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Lawsoniainermis L. LYTRHRACEAE and *Cassia obovata*Collad. FABACEAE growing in Madagascar: Ethnopharmacologicalsurvey, phytochemical screening and coloring properties

F. MIANDRIMANANA[a], H. RIM FARASOA[a], J.M. RAZAFINDRAKOTO [b]*

[a]*University of Antananarivo, Faculty of Science, PO Box 906, 101 Antananarivo, Madagascar*
[b]*University of Antananarivo, High School of Agronomic Sciences, PO Box 175, 101 Antananarivo, Madagascar*

A B S T R A C T

This study aims at investigating the ethnopharmacological and local tinctorial uses of two dyeing plants *Lawsoniainermis* and *Cassia obovata* growing in Madagascar. Phytochemical screening of main compound families and study of the coloring of their extracts were also carried out with comparison with an imported Pakistani *Lawsoniainermis*leaf powder. Both plants are used by local people to treat gynecological ailments. *Lawsoniainermis* is more frequently used as tinctorial plant than *Cassia obovata*. This latter has a traditional use in the treatment of burns, ulcers, fever and urinary tract infections. *Lawsoniainermis* is commonly used for cosmetic purposes by local people. No local use for dyeing fabrics has been reported, however both plants are used for handicrafts to dye raffia. Compared to plants from other countries, both *Cassia obovata* and *Lawsoniainermis* growing in Madagascar have shown a change in their phytochemical constituents. This change affects the dye properties of the Madagascan *Lawsoniainermis* even though the main coloring compound Lawsone has been identified in its aqueous extract. Lowering the pH resulted in an increase in staining intensity, whereas the opposite was the case for the Pakistani extract, and literature data confirms that the coloring activity of lawsoneis reduced if the pH is more acidic. Both plants give a yellow to orange tone.

Keywords:*Lawsoniainermis*; *Cassia obovata*; traditional use; lawsone; dyeing properties.

1. Introduction

Secondary metabolites in plants are affected by internal and external factors.A change in plant growing environment could result in a change on its metabolites, and thus, a variation of its pharmacological properties (Li, 2020). *Lawsoniainermis*L. LYTRHRACEAE, also called hennais native to North Africa and South West Asia (Chaudhary, 2010), but it is naturalized in many tropical and subtropical regions including Madagascar (Zavada, 1993). *Cassia obovata*Collad. FABACEAE, known as neutral henna is native to tropical Africa (Ghassemi-Dehkordi, 2014). These two plants are well known for their phytochemical and pharmacological properties in many countries(Lavanya, 2018,Al-Snafi, 2019). However, henna and neutral henna of Madagascan origin are poorly documented. The aim of this work is to investigate the chemical profiles and the traditional uses of these two plants grownin Madagascar. A sample of henna leaf powder from Pakistan was purchased and used in the study for comparison.

* *Corresponding author.* Tel.: +0-000-000-0000 ; fax: +0-000-000-0000.
E-mail address: author@institute.xxx

2. Materials and methods

2.1. Ethnopharmacologicalsurvey

2.1.1. Study area

Survey was conducted in the Northern Region of Madagascar, in the Bongolava forest corridor between 15° 25’ 09’’ and 16° 10’ 05’’ South latitude, and between 47° 15’ 10’’ and 47° 39’ 04’’ East longitude. Figure 1 shows the localization of the study area.

