International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
June - July, 2013, Vol. 2, No.4, pp 519-526
ISSN: 2278-0238

Research Article

COSMOMEDICAL POTENTIALS OF BLACK MAHLAB SEEDS GROWING IN SUDAN

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(Received: March 11, 2013; **Accepted:** May 05, 2013**)**

ABSTRACT

The seeds of black mahlab are used widely in the Sudanese traditional medicine as cosmomedical preparations for skin and mucous membrane disorders. Therefore, the objective of the present study is to verify this claim in scientific manner. Water and methanol extracts were tested against common pathogens for skin and mucous membrane infections. Assays were performed using extract concentrations of 25, 50, 100 and 200mg/mL, and agar well diffusion method was utilized. Results obtained, revealed a significant difference ($P<0.05$) in inhibition zone diameters between bacteria and fungi used. Phytochemical screening revealed the presence of some phytochemicals which have been known to have antimicrobial and astringent effects. The findings from these studies provide evidences for the ethnomedicinal uses of the seeds for skin and mucous membrane disorders and beautifulness.

Keywords: Bacteria, extract, fungi.

INTRODUCTION

Humans everywhere in the world are still plagued by amyriad of ailments and infections with microorganisms. Inspite of the availability of drugs for treatment, diseases of microbial origin remain a scourge. The use of herbal medicine in the treatment of infection with microorganisms predates the introduction of antibiotics Herbalists have claimed that certain ailments and infections which have defied western medicine can be cured with local herbs. As such they have used different plant parts in the treatment of various infections (Owoyale et al., 2005).

Black mahlab or *Monechma ciliatum* (scientific name) belongs to the family *Acanthaceae*. It is annual hispid- scabrous or almost glabrous herb that grows up to 1- 2 feet high tall. It is restricted to Sudan; the seeds (Figure 1) are used as an effective remedy for common cold and other chest allergic, it also extensively used for pain relief and stomach problems. The therapeutic potentials of the herb, black mahlab have been studied by researchers such as (Abdelmoneum, 2008) and (Uguru and Evan 2002).

Figure 1: The seeds of Black mahlab.

The skin is the largest organ of the human body, both in terms of surface area and weight. It accounts for 15% of total body weight. Infectious diseases, particularly skin and

mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. One of the common causes of skin and soft-tissue infections is the occurrence of secondary microbial infection that complicates skin lesions. The skin lesions that can be secondarily infected with microbes are scabies, psoriasis, poison ivy, atopic dermatitis, eczema herpeticum and kerion (Brook, 2009).

Many hundreds of medicinal plant species worldwide are used in the traditional medicine as treatment for skin diseases caused by bacteria and fungi. The seed's powder and aqueous paste of black mahlab are used for skin and mucus membrane disorders, e.g. skin rashes, oral thrush, and gingivitis in different parts of Sudan (personal communication). The seeds are also used in traditional Sudanese fragrances, lotions, and other cosmetics for wedding preparation and childbirth rituals (Mariod et al., 2009).

This study was therefore designed to evaluate these claims scientifically by studying the antimicrobial activities of the seeds against pathogenic bacteria and fungi causing skin and mucus membrane infections. Investigations of herbal medicines involve the selection of test crude extracts based on a combination of ethnopharmacology and daily native's practices. Therefore, the extracts used in this research are closely resembled to the traditional preparations.

MATERIALS AND METHODS

Materials

1. Collection of the seeds

The seeds of black mahlab used in this study were obtained from Nubia Mountains region (South Kordofan State, Sudan). It authenticated at the Department of Pharmacognosy, Omdurman Islamic University and a voucher specimen with herbarium number (31) was deposited in the herbarium of the same Department for further referencing.

2. Test microorganisms

Test microorganisms *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (ATCC 53657), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 7596), *Aspergillus niger* (ATCC 9763) and *Aspergillus flavus* (ATCC13073) were laboratory isolates obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Omdurman Islamic University.

