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Chemical composition by PTP 1B inhibitory activity guided analysis of a plant-based antidiabetic polyherbal drug used in ethnomedical systems

Pardon Mugari^{1,3}, Pamhidzai Dzomba^{2*}  and Stephen Nyoni¹

Abstract

Background Due to growing resistance and continued depletion of drugs that can be used as replacements, people turn to herbal antidiabetic medicines. A polyherbal drug consisting of a mixture of two herbal plants, *Zanthoxylum chalybeum* and *Xeroderris stuhlmannii* root powder mixed in equal amounts using a tea cup, was studied to investigate its chemical composition and possible mode of action.

Methods Phytochemicals were isolated using Column and Thin Layer Chromatography, and antidiabetic activity was ascertained using an enzyme inhibition model. Active compounds identity was established using ¹HNMR, ¹³CNMR, HSQC-NMR, UV, FT-IR and MS spectroscopy.

Results Results showed that the polyherbal drug consisted of two alkaloids and three anthocyanins as the major active components. PTP 1B inhibition of alkaloids was not significantly different from that of metformin with percentage inhibition of 78.6 ± 3.2 and $79.6 \pm 2.3\%$. The anthocyanins inhibited PTP 1B enzyme significantly better than metformin with percentage inhibition of 88.2 ± 3.2 , 90.3 ± 2.3 and $93.6 \pm 2.9\%$. One new compound, an alkaloid, 1, 2-dimethoxy-12-methyl-7-(3-methylbut-2-en-1-yl)-12, 13-dihydro [1,3] benzodioxolo [5,6-c] phenanthridin-13-ol, one previously reported alkaloid berberine, IUPAC name, 9,10-dimethoxy-5,6-dihydro[1,3] dioxolo [4',5':6,7] isoquinolino [2,1-b][2] benzazin-7-ium and three anthocyanins, malvidin 3-o-glucoside, pelargonidin-3-O-gucoside and malvidin 3, 5-O-diglucoside were identified as the active compounds in the polyherbal drug extract.

Conclusion Presence of alkaloids and anthocyanins that have PTP 1B inhibitory activity showed that the polyherbal drug is a potential medicine for managing diabetes.

Keywords Polyherbal drug, Antidiabetic activity, PTP 1B, Diabetes mellitus 2

Background

Polyherbal drugs comprising of two or more herbs are considered to be effective and safe natural therapeutics for many diseases (Suvana et al. 2023; Vikhe et al. 2022). Over the years studies investigating antidiabetic activities of polyherbal drugs of an ethnomedicinal origin have been rising (Gopalakrishnan et al. 2020; Wanjari et al. 2021). Thus herbal medicines from different plants have been integrated into various formulations which are now available in the form of capsules, tablets and powders

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(Tchamadeu et al. 2023; Alhamhoom et al. 2023). A polyherbal drug consisting mainly of equal amounts of *Zanthoxylum chalybeum* and *Xeroderris stuhlmannii* root bark powder and minor amounts of *Zanthoxylum chalybeum* leaf powder added for taste is very popular locally in traditional medicine practices as potent remedy for diabetes type 2. *Zanthoxylum chalybeum* and *Xeroderris stuhlmannii* are widely distributed plants in Southern Africa in countries such as, South Africa, Zambia, Botswana, Mozambique and Zimbabwe. The polyherbal drug is prepared by mixing mainly pulverized root bark powders which is taken orally as it is, in half teaspoon measure, approximately 0.852 g. Alternatively the powder is infused in corn meal porridge and administered orally. Other preparation modes include preparing the same amount in decoctions in cold or warm water. The water is then orally taken periodically 3 times a day until the patient feels well. *Z. chalybeum* and *X. stuhlmannii* have been reported to exhibit antidiabetic activity as shown by inhibiting alpha amylase and glucosidase in previous studies (Agwaya et al. 2016; Kimani et al. 2013; Musabayane et al. 2005). Despite the polyherbal drug being popular as antidiabetic remedies among many local people, little information is known about the chemical composition and the mode of action of the polyherbal drug.

In limited previous studies, Nyathi et al. (2023) investigated *X. stuhlmannii* inhibitory activity of stem bark extract using enzymes, α -amylase and glucosidase and discovered that the crude extract was a good inhibitor of the enzymes. By screening phenolics and alkaloids in *X. stuhlmannii*, Selemani et al. (2021) established myricetin as the novel inhibitor. In another study, the investigation of leaf extract of *X. stuhlmannii* yielded isoflavones and rotenoids with significant antibacterial and anti-fungal activity (Mekute et al. 2023). Agwaya, et al. (2016) reported in vivo antidiabetic activity of water extracts of *Z. chalybeum*. A systematic investigation of *Z. chalybeum* root extract revealed presence of various phytochemicals including chaylbemides, fagaramide, skimmianine, norchelerythrine, 6-acetyl dihydrochelerythrine, 6-hydroxy-N-methyl decarine, aianthoidol, 2,3-epoxy-6,7-methylenedioxy coniferyl alcohol, sesamine, lupeol, lupanone, 3 α ,20-dihydroxy-28-lupanoic acid, 20-hydroxy-3-oxo-28-lupanoic acid and 3 α ,20,28-trihydroxylupane. The Chaylbemides fagaramide, skimmianine, norchelerythrine, 6-acetyl dihydrochelerythrine and 6-hydroxy-N-methyl decarine were the best inhibitors of α -amylase and α -glucosidase (Ochieng et al. 2020).

The major classes of phytochemical compounds that have portrayed remarkable antidiabetic activity in previous studies include flavonoids, triterpenoids and alkaloids (Uvarani et al. 2014; Choi et al. 2014). In an effort

to understand the chemical composition and mode of action of the polyherbal drug formulation, this work used, PTP 1B (protein tyrosine phosphatase 1B) inhibitory activity directed assay to isolate the active phytochemicals. PTP 1B is considered to play a major role in the regulation of insulin signal transduction, therefore it is considered to be an important target in diabetes mellitus type 2 management (Shah et al. 2024). The aim of the this study was to find out if active compounds that can inhibit PTP 1B are present in the polyherbal mixture which was appraised by many subjects during an ethnobotanical survey we conducted. Currently drugs that targets PTP 1B have not been found despite it being a good target for diabetes mellitus type 2 treatment. The few drugs that entered the clinical trial, ertiprotafib, trodusquimine and JTT-551 were discontinued due to serious side effects and poor selectivity (Liu et al. 2023). Thus more studies are required to find safe and more efficacious drugs which targets PTP 1B.

