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Healing Activity of Sepia Officinalis Bone

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Abstract – Madagascar is a country renowned worldwide for its exceptional biodiversity and high level of endemism. Its fauna is of particular scientific interest in the search for new molecules of biopharmaceutical interest. Surveys carried out in the south-west region of this large island have revealed that S. officinalis bones have been used in this region to treat wounds. Thanks to a number of biological models, this information on ethno-medical uses has been scientifically validated. The results of scientific studies show that it has two effects: healing and anti-healing.

Keywords – Bone S. officinalis, Sepiidae, Healing.

I. Introduction

In underdeveloped countries, access to modern medical care is becoming increasingly difficult due to low purchasing power. Even if there are possibilities, the inadequacy of the medical infrastructure, including materials, and the lack of resource persons contribute to these problems **[1-4]**.

On the other hand, given the importance of traditional customs in society, and the natural wealth of flora and fauna in Madagascar **[6-7,23-24]**, people use their natural resources to treat themselves and eliminate their suffering **[5, 8-10]**. During in ethno-medical survey, 80% of traditional interviewed reported that, the bone of *Sepia Officinalis* (Sepiidae) known Angisy in Malagasy language (Vezo tribe) is used by the locally communities to heal the wounds.

The aim of the present study to verify by scientifically methods the wound-healing effect of *Sepia officinalis bone* and to formulate an ointment based on the aforementioned animal.

II. Materials And Methods

Ethno-medical survey

Ethno-medical information about the animal matrix selected for this study was obtained by interviewing traditional healers during field work which was conducted in the South-west of Madagascar. Surveys were conducted from April to Jolay 2022 in seven villages one the sea of the South-west of Madagascar. These villages are Beheloke, Saint-Augustin, Ifaty, Mangily, Ambolimailake, Manombo and Andavadoake. There are four different ethnic groups inhabiting the South-west of Madagascar: Mahafaly, Tagnalana, Vezo and Masikoro. They all share a common language Malagasy, which is the unique characteristic of this island.

A total of 30 traditional healers were interviewed. Informants were selected for their authentic knowledge on the utilization of animal product basis. Malagasy, the national language of Madagascar was used during anthropological interviews. Traditional healers were interviewed on a voluntary basis. The study followed principles laid out in the declaration of Helsinki as previously reported [10-12]. The questionnaires were divided into three sections: (a) personal information such as name, age, sex, marital status and studies level; (b) traditional medicine practice (including knowledge of diseases and symptoms); (c) the first matter employee (plants, animals and vernacular name), part used, preparation methods and administration route of remedies.

Selection and collection of animal matrix

The animal matrix (*Sepia officinalis*) was selected based on its relative citation frequency (use value =0.68) and the informant consensus factor value (0.49). The *Sepia officinalis bone*s were collected in Ambolimailake village, district of Toliara-II (South-west Madagascar) on Jolay 2022 (fig.1). The animal species was identified by comparison with reference specimens available at the Department of Biology, Faculty of Sciences, University of Toliara BP Toliara 601, Madagascar. Voucher specimens with assigned sample number SO-001 was deposited at the herbarium of the laboratory of Applied Chemistry, Layflaylle Street, University of Toliara Madagascar.

Figure 1: Extract of technical of the bones of *Sepia officinalis*

Systematic presentation of Sepia officinalis

Phylum: Mollusks
Class: Cephalopoda
Order: Coleoidae
Family: Sepiidae
Genus: Sepia
Species: Officinalis

Figure 2: Photo of S Officinalis

Characteristics of Sepia Officinalis bone

Sepia Officinalis bone is oval-shaped and pointed at both ends. The top part of its inner surface is marked with several stripes, while the other part is smooth.

Figure 3: Characteristic of bones extracted from the back of *S. Officinalis*

Extraction and chemical screening

The dried and powdered first matter (2 kg of the animal matrix) was repeatedly extracted by cold percolation with 95% ethanol and water (2 l x 3) for 48 hours at room temperature on a shake. Pooled organic solvent was dried over Na_2SO_4 and evaporated until dryness at 40°C, under reduced pressure to yield 49, 56 g of crude extract. Chemical screening was done according to a well-known protocol as previously reported [21-22, 25-26].

