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Research Article

Phytochemical, Acute Toxicity and Tolerance Evaluation of *Solanum rugosum* (Solanaceae) on Skin and Eye

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Abstract

Background and Objective: The root bark of *Solanum rugosum* is used in traditional Ivorian medicine to treat certain eye diseases such as allergic, bacterial and viral conjunctivitis. Thus, the objective of this study was to highlight the presence of alkaloids in the root bark of *Solanum rugosum* and evaluate *in vivo* the acute oral toxicity as well as the skin and eye tolerances of the extract.

Materials and Methods: The powdered root bark was refluxed for 15 min in distilled water. The chemical study of the extract was made by LC-ESI-Q-TOF-MS. For acute oral toxicity in mice, skin and eye tolerance OECD methods 423, 404, 405 were used, respectively.

Results: Four alkaloids including lycorine, corynanthine, corynantheine and mitrinermine were found in the root bark. The cutaneous and ocular tolerance tests carried out demonstrated good cutaneous and ocular tolerances of the extract. **Conclusion:** The findings of the study revealed that the total aqueous extract of the root bark of *Solanum rugosum* is not toxic and is well tolerated.

Key words: *Solanum rugosum*, Solanaceae, LC-ESI-Q-TOF-MS, acute toxicity, skin and eye tolerances

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In many pharmacopoeias, there is a large number of plant species used in the treatment of eye conditions. These plants for ocular purposes must be the subject of special attention because of the irreversible lesions that can occur following the use of toxic plants^{1,2}.

The Solanaceae family is typically ethnobotanical and it is widely used for food and health care. Because of their alkaloid content, many species provide stimulants, poisons, narcotics, analgesics, for example, atropine poisoning can cause loss of consciousness, delirium, headache, visual disturbances, increased heart rate³.

In both traditional and modern ophthalmology, Solanaceae are important. Indeed, the Romans used since Antiquity the juice of the berries of belladonna (*Atropa belladonna*) to dilate their pupils and make the eyes more attractive⁴. In Senegal, the maceration of the leaves of *Solanum incanum* L. is instilled in the eye to treat inflammatory diseases while in Tanzania it is the juice of the leaves that are used. In Malawi, juice from the fruit of the same plant is applied to scarifications around the eye to treat conjunctivitis^{5,6}.

The alkaloids of Solanaceae occupy an important place in modern ophthalmology mainly the atropine contained in belladonna, datura, henbane and mandrake.

In addition to mydriasis, atropine helps to obtain, transient paralysis of the ciliary muscles. It is used for intraocular inflammation (uveitis, trauma, surgery etc.) to limit the risk of synechia formation. Atropine has an analgesic action by resting the intrinsic oculomotor muscles, it is useful to explore certain hypermetropia compensated in part by the accommodative efforts in the young subject, finally, it's essential in the exploration of the posterior pole of the eye^{4,7}. An ethnobotanical survey carried out in Abié (southern Côte d'Ivoire) by a known traditional therapist to treat eye diseases, mainly allergic conjunctivitis identified *Solanum rugosum* in its recipe.

The main objective was to identify the alkaloids present in decocted and to evaluate the acute toxicity and cutaneous and ocular tolerances of decocted of the plant used for the treatment of eye diseases by the population.

MATERIALS AND METHODS

Plant material: The bark of the root of *Solanum rugosum* is used. The plant was harvested from 09-14 February, 2020 in Abié, a village in the Yakasse-Mé sub-prefecture in the Mé

region. The species was identified at the National Floristic Center of the University Félix HOUPHOUËT-BOIGNY N°UCJ016910. The bark was taken from the roots and then cut into small pieces. They were dried at room temperature in the Pharmacognosy Laboratory of UFR SPB (around 22°C) for one month. They were then pulverized using a mechanical grinder of the GM 300 type, sieved and then stored in hermetically sealed jars. The powder thus obtained made it possible to carry out the various tests.

Animals: The experiments were carried out on female albino white mice of the Swiss-type and New Zealand male rabbits. These animals come from the UFR SPB animal facility. The ventilation conditions were good. The lighting was natural. The animals received granules supplied by IVOGRAIN and drinking water as a drink. The selected females were nulliparous and not pregnant. None of these animals had been subjected to previous experiments.

Equipment: For this study, equipment used for the extraction is a mechanical grinder type GM 300, a heating flask, a precision balance of type Si-602, a freeze-dryer type Alpha 12 (Christ) and a refrigerator (Liebherr Premium, France). A hot sand bath and test tubes were used for the characterization of the alkaloids. For the study of acute oral toxicity, a mouse scale as well as a mini-vortex was used. For the study of tolerances, rabbit restraints, a clipper, magnifying glasses and an ophthalmoscope of the HEINE BETA 200 type were used.

