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Acute Toxicity and Irritancy of the Essential Oil of the Leaves of *Vitex simplicifolia* Oliv. (Verbenaceae) in Burkina Faso

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ABSTRACT

The main goal of this study was to determine the acute toxicity and irritancy of the leaf of essential oil of *Vitex simplicifolia* Oliv. (Verbenaceae) growing in Burkina Faso and whose therapeutic use is multiple. The acute toxicity of the essential oil was determined by Litchfield method. Skin and eye irritancy was performed by Draize method. The acute toxicity of essential oil was evaluated to give a LD_{50} = 2830.77±299.22 mg kg^{-1} and showed that the essential oil of *Vitex simplicifolia* is slightly toxic. Skin and eye irritation was evaluated and showed that essential oil of this plant is slightly irritating with a primary irritation index PI = TEO/16 = 0.75, an Acute Ocular Index, A_c.O.I. =12.83, an Average Ocular Index, A_v.O.I. of less than 5 after 48 h. The results suggest that the leaf essential oil of *Vitex simplicifolia* Oliv. possessed slightly toxic and slightly irritancy activities. It could be used as natural potential ingredient for pharmaceutical industry.

Key words: Verbenaceae, *Vitex simplicifolia* oliv., acute toxicity, skin irritation, ocular irritation

INTRODUCTION

Vitex simplicifolia oliv. (Verbenaceae) is a species of tropical Africa extends to Egypt. It is a plant of the Sudanian and Guinean savanna woodlands, slopes, fallow and rocky soils (Eyog *et al*., 2006).

In Burkina Faso is a plant whose use of traditional medicine is multiple. Studies by Nacoulma-Ouedraogo (1996) showed that this plant is used in the treatment of dermatoses, dermatitis, bilharzia, headaches, fever, muscle aches, amoebiasis, toothache, colic. *Vitex simplicifolia* Oliv. is also used in the treatment of malaria and infant tetanus. The study of the chemical composition of the essential oil of the leaves (Ouoba *et al*., 2009) has shown that it is rich in hydrocarbon monoterpenes and sesquiterpenes, however, very few works have been done on the pharmacological properties of this plant (Ouoba *et al*., 2012). The aim of this study is to evaluate the acute toxicity, skin and eye irritant activities of the essential oil of the plant in order to allow better use.

MATERIALS AND METHODS

Toxicity study

Acute: The LD_{50} of the essential oil of *Vitex simplicifolia* Oliv. was determined on five groups of six albino rats each (Litchfield and Wilcoxon, 1949). Five batches are composed of male and female rats aged three months whose body weights are between 47.5 and 128 g. Animals receive essential oil orally (esophageal gavage) with increasing dose (Table 1).

The results are obtained by the method of Litchfield and Wilcoxon (1949). These were by:

- The manual process
- Data processing software on a suitable method (Pharmacological Calculation System PCS)

The class of toxicity was assessed according to the WHO (2002), adaptation of the current method of Hodge and Sterner (1943).

Tests of skin and ocular irritations

Determination of skin irritancy: The assessment of skin irritancy of the essential oil of leaves of *Vitex simplicifolia* oliv. (Verbenaceae) was performed by the Draize rabbit method (Draize *et al.*, 1944) revised in the Official Journal of the French Republic on 21/04/1971. With 0.5 mL of essential oil compress is soaked and secured by a non-irritating tape stuck to one side flank not incised and another on the other flank incision.

Determination of ocular irritation: Highlighting the irritancy of the essential oil of leaves of *Vitex simplicifolia* oliv. (Verbenaceae) on the eyes was performed by the method of Draize *et al.* (1944), revised in the Official Journal of the French Republic on 21/04/1971. The 0.1 mL of the essential oil is introduced into the conjunctival sinus in one eye, the other serving as a control. After administration of the eye lids are held closed for 60 sec, then the animals are returned to their cages. The ocular examination was carried out at both treated eyes compared to controls and made an observation after: 1, 24, 48, 72, 96 h and 7 days.

RESULTS AND DISCUSSION

Acute: Signs of toxicity (toxidrome) are observed during the experiment, during 2 h following the administration of the oil, 24, 48 and 72 h after. These signs are: a decrease in tensile strength and a decrease in exploration instinct; neurobiological impairment with apparent effects type cholinergic (nasal hyper secretion, diarrhea), dyspnoea and death of the animal depending on the effect of the administered dose.