Methods

Plant samples collection

The polyherbal drug was given to us as a gift by a local traditional practitioner. The researchers witnessed the collection and preparation of the drug. The polyherbal drug consisted of equal amounts of powdered root bark of *Z. chalybeum* (200 tea cups) and *X. stuhlmannii* (200 tea cups) and little amounts of *Z. chalybeum* leaf powder for taste (1 tea cup). One tea cup full of each powdered plant material weighed approximately 127 g. The mixture was prepared by adding the powdered plant material a little each at a time while stirring using a wooden spoon specifically prepared for the job. Plant samples that were used in the preparation of the polyherbal drug were further authenticated using taxonomists at the National Herbarium. Voucher specimens were then deposited at the Hilbright Science Education College herbarium, voucher specimen numbers 2023/01-30DMT2.

Chemicals

Protein Tyrosine Phosphate 1B, p-nitrophenyl phosphate (pNPP), metformin, solvents were purchased from Merck SA. All other chemicals used for the study were purchased locally from industrial sources. A UV-Vis spectrophotometer, GENESYS 10S UV-Vis v4.003 2L9Q129001 Thermofisher scientific, (USA) was utilized to collect absorbances.

Extraction of crude sample

The herbal drug sample, 100 g was extracted three times using 1L of absolute ethanol on a shaker over 24 h. The contents were then combined and filtered using cheese cloth first and then filter paper. Vacuum evaporation of

solvent yielded 32 g of extract. This was dried and kept at room temperature until used to imitate conditions that the polyherbal drug is usually kept.

Test for flavonoids

Presence of flavonoids was investigated using Shinoda reagent test by dissolving 0.5 g of sample in absolute ethanol as reported by Qasim and Al-Salman, (2023). Magnesium ribbon pieces were then added in the tube followed by drop wise addition of concentrated hydrochloric acid. A positive result was marked by an intense cherry red color.

Test for alkaloids

Mayer's reagent test for alkaloids was performed as described by Ali et al., (2018) by freshly preparing a mixture of mercuric chloride (1.36 g) and potassium iodide (5.00 g) dissolved in 100 ml of distilled water. 1 ml of the solution was added to 1 ml of the polyherbal extract dissolved in ethanol. A cream colored precipitate showed the presence of alkaloids.

Test for terpenoids

Salkowski test was used to check the presence of terpenoids following a method reported by Das et al. (2014). Polyherbal extract (5 ml) was mixed with 2 ml of chloroform and then 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish-brown coloration of the inter face showed positive results for the presence of terpenoids.

Extraction of alkaloids

Total alkaloid extraction followed a slightly modified method by Ochieng et al. (2020). A 10 g portion of the polyherbal drug was extracted using 200 ml of 2% of 0.1 sulphuric acid in absolute ethanol by shaking for 40 min and repeating the process four times. The non-polar extracts were combined and extracted three times in n-hexane (300 ml) using a separating funnel. To neutralize the acidic portions 0.1 M ammonium hydroxide solution was added and the resulted mixture extracted exhaustively using 200 ml CH_2Cl_2 as shown by a negative Dragendorff's reagent. The CH_2Cl_2 fraction was water dried using anhydrous Na_2SO_4 and concentrated under vacuum to a 2.6 g (26% DW). A normal phase silica gel packed column was then used to partition the alkaloid fraction. Elution was done with increasing polarity (v/v) ratio of; CH_2Cl_2 : $\text{CH}_3\text{CH}_2\text{OH}$ 10:0 (500 mL), 9:1 (500 mL), 8:2 (500 mL), 7:3 (500 mL), 1:1 (500 mL) and final 100% $\text{CH}_3\text{CH}_2\text{OH}$ (300 mL). A total of 28 fractions were obtained which were reduced to 5 fractions by combining following similar color and TLC profiles as monitored by spraying Dragendorff's reagent solution

developed using CH_2Cl_2 : $\text{CH}_3\text{CH}_2\text{OH}$: 10% ammonium hydroxide 89:10:1 as mobile phase. Fractions 3, (10–16 combined) (50 mg) and 5 (22–28 combined) (72 mg) showed significant PTP inhibitory activity (significantly not different to that of metformin, SPSS version 21, ANOVA test, $p=0.05$). The 2 fractions were mixed and chromatographed using preparative thin layer chromatography developed utilizing $\text{CH}_3\text{CH}_2\text{OH}$ (8%) in dichloromethane to further purify them. Bands showing positive for alkaloids were scrapped of, dissolved in CH_2Cl_2 , filtered and recrystallized in n-hexane afford two very bright yellow crystalline compounds 14 mg and 86 mg.

Extraction of flavonoids

Dried polyherbal crude extract, 15 g was first defatted using petroleum ether (200 ml) and then thoroughly extracted with 95% ethanol at 25 °C on a shaker for 24 h. The resultant sample was concentrated under vacuum. A 3.5 g aliquot was then suspended in 250 ml of distilled water (1.0 L) followed by partitioning successively with hexane, ethyl acetate and ethanol to yield hexane (0.3 g), ethyl acetate (1.2 g) and ethanol (0.5 g) fractions respectively. The extracts were then investigated for their enzyme inhibitory activity. The ethyl acetate fraction exhibited the most significant activity. Therefore, this fraction was subjected to column chromatography on Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Ethyl acetate/ethanol (100:1) in an increasing polarity gradient elution up to ethyl acetate/ethanol (1:100) ratio was use as eluting solvent.

Separation of compounds

The ethyl acetate fraction exhibiting significant inhibitory activity (1.2 g) was used for separation of flavonoids by using column chromatography over Sephadex LH-20. Initially, petroleum ether was used to pack the column and then 1.2 g extract was loaded to the column and twenty test tubes with 3 ml eluents were collected. The varied ethanol-ethyl acetate solvent system of decreasing polarity was used.

PTP1B inhibitory assay

Enzyme inhibition activity using protein tyrosine phosphatase 1B of crude extracts and column fractions were evaluated using p-nitrophenyl phosphate (p-NPP) as a substrate according to a protocol reported by Ha et al. (2020) and Paudel et al. (2016).

Active compounds structural elucidation in the polyherbal drug

This was performed by analyzing the samples on an AB Sciex LC–MS/MS QTRAP 5500 to obtain molecular

mass and fragments, a Joel 400 MHz Year Hold Magnet spectrometer, Tokyo, Japan to record NMR spectra (^1H , ^{13}C DEPT-135, HMQC and HMBC. Chemical shifts were referenced to residual solvent signal CDCl_3 and DMSO. FT-IR and UV spectra were collected on a Nicolet iS10 spectrometer (Thermo Fisher Scientific, Waltham, MA USA) and UV-Vis spectrophotometer, (GENESYS 10S UV-Vis v4.003 2L9Q129001 Thermo Fisher scientific, USA) respectively.