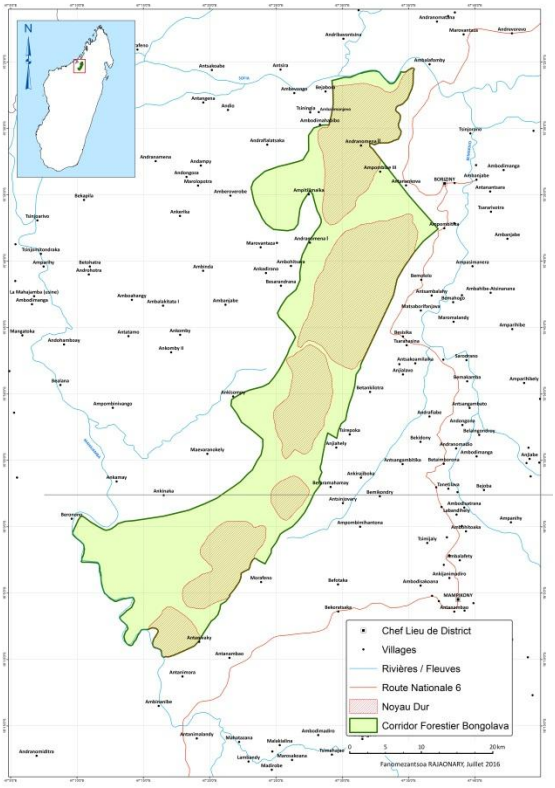

Fig. 1–Map of the ethnopharmacological study area.

2.1.2. Selection of participants

154 people participated in the inquiry, including traditional healers, herbalists, craftsmen and people using the two plants for personal care, aged over 15, males and females. No incentives were offered and people were free to answer or not to the questionnaire. A face to face interview was conducted, but the study was anonymous.

2.1.3. The questionnaire

Information on the vernacular names of the plants and their medicinal uses were collected. If the plants were used as tinctorial, process for dye extraction was asked. They were also requested to give more details on the application method and the purpose of the coloring.

2.2. Plant material

Leaves of *Lawsoniainerms* were collected in Port Bergé, in the Region of Sofia, Northern Madagascar. Leaves of *Cassia obovata* were from Antananarivo, Analamanga Region, and Capital city. Leaves were shadow dried until complete dryness. After grinding, the powders were carefully stored in air-tight containers away from light and humidity.

2.3. Phytochemical screening

Leaf powders of the two plants together with the Pakistani henna powder were extracted according to the method described by C. BEKKOUR, 2019 with slight modifications. 50g of each plant powder were macerated in distilled water during 24 hours with stirring. After filtration, extracts were evaporated under vacuum pressure at 40°C to 50°C.

2.3.1. Test for saponins

Aqueous extracts were prepared by stirring 1g powder of each plant in 10 ml of distilled water for 2 minutes. The appearance of foam that persists more than 15 min indicated the presence of saponins (Karumi, 2004).

2.3.2. Test for tannins

A few drops of 0.1% ferric chloride solution were added to 2 ml of each aqueous extract in test tubes. Apparition of greenish blue color indicated the presence of condensed tannins. A bluish black color indicated the presence of hydrolysable tannins (Gull, 2013).

2.3.3. Test for steroids and terpenoids

Aqueous extracts were dissolved in 1ml of acetic anhydride and 1.5 ml of concentrated sulfuric acid was added to form a layer. Appearance of a reddish brown color at the interface indicated the presence of steroids and terpenoids (Gull, 2013).

2.3.4. Test for flavonoids

Each of the extracts was dissolved in 5 ml of concentrated chlorhydric acid and a piece of Magnesium ribbon was added. A red to purple color indicated the presence of flavonoids (Praktash&Kontu, 2019).

2.3.5. Test for leucoanthocyanins

1 ml of concentrated chlorhydric acid was added to the extracts and samples were given steam bath during 30 min. After cooling, a color change to reddish purple indicated the presence of leucoanthocyanins.

2.3.6. Test for alkaloids

10 mg of extract was dissolved in 5 ml of 1% aqueous chlorhydric acid and boiled in a water bath during 5 min. After cooling, the solution was divided into three samples. One sample served as a reference. For the two other samples, Mayer's reagent and Dragendorff's reagent were added respectively. The presence ofyellowishto orange precipitate indicated the presence of alkaloids (Maria, 2018).

2.3.7. Test for quinones

5 ml of concentrated ammonium hydroxide was added to each extract which was previously treated with a mixture of ether and chloroform (3:1 V/V). A color change to reddish purple indicated the presence of quinones (Maria, 2018).

