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PHYTOCHEMICAL SCREENING OF *SIDA RHOMBIFOLIA* KNITTING EXTRACT MACERATED WITH *ALOE VERA* GEL TO COMBAT MOSQUITO ATTACKS

BY

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Abstract. The objective of this study is to identify the metabolites in the extract of *Sida rhombifolia* fibers treated with *Aloe vera* gel using colored reactions and thin-layer chromatography, following analytical techniques. The results show that the three samples fibers not treated with *Aloe vera* gel, fibers treated with *Aloe vera* gel, and washed treated fibers contain flavonoids in low quantities. The first two samples also present sterols and triterpenes in medium quantities, while the washed treated fibers contain these in low quantities.

Based on the chromatographic profiles, it can be concluded that the untreated fibers, treated fibers, and washed treated fibers extracts have nearly identical

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constituents, as the Rf values of the different spots are the same. The UV light absorption profile indicates the presence of chromophores responsible for this absorption, specifically double and triple bonds, which are also confirmed by fluorescence (π-π* transition). The different colors observed include yellow for flavonoids, blue for triterpenes, black for alkaloids, and orange for sterols.

Looking ahead, the experiments recommended by the World Health Organization (WHO) on the effectiveness of impregnated curtains in combating mosquito attacks (cone test, cylinder test, and tunnel test) will be the focus of future work**.**

Keywords: Knitting, phytochemical, screening, *Sida rhombifolia*.

1. Introduction

Sustainable development would require us to replace non-biodegradable products and materials with biomaterials, specifically eco-friendly materials (Aoun, 2010; Islam *et al*., 2017; Pasteur Institute of Lille, 2022). The majority of textiles used in the fight against mosquito attacks are polyethylene and polyesters treated with chemicals from the pyrethroid family (Kakou, 2021; Yadouleton *et al.*, 2018; WHO, 2016).

In Cameroon, three brands of long-lasting insecticidal nets (LLIN) are commonly used: Olyset Net which has polyethylene as its textile support and the insecticide is alpha cypermethrin, Perma Net 3.0 which has polyethylene as its textile support and insecticide is deltamethrin and Mag Net which has polyester as textile support and alphacypermethrin as insecticide mixed with PBO (WHO, 2016). These nets can cause skin irritation upon contact. Replacing them with a natural product and natural treatment are major concerns of the moment. This study focuses on investigating the metabolites of the extract of *Sida rhombifolia* fibers treated with *Aloe vera* gel.

Sida rhombifolia is a plant used empirically to relieve certain ailments including fatigue of wounds until they close, boils, ulcers Youssouf *et al.*, 2020; Nsangou *et al*., 2022a; Nsangou *et al*., 2022b). In Cameroon, there are three types or brands of LLIN which are: Olyset Net which has polyethylene as its textile support and the insecticide is alpha cypermethrin, Perma Net 3.0 which has polyethylene as its textile support and insecticide is deltamethrin and Mag Net which has polyester as textile support and alphacypermethrin as insecticide mixed with PBO (WHO, 2016). *Aloe vera* gel is an antimicrobial thanks to fumaric acid (Nsangou *et al*., 2022b and 2023). It has been tested and shown to be effective against four common bacteria: *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli* and *Salmonella* (Nsangou *et al*., 2022b; Sánchez *et al.*, 2020).

Phytochemical screening is a way to highlight the presence of groups of chemical families present in a given drug. This work aims to know the metabolites of the mixture of the two natural products (*Sida rhombifolia* extract

and *Aloe vera* gel). Phytochemical screening was done using colour reactions and thin layer chromatography (TLC) was carried out according to analytical techniques, Hamid *et al*., 2018; Mboussy *et al*., 2021).

2. Methodology

2.1. Materials

The *Sida rhombifolia* selected for this study is harvested from a swamp on the campus of the University of Yaoundé I in the Ngoa-Ekélé district of Yaoundé is in according to the National Herbarium of Cameroon. The rods were soaked in water at the ambient temperature of 32.7°C. 6 days later, we noticed the softening of the bast and the fibres were extracted by hand.