Results

Phytochemical test

Results of the Shinoda, Mayer and Salkowski test are shown in Table 1 and Fig. 1. An intense cherry red color was observed with Shinoda test Fig. 1 showing the presence of flavonoids in the polyherbal drug. A cream precipitate was observed with Mayer’s reagent showing the presence of alkaloids and a negative test was observed with the Salkowski test indicating absence of terpenoids.

Table 1 Phytochemical test

Test	Observation	Conclusion
Shinoda	Intense cherry red color	Flavonoids present
Mayer’s reagent	Cream precipitate	Alkaloid present
Salkowski	–	No terpenes and terpenoids

Extraction of alkaloids

Mayer’s reagent test showed that the polyherbal crude extract was rich in alkaloids as shown by formation of a cream precipitate Fig. 1 therefore a dedicated protocol to extract alkaloids was carried out. Chromatographic separation (silica gel 60 mesh) of the alkaloid-rich extract yielded 5 different compounds from which only 2 showed significant PTP IB inhibitory activity (Table 2). Further purification of the active fractions by preparative TLC (Silica gel, 1000 μm glass plates UV254, 20 $\times$ 20 cm) and recrystallization in CH_2Cl_2 : n-hexane gave 2 very bright yellow crystalline compounds, 14 mg and 86 mg (Fig. 2), which tested positive for alkaloids with Dragendorff’s reagent and negative for flavonoids with Shinoda test showing that the compounds were alkaloids. Their

Table 2 PTP IB inhibitory activity of alkaloid column chromatography isolated fractions

Fraction	Combination number	Color	% PTP inhibition $\pm$ SD
1–6	1	Colorless	–
6–9	2	Light yellow	10.3 $\pm$ 2.2
10–16	3	Light Yellow	78.6 $\pm$ 3.2*
17–21	4	Brown	–
22–28	5	Yellow	79.6 $\pm$ 2.3*
Metformin	Reference	–	82.1 $\pm$ 1.2*