2.4. TLC profile

Extracts were dissolved in methanol. Thin Layer Chromatography on Silica gel was developed with different solvent systems:

- Hexane-dichloromethane (70:30 V/V).
- Hexane-dichloromethane (20:80 V/V)
- Ethyl acetate-methanol (80:20 V/V)
- Ethyl acetate-methanol (20:80 V/V)

2.5. Coloring properties

The test was carried out according to a method inspired from K.C. JAMES, 1986 with modifications. 90 ml of distilled water was added to 30 g of plant powder and the mixture was heated to 70 °C in a water bath during 30 min. After cooling, the extracts were filtered and divided into two parts. Lemon juice was added to one part in order to scientifically verify the traditionalpractice. 2 cm square wool pieces were dipped into the extracts overnight and were dried in the shade after removal. Dye color and intensity were analyzed for RGB color histogram (Red Green Blue) using Image J software.

3. Results and discussion

3.1. Ethnopharmacological survey

The percentages of uses of the two plants during the survey areshown in figure 2. *Lawsoniainermis* is more frequently used as a tinctorial plant while *Cassia obovata* is mainly used as a medicinal plant. Only 5% of the participants use it as a dye plant. Nearly half of the participants declare using *Lawsoniainermis* for its branches for the making of tool handles.*Cassia obovata* leaves are used to cover banana and mango fruits to speed up their ripening.The seeds of this plant are used like the seeds of*Tephrosia*as a coffee substitute. This use is very common in other African countries (Lavanya, 2018).

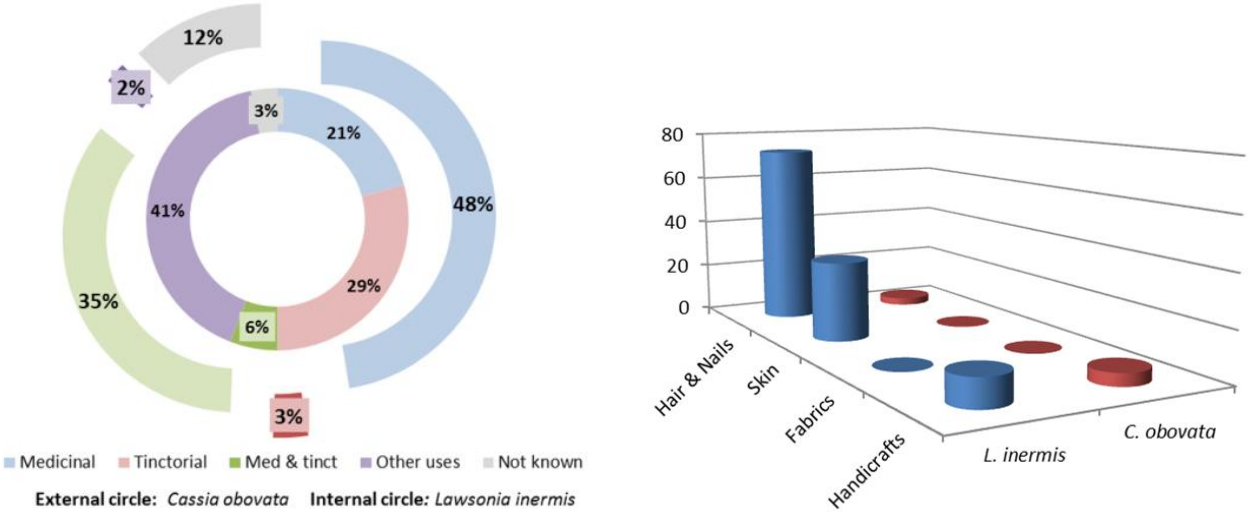

Fig. 2–Uses of the two plants- (a) General uses; (b) Uses as dye plants.