Extraction: The total aqueous extract of the root bark was prepared as follows: 100 g of the root bark powder was dissolved in 1000 mL of distilled water. The mixture was refluxed under a magnetic stirrer for 15 min. After cooling, the decoct was filtered through Whatman filter paper. The filtrates were put in the various ground and frozen flasks. They were then placed in the freeze dryer for 4 days. The lyophilisates obtained were put in pillboxes to carry out various tests.

Preparation of eye drops: The 5 mg of the lyophilisate of the root bark of *S. rugosum* were placed in a flask in which 50 mL of sterile and homogenized distilled water were added.

Tube characterization of alkaloids: The characterization in the tube of the alkaloids was carried out from decocted, 6 mL of the decocted was evaporated to dryness. The residue was taken up in 6 mL of alcohol at 60. The revelation was carried out using 2 drops of Dragendorff's reagent on the alcoholic solution causing an orange colour. Adding 2 drops of

Bourchardat's reagent to the alcoholic solution caused a reddish-brown precipitate indicating a positive reaction⁸.

LC-ESI-Q-TOF-MS analysis: The analysis was performed on an HPLC line (Agilent 1260 Infinity) coupled to a mass spectrometer (Q-TOF-MS Agilent 6530) equipped with an ESI source. A C18 Sunfire® Water's column of length 150 mm, diameter 2.1 mm and particle diameter 3.5 µm was used. The mobile phase consists of a gradient of solvents A (H₂O+0.1% HCO₂H) and B (CH₃CN). A linear gradient was used for 41 min in the following proportions: 0-5% B (0-5 min), 5-95% B (5-15 min), 95-100% B (15-25 min), 100% B (25-30 min), 100-0% B (30-32 min) and 0% B (32-41 min). The sample injection volume and flow rate were set at 5 µL and 250 µL min⁻¹, respectively. Data analysis was performed using Agilent Mass Hunter Workstation software⁹.

Assessment of acute oral toxicity in mice (OECD no. 423):

The animals were deprived of food but no water during the night before the test. After the fast, the animals were weighed and the test substance was administered orally by gavage.

Five groups of 3 mice were made up to receive the following solutions:

- Lot 1 control: Physiological serum
- Lot 2: Extract at 5 mg/kg/b.wt.
- Lot 3: Extract at 50 mg/kg/b.wt.
- Lot 4: Extract at 300 mg/kg/b.wt.
- Lot 5: Extract at 2000 mg/kg/b.wt.

After treatment, the animals were observed individually at least once during the first 30 min and regularly for the first 24 hrs after the treatment with particular attention during the first 4 hrs. Then, they were observed daily for 14 days after administrating the substance. Observations were focused on the changes in skin, body, hair and somatic disorders. Attention was paid in particular to the observation of the various manifestations of tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma¹⁰.

Skin tolerance

Acute irritant/corrosive effect on the skin of rabbits (OECD no. 404):

Two areas of the trunk are shaved 24 hrs before the experiment. The test substance, which was 0.5 mg, was applied to one of the mowed areas and covered with a compress of gas, secured with a non-irritating plaster. Care should be taken to ensure that the animal did not have access to the pad and cannot ingest or inhale the test substance. The

other shaved area serving as a witness. At the end of the exposure period, which lasts 4 hrs, the remaining test substance was removed with water or an appropriate solvent without interfering with the reaction or damaging the integrity of the epidermis. The test area is observed 1, 24, 48 and 72 hrs after application of the test substance. Reversibility of effects is determined by observation of the animals over up to 14 days after patch removal¹¹.

The rating of the lesions observed was as follows:

- No lesion = 0
- Slight lesion = 1
- Moderate injury = 2
- Severe injury = 3
- Very serious injury = 4

Eye tolerance

Acute irritant/corrosive effect on the eyes of rabbits (OECD no. 405):

A drop of the *S. rugosum* solution was instilled into the right eye of the rabbit, the left eye serving as a control. The duration of the observation period should be sufficient to allow a full assessment of the magnitude and reversibility of the effects that can be observed. Animals should normally be observed for 15 days after administration of the test substance. If the reversibility is observed before this time, the experiment was over at that time. The eyes are examined 1, 24, 48 and 72 hrs after application of the test substance¹¹.

The rating of the lesions observed was as follows:

- No lesion = 0
- Slight lesion = 1
- Moderate injury = 2
- Severe injury = 3
- Very serious injury = 4

Ethical approval: The experimental procedures were carried out according to the ethical guidelines of the Félix HOUPOUËT-BOIGNY University relating to animal resources in Côte d'Ivoire. All procedures performed complied with European Union guidelines and statements regarding the handling and care of laboratory animals¹². Animal Ethics committee approval has been collected and preserved by the author(s).

Statistical analysis: The results of the toxicological study are analyzed by Student's t-test for single comparisons and univariate ANOVA followed by Tukey's test for multiple comparisons and determination of significance rates. The

p-values less than 0.05 ($p < 0.05$) are considered statistically significant. The comparison of means and variances is determined using XLSTAT software version 2014 and GraphPad Prism version 5.0. The values are expressed as the Mean \pm Standard deviation.