Table 1: Determination of LD_{50} of *Vitex simplicifolia* essential oil

Lots	Dose (mg kg^{-1})	No. of rats
I	2000	6
II	2500	6
III	3000	6
IV	4000	6
V	Control	6

The rats were kept under observation for 2 h for the immediate toxicity and up to 72 h after having re-established a normal diet (water, granules)

General signs of intoxication occur in the first moments after the administration of the oil and can last for 72 h. Surviving animals recover with time. Acute clinical signs observed in different doses are attributed to intense biological action of certain families of compounds. Ketone compounds are physiologically very active, they are the cause of seizures preceded by salivation observed in rats, within 2 h after the administration of the essential oil at doses of 3000 and 4000 mg kg^{-1}. This action is of central origin (central nervous system) and could thus lead to death (Bruneton, 1993a).

Evaluation of LD_{50}: The result of acute toxicity was summarized in Table 2 and the dependance of animal mortality with the extract dose in Fig. 1.

Equation of regression line giving the animal mortality in the logarithm of the dose of essential oil is indicated as well as the LD_{50} value. By data mining with the PCS we obtained the following results:

- There is a good correlation (R_2 = 0.96) between mortality rates and doses recorded. Indeed, the equation of the regression allows us to estimate the LD_{50} of the essential oil of *Vitex simplicifolia* oliv. to 2830.77±299.22 mg kg^{-1}. The dose used to avoid animal mortality is less than 2109.72 mg kg^{-1} (DL_1), while a dose of approximately 3798.26 mg kg^{-1} (DL_{99}) leads to almost certain death

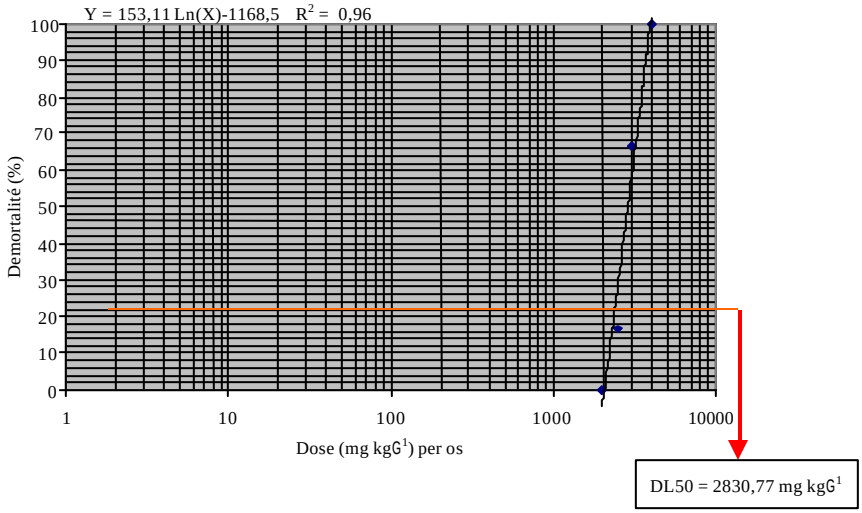

Fig. 1: Graph of acute toxicity, the cumulative mortality within 72 h after oral administration (digital process)

Table 2: Data processing of acute toxicity

Lots	Dose (mg kg^{-1})	Log doses	Deaths (%)	Probits
I	2000	3.301	0	2.251
II	2500	3.397	16.66	4.032
III	3000	3.477	66.66	5.430
IV	4000	3.602	99.70	7.748

DL_1: 2109, 73 mg kg^{-1} LD_{50} = 2830. 77 mg kg^{-1} DL_{99} = 3798. 26 mg kg^{-1}, ● LD_{50} and its confidence limits: LD_{50} = 2830. 77 mg kg^{-1} (270; 61; 299; 21), ● Confirmation of the validity of the data obtained: DL_{50}/DL_1 = 1. 34 DL_{99}/DL_1 = 1. 34, ● Security Index essential oil tested DL_{99}/DL_1 = 1. 80

- Reports and DL_{50}/DL_1 DL_{99}/DL_{50} are equal (1.34), which demonstrates the validity of the slope of the regression line. The report indicates DL_{99}/DL_1 narrow safety index of 1.80. The low acute toxicity on rats also apply to humans, according to the toxicity scale of Hodge and Sterner (1943) and the WHO (2002)

Skin irritant activities: Table 3 showed observations of incised parties and non incised, the Primary Irritation Indice (PI) and the irritant activities of essential oil. The primary irritation indice (PI) obtained was 0.75 and between 0.5 and 2. In accordance with the scale of Draize *et al.* (1944), we can consider that extract of essential oil *Vitex simplicifolia* Oliv. was slightly irritating dermal rabbits (Draize *et al.*, 1944). An amount of 550 mg (0.5 mL) of essential oil caused slight irritation to the skin of rabbits during the first 24 h and then regressed this irritation over time for 48 and 72 h.