SD= standard deviation, *=no significant difference, ANOVA analysis at p=0.05

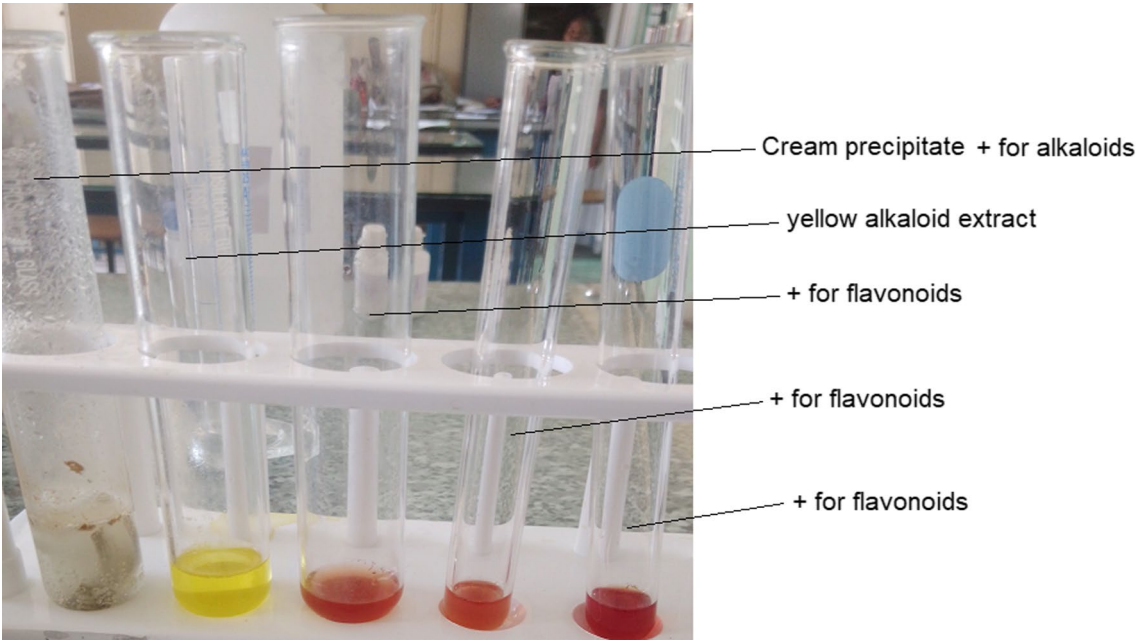


Fig. 1 Phytochemical test results

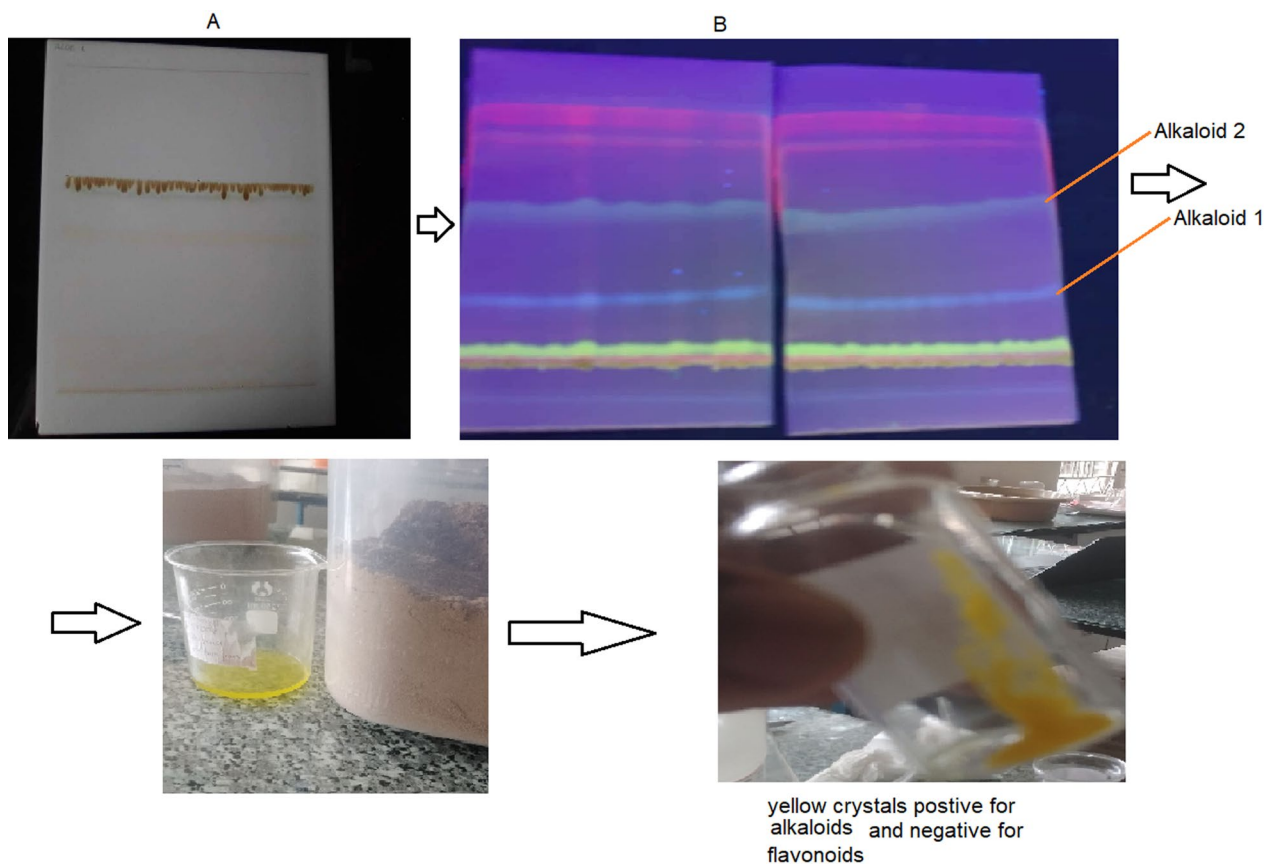


Fig. 2 Preparative TLC for combined fractions 3 and 5 and filtered and recrystallized scratched band of alkaloid 2. **A** = preparative TLC under visible light after spraying Dragendorff's reagent, **B**=preparative TLC under UV 366 nm light

Table 3 PTP 1B inhibitory activity of ethanol, ethyl acetate and hexane extracts

Extract	% PTP inhibition $\pm$ SD
Ethyl acetate	92.6 $\pm$ 3.2*
Ethanol	48.6 $\pm$ 1.6
Hexane	12.6 $\pm$ 3.3
Metformin	83.1 $\pm$ 2.1

* Significantly greater

eluting at 2 different Rf values, 0.46 and 0.68 showed that they were different though producing the same yellow colored crystals.

Extraction of flavonoids

Results of the PTP 1B inhibition of ethanol, ethyl acetate and hexane extracts are shown in Table 3. Ethyl acetate extract of the polyherbal drug showed significant inhibitory activity that was greater than

metformin, $p = 0.00$, ANOVA analysis SPSS version 21. Therefore, the ethyl acetate extract was subjected to column and TLC chromatography purification. Fractions (32) were collected in test tubes. All fractions were examined by analytical thin layer chromatography and differences in color. Fractions with similar Rf values and color were combined together. After combining a total of 9 fractions were obtained. Out of the 9 fractions 4 fractions tested positive for flavonoids using Shinoda test (Table 4 and Fig. 3). From the 4 fractions only 3 showed significant PTP 1B inhibitory activity (Table 4). The active fractions were further purified using preparative TLC by scratching bands showing positive test for flavonoids and respective Rf values to yield 2 active fractions producing pink and 1 reddish solution in ethanol which were dried under vacuum to yield 2 pink amorphous compounds, 22 and 35 mg each, and 1 red brown amorphous product, 26 mg. The process was repeated many times so that a 200 mg yield of each compound was obtained for further analysis.

Table 4 PTP 1B inhibitory activity of ethanol, ethyl acetate and hexane extracts

Fraction	Color	Shinoda test observation	% PTP inhibition ± SD
1	Light yellow	–	–
2	Light yellow	–	–
3	Pink	+	88.2 ± 3.6
4	Red	+	90.3 ± 2.3
5	Red	+	
6	pink	+	93.6 ± 2.9
7	brown	–	–
8	orange	+	22.4 ± 3.3
9	Light yellow	–	–
Metformin		–	80.2 ± 2.7

Fraction 4 and 5 showed that they were similar by TLC Rf value 5.2 therefore they were combined. –negative results, + results

Structural elucidation of active compounds in the polyherbal drug

The phytocompounds isolated from the polyherbal extract identities, were determined by ¹HNMR, ¹³CNMR, HSQC, MS, FT-IR and UV absorption spectroscopy. The constituents of the polyherbal drug used

to manage diabetes by the local people has not been reported before.

Alkaloid 1

Yellow crystalline powder giving yellow solution in ethanol and ethyl acetate, MS, M + , 433.49628, molecular formula C₂₆H₂₇NO₅, FT-IR, 3529.3 cm⁻¹ (–OH), 2926.80 cm⁻¹ (C=C), 3019.20 cm⁻¹ (C–H alkane), ¹H NMR: (400 MHz, CDCl₃), δ 1.52–1.62 (6H, 1.57 (s), 1.57 (s)), 3.24 (2H, d, J=7.3 Hz), 3.45 (3H, s), 3.58 (3H, s), 3.84 (3H, s), 5.33 (1H, t, J=7.3 Hz), 6.13 (2H, d, J=15.5 Hz), 6.52–6.70 (2H, 6.57 (s), 6.64 (d, J=8.7 Hz)), 6.94 (1H, d, J=0.4 Hz), 7.11 (1H, dd, J=8.5, 0.4 Hz), 7.36 (1H, d, J=8.7 Hz), 7.80 (1H, d, J=8.5 Hz). ¹³C NMR: (400 MHz, CDCl₃), δ 17.9 (1C, s), 25.1 (1C, s), 25.8 (1C, s), 39.3 (–C–N), 56.0–56.0 (2C, 56.0 (C–O), 56.0 (C–O)), 77.5 (>C–O), 100.2 (1C, s), 101.3 (OOC), 111.2 (1C, s), 116.2 (1C, s), 121.0 (1C, s), 122.5 (–C=), 124.0 (1C, s), 124.2 (1Cs), 124.5 (1C, s), 124.8 (1C, s), 128.0 (1C, s), 130.6 (1C, s), 132.0 (>C=), 134.1 (1C, s), 139.0 (1C, s), 142.8 (=C–O), 144.0 (=C–O), 151.9 (=C–O), 152.7 (1C, s). The HSQC, (400MHZ) correlation show correlation including 2 methyl protons δ_H1.52–1.62 with δ_C25.1; δ_H3.84 with δ_C56.0; δ_H5.33 with δ_C122.5; δ_H6.13 with δ_C101.3; δ_H7.36 with δ_C100.2.

