International Journal of Medicine Research
www.medicinesjournal.com
ISSN: 2455-7404
Received: 08-10-2021, Accepted: 23-10-2021, Published: 09-11-2021
Volume 6, Issue 4, 2021, Page No. 14-18

Thin layer chromatography fingerprints of some ameliorated medecines based medicinal plants extrait in the democratic republic of the Congo: A preliminary study

Mbenza P A[1, 3], Nsangu M J[1], Kimbeni M T[3], Mbala M[4], Cimanga K R[2]

[1] Department of Basic Sciences, Faculty of Pharmaceutical Sciences, University of Kinshasa, Democratic Republic of the Congo

[2] Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences. University of Kinshasa, Democratic Republic of the Congo

[3] Faculty of Pharmaceutical Sciences, University of Kinshasa, Democratic Republic of the Congo

[4] Department of Chemistry, Faculty of Sciences, University of Kinshasa, Democratic Republic of the Congo

Abstract

A program on the standardization of prepared medicine based on different plants extracts was initiated in RD Congo by the Faculty of pharmaceutical sciences of the University of Kinshasa. The studies conducted in the program aimed the establishment of chromatographic fingerprints of these preparations in the present study, five preparation including Diazostimul (tablets), Manadiar (tablets and suspension), Meyamycin (tablet and suspension), Manalaria (tablets and suspension) and N'sansiphos (suspension) were investigated.

These thin layer chromatographic fingerprints were recorded on which major compounds were detected. These compounds showed different intensity according to the type of preparation. For the some preparation in tablets or suspension, these majors' compounds were present with different intensity of fluorescence under UV at 361nm.

These TLC fingerprints had specific characteristic related to each analyzed medicine. They showed uniformity and homogeneity of analyzed medicinal based on phase finishing, some analyzed ameliorated medicines were detected as standardized.

Keywords: herbal medicines, standardization, TLC fingerprint

Introduction

According to the World Health Organization (WHO), a herbal medicine is a finished medicinal product, labeled, which contains as active ingredients exclusively extracts from plants (underground or aerial parts), other plant materials or combinations of extracts from plants in the raw state. Plant products include juices, gums, fatty oils, essential oils or any other substance of a natural plant nature, in a standardized state (WHO, 1998).

The drugs that are locally produced are often prepared outside of an official framework regulating manufacturing and quality control standards and do not always meet requirements for chemical and pharmacotherapeutic reproducibility. For this purpose, WHO suggests reference to guidelines for their evaluation, in particular the use of chromatographic fingerprints to guarantee the constant quality of a preparation. (WHO, 1998).

In this study, we established the thin layer chromatographic fingerprints of some herbal medicines registered in the DRC. These fingerprints were established on the basis of the results of the phytochemical screening of reactions in solution.

They will be useful to manufacturers, analysts and regulatory authorities who may use them in compiling analytical quality assurance dossiers and in renewing and granting marketing authorizations.

Materials and methods

Ameliorated preparation

Different batches of five herbal medicines registered at the DPM (Department of Pharmacy and Medicines) of the Ministry of Public Health were collected from different manufacturers and purchased in a few pharmacies open to the public for our research in different pharmaceutical forms. These are Diazostimul (tablets), Manadiar (tablets and suspension), Manalaria (tablets and suspension), Meyamycin (tablets and suspension), and N'sansiphos suspension.

Fig 1: Registered medicines collected in the city of Kinshasa

Phytochemical screening

The search for secondary metabolites was carried out by means of chemical methods (reagents in solution) and TLC according to the methodology proposed by Marini-Bettolo *et al*. (1981) [4] and Harborne 1998 [3]. The following metabolic groups were investigated in each batch: Alkaloids, polyphenols, flavonoids, tannins, coumarins, quinones, cardiotonic heterosides, anthocyanins, saponins, steroids and terpenoids. All analyzes were performed in triplicate.

Preparation of extracts

Extraction of flavonoids in tablets and suspension

After grinding 10 tablets from each batch with a mortar, the obtained powder were weighed, dispersed in 20 mL of methanol and then heated in a bain-marie at 60°C for 10 minutes. The resulting dispersions were filtrate for analysis. One hundred milliliters of suspension were evaporated in an oven under ventilation at 40 ° C. The dried residues were dispersed in 20 mL of methanol and then heated in a bain-marie at 60°C for 10 minutes. The resulting dispersions were filtrate for the analysis of flavonoids.

