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Original Article

Pattern Recognition and Chemometrics for Qualitative Pharmacological Indication of Moringa Oleifera Lam. Leaves Commercial Products

Kokoette Bassey*

ABSTRACT

Background: *Moringa oleifera* Lam leaves are known for their nutritional, pharmacological, and related biological 3 characteristics across the Globe. Its commercial products are marketed across Pretoria, South Africa. The label claims portray 4 such products contain *Moringa oleifera* leaves powder, extracts, or compounds inherent in the plant, and that pharmacological 5 activities of such products are intrinsically linked to them. **Methods:** We investigated ($n = 29$) commercial products using affordable, spectrophotometric UV-vis, high performance thin layer chromatography (HPTLC), high performance liquid chromatography finger printing and chemometrics principal component analysis. The aim of the investigation was to recognize the phytochemical patterns similarity between the plant extracts and commercial products, to ascertain which product contains quercetin-3-*O*-glycoside or kaempferol-3-*O*-glycoside marker compounds known to be present in *M. oleifera* leaves that should mitigate the pharmacological properties of the products. **Results:** The pattern of quercetin-3-*O*-glycoside and kaempferol-3-*O*-glycoside standards conform to a peak in the ethanol extract to suggest that both compounds are present in the ethanol but not in the dichloromethane extract. The HPTLC analysis also indicated the presence of the quercetin and kaempferol glycosides in the ethanol extract at Rf of 0.18 and 0.28 respectively. As for the commercial products, only $n = 3$ of the 29 (≈10%) revealed patterns that were like that of the ethanol extracts. A principal component analysis with $R^2 = 0.97$ for the DCM extract and 0.89 for the ethanol extract highlighted that commercial products P1, P7 and P24 as indicating good quality. The good quality commercial products clustered together with the quercetin-3-*O*-glycoside, kaempferol-3-*O*-glycoside, ethanol, or dichloromethane extracts while the poor-quality products were placed in a separate cluster in the PCA analysis conducted. **Conclusion:** This implies that only these three products will exhibit expected pharmacological and other biological activities displayed on the product labels.

Keywords: Pattern recognition, *Moringa oleifera* leaves, Commercial products, Quality indication.

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INTRODUCTION

Moringa oleifera Lam. (*M. oleifera)* is a nutritious medicinal plant that is naturalized in South Africa as medicine and food. It is one of the most studied, widely distributed, and commercialized species of a monogeneric family Moringaceae[1]. Its multiple uses pharmacological relevance and disease mitigating potentials attracted the attention of farmers and researchers

in the past and Ayurvedic traditional medicine postulates that *M. oleifera* can prevent up to 300 diseases[2]. *Moringa oleifera* Lam. leaves (MOL), are the most exploited and widely used for nutritional, preventive, and curative purposes. Several products claiming to contain any of the plants part - flowers, leaves, seeds, seed coats, stem and stem bark are marketed in health shops across South Africa and on the Worldwide web[2,3]. A search lead termed "*moringa oleifera* leaves commercial products "in a price comparison site-priceCheck turn in 300,000 results within 0.59 seconds to underscore the unscrupulous commercialization of the plant leaves in different dosage forms[4]. The disheartening facts remains that at the exorbitant cost of *Moringa oleifera* leaves based products available in the South African market and online, there is insufficient scientific validated studies for standardization and quality control of these products[5]. The labels on the *M. oleifera* leaves based commercial products (whether in capsule, tablet, tincture, infusion, cream) just to mention but a few used in this study tells the potential user that the products contain *M. oleifera* leaves, extracts or at least one of the isothiocyanates or flavonoids including quercetin-3-*O*-glycoside and kaempferol-3-*O*-glycoside that has been identified as marker compounds in most *M. oleifera* leaves. The pharmacological and biological benefits of using such commercial products are intrinsically linked to the presents of these compounds in the plant leaves extract. A survey of local *muthi* markets, health shops and pharmacies across Pretoria indicated to us that most commercial products are purportedly formulated using *M. oleifera* leaves as evident on the label claims. Consequently, this study investigated such commercial products sold across Pretoria. The aim of the study includes comparing the phytochemical pattern or fingerprint of the *M. oleifera* dichloromethane and ethanol extracts with that of the commercial products. Secondly, we aimed to identifying, products with good quality by way of establishing if such products contain either the plant extracts, using similarity in patterns or any of querceting-3-*O*-glycoside or kaempferol-3-*O*-glycoside that are standard pharmacological and biochemical markers of *M. oleifera* leaves[6]. Our investigations were conducted using cost effective UV-vis spectrophotometry, high performance

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thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC) and chemometrics principal component analysis (PCA), that is capable of recognizing pattern in multivariate data and clustering such datasets based on similarity in their phytochemical content[7]. This combination has reportedly been used for the authentication of the quality of apple vinegar[8].

MATERIALS AND METHODS

All solvents used in this study were of analytical grades and were purchased from either Rochelled Laboratory Services or

Sigma-Aldrich, Johannesburg, South Africa. The quercetin-3-*O*-glycoside, kaempferol-3-*O*-glycosides standards, and TLC plates (F_{254}-uv grade) were supplied by the same companies. Derivatization of the developed TLC plates were done using polyethylene glycol solution (10 g/200 mL dichloromethane) while viewing of the plates were carried using a TLC viewing chamber (Spectronics Corporation, Westbury, New York, USA) set at either at 245 or 366 nm.

Plant collection, commercial products purchasing and extraction

Briefly, 10.0 g green powder of *M. oleifera* leaves were collected as a donation from a master's student in the phytochemistry laboratory of Sefako Makgatho health Sciences University (SMU). The sample was already identified during harvesting in Limpopo by a local farmer and voucher specimen number MOL01 was allocated by the South African National Biodiversity Institute (SANBI), Pretoria. The powder was divided into two equal portions of 5.0 g each and ultrasonication assisted extracted with dichloromethane and ethanol respectively. The 5.0 g powder was placed in a 200 mL Erlenmeyer flask and to it was added 50 mL of ethanol. The Flask with its contents was then placed into an Ultrasonic bath (Rochelle Chemicals and Lab Equip, South Africa) and sonicated at 40 °C for 25 minutes. The sample mixture was later filtered using Whatman No 1 filter paper. The extraction was repeated two more times, filtered and the pooled filtrate evaporated with rotary evaporator (Stuart evaporator Cole Parmer Ltd., UK) connected to a Vacuubrand MZ 2C NT pump (Vacuubrand GmBH + Co Kg, Wertheim, Germany) to afford dry mass of ethanol extract. The same protocol was used to afford the dry dichloromethane extract. As for the commercial products (Table1), n = 29 were purchased across Pretoria. 5.0 g of solid commercial products including in-capsule powder, powders, creams, bar soap and tea bags were each weighed and placed in separate Erlenmeyer flask. In the case of decoctions and infusions, the in-product solvent was evaporated, and 5.0 g of the dry residue weighed. Each of the commercial products was separately extracted using the same extraction protocol to give the dry ethanol and dichloromethane extract respectively.

Standard solution preparation

Briefly, 5.0 mg of quercetin-3-*O*-glycoside was dissolved in 10 mL of methanol and 5.0 mL of kaempferol-3-*O*-glcoside was placed in 10.0 mL of hot ethanol. The mixture in a 25 mL Falcon tubes were respectively vortex for 10 min to allow the formation of a homogenized solution. Each stock standard solution was diluted to 1.0 mg/mL and used for the HPTLC analysis when required.