These two plants are not used to dye fabrics in Northern Madagascar. *Lawsoniainermis* is mainly used to dye hair and nails and to a lesser extent, to dye skin. *Cassia obovata* is used more for crafts than for cosmetics. Figure 3 shows the preparation methods and the color obtained. The only solvent used for dye extraction is water. For more than 90% of respondents, maceration at room temperature is the chosen method. For *Lawsoniainermis* this gives a reddish orange color. Adding a few drops of lemon juice to the plant extracts allows a more intense coloration. Extraction time varies from 1 hour to 24 hours. The aspect and the intensity of the coloring change according to extraction temperature and duration. These parameters also depend on application time.

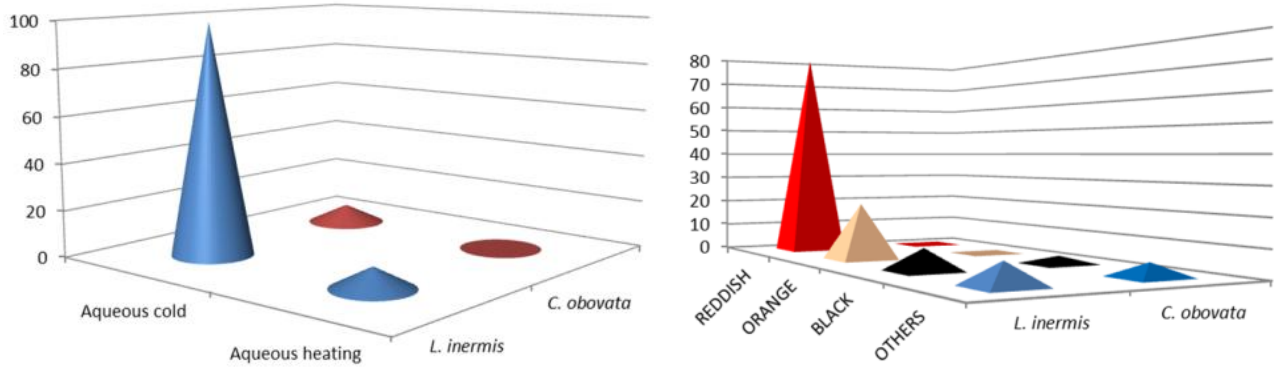

Fig. 3–Dye extraction (a) Method used; (b) Color obtained.

This traditional method of dyeing is consistent with scientific results demonstrated byKannanmarikani*et al*., 2015. Color strength increases with the dyeing time, and 24 hours is the optimal duration for application. All the medicinal uses cited in the table 1 below, have been demonstrated scientifically by researchers of other countries. Henna is used for its cosmetic properties on hair and nail growth. This is very common for many popular uses in other countries of Africa or India, (BadoniSemwal, 2014) and henna activity on hair growth is scientifically well established (Sadeghinia&Sadeghinia, 2011). Skin disease or skin cancer protection using topical application of *Lawsoniainermis* have been demonstrated by many authors(Kapadia, 2013,Niazi, 2020).

The two plants are both used in problems of female reproducing organ. In vivo experimentation of *Lawsoniainermis*Hydroalcoholic extract in mice has revealed that it affects the serum levels of estrogen and progesterone (Esteki, 2016). *Cassia obovata* has nociceptive and sedative activities (Ali, 1997) which may explain its use to relieve menstrual pains. It has been proven that *Lawsoniainermis* has antibacterial activity (Christy Jeyaseelan, 2012), and leaf extract is astringent (Gozubuyuk, 2014, Sultana, 2009). This may explain its traditional use against diarrhea.*Cassia obovata* leaf extract being antibacterial, analgesic, antipyretic, anti-inflammatory and cytogenetic (VijayaBharathi, 2018,Al-Naimy, 2010), this plant is traditionally used for relieving fever, burn and ulcer and also for the treatment of urinary tract infection.

Table 1 –Medicinal and cosmetic uses of*Lawsoniainermis* and *Cassia obovata*.

