

Bioactive composition and TLC profile data on Pax Herbal Malatreat Tea

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Bioactive components of the plant(s) have been linked to most pharmacological activities of herbal drugs, these substances are affected by the quality control system adopted for the drug production processes. Thin Layer Chromatography is one of such quality control parameters that demonstrate uniqueness and uniformity between various substances, thus serving as an identity for such products. Pax Herbal Malatreat tea is a polyherbal drug used in the management and treatment of malaria symptoms. This study evaluated the different phytoconstituents present and developed fingerprint profiles (TLC) for the drug (Pax Herbal Malatreat Tea) to serve as a quality control check during the production consistency and market uniqueness. Qualitative phytochemical and chromatographic analyses were carried out using standard methods. The qualitative test revealed the presence of Tannin, Flavonoid, Saponin, Steroids, Reducing sugar, and Terpenoids, and the finger-print chromatograms after development with chloroform-ethyl acetate (9.5:0.5) showed five distinct components at 365 nm and four spots when sprayed with 20% methanolic sulphuric acid and viewed under visible light. From this present report, an identity card has been developed for the drug (Pax Herbal Malatreat Tea) via bioactive composition and TLC profiles which can be used in accessing the quality and consistency of the product.

Keywords: Bioactive composition; Thin Layer Chromatography (TLC); herbal medicine; quality control; Pax Herbal Malatreat Tea.

Article received at 04/06/2022 and accepted at 11/07/2022.

<https://doi.org/10.22456/2527-2616.125038>

Introduction

Sustainable operation of traditional medicinal factories' coffers is important, not only because of their value as an implicit source of new medicines but due to reliance on traditional medicinal companies for health. The vast majority (70-80%) of people in Africa consult traditional medical practitioners for healthcare (1). Traditional medicine-producing companies have been considered to be veritably successful in addressing several infections and habitual conditions with the advantage of being completely natural (2). Plants have numerous bioactive constituents like tannins, alkaloids, steroids, glycoside, fixed oils, phenols etc that are contained in different parts of the plant. The therapeutic effects of plants typically occurred from one or a combination of these bioactive constituents (3).

The increasing public demand for natural medicines has resulted in increased commercial activity and production of these medicines. This has led to growing concern about ensuring the consistency and quality of herbal drugs (4). To effectively coordinate the quality of raw materials, processing of materials, and the final products, it has become essential to develop reliable, specific, and sensitive quality control. Thus, thin-layer chromatography (TLC), has arrays of usefulness. The technique is simple, cost-effective, and versatile. The uses of TLC in quality control of plant materials include fingerprint profiling for

the assessment of chemical constituents of an extract and quantitative analysis of markers in plant drugs (5).

Pax Herbal MALATREAT TEA, is a poly-herbal drug developed by Pax-herbals, of the Benedictine monastery in Nigeria, for the management and treatment of malarial symptoms, and registered by, **NAFDAC**, with **REG. NO.: A7-04S2L** sells and distributed across Nigeria and abroad for over 20 years.

The drug is presented as a Light brown powder with a bitter taste and aromatic smell, teabagged in a filter paper, enveloped in customized transparent nylon, and packed in 25 tea bags per box. The drug consists of well-researched medicinal plants which include; *Sida acuta* (Bariyara), *Tridax procumbens*, *Alstonei boonei* (stool wood), *Phyllanthus amarus*, *Citrus sinensis* (Sweet orange).

It has been established that the exerted biological activity of any plant is the properties of its phytochemicals. This current study aimed at profiling the phytoconstituents of pax herbal MALATREAT TEA.

Materials and methods

Chemicals

All chemicals and reagents used were of analytical grade and the water used was glass distilled.

Extraction of the drug for phytochemical screening

A pack of Pax herbal Malatreat tea from four different production batch were obtained from the finished goods store of Paxherbal clinic and research laboratories, each batch was treated separately as follows; 20 Teabags were tour open and the powder drug was poured into a clean dried beaker and weighed and processed following the manufacturers' instructions. Briefly, 100 g of this material was soaked in warm water (40°C) for 45 minutes with constant agitation, this was filtered using a muslin cloth, and the filtrate was allowed to cool and stored in the refrigerator (at 4°C) for the analysis.

Qualitative phytochemical screening

The extracted filtrate was tested for the presence and absence of bioactive compounds by using the standard methods (6,7) as simplified by (8):

1. Test for alkaloid

Filtrate (3mL) + 3mL of 1% HCl + steam (30 minutes) + cooling + centrifuging at 2000-3000rpm for 10 minutes

- a) 1mL of supernatant + 1mL of Drangedroff reagent
- b) 1mL of supernatant + 1mL of Mayer's reagent
- c) 1mL of supernatant + 1mL of Wayner's reagent

Results for confirmation:

Dangedroff reagent - Orange ppt

Mayer's reagent - Creamy ppt

Wagner's reagent - Reddish brown ppt

2. Test for cardiac glycoside

Two mL of filtrate + 2 mL of glacial acetic acid + 1 mL of 0.1% FeCl₃ + 1 mL of Conc. H₂SO₄ acid. Green-blue colouration indicates the presence of cardiac glycoside.