2.3 HPTLC, Spectrophometric and HPLC-PDA analysis of the extracts, standards, and commercial products

The HPTLC consisting of a CAMAG® (Switzerland) semi-automated HPTLC system was used to obtain a characteristic fingerprint for each sample. The prepared extracts (10.0 mg/mL) and 1.0 mg/mL of the standards were analysed on the instrument using the conditions displayed in Table 2. Visualization of the compounds on the developed plate was done by dipping the developed and dry plates into

Table 1. Moringa oleifera leaves commercial products purchased across health shops, pharmacies and muti markets across Pretoria, South Africa.

Product (P)	MOL product type	Sample location/GPS	Voucher specimen
P1	Powder#	Marabastad /25.7394° S, 28.1759° E	K2*
P2	Capsules	Marabastad /25.7394° S, 28.1759° E	K1
P3	Bar soap	Marabastad /25.7394° S, 28.1759° E	K3
P4	Powder	Marabastad /25.7394° S, 28.1759° E	K4
P5	Powder	Marabastad /25.7394° S, 28.1759° E	V12
P6	Powder	Marabastad /25.7394° S, 28.1759° E	V13
P7	Powder	Marabastad /25.7394° S, 28.1759° E	V14
P8	Energy drink	Marabastad /25.7394° S, 28.1759° E	V16
P9	Powder	Lynwood / 25.7643° S, 28.2673° E	B28
P10	Tincture	Lynwood / 25.7643° S, 28.2673° E	B29
P11	Cream	Lynwood / 25.7643° S, 28.2673° E	B30
P12	Cream	Lynwood / 25.7643° S, 28.2673° E	B31
P13	Tea bags	Lynwood / 25.7643° S, 28.2673° E	B32
P14	Capsule	Karen Park / 25.4329°S, 28.0715°E	N17
P15	Cream	Karen Park / 25.4329°S, 28.0715°E	N18
P16	Powder	Karen Park / 25.4329°S, 28.0715°E	N19
P17	Powder	Karen Park / 25.4329°S, 28.0715°E	N20
P18	Decoction	Karen Park / 25.4329°S, 28.0715°E	N21
P19	Cream	Pretoria CBD/25.7489°S, 28.1854° E	N22
P20	Powder	Pretoria CBD/25.7489°S, 28.1854° E	KT7
P21	Capsule	Pretoria CBD/25.7489°S, 28.1854° E	KT8
P22	Powder	Pretoria CBD/25.7489°S, 28.1854° E	KT9
P23	Tea bags	Pretoria CBD/25.7489°S, 28.1854° E	KT10
P24	Capsule	Pretoria CBD/25.7489°S, 28.1854° E	KT11
P25	Powder	Marabastad /25.7394° S, 28.1759° E	M23
P26	Powder	Marabastad /25.7394° S, 28.1759° E	M24
P27	Bar soap	Marabastad /25.7394° S, 28.1759° E	M25
P28	Decoction	Marabastad /25.7394° S, 28.1759° E	M26
P29	Powder	Marabastad /25.7394° S, 28.1759° E	M27

Usually added to porridge served as breakfast to majority of children and adults alike.

*Randomly selected for the pilot study and allocated P1 instead of P2.

Table 2. The HPTLC conditions used for the analysis of the extracts and standards.

System set	Server Deaktop-H3E9P9H, Version3.121109.3
up	
Software	S/N: 281364
ADC 2	S/N:200923
ATS 4	N/A
Chamber TLC visualiser 2 **Chromatography plate layout**	S/N: 290053
Stationary phase	Merck, HPTLC Silica gel 60 F_{245}
Plate format	200 x 100 mm
Application type	Band
Application	Position Y: 8 mm, length: 8 mm, width: 0 mm
Track	First position X: 43 mm, distance: 11.4 mm
Solvent front **Application - ATS 4 (S/N: 200923)**	70 mm
Spay gas	Air
Sample solvent type	Methanol
Filling speed	15 µL/s
Predosage volume	200 nL
Retraction volume	200 nL
Dosage speed	150 nL/s
Filling quality	Standard
Rinsing cycles/volume	¼ s
Filling cycles/volume	¼ s
Rinsing solvent	Methanol
Nozzle temperature **Development – ADC 2 (S/N: 281364)**	unheated
Development type	Activated and saturated
Saturation	On: 25 mL, 20 min, with pad
Activation	On: $MgCl_2$ 933% RH), 10 min
Drying	On: 5 min, with pre-drying
Pre-conditioning	Off
Mobile phase	10 ml: (DCM, EtOAc:MeOH: FA (90:10:20:0.5 v/v/v/v)
Tank	TTC 20x10
Saturation time	20 min
Volume front through	10 mL
Volume rear through	20 mL
Drying time	5 min
Drying temperature	Room temperature

polyethylene glycol (PEG) solution, using a CAMAG® Chromatogram Immersion Device 3. Scanning of the 5 mg/mL *M. oleifera* leaves dichloromethane and ethanol extract solution and each of the commercial product to register their absorbance fingerprint was made possible using Nanocolor UV/VIS II spectrophotometer (Macherey-Nagel, Landsberger, Berlin). A Shimadzu® HPLC-PDA (Shimadzu Corporation, Japan) ystem that was used to generate the multivariate absorbance data was equipped with a photodiode array detector, which was sed to optimize the separations during the initial analyses. The plant extracts and pure compounds were separated on ShimPack (4.6 x 150 mm, i.d., 5 mm particle size; Shimadzu) maintained at 40 °C. The mobile phase consisted of 0.1% aqueous formic acid (Solvent A) and HPLC grade MerckTM, (Germany) acetonitrile (Solvent B), at a flow rate of 0.3 mL/min. Gradient elution was applied as follow: 35% B in 7 min, changed to 50% B in 1 min (held for 2.5 min), before returning to the initial ratio in 0.5 min and held for 9 min in a total run time of 20 mins. Principal component analysis (PCA) of the spectrophotometric and HPLC data was performed using Simca 15® (Umetrics, Sweden).

RESULTS

Phytochemical pattern recognition in *M. oleifera* leaves extracts and standards by HPTLC Analysis

From the HTLC profiles (Figure 1), one could hypothesize that any commercial product with good quality potential should have identical phytochemical pattern like either dichloromethane (track 1) or ethanol extracts (track 4). Such products should also consist of either quercetin glycoside (track 3), kaempferol glycoside (track 2) or both. This is WHO pre-requisite for good quality herbal medicines and other related products[9].

The HTLC analysis of the plant leaves extracts, commercial products, and standards – quercetin and kaempferol glycosides afforded a visual comparison of the profiles of the analyte. It is evident from results obtained (Figure 1), that the *M. oleifera* leaves ethanol extract indeed contains the standards quercetin at Rf = 0.18 and kaempferol glycosides at Rf = 0.28. The 111 HPTLC profiles of the two extracts displayed a somewhat identical phytochemical profiles between the Rf region 0.5 – 0.9, 112 but with a huge variation between 0.1 and 0.49 retardation factors. The HPTLC fingerprints or pattern (Figure 2) suggest that 113 products (P1, P6 – P8, P20-P24 and P27) as indicating similarity with ethanol, dichloromethane extracts or containing kaempferol glycoside or quercetin glycoside of both. Scrutinizing these suggested products physically reveals that the majority were either powder, capsules, decoction, or a teabag. Other products , such as bar soaps, creams, energy drink, and tincture displayed a large variation in their pattern compared with the extracts and standards.