Plants	Uses	Parts used	Preparation
Lawsoniainermis	Skin disease	Leaves	Decoction / Topical poultice
Lawsoniainermis	Hair and nails growth	Leaves	Topical poultice
Lawsoniainermis	Irregular periods	Flowers, fruits, roots	Decoction
Lawsoniainermis	Diarrhea	Roots	Decoction
Cassia obovata	Burn, ulcer	Leaves	Local application
Cassia obovata	Painful periods	Roots	Decoction
Cassia obovata	Fever	Roots, leaves, pods	Decoction
Cassia obovata	Urinary tract infection	Roots	Decoction

3.2. Phytochemical screening

Table 2 shows the result of the phytochemical screening for the two *L. inermis* leaf extracts. Madagascan henna extract differs from Pakistani henna in its saponin and leucoanthocyanin contents. Madagascan henna also contains a higher amount of tannins and flavonoids. Both extracts are rich in quinones.

Table 2–Phytochemical screening for *Lawsoniainermis* leaf extracts from Madgascar and from Pakistan.

Metabolites	Madagascan *L.inermis*	Pakistani *L.inermis*
Saponins	-	+
Tannins	++	+
Steroids and terpenoids	+	+
Flavonoids	++	+
Leucoanthocyanins	+	-
Alkaloids	-	-
Quinones	++	++

Key: (-) Absence, (+) Moderate, (++) Abundant

Unlike*C. obovata* from Nigeria and from Benin, Madagascan *C. obovata* leaf extract contains saponins and reveals the absence of alkaloids and leucoanthocyanins (Table 3). These three extracts showing different metabolic profiles, it could be deduced that studies on the impacts of edaphic and climatic conditions are important prior to the exploitation of this plant, because of the possible modification in its phytochemical and pharmacological properties.

Table 3–Phytochemical screening from Madagascan *Cassia obovata*leaf extract compared to literature data.

Metabolites	Madagascan *C. obovata*	Nigerian *C. obovata* (Dabai, 2012)	Beninese *C. obovata*(Adjou, 2021)
Saponins	+	-	-
Tannins	+	-	+
Steroids and terpenoids	+	+	
Flavonoids	+	+	+
Leucoanthocyanins	-		+
Alkaloids	-	+	+
Quinones	+		+

Key: (-) Absence, (+) Presence

3.3. TLC profile

As shown in Tables 4 and 5, best separations of compounds were obtained using Hexane-dichloromethane (20:80 V/V) as solvent systems. As compared to the description by A.E. Musa, 2012, the compound revealing an orange spot in visible light, with Rf Values of 0.59 and 0.57 for Madagascan *L. inermis* and Pakistani *L. inermis* respectively in the solvent system Ethyl acetate-methanol (80:20 V/V) could be attributed to Lawsone.For Hexane-dichloromethane solvent systems Pakistani *L. inermis* shows the presence of a compound which is absent in Madagascan *L.inermis*. This would reflect a change in the composition due to the difference of growing sites.

Table 4–TLC profile of *L. inermis* from Madagascar and from Pakistan.

Solvent system	**Rf Values**	
	Madagascan *L.inermis*	**Pakistani *L.inermis***
Hexane-dichloromethane (70:30 V/V)	0.05	0.08
		0.76
Hexane-dichloromethane (20:80 V/V)	0.42	0.21
	0.63	0.46
	0.72	0.63
		0.72
Ethyl acetate-methanol (80:20 V/V)	0.59	0.57
	0.64	0.64
Ethyl acetate-methanol (20:80 V/V)	0.58	0.60
	0.66	0.69

Table 5–TLC profile of *Cassia obovata* from Madagascar.

