Micrography characterization and chemical analysis of a traditional eye drops sold in town Abidjan of Côte d'Ivoire

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Abstract:
It is important to evaluate traditional eye medicine because of their potential iatrogenic properties linked to their composition and method of storage, which can lead to harmful effects that are sometimes irreversible on the eye. Thus, the objective of this work was to study the phytochemistry and characterization of traditional eye drops (TED) of unknown composition. The tests were carried out from a preparation 10% of TED extract. Macroscopic and microscopic characterizations were performed as well as chemical analysis by HPLC-QTOF-MS. Results showed that TED has a cloudy reddish appearance, the smell is flavored somewhat reminiscent of lemon with a slightly sour taste. Thick branched fibers, covering hairs, calcium oxalate and starch grains were observed under the microscope. Molecules such as burnamine, picralin, uncarin C; quercetin 3-O-glucuronide, apigenin 7-O-methylglucuronide, luteolin 7-glucuronide and 3,4-dimethoxycinnamic acid have been identified in TED.

Keywords: Traditional eye drops, Characterizations, Micrography, Phytochemical screening, HPLC-QTOF-MS, Medicinal plants

Caractérisation micrographique et analyse chimique d'un collyre traditionnel vendu dans la ville d'Abidjan en Côte d'Ivoire

Résumé :
Il est important d'évaluer les médicaments traditionnels oculaires à cause de leur iatrogénie potentielle liée à leur composition et mode de conservation pouvant entrainer des effets néfastes parfois irréversibles au plan oculaire. Ainsi, l'objectif de ce travail était d'étudier la phytochimie et la caractérisation d'un collyre traditionnel (TED).
Pour exécuter les différents tests, nous avons réalisé une préparation à 10 % (m/v) à partir du lyophilisat de TED de composition inconnue. Des caractérisations macroscopique et microscopique ont été effectuées de même qu'une analyse chimique par HPLC-QTOF-MS. Les résultats ont montré que TED a un aspect trouble de couleur rougeâtre, l'odeur est aromatisée rappelant un peu celle du citron au goût légèrement aigre. Des fibres épaisses et ramifiées, des poils tecteurs, de l'oxalate de calcium et des grains d'amidon ont été observés au microscope. Des molécules telles que la burnamine, la picraline, l'uncarine C ; la quercétine 3-O-glucuronide, l'apigénine 7-O-méthylglucuronide, la lutéoline 7-glucuronide et l'acide 3,4-diméthoxycinnamique ont été identifiées dans TED.

Mots clés : Collyre traditionnel, Caractérisations, Micrographie, Tri phytochimique, HPLC-QTOF-MS, Plantes médicinales

Introduction

In Côte d'Ivoire, despite the modernization of health structures authorized for primary eye consultations, traditional medicine sometimes remains the main first-line remedy for a large part of the population. This herbal medicine is practiced in both rural and urban areas because of their accessibility and acceptability (Adiko *et al.*, 2014); for many infectious, parasitic and inflammatory pathologies.

The remedies are prepared from all the organs of plants, leaves, stems, bark, roots or sometimes the whole plant. The leaves are the most used part of the plant, followed by the bark, stems and roots (Adiko *et al.*, 2014). Decoctions represent 56 % of the preparation methods, they are made in kitchen pot. The juices (35 %) are generally made from the fresh leaves, passed over low heat and crumpled in the palms of the hands; the extract obtained is instilled directly into the eyes, the preparation is renewed every day. The sap or latex (9 %) of the fresh organs is also put in direct contact with the eyes. The various drugs prepared in the form of decoctions, macerates or juices, are used in instillation, in eye baths, sometimes in washing the face. Applications are made 2 to 3 times a day, especially in the morning and evening (Adiko *et al.*, 2014). Regarding the indications, the eye conditions cited are "sticky eyes" to designate

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conjunctivitis, redness of the eyes due to the projection of snake venom, white spots on the cornea (corneal pillowcases), microbial or allergic conjunctivitis.