RESULTS AND DISCUSSION

The characterization of the aqueous extract of the root bark of *Solanum rugosum* was conducted with the reagents of Dragendorff's and Bouchardat's. The obtained results shown in Table 1 revealed a high content of alkaloids in the extract of the plant. These data are confirmed by LC-ESI-Q-TOF-MS analysis. Indeed, four alkaloid compounds have been detected in the alkaloid extract of the bark of the root of *Solanum rugosum*. These were lycorine (1), corynanthine (2), corynantheine (3) and mitrinermine (4) given in Fig. 1 and 2.

Lycorine (1): For this compound, fragments are observed at m/z 288.1212, 268.0817, 250.0684, 222.0742, 147.0298 and 119.0356. The various fragments observed are identical to those identified by Ptak *et al.*¹³ in Fig. 3. This molecule is identified for the first time in the species. Lycorine has already been identified in *Leucojum aestivum*.

Corynanthine (2): Figure 4 indicated a molecular ion at m/z 355 [M+H]⁺. Fragment ions at m/z 306.1564, 279.1484, 246.1426, 144.0804 (100%) [M-210]⁺ and 117.0693, allowed us to identify this compound as corynanthine¹⁴.

Table 1: Results of the characterization of alkaloids

	Colouring	Result
Reagent of Dragendorff's	Orange	+
Reagent of Bouchardat	Reddish-brown	+

+: Presence of alkaloid compounds

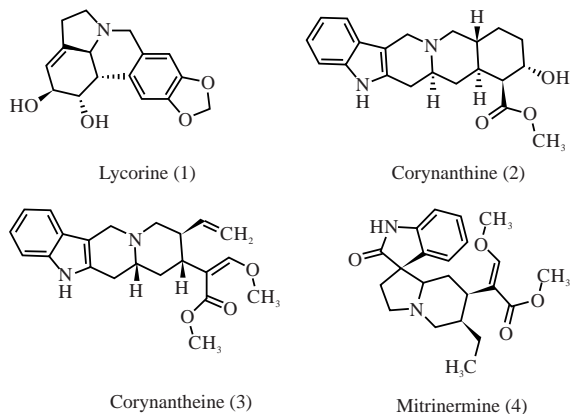


Fig. 1: Compounds isolated from the alkaloid extract of the root bark of *Solanum rugosum*

Corynantheine (3): Regarding to this compound, fragmentations are observed at m/z 367.2001, 298.1346, 144.0785 (100%), 108.0794 (11.13%). The different fragmentations directed us towards the structure of corynantheine in Fig. 5¹⁵.

Mitrinermine (4): The various fragments observed were similar to those obtained by Long *et al.*¹⁵ at m/z 385.2113 (92.53%), 269.1633, 241.1324, 187.119 (10.66%), 160.0755 (97.03%). Thus, the compound is identified as mitrinermine in Fig. 6¹⁵.

Concerning acute oral toxicity and both skin and eye tolerances, we referred to the OECD guidelines. During the acute phase, no clinical signs of intoxication were recorded for the doses of 5 and 50 mg kg⁻¹ (batches 2 and 3) as well as in the control batch. Apathy was observed at a dose of 300 mg kg⁻¹ for 40 min after administration of the aqueous extract of the root bark of *S. rugosum* in the mice of group 4.

Then, noted a seizure in one of the mice of batch 5, 20 min after administrating the extract. Reversibility was noticed of the behavioural problems after 4 hrs. No mouse deaths were recorded throughout the experiment. The total aqueous extract of the root bark of *S. rugosum* was not harmful, the LD₅₀ is estimated to be greater than 2000 mg/kg/b.wt. in mice.

The mean weights of the animals during the observation period varied from 22.33 \pm 1.15 g for batch 1 (control) to 37.33 \pm 1.53 g, with an increase of weight of 15 g. On the other hand, for the dose of 2000 mg kg⁻¹, the average weight of the batch varied from 18 \pm 2.00-22.33 \pm 2.31 g, with an increase of weight of 4.33 g. During 14 days of observation, oral administration of the extracts at different doses resulted in non-significant weight loss compared to the control group. The variation of the average weights (g) for each batch after testing is shown in Table 2.

The acute *in vivo* toxicity study of the total aqueous extract of the root bark of *S. rugosum* by oral route in female mice did not reveal any animal deaths at the dose of 2000 mg/kg/b.wt. The LD₅₀ of the total aqueous extract of *S. rugosum* was greater than 2000 mg/kg/b.wt. According to the globally harmonized classification system of the OECD 423, this extract could be classified in category 5 no toxic.