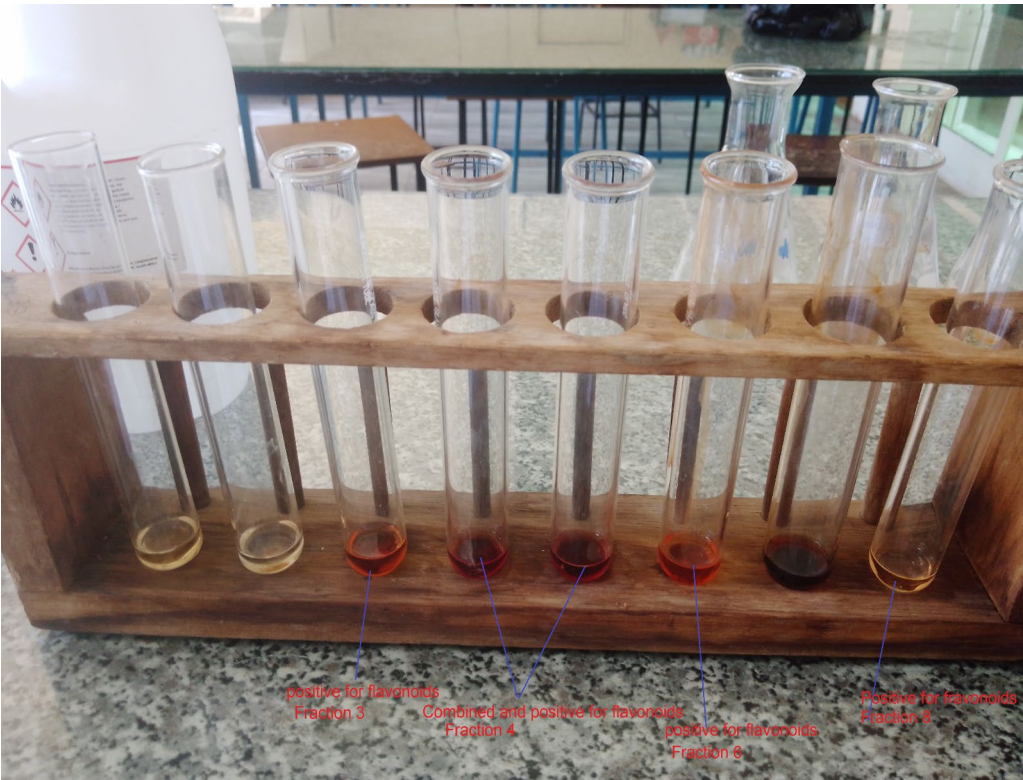


Fig. 3 Colors of fractions purified by column chromatography in an increased polarity gradient elution

The compound was determined as hydroxylated and prenylated Chelerythrine, 1, 2-dimethoxy-12-methyl-7-(3-methylbut-2-en-1-yl)-12, 13-dihydro[1,3] benzodioxolo[5,6-*c*]phenanthridin-13-ol Fig. 4. The isoprene unit is attached at carbon 18 while the hydroxyl group is attached at carbon 7.

Alkaloid 2

It was isolated as a very bright yellow crystalline powder dissolving well in warm ethanol and water. MS, M^+ , 336.3607 g, molecular formula $C_{20}H_{18}NO_4$, FT-IR, 3055 cm^{-1} (C–H aromatic), 2928.60 cm^{-1} and 2856.50 cm^{-1} (C–H alkane) ^1H NMR: δ 3.01 (2H, ddd, $J=16.1, 6.8, 2.9\text{ Hz}$), 3.60 (3H, s), 3.81 (3H, s), 4.21 (2H, ddd, $J=18.1, 6.8, 2.9\text{ Hz}$), 6.45 (2H, d, $J=12.6\text{ Hz}$), 6.92 (1H, dd, $J=8.9, 0.5\text{ Hz}$), 7.18 (1H, d, $J=0.5\text{ Hz}$), 7.33–7.55 (2H, 7.38 (d, $J=0.5\text{ Hz}$), 7.48 (ddd, $J=8.9, 2.0, 0.5\text{ Hz}$), 7.71 (1H, dt, $J=2.0, 0.5\text{ Hz}$), 9.71 (1H, t, $J=0.5\text{ Hz}$). ^{13}C NMR: δ 28.9 (1C, s), 56.0–56.0 (2C, 56.0 (s), 56.0 (s)), 58.4 (1C, s), 101.3 (1C, s), 105.5 (1C, s), 108.3 (1C, s), 120.2 (1C, s), 121.5 (1C, s), 121.8 (1C, s), 122.0 (1C, s), 129.4 (1C, s), 130.6 (1C, s), 135.6 (1C, s), 137.0 (1C, s), 145.0 (1C, s), 145.3 (1C, s), 146.0 (1C, s), 147.4–147.5 (2C, 147.4 (s), 147.4 (s)). HSQC, (400MHz) correlation including 2 methyl protons $\delta_{\text{H}}3.60$ with $\delta_{\text{C}}56.0$; $\delta_{\text{H}}3.81$ with 58.4; $\delta_{\text{H}}3.01$ with $\delta_{\text{C}}28.9$. The compound was identified as berberine Fig. 4, IUPAC name, 9,10-dimethoxy-5,6-dihydro[1,3] dioxolo[4,5':6,7]isoquinolino[2,1-*b*][2]benzazin-7-ium.