Phytochemical pattern recognition in *M. oleifera leaves extracts* and standards by Spectrophotometric Analysis

Based on the information available in literature, most *M. oleifera* leaves predominantly contain at least one of quercetin-3123 *O*-glycoside and kaempferol-3-*O*-glycoside[10]. After the spectrophometry data was exported to Excel®, a graph of absorbance versus wavelength (Figure 3) indicated that both quercetin-3-*O*-glycoside and kaempferol-3-*O*-glycoside are present in the ethanol extract of *M. oleifera* leaves in agreement with the HPTLC results. As evident in Figure 3, the ethanol extract revealed absorbance peaks and the first peak designated peak number **1** corresponded to the peak of the two compounds, quercetin-3-*O*-glycoside, and kaempferol-3-*O*-glycoside at 0 – 410 nm. From the Figure, one can also observe that both compounds contain a single peak to confirm their respective purity.

On the other hand, even though the dichloromethane extract did not contain the quercetin-3-*O*-glycoside and kaempferol-3-*O*-glycoside due to a lack of peak alignment between 0 – 300 nm, it also displayed four peaks between 305 – 800 nm. This observation suggests that quercetin-3-*O*-glycoside, kaempferol-3-*O*-glycoside, and the *M. oleifera* leaves ethanol and dichloromethane extract as suitable markers to use as standards for qualitative analysis of *M. oleifera* leaves based commercial products patter recognition[9] in other to qualitatively evaluate the pharmacological relevance of the commercial products claiming to contain them. According to the World Health Organization, a commercial herbal product is indicative of good quality if it contains at least one compound or extracts of the raw plant or herbal material that it is formulated from. In addition, spectrometric fingerprinting or pattern marching is recommended as a critical tool in evaluating the quality of herbal medicine and other herbal based products[9]. This is because such products usually contain a matrix of active components. It is also a scientific fact that the quality and pharmacological efficacy of a commercial herbal product is intrinsically linked to active compound,

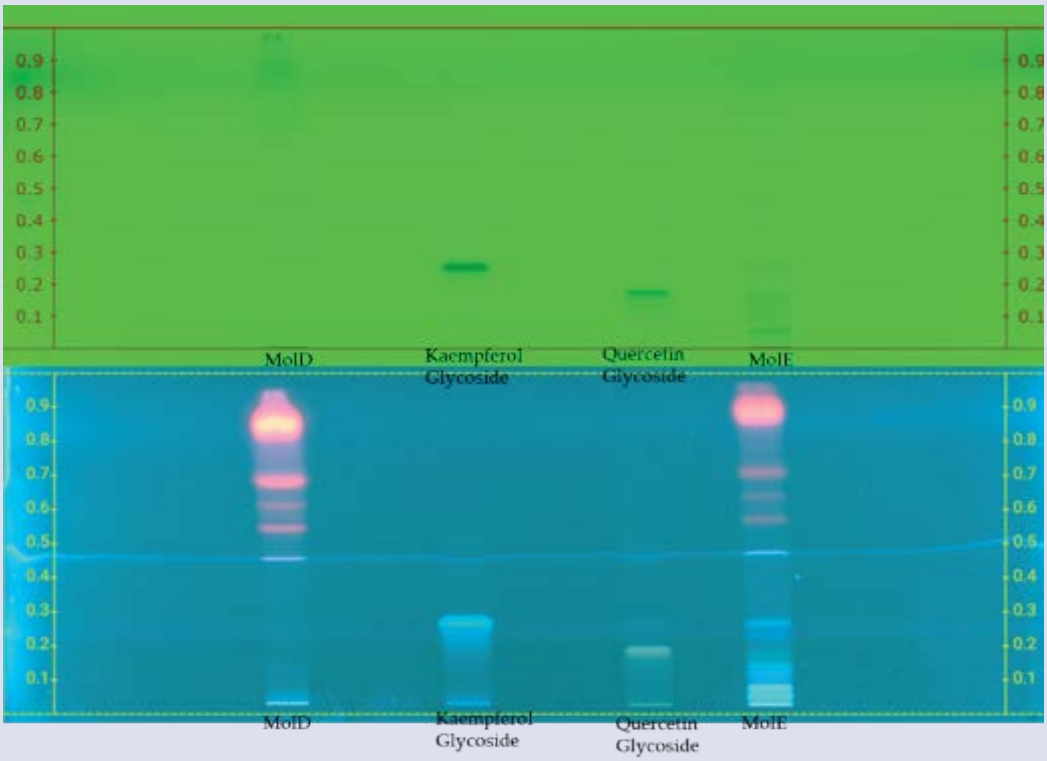

Figure 1. HPTLC profile of the *M. oleifera* leaves dichloromethane (track 1), ethanol extract (track 4), kaempferol glycoside (track 2) and quercetin glycoside (track 3). Plate A captured at 254 nm and B captured at 366 nm. Mobile phase: dichloromethane, ethyl acetate: formic acid (90:20:20:0.5 v/v/v).

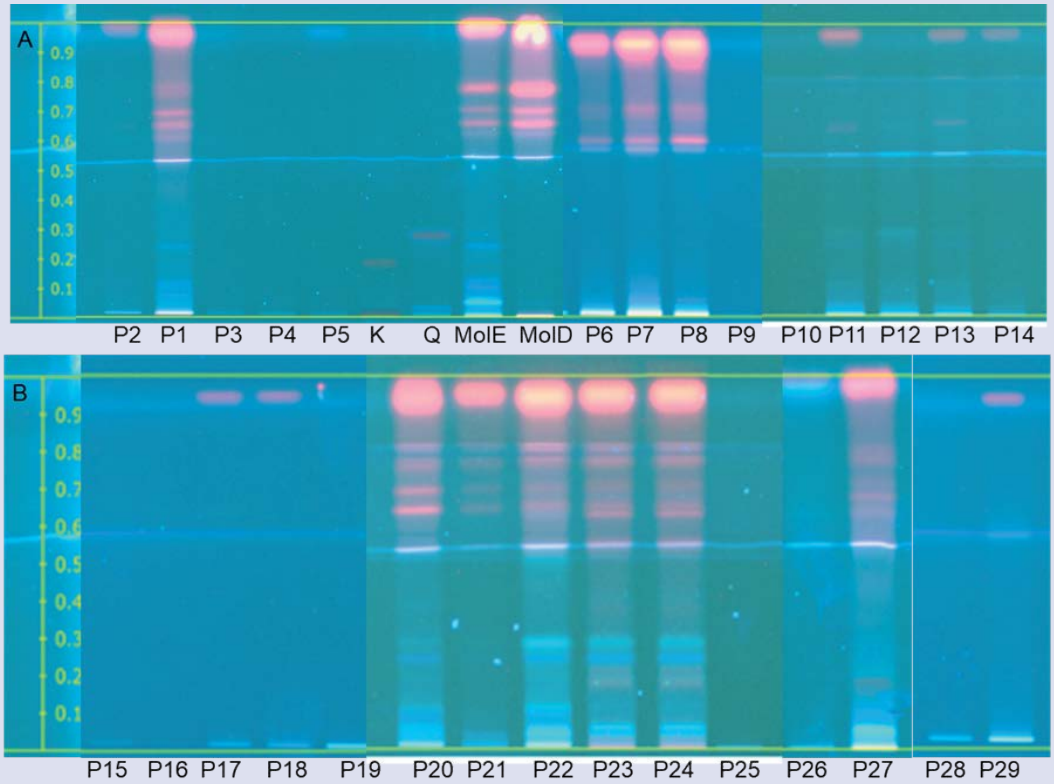

Figure 2. HPTLC profile of the 29 commercial products (tracks 1-5 and 10 - 29), *M. oleifera* leaves ethanol extract (track 119 8), dichloromethane extract (track 9), kaempferol glycoside (track 6) and quercetin glycoside (track 7). Plates were captured 120 at 366 nm and derivatized with PEG. Mobile phase: dichloromethane, ethyl acetate: formic acid (90:20:20:0.5 v/v/v).

