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Analysis of phytochemical profile, ferric reducing power, H₂O₂ scavenging activity and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of extracts from aerial parts of *Pseudognaphalium undulatum*

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Abstract

In this study, the antioxidant activity and phytochemical analysis of dichloromethane (DCM) and methanolic extracts of *Pseudognaphalium undulatum* (L.) Hillard & B.L. Burt were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, hydrogen peroxide (H₂O₂) assay, ferric reducing power assay and standard analytical methods. Out of nine phytochemical classes determined, the DCM extract showed the presence of 33.3% of them while the methanolic extract showed the presence of 88.9%. The extracts showed significant antioxidant properties of which the methanolic extract showed the DPPH and H₂O₂ IC₅₀ values of 706.38 and <50 µg/ml, respectively. Similarly, the methanolic extract showed the highest total phenolic and flavonoid contents of 213.03±0.82 mg GAE/g DW and 43.81±0.93 mg QE/g DW, respectively. The ferric reducing powers of both extracts showed a linear correlation with the ascorbate standard. Further studies such as bio-guided fractionation are required to explore in depth the pharmacological and ethnomedicinal properties of pure compounds from this plant.

Keywords: 2, 2-diphenyl-1-picrylhydrazyl radical, dichloromethane extract, methanolic extract, Asteraceae, *Pseudognaphalium undulatum*

1. Introduction

Free radicals cause oxidative damage to lipids, proteins, and DNA, ultimately resulting in chronic diseases, such as diabetes, cancer, diabetes, and cardiovascular as well as neurodegenerative diseases [1-3]. That happens when the bodily cellular antioxidant defense systems are weakened or insufficient. Therefore dietary intake of antioxidant supplements is needed to protect cells from damage caused by free radicals [2]. The majority of antioxidants occur naturally in resources such as plants in good concentrations and have synergistic actions [3]. Recent research has paid much attentions towards the development of traditional medicines as they contain phenols and flavonoids as well as other pharmacologically active phytochemicals. According to 2019 World Health Organization (WHO) Global Report on Traditional and Complementary Medicine, 88% of WHO Member States are using Traditional and Complementary Medicines [4]. In 2019, the global Herbal Medicine market size was US\$ 138350 million and it is expected to reach US\$ 218940 million by the end of 2026, with a Compound Annual Growth Rate (CAGR) of 6.7% during 2021-2026 [5].

Pseudognaphalium undulatum (L.) Hilliard & B.L. Burt, vernacularly known as mosuoane/manku in Sesotho, belonging to the Compositae-Gnaphalidae family, is one the 126 species of the *Pseudognaphalium* genus [6-9]. *P. undulatum*, formerly known as *Gnaphalium undulatum*, is a bushy herb with greyish-white wholly stems and height of about 60cm [6, 8, 10]. *P. undulatum* is characterized by its several, tight clustered heads of about 3mm in length with small, wholly based, whitish flowers [6, 9]. The reported literature on the ethnomedicinal use of *P. undulatum* as well as ethno pharmacological studies are limited possibly because previous studies focused on ethnobotanical studies, however, *P. undulatum* has various therapeutic and commercial applications in Lesotho. *P. undulatum* is used as a fumigant to relieve fever in children [7]. Decoction of whole plant of *P. undulatum* is used to relieve kidney related ailments, profuse menstrual cramps, sore throat and ulcers. A local company called Kingdom Skylite Hub makes herbal and ice teas with *P. undulatum*.

Despite the recent advances in modern medicine, scientific screening of medicinal plants exploited to make traditional medicines is required in order to validate their respective healing properties, trace possible adverse effects or their cytotoxic nature and even discover other probable ethnomedicinal uses as well as their effective doses of usage. To the best of our knowledge, *P. undulatum* species have not been largely explored for its pharmacological and therapeutic applications, particularly the species collected from the Kingdom of Lesotho. As such, this study seeks to investigate the phytochemical profile and antioxidant activities of dichloromethane and methanolic extracts of *P. undulatum*. The results are communicated in this study.

2. Materials and Methods

2.1 Chemicals and Reagents

Chloroform, Dichloromethane and methanol (AR Grade, 99%) were purchased from Sigma Aldrich. Tris (hydroxymethyl) aminomethane (THAM), sulphuric acid, hydrogen peroxide and hydrochloric acid were purchased from Minema chemicals. Ascorbic acid, tri-sodium hydrogen phosphate, sodium dihydrogen phosphate, ferric chloride, potassium ferri cyanide, magnesium powder and sodium hydroxide were purchased from Merck. Benedict's solution was purchased from Prestige Laboratory Supplies.

