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Investigation of pharmacognostical, phytochemical and antioxidant activity of aerial part of *Parthenium hysterophorus* (Asteraceae)

Saleh Kassem Algfri[1*], Gulia Ahmed Naser[1], Rashid Saeed Rageah[2]
[1] Department of Pharmacognosy, Faculty of Pharmacy, University of Aden, Yemen
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Aden, Yemen

Abstract
Parthenium hysterophorus L. (Asteraceae) grows in Yemen and is widely used by the local people to treat various diseases including rheumatic pain and cold. There is a lack of pharmacognostic, phytochemical, and pharmacological information about this plant in the available literature, so the present research aims to evaluate the pharmacognostic characteristics, chemical constitution, and antioxidant activity of extracts obtained from the aerial parts. The macroscopic and microscopic characteristics as well as physicochemical parameter were established; phytochemicals such as carbohydrates, amino acid, protein, saponins, triterpenes, sterols, polyphenols, flavonoids and alkaloids were identified in the studied extracts. The total phenolic content was 121.00, 95.30 mg/g in 80% methanol and water extracts respectively, but the total flavonoid content was 81.85, 23.00 mg/g in 80% methanol and water extracts respectively. TLC profile was performed for petroleum ether, 80% methanol and water extracts as well as for the chloroform and ethyl acetate fractions obtained from the 80% methanol extract. Ethyl acetate and chloroform fractions were exhibited antioxidant activity by dot-blot DPPH method and by TLC bioautography assay. The antioxidant activity was 28.74±5.21 % and 78.77±4.40 % for the chloroform and ethyl acetate fractions respectively. The aerial parts of *Pisadia punctulata* are considered a promising candidate for the production of natural antioxidant agents due to the many compounds they contain.

Keywords: Yemen, *Parthenium hysterophorus*, pharmacognostical, phytochemical**,** antioxidant

Introduction
Particularly in developing nations that continue to employ plant-based traditional medicine (TM) for their healthcare, plants are still vital sources of medications (Cohen *et al*., 1996) [6]. For the treatment of common ailments, over 60-80% of the world's population still uses traditional medicines (Rajalakshmy *et al*., 2010) [25]. In recent years, there has been a growing interest in using natural materials as substitutes for synthetic additions in pharmacologically relevant medications (Turek *et al*., 2013.) [32]. *Parthenium hysterophorus* (Asteraceae) is distributed in different part of the world and historically has been used as an emmenagogue, to treat fever, diarrhea, neurologic diseases, urinary tract infections, dysentery and malaria (Surib-Fakim *et al*., 1996) [30]. Studies have indicated that it contains several important chemical constituents mainly histamine, sesquiterpenes, flavonoids and alkaloids (Adkins *et al*., 2018) [1]. Yemen has a long history of using herbal medicine to treat a variety of illnesses, such as infections, inflammations, and other ailments (Al-Dubai and Al-khulaidi, 1996; Fleurentin and Pelt, 1982; Schopen, 1983) [3, 9, 27]. Despite various studies conducted over the past ten years, the pharmacological and chemical properties of only a small group of traditionally used medicinal herbs have been evaluated. (Awadh *et al*., 2001; Mothana and Lindequist, 2005; Mothana *et al*., 2009) [4, 23, 22]. *Parthenium hysterophorus* grows in southern Yemen and is widely used by the local population to treat various ailments, including rheumatic pains and colds. Not enough pharmacognostical, phytochemical and pharmacological information about this plant was found in the available literature; therefore, its study is an important and necessary to document the traditional use, establish its chemical composition and therapeutic effect. To achieve this the aerial parts we select for present research, and the results will thus significantly improve our understanding about it.

Materials and Methods
Collection and identification of plant material
The aerial parts (leaves, soft stems) of *Parthenium hysterophorus* were collected in July 2020 from Al Dhalea, Republic of Yemen, dried in the shaded area and then manually grinded and stored at room temperature for further analysis. The plant sample was identified by taxonomist, Associate Professor Othman S. Alhawshibi of the faculty of Science, University of Aden, Yemen.

Pharmacognostic study
Macroscopic evaluation
Morphology of studied plant material was observed with the help of the magnifying lens. Parameters like shape, colour, odor and taste were evaluated for each plant sample. The macroscopic characters were studies as per the produce given in WHO guidelines (WHO 1998) [35].

Microscopic studies
Free hand sections of leaves, petiole and of stem of *Parthenium hysterophorus* were studies as per the produce given in WHO guidelines (WHO 1998) [35]. Sections were cleared by heating with chloral hydrate solution and examined under microscope. Photomicrographs were taken with Leica USA model 2000ATC (ocular: CPL W10X; objective: 4X, 10X and 40X). Various identifying characters, such as type of trichomes, type of stomata and

epidermal cells were recorded, and then photomicrography was done (Kokate, 1994; Kokate, 2003) [17, 18]. Photographs were taken with the help of digital camera (Sony 16 MP).

Physicochemical parameters

The physicochemical parameters like moisture content, percentage extractives in different solvents, ash content, acid insoluble ash, water soluble ash and moisture content by loss on drying were determined by standard methods as in WHO guidelines (WHO 1998) [35].

Phytochemical analysis

Extraction

The extract was prepared and fractionated by standard methods (Harborne, 1998; Sass, 1940; Wagner and Bladt, 1996) [11, 26, 33]. The dried powder of the aerial part (50 gm) was subjected to successive soxhlet extraction using petroleum ether, 80% methanol and water respectively until extraction was complete. The extracts were filtered using Whatman No. 1 filter paper, the filtered extracts were concentrated by a rotary evaporator, and the residual extracts were dried. The percentage yield was obtained using dry weight. Part of 80% methanol extract (80%) was suspended in water, extracted successively with chloroform and ethyl acetate (6×300 ml each) and then resulting extracts were concentrated to provide chloroform and ethyl acetate. The rest of methanol extract (20%) was used for further phytochemical analysis.