3. Test for flavonoid

Two mL of the filtrate + 2 mL dilute ammonia, add 1mL of Conc. H₂SO₄ acid. Yellow coloration reveals the presence of flavonoid, which disappears upon standing.

4. Test for phlobatannins

Two mL of filtrate + 2 mL of 1% HCL acid + steam for 30 minutes. A red deposit at the base of the test tube shows the presence of phlobatannins.

5. Test for reducing sugar

Two mL of filtrate + 2 mL (Fehling solution A & B) + steam for 30 minutes. Red coloration reveals the presence of reducing sugar.

6. Test for saponin

0.5 mL of the filtrate + 5 mL of distilled water and shake vigorously. Persistent fronting means saponin is present.

7. Test for starch/polysaccharide

Two mL of filtrate + 6 drops of iodine solution. The blue-black coloration reveals the presence of starch.

8. Test for steroid

0.5 mL of sample + 0.5 mL of acetic acid anhydride + cool in ice + 0.5 mL chloroform + 1 mL conc. H₂SO₄ acid is added carefully using a pipette. A reddish-brown ring at the interphase of the two liquids reveals the presence of steroids.

9. Test for tannins

Two mL of filtrate + 2 mL of 0.1% FeCl₃ solution. A blue-black coloration indicates the presence of hydrolyzable tannin and brownish-green color indicates the presence of condensed tannin.

10. Test for terpenoid

Two mL of filtrate + 6 drops of Brady's reagent. A yellowish-orange coloration reveals the presence of terpenoid.

Extraction for TLC

Five tea bags of Paxherbals Malatreat tea (10 g) from four different production batches were extracted separately with 50 mL of methanol for 10 mins with constant shaking, the mixture was filtered through a muslin cloth. The resultant filtrate was added to 50 mL of deionized water and the mixture was partitioned with 50 mL of n-Hexane. The n-hexane phase was collected for the TLC and coded 1, 2, 3, and 4 respectively.

Thin-layer chromatography

The TLC was carried out on an analytical pre-coated TLC plate (silica gel, 60 F254, Sigma Aldrich, Germany). Samples (1-4, representing different production batches) were loaded with a micro-capillary tube on the TLC plate and developed in a tank (Shandon Southern T.L.C Chromatank, Unikit) with mobile phase chloroform: ethyl acetate (9.5:0.5).

Observation of separation

Plates were viewed under 350 nm UV light (ZF-1, niusiwen UV lamp, China) sprayed with 20% methanolic sulphuric acid, heated at 105°C for 30 mins, and also iodine vapor was used for derivatization.

Recording chromatograms

Fluorescence and non-fluorescence under UV light were recorded with a digital camera and visualization was improved by changing contrast, intensity, and/or brightness using picture editing software such as Microsoft Picture Editor.

Results and Discussion

Phytochemical screening results

Out of the ten groups of phytoconstituents that were screened for in the filtrate of the drug, and results obtained are presented in Table 1 below.

Table 1: Results of the qualitative phytochemical screening of Pax herbal MALATREAT TEA.

Parameter	Batches			
	1	2	3	4
Cardiac Glycoside	-	-	-	-
Saponin	++	++	++	++
Tannin(Hydrolysable)	++	++	++	++
Phlobatannin	-	-	-	-
Flavonoid	++	++	++	++
Steroid	++	++	++	++
Alkaloid	-	-	-	-
Reducing sugar	++	++	++	++
Terpenoid	++	++	++	++
Polysaccharide/Starch	-	-	-	-

Key: (+) = Mildly present; (++) = Moderately present; (+++) = Abundantly present; (-) = Absent

Phytochemicals are the essence of any medicinal plant, the presence of these constituents has a direct link to the pharmacological activities of such plant that contains them. Many researchers have reported the importance of phytochemicals and their pharmacological effects (9). The reported phytochemicals in this study (Table 1) include phenolics, this class of constituents has been reported to exhibit a wide range of activities including antimalarial effects (10) which is the activity indicated by the manufacturer of the drug investigated (Paxherbal Malatreat tea).

Thin Layer Chromatography results

Five conspicuous components were observed under UV light with respective distinct colors (Figure 1a), these components are the fluorescent ones and thus were captured at a wavelength of 365, their respective color, and R_f values are presented below (Table 2). However, only four components were seen 20% methanolic sulphuric acid sprayed plate under visible light (Figure 1b), and only two components with iodine vapor (Figure 1c).