2.2 Sample collection

Fresh plant materials of *P. undulatum* were collected in August 2021 inside Roma campus of the National University of Lesotho (29.4503° S, 27.7205° E). The plant species was authenticated at the herbarium of the Department of Biology, National University of Lesotho. A voucher specimen *viz.* Matamane/PU/2021 was deposited at the Organic Research Laboratory, National University of Lesotho, Roma Campus.

2.3 Sample processing

The plant materials were dried in the shade at room temperature for two weeks. The air-dried whole plant materials were then pulverized using a laboratory blender (Waring Blender, Blender 80119, Model HGB2T93, 240V, 3.5 AMPs). A total mass of 49.0377g of powdered sample was obtained and it comprised of both the leaves and stems of *P. undulatum*.

2.4 Preparation of extracts

A mass of 22.5419g of the powdered sample was macerated with 200ml of dichloromethane (DCM) for three days with occasional agitation. The extract solution was filtered off through a Whatman No. 1 filter using a vacuum pump. The extract was concentrated *in vacuo*. The extract was then collected in a pre-weighed, clean and dry beaker. The exact procedure was duplicated and 3.8914g of the combined DCM extract was obtained.

The same procedure was repeated but instead macerating 26.4281g of the powdered sample with 200ml of methanol. A mass of 8.6744g of the combined methanolic extract was obtained.

2.5 Phytochemical screening

Preliminary phytochemical screening of the DCM and methanolic extracts of *P. undulatum* for the presence of saponins, tannins, phlobatannins, terpenoids, phenolics, flavonoids, quinones, sterols and reducing sugars was carried out using standard methods reported in literature [11, 12].

2.5.1 Detection of saponins

An amount of 0.1g of the sample was dissolved in 2.0ml distilled water followed by vigorous shaking of the contents for 15 minutes. The formation of 1cm layer of foam confirmed the presence of saponins.

2.5.2 Detection of tannins

A volume of 2.0ml of distilled water was used to dissolve 0.2g of the sample and the contents were heated in a water bath (95°C). After filtering the mixture, few drops of concentrated sulphuric acid were added and lastly, 5% freshly prepared ferric chloride was added. The deposition of blue black, green or blue-green precipitate was taken as evidence for the presence of tannins.

2.5.3 Detection of phlobatannins

A mass of 0.1g of the sample was boiled in 3.0ml of 1% hydrochloric acid. The deposition of red precipitate confirmed the presence of phlobatannins.

2.5.4 Detection of terpenoids

An amount of 0.25g of the sample was dissolved in 0.25ml of distilled water. A volume of 1.0ml of chloroform was then added to the extract solution followed by addition of 3.0ml of concentrated sulphuric acid. The presence of terpenoids was indicated by the formation of a reddish brown colouration on the interface.

2.5.5 Detection of phenolics (Phenols and Polyphenols)

A mass of 0.5g of the sample was boiled in 1.0ml of distilled water for 30 minutes in a water bath maintained at 95°C. Upon filtration, 3.0ml of 5% (w/v) freshly prepared ferric chloride was added to the filtrate. Lastly, a volume of 1.0ml of 1% (w/v) potassium ferricyanide was added. The formation of green colouration was taken as evidence for the presence of simple phenols and the formation of blue colouration was taken as evidence for the presence of polyphenols.

2.5.6 Detection of flavonoids

A volume of 2.0ml of distilled water was used to dissolve 0.50g of the sample and the contents were heated in a water bath followed by addition of small amounts of powdered magnesium. Few drops of concentrated hydrochloric acid were then added and the presence of flavonoids were indicated by the formation of dark brown colour which steadily shades to dark red or pink colour.

2.5.7 Detection of quinones

A volume of 0.5ml of distilled water was used to dissolve 0.25g of the sample. The formation of green or white precipitate upon addition of 1.5ml of concentrated hydrochloric acid confirmed the presence of quinones.

2.5.8 Detection of sterols

A volume of 2.0ml of chloroform was used to dissolve 0.3g of the sample. Upon filtration, a volume of 1.0ml of concentrated sulphuric acid was added to the filtrate and the formation of two layers in which the chloroform phase turn red was evident of the presence of sterols.

