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HPTLC Fingerprint Profiling of *Protorhus longifolia* Methanolic Leaf Extract and Qualitative Analysis of Common Biomarkers

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Abstract*—Protorhus longifolia* is known as a medicinal plant that has been used traditionally to treat various ailments such as hemiplegic paralysis, blood clotting related diseases, diarrhoea, heartburn, etc. The study reports a High-Performance Thin Layer Chromatography (HPTLC) fingerprint profile of *Protorhus longifolia* methanolic extract and its qualitative analysis of gallic acid, rutin, and quercetin. HPTLC analysis was achieved using CAMAG HPTLC system equipped with CAMAG automatic TLC sampler 4, CAMAG Automatic Developing Chamber 2 (ADC2), CAMAG visualizer 2, CAMAG Thin Layer Chromatography (TLC) scanner and visionCATS CAMAG HPTLC software. Mobile phase comprising toluene, ethyl acetate, formic acid (21:15:3) was used for qualitative analysis of gallic acid and revealed eight peaks while the mobile phase containing ethyl acetate, water, glacial acetic acid, formic acid (100:26:11:11) for qualitative analysis of rutin and quercetin revealed six peaks. HPTLC sillica gel 60 F254 glass plates (10 × 10) were used as the stationary phase. Gallic acid was detected at the R_f = 0.35; while rutin and quercetin were not evident in the extract. Further studies will be performed to quantify gallic acid in *Protorhus longifolia* leaves and also identify other biomarkers.

***Keywords*—**Biomarkers, fingerprint profiling, gallic acid, HPTLC, *Protorhus longifolia.*

I. Introduction

OVER the past years, medicinal plants have been used by human beings to treat various diseases such as cancer, etc. [1]. They are an essential source of secondary metabolites such as terpenoids, tannins, vitamins, alkaloids, polyphenols, quinones, lignins, coumarins and other classes of natural compounds with great therapeutic properties [2], [3]. In the past few years, the use of medicinal plants or herbal drugs for the treatment of various diseases has received a lot of interest with recent numerous medicines isolated from them, because of easy accessibility, affordability, safety and efficiency of plant-based medicines [4]. Furthermore, South Africa's plant species diversity is envisaged to offer alternative ways to treat diseases using some of the medicinal properties, and traditional cultural healing practises [5]. World Health Organization (WHO) has estimated that 80% of the residents in the developing countries are still dependent on the use of medicinal plants for their primary health care needs [6].

Standardization, identification and quality control analysis of chemical constituents of medicinal plants is a challenge and remains an important need to address problems such as efficacy, safety, lack of consistency and their quality [7]. The process is usually cumbersome because plant extracts constitute an enormous variety of chemical constituents [8]. Modern techniques such as chromatographic fingerprinting which include High Performance Thin layer Chromatography (HPTLC) plays a significant role in assisting with standardization, identification, and quality control analysis of complex plant extracts [9].

HPTLC is an important chromatographic fingerprinting technique due to its advantages that include; its ability to use relative small volumes of mobile phase to run several samples simultaneously on the same plate, reduced cost per analysis and analysis time. In addition, it offers improved resolution and estimation of chemical constituents can be achieved with accuracy that is reasonable [10].

Fig. 1 Leaves of *Protorhus longifolia*

Protorhus longifolia (Fig. 1) also known as red Cape beech belongs to the Anacardiaceae family. It is an evergreen tree that can reach up to 15 m in height and is found in Southern Africa [11]. This species has been used traditionally to treat different types of diseases such as hemiplegic paralysis, heart burn, bleeding from the stomach, blood clotting related diseases, heart water, dysentery and diarrhoea in cows [12]. Studies have been done on this species which reported activities such as antimicrobial and antioxidant activities [13], [14]. In this study, HPTLC method is used to develop fingerprint profiles of *Protorhus longifolia* methanolic extract and to also identify some of common biomarkers.

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II. Materials and Method

A. Collection of Plant Material

Fresh leaves of *Protorhus longifolia* were collected from Pietermaritzburg botanical garden KwaZulu-Natal, South Africa.