3. Chemicals

Mueller-Hinton agar and Mueller-Hinton broth: HiMedia Laboratories Pvt. Ltd, India. Ampicillin: Bristol –Mayers Squibb, USA. Clotrimazole: General Medicine Co., Sudan. Methanol: Scharlau Chemie S.A, Spain. Gentamicin: Roussel, UK. Methanol: BDH laboratory, UK. Nystatin: Sigma Medical Company, USA. Sabouraut Dextrose agar and Sabouraut Dextrose broth: HiMedia Laboratories Pvt. Ltd, India.

Methods

1. Preparation of the extracts

The seeds were dried at room temperature (30°C) and crushed using the mortar and pestle to coarse powder. 100g each of the coarse powder s were macerated separately in distilled water and 70% (v/v) methanol, for 24 h, with periodic stirring. The extracts were passed through a sterile muslin cloth and the solid residue (marc) pressed (screw press).The strained and expressed liquid obtained were mixed, put for 12h for clarification and filtrated by Whatman filter paper No 42 (125 mm). The aqueous filtrate was concentrated using a lyophilizer. Methanolic filtrate was concentrated in vacuo at 40°C, using a rotary evaporator. Both extracts were stored in a refrigerator at 4°C until used. The percentage yield of each extract was also determined.

2. Preparation of bacterial and fungal suspension

Standard microorganisms were prepared according to method described by Lopez et al. (2003). Briefly, a loopful of test bacteria were transferred separately to 10mL Mueller-Hinton broth and incubated for 24h at 37 ☐ 1°C. A loopful of test fungi were transferred separately to 10mL Sabouraut dextrose broth and incubated for 24h at 30 ☐ 1°C for yeast. Optical densities (OD) of the 24h cultures (bacteria and fungi) were measured using a spectrophotometer. OD was then adjusted to 0.5 by adding Mueller-Hinton broth and Sabouraut dextrose broth for bacteria and fungi suspensions respectively. The concentration of bacterial suspensions were adjusted to 10^8 cells/mL, and that of fungi to 10^7 cells/mL.

3. Testing of the extracts for antibacterial activity

Both extracts were screened for antibacterial activity using the agar well diffusion method described by (Lino and

Deogracious, 2006). Briefly, 0.01 mL of standardized inoculum of each test bacteria was spread on to sterile Mueller-Hinton agar plate so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter10mm was used to bore wells in the agar plates. From a stock solution, different concentrations of the extracts were prepared (25, 50, 100 and 200 mg/mL) and were separately introduced into the wells. Each extract concentration was replicated thrice. The plates were allowed to stand for 1h for diffusion to take place and then were incubated at room temperature (30°C), for 24 h. The external diameters of visible zones of growth inhibition were measured after incubation. A positive control was set up using separate plate. The assay in this case consisted of each test organism and the drugs gentamicin and ampicillin (0.005, 0.01, 0.02, 0.04mg/mL). Negative control consisted of test bacteria each in separate plate and distilled water. These plates were also incubated at room temperature (30°C) for 24 h. These controls were set up in triplicate too. The mean inhibition zone diameter was determined in each case.

4. Testing of extracts the for antifungal activity

Both extracts were screened for antifungal activity using the agar well diffusion method described by (Lino and Deogracious, 2006). Briefly, standardized inoculum (10^7cells/mL) of each test fungus was spread on to sterile Sabouraud dextrose agar plate so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 10 mm was used to bore wells in the agar plates. From a stock solution, different concentrations of the extracts were prepared (25, 50, 100 and 200 mg/mL) and were separately introduced into the wells. Each extract concentration was replicated thrice. The plates were allowed to stand for 1h for diffusion to take place and then were incubated at room temperature (30°C), for 48 h. The external diameters of visible zones of growth inhibition were measured after incubation. A positive control was set up using separate plate. The assay in this case consisted of each test organism and the drugs nystatin and clotrimazole (0.0125, 0.025 and 0.05mg/mL). Negative control consisted of test organism each in separate plate and distilled water. These plates were also incubated at room temperature (30°C) for 48 h. These controls were set up in triplicate too. The mean inhibition zone diameter was determined in each case.