2.5.9 Detection of reducing sugars

A volume of 2.5ml of distilled water was used to dissolve 1.0g of the sample. Aliquot of 2.0ml of the extract concentration was added to 2.0ml of Benedict's solution in a

test tube. The contents were heated in water bath (95 °C) for period of 5 minutes. The deposition of brick-red colour was indicative of the presence of reducing sugars.

2.6 Antioxidant analysis

2.6.1 DPPH radical scavenging activity

DPPH radical scavenging activity of DCM and methanolic extract from *P. undulatum* was performed according to the method described in literature [13, 14]. Briefly, a stock solution of each extract was prepared by dissolving 3.0 mg of the extract in 1 ml of 50% methanol. Serial dilutions with the concentrations of 3000, 2000, 1500, 1000, 800, 500 and 200 µg/ml were prepared separately from the stock solutions. The 0 µg/ml solution (solution without extract) served as negative control. A 0.1 mM DPPH solution, which served as oxidant, was prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol and was then stored in a dark cupboard to minimize chemical degradation. Aliquots of 100 µl of the extract solutions were mixed separately with 1.0 ml of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris-HCl buffer maintained at pH 7.40. The mixed contents were shaken vigorously and then incubated in the dark cupboard for 30 minutes. The optical density of the incubated contents was measured at 517.0 nm using a spectrophotometer (MRS Spectro UV – 11). Similarly, a stock solution of ascorbic acid, which served as positive control, was also prepared by dissolution of 3.0 mg of ascorbic acid in 1 mL of 50% methanol. Serial dilutions of concentrations of 3000, 2000, 1500, 1000, 800, 500, and 200 µg/ml were also prepared accordingly. The solution without the ascorbic acid (0 µg/mL) also served as the negative control and the optical densities (Absorbances) were measured at 517.0 nm. The ability of the extracts and/or ascorbate standard to scavenge DPPH radical was calculated using the given equation below:

$$\text{DPPH radical scavenging activity (\%)} = ((A_{\text{cont}} - A_{\text{test}})/A_{\text{cont}}) \times 100$$

A_{cont} = Absorbance of the negative control

A_{test} = Absorbance in the presence of the extract or positive control.

The concentration of the extract that yields 50% inhibition is known as the IC₅₀ value [15]. The IC₅₀ values were generated using Microsoft Excel by plotting the extract concentration versus % inhibition of DPPH free radical. An IC₅₀ value of low concentration represents a higher antioxidant activity and that of high concentration represents a lower antioxidant activity [16].

2.6.2 Hydrogen peroxide scavenging activity

The ability of the DCM and methanolic extracts of *P. undulatum* to scavenge hydrogen peroxide was measured using a method reported in literature but with modifications [12]. Briefly, 50 mM phosphate buffer solution maintained at pH 7.4 was prepared accordingly. A 2 mM solution of hydrogen peroxide was prepared using the 50 mM phosphate buffer. Serial dilutions with concentrations of 50, 100, 200, 300, 400 and 500 µg/ml were prepared separately from stock solutions of 500 µg/ml of the extracts. The 0 µg/ml solutions served as the negative control and the ascorbic acid maintained at similar dilutions served as the positive control. Aliquots of 100 µl of the extracts or the ascorbate standard were mixed separately with 300 µl of 50 mM phosphate buffer (pH 7.4) in clean and dry test tubes. 600 µl of the H₂O₂ solution were added to each test tube and the contents were

vortexed for 10 seconds then incubated at room temperature for 10 minutes for colour development. After the incubation period, the absorbance of the contents was taken at 230.0 nm using a spectrophotometer (MRS Spectro UV-11). The H₂O₂ scavenging capacity of the extracts was estimated using the following equation:

$$\text{H}_2\text{O}_2 \text{ scavenging activity} = (1 - \text{absorbance of sample/absorbance of blank}) \times 100$$

The IC₅₀ values of the extracts were also generated using MS Excel. In the same way, an IC₅₀ value of low concentration signify a higher antioxidant activity and that of high concentration represents a lower antioxidant activity.