B. Fingerprint Analysis by HPLC

The leaves were dried at room temperature and grinded to powder, the powdered leaves were then stored at room temperature in an airtight bottle until further use. 100 mg of pulverised *Protorhus longifolia* was dissolved in 10 ml of methanol and sonicated for 10 minutes using Branson 3510-DTH Ultrasonic Cleaner and centrifuged at 6000 rpm for 10 minutes. The supernatant was used as a test solution for analysis.

C. Standards Preparation

1 mg of gallic acid, rutin and quercetin were individually dissolved in 10 mL of methanol.

D. Sample Application

2 µL of the samples and standards dissolved in methanol were applied in a form of a band using spraying technique with 100 µL syringe on HPTLC sillica gel 60 F254 glass plates (10 × 10) (Merk, Darmstadt, Germany) using automatic sampler TLC 4 (CAMAG, muttenz, Switzerland).

E. Chromatogram Development

After application of the samples and standards, the chromatogram was developed in a saturated CAMAG ADC2 automatic developing chamber, using mobile phase containing toluene, ethyl acetate, formic acid (21:15:3) (system 1) for gallic acid identification and ethyl acetate, water, glacial acetic acid, formic acid (100:26:11:11) (system 2) for rutin and quercetin identification. The developing distance was 70 mm from the lower edge of the plate. The developed plates were dried using hot air to remove the solvents from the plate.

F. Detection of Spots

The documentation of the plate was carried out using TLC visualizer 2 (CAMAG, Muttenz, Switzerland) before and after derivatization with *p*-anisaldehyde-sulphuric acid reagent in white light, UV 254 and UV 366 illumination mode, the plate was also scanned using TLC scanner (CAMAG, Muttenz, Switzerland) at 280 and 366 nm. Lastly, R_f values and fingerprint profiles were recorded by visionCATS CAMAG HPTLC software.

III. Results

The results from HPTLC fingerprint using the mobile phase system 1 and scanned at 280 nm revealed the presence eight of phytoconstituents with R_f values ranging from 0.035 to 0.647 (Table I and Fig. 2). HPTLC fingerprinting images of gallic acid and *Protorhus longifolia* powder in MeOH (Track 1, 4-gallic acid, track 2, 3- *Protorhus longifolia*) a) underivatized, under UV 254 nm; b) underivatized, under UV 366 nm; c) derivatized, under white light and d) derivatized, under UV 366 are shown in Fig. 3. The presence of gallic acid in the sample was identified and confirmed by relating the gallic acid band to that of the sample and also by relating the chromatograms (Figs. 4, 5) obtained from gallic acid and the sample together with their R_f values. The R_f value of standard gallic acid is 0.36, whereas the R_f value of gallic acid in *Protorhus longifolia* methanolic extract is 0.35 (Fig. 2), two R_f values almost correspond with each other.

TABLE I
R_F Values of Peaks for *Protorhus longifolia* Methanolic Extract for Mobile Phase System 1

Peak	Start		Max			End		Area	
	R_f	Height	R_f	Height	%	R_f	Height	A	%
1	0.005	0.0000	0.019	0.0222	1.52	0.035	0.0000	0.00035	0.66
2	0.039	0.0000	0.074	0.0686	4.71	0.97	0.0000	0.00187	3.51
3	0.102	0.0000	0.142	0.0936	6.42	0.171	0.0000	0.00247	4.63
4	0.171	0.0000	0.239	0.3713	9.49	0.265	0.0510	0.00602	11.30
5	0.265	0.0510	0.327	0.3713	25.48	0.344	0.3096	0.01586	29.78
6	0.346	0.3103	0.352	0.3177	21.80	0.375	0.0499	0.00714	13.41
7	0.382	0.0236	0.423	0.3877	26.60	0.476	0.0207	0.01456	27.35
8	0.550	0.0432	0.600	0.0580	3.98	0.647	0.0444	0.00499	9.36

