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Toxicological Analysis by Computational Assessment Tools of Herbal Medicine Compounds Urushiol II and Bhilawanol-B from Semecarpus Anacardium

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Abstract

Background: *Semecarpus anacardium* used in many Indian folklore medicine as 'Ballataka' or 'Bhilwa'. They are also known to cause pathological conditions by the presence of toxic principles in nut and roots of plants and possible pharmacokinetic and toxicological profiling of known toxic principles are explored by computational tools.

Aims & Objectives: The aims of the study were to study toxicological active principle Urushiol II and Bhilawanol-B by computational analysis and prediction tools.

Materials & Methods: This study was investigated on web-based tools PubChem to extract the chemical structure, followed by authentication and validation with the chemical formula. The two-dimensional structures are further converted to three-dimensional (3D) structure with ChemSketch software; the derived 3D structures are then screened for molecular properties followed by absorption, distribution, metabolism, elimination, and toxicity through admetSAR software. The reports are analyzed and predicted for pharmacokinetic and toxicity characters of Urushiol II and Bhilawanol-B from *S. anacardium*.

Results: The compounds screened Urushiol II and Bhilawanol-B from *S. anacardium* both had drug likeness score of MLOGP>4.15, the LD 50 values of urushiol II is comparatively more 4000 mg/kg/d than bhilawanol 2700 mg/kg/d for rat oral route testing and positive predictive results for skin irritation and allergic dermatitis. The genotoxicity battery assay was negative for both Urushiol II and Bhilawanol-B.

Conclusion: The computational analysis predicts the uroshiol II and bhilawanol-B present in *S. anacardium* belongs to class II Toxic hazard classification.

Keywords: *Semecarpus anacardium*; folklore; toxicology; urushiol II; bhilawanol-B.

Introduction

In folklore and traditional medicine, mostly many medicinal preparations are extracted from various plant sources.[1] Complementary and Alternative Medicine (CAM) plant preparations are widely used when disease treatment not affordable and amenable to treatment by modern method[2].

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Semecarpus anacardium Linn. Belongs to family: *Anacardiaceae* distributed in sub-Himalayan region, tropical and central parts of India. The plant well-known for its medicinal value in Ayurvedic and Siddha system of medicine utilizes this plant for its medicinal value and commonly known as marking nut (dhobi nut) with vernacular names as 'Ballataka' or 'Bhilwa'.[3,4] The available literature review revealed presence of various components from *S. anacardium* such as phenolic compounds, biflavonoids, sterols and glycosides.[5-8]

The constituents of our study interest is present in the fruit pericarp called as marking nut include 1-(2,3-dihydroxyphenyl)-8-pentadecene called Urushiol II and o-dihydroxy compound called bhilawanol. Previously reported pharmacological activity of *S. anacardium* includes anti-atherosclerotic activity[9], anti-inflammatory activity of SA for both immunological and non-immunological origin[10], antioxidant activity[11], hypoglycemic effect[12] and anti-spermatogenic effect[13] in various animal published studies. The present study is an attempt to explore toxicological aspects and pathogenesis predictions of Urushiol II and Bhilawanol-B using standardized computational tools. Utilization of computational research methods and analysis is economical, time saving with advantage to possibilities to predict toxicological properties avoiding animal and humans exposure and provide results to understand pathophysiological reasons to guide further wet lab research.

Aims and Objectives

The Study aims to screen pharmacokinetic and toxicological properties of known biological active compounds Urushiol II and Bhilawanol-B from *S. anacardium* by standardized computational analysis tools.

Materials and Methods

Hardware and Software

The selected compounds molecular properties of chemical structure from leaf extract of *A. paniculata* extract are carried out in Lenova 2021 Model installed with windows 11 software, java enabled with updated plugins.

Data Set

The chemical structures of Urushiol II and Bhilawanol-B from *S. anacardium* with two-dimensional (2D) pictures were collected from accredited indexed published journals and other sources such as PubChem, Chembank, ChemPDB, and Asinex Ltd. After a detailed review, the structures are developed with ChemSketch, followed by PHASE software module was used to convert the 2D structures into three-dimensional (3D) structures.[14]

Virtual Screening

Pharmacokinetic and Toxicological Prediction

The 3D structures developed are explored virtually using online prediction software[15]. Results from computational analysis of compounds on absorption, distribution, metabolism, excretion, and toxicity are acquired in the virtual screening workflow protocol.