This essential oil could therefore be used without any risk of dermal toxicity or potential damage to the skin. Irritation characterized by erythema (skin redness) was observed just after 24 h. Skin forming an effective barrier between the body and external aggressions, the absorption of certain chemicals can produce skin effects. The slight irritation of *Vitex simplicifolia* Ohv. essential oil could be due to the presence of nonanal (in small quantities) and of terpenes revulsive properties that may cause an increase in microcirculation, warmth and low reddening (Bruneton, 1993b). Terpene alcohols with low molecular weight having a higher degree of unsaturation facilitate the penetration of hydrophilic drugs across the stratum corneum (Ghafourian *et al.*, 2004). Xiong *et al.* (1996) showed that the activity of nerolidol was diffusive due to its amphiphilic structure that allowed it to pass through the lipid layer of the stratum corneum (horny layer) of the epidermis. However, the irritant effect of the essential oil on the skin of rabbit was local and reversible, because it is at the contact area and disappears when exposure to the oil ceases (for 24 h maximum).

Table 3: Result of the skin irritant test of essential oil of *Vitex simplicifolia* oliv.

		24 h		72 h		
						Total (24+72 h)
Rabbit No.	Flank	Erythema	Oedema	Erythema	Oedema	Erythema+Oedema
1	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
2	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
3	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
1	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
2	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
3	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
4	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
5	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1
6	Non incised	1	0	0	0	1
	Incised	1	0	0	0	1

Total (TEO), 12IP: TEO/16 = 0.75, Conclusion: Slightly irritant

Table 4: Results of ocular irritation test of *Vitex simplicifolia* essential oil oliv

	Individual eyepiece index (IOI) per observation period					
Rabbit No.	1 h	J1	J2	J3	J4	J7
1	16	4	0	0	0	0
2	14	4	0	0	0	0
3	16	4	0	0	0	0
4	11	2	0	0	0	0
5	11	2	0	0	0	0
6	9	2	0	0	0	0
Total	77	18	0	0	0	0
Average =	12.83	3	0	0	0	0
A_v.O.I.	3					
Highest Av.O.I. = Ac.O.I.	12.83					
Conclusion	5<12.83<15 Av.O.I. and <5 to J2 ⇒ slightly irritant					

Av.O.I.: Average ocular index, Ac.O.I.: Acute ocular index

Eye irritant: Table 4 showed the indices for individual eye observation period for the determination of the irritant essential oil.

The highest acute ocular index is 12.83 and the average ocular index is less than 5 after two days (5<12.83<15 Av.O.I. and <5 to J2). In accordance with the scale of Draize *et al.* (1944) ICCVAM Summary Review Document (2010), we can consider that extract essential oil *Vitex simplicifolia* oliv. (Verbenaceae) is slightly irritating to rabbit eye. Then, 0.5 mL of either 550 mg of essential oil of *Vitex simplicifolia* Oliv. causes slight ocular irritation in rabbits. This irritation is observed from the first moment that is within 60 min of administration up to 24 h and then disappears and regress from 48 h until the end of the experiment.

Low irritation to rabbit eye shows that this product will cause less damage in a usage error. So, we could easily reduce or neutralize its effect by appropriate solvents (simply by flushing the eye with plenty of water).

In fact, the local effect of the essential oil causes a reversible redness of the conjunctiva and it would be caused by the action of terpenes repellents that increases the microcirculation and cause slight ocular rubéfactions (Bruneton, 1993a). However, the aggressiveness of the essential oil of *Vitex simplicifolia* Oliv. on rabbit eye does not affect the structure and functioning of the conjunctiva and iris.

CONCLUSION

The preliminary study on the acute toxicity of the essential oil has ranked among the products of low toxicity according to the WHO scale. Tests of skin and ocular irritation were used to classify the essential oil from the mild irritant according to the scales of Draize *et al.* (1944) and Kay and Calandra (1962).

The overall results of the toxicological study justify the traditional uses of the plant. Further work will be considered in irritation to thoroughly check the healing properties of essential oil on the deeper scarring.

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