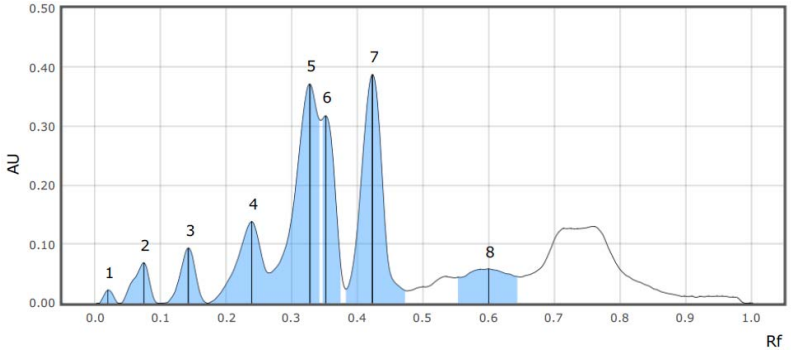


Fig. 2 Chromatogram of *Protorhus longifolia* methanolic extract for mobile phase system 2

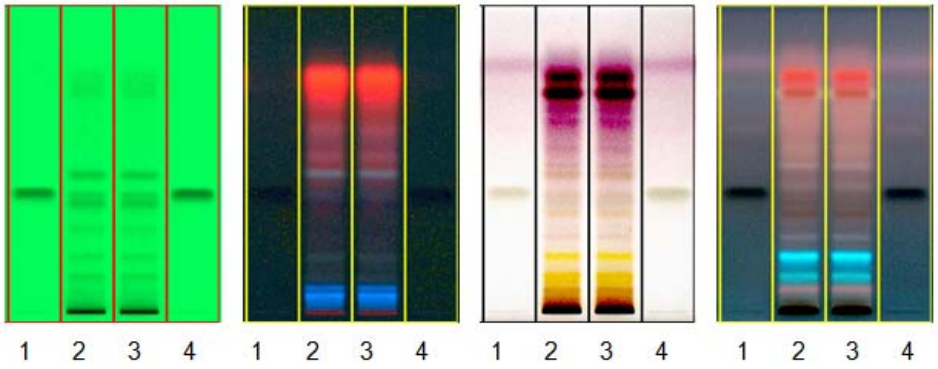


Fig. 3 HPTLC fingerprinting images of gallic acid and *Protorhus longifolia* powder in MeOH (Track 1,4-gallic acid, track 2,3 *Protorhus longifolia*) (a) underivatized, under UV 254 nm; (b) underivatized, under UV 366 nm; (c) derivatized, under white light and (d) derivatized, under UV 366

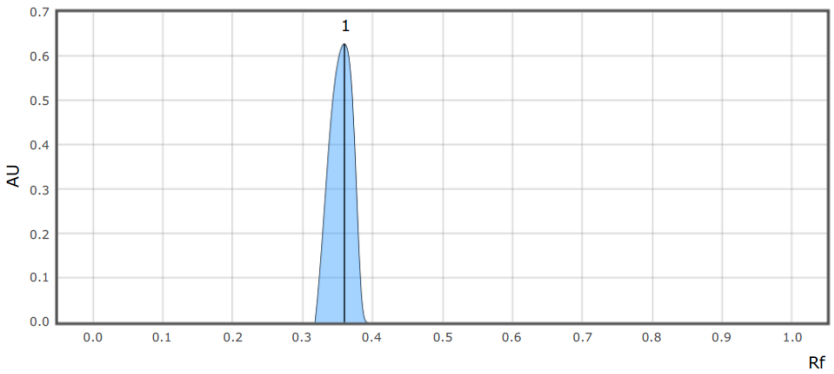


Fig. 4 Chromatogram of gallic acid scanned at 280 nm

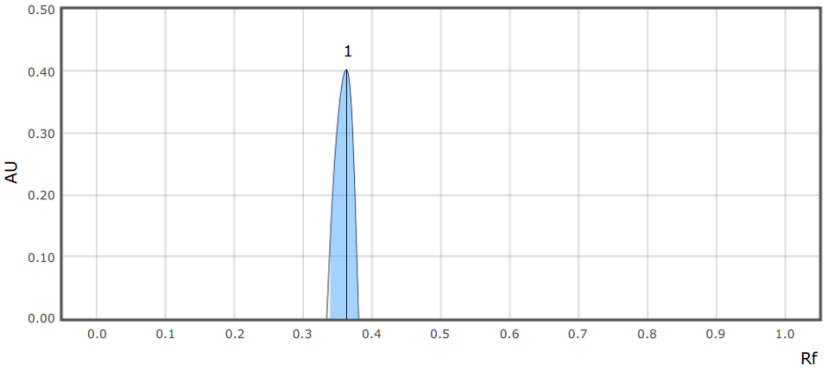


Fig. 5 Chromatogram of gallic acid in methanolic extract of *Protorhus longifolia* scanned at 280 nm

