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Research Article

PHARMACOLOGY AND TOXICOLOGICAL ANALYSIS IN SILICO AND HEALING ACTIVITY IN VITRO OF FLAVONOID LUTEOLIN ISOLATED FROM *PROSOPIS JULIFLORA*T

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ABSTRACT

Introduction: the Flavonoids are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings, are present in plants and are synthesized by phenylpropanoid pathway. Luteolin (3',4',5,7-tetrahydroxyflavone) is a flavonoid exerts a variety of pharmacological activities including anti-oxidant properties. **Objective:** the aimed to analyze pharmacology and toxicological in silico and evaluate the in vitro wound healing potential of Luteolin. Methods: The in silico study were performed with the software Passonline, Molinspiration Cheminformatics and admetSAR. The in vitro study was using the spreading and migration capabilities of fibroblast cell line.**Results and discussion**:the luteolin isolated from *Prosopis juliflora* showed 25 possible pharmacological activities and adverse and toxic effects with genotoxic, nephrotoxic, neurotoxic. Was not classified as carcinogenic, and the acute oral toxicity. Conclusion: strach assay showed that the luteolin from P. juliflora allowed the cellular migration in 45% more than the control.

Keywords: In silico study pharmacology and toxicology. In vitro study for wound healing. *Prosopis juliflora*. Flavonoids. Luteolin.

INTRODUCTION

The Flavonoids are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings, are present in plants and are synthesized by phenylpropanoid pathway. Inflammatory activity and healing wound, antioxidant, antimicrobial, antifungal and anticancer activit are reported how biological, pharmacological and medicinal properties of flavonoids [1].

Luteolin (3',4',5,7-tetrahydroxyflavone) is a flavonoid exerts a variety of pharmacological activities including antioxidant properties associated with its capacity to scavenge oxygen and potent anti-inflammatory. Theseevidences indicate a Luteolin can be supported for ideal wound healing process [2, 3].

Wound healing is one of the most complex processes in the human body. It involves the spatial and temporal synchronization of a variety of cell types with distinct roles in the phases of hemostasis, inflammation, growth, re-epithelialization, and remodeling [4].

Prosopis juliflora belongs to the family Leguminosae (Fabaceae), sub-family Mimosoideae, and it has 44 species of which 40 are native to the Americas, three to Asia and one to Africa. The phytoconstituents present in this plant are tannins, phenolics, flavonoids, alkaloids, terpenes and steroids. This plant shows various pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial activity [3, 5].

The present study aimed to analyze pharmacology and toxicological *in silico* and evaluate the *in vitro* wound healing potential of Luteolin by using the spreading and migration capabilities of fibroblast cell line.

Material and Methods

Obtainment of luteolin

The experimental part was carried out at the Pernambuco Institute of Technology (ITEP) in Biotechnology laboratory.

The leaves of *P. juliflora* was collected of trees localized in the forest nursery of Rural Federal University of Pernambuco (UFPE), a kindness of Forest science department. The luteolin was isolated from leaves and extraction of plant material and chromatographic procedures were employed and the identification by comparison described by Preeti et al.[6] and Prabha et al. [7]. The Voucher of specie was deposited in the UFRPE.

Pharmacology and toxicological analysis

Initially the chemdraw software was used for the design of the molecule, then the analysis of the probability of the activity of the molecule was performed with the software *Passonline*. In the analysis of pharmacological parameters, the theoretical oral bioavailability of the product was evaluated by Lipinski's Rule of five with *Molinspiration Cheminformatics* software. The toxicological parameters as well as the theoretical study on the carcinogenic effect, the AMES test and the acute oral toxicity were carried out in the *admetSAR* program. All programs are freely available on the internet.

*Cell culture*and Cell viability assay.