The *Aloe vera* sample from our work is picked from the Biyem-Assi district in Yaoundé also, in according to the National Herbarium of Cameroon. To obtain the gel the leaf was opened using a knife and the contents which are the gel were scraped out.

2.2. Extract Preparation for Phytochemical Screening

Five hundred grams of each fibre not treated with *aloe vera* gel (NNT), fibres treated with *aloe vera* gel (NT) and washed treated fibres (UL) were crushed, and the powder obtained was macerated in 2 L of methanol-water mixture (80:20), at room temperature, for 24 hours. The mixture was stirred for 10 minutes. The macerate is then filtered using hydrophilic cotton and the filtrate was evaporated using a rotary steamer under pressure reduced to 65°C relative to the boiling point of methanol; Then drying in a 45°C oven for 2 days to allow total removal of the solvent (Aoun, 2010; Islam *et al*., 2017; Makoundou *et al*., 1995).

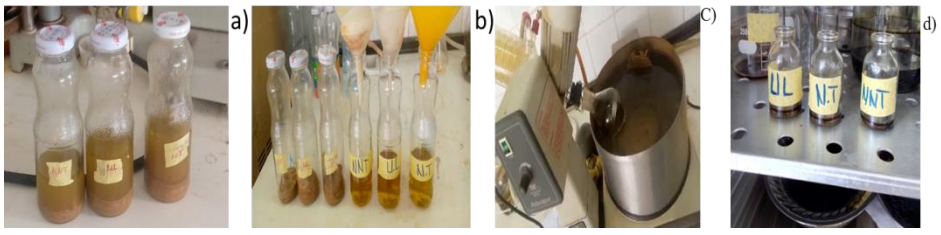


Fig. 1 − Preparation of the extract for phytochemical screening: a) Maceration of the powder in the water-methanol mixture; b) Filtration of the extract; C) evaporation of the solvent using a rotary steamer and d) drying in an oven.

2.2.1. Phytochemistry screening by colour reaction

a - Flavonoid test

500 mg of extract are dissolved in 5mL of 1N NaOH. The discoloration of the yellow and orange-yellow colour obtained after addition of 1N hydrochloric acid indicates the presence of flavonoids (Tia *et al.*, 2019).

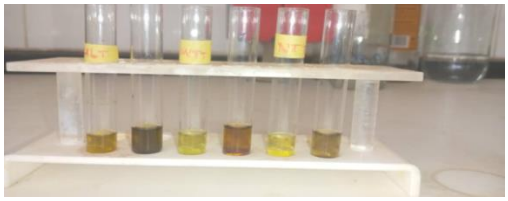


Fig. 2 − Identification of flavonoids.

b - Shinoda test

Procedure: In a test tube, dissolve 1 mg of compound in 2 mL of ethanol and add 1 mL of hydrochloric acid then 2 magnesium shavings. A brick red colour is formed, a sign of the presence of flavonoids (Enan, 2005a; 2005b).

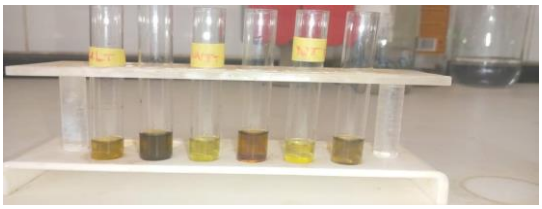


Fig. 3 − identification of flavonoids

c - Alkaloid Test

500 mg of extract are heated in 10 mL of one percent sulfuric acid for 2 to 3 min, then filtered. One mL of the filtrate was mixed with a few drops of Mayer's reagent. The formation of a white precipitate indicates the presence of alkaloids. No precipitation was observed during this test (Tia *et al*., 2019).

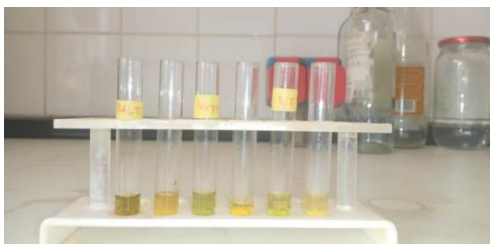


Fig. 4 − identification of alkaloids.

d - Triterpene and sterols test

500 mg of extract were dissolved in 20 mL of methylene chloride. Four drops of acetic anhydride and sulfuric acid were successively added. The presence of triterpenes is indicated by a purplish-red color, while a greenish-blue color is characteristic of sterols (Rikisahedew *et al*., 2023).