TABLE II
R_F VALUES OF PEAKS FOR *PROTORHUS LONGIFOLIA* METHANOLIC EXTRACT FOR SYSTEM 2

Peak	Start		Max			End		Area	
	R_f	Height	R_f	Height	%	R_f	Height	A	%
1	0.058	0.0000	0.084	0.0402	4.07	0.115	0.0015	0.00079	1.68
2	0.487	0.0458	0.555	0.0991	10.05	0.603	0.0727	0.00905	19.21
3	0.603	0.0727	0.639	0.1834	18.59	0.673	0.0388	0.00797	16.91
4	0.674	0.0384	0.710	0.1634	16.56	0.745	0.0331	0.00656	13.92
5	0.745	0.0331	0.829	0.0964	9.77	0.877	0.0494	0.00944	20.03
6	0.879	0.0494	0.953	0.4042	40.96	0.974	0.0008	0.01331	28.25

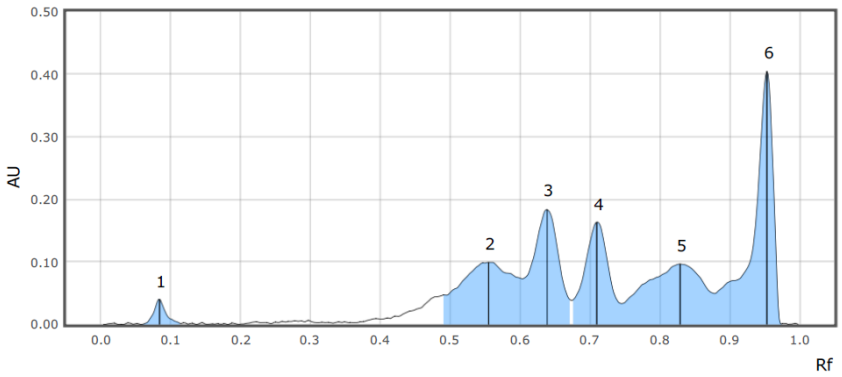
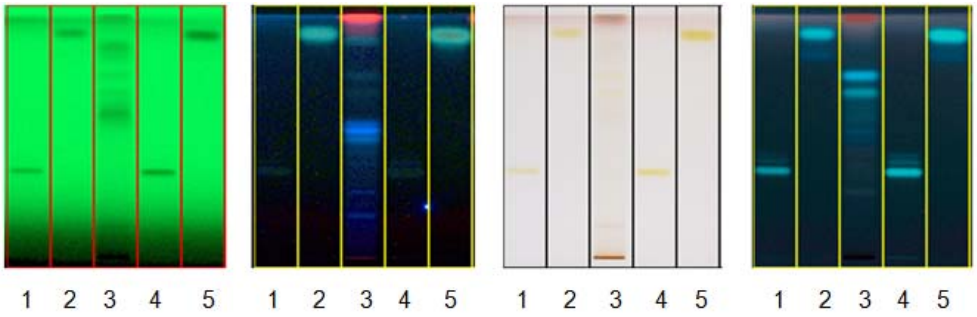


Fig. 6 Chromatogram of *Protorhus longifolia* methanolic extract for mobile phase system 2



(a) (b) (c) (d)

Fig. 7 HPTLC fingerprinting images of rutin, quercetin and *Protorhus longifolia* powder in MeOH (Track 1,4-rutin, track 2,5-quercetin, track 3 *Protorhus longifolia*) (a) underivatized, under UV 254 nm; (b) underivatized, under UV 366 nm; (c) derivatized, under white light and (d) derivatized, under UV 366