Traditional eye care should be given special attention due to complications to its potential iatrogenicity, composition, method of preparation, storage and use of toxic plants. The complications observed are conjunctival irritations, blepharitis and keratitis. Corneal ulcerations are frequent and their healing leads to corneal dystrophies responsible for visual impairments, low vision or even blindness, especially in children (WHO, 2004; Ouattara, 2002; Mwanza *et al*., 2001). Based on this observation, traditional eye recipes must be evaluated in order to identify possible risks, but very little work has addressed them. This research work is part of this process.

This study targeted a traditional eye drops coded TED offered to populations in Côte d'Ivoire for the treatment of conjunctivitis. The recipe was packaged in a modern eye drops bottle, however, the concentration and composition were unknown. Indeed, no indication mentioning the composition appeared on the bottle. Thus the objective of this work was to determine the macroscopic and microscopic characteristics of TED and from the HPLC-QTOF-MS method, to identify chemical molecules contained in the remedy.

Material and methods

Material

-Drug product: It consists of the traditional eye drops coded TED bought in a center run by traditional healers in Plateau town of Abidjan (**Figure 1**).

Traditional Eye Drops (TED) **TED lyophilisate**

Figure 1 : Traditional Eye Drops (TED)

Methods

▪ ***Preparation of dry extract:***

In a ground-flask weighed empty, 100 ml of TED were introduced and weighed again; then the balloon was put in the freezer then freeze-drying for 72 hours. After lyophilization, the flask was weighed again.

▪ ***Preparation of TED extract:***

We made preparations TED 10% to lead our various tests; 5 g of dry extract was dissolved in 50 mL of sterile distilled water.

▪ ***TED characterizations:***

Macroscopic evaluation: It was carried by sensory analysis based on the perception of the following various parameters:

- Color: appreciated by the eyes;
- Aspect: homogeneity or heterogeneity is assessed;
- Smell: perceived by drawing inspiration from certain volatile substances;
- Taste or flavor: acid, sour, bitter, astringent, salty or sweet.

Microscopic evaluation, concerns botanical identification by micrographic study. A small amount of TED dry extract was removed with forceps and mixed with a drop of KOH (5 %). The preparation obtained was observed under an optical microscope at magnifications 100 and 400 in order to observe fragments of tissues and cells generally present in herbal drugs (Domange, 2005).

▪ ***Physico-chemical study:***

Several parameters were evaluated according to methods describe by Soro *et al*. (2018).

▪ ***Determination of yield:***

The freeze-drying yield is the ratio expressed as a percentage between the quantity of material actually obtained and the theoretical maximum quantity.

$$R(\%) = \frac{mf}{mi} x100$$

R: lyophilization yield
mf: Final mass dry matter
mi: Test mass.

▪ ***Determination of dry matter:***

The determination of the solids content of the solutions is based on the measurement of the mass of the product resulting from the desiccation of the sample, expressed as percentage (Kouamé *et al.*, 2005).

$$M(\%) = \frac{mf}{vi} x100$$

M: Dry matter rate
vi: Test sample volume
mf: Final mass dry matter.

In a porcelain crucible, collect 2 g or 2 mL of the sample. Incinerate in a muffle furnace at a temperature of 600 °C ± 25 °C for 6 hours. Then weigh (mf).

$$Ct(\%) = \frac{mf}{mi(or\ vi)} x100$$

Ct: Total ash.

The pH measurement was performed directly on the 10 % TED. Note the pH value read and the temperature.

▪ ***Chemical study***

- ***Phytochemical screening:***

Characterization was made by colorimetric methods using standards reagents shown in **Table I** (Bruneton, 2009 and Yapi *et al.*, 2018).

Table I: Colorimetric characterization

Chemical groups	Identification reagents	Positive reaction
Sterols and polyterpenes	Acetic anhydride Concentrated sulfuric acid	Appearance at the interphase of a purple ring; or purple, turning blue then green
Polyphenols	Ferric chloride $FeCl_3$ (2 %)	Appearance of a blackish blue or more or less dark green color
Flavonoids	Hydrochloric alcohol, Magnesium shavings, Isoamyl alcohol	Release of heat then orange-pink or purplish color
Tannins Catechic Gallic	Formaldehyde Concentrated hydrochloric acid Sodium acetate Ferric chloride	Gelatinous precipitate (in large flakes) Intense blue-black coloring
Quinones	Ammonia	Appearance of a color ranging from red to purple
Saponosides	Foam index	Appearance of a persistent foam of at least 1 cm
Alkaloids	Potassium iodo-bismuthate Iodine-iodine reaction	a reddish-brown precipitate a reddish-brown precipitate

