoxy 11, 12 didehydroandrographolide	-8.1
Andrograpanin	-7.2
Andrographiside	-8.4
Apigenin	-8.8
Diclofenac	-8.1

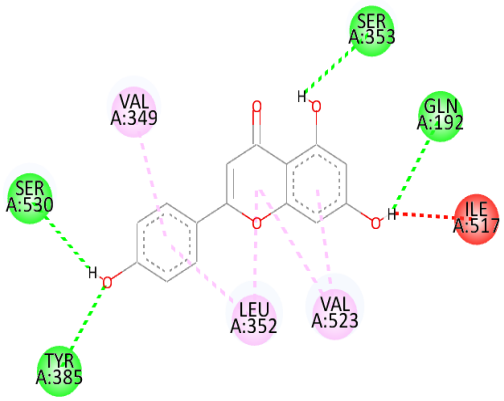

Fig.2 : Interaction diagram of Apigenin

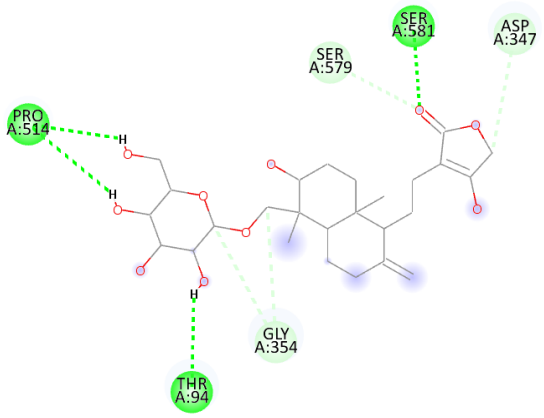

Fig. 3: Interaction diagram of Andrographiside

Anti-inflammatory activity of *A. paniculata* was evaluated against denaturation of BSA method. The highest inhibition rate was observed in the extract at the concentration of 0.25 mg/ml. µg/ml. All inhibitions are detailed in Table 2 given below. With the comparison of *A. paniculata* extracts (0.284 mg/ml), the inhibition rates of reference drug were found to be lower (0.47 mg/ml). Thus this indicates a potential anti-inflammatory property of *A. paniculata* extract [12].

Table:2: Anti-inflammatory activity of standard drug

Concentration(mg/ml)	**% inhibition**	**IC50 value**
0.1	0.12	
0.25	23.37	0.47 mg/ml
0.5	56.14	
1	83.92	

Table:3 Anti-inflammatory activity of *A. paniculata* extract

Concentration(µL)	**% inhibition**	**IC50 value**
0.1	1.7	
0.25	7.01	0.284 mg/ml
0.5	84.83	
1	61.12	

From the table 4 it can be observed that the result of all four formulations were stable and with the addition of extracts F2, F3 and F4 were found to be showing very smooth consistency. That may be due to the nature of the extract. Spredability of F3 was stand out indicating that F3 containing 20mg/ml was found to be having the best results and a similar trend was observed for pH. All the formulations were found to be non-irritant in nature [13].

Table:4 Evaluation of cream formulation

Sl.No	Parameter	F1	F2	F3	F4
1	Colour	Buff	Olive Green	Olive Green	Dark Olive Green
2	Odour	Yes (clove)	Yes (clove)	Yes (clove)	Yes (clove)
3	State	Semi solid	Semi solid	Semi solid	Semi solid
4	Consistency	Gritty	Smooth	Smooth	Smooth
5	Ph	7.12	7.08	7.01	7.05
6	Spredability	0.65	0.71	0.77	0.72
7	Washability	Easy washable	Easy washable	Easy washable	Easy washable
8	Non-irritancy test	Non-irritant	Non-irritant	Non-irritant	Non-irritant
9.	Stability	Stable	Stable	Stable	Stable

4. Conclusion

Through this study, an excellent anti inflammatory activity of *A. paniculata* leaf extracts were observed by in vitro process. More over GCMS studies showed around eight bioactive agents present in the extracts including Apigenin, Andrographolide, Neoandrographolide, Isoandrographolide, Dehydroandrographolide, Andrograpanin, Deoxyandrographolide and Andrographiside. To increase the confidence of the obtained result, *in silico* analysis was performed, which included toxicology analysis, and molecular docking which also ensured the anti inflammatory properties of this plant. In silico studies on these flavonoid compounds yielded that Apigenin (-8.8 kcal/mol), and Andrographoside (-8.4 kcal/mol), have the best binding energy as compared to the standard diclofenac indicating that the extract can find its usage as anti inflammatory drug. Further the claim was confirmed by in vitro study using BSA method. As compared to standard diclofenac (IC50: 0.47 mg/ml) the IC50 value of papaya extract was found to be only 0.284 mg/ml indicating a significant increase in inhibition at low concentration of the extract. Thus all the evidence confirm that the presence of flavanoid compounds prompts the extract to have anti inflammatory properties. A set of topical formulation was designed using concentration of extract with all formulation had high stability but F3 had the best pH and spreadability indicating 20mg/ml as the ideal concentration for preparation of formulation.

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