2.6.3 Determination of total phenolic content

The total phenolic content, TPC, of the dichloromethane and methanolic extract of *P. undulatum* was evaluated using a method described in literature [11]. Briefly, 10.0 mg of gallic acid was dissolved in 10.0 ml of absolute methanol to form a 1000 µg/ml stock solution. Two-fold serial dilution of 1000, 500, 250, 125, 62.5, 31.25 and 15.625 µg/ml were prepared from the 1000 µg/ml stock solution. The extracts were maintained at a 1000 µg/ml separate solutions. Aliquots of 2.0 ml of the gallic acid or extract solutions were mixed with 1.0 ml of 1% (v/v) Folin-Ciocalteu reagent separately. The contents were shaken for a few seconds and left to stand at room temperature for 4 minutes. A volume of 1.0 ml of 0.7 M sodium carbonate was added to the contents followed by vortex mixing the mixtures for about 15 seconds. The mixed contents were incubated in the dark cupboard for 15 minutes with intermittent agitation. The absorbance of the reaction mixtures was taken at 765 nm with a spectrophotometer. The determinations were done in triplicates.

2.6.4 Determination of total flavonoid content

The total flavonoid content, TFC, of the dichloromethane and methanolic extract of *P. undulatum* was determined using a method reported in literature [11]. The method involved the use of aluminum chloride calorimetric method whereby, various dilutions; 25, 20, 15, 10 and 5 µg/ml, of quercetin standard were obtained from a 25 µg/ml stock solution prepared by dissolving 25.0 mg with 1.0 ml of absolute methanol. The extracts were maintained at respective concentration of 1000 µg/ml. A volume of 3.0 ml of the quercetin standard or extract was mixed separately with 1.0 ml of 2% aluminum chloride in clean test tubes. After incubating the contents at room temperature for 15 minutes, the optical densities (absorbances) were measured at 420.0 nm using a spectrophotometer. The determinations were done in triplicates.

2.6.5 Evaluation of ferric reducing power

Ferric reducing power of the dichloromethane and methanolic extract of *P. undulatum* was determined using a method described in literature but with slight modifications [17]. The ability of the extract to transform Fe (III) to Fe (II) was regarded as its reducing power [17]. This property was tracked by measuring the formation of Prussian blue colour at 700.0 nm, characteristic of the formation of Fe (II). Briefly, stock solutions of 0.2 mg of the sample in 1 ml of methanol were prepared separately. Two-fold serial dilutions of 200 to 0 µg/ml were prepared and the 0 µg/ml solution served as the negative control while ascorbic acid was maintained as the positive control. Aliquots of 2.0 ml of extract solutions at

various concentrations were mixed separately with 2.0 ml of 0.2M phosphate buffer (pH 6.6), 2.0 ml of 0.01% (w/v) potassium ferricyanide solution and the resulting mixtures were incubated at 50°C. Aliquots of 2.0 ml of 0.1% (w/v) trichloroacetic acid were added to the mixtures and the contents were centrifuged at 3000 rpm for a period of 10 min. The upper layer was collected thereafter and aliquots of 2.0 ml of the supernatant liquid were mixed further with 0.4 ml of 0.1% (w/v) freshly prepared ferric chloride solution and 2.0 ml of distilled water. The optical density of the contents was taken at 700.0 nm using a spectrophotometer (MRS Spectro UV-11) after allowing the reaction to occur for 10 min. The higher the absorbance of the reaction, the higher the reducing ability of the extract [12].

2.7 Statistical analysis

All determinations were performed in triplicates and the results were expressed as mean±SD. Data analysis was performed using SPSS v16.0 software application and the differences were considered statistical significant when $p \leq 0.05$.

3. Results and Discussion

3.1 Preliminary phytochemical screening

Table 1 summarized the qualitative phytochemical screening of dichloromethane extract of *P. undulatum* (DPU) and methanolic extract of *P. undulatum* (MPU). DPU demonstrated the presence of terpenoids, phenolics and sterols. MPU demonstrated the presence of saponins, tannins, phlobatannins, terpenoids, phenolics, flavonoids, sterols and reducing sugars.