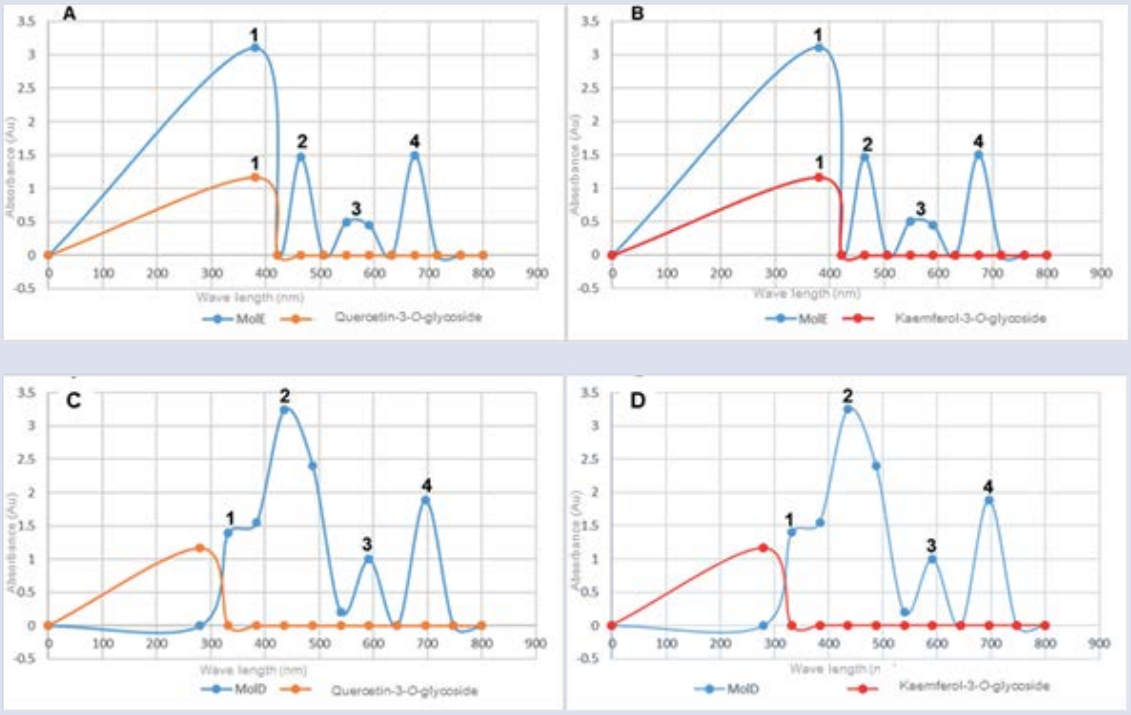

Figure 3. UV vis absorbance peak indicating the presence (A and B) and absence (C and D) of the quercetin-3-*O*-glycoside and kaempferol-3-*O*-glycoside standard compounds in *M. oleifera* dichloromethane (MolD) and ethanol (MolE) extracts.

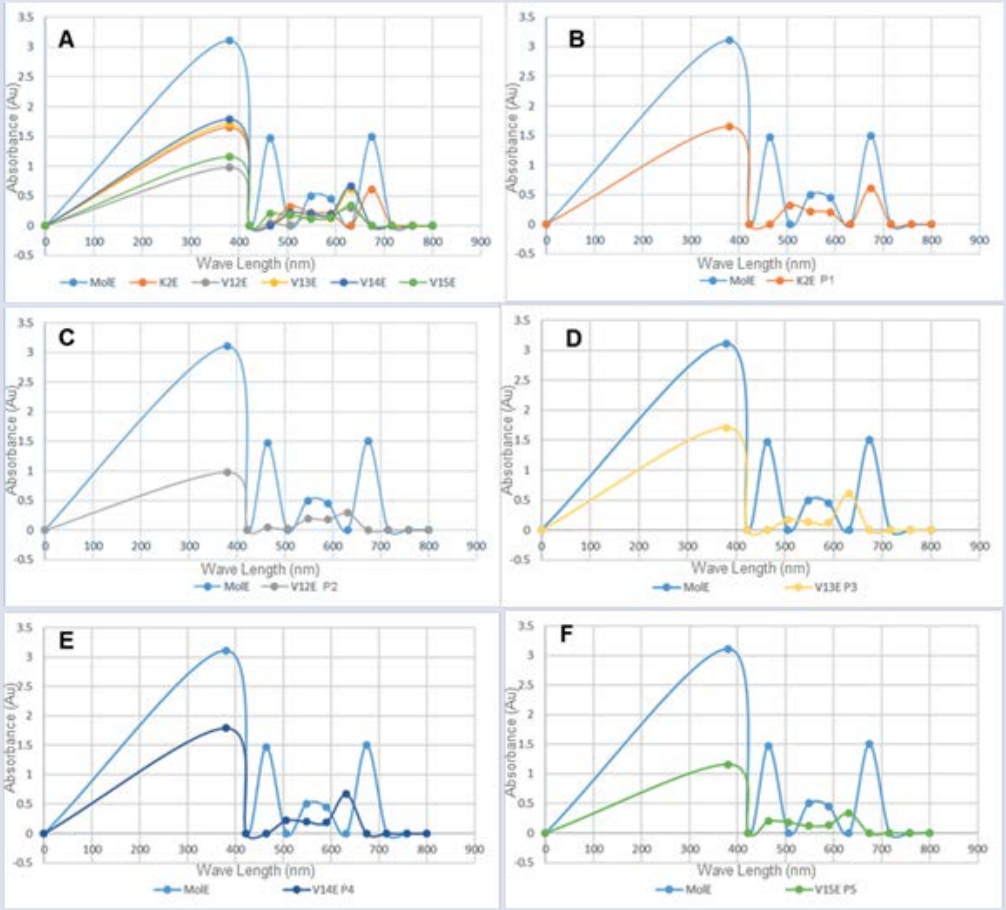

Figure 4. Pattern matching of *M. oleifera* leaves ethanol extract (MolE) with those of five randomly selected commercial products (P1 – P5) ethanol extract.

plant extract or the actual plant powder thereof present in the over-the-counter product[10]. Furthermore, all the quality control approaches for herbal medicines were narrowed down into marker-compound and pattern-fingerprinting strategy by[11]. Both approaches were applicable in this study. All the commercial products (n = 29) under investigation were scanned using the spectrophotometer and their absorbance, plotted alongside the *M. oleifera* ethanol and dichloromethane extracts on the same axes using Excel. The results obtained for five randomly selected commercial products (P1 – P5) are displayed in Figure 4.