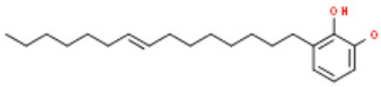
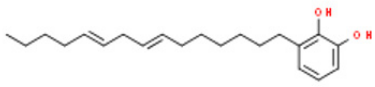
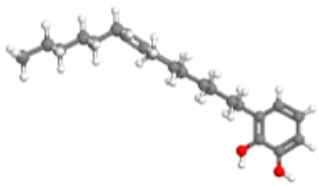
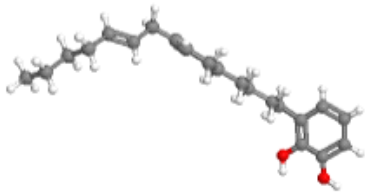
Statistical Methods and Calculation

Interactive molecular properties calculator applet (MolSoft L.L.C. San Diego, CA, USA) is used for molecular volume and drug-likeness score. The study is done in the department of Forensic medicine, Pharmacology and the college digital library using online tools during Dec 2021 to Feb 2022. The study was self-funded conducted at SVIMS University, Tirupati, Andhra Pradesh, India. The study is considered under the category for exemption from institutional ethics committee approval as it does not involve animals and humans and done by computational bioinformatics tools.

Results and Discussion:

The present study is designed to study and analyze Toxicological properties of Urushiol II from *S. anacardium* followed by a prediction of pharmacokinetic parameters based on results obtained by bioinformatic experimental models. From the detailed review of literature from earlier studies Urushiol II and Bhilawanol-B from *S. anacardium,* the 2D chemical structure were retrieved from PubChem online compound database platform and the 2D structures converted to 3D structure by ChemSketch software [Table 1].[16] The 3D structures are the processed with MOLSOFT L.L.C Software, and the molecular properties are predicted [Table 1].

Table 1: *S. anacardium* compounds 2D, 3D structure & Physiochemical properties

Sl.No	Characters	Urushiol II	Bhilawanol-B
1.	2D Structure		
2	3D Structure		
3	Chemical Formula	C21H34O2	C21H32O2
4	Molecular weight	318.49 g/mol	316.48 g/mol
5	Num. H-bond acceptors	2	2
6	Num. H-bond donors	2	2

The results predict 2 hydrogen bond acceptor and donor site in the structure of both molecules which could be analysed to have better absorption and permeation. The Pharmacokinetics prediction profile of Urushiol II revealed similarly to have high gastro enteral absorption but cannot cross the blood brain barrier and not a substrate of P-Glycoprotein [Table 2]. With metabolism prediction with CYP4503A4 a most common pathway for xenobiotics Uroshiol II did not inhibit where as Bhilawanol –B found to enzyme inhibitor, which may be expected to cause drug interactions.

Table 2: ADME predicted profile of *S. anacardium* nut active compounds

Sl.No	Pharmacokinetic Parameters	Urushiol II	Bhilawanol-B
1.	G.I Absorbtion	High	High
2	BBB	No	No
3	P-gp Substrate	No	No
4	CYP450 3A4 Inhibitor	No	Yes
5	Log K_P	-2.90cm/s	-3.37cm/s
6	Druglikeness	Yes; 1 violation: MLOGP>4.15	Yes; 1 violation: MLOGP>4.15
	Bioavailability Score	0.55	0.55

This prediction of permeability coefficient (K_p) for the transport of compounds through mammalian epidermises is based on the linear model by Potts RO. and Guy RH. Based on the Computational analysis the results of uroshiol II and bhilawanol-B were -2.90cm/s and -3.37 cm/s respectively[Table 2]. The analysis of reports shows very high penetrability over topical application of both molecules.[17] The Predictions results revealed drug-like physicochemical properties maintained by both uroshiol II MLOGP>4.15 and bhilawanol-B MLOGP>4.15 with good bioavailablity score of 0.55 [Table 2] which is analyzed as sufficient for oral absorption as described by Lipinski's rule (*Lipinski CA (December 2004).*[18]

Table 3A: Toxicology prediction profile of *S. anacardium* nut active compounds

Sl.No	Species	LD50(mg/Kg/d)		Reliability Index	
1.	Rat Oral	**Urushiol II**	**Bhilawanol-B**	**Urushiol II**	**Bhilawanol-B**
		4000	2700	0.52	0.53
2.	Rat Intraperitoneal	180	130	0.28	0.39
3.	Mouse Oral	790	520	0.44	0.27
4.	Mouse Intraperitoneal	130	190	0.77	0.81
5.	Mouse Intravenous	31.91	6.92	0.28	0.29
6.	Mouse Subcutaneous	180	140	0.25	0.29

Reliability index: <0.3 = Not reliable prediction quality; 0.3-0.5 = borderline prediction quality; 0.5-0.75 = moderate prediction quality; >0.75 = high prediction quality. LD-Lethal dose.