5. Minimum inhibitory concentration

The Minimum inhibitory concentration (MIC) of the both extracts was determined by using the method described by Fabry et al. (1996). Briefly, solutions containing reconstituted extracts (3.125, 6.25, 12.5, 25, 50mg/mL) were incorporated into sterilized pre-poured medium of 20 mL of Mueller-Hinton agar (for bacteria) or Sabouraut Dextrose agar (for fungi), the medium poured and the agar in the plates allowed to set. The plates were then inoculated with the test bacteria or fungi and incubated at 37°C for 48 h. Control plates, which contained no plant extracts, were also made with the test. The MIC of each plant was determined after 48h (lowest concentration at which no visible growth was observed).

6. Phytochemical screening

The black mahlab seeds were screened for their phytochemical components, using the methods described by (Sofowora, 2006) and (Karou et al., 2006). The plant metabolites that were tested for were alkaloids, anthraquinones, triterpens, unsaturated sterols, cyanogenic glycosides, flavonoids, saponins, coumarins, and tannins.

7. Statistical analysis

Data obtained from this study were statistically analyzed using SPSS (version 15.0) computer program. The values are expressed $p< 0.05$ was considered significant in all comparisons.

RESULTS

The yields of the water and methanol extracts of the seeds were 9.05 and 7.40% (w/w) respectively as shown in **Table 2**. Phytochemical analysis of the black mahlab seeds showed the presence of flavonoids, tannins, anthraquinones, unsaturated sterols and triterpens (**Table 1**).

The antibacterial activities of the extracts of black mahlab on the bacterial species tested were tabulated in **Table 2** and figured in **Figures 2** and **3**.

The antifungal activities of extracts of black mahlab on the fungal species tested are shown in **Table 3** and figured in **Figure 4**.

The results of test of determining the minimum inhibitory concentrations (MIC) for water extract against *Bacillus*

subtilis, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*, were summarized in **Table 4.**

The results of test of determining the minimum inhibitory concentrations (MIC) for methanol extract against *Candida albicans* and *Aspergillus niger*, were summarized in **Table 5.**

extract yield compared to soxhlet and other methods of extraction. Maceration method was preferred in this research because it does not require heating, thus preserving thermolabile compounds. The water extract had higher yield than the methanol extract (**Table 2**), suggesting a higher

Table 1: Phytochemical test on seeds of black mahlab

Test Name	Observations	Result
Test for alkaloids	No color change	-
Test for anthraquinones	Pink precipitate	++
Test for coumarins	No color change	-
Test for cyanogenic glycosides	No color change	-
Test for flavonoids	Red colouration	++
Test for saponins	No color change	-
Test for tannins	Green precipitate	+
Test for triterpens	Pink to purple precipitate	+++
Test for unsaturated sterols	Green to purple precipitate	+++

(+++): appreciable amount; (++): moderate amount; (+): minute amounts; (-): not detected.

Table 2: Comparative antibacterial activity of extracts and standard drugs against test- bacteria

Extract	Yield (±0.5%)	Conc. mg/mL	Standard Bacteria				
			S. *aureus*	B. *subtilis*	K. *pneumoniae*	E. *coli*	P. *aeruginosa*
Water	9.50	25	-	16	12	-	–
		50	13	16	15	15	–
		100	18	25	18	17	–
		200	20	33	21	18	–
Methanol	7.40	25	-	-	-	12	–
		50	11	-	14	15	–
		100	13	18	21	17	–
		200	17	20	21	19	–
Gentamicin		0.005	12	16	12	11	–
		0.01	14	16	15	15	–
		0.02	16	22	19	18	–
		0.04	20	30	33	22	–
Ampicillin		0.005	–	16	–	–	–
		0.01	–	14	–	–	–
		0.02	–	13	–	–	–
		0.04	–	12	–	–	–
D. Water		–	–	–	–	–	–

Data are presented as Mean Diameter of Growth Inhibition Zone (mm), Average of three replicates, inhibition zone 15mm: sensitive, 14-15mm: intermediate, <15mm: resistant, - : no inhibition zone.