Regarding skin tolerance, 0.5 mg of the moistened lyophilisate is applied to the skin of rabbits. Figure 7a exposes the area to be tested, the extract of *S. rugosum* is then applied to the skin of the animals in Fig. 7b and the drug is protected by a dressing in Fig. 7c. After 4 hrs, the extract is cleaned in Fig. 7d and noted an absence of Edema and erythema in the tested area one hour after the cleaning of *S. rugosum*, this

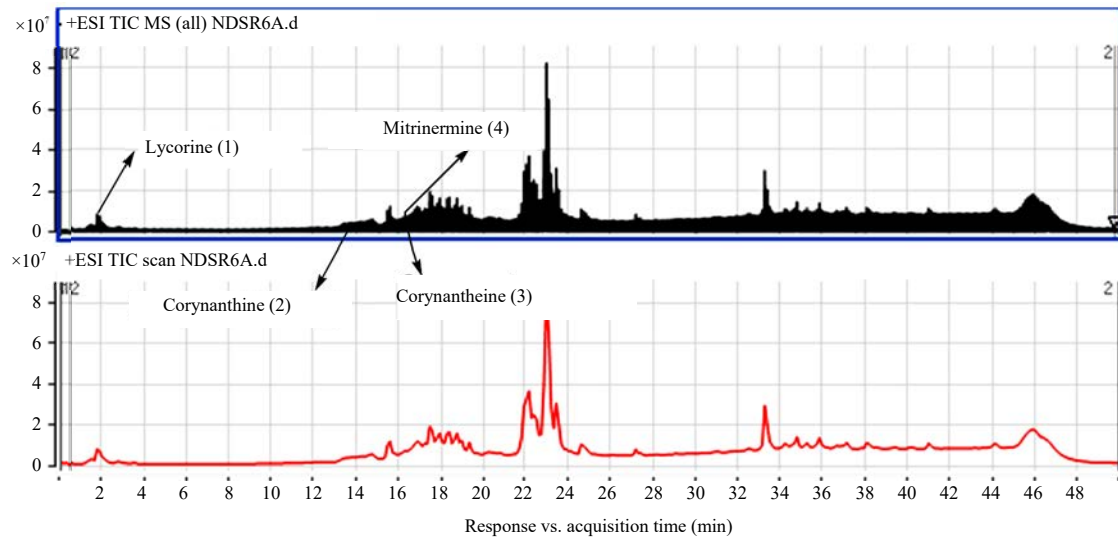


Fig. 2: ESI-MS spectrum of the alkaloid extract of the root bark of *S. rugosum*

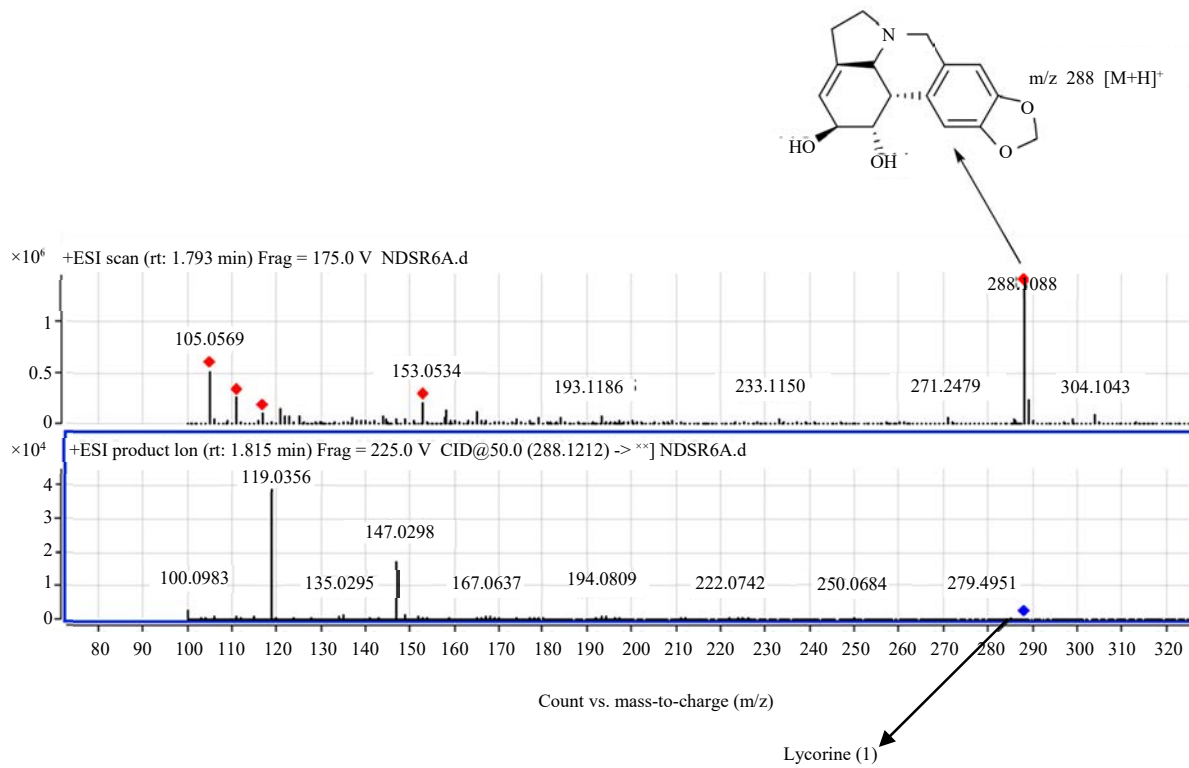


Fig. 3: ESI-MS spectrum of Lycorine (1)