Detection of phenols (Ferric Chloride Test)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated indicates the presence of phenols.

Detection of flavonoids

The ethanol extract (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

Detection of anthocyanosids

The presence of anthocyanosids is revealed by a color change as a function on pH due to titration of the acidic aqueous solution with a solution of NaOH. If the solution turns a red color, the pH is less than 3, if against a blue color; the pH is between 4 and 6.

Detection of tannins

Two methods were used to test for tannins. First, about 1 ml of the ethanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution were added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration. Second, 2 ml of the aqueous extract was added to 2 ml of water, a 1 to 2 drops of diluted ferric chloride solution were added. A dark green or blue green coloration indicates the presence of tannins.

Detection of leucoanthocyanins

To 2 ml of aqueous extract was added few drops of Shinoda regeant in a test tube and then boiled. A red purple coloration in the supernatant indicates the presence of leucoanthocyanins.

Detection of saponins

To 1 ml of aqueous extract was added few volumes of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

Detection of alkaloids

Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

Detection of coumarins

Evaporate 5 ml of ethanolic solution, dissolve the residue in 1-2 ml of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 ml 10% NH_4OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.

Detection of free quinones

To 1 ml of organic extract was added few drops of Borntrager reagent (NaOH 10% ou 10% NH_4OH) in a test tube. The solution was and then shaken vigorously. A sharp red or orange coloration indicates the presence of free quinones.

Detection of steroids

One milliliter (1 ml) of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube.

The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Detection of diterpenes (Copper acetate Test)

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Detection of triterpenoids

Ten milligram (10 mg) of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

Evaluation of the healing effect of the hydroalcoholic extract of Sepia officinalis Bone [14-17, 19-20]

The healing activity of *Sepia officinalis* Bone was evaluated on the basis of wound healings created on rabbits treated with ointment prepared from crude extracts of Sepia officinalis Bone.

Animal preparation

Six-month-old rabbits of either sex, averaging 1,300kg ± 0.005 kg, were conditioned for 15 days on a normal diet. The conditioned animals were subjected to general anesthesia (fig. 4a) using chloroform via the respiratory route. If unconscious, each animal's coat was shaved approximately 15 cm in diameter (fig. 4b). After removal of their fur, the shaved area was cleaned with 80° ethanol and then the back of each rabbit was incised to create a wound approximately 5 cm in diameter (fig.4c).

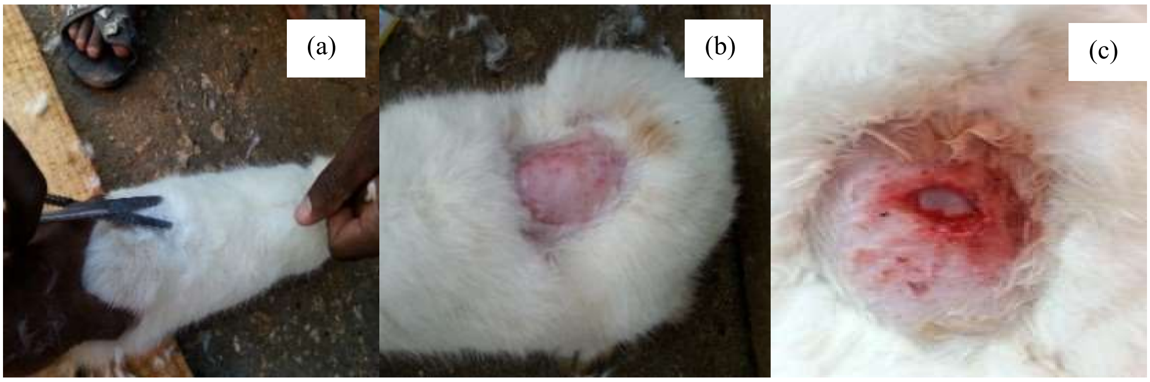


Figure 4: The wound created on the back of each rabbit

Treatment method [18]

The wound created on the back of each animal was cleaned with 75° ethanol to avoid the risk of bacteriological infection, and then treated with ointments formulated from *S. officinalis bone* extracts. A precise quantity of extract and excipient was introduced into the mortar and crushed with the pestle (Figure 5). Three types of ointment were prepared:

Ointment A: Extract plus 50% Vaseline by weight of mixture

Ointment B: Mixed extract with 50% fat by weight of the extract used

Ointment C: Unblended extract

Evaluation of the healing activity of ointments derived from *S. Officinalis* bone extracts

The effect of each ointment was assessed on the basis of wound shrinkage measured before each dressing for each 48-hour period. Treatments lasted two weeks and Betadine was used as the reference product.