DISCUSSION

The use of water for extraction in this research is in harmony with folkloric practice. The percentage yield of extracts (**Table 2**) was varied with the solvent used for extraction. However, the yield was low when compared with the amount of seeds powder used for extraction. This is ascribable to the method of extraction employed (maceration). Ibrahim et al. (1997) state: maceration has been reported to result in low proportion of water-soluble components in the seeds of black mahlab.

The seeds were found to contain flavonoids, tannins, anthraquinones, unsaturated sterols and triterpens, which have been reported in other studies to elicit antimicrobial and astringent effects. Doss et al. (2009) report: tannins have antibacterial activity against *Staphylococcus aureus* and

Table 3: Comparative antifungal activity of extracts and standard drugs against test- fungi

Extract	Yield (± 0.5 %)	Conc. (mg\mL)	Standard fungi		
			C. albicans	*A. niger*	*A. flavus*
Water	9.05	25	13	-	–
		50	15	12	–
		100	21	14	–
		200	23	20	–
Methanol	7.40	25	–	–	–
		50	–	19	–
		100	–	23	–
		200	–	26	–
Nystatin		0.0125	23	17	–
		0.025	26	14	–
		0.0125	28	–	–
Clotrimazole		0.0125	33	17	–
		0.025	30	29	–
		0.0125	34	24	–
D. water		–	–	–	–

Data are mean diameter of growth inhibition zone (mm), average of three replicates.
Inhibition zone 15mm: sensitive, 14-15mm: intermediate, <15mm: resistant, - : no inhibition zone.

Table 4: Minimum inhibitory concentration of water and methanol extracts against selected pathogenic bacteria

Conc. mg/mL	Medium Added (mL)	Inoculum Added (mL)	Water Extract				Methanol Extract			
			Sa	Bs	Kp	Ec	Sa	Bs	Kp	Ec
3.125	20	0.01	+	+	+	+	+	+	+	+
6.25	20	0.01	+	+	+	+	+	+	+	+
12.5	20	0.01	+	*	+	+	+	+	*	+
25	20	0.01	*	-	*	*	*	*	-	*
50	20	0.01	-	-	-	-	-	-	-	-

(+): Growth; (-): No growth; (*): MIC; All determinations were done in triplicates. Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Kp: *Klebsiella pneumoniae*; Ec: *Escherichia coli*.

Table 5: Minimum inhibitory concentration of water and methanol extracts against selected pathogenic fungi

Conc. mg/mL	Medium Added (mL)	Inoculum Added (mL)	Water Extract		Methanol Extract
			Ca	An	An
3.125	20	0.01	+	+	+
6.25	20	0.01	+	+	+
12.5	20	0.01	+	+	*
25	20	0.01	*	*	-
50	20	0.01	-	-	-

(+) Growth; (-) No growth; (*): MIC; All determinations were done in triplicates. Ca: *Candida albicans*; An: *Aspergillus niger*.

Pseudomonas aeruginosa. Rhama and Madhavan (2011) report: flavonoids have antibacterial activity against *Escherchia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Also, Reyes et al. (1998) report: flavonoids possess antifungal property. Earlier, Baba-Moussa et al. (1999) report: tannins present in some plant species possess antifungal property. These phytochemicals may have caused inhibitory effect with synergistic effects. Padmanabhan and Jangle (2012) report: the crude extracts of plants are pharmacologically more active than their isolated active principles due to the synergistic effects of various components present in the whole extract.