Table 2: Variation in the mean weight of mice

Doses administered (mg kg ⁻¹)	Average animal weights (g)		Variation (g)	Student test-t (5%)
	J0	J14		
Control (distilled water)	22.33±1.15	37.33±1.53	+15.00	-
5	21.67±2.31	34.33±0.58	+12.66	NS
50	21.33±5.03	31.33±1,00	+10.00	NS
300	20.00±2.64	25.80±1.73	+5.80	NS
2000	18.00±2.00	22.33±2.31	+4.33	NS

Values are expressed as Mean±Standard deviation, variation, NS: Not significant, p>0.05

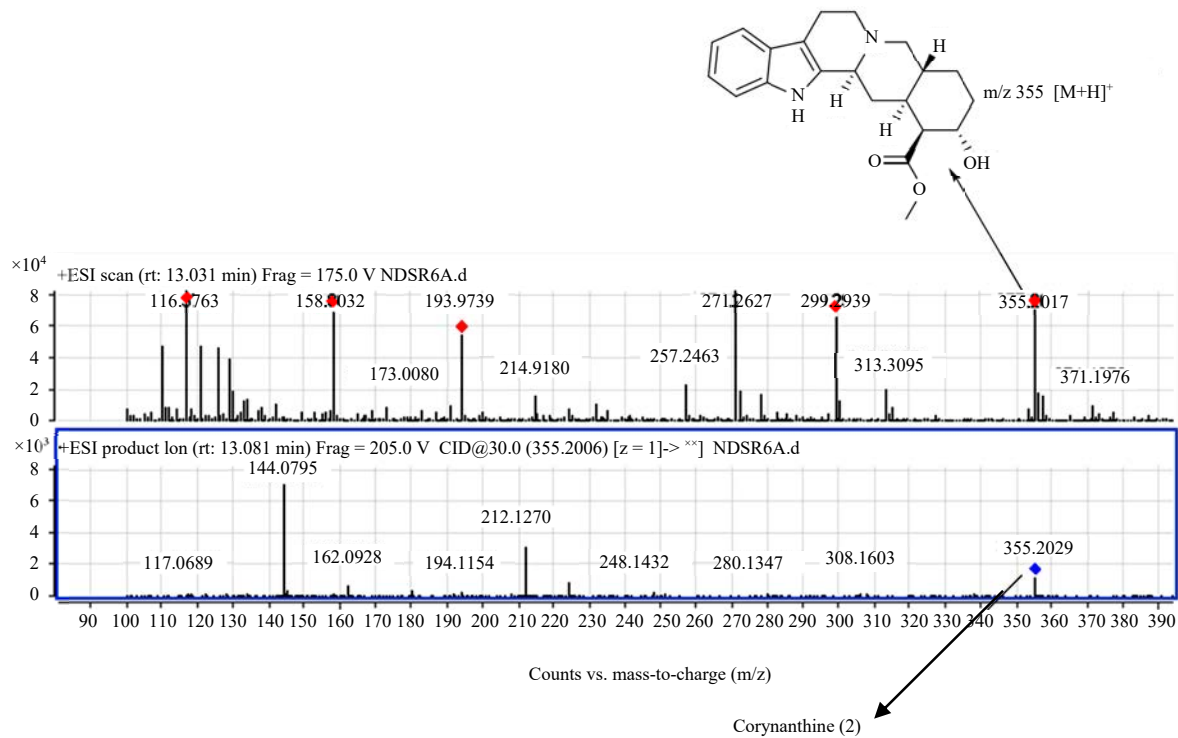


Fig. 4: ESI-MS spectrum of Corynanthine (2)