Figure 5: Ointment preparation technique

III. Results And Discussion

Ethno-medical survey

During survey, thirty traditional healers were interviewed about animal matrix used in folk medicine to treat wounds. The most cited animal was *S. Officinalis* bone with the use value and information consensus factor of 0.68 and 0.49 respectively.

Extraction and chemical screening

The yield of the bones crude extract of *S. Officinalis* obtained by cold percolation with 95% ethanol and water was 4.478%. The results of chemical screening of the bones crude extract of *S. Officinalis* are presented in Table 1. It is deduced from the table 1, that bone of *S. Officinalis* contains phenols, flavonoids, saponins, coumarins, free quinones, steroids, diterpenes and triterpenoids. However, we also note that compounds such as anthocyanosids, tannins, leucoanthocyanins and alkaloids are not found in the bone of *S. Officinalis*. The presence of various secondary metabolites in the first matter could justify its medical use. These compounds, which are significantly present in the animal matrix, are well known for their large spectrum of pharmacological properties.

Table 1: Chemical screening of the bone's crude extract of *S. Officinalis*

Secondary metabolites	The bones crude extract of *S. Officinalis*
Phenols	+
Flavonoids	+
Anthocyanosids	-
Tannins	-
Leucoanthocyanins	-
Saponins	+ +
Alkaloids	-
Coumarins	+++

Free quinones	++
Steroids	+
Diterpenes	+
Triterpenoids	-/+

(-): absent; (+/-): trait; (+): weak; (+ +): middle; (+++): richness

Results of biological studies

The healing effects of ointments formulated (table 2) from the hydroalcoholic extract of S. officinalis bone powder were assessed on the basis of wound healing in wounds treated with these ointments (fig. 5).

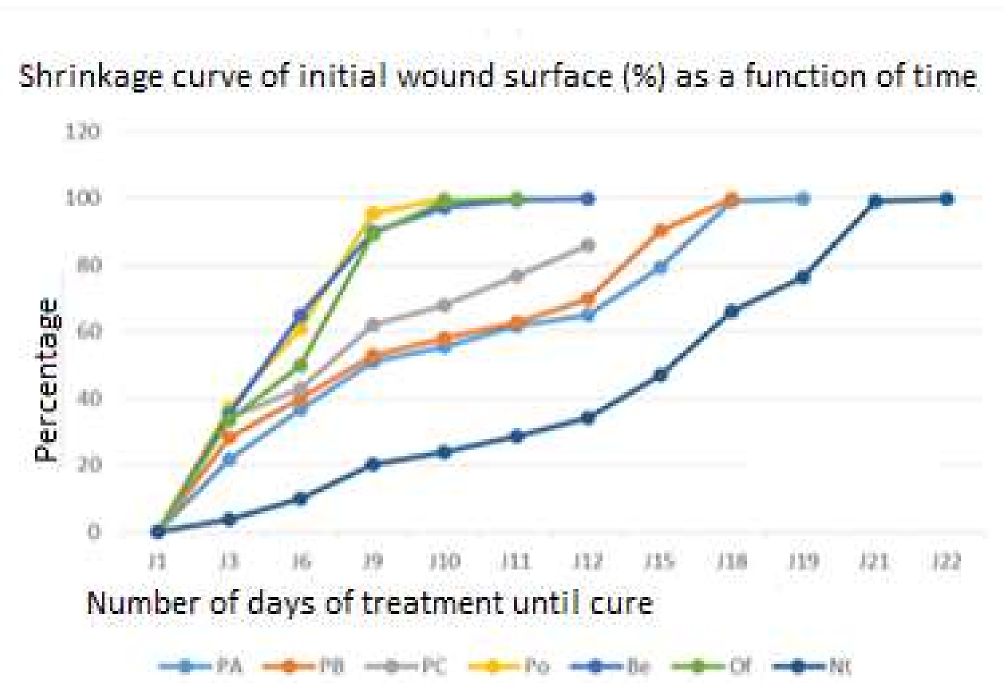
Figure 5: The treatment of first day

Table 2: Three types of ointment were prepared

		Ointments		
		A	**B**	**C**
Composition	Quantity of extract	3g	3g	3g
	Beef oil fat	-	3g	-
	Vaseline	3g	-	-
Forme		Fatty body	Fatty body	Fatty body
Color		Beige	Yellowish	Yellowish