The Mouse fibroblast cell line (L929) was purchased from American Type Culture Collection (ATCC) obtained from the Tecpar Laboratory. Cells were maintained at Roswell Park Memorial Institute medium (RPMI1640, Sigma-Aldrich), L-Glutamine and supplemented with 10% of Fetal Bovine Serum (FBS, Sigma-Aldrich), 2 g/L sodium bicarbonate, 10 ml/L penicillin and streptomycin (PSA), 5 ml/L 4- (2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Cell cultures were maintained at 37 °C in a humidified 5% CO_2 incubator.

L929 cells were seeded in 96-well plates at a concentration of 2X10 5 cells/well and incubated for 48 hours at 37 ° C. Then a series of ten luteoline dilutions were added per well and incubated for an additional 24 hours. Three replicates were used for each concentration. Negative and positive control tests were also prepared using distilled water and ethanol (70%), respectively. 24 hours later, the treatment solutions were removed from the wells which were washed again with PBS to remove any remaining traces. 10µl of MTT (5 mg/ml in PBS) solution and 90µl ascorbate free cell culture media, RPMI 1640 was added and incubated for another 4 hours at 37 °C. Reaction was stopped by adding 100 µl Dimethylsulfoxide (DMSO, Spectrophotometric grade) and finally absorbance was measured using ELISA reader at wave lengths: reference, 630nm and test, 570 nm.*Invitro* cytotoxic activity was

measured by the MTT assay as previously describedMosmann[8]. Allexperiments were carried out in triplicate.

Healing activity in vitro

The spreading and migration capabilities of fibroblast cell line (L929) were assessed using a scratch wound assay which measures the expansion of a cell population on surfaces. The cells wereseeded into 24-well tissue culture dishes containing coverslips-coated with collagen type I (40 µg/ml) for 2h at 37 °C, at a concentration of 3×10^5cells/ml and cultured in medium containing10% FBS to nearly confluent cell monolayers, performed Liang et al. [9] and Bobadilla et al.[10].Then, a linear wound was generated in the monolayer with sterile 100µl plastic pipette tip. Any cellular debris was removed by washing the coverslips with phosphate buffer saline (PBS). DMEM medium with dimethylsulfoxide (0.25%) (control group), platelet derived growth factor (2ng/ml) (as positive control), the luteolin (10 and 50 µg/ml), the commercial *Hypericum perforatum* oil (0.5 µg/ml) was added to a set of 3 coverslips per dose and incubated for 12h at 37 ºC with 5% CO_2. Thecells were fixed with 4% paraformaldehyde for 15min and stainedwith 4′,6-diamino-2-phenylindole (DAPI) overnight. Three representative images from each coverslip of the scratched areas underreach condition were photographed to estimate the relative migration cells. The data were analyzed using NIS elements F 3.2 software. The experiments were performed at least in duplicate.

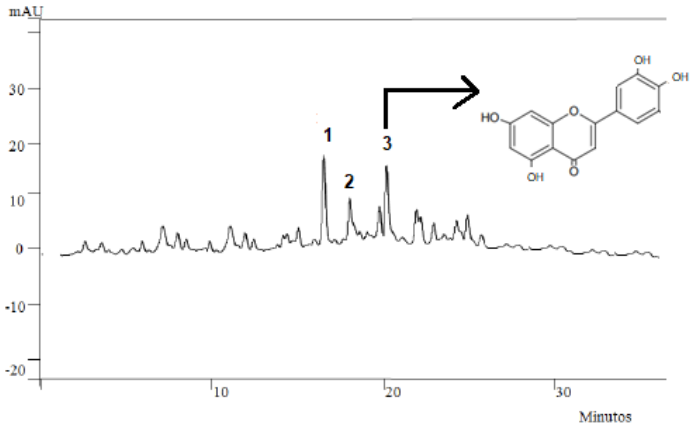
Statistical analysis

BioEstat 5.0 software was used for all statistical analyzes. Data are expressed as values: mean ± SE of eight repetitions andanalyzed by one-way analysis of variance with p <0.05 when compared to control indicated statistically significant difference in relation to the respective group using ANOVA,followed by Tukey's comparison test (p> 0.05).