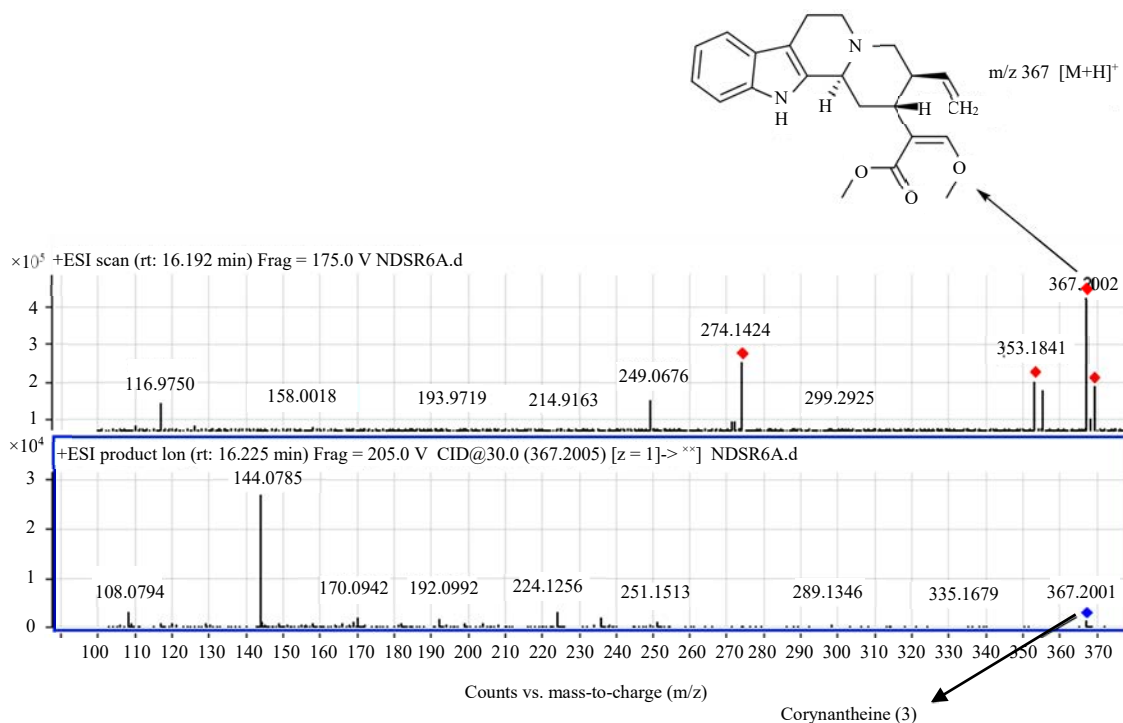


Fig. 5: ESI-MS spectrum of Corynantheine (3)

was a good skin tolerance of the aqueous extract of the bark of *S. rugosum* according to OECD 404 in Fig. 7e. The different scores of the lesions observed are reported in Table 3. Indeed,

all scores were 0 from the 1-72 hrs and then 15th day (D15) observations of experimentation. 0 as the score indicates an absence of skin lesions in rabbits.

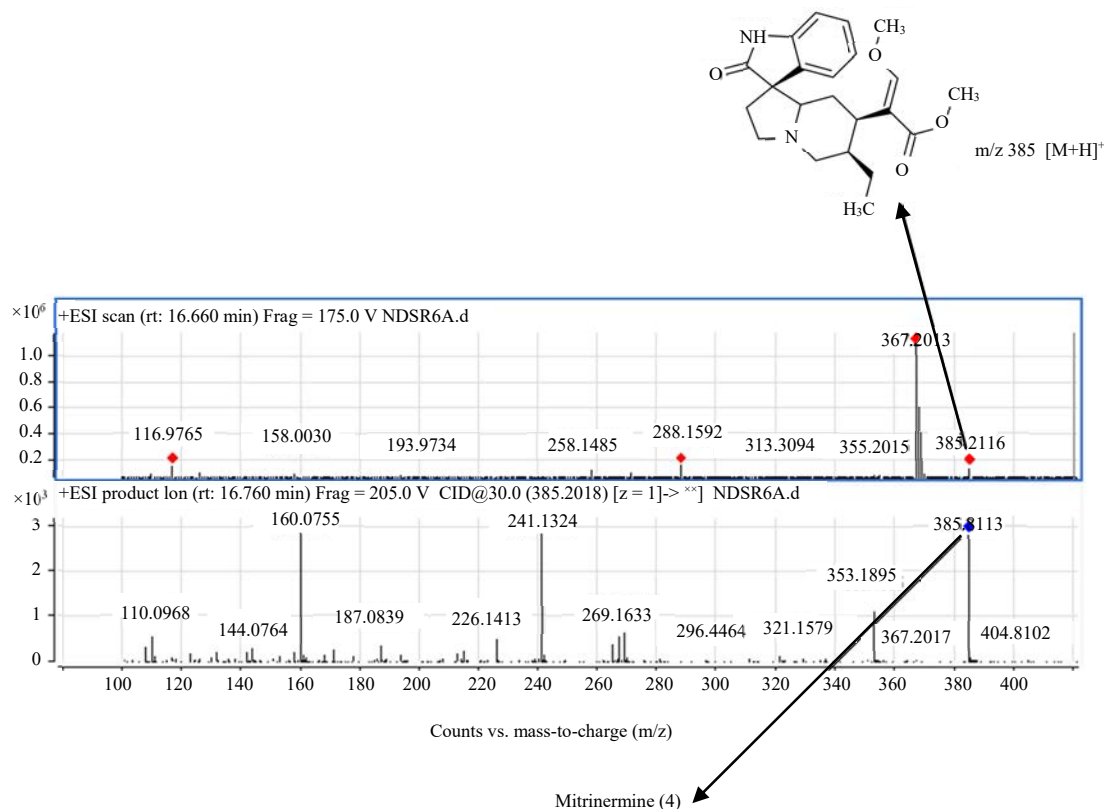


Fig. 6: ESI-MS spectrum of Mitrinermine (4)