Extraction des tanins

For extraction of tannins, 10 g of each tablet powders (or 100 ml of suspension) were mixed with 20 ml methanol 80 % for 24 hours. After filtration on paper filter Whatman n°1, each filtrate was evaporated in vacuum (40 °C) yielding dried extracts. The residue was dissolved in sulfuric acid solution (1N, XXX ml). The extraction of tannins was then performed thrice with 15 ml of ethyl acetate. After concentration, the obtained extract was used for analysis.

- For the extraction of alkaloids, the dried residues were firstly dissolved in aqueous solution containing 10 % ammonia. The extraction was fulfilled by using chloroform (3 × 15 mL). After concentration, the chloroformic extract was used for analysis.

The chromatographic fingerprints of each drug were established according to its metabolites revealed during a prior phytochemical screening (Brain and Turner, 1975; Harbone, 1998; Trease and Evan, 2002; Marini *et al*. 1981; Ngombe, 2010) [2, 8, 3, 4]. All analyzes were performed three times. All the chromatographic conditions were adapted in relation to the specificity of each group of substances analyzed.

Results and discussion

Results

The results of the chemical screening are shown in Table I.

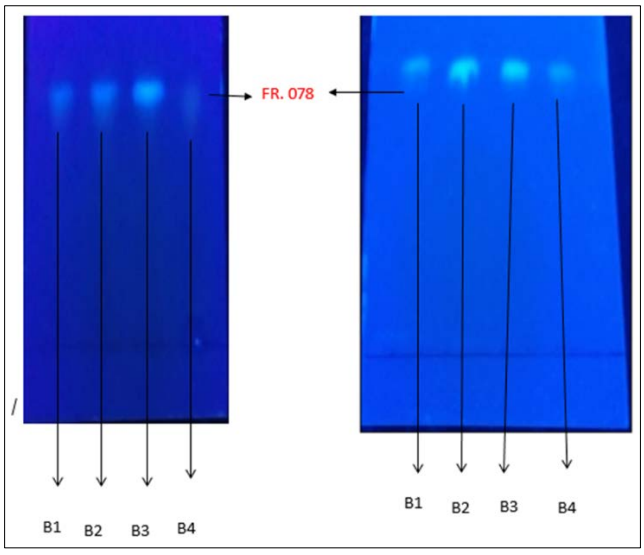
Table 1: Phytochemical screening results

Samples Chemical groups	Meyamycin (tablets and suspension)				Manadiar (tablets and suspension)				Manalaria (tablets and suspension)				Diazostimul (tablets)		N'sansiphos (suspension)	
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 1	Batch 2
Alkaloids	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Coumarins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinones	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
Cardiotonic hetersoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids terpenoids	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+
Anthocyanins	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Saponins	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-

+: Presence

-: Absence

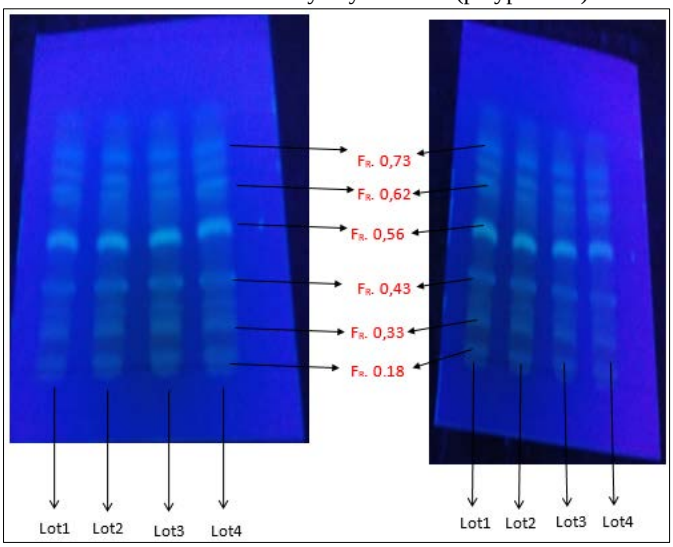
Chromatographic fingerprints of the different products are shown in Figures 3 to 5. In each case, the chromatographic fingerprint is only for the most dominant metabolic group found in the filtrate.