Results and Discussion

The extensive HPLC-DAD analysis showed luteolin (3) identified at 21.04 minacording of figure 1.The retention times (tR) of the luteolin-related indicators were compared(tR: 2.,577min) with chromatograms Shanmugam et al. [11].

Fig. 1: Cromatogram HPLC-DAD of *Prosopis juliflora*.



Acoording of Preeti et al. [6], flavonoids are used commercially as biologically active compounds and generally high value-low volume products than the primary metabolites, which are used in drug manufacture by the pharmaceutical industries.

The pharmacological activities were detected in the *Pass online*. Were detected 25 possible pharmacological activities among them, antioxidant, antimutagenic, kinase inhibitor (Table 1). The analyzing the probability of developing pharmacological substances and predicting the biological activity potential of a molecule, it was possible to use as composite resources, through the Pa (Probability of being active) and Pi (Probability of being inactive) indices, using a description by Berlink et al [12].

Table 1: *In silico* prection of biological activities of the flavonoid luteolin isolated from *P. juliflora* using *Pass online* (http://www.pharmaexpert.ru).

All Pa>Pi Pa>0,3 Pa>0,7 ok

Pa	Pi	Activity
0,978	0,001	Chlordecone reductase inhibitor
0,965	0,003	Membrane integrity agonist
0,964	0,003	HIF1A expression inhibitor
0,953	0,002	Membrane permeability inhibitor
0,952	0,002	2-Dehydropantoate 2-reductase inhibitor
0,947	0,001	Aryl-alcohol dehydrogenase (NADP+) inhibitor
0,947	0,003	Aldehyde oxidase inhibitor
0,942	0,001	P-benzoquinone reductase (NADPH) inhibitor
0,940	0,001	Antimutagenic
0,940	0,002	Kinase inhibitor
0,942	0,005	CYP2C12 substrate
0,936	0,002	Peroxidase inhibitor
0,935	0,002	HMOX1 expression enhancer
0,932	0,002	CYP1A inducer
0,927	0,001	NADPH-ferrihemoprotein reductase inhibitor
0,923	0,002	UGT1A6 substrate
0,918	0,003	Anaphylatoxin receptor antagonist
0,914	0,001	SULT1A3 substrate
0,916	0,004	TP53 expression enhancer
0,913	0,001	CYP1A1 inducer
0,912	0,002	UGT1A9 substrate
0,909	0,002	Histidine kinase inhibitor
0,808	0,016	Mucomembranous protector
0,791	0,007	CYP3A4 inducer
0,783	0,002	CYP19A1 expression inhibitor
0,777	0,004	MMP9 expression inhibitor
0,775	0,004	Antioxidant
0,783	0,014	Antineoplastic
0,772	0,003	UGT1A8 substrate
0,769	0,001	Iodide peroxidase inhibitor
0,771	0,009	Alkane 1-monooxygenase inhibitor
0,764	0,002	CYP1B1 inhibitor
0,762	0,002	1-Alkylglycerophosphocholine O-acetyltransferase inhibitor

Legende: Pa (Probability of being active); Pi (Probability of being inactive)

The possible adverse and toxic effects were based onmanifestations, sometimes seen in few or even a single patient, are shown in table 2.

Table 2: In silico prediction of adverse and toxic effectsof the flavonoid luteolin isolated from *P. juliflora* using *Pass online* (http://www.pharmaexpert.ru).