Table 1: Preliminary phytochemical screening of DCM and methanolic extracts of *P. undulatum*

Test no.	Phytochemical class	Extract	
		DPU	MPU
1	Saponins	-	+
2	Tannins	-	+
3	Phlobatannins	-	+
4	Terpenoids	+	+
5	Phenolics	+	+
6	Flavonoids	-	+
7	Quinones	-	-
8	Sterols	+	+
9	Reducing sugars	-	+

DPU – Dichloromethane extract of *P. undulatum*; MPU – Methanolic extract of *P. undulatum*; (+) and (-) signs indicates the presence and absence of the compound

There are no previous studies carried out for preliminary phytochemical screening of *P. undulatum* species. However, phytochemical characterization of *P. undulatum* (formerly known as *G. undulatum*) has been previously conducted [8]. One flavonoid *viz.* 5-hydroxy-3,7,8-tetramethoxyflavone and eight terpenoids (diterpens) *viz.* 8-*epi*-sclareol, 13-*epi*-cyclosclareol, *ent*-Kauran-16-ene, *ent*-Kaura-16-en-19-al, *ent*-Kaur-16-19-oic acid, 15 α -hydroxy-*ent*-Kaur-16-ene-19-oic acid, *ent*-Kaur-9(11),16-dien-19-oic acid and 15 β -hydroxy-wedeliassecco Kaurenolide were identified [8]. Flavonoids and terpenoids were qualitatively detected in our study. Steroidal alkaloids have been found to exhibit various, protective metabolic functions, tannins possess remarkably potent antifungal activities and flavonoids have found to possess anti-tumor and antioxidant properties [18]. Our study confirmed the presence of tannins and flavonoids in *P. undulatum* species collected from the Kingdom of Lesotho

and so *P. undulatum* has antifungal, antioxidant and anti-tumor potential.

Table 2: Percentage inhibition of DPPH free radical activity of extracts from *P. undulatum* relative to the ascorbate standard

Concentration (μ g/ml)	Material		
	DPU	MPU	Asc. Acid
200	14.31±0.88 ^{ac}	35.59±1.06 ^{bde}	53.11±0.52 ^b
500	29.09±2.95 ^f	44.75±1.64 ^{be}	56.14±0.44 ^{ab}
800	31.96±0.08 ^{abd}	52.44±0.21 ^a	64.95±1.14 ^{cde}
1000	35.74±3.17 ^h	55.79±1.92 ^{cf}	67.48±1.70 ^{bd}
1500	38.34±1.51 ^c	59.69±1.11 ^{cdf}	74.47±0.46 ^a
2000	42.03±0.87 ^a	62.62±0.33 ^a	79.17±2.01 ^{cg}
3000	44.20±0.17 ^a	66.85±0.06 ^e	84.02±1.07 ^{cd}

DPU – Dichloromethane *P. undulatum* extract, MPU – Methanolic *P. undulatum* extract, Asc. acid – Ascorbic acid (positive control). Values with different superscripts (^{a-h}) in the same column are significantly different ($p < 0.05$). The experiments were performed in triplicates ($n = 3$).

3.2 DPPH radical scavenging activity

Table 2 summarizes the DPPH radical scavenging potential of dichloromethane *P. undulatum* extract (DPU) and methanolic *P. undulatum* extract (MPU) relative to that of the ascorbate standard. DPU exhibited DPPH radical scavenging activity of 14.31±0.88, 29.09±2.95, 31.96±0.08, 35.74±3.17, 38.34±1.51, 42.03±0.87 and 44.20±0.17% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 μ g/ml, respectively. On the other hand, the ascorbate standard showed 53.11±0.52, 56.14±0.44, 64.95±1.14, 67.48±1.70, 74.47±0.46, 79.17±2.01 and 84.02±1.07% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 μ g/ml, respectively. This result showed that, compared with ascorbic acid, DPU exhibited a weak radical scavenging activity.

Table 3: The percentage inhibition of hydrogen peroxide scavenging activity of extracts from *P. undulatum* at various concentrations

Concentration (μ g/ml)	Material		
	DPU	MPU	Asc. Acid
50	38.61±0.67 ^{af}	61.71±2.44 ^d	77.23±0.24 ^b
100	41.51±1.59 ^c	65.49±0.19 ^a	79.51±0.64 ^a
200	44.02±2.16 ^{dffg}	69.99±1.01 ^{ad}	81.27±0.82 ^a
300	46.82±0.93 ^e	74.36±0.72 ^b	86.91±2.10 ^{dffg}
400	50.95±1.78 ^d	74.68±3.17 ^{cdh}	88.42±0.36 ^e
500	53.26±0.37 ^a	77.42±2.00 ^f	94.08±1.11 ^{ce}

DPU – Dichloromethane *P. undulatum* extract, MPU – Methanolic *P. undulatum* extract, Asc. acid – Ascorbic acid (positive control). Values with different superscripts (^{a-h}) in the same column are significantly different ($p < 0.05$). The experiments were performed in triplicates ($n = 3$).