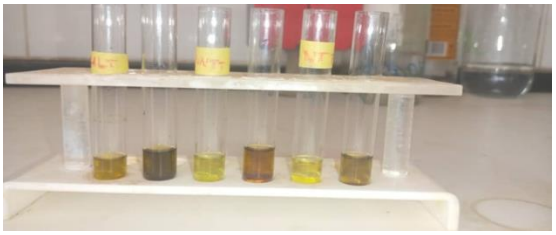


Fig. 5 − identification of triterpene and sterols alkaloids.

e - Saponin test

500 mg of extract were added to 5 mL of distilled water. After homogenization, the mixture was heated for 5 min until boiling. The presence of saponins results in the appearance of a persistent foam after 1 min. These results are further confirmed by TLC thin layer chromatography (Rikisahedew *et al.*, 2023; 2024).

2.2.2. Phytochemical screening by thin layer chromatography

It is based on the separation of chemical substances by migration and adsorption on a support or polar stationary phase, in a mobile or eluting phase, depending on their nature, the eluting power of the mobile phase, the adsorbent power of the support (Aoun, 2010; Islam *et al*., 2017; Makoundou *et al.*, 1995).

This technique allows the identification of several groups of secondary metabolites by the specific colorations of the spots, visible under certain wavelengths. A solution with a concentration of 10 mg/mL was prepared by dissolving 10 mg of each extract in 1 mL of absolute methanol. 10 microliters of each compound were spotted on F254 silica gel plates (stationary phase) using a micropipette. The plates were placed in tanks previously saturated with eluent or mobile phase and then dried (Sánchez *et al.*, 2020).

These plaques were observed before and after development either in the visible 254 nm or under a UV lamp at 366 nm. The frontal ratios (Rf) of the different tasks observed were calculated according to the formula:

$$Rf = \frac{Distance\ traveled\ by\ the\ compound}{Distance\ traveled\ by\ the\ solvent} \quad (1)$$

f - Flavonoids

Flavonoids were detected with Godin's reagent. After spraying the plate with Godin's reagent followed by heating at 100°C for 10 min; colouring is observed. Under 366 nm UV light, yellow or orange fluorescence indicates the presence of flavonoids (Aoun, 2010; Islam *et al*., 2017; Makoundou *et al*., 1995).

g - Alkaloids

After spraying with Dragendoff's reagent and heating the chromatogram at 100°C for 10 minutes, the alkaloids appear as orange spots in the visible (Enan, 2005a; 2005b).

h - Sterols and Polyterpenes

In general, sterols fluoresce under UV light at 366 nm in shades of blue, yellow, and green, while terpenes fluoresce in blue, yellow, green, and purple. Sterols are detected under UV light at 366 nm with the Liebermann-Bürchard reagent, showing yellow and yellow-green colors. For polyterpenes, this reagent classifies them into triterpene genins if the spots are visible in blue or purple. It also classifies them as oleanane and ursane-type triterpenes if the spots are red, or as lupane-type triterpenes if the spots exhibit yellow-orange fluorescence. Some sterols and terpenes are revealed in blue and purple, respectively, by Godin's reagent (Tia *et al*., 2019).

3. Results

3.1. Phytochemical Screening by Colour Reactions

After evaporation to dryness of the hydro-alcoholic extract, 65 g of extract were obtained, yielding 13% of the initial material The chemical groups highlighted are alkaloids, flavonoids, saponins, sterols and triterpenes. The results of the different tests are grouped in the table below.