- 3g of Vaseline was mixed with 3g of extract to form fat called pomade A.
- 3g of beef oil fat was mixed with 3g of extract to form an ointment B.
- A total extract-based ointment was named Ointment C.

Wound healing rates for the different types of ointment are determined by restricting wound diameter as a function of the number of days of treatment (fig.6, table 3).



OA: Ointment A   OB: Ointment B OC: Ointment C BP: Bone Powder   Be: Betadine

Of: Ody fery Ratsimamanga   Un: Untreated

Figure 6: Healing effects of different ointments

Table 3: Wound shrinkage

%	Number of treatment days										
	D3	D6	D9	D10	D11	D12	D15	D18	D19	D21	D22
OA	21,8	36,8	51	55,4	61,8	65	79,4	99,1	100		
OB	28,4	40	53	58,2	63	69,8	90,2	100			
OC	34,3	43	62,2	68	76,8	86					
BP	37,2	61	95,5	100							
Be	35,3	65	90	97,5	99,4	100					
Of	33,6	50	89,3	99,2	100						
Un	3,8	10	20,3	24	28,8	34	47	66,2	76,3	99,2	100

After a fortnight's treatment, the wound treated with ointment C was completely healed, while the other wounds treated with ointments A and B were healing (fig.7).

Figure 7: The Wound-graph of fifteen days after treatments

By the twentieth day of treatment, all wounds had healed, but the wound treated with ointment "C" had not, and their fur had grown back completely (fig. 8). Taken together, the results of the pharmacological studies justify the traditional uses of S.officinalis bone to treat wounds.

Figure 8: The heal wound

Discussion

The ethno-medical results obtained during the surveys carried out in the south-west region of Madagascar revealed that *S. Officinalis bones* are used by the Vezo tribe to heal wounds of all kinds.

The results of phytochemical screening (Table 1), revealed also of the various types of chemical compounds found in the studies animal matrix extract have a broad range of biological properties [29]. For example, phenolic compound reported to have antibacterial activities [27]**.** The users would profit from it if the action the preparation is limited only to the pathogenic microbes. While others revealed that the flavonoids preset of radical scavenging properties [6-9]. Many reports revealed that saponins possess anti-inflammatory. The presence of saponins in some screened of the bones crude extract of *S. officinalis* could also induce the cicatrization. Saponins being used as detergent for their surface-active properties could also cause the inhibition of Lactobacillus [28]. The results of phytochemical screening revealed also the presence of coumarins only in the bone crude extract of *S. officinalis*. These compounds were reported to stop the haemorrhages after delivery [30]**.** The various types of the chemical molecular families present in the animal matrix justify their biological activities of the bone crude extract of *S.officinalis* [31].

Four types of ointment formulated from *S. Officinalis* bones were tested on incised wounds, with Ody Fery IMRA ointment and Betadine as reference products. The aim of this test was to detect the active preparation(s) based on information concerning traditional uses among the local population in this region. The results showed that all ointments were active, but ointment C was the most active compared with the other ointments [fig.7].

After fifteen days of treatment, scarring of the wound treated with ointment C had gradually disappeared, and at the same time the animals' fur was growing back very rapidly (fig.8). So, this ointment helped restore the skin to its original shape.

IV. Conclusion

Ethnomedical surveys play an important role in scientific research. They have revealed nature's secrets about traditional uses. The results of these surveys have shown that *S. officinalis bones* are used by the Vezo tribe to heal wounds. The results of scientific studies justified the ethnomedical data disproving the empirical uses of this animal matrix. During treatment, the effects of these ointments are twofold. The bones of S. officinalis have healing properties, while at the same time exhibiting anti-healing effects.

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