MPU showed 35.59±1.06, 44.75±1.64, 52.44±0.21, 55.79±1.92, 59.69±1.11, 62.62±0.33 and 66.85±0.06% of radical scavenging activity at subsequent concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 μ g/ml, respectively. Comparative to the results obtained for the ascorbate standard, this result revealed that MPU is relatively weak at concentrations <800 μ g/ml but shows significant radical scavenging activity at concentrations \geq 800 μ g/ml. Table 4 summarized the IC₅₀ values of the DCM and methanolic extract of *P. undulatum* under DPPH test. DPU and MPU exhibited IC₅₀ values of >3000 and 706.38 μ g/ml respectively whereas that of the ascorbate standard was <200 μ g/ml. This result showed that DPU has a weak antioxidant activity and MPU has moderate antioxidant activity relative to

the ascorbate standard.

There is no comparable data on the antioxidant activity of *P. undulatum* (*G. undulatum*). However, the antioxidant activity of some species of the *Pseudognaphalium* genus has been reported. Under DPPH test, the ethanolic extract of *G. uniflorum* (*P. uniflorum*) has been reported to exhibit a strong inhibitory activity of about 14.86 $\mu\text{g/ml}$ inhibitory concentration (IC_{50}) [8]. The highest IC_{50} value obtained in our study is 706.38 $\mu\text{g/ml}$ which implies that, *P. undulatum* is a weaker antioxidant source compared to *P. uniflorum*. Ethanolic extract of *Pseudognaphalium elegans* at concentration of 2500 $\mu\text{g/ml}$ was reported to inhibit 56.38% of 2,2-diphenyl-1-picrylhydrazyl radical [19]. In our study, the methanolic extract of *P. undulatum* at 2000 $\mu\text{g/ml}$ surpassed the ethanolic extract of *P. elegans* in scavenging the DPPH radical, hence it can be deduced that *P. undulatum* is a more potent antioxidant source than *P. elegans*.

3.3 Hydrogen peroxide scavenging activity

Table 3 summarizes the hydrogen peroxide scavenging activity of dichloromethane and methanolic extracts of *P. undulatum* relative to ascorbic acid. DPU showed 38.61 \pm 0.67, 41.51 \pm 1.59, 44.02 \pm 2.16, 46.82 \pm 0.93, 50.95 \pm 1.78 and 53.26 \pm 0.37% of hydrogen peroxide scavenging activity at concentrations of 50, 100, 200, 300, 400 and 500 $\mu\text{g/ml}$, respectively. On the other hand, the positive control, ascorbic acid, exhibited hydrogen peroxide scavenging activity of 77.23 \pm 0.24, 79.51 \pm 0.64, 81.27 \pm 0.82, 86.91 \pm 2.10, 88.42 \pm 0.36 and 94.08 \pm 1.11% at corresponding concentrations of 50, 100, 200, 300, 400 and 500 $\mu\text{g/ml}$, respectively. This result shows that DPU is weakly active at concentrations below 400 $\mu\text{g/ml}$ but shows promising hydrogen peroxide scavenging activity at concentrations \geq 400 $\mu\text{g/ml}$ relative to the ascorbate standard. MPU showed 61.71 \pm 2.44, 65.49 \pm 0.19, 69.99 \pm 1.01, 74.36 \pm 0.72, 74.68 \pm 3.17 and 77.42 \pm 2.00% of the hydrogen peroxide scavenging activity at concentrations of 50, 100, 200, 300, 400 and 500 $\mu\text{g/ml}$, respectively. Comparative to the ascorbate standard, this result shows that MPU exhibited significant hydrogen peroxide scavenging activity at all corresponding concentrations. Similarly, table 4 also summarized the IC_{50} values of the DCM and methanolic extract of *P. undulatum* under H_2O_2 test. The DPU and MPU exhibited IC_{50} values of 388.31 and $<$ 50 $\mu\text{g/ml}$ respectively. On the other hand, the ascorbate standard an IC_{50} value of $<$ 50 $\mu\text{g/ml}$, which is the same as that portrayed by MPU. This result showed that, though both DPU and MPU showed relatively strong H_2O_2 scavenging activity, MPU emerged as the most potent H_2O_2 scavenging agent than DPU.