Flavonoid identification

Flavonoid 1, was extracted as a pink amorphous powder, MS, M^+ , 491.464 g, molecular formula $C_{24}H_{27}NO_{11}$, UV (EtOH) λ_{max} , 510 nm. ^1H NMR: δ 3.20–3.60 (10H, 3.26 (dd, $J=10.2, 2.6\text{ Hz}$), 3.34 (dd, $J=3.5, 2.7\text{ Hz}$), 3.39 (dd, $J=10.2, 3.5\text{ Hz}$), 3.48 (td, $J=4.6, 2.7\text{ Hz}$), 3.55 (s)), 3.82 (2H, d, $J=4.6\text{ Hz}$), 6.01 (1H, d, $J=2.6\text{ Hz}$), 6.46 (1H, dd, $J=1.5, 0.5\text{ Hz}$), 7.18 (1H, dd, $J=1.5, 0.4\text{ Hz}$), 7.33 (2H, d, $J=1.6\text{ Hz}$), 7.64 (1H, t, $J=0.4\text{ Hz}$). ^{13}C NMR: δ 56.0 (2C, s), 62.3 (1C, s), 71.1 (1C, s), 74.0 (1C, s), 76.7 (1C, s), 78.1 (1C, s), 96.5 (1C, s), 101.9 (1C, s), 103.3 (1C, s), 110.6 (2C, s), 113.3 (1C, s), 126.3 (1C, s), 136.3 (1C, s), 137.0 (1C, s), 144.4 (1C, s), 147.6 (2C, s), 156.3 (1C, s), 157.9 (1C, s), 163.3 (1C, s), 168.8 (1C, s). HSQC, (400MHz) correlation including 2 methyl protons $\delta_{\text{H}}3.55$ with $\delta_{\text{C}}56.0$; $\delta_{\text{H}}3.55$ with 56.0; $\delta_{\text{H}}3.35$ with $\delta_{\text{C}}71.1$; $\delta_{\text{H}}3.38$ with $\delta_{\text{C}}76.7$; $\delta_{\text{H}}3.48$ with 76.7; $\delta_{\text{H}}3.82$ with $\delta_{\text{C}}62.3$; $\delta_{\text{H}}3.82$ with $\delta_{\text{C}}62.3$; $\delta_{\text{H}}6.01$ with $\delta_{\text{C}}103.3$; $\delta_{\text{H}}6.46$ with $\delta_{\text{C}}103.3$; $\delta_{\text{H}}6.46$ with $\delta_{\text{C}}96.5$; $\delta_{\text{H}}7.33$ with $\delta_{\text{C}}110.6$ and $\delta_{\text{H}}7.64$ with $\delta_{\text{C}}136.3$. The pink amorphous flavonoid was identified as malvidin 3-*o*-glucoside Fig. 5.

Flavonoid 2 was extracted as a reddish amorphous powder, MS, M^+ , 433.3848 g, molecular formula $C_{21}H_{21}NO_{10}$, UV (EtOH) λ_{max} , 502 nm. ^1H NMR: δ 3.20–3.54 (4H, 3.26 (dd, $J=10.2, 2.6\text{ Hz}$), 3.34 (dd, $J=3.5, 2.7\text{ Hz}$), 3.39 (dd, $J=10.2, 3.5\text{ Hz}$), 3.48 (td, $J=4.6, 2.7\text{ Hz}$), 3.82 (2H, d, $J=4.6\text{ Hz}$), 6.02 (1H, d, $J=2.6\text{ Hz}$), 6.70 (1H, dd, $J=1.5, 0.4\text{ Hz}$), 7.04 (2H, ddd, $J=8.9, 1.4, 0.5\text{ Hz}$), 7.29 (1H, dd, $J=1.5, 0.4\text{ Hz}$), 7.64–7.77 (3H, 7.70 (ddd, $J=8.9, 1.5, 0.5\text{ Hz}$), 7.71 (t,