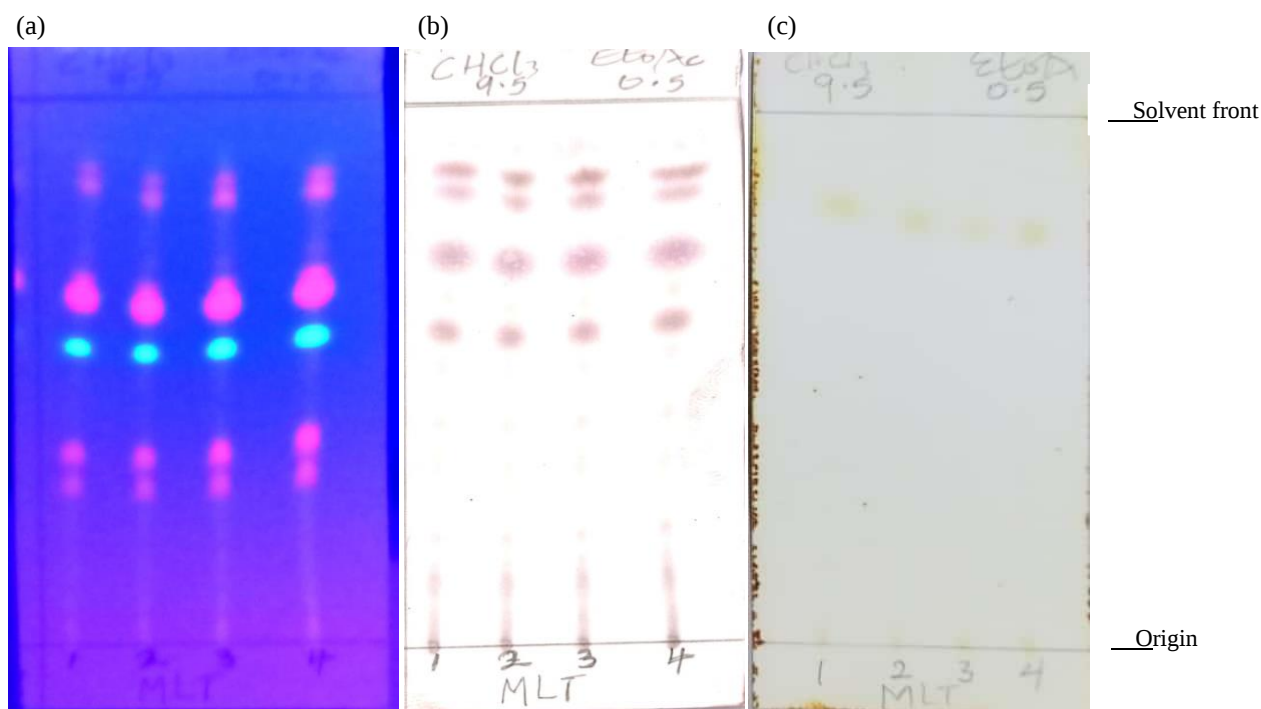


Figure 1: Pax Herbal Malatreat Tea Chromatograms: Adsorbent – Silica gel GF₂₅₄, Solvent systems: Chloroform:ethylacetate (9.5:0.5); (a) Viewed under 365 nm, (b) Sprayed with 20% methanolic H₂SO₄, Viewed under visible light, (c) Sprayed with Iodine vapor, Viewed under visible light.

Table 2: Chromatograms under UV lamp (365 nm)

Components	Colour	Rf
1	Pink	0.875
2	Pink	0.6875
3	Blue	0.6
4	Pink	0.3875
5	Pink	0.3125

Table 3: Chromatograms under visible light (sprayed with 20% methanolic sulphuric acid).

Components	Colour	Rf
1	Brown	0.9
2	Brown	0.8625
3	Brown	0.725
4	Brown	0.6

The usefulness of TLC in the quality control of herbal medicine cannot be overemphasized (11). Its merit includes but is not limited to low cost, simplicity, and reproducibility. The appearance of chromatograms in common and different bands can be useful for the identification and authentication of medicinal herbs. The TLC profiles of the drug studied (Paxherbal Malatreat tea) shown in figure 1a-c showed that the drug investigated has some fluorescent and non-fluorescent constituents with aligned bands and the same Rf values for all the different production batches. All identifications in the TLC are grounded on a comparison of the migration distances (Rf values), and the color of the spots between the sample when the TLC plate is sprayed with a specific chromogenic reagent. The quality of the analysis depends on the correct positioning of the samples used in the TLC (12).



Figure 2: Paxherbal Malatreat tea.

Conclusions

Phyto-constituents of Paxherbals Malatreat tea are limited to saponin, hydrolysable tannins, Flavonoid, Reducing sugar, and Terpenoid and established the identity of the drug to contain only five conspicuous spots on the TLC

plate under the above conditions. Thus the uniqueness and consistency of the herbal drug can be monitored through qualitative phytochemical screening and TLC profiling.

Acknowledgments

We wish to acknowledge the support of Pax Herbal Clinic and Research Laboratories management for providing the product for the study.

Conflict of interest

The authors declare no conflicts of interest.

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