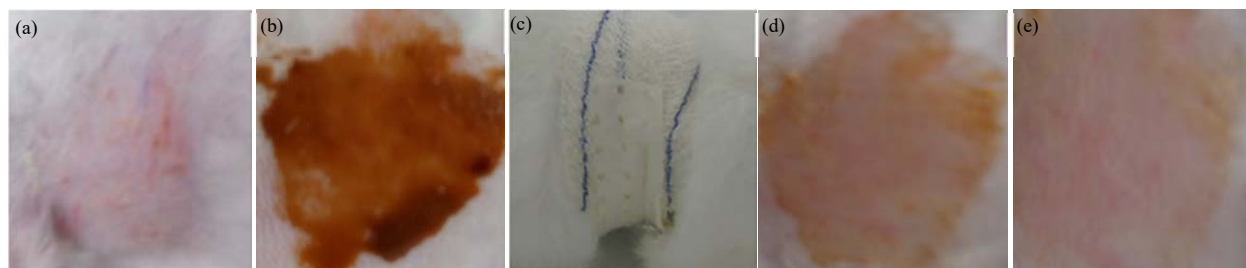


Fig. 7(a-e): Skin tolerance test, (a) Shaving of the area to be tested 24 hrs before the experiment, (b) Dressing 4 hrs after applying the plant extract, (c) Protection of the tested area, (d) Cleaning of the tested area and (e) Absence of skin lesions no erythema and no edema 24 hrs later

Table 3: Assessment of skin irritation

Observation period	Erythema					Edema				
	1 hr	24 hrs	48 hrs	72 hrs	D15	1 hr	24 hrs	48 hrs	72 hrs	D15
Rabbit 1 (initial test)	0	0	0	0	0	0	0	0	0	0
Rabbit 2 confirmation test	0	0	0	0	0	0	0	0	0	0
Rabbit 3 confirmation test	0	0	0	0	0	0	0	0	0	0

For the evaluation of ocular tolerance made a 10% solution from the lyophilisate of the root bark of *S. rugosum* and sterile distilled water. A drop of the extract is instilled into

the right eye of the rabbits, the left eye serving as a control. Figure 8a indicates the rabbit eye before instillation. Figure 8b showed the stage of instillation and the condition of the

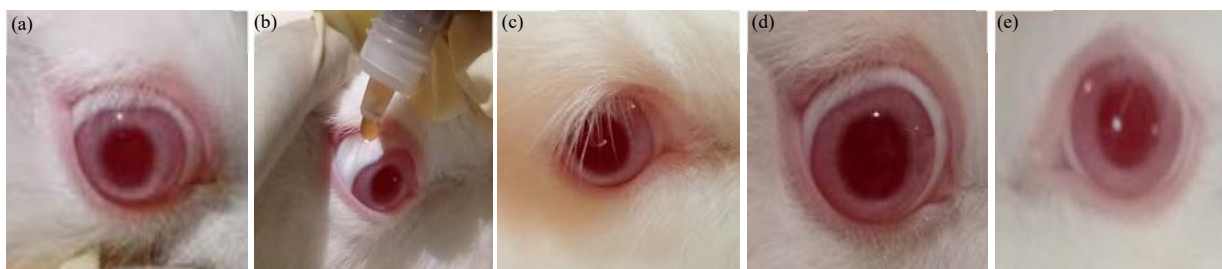


Fig. 8(a-e): Eye tolerance test, (a) Eye before instillation, (b) Instillation of *S. rugosum* extract, (c) Eye condition 1 hr later, (d) Eye condition 24 hrs later and (e) Left eye witness

Table 4: Assessment of eye irritation

	1 hr	24 hrs	48 hrs	72 hrs	D 15
Cornea	0	0	0	0	0
Conjunctiva	0	0	0	0	0
Secretions	0	0	0	0	0
Chemosis	0	0	0	0	0
Eyelids	0	0	0	0	0

eye 1 and 24 hrs later in Fig. 8c and d, compared to the left eye in Fig. 8e. The observations 24 hrs after eye contact with *S. rugosum* were as follows:

- Absence of corneal damage
- Absence of chemosis
- No eyelid tumefaction
- No conjunctival secretions

Table 4 showed the scores for lesions at the level of the different organs (cornea, eyelids and conjunctiva) of the eye. Note also that the scores were 0 throughout the 15 days of observation (D15).