- ***Chemical composition:***

The determination of the chemical composition of TED began with a dereplicative study, without prior purification. Indeed, dereplication is a process which makes it possible to identify, in a complex mixture (raw natural extract), the presence of compounds already described in the literature even before their physical isolation. Dereplication strategies are based on analytical techniques and database searches to identify secondary metabolites (Michel *et al.*, 2013).

Among the analytical techniques, the ESI-QTOF-MS (electrospray ionization-quadrupole-time of flight-mass spectroscopy) technique could provide valuable information on the chemical structures of secondary metabolites (Ahn *et al.*, 2017). Thus, the extracts were analyzed by liquid chromatography coupled with high resolution mass spectrometry (HPLC-QTOF MS) in positive ionization mode in order to identify the secondary metabolites contained in TED.

Results and discussion

1. Macroscopic characteristic:

TED has a cloudy reddish appearance, the smell is flavored somewhat reminiscent of lemon with a slightly sour taste.

2. Physico-chemical study:

The results of the physico-chemical tests were reported in **Table II and III.**

The dry extract yield of TED is 52 %, while the level of dry residue obtained is 4.5 %. The total ash content was 0.10 % and the pH was 6.04 at 28.5 °C.

The pH of tear fluid and blood plasma are around 7.4. The pH of the eye drops should approach this value in order to avoid any discomfort or unpleasant or even painful stinging, which could compromise adherence to treatment. However, sometimes the buffering power of tears is sufficient to instill a solution with a pH deviating from neutral. Thus, overall, ophthalmic solutions generally have a pH of between 3 and 10 (Tuil, 2009).

Table II : Macroscopic characterization

Organoleptic characteristics of TED			
Aspect	Color	Odour	Taste
Trouble	Reddish	Lemon flavored	Sour astringent

Table III: Physico-chemical study of TED

TED physico-chemical study			
Yield (%)	Dry matter (%)	Total ash (%)	pH at 28.5°C
52	4.5	0.10	6.04

The pH of TED being 6.4 is within the range of tolerable values for the eyes. In addition, TED had a cloudy reddish appearance whereas the ophthalmic preparations must be clear (Afssaps, 2004).

3. Microscopic characteristic:

The micrography of the TED dry extract was shown in **Figure 2**, observed elements were branched fibers, thick fibers, punctate vessels, starch grains, octahedral calcium oxalate, and covering hairs.

Generally, starch grains are found in cereals, tubers, legumes and roots; covering hairs in the leaves; thick fibers in the stems and branched fibers in the roots (Domange, 2005).

The presence of these elements in TED makes it possible to certify the presence of leaves, stem and root in the preparation of this traditional eye drops. However, the presence of large plant particles may impair tolerance through feelings of irritation.