For bacteria, collectively, the zone of inhibition increased with increase in concentration of extract (**Table 2** and **Figures 2** and **3**). The water extract inhibited *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* with inhibition zone diameter 13 to 20, 16 to 33, 12 to 21 and 15 to 18mm respectively. For *Staphylococcus aureus* and *Escherichia coli* the least extract concentration (25 mg/mL) did not show any inhibition as shown in **Table 2.** The

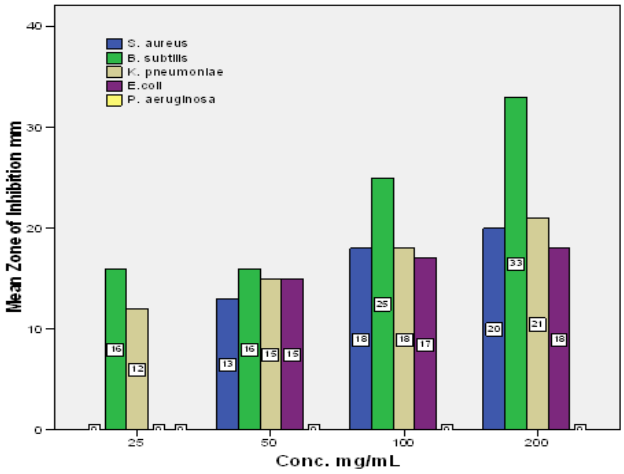

Figure 2: Effect of different concentrations of water extract on test- bacteria

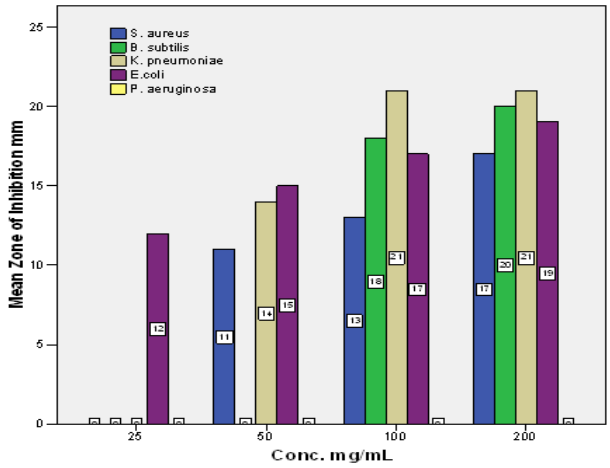

Figure 3: Effect of different concentrations of methanol extract on test- bacteria

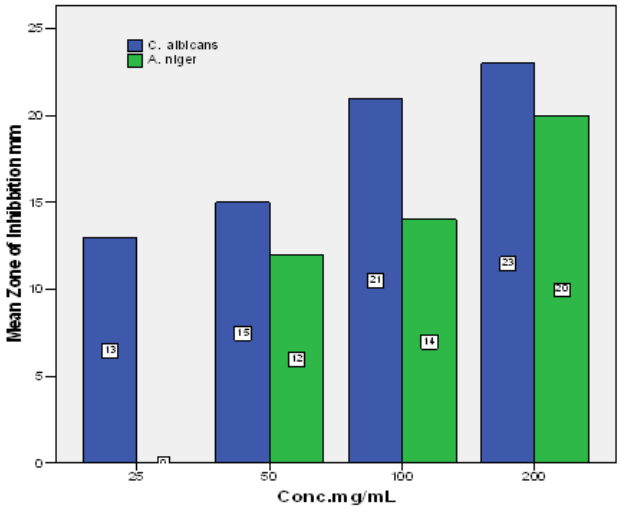

Figure 4: Effect of different concentrations of water extract on test- fungi

methanol extract inhibited *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* with inhibition zone diameter11to17, 18 to 20, 14 to 21 and 12 to 19mm respectively. For *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae* the least extract concentration (25 mg/mL) did not show any inhibition as shown in **Table 2**. Inhibition of test bacterial species was observed in *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* with the use of the standard drugs (gentamicin and ampicillin) as positive control. No inhibition was observed in the negative control (distilled water).

Pseudomonas aeruginosa neither was sensitive to both extracts nor to standard antibiotics used gentamicin and ampicillin (**Table 2**). Omolola (2011) state: resistance of *Pseudomonas aeruginosa* to extracts may be due to its the permeability barrier afforded by its outer membrane lippolysaccharide and its tendency to colonize surfaces in the biofilm form makes the cells impervious to therapeutic concentrations of antibiotics.