It is perceptible from the Figure 4A, that the absorbance pattern of all the five-products ethanol extract may contain quercetin-3-*O*-glycoside standard compounds due to the pattern peak matching with the *M. oleifera* leaves ethanol extract at between 0-410 nm. Above this region, ranging from the region 411 – 610 nm, no matching patterns or fingerprints were observed between the five commercial products and the standard ethanol extract. However, product P1 indicated a match with the ethanol extract between 610 – 710 nm.

This will translate to P1 indicating good quality product because it is either having quercetin-3-*O*-glycoside or kaempferol-3-*O*-glycoside at 0 – 410 nm and another unknown compound at 610-710 nm to meet the criteria of containing at least two compounds for a good product[9]. To further investigate on the possibility of product P1 being of good quality, each of the five commercial products were compare with the ethanol extract (Figure 5B – 5F) to better visualized on the number of peaks in P1 that matches those in the ethanol extract. From the one-on-one analysis, product P1 indeed revealed two matching peaks at 0-410 and 610-710 nm and is therefore the only product indicating good quality from the five analysed. Using the same commercial products (P1 – P5), similar analysis was conducted for the dichloromethane extract. Results obtained (Figure 5A-F) concur with that obtained for the products extracted with ethanol, the observation that only product P1 (Figure 5B) indicated good quality. In addition, the analysis also pointed at the observation that P4 may also be of good quality due to the partially aligned peaks of the extract and compound in P4 at 650 – 750 nm. Figure 5. Pattern matching of M. oleifera leaves dichloromethane (MolD) with those of five selected products dichloromethane (P1 – P5) extracts.So regardless of the extraction solvents, product P1 may be considered as indicating good quality because it has peaks or phytochemicals that match those present in the *M. oleifera* leaves. The pattern or fingerprint matching was extended to the all the n = 29 commercial samples. The result, Figure 6, unravelled that about 6 products (P1, P3, P4, P7, P9, P15, P24) between 400 – 500 nm and 3 products (P1, P4, P9) at 700 – 800 nm displayed matching pattern with the dichloromethane extract (MolD), Figure 6A. On the other hand, all except one product (P23) at 0 - 410 nm and 7 products (P1, P3, P4, P7, P9, 178 P15, 24) at 600 – 700 nm had matching pattern with the ethanol extract (MolE), Figure 6B.

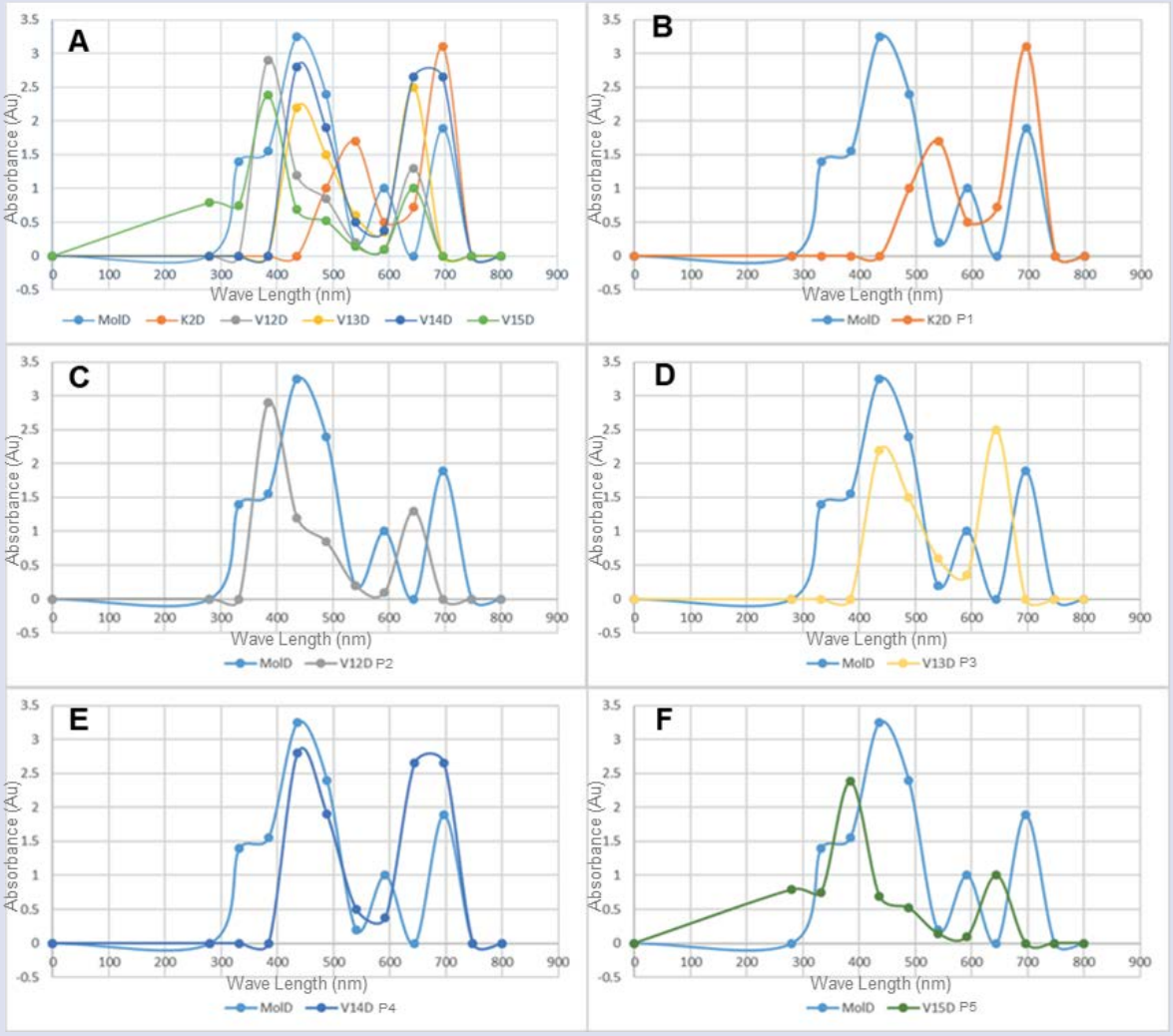

Figure 5. Pattern matching of M. oleifera leaves dichloromethane (MolD) with those of five selected products dichloromethane (P1 – P5) extracts.