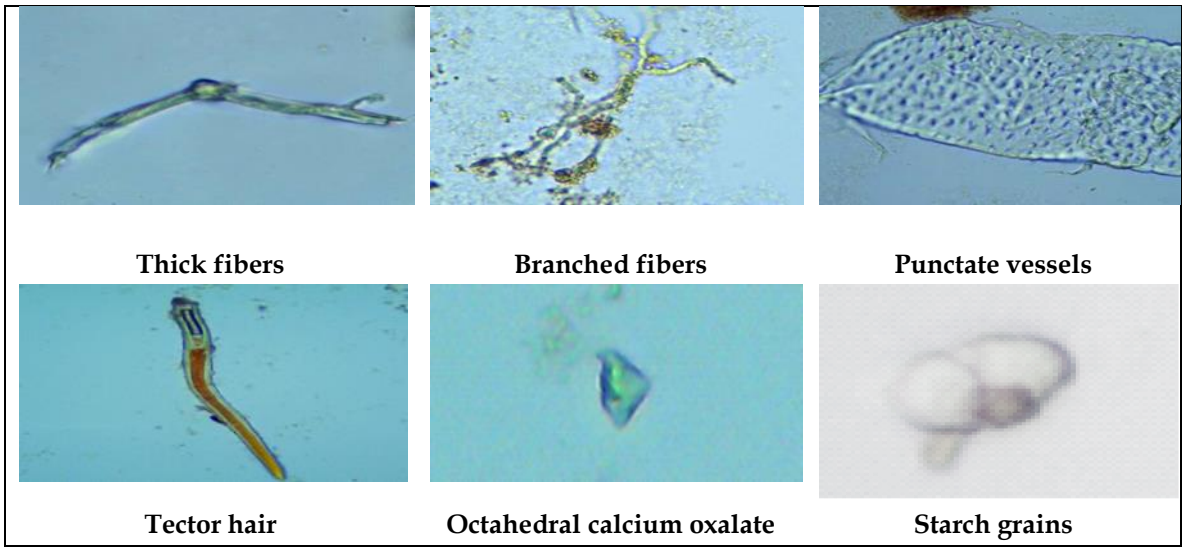

Figure 2: Microscopic characteristics of TED.

4. Chemical study:

▪ ***Phytochemical screening***

Colorimetric characterization in a tube revealed the presence of polyphenols, flavonoids, sterols and polyterpenes and alkaloids in TED (**Table IV**).

Table IV: Colorimetric results

Polyphenols	Flavonoids	Saponosides	Tannins		Quinones	Sterols and polyterpenes	Alkaloids
			Gal.	Cat.			
++	++	-	-	-	-	++	+++

(+) positive test; (-) negatif test; (+++) abundant; Gal: gallic; Cat: Catechetical; TED

▪ ***Chemical composition***

- ***Analysis of TED chromatographic profiles:***

The MS chromatographic profile (**Figure 3A**) of TED contains several secondary metabolites, some of which are in large quantities marked by the two peaks (2 and 7) of high intensity which correspond respectively to 3,4-dimethoxycinnamic acid and luteolin 7 - glucuronide. The other peaks are low intensities. The HPLC / DAD-UV analytical chromatogram at 254 nm gives a large peak retention time at approximately 16.53 minutes and in the retention time intervals 17-37 minutes appear weak peaks (**Figure 3B**).

The HPLC / DAD-UV analytical chromatogram at 280 nm shows major peaks that appear at different analysis times in the 14-33 minute retention time intervals (**Figure 3C**).

- ***Identification of compounds by the HPLC-QTOF-MS method:***

The molecules identified in TED are listed in order of retention time and an identification number has been assigned to each compound (**Table V and Figure 4**). The molecules listed in the table were identified in the online Global Natural Products Social Molecular Networking (GNPS) database.