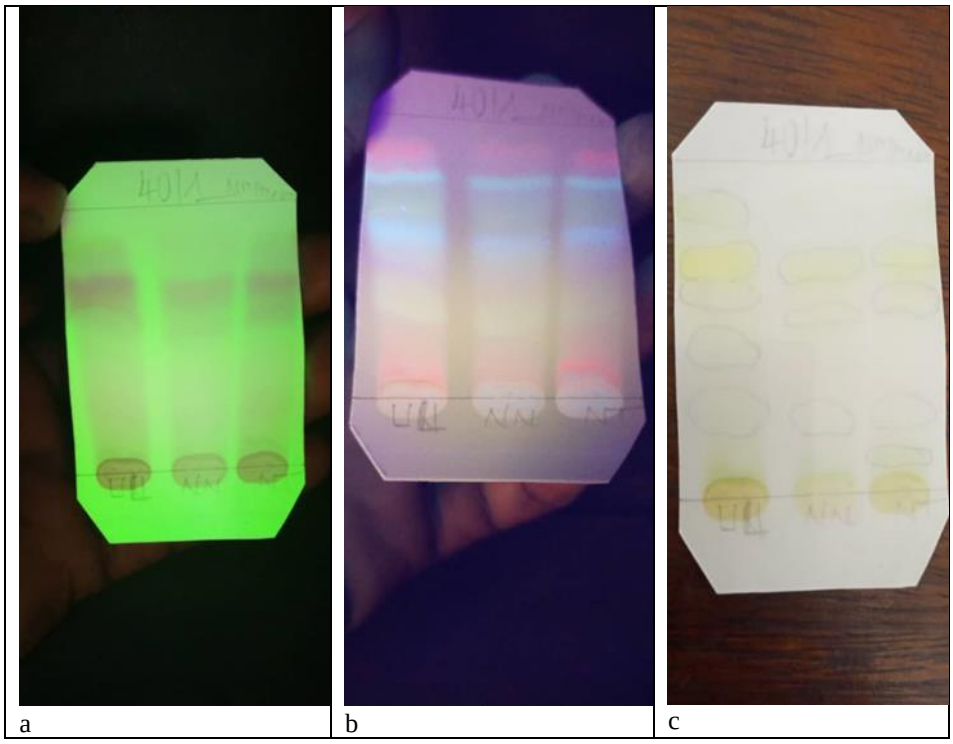
Table 1
Contents of the different chemical families present in the extract

Test	UL	NNT	NT
Flavonoids	+	+	+
Sterols and triterpenes	+	++	++
Saponin	-	-	-
Alkaloids	-	-	-

(+++): High content, (++): Medium content, (+): Low content

The three sample fibers—fibers not treated with *Aloe vera* gel (NNT), fibers treated with *Aloe vera* gel (NT), and washed treated fibers (UL)—contain flavonoids in low quantities. Additionally, the first two samples (NNT and NT) contain sterols and triterpenes in medium quantities, while the washed treated fibers (UL) contain these compounds in smaller amounts.

3.2. Phytochemical Screening By TLC



a b c

Fig. 6 − a) Chromatograms detection of sterols revealed by Godin's reagent in the visible. b) Chromatograms of polyterpenes revealed by Godin's reagent under UV/366 nm. Detection of c) Chromatograms for detection of flavonoids revealed by $AlCl_3$ in the visible.

Based on the chromatographic profiles, it can be concluded that the UL, NT, and NNT extracts contain nearly identical constituents, as the frontal ratios of the different spots are the same. The same profile, which shows UV light absorption, suggests the presence of chromophores responsible for this absorption, specifically double and triple bonds, which are further confirmed by fluorescence (π-Transition). The different colors observed include yellow for flavonoids, blue for triterpenes, black for alkaloids, and orange for sterols.