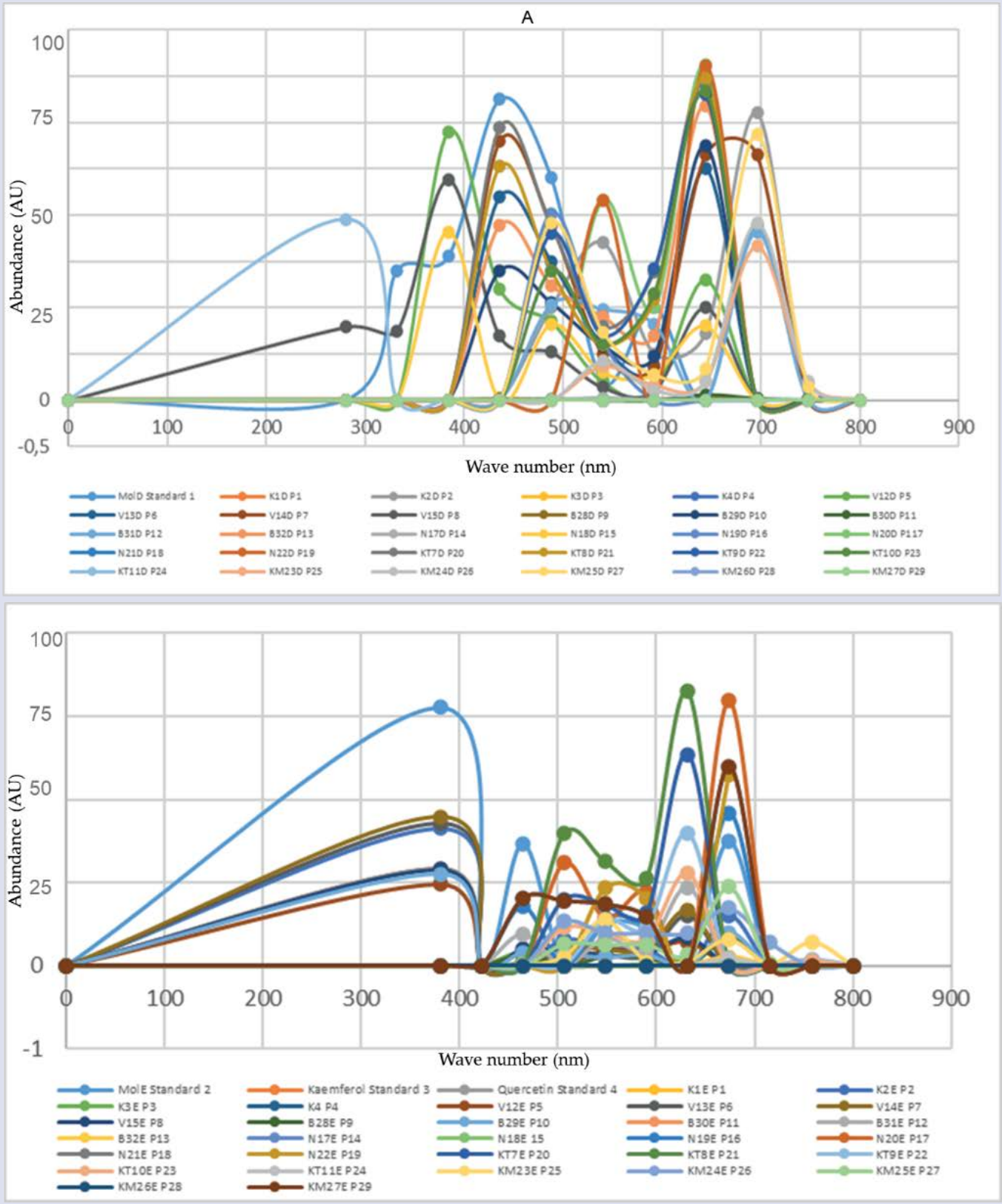

Figure 6. Stacked dichloromethane (A) and ethanol (B) spectra of the spectrophotometric data obtained for n = 29 commercial products versus M. oleifera leaves dichloromethane, ethanol extracts, quercetin-3-O-glycoside, and kaempferol-3-O182 glycoside'

Moringa oleifera leaves commercial products quality indicators by chemometrics principal component analysis(PCA)

Principal component analysis (PCA) is a statistical technique that is often deployed and used to analysed multivariate datalike spectrophotometric, chromatographic and even spectroscopic data. The PCA is widely used in grouping and recognizing patterns in such data based on similarity or differences in the phytochemical composition of the data among other factors[7]. In addition, the data from each sample can be visualized in a scatter plot of any two principal components to reflect the relative variation of the multidimensional data. The samples sharing similar characteristics are expected to be located near each other in a cluster of the scatter plots. Therefore, the PCA can be used to separate the data into subgroups when available 192 and identify the subsets of samples that may be associated with different phenotypes[12].

The ultraviolet visible spectrophotometric data were converted to excel data point and made compatible for PCA analysis using Simca-15. A four-component model with R^2 = 0.89 was constructed and used for the analysis of the *M. oleifera* ethanol extract and the five commercial products P1 – P5 for the test analysis as was done with the pattern matching. However, only the first 2 components (t1 and t2), explained the variations in the samples better. As for the *M. oleifera* dichloromethane extracts and commercial products P1 – P5, a five-component model was constructed but only component 4 and 5, (t4 and t5) with R^2 = 0.97, best explained the variations in the data. The PCA score scatter plot (Figure not shown) in both analyses further underscored the earlier observation recorded using the phytochemical matching approach. That is, whether the commercial samples were extracted with dichloromethane or ethanol, commercial product P1 was clustered with either the dichloromethane or ethanol extract to confirm sharing similar chemical characteristics as the extracts. In other words, only that product out of the five contains the *M. oleifera* leaves plant material and should exhibit a relatively better quality in comparison to the other four products. Following the consistency in results obtained for the 5 samples, a PCA analysis was used for the analysis of all the commercial products. The PCA score scatter plot for the products extracted with dichloromethane (Figure 7A) nominated products P1, P4 and P24 has having the best quality because these products clustered (red perforated oval) with the dichloromethane