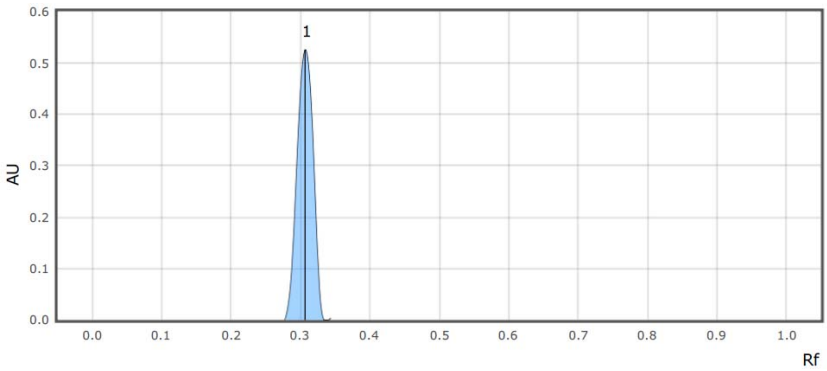


Fig. 8 Chromatogram of rutin scanned at 366 nm

The results from HPTLC fingerprint using the mobile phase system 2 and scanned at 366 nm revealed the presence of six phytoconstituents with R_f values ranging from 0.115 to 0.974 (Table II and Fig. 6). The obtained results from Figs. 6 and 7 revealed that fingerprint images of rutin (R_f = 0.31) and quercetin (R_f = 0.88) standards were absent from *Protorhus longifolia* methanolic extract.

IV. Discussions

Medicinal plants contain many secondary metabolites, as a result, there is a need for reliable and consistent techniques that can be used to analyze and separate them effectively. For the past two decades, HPTLC technique has arisen as an essential tool for quantitative, qualitative and identification of secondary metabolites in medicinal plants [15], [16]. In this study, the HPTLC fingerprint profile of *Protorhus longifolia* extract was established and common biomarkers (gallic acid, rutin, and quercetin) were examined for their presence.

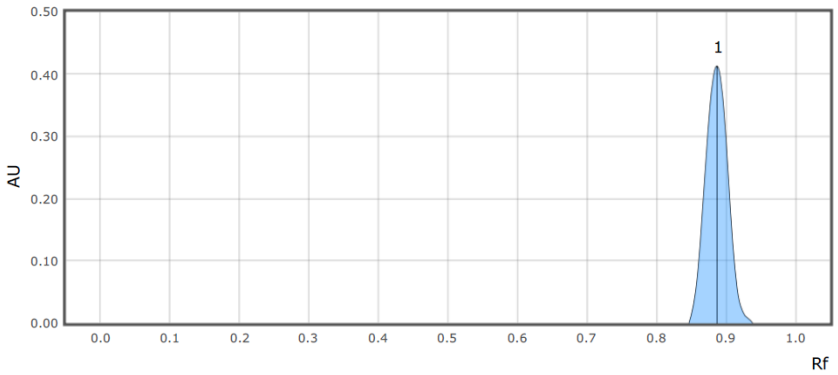


Fig. 9 Chromatogram of quercetin scanned at 366 nm

The results obtained in this study illustrated that eight peaks were present in *Protorhus longifolia* methanolic extract when the mobile phase containing toluene, ethyl acetate, formic acid (21:15:3) was used, while six peaks were present for the mobile phase containing ethyl acetate, water, glacial acetic acid, formic acid (100:26:11:11). The peaks are indicative of the presence of phytoconstituents. It was also observed in this study that gallic acid which is known for various biological properties, including anticancer and antioxidant [17], is present in the extract while rutin and quercetin were absent. As a result, further studies are necessary to quantify gallic acid in *Protorhus longifolia* leaves and identify other phytoconstituents that are responsible for making *Protorhus longifolia* a medicinal plant. The present work illustrates that HPTLC is a quick and reliable technique for the identification of biomarkers in medicinal plant extracts and provide valuable information for quality analysis of *Protorhus longifolia.*

Acknowledgments

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