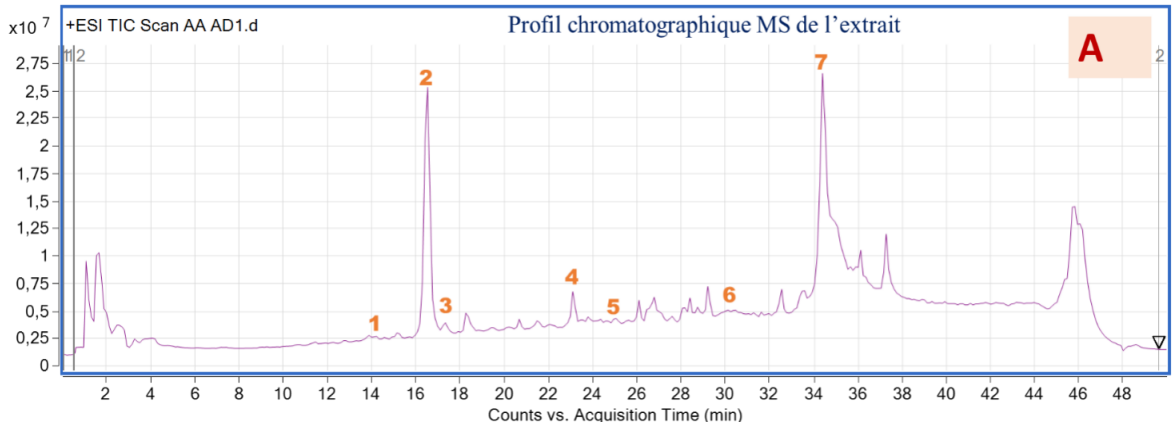

Figure 3A: MS chromatographic profiles of TED

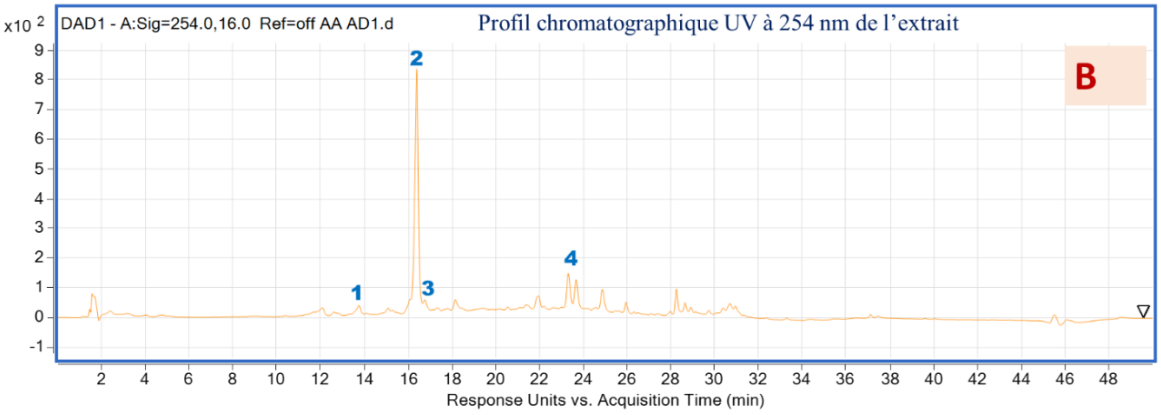

Figure 3B: MS chromatographic profiles of TED

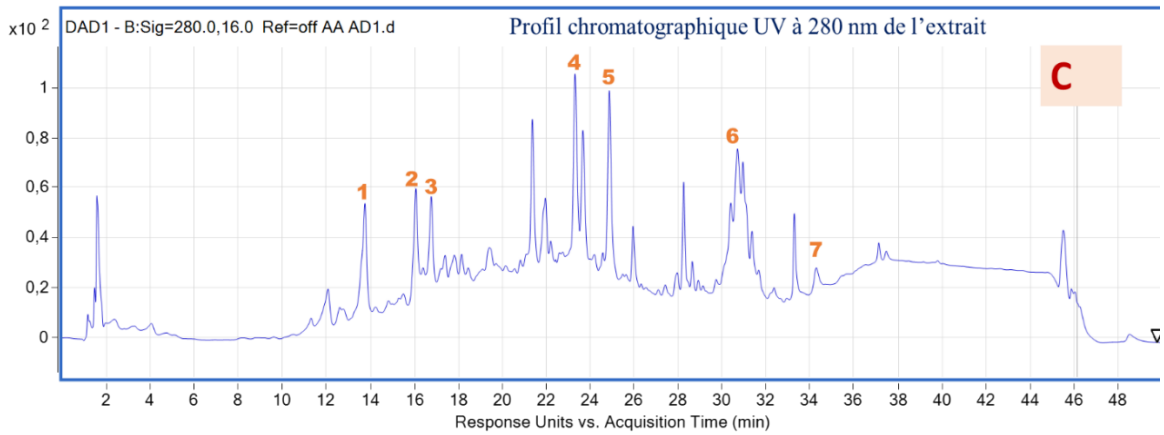

Figure 3C: MS chromatographic profiles of TED
Sunfire® C18 (150×2.1 mm; 3.5 µm, H2O+0.1% HCOOH/ACN (95:5 to 5:95, v/v), output: 0.25 mL/min, injected volume: 5 µL. Concentration: 1 mg/mL.

Figure 3: Chromatographic profiles of TED 10 %.