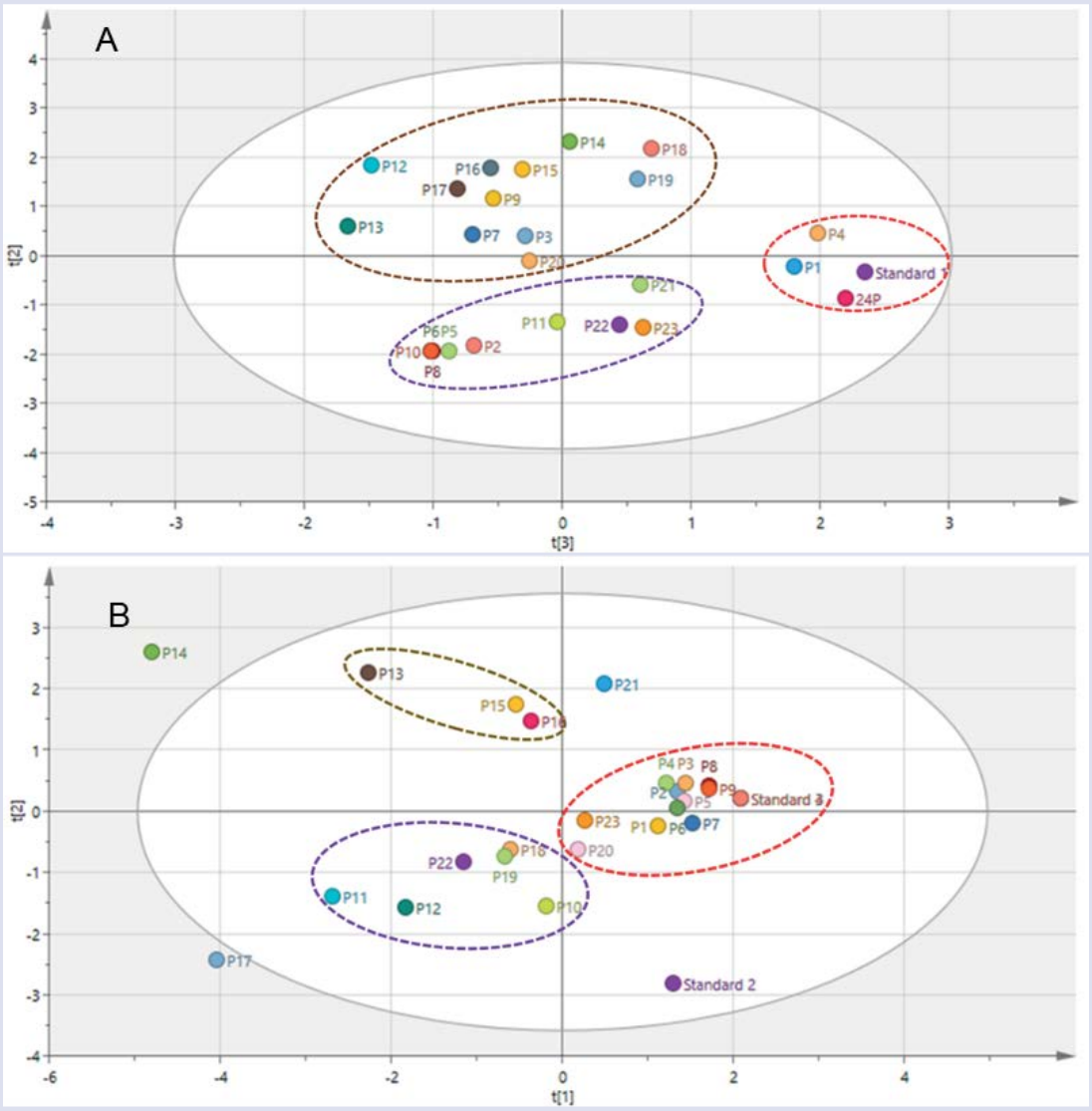

Figure 7. PCA Score scatter plot of the dichloromethane (A) and the ethanol (B) spectrophotometric data of extracts of n = 29 commercial products and standards.

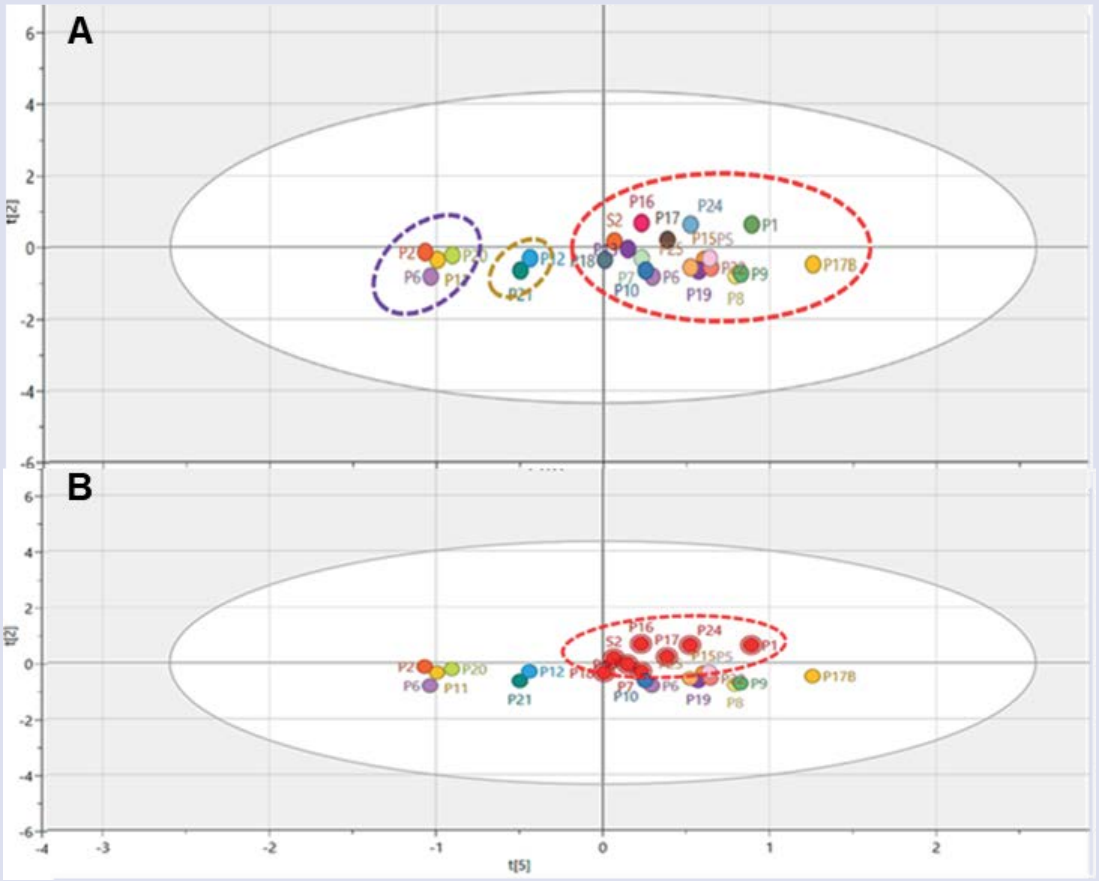

Figure 8. PCA scatter score plot indicating the three clusters of the commercial products (A) and the products (red) grouped with the standard M. oleifera extract (s) indicating possible good quality (B). P13 and P16 at peak numbers 13 and 14.

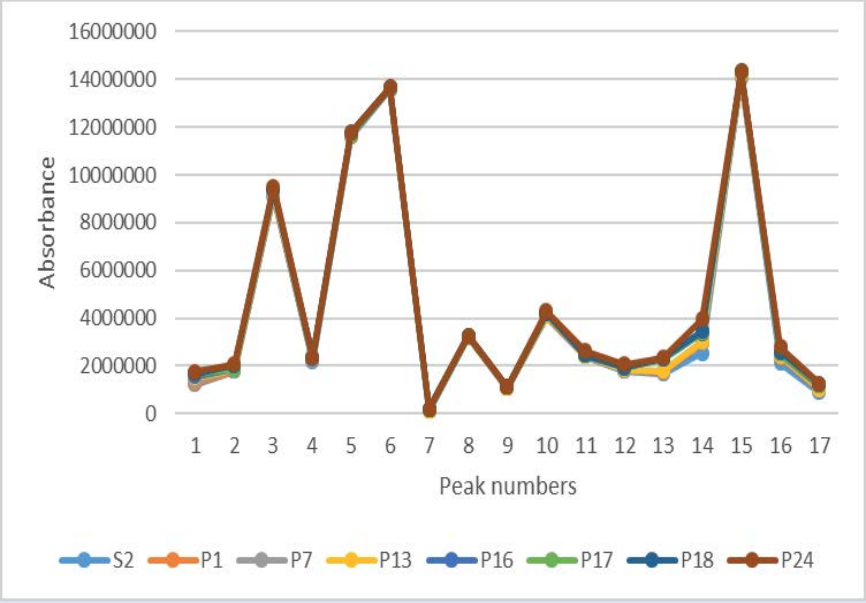

Figure 9. Using a Venn diagram, all the different commercial products with potentials for good quality as suggested by the different analysis were evaluated. From the HPTLC fingerprints, P1, P6, P7, P8, P20, P21, P22, P23, P24 and P27 were suggested as having potentials for good quality, while from the dichloromethane UV-vis PCA analysis P1, P4 and P24 were suggested. Whereas the ethanol UV-vis PCA analysis nominated P1, P3, P4, P7, P9, P15, P24 and P24, the ethanol HPLC PCA underscored P1, P3, P4, P5, P7, P8, P9, P20, P23 and P24 as having potentials for good quality. Figure 11. Venn diagram used in nominating the M. oleifera commercial products P1 and P24 with best quality potentials. However, products P1, P7 and P24 should have the best quality because they were nominated by all the method of analysis regardless of the solvent used for M. oleifera leaves extraction. This should then be followed by products P4 and P23 that was nominated by two of the methods used for the analysis and then the rest.