Solvent system	**Rf Values**
	Madagascan *Cassia obovata*
Hexane-dichloromethane (70:30 V/V)	0.08
	0.10
	0.75
Hexane-dichloromethane (20:80 V/V)	0.17
	0.25
	0.63
	0.72
Ethyl acetate-methanol (80:20 V/V)	0.46
	0.66
Ethyl acetate-methanol (20:80 V/V)	0.67

3.4. Coloring profile

The color histogram shows the dominance of red and green channels. This is characteristic of yellow to orange tone. *Cassia obovata* gives the strongest color intensity and the Madagascan *L.inermis* gives the least intense color. For *Cassia obovata*, the green channel and the red channel show approximately the same intensity, thus, this extract gives a yellow color. For all the other extracts, red is more intense than green, showing an orange tone. For *C. obovata* and the Pakistani *L. inermis*, adding a small amount of lemon juice to the extract decreases the color intensity but the color tone does not change. The opposite effect is obtained for the intensity of the Madagascan *L. inermis* extract: lemon juice reinforces the color intensity. The color tones for the Madagascan and the Pakistani *L. inermis* extracts are the same but the Pakistani one gives a more intense coloration.

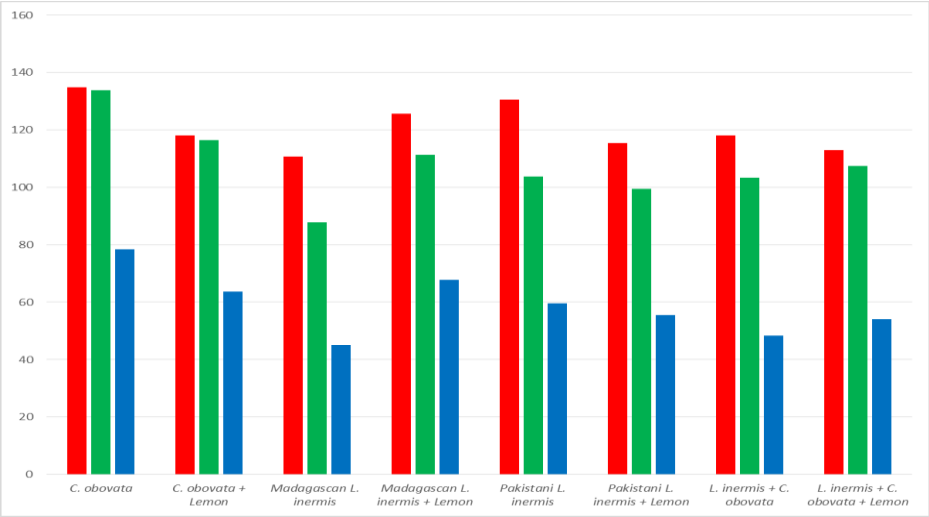

Fig. 4–Dye extraction (a) Method used; (b) Color obtained.

3.5. Discussion

Madagascan *L. inermis* and *C. obovata* have many common traditional uses with plants of these two species growing in other countries and most of these uses have been scientifically substantiated. As a result of TLC profile, Lawsone could be identified as the main dyeing compound in *L. inermis* from Madagascar. This latter has a different phytochemical profile compared to Pakistani *L. inermis*. The Madagascan plant is richer in Flavonoids, tannins and leucoanthocyanins which have antioxidant properties. The phytochemical difference has an impact on dyeing properties of the local *L. inermis*. The enhancement of tincture intensity with a mild lowering of the pH bythe addition of a small amount of lemon juice is peculiar to the Madagascan *L. inermis*.The popular use of this practice is experimentally demonstrated here. Other compounds like tannin, xanthone and gallicacid also contribute to the dye property of *L. inermis*. While Lawsone becomes colorless with acidic pH (Bhuiyan, 2017), the enhancement of color intensity observed in the Madagacan*L. inermis* extract could possibly be explained by the release of some other compounds that may be responsible for the dyeing property. A complementary study should then be carried out to analyze and to investigate all the compounds responsible for the coloring activity of Madagascan *L. inermis*. Another explanation would be the existence of some chemical reactions between the lemon juice compounds and the extract components. The confirmation of this hypothesis would require a further investigation.

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