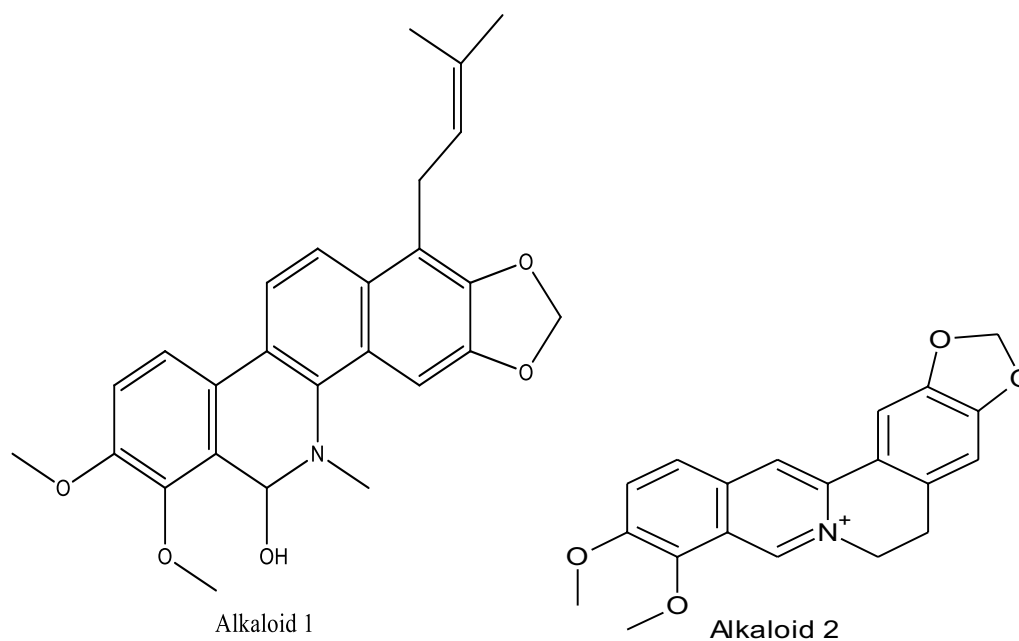


Fig. 4 Structures of active alkaloids in the polyherbal extract

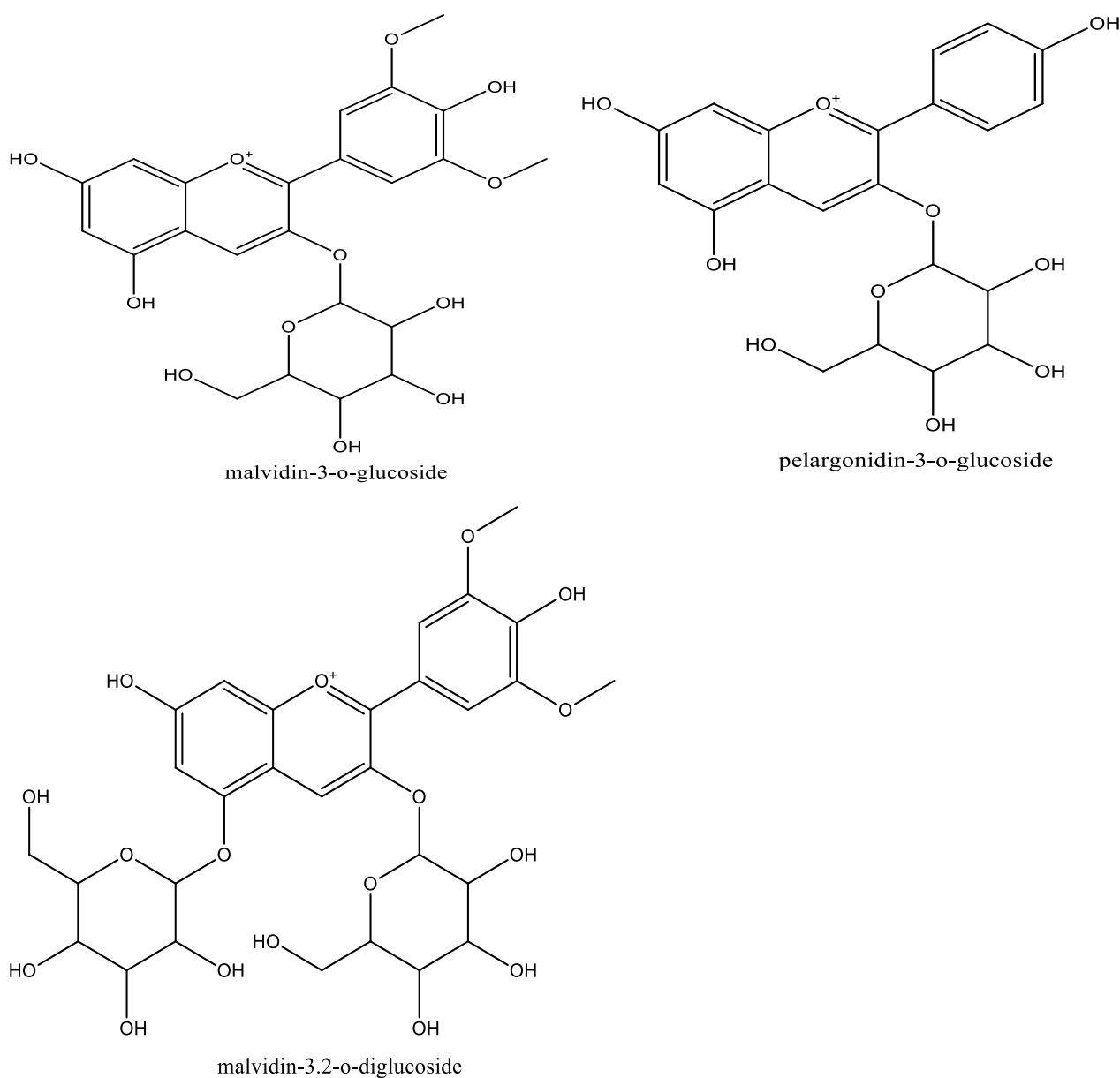


Fig. 5 Structures of active flavonoids in the polyherbal extract

$J=0.4$ Hz)). ^{13}C NMR: δ 62.3 (1C, s), 71.1 (1C, s), 74.0 (1C, s), 76.7 (1C, s), 78.1 (1C, s), 96.5 (1C, s), 101.9 (1C, s), 103.3 (1C, s), 113.3 (1C, s), 115.7 (2C, s), 119.4 (1C, s), 135.0 (2C, s), 136.3 (1C, s), 144.4 (1C, s), 156.3 (1C, s), 157.4 (1C, s), 157.9 (1C, s), 163.3 (1C, s), 168.8 (1C, s). HSQC, (400MHZ) correlation including 2 methylene sugar protons δ_{H} 3.82 with δ_{C} 62.3; δ_{H} 3.82 with 62.3; δ_{H} 3.27 with δ_{C} 71.1; δ_{H} 3.34 with δ_{C} 76.7; δ_{H} 3.38 with δ_{C} 74.0; δ_{H} 3.48 with δ_{C} 78.1; δ_{H} 6.01 with δ_{C} 103.42; δ_{H} 6.69 with 96.5; δ_{H} 7.33 with δ_{C} 101.9; δ_{H} 7.70 with δ_{C} 115.7; δ_{H} 7.71 with δ_{C} 136.3; δ_{H} 7.92 with δ_{C} 135.4.

The reddish amorphous flavonoid was identified as pelargonidin-3-O-gucoside Fig. 5.

Flavonoid 3 was extracted as a pink amorphous powder, MS, M^+ , 655.5775 g, molecular formula $\text{C}_{29}\text{H}_{35}\text{O}_{17}$, UV (EtOH) λ_{max} , 512 nm. ^1H NMR: δ 3.17–3.62 (14H, 3.24 (dd, $J=10.2$, 2.6 Hz), 3.26 (dd, $J=10.2$, 2.6 Hz), 3.34 (dd, $J=3.5$, 2.7 Hz), 3.34 (dd, $J=3.5$, 2.7 Hz), 3.38 (dd, $J=10.2$, 3.5 Hz), 3.39 (dd, $J=10.2$, 3.5 Hz), 3.48 (td, $J=4.6$, 2.7 Hz), 3.48 (td, $J=4.6$, 2.7 Hz), 3.57 (s)), 3.76–3.88 (4H, 3.82 (d, $J=4.6$ Hz), 3.82 (d, $J=4.6$ Hz)), 5.72 (1H, d, $J=2.6$ Hz), 6.00 (1H, d, $J=2.6$ Hz), 6.55 (1H, dd,

$J=1.6, 0.4$ Hz), 7.20 (1H, dd, $J=1.6, 0.4$ Hz), 7.33 (2H, d, $J=1.6$ Hz), 7.65 (1H, t, $J=0.4$ Hz). ^{13}C NMR: δ 40.7 (1C, s), 56.0 (2C, s), 62.3 (1C, s), 64.4 (1C, s), 71.0–71.1 (2C, 71.0 (s), 71.1 (s)), 73.3 (1C, s), 74.0 (1C, s), 76.7 (1C, s), 78.1 (1C, s), 92.7 (1C, s), 96.5 (1C, s), 96.8 (1C, s), 101.9 (1C, s), 102.7 (1C, s), 110.6 (2C, s), 112.0 (1C, s), 126.3 (1C, s), 136.3 (1C, s), 137.0 (1C, s), 144.4 (1C, s), 147.6 (2C, s), 155.6 (1C, s), 156.3 (1C, s), 163.3 (1C, s), 168.8 (1C, s). HSQC, (400MHz) correlation including 2 methyl protons $\delta_{\text{H}}3.57$ with $\delta_{\text{C}}56.0$; $\delta_{\text{H}}3.57$ with 56.0; $\delta_{\text{H}}3.24$ – 3.26 with $\delta_{\text{C}}74.0$; $\delta_{\text{H}}3.34$ with $\delta_{\text{C}}71.1$; $\delta_{\text{H}}3.38$ with 76.7; $\delta_{\text{H}}3.48$ with $\delta_{\text{C}}78.1$; $\delta_{\text{H}}3.82$ with $\delta_{\text{C}}62.3$; $\delta_{\text{H}}5.73$ with $\delta_{\text{C}}101.9$; $\delta_{\text{H}}6.56$ with $\delta_{\text{C}}102.7$; $\delta_{\text{H}}7.34$ with $\delta_{\text{C}}110.58$; $\delta_{\text{H}}7.20$ with 96.5; $\delta_{\text{H}}7.65$ with $\delta_{\text{C}}136.3$. The pink amorphous flavonoid was identified as malvidin 3, 5-O-diglucoside Fig. 5.