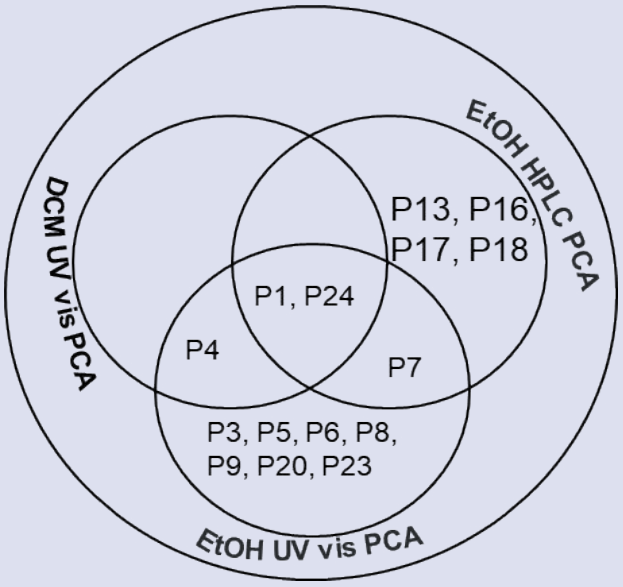

Figure 10. Matching profile of the standard M. oleifera ethanol extract (S2) with those of potentially good products (P1, P7, P13, P16, P17, P18 and P24) from HPLC-PCA of ethanol extract.

crude extract (Standard 1). In comparison, the result of a similar analysis done for the commercial products extracted with ethanol nominated products P1, P3, P4, P5, P6, P7, P8, P9, P20, P23 and P24 for good quality potential. This again is because these products clustered in red together with the crude *M. oleifera* ethanol extracts (standard 2) and quercetin-3-O-glycoside (standard 3). The similarity in the chemistry of the ethanol extract in comparison to that of commercial products that clustered with the quercetin-3-*O*-glycoside may be drawn from the fact that they are all placed in the same quadrant.

HPLC finger printing and Chemometrics PCA analysis of the MOL products

The HPLC retention time versus area under the peak plots were extracted unto excel 2016. From the HPLC fingerprints (Figure 9), the were no clear patterns match between the five. randomly selected commercial products (P1 – P5) compared to the reference *M. oleifera* crude ethanol extract. A detailed observation highlighted the fact that compound peaks 8, 9 and 10 were present in various amount in products P3 – P5 while compound peaks 9 and 10 were predominantly found in P1 and P2. This observation tends to support the nomination of products P1, P3, P4 and P5 from the PCA analysis as having potential for good quality. Prior to the PCA analysis of the HPLC data, solvent peaks that appeared between 1.333 - 2.33 mins were removed from the spreadsheet before making the data compatible for chemometrics computation by aligning the peak area against the respective retention time. A five component PCA analysis with $R^2 = 0.78$ was constructed using the data.From the second and the third components (t1 and t3), a score scatter plot, Figure 9 afforded three clusters just as was observed using the spectrophotometric data. The commercial products placed in the red cluster with the crude ethanol extract exhibited potential for good quality. However, products including P1 and P24 that weighed the most with the largest contribution to the plots (highlighted in red) were considered the ones with best quality. The phytochemical pattern of these products with that of the *M. oleifera* ethanol extract were compared and the results (Figure 10) shows a complete match with slight variations for products

DISCUSSION

Over 80% of South Africans rely on herbal or traditional medicines to cater for their primary health care needs[13]. Affordability and cultural beliefs, among other factors, are partly the reason for the afore mentioned[14]. In addition, the belief that "if it is green then it is fine' and side effects associated with the use of orthodox medicines further drives humans towards the consumption of herbal medicines. Furthermore, the validation of herbal, alternative and other complimentary medicines by the World Health Organization as well as several governmental organizations both in the West and other countries has catalysed the boom in the sales and marketing of herbal medicines that was worth USD 151.91 billion in 2021 and projected to grow from USD 165.66 billion in 2022 to USD 347.50 billion by 2029, with a compound annual growth rate of 11.16% during the forecast period. Just as the case of drug discovery and development that requires billions of British pounds, and highly exorbitant equipment that are usually only afforded by the Western business tycoons, the third world entrepreneurs can only distribute herbal and other related natural products to their poor masses without appropriate quality control measures in place including the evaluation of the pharmacological and related health claims on the labels of the herbal products[15]. Whereas the herbal medicines market is well regulated in most Asian and the Western countries, the contrary is the case for most countries in the African continent[16]. Consequently, what may appear on the labels of most herbal medicines found on the shelves of most pharmacies and other health shops is in most cases not what one may find in the product tablet, capsules, powder, cream, porridge just to mention, but a few. The dire health implications of inappropriate quality control measures to guarantee the pharmacological relevant safety and good quality of such products could include the loss of lives[17]. The exposé on such loopholes, that only three out of twenty-nine commercial products purportedly formulated from *Moringa oleifera* Lam. leaves., with label claims of good pharmacological health benefits is what this study has unravelled.

CONCLUSIONS

From the twenty-nine commercial products investigated, three products and P1, P7 and P24 indicated good quality from the pattern recognition and chemometrics analysis and in line with World Health (2018) Organization recommendations for herbal products.

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AUTHOR CONTRIBUTIONS

Conceptualization -KB , Methodology - KB, Formal Analysis - KB, Investigation-KB , Resources - KB, Project Administration - KB, Visualization - KB, Writing - KB, Editing - KB.

CONFLICTS OF INTEREST

The author has no conflicting interest whatsoever to declare.

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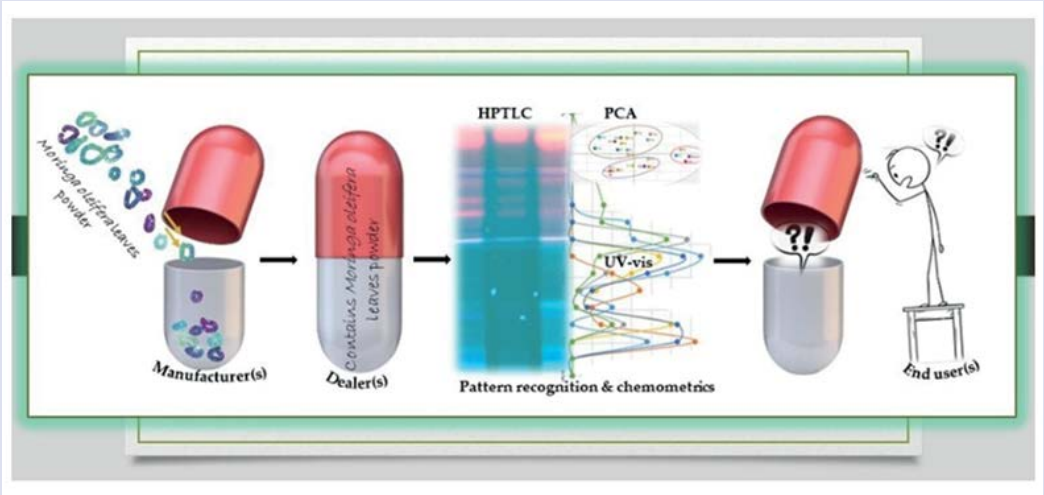
GRAPHICAL ABSTRACT



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