The characterization tests performed on the total aqueous extract of the root bark of *Solanum rugosum* revealed the presence of alkaloids. Indeed, these secondary metabolites were present in the genus *Solanum*. Research work has revealed the presence of these compounds in *Solanum dulcamara* L., *Solanum nigrum* L., *Solanum torvum*¹⁶⁻¹⁸. According to these authors, the Solanaceae contain a variety of alkaloids.

Four alkaloids were identified through analysis performed in LC-ESI-Q-TOF-MS. Lycorine (1) is an alkaloid extracted from several genera of the Amaryllidaceae family. It has anti-tumour activity. It is endowed with significantly higher anti-proliferative activity in cancer than in normal cells.

Empirical data from the case reports suggested that lycorine may be the toxic component of the multicomponent mixture responsible for symptoms such as nausea and vomiting. However, no systematic study of the *in vivo* effects

of Amaryllidaceae alkaloids is available. Therefore, in an open-label, prospective, randomized, controlled trial, the dose-response relationship of lycorine-induced nausea and vomiting and Lycorine toxicokinetics in dogs was investigated. The maximum emetic dose of lycorine was 2 mg/kg/b.wt. There was a correlation between dose and onset of nausea as well as between dose and number of emetic events induced. Nausea and vomiting were short-lived and occurred no later than 2.5 hrs after the dose. The biochemical and haematological safety parameters showed no pathological signs. The results proved that lycorine can be considered as the main constituent, if not the crucial constituent responsible for nausea and vomiting in humans and animals in the poisoning was due to ingestion of plant material in Amaryllidaceae¹⁹⁻²¹.

Corynanthine, also known as rauhimbine, is an alkaloid found in the genera *Rauvolfia* and *Pausinystalia*. This molecule is used as an antagonist of α 1-adrenergic and α 2-adrenergic receptors with approximately 10-fold selectivity for the first site over the last. This compound is not a stimulant but a depressant and probably plays a role in the antihypertensive properties of *Rauvolfia* extracts²².

Corynantheine showed activity against *Leishmania major* promastigotes but no significant antiplasmodial activity *in vitro* against *Plasmodium falciparum*²³.

Mitrinermine or rhynchophylline is a natural substance used to treat cardiovascular disease. It also has anti-hypertensive properties²⁴.

Indeed, the presence and synergy between these different alkaloids could allow the plant to have some interesting biological activities. The study of the acute *in vivo* toxicity of the total aqueous extract of the root bark of *Solanum rugosum* by the oral route in female mice did not reveal any animal deaths at the dose of 2000 mg/kg/ b.wt. The LD₅₀ of the total aqueous extract of *Solanum rugosum* was greater than 2000 mg/kg/b.wt. According to the globally harmonized classification system of the OECD, this

extract can be classified in category 5 and considered as an oral non-toxic substance. The LD₅₀ was obtained from the limit test of modified OECD recommendations 423. These results corroborated with the oral toxicity of certain species of the genus *Solanum*, namely *Solanum paniculatum* L.²⁵ and *Solanum betaceum* Cav²⁶.

Seventy-two hours after application of the total aqueous extract of the root bark of *Solanum rugosum* to the skin and in the eye, there is no erythema, edema and eye damage. (Conjunctivitis, swelling of the eyelids, damage to the cornea and iris), thus, reflecting the absence of toxicity and the good cutaneous and ocular tolerances of the total aqueous extract of the bark of the root of *Solanum rugosum*. Similar results were obtained by Kouao *et al.*²⁷ and Fokou *et al.*²⁸, who studied the healing properties of *Solanum rugosum* leaves in the treatment of Buruli ulcers.

CONCLUSION

A chemical study of aqueous extract of root bark of *Solanum rugosum* carried out in LC-ESI-Q-TOF-MS allowed us to identify 4 alkaloids in alkaloid extract. These compounds are identified for the first time in the plant.

The evaluation of acute toxicity and the skin and eye tolerance of total aqueous extract of root bark of *Solanum rugosum* allowed us to conclude that the determined LD₅₀ is greater than 2000 mg/kg/b.wt. No animal deaths were observed after 14 days of observation. This extract can be classified in category 5 of non-toxic substances. The extract is well tolerated by the skin and eyes.

The results obtained are very interesting, however further toxicity tests must be carried out by studying chronic and subchronic toxicities for better safety of use and to justify its use in traditional Ivorian medicine in the treatment of eye diseases.

SIGNIFICANCE STATEMENT

This study discovered that the total aqueous extract of the root bark of *Solanum rugosum* is not toxic and is well tolerated which can be beneficial for being used to cleanse the eyes and maintain good eyesight. This study will help the researchers to uncover plants to cure eye diseases that many researchers were not able to explore. Thus, a new theory on plants used to treat inflammatory diseases of the eye may be arrived at.

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