2a 2b

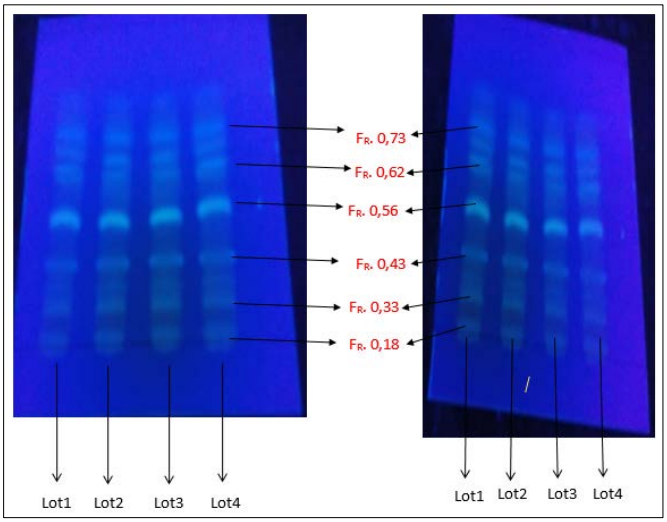
Fig 2a: Chromatographic fingerprints of four different batches of meyamycin syrup (polyphenols), Fig 2b**:** Chromatographic prints of four

different batches of Meyamycin tablet (polyphenols)



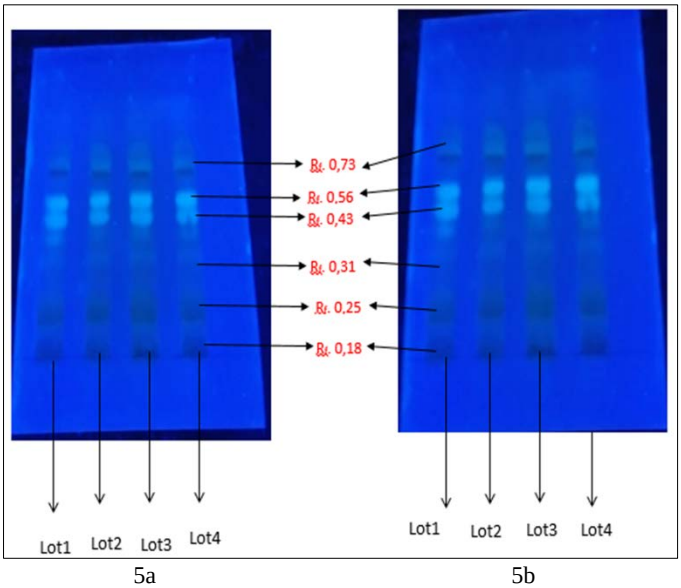
3a 3b

Fig 3a: Chromatographic fingerprints of four different batches of Manalaria tablets (tablets) Fig 3b: Chromatographic fingerprints of four different batches of Manalaria suspension (flavonoids)



4a 4b

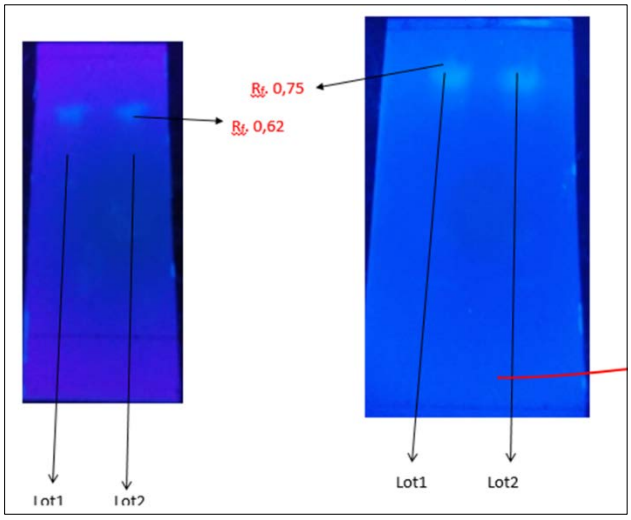
Fig 4a: chromatographic fingerprints of four different batches of four different batches of manalaria tablets (tablets) Fig 4b: Chromatographic fingerprints of four different batches of Manalaria suspension (flavonoids)



5a 5b

Fig 5a: chromatographic fingerprints of four different batches of manadiar suspension (flavonoids) Fig 5b: Chromatographic fingerprints of

four different batches of Manadiar tablets (flavonoids)



6 7

Fig 6: EChromatographic fingerprints of two differents batches of daizostimul tablets (alkaloids) Fig 7: Chromatographic fingerprints of two different batches of N'sansiphos suspension (Alkaloids)

Discussion

After treatment of the sample by TLC, the batches of the standardized drugs exhibited spots with the same frontal ratio, the same dimensions and the same intensities after development by the appropriate chemical developer or by UV (WHO, 1998). The chromatographic fingerprints of the standardized batches are superimposable. Thereby, Meyamycin suspension and tablets: For the suspension form, the different batches are not standardized. The different spots of four batches do not have the same intensities although they have the same Rf while for the tablet form, the batches are identical in pairs. Manadiar suspension and tablets: Medicines standardized with identical TLC for the four batches. Manalaria suspension and tablets: Medicines standardized with identical TLC for the four batches. Diazostimul tablets: Medicinal product standardized with two identical batches. N'sansiphos suspension: Medicinal product standardized with two identical batches.