Acute toxicity LD50 prediction models over rat oral and mouse intra peritoneal analysis revealed highly predictable index and the LD 50 values of urushiol II is comparatively more 4000 mg/kg/d than bhilawanol 2700 mg/kg/d for rat oral route testing[Table 3A].

Table 3B: Toxicology prediction profile of *S. anacardium* nut active compounds

Sl.No	Toxicology Profile	Urushiol II	Bhilawanol-B
1.	Toxic hazard classification by Cramer	Class -II	Class -II
2.	Skin irritation(rabbits)	Positive	Positive
3.	Allergic contact dermatitis (human & guinea Pig)	Positive	Positive
4.	Respiratory Sensitization	Negative	Negative
5.	Teratogenic potential (human Model)	Negative	Negative

Table 4: Genotoxicity prediction profile of *S. anacardium* nut active compounds

Sl.No	Parameters	Urushiol II	Bhilawanol-B
1.	Structural Alerts for DNA Reactivity	Negative	Negative
2.	Ames test in S. typhimurium (in vitro)	Negative	Negative
3.	Mutagenicity consensus	Negative	Negative
4.	Micronucleus Test in Mouse Erythrocytes	Negative	Negative
5.	Comet Assay in Mouse	Negative	Negative

The toxicological prediction of skin irritation (rabbit model) and allergic contact dermatitis(in human & guinea Pig model) was positive as compared with literature review [Table 3B]. This explains that when applied externally, the juice causes irritation and a painful blister, which produces eczematous eruptions of the neighboring skin with which it comes into contact, and there is itching. The lesion resembles a bruise (artificial bruise). To fabricate wounds (usually bruise) by external application of juice over the skin is a common criminal practice by malingers.[19-21]

Histopathologic study is considered gold-standard for diagnosis of skin lesions, either neoplastic or inflammatory, most of the times macroscopically when a blister is less than 0.5 cm, it is called a vesicle and when it's greater than 0.5 cm, it is called a bulla. In humans, high concentrations of an irritant will produce marked ballooning of keratinocytes in the upper epidermis with variable necrosis ranging from a few cells to confluent areas of the epidermis.

Neutrophils are found in the areas of ballooning and necrosis, and mild spongiosis is also present in the adjacent epidermis. If low and medium concentrations of an irritant are applied, the histopathological spectrum of the reactions produced often mimics that seen in allergic contact dermatitis, with epidermal spongiosis, mild superficial dermal edema, and a superficial, predominantly perivascular infiltrate of lymphocytes. Pustular reactions show sub corneal vesicles with neutrophils, cellular debris, and a fibrinous exudate. There are also some neutrophils in the upper dermal infiltrate.[22]

Both uroshiol II and bhilawanol-B prediction analysis revealed negative to respiratory sensitization and Tearatogenic potential to human models and the battery of genotoxicity profiling was also negative and falling in class II hazardous chemicals probably explains the use in traditional medicines with the indigenous ways of detoxification and use as medicinal substances.[Table 3B & 4].

Conclusion

The use of computational bioinformatics tool is a test method that substitutes traditional animal models with non-animal systems such as *insilico* standardized models benefits in the Reduction, Refinement, and Replacement of animals used in research and testing. Skin contact with the acrid juice *Semecarpus anacardium* is known to cause skin and mucosal irritation, inflammation, vesication, and ulceration[23]. The present computational analysis predicts the presence of uroshiol II and bhilawanol-B in juice can cause skin sensitization with better dermal penetrability and ability to cause allergic dermatitis A variety of nut extract preparations from this source are effective against many diseases, viz., arthritis, tumors, and infections and so on in ayurveda and siddha medicine. The negative teratogenic ,genotoxic potential and compounds are predicted to be class II toxic hazard classification by Cramer. However, the mechanism of the pharmacological action and its scientific validation in traditional use of its nut can be greatly aided by the isolation of its active principle and determination of structure–function relationship for pharmacological and toxicological activity by further wet lab studies.

Conflict of Interest: None declared.

Ethical clearance: No Humans or animals involved in study.

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