Excerpts	Godin reagent in the visible		Godin reagent under UV/366 nm		$AlCl_3$ in the visible		Secondary metabolite substances
	RF	Colour	RF	Colour	RF	Colour	
UL	0.80	Black	0.91	Yellow-orange	0.88	Yellow	Sterols Flavonoids and triterpenes
			0.88	White			
			0.65	White			
	0.32	Black	0.32	Yellow-orange	0.32	Yellow	
NNT	0.80	Black	0.91	Yellow-orange	0.88	Yellow	Sterols Flavonoids and triterpenes
			0.88	White			
			0.65	White			
	0.32	Black	0.32	Yellow-orange	0.32	Yellow	
NT	0.80	Black	0.91	Yellow-orange	0.88	Yellow	Sterols Flavonoids and triterpenes
			0.88	White			
			0.65	White			
	0.32	Black	0.32	Yellow-orange	0.32	Yellow	

Phytochemical screening of the extract revealed the presence of several compound groups, including flavonoids, sterols, and polyterpenes. The abundance of these molecular groups, identified both in visible light and under UV at 366 nm, and further confirmed through various reagents, underscores the richness of the extracts in bioactive chemical substances. This suggests that the plant may be effective in combating mosquitoes, either through the action of these chemical families individually or through their synergistic effects.

Given the complexity of plant secondary metabolites, whose biological activity often specific to mosquitoes can vary depending on the radicals associated with their basic structure (Tia *et al*., 2019), further testing on treated, washed, and untreated Anopheles gambiae fabrics is necessary to obtain reliable information on their effectiveness against mosquitoes.

3. Conclusions

The present study aims to identify the metabolites in *Sida rhombifolia* fiber treated with *Aloe vera* gel through colorimetric reactions and thin layer chromatography (TLC) using analytical techniques. The results show that the three sample groups—fibers not treated with *Aloe vera* gel (NNT), fibers treated

with *Aloe vera* gel (NT), and washed treated fibers (UL)—contain flavonoids in low quantities. Additionally, sterols and triterpenes were present in medium quantities in the first two samples (NNT and NT), while UL exhibited these compounds in smaller quantities.

Based on the chromatographic profiles, it can be concluded that the UL, NT, and NNT extracts have nearly identical constituents, as indicated by the consistent frontal ratios of the various spots. This profile, which demonstrates the absorption of UV light, suggests the presence of chromophores, such as double and triple bonds, further confirmed by fluorescence (π-Transition). The observed colors, including yellow for flavonoids, blue for triterpenes, black for alkaloids, and orange for sterols, corroborate these findings.

In future work, experiments recommended by the WHO, such as corn tests, cylinder tests, and tunnel tests, will be conducted on impregnated curtains to evaluate their effectiveness in combating mosquito attacks.

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SCREENING FITOCHIMIC AL EXTRACTULUI MACERAT DIN *SIDA RHOMBIFOLIA* CU GEL DE *ALOE VERA* PENTRU COMBATEREA ATACURILOR DE ȚÂNȚARI

(Rezumat)

Obiectivul acestui studiu a fost identificarea metaboliților din extractul de fibre de *Sida rhombifolia* tratate cu gel de *Aloe vera*, prin reacții colorimetrice și cromatografie pe strat subțire conform tehnicilor analitice. Rezultatele obținute arată că cele trei probe - fibre netratate cu gel de *Aloe vera,* fibre tratate cu gel de *Aloe vera* și fibre tratate și spălate - conțin flavonoide în cantități mici. Primele două probe prezintă, de asemenea, steroli și triterpene în cantități medii, în timp ce fibrele tratate și spălate conțin aceste substanțe în cantități reduse.

Pe baza profilurilor cromatografice, se poate concluziona că extractele din fibre netratate cu gel de *Aloe vera,* fibre tratate cu gel de *Aloe vera* și fibre tratate și spălate au practic aceiași constituenți, deoarece rapoartele frontale ale diferitelor pete sunt identice.

Pornind de la acest profil, care indică absorbția luminii ultraviolete, putem concluziona că există cromofori responsabili de această absorbție, și anume legături duble și triple, confirmate și de fluorescență (tranziție -π). Culorile diferite, cum ar fi galben pentru flavonoide, albastru pentru triterpene, negru pentru alcaloizi și portocaliu pentru steroli, susțin aceste concluzii.

În perspectivă, experimentele recomandate de Organizația Mondială a Sănătății, cum ar fi testul corn, testul cilindru și testul tunel, vor fi realizate în viitor pentru a evalua eficacitatea perdelelor impregnate în combaterea atacurilor de țânțari.