Table IV: IC_{50} values of dichloromethane and methanolic extracts from *P. undulatum* at various concentrations

Material	IC_{50} value ($\mu\text{g/ml}$)	
	DPPH test	H_2O_2 test
DPU	$>$ 3000	388.31
MPU	706.38	$<$ 50
Asc. Acid	$<$ 200	$<$ 50

DPU – Dichloromethane *P. undulatum* extract, MPU – Methanolic *P. undulatum* extract, Asc. acid – Ascorbic acid (positive control). The experiments were performed in triplicates ($n = 3$).

There is no comparable literature on the hydrogen peroxide scavenging activity of extracts from *P. undulatum* species. In the body, hydrogen peroxide is generated in minimal quantities and can undergo several chain reactions which generate the hydroxyl radical ($\text{HO}\cdot$) [12]. The hydroxyl radical

is responsible for lipid peroxidation which in turn can pose detrimental effects on the DNA [20]. In our study, MPU was found to possess phytoconstituents with potent hydrogen peroxide scavenging potential. Consequently, *P. undulatum* emerges with great ethnomedicinal potential to alleviate ailments related to lipid peroxidation.

3.4 Ferric reducing power analysis

Figure 1 depicts the ferric reducing power of dichloromethane and methanolic extracts of *P. undulatum* relative to the ascorbate standard. The reducing power of the extracts was found to be dose-dependent. MPU exhibited a higher reducing power than DPU relative to the ascorbate standard. At the highest concentration, 250 $\mu\text{g/ml}$, MPU showed a significantly promising reducing power of 1.502 \pm 0.026.

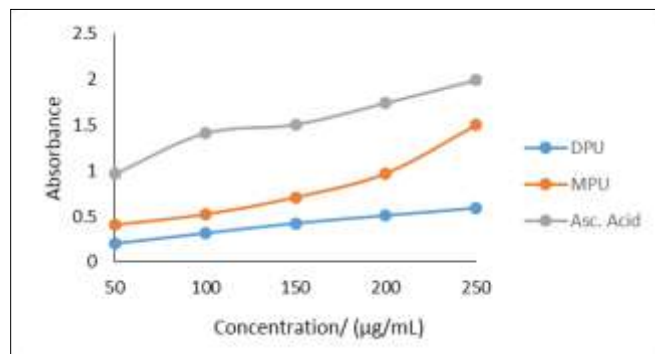


Fig 1: Ferric reducing power of extracts from *P. undulatum*

There is no comparable literature on the ferric reducing power of extracts *P. undulatum*. However, previous studies revealed that tannins do not only possess vital anti-inflammatory properties by their ability to inhibit the 5-lipoxygenase enzyme in the arachidonic acid metabolism but are also one of the potent ferric reducing agents [21]. As a result, the appreciably high ferric reducing power observed in MPU is attributed to the presence of tannins and their derivatives in MPU.

3.5 Total phenolic and flavonoid content

Figure 2 depicts the Gallic acid calibration curve used to estimate the total phenolic content of DPU and MPU using the regression equation ($y = 0.0011x + 0.2884$, $R^2 = 0.981$) and the result was expressed as mg of Gallic acid equivalents per gram of sample in dry weight (mg GA/g DW). The TPC of DPU and MPU were determined to be 93.46 \pm 0.15 and 213.03 \pm 0.82 mg GAE/g DW, respectively.

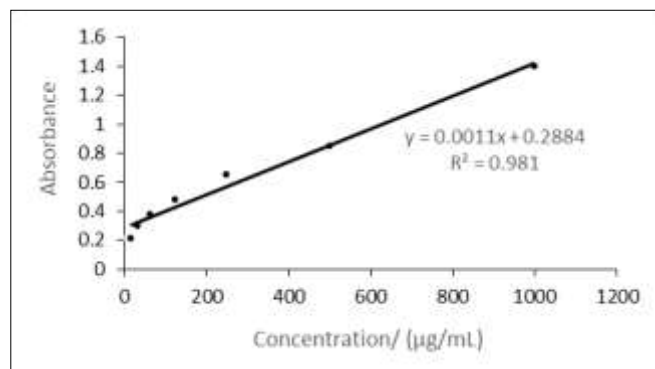


Fig 2: Graph for estimation of total phenolic content MPU exhibited relatively higher TPC owing to the presence of about 88.9% of the determined phytochemical classes whereas DPU only possess 33.3%.