- ***Identification of compounds by the HPLC-QTOF-MS method:***

The molecules identified in TED are listed in order of retention time and an identification number has been assigned to each compound (**Table V and Figure 3**). The molecules listed in the table were identified in the online Global Natural Products Social Molecular Networking (GNPS) database.

Table V: List of some molecules identified in the TED

N° Pic	Identified compounds	Family of compounds	Retention time (min)
1	Burnamine	Alkaloid	14.68
2	3,4-Dimethoxycinnamic acid	Acid	16.53
3	Picralin	Alkaloid	17.56
4	Quercetin 3-O-Glucuronide	Flavonoids	23.90
5	Apigenin 7-O-Methylglucuronide	Flavonoids	25.17
6	Uncarin C	Alkaloid	31.35
7	Luteolin 7-Glucuronide	Flavonoids	34.32

These are alkaloids such as burnamine, picralin, and unarine C; flavonoids such as quercetin 3-O-glucuronide, apigenin 7-O-methylglucuronide and luteolin 7-glucuronide; and 3,4-dimethoxycinnamic acid have been revealed. TED was rich in chemical compounds due to the use of plant totum or a mixture of several plants. As a result, many compounds have been revealed by HPLC-QTOF-MS in TED.

The alkaloids identified were burnamin, picralin, and uncarin C. Burnamin and picralin are indole-type alkaloids reported in various parts of plants, especially in Apocynaceae family in genus such as Picralima, Alstonia, Rauvolfia and Aspidosperma. Indeed, these two molecules have been identified in *Picralima nitida* (Creed, 2021; Li-Mei, 2019; Ndukwu, 2019 and Erharuyi, 2014). In the genus Alstonia, *Alstonia macrophylla* (Arai, 2010); *Alstonia scholaris* (Pandey, 2020 and Jing, 2016) and *Alstonia boonei* (Creed, 2021) could be cited. Regarding Rauvolfia, *Rauvolfia yunnanensis* (Gao, 2011 and Cheng, 2009), *Rauvolfia serpentina* (Coulerie, 2016) and *Rauvolfia tetraphylla* (Gao, 2012). *Aspidosperma rigidum* (Vieira, 2013) were cited. These plants were widespread used in ivorian traditional medicine (N'dri, 2015; Adjoumani, 2018).

Regarding uncarin C, a biologically active alkaloid isolated from *Uncaria*, the species inventoried in Côte d'Ivoire are *Uncaria africana* and *Uncaria talbotii*, used by the Gouro to treat otitis externa (Tuo, 2017; Heitzman et *al.*, 2005). 3,4-Dimethoxycinnamic acid has been reported in *Nauclea officinalis* (Muronetz, 2020) and in rhizomes of *Curcuma longa* (Khan, 2008). The rhizomes of *Curcuma longa* are increasingly cultivated and used by the Ivorian populations for use in cosmetics, food and therapy.

In general, flavonoids are credited with antioxidant, anti-inflammatory and antibacterial properties. Indeed, Luteolin 7-Glucuronide which constitutes a major compound in this traditional recipe has been reported in many species such as

Oenothera biennis (Kim, 2021), *Carduus sp* (Kozyra, 2019), lavenders (Ez zoubi, 2020), *Salvia officinalis* (Dent, 2013), *Lippia alba* (Hennebelle 2008) and *Euphorbia hirta* (Al-Snafi, 2020). Thus, considering the results, TED contained plant particles such as leaves, roots or stems; and some local plants from genus *Picralina*, *Alstonia*, *Rauvolfia* etc. were probably used in the preparation of this traditional eye drops.

Conclusion

The study made it possible to characterize and identify elements of herbal drugs and chemical molecules in a traditional eye remedy of unknown composition. TED has a cloudy reddish appearance, the smell is flavored somewhat reminiscent of lemon with a slightly sour taste with the pH at 7.4. The micrographic study made it possible to reveal elements of plants. Chemistry has revealed the presence of alkaloids, flavonoids, polyphenols, acids, sterols and polyterpenes. Then it would be interesting to study the pharmacological properties of TED by evaluating the antibacterial and anti-inflammatory activity.

Competing interests

The authors declare that there are no known conflicts of interest associated with this publication.

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