For fungi, generally, the zone of inhibition increased with increase in concentration of extract (**Table 3** and **Figure 4**). The aqueous extract inhibited *Candida albicans* with inhibition zone diameter ranging from 13 to 23 mm. For *Aspergillus niger* it was 12 to 20 mm, but the least extract concentration (25 mg/mL) did not show any inhibition. *Aspergillus flavus* was found to be insensitive to water extracts used as shown in **Table 3**.The methanol extract inhibited *Aspergillus niger* with inhibition zone diameter ranging from 19 to26 mm. The least extract concentration (25mg/mL) also did not inhibit the growth. *Candida albicans* and *Aspergillus flavus* was found to be insensitive to methanol extracts used as shown in **Table 3**. Inhibition of test fungal species was observed in *Candida albicans* and *Aspergillus niger* with the use of the standard drugs (nystatin and clotrimazole) as positive control. *Aspergillus flavus* also resisted the standard drugs used in all concentrations (**Table 3**). No inhibition was observed in the negative control (Distilled water). It was observed that *Candida albicans* and *Aspergillus niger* used in this research exhibited varying degrees of susceptibility to the extracts. Thus, the values obtained for the zones of inhibition differed, for each test organism could be attributed to the inherent resistance factor of the test organisms. This study has

revealed that the extracts of black mahlab seeds hold antifungal potential, which can be further explored in the treatment and control of some fungal infections.

For methanol extract the MIC were 12.5mg/mL for *Klebsiella pneumoniae* and 25mg/mL for *Staphylococcus aureus, Bacillus subtilis*, and *Escherichia coli*. The lowest MIC value of 12.5 mg/ml was obtained by water and methanol extract for *Bacillus subtilis* and *Klebsiella pneumoniae* respectively (**Table 4**). The relatively low MIC values (12.5 and 25mg/mL) against tested bacteria means that the seeds have the potential to treat any ailments associated with these bacterial pathogens effectively.

The minimum inhibitory concentrations (MIC) of water extract were 25mg/mL for *Candida albicans* and *Aspergillus niger*. For methanol extract the MIC was 12.5mg/mL for *Aspergillus niger*. The lowest MIC value of 12.5 mg/ml was obtained by methanol extract for *Aspergillus niger* (**Table 5**). The relatively low MIC values (12.5 and 25mg/mL) against tested bacteria means that the seeds have the potential to treat any ailments associated with these fungal pathogens effectively.

In this study, the water extract demonstrated a higher activity on the test organisms than the methanol extract (**Figures 2, 3** and **4**). This suggests that the active compound(s) were more soluble in water than in the methanol. The antibacterial activities of the extracts were attributed to flavonoids and tannins, that acted by different mechanisms comparable to the standard antibiotics, and with large margin of safety. Results obtained from this study were statistically analyzed using SPSS. SPSS was used to determine the significance of difference in inhibitory activity of the extracts on the test microorganisms. Analysis of results of antibacterial assays involving the water and methanol extracts revealed that there was significant difference ($P<0.05$) in inhibition zone diameter between *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli*. Analysis of results of antifungal assays involving the water extract revealed that there was significant difference ($P<0.05$) in inhibition zone diameter between *Candida albicans* and *Aspergillus niger*.

The traditional uses of the seeds for cosmetics purposes were attributed to antioxidant activity of flavonoids, triterpens and tannins. Sundang et al. (2012) report: antioxidant compounds (flavonoids and tannins) play an important role in limiting the damaging effect of free radicals of skin. Saeidnia et al. (2009) report: the triterpens and sterols are aromatic constituents of medicinal plants.

The black mahlab seeds in this study exerted a relatively high antibacterial activity against *Staphylococcus aureus, Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The study provides a scientific basis for the traditional application of black mahlab seeds as cosmomedical for skin and mucous membrane disorders. Therefore, this herb can be used for the treatment of such disorders caused by these bacteria and fungi. Further investigations of its activity against a wider range of bacteria and fungi and elucidation of the structure of the active principle(s) and its/their subsequent use in antibacterial and antifungal investigations and skin toxicological investigations of the extracts are strongly recommended.

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