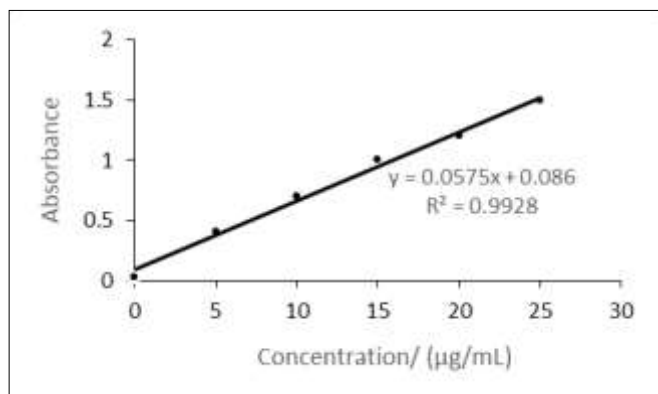


Fig 3: Graph for estimation of total flavonoid content

Figure 3 shows the quercetin calibration curve used to estimate the total flavonoid content of DPU and MPU using the regression equation ($y = 0.0575x + 0.086$, $R^2 = 0.9928$) and the result was expressed as mg of quercetin equivalents per gram of sample in dry weight (mg QE/100g DW). The TFC of DPU and MPU were determined to be 1.60 ± 1.27 and 43.81 ± 0.93 mg QE/g DW, respectively. In the preliminary phytochemical screening of DPU, there were no observable changes to qualify a positive test (+) for flavonoid in the extract but quantitative determination revealed the presence of flavonoids. The discrepancy is attributed to the very low concentration, 1.60 ± 1.27 mg QE/g DW, of flavonoids in DPU which can be assumed as below detectable limits.

In the same manner, there exist no comparable literature reporting the total flavonoid and phenolic contents of extracts from *P. undulatum*. However, the total phenolic and flavonoid contents of other species of *Pseudognaphalium* genus have been previously evaluated. The TFC of the ethanolic extract of *P. elegans* have been reported to be 130.69 ± 5.70 mg QE/100g DW^[19]. The flavonoid content of *P. elegans* was found to be higher than that in *P. undulatum* determined in this study. The extracts were derived from solvent systems of different polarity index, hence the incongruity in their respective TFCs. Flavonoids exhibit a broad spectrum of medicinal significance. Medicinal responses such as cellular health, antioxidant activity, tissue growth and repair are some of the few functions initiated by flavonoids^[22].

4. Conclusion

This is the first report exploiting the DCM and methanolic extracts of *P. undulatum* for their antioxidant capacity and phytochemical profile. The DCM extract showed the presence of about 33.3% of the analyzed phytochemical classes whereas the methanolic extract exhibited 88.9%. DCM extract exhibited DPPH radical scavenging activity in the range 14.31 ± 0.88 to $44.20 \pm 0.17\%$ with an IC_{50} value of >3000 µg/ml. Methanolic extract exhibited DPPH radical scavenging activity in the range of 35.59 ± 1.06 to $66.85 \pm 0.06\%$ with an IC_{50} value of 706.38 µg/ml. Methanolic extract of *P. undulatum* showed promising antioxidant properties. Moreover, DCM extract showed H_2O_2 scavenging activity in the range 38.61 ± 0.67 to $53.26 \pm 0.37\%$ while that of the methanolic extract was found between 61.71 ± 2.44 to $77.42 \pm 2.00\%$. The IC_{50} values of DCM and methanolic extract were found to be 388.31 and <50 µg/ml, respectively, under the H_2O_2 test. The total phenolic content of the DCM and methanolic extracts were found to be 93.46 ± 0.15 and 213.03 ± 0.82 mg GAE/g DW, respectively. On the other hand, the total flavonoid content of the DCM and methanolic extract were found to be 1.60 ± 1.27 and 43.81 ± 0.93 mg QE/g DW,

respectively. Ferric reducing power of both extracts was found to increase with increasing concentration. Further studies are required on this plant in order to explore in depth its biological and pharmacological properties.

5. Conflict of interest

The authors declare no conflict of interests.

6. References

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