TLC Fingerprints of Manadiar tablets and suspension in 4 lots recorded in EtAc/Formic acid/H2O : 88/6/6 with new reagent revelated the presence of two major compounds with Rf= 0,56 and 0,43 identified as flavonoids (Fig. 3A and B) in they gave intense yellow after visualization and observation under UV at 366 nm. Other minor compounds were detected in both fingerprint indicating that they were will superposable since the composition was the same. This intensity of yellow color and fluorescence developed by these two compounds were identical in Manadiar tablets and suspension. This Manadiar tablets and suspension can be considered as standardized medicine i.e. the 4 lots of Manadiar tablets and suspension are uniforme.

The last fingerprints recorded in this study concerned Diazostimul tablets and N'sansiphos suspension.

Resultats indicated the presence of major compound in each fingerprint identified as alkaloid with Rf=0,62 in Diazostimul and Rf=0,75 in N'sansiphos suspension indicating that these two alkaloids were different. In Diazostimul, the intensity of spot of the alkaloids is ??? and in N'sansiphos, it is intense in both lots of treated medicine suggesting also the uniformity and homogeneity of these ameliorated preparation based on the results, it can be concluded that these preparations were standardized.

The appreciated of intensity of spot of major compound uniformity and homogeneity was also plant extract (Mbenza *et al*., 2012; Bakana *et al*, 2003) [5]. These reported TLC fingerprints results constituted a refutable scientific basis confirming in standardization of these analyzed ameliorated medicines. It can also more infirm by this evaluation of their biological activities and acute, subacute and subchronic toxicity as proposed by Ch *et al* 2012;

Conclusion

The present study had reported for the first time TLC fingerprints of some ameliorated medicines found in RD Congo. These described analytical elements were with great importance in the control of the conformity of these medicines.

They will be used in future as reference documents for the quality control of these medicine. They were declared standardized based on their homogeneity, uniformity and identification of major compounds with particular characteristics.

These parameters were also important in the reproducibility and repeatability of therapeutic effects in humans. These TLC fingerprints can serve to detect falsification of these medicines since these analytical documents possessed unfalsifiable specific characteristics related to definite medicine. They can be help in future as reference documents for the analysis of new same ameliorated medicine in future.

References.

1. Bakana DP, Mbenza AP, Matondo NS et Kalenda T.D. Etat de standardisation de la Boisson Médicinale Populaire Tangawisi. (Annale de Pharmacie,2005:3:159-165.
2. Brain KR, Turner TD. The pratical evaluation of evaluation of phytopharmaceuticals, Weight-Science technical. Bristol, Britain, 1975, 56-64.
3. Harbone JB. Phytochemical Methods.A guide to modern techniques of plant analysis.Chapman& Hall Ltd. London, 1998, 40-96.
4. Marini – Betollo GB, Nicoletti M, Patamia M. Plant screening by chemical and chromatography procedure under field conditions. J. Chrom,1981:218:1.

5. Mbenza AP, Kalenda PT, et Bakana DP. Empreintes chromatographiques sur couche mince de quelques Phytomédicaments commercialises en RDC. (Annales de Pharmacie,2012:4(1):35-56.
6. Ngombe KN. Contribution à l'étude phytochimique et détermination de quelques activités bologiques *d'Anthocleista Schweinfurtii* (Gilg) Gentianaceae. Thèse de doctorat, en Sciences Pharmaceutiques. Université de Kinshasa, 2010.
7. OMS. Assurance de la qualité des produits pharmaceutiques,1998:1:38-51.
8. Trease GE, Evans C. Text Book of pharmacognosy 14thed. London, Bailière Tindall WB Saunders. Company Ltd, 2002, 13-58.
9. Wagner and Bladt S. Plant drug analysis: a thin layer chromatography. Atlas 2ème edition springer Verlag, 1995.