All Pa>Pi Pa>0,3 Pa>0,7 ok

Pa	Pi	Activity
0,824	0,004	Genotoxic
0,819	0,016	Reproductive dysfunction
0,823	0,028	Shivering
0,804	0,017	Toxic, vascular
0,752	0,014	Urine discoloration
0,734	0,012	Endocrine disruptor
0,713	0,039	Ulcer, aphthous
0,693	0,022	Allergic contact dermatitis
0,675	0,020	Panic
0,658	0,021	Hypothermic
0,654	0,030	Inflammation
0,699	0,100	Twitching
0,648	0,053	Hematotoxic
0,585	0,011	Acidosis, lactic
0,613	0,047	Nephrotoxic
0,606	0,043	Neurotoxic
0,595	0,034	Fibrosis, interstitial
0,610	0,054	Hepatotoxic
0,570	0,020	Photoallergy dermatitis
0,603	0,055	Excitability
0,594	0,056	Occult bleeding
0,561	0,027	Non mutagenic, Salmonella

Legende: Pa (Probability of being active); Pi (Probability of being inactive)

The flavonoid under study does not go against any of Lipinski Five Rules, is the product has a good therorical oral bioavailility (Table 3). The flavonoid was also non-toxic by the AMES test (software *admet*SAR), not classified as carcinogenic, and the acute oral toxicity category III acoording Oliveira et al. [12].

Table 3:*In silico* therorical oral bioavailility by Lipinski's rulesof luteolin isolated from *P. juliflora* using*Molinspiration*® (http://www.molinspiration.com/cgi-bin/properties).

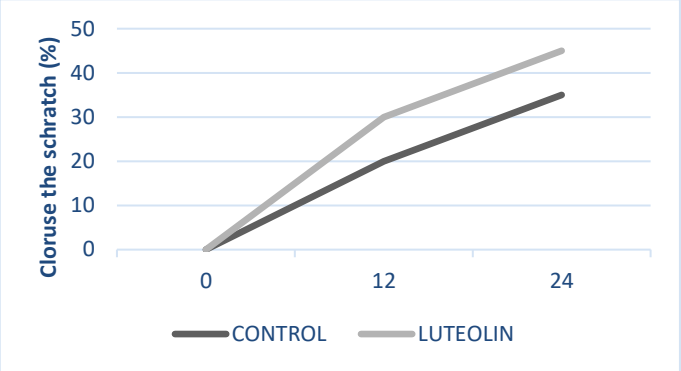
Flavonoid	miLogP	MW	HBD	HBAs	Rot B
Luteolin	1.97	286.24	5	4	1

Legende: miLop (physicochemical property parameters); MW (molecular weight); HBD (number of hydrogen bond donors); HBAs (hydrogen bond acceptors); Rot B (rotatable bonds)

An ideal drug molecule would comply with the physicochemical property guidelines of Lipinski's Rule ofFive (RO5). It predicts the drug likeness of a chemical compound with a certain biological activity designedfor oral route of administration. According to the RO5, a drug-like compound shouldhave a molecular weight (MW) of <500 g/mol, a log p value of <5 representing its hydrophobicity, no >5hydrogen bond donors (HBDs), and no >10 hydrogen bond acceptor (HBA) and <10 rotatable bonds (RotB)[13]. The luteolin from *P. juliflora* showed physicochemical property according of RO5.

The *in vitro* scratch assay is the metod based on the observation that, upon creation of a new artificial gap, so called "scratch", on a confluent cell monolayer, the cells on the edge of the newly created gap will move toward the opening to close the "strach" until neu cell-cell contacts are estabilished again [9]. Strach assay showed that the luteolin of *P. juliflora* allowed the cellular migration in 45% more that the control, acoording of Figure 2. The luteolin not toxic in citotoxic assay.

Fig. 2: Cell migration *in vitro* of fibroblast tratated and non-tratated with luteolin from *P. juliflora* using by hours using *in vitro* scratch assay.



CONCLUSION

Pharmacology and toxicological analysis *in silico* and healing activity *in vitro* of flavonoid luteolin isolated from *Prosopis juliflora* showed 25 possible pharmacological activities among them, antioxidant, antimutagenic, kinase inhibitor; adverse and toxic effects with genotoxic, nephrotoxic, neurotoxic and was not classified as carcinogenic, and the acute oral toxicity. Strach assay showed that the luteoli from *P. juliflora* allowed the cellular migration in 45% more than the control.

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