Discussion

Polyherbal drugs are now very important as alternative arsenals during clinical failure or cases of severe side effects of conventional medicines. A polyherbal drug widely commended in an ethnobotanical survey we conducted was tested to investigate its mode of action and therefore provide an explanation for its great efficacy and popularity in traditional practices. In the present study alkaloid extracts of the polyherbal drug made from *Z. chalybeum* and *X. stuhlmannii* root bark powders gave 2 significantly active alkaloids against PTP 1B enzyme, Alkaloid 1 and 2. Alkaloid 1, confirmed by testing positive for alkaloids using Dragendorff's reagent, is a derivative of Chelerythrine named 1, 2-dimethoxy-12-methyl-7-(3-methylbut-2-en-1-yl)-12, 13-dihydro[1,3]benzodioxolo[5,6-c]phenanthridin-13-ol and alkaloid 2 is berberine, IUPAC name, 9,10-dimethoxy-5,6-dihydro[1,3]dioxolo[4',5':6,7]isoquinolino[2,1-b][2]benzazin-7-ium. While berberine and Chelerythrine have been reported before (Carromberth et al. 2009), the prenylated and hydroxylated Chelerythrine is reported here for the first time. Prenylated and hydroxylated Chelerythrine was obtained as a yellow crystalline powder that dissolved in ethanol and ethyl acetate giving yellow solution. The ^1H NMR of alkaloid 1 exhibited 5 signals corresponding to aromatic protons pointing to presence of a benzophenanthridine system (Nissanku et al. 2001). Basing the aromatic region of alkaloid 1, ^1H NMR spectrum consist of signals of 2 pairs of hydrogen doublets, $\delta_{\text{H}}7.11$ (1H), 7.80(1H) and 7.36(1H), 6.64(1H), and two one-hydrogen singlets, $\delta_{\text{H}}6.94$ (1H) and 6.57(1H). The ^1H NMR spectrum also showed the existence of the methylene dioxide group at $\delta_{\text{H}}6.13$ (2H), hydroxyl group at $\delta_{\text{H}}6.57$ (s), methoxy groups at $\delta_{\text{H}}3.58$ (3H), 3.83(3H) and N-methyl group at $\delta_{\text{H}}3.45$ (3H). Furthermore ^1H NMR spectrum exhibited the presence of an isoprene unit

attached to C18 indicated by 2 methyl groups at $\delta_{\text{H}}1.56$ (6H), methylene proton (1H) at $\delta_{\text{H}}5.33$ (t, $J=7.3$ Hz) and CH_2 group at $\delta_{\text{H}}3.24$ (2H, d, $J=7.3$ Hz). The ^{13}C NMR spectrum confirmed the existence of the isoprene unit with signals at $\delta_{\text{C}}25.1$ (CH_2), 19.9 ($=\text{C}-\text{CH}_3$), 25.8 ($=\text{C}-\text{CH}_3$), 132 ($=\text{CR}_1\text{R}_2$, s) and 122.5 (CH) therefore the structure of alkaloid 1 was established as 1, 2-dimethoxy-12-methyl-7-(3-methylbut-2-en-1-yl)-12, 13-dihydro[1,3]benzodioxolo[5,6-c]phenanthridin-13-ol, a new compound which is a derivative of Chelerythrine. However previous studies have reported isolation of 6-acetyl-N-methyldihydrodecarine, a decarine derivative from *Zanthoxylum riedelianum* (Mekute et al. 2023).

Isolation and identification of flavonoids yielded 3 already known anthocyanins, malvidin 3-O-glucoside, pelargonidin-3-O-glucoside and malvidin 3, 5-O-diglucoside, Fig. 5, from the polyherbal drug extracts. These also showed remarkable PTP 1B inhibition activity that was better when compared to that of metformin (Table 4). The present study is the first publication in trying to understand the chemical composition of the polyherbal drug and its mode of action. However, previous studies report biological activities including antibacterial activity of rotenoids and Isoflavones isolated from *X. stuhlmannii* and *Z. chalybeum* (Mekute et al. 2023). Inhibition of PTP 1B by malvidin 3-O-glucoside has been reported before by Xue et al. (2022). From the phenolics which were tested, malvidin-3-glucoside showed the greatest inhibition in a competitive inhibition mode. In another study by Ye et al. (2024) and Jayaprakasam et al. (2005) anthocyanins significantly reduced sugar levels in rats by stimulating the secretion of insulin. Protein tyrosine phosphatase 1B (PTP1B) is an important target to treat diabetes mellitus 2, due to its deep correlation with insulin signaling, and glucose regulation (Tovara et al. 2023; Nandi and Saxena 2020). The presence of the 3 anthocyanins, malvidin 3-O-glucoside, pelargonidin-3-O-glucoside and malvidin 3, 5-O-diglucoside therefore makes the polyherbal drug an important remedy for managing diabetes. Over the years polyherbal drugs have gained popularity because of the belief that they are natural and safe and also are cheap and readily available.

Conclusions

The polyherbal crude extract was obtained by extraction with absolute ethanol. One new compound, 2-dimethoxy-12-methyl-7-(3-methylbut-2-en-1-yl)-12,13-dihydro[1,3]benzodioxolo[5,6-c]phenanthridin-13-ol, one previously reported compound berberine, and three anthocyanins were isolated from the polyherbal extract. Both the alkaloids and the anthocyanins showed significant PTP 1B inhibitory activity showing

that the polyherbal drug is a potential medicine for managing diabetes.

Abbreviations

PTP 1B	Protein tyrosine phosphate 1B
pNPP	P-nitrophenyl phosphate
¹ H NMR	Hydrogen nuclear magnetic resonance
¹³ C NMR	Carbon 13 nuclear magnetic resonance
HSQC-NMR	Heteronuclear single quantum coherence nuclear magnetic resonance
UV	Ultraviolet
FT-IR	Fourier transform infrared
MS	Mass spectrometry

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Author contributions

All author contributed in the design of the study. PM carried out the experiments, data analysis and wrote the first draft. PD and SN wrote the second draft. PM wrote the final draft. All authors read and approved the final draft.

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Data availability

Raw spectra data available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

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Competing interests

Authors state no conflict of interest.

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