Qualitative phytochemical analysis

Various qualitative tests were performed to identify the nature of the chemical composition of extracts according to standard methods (Peach and Tracey, 1956; Harborne, 2007; Trease and Evans, 2002) [24, 12, 31].

Determination of total phenolic and flavonoid contents

The total phenolic content (TPC) of the aerial parts of *Parthenium hysterophorus* extract was determined using the modified Folin-Ciocalteu method (Ainsworth and Gillespie, 2007) [2], using gallic acid as the standard and expressing results as mg gallic acid equivalent (GAE) per gram of sample. Different concentrations (40–100 µg/mL) of gallic acid were prepared in methanol. Aliquots of 0.5 ml of the test samples and the standard solution were mixed with 2 ml of Folin–Ciocalteu reagent and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were incubate at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using UV-VIS spectrophotometer. All the samples were analyzed in three replications. The total phenol was determined with the help of standard curve prepared from pure phenolic standard (gallic acid).

The total of flavonoid content (TFC) of the aerial parts of *Parthenium hysterophorus* extract was determined by aluminum chloride colorimetric method (Zhishen *et al.*, 1999) [37] with modification. Different concentrations (20–100 µg/mL) of the extract and standard solution (quercetin) were added with 2 ml of distilled water and subsequently with 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) solution and mixed. After 6 minutes, 0.15 ml of (10% $AlCl_3$, w/v) solution was added. The solutions were allowed to stand for further 6 min and after that, 2 ml of sodium hydroxide (4% NaOH, w/v) solution was added to the mixture. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm on UV spectrophotometer. TFC was determined as mg quercetin equivalent per gram of sample with the help of calibration curve of quercetin. Determination performed in triplicates (n=3).

Thin layer chromatography

Thin layer chromatography (silica gel 60 F254; Merck) of prepared extracts and fractions was performed to determine Rf values (Wagner and Bladt, 1996) [33]. Various solvent systems were tested to obtain best results. TLC plates were viewed in UV chamber and Rf of all were calculated.

Antioxidant studies of chloroform and ethyl acetate fractions

Rapid screening of antioxidant by dot-blot and DPPH staining

Rapid screening of antioxidant using Dot-Blot assay with DPPH staining was adopted with slight modification. Drops of each sample of chloroform and ethyl acetate fractions were loaded onto a TLC layer in order of decreasing concentration (of 500, 250, 125, and 62.5 µg/ml), along the row and dried for 10 min. The sheet bearing the dry spots was placed upside down for 10 s in a 0.05% 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution and the layer was dried. Stained silica gel layer revealed a purple background with yellow to white spots at the location where radical scavenging capacity observed. The intensity of the yellow color depends on the amount and nature of radical scavenger present in the sample (Soler-Rivas *et al.*, 2000) [29].

TLC bioautography assay

About 2 µg of each fractions were loaded on TLC plate. The plate was developed in solvent system toluene-ethylformate-formic acid (5:4:1) to separate different constituents. Development plate sprayed with 0.05% DPPH reagent to give antioxidant constituents. The antioxidant constituents were analyzed by DPPH technique (Kannan *et al.*, 2010; Chan *et al.*, 2007) [15, 5]. The active antioxidant constituents of the extracts were detected in sunlight as yellow spots produced via reduction of DPPH by resolved bands against purple back ground on the TLC plate. The colour of the spots was noted and Rf values were calculated (Wagner and Bladt, 1996; Waksmundzka-Hajnos *et al.*, 2008) [33, 34].

DPPH radical scavenging activity assay

The DPPH radical scavenging activity assay of chloroform and ethyl acetate fractions was carried out according to the method of Chan *et al* with slight modification (Chan *et al.*, 2007) [5]. Different dilutions of the fractions and standard quercetin (100-500 µg/ml) were prepared. DPPH solution was also prepared by dissolving 5.0 mg of DPPH in 100 mL methanol. Then, 1 mL of fraction from each dilution was added into the test tube containing 2 mL of DPPH solution. Control was prepared by adding 1 mL of methanol to 2 mL of DPPH solution. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured using UV-VIS spectrophotometer at 517 nm. The scavenging activity of extract on DPPH radical was calculated using the following equation:

Inhibition % = [(A0 –A1) /A0] x 100;

A0 is the absorbance of control and A1is absorbance of test. Antioxidant activity of chloroform and ethyl acetate fractions expressed as IC50 values and compared with standard. The 50% inhibition (IC50) of antioxidant activity was calculated as the concentrations of samples that inhibited 50% of scavenging activity of DPPH radical's activity under these conditions (Huang *et al.*, 2007) [13]. The data were presented as mean values ± standard deviation (n = 3).

Result and Discussion

Pharmacognostic study

Macroscopic study of aerial part

Before any tests are carried out for any plant, it is necessary to determine the macroscopic and microscopic characteristics to establish its identity and level of purity (WHO 1998) [35]. *Parthenium hysterophorus* is a branched, erect herbaceous plant. It usually grows 0.5-1.5 m tall; it is unbranched in lower part and branched in upper part, octangular and longitudinally striated (Figure 1A). The leaf is green in colour and has aromatic odour, bitter taste, basal rosette, bipinnatifid or pinnatifid, 3-25 cm long, 2-13 cm wide. The lower leaves are petiolate but the upper leaves are sessile, their lobules oblong-lanceolate, short-acuminate, entire. The leaves are arranged alternately (Figure 1 A, B). Flowers are whitish cream in colour, with sweet odour and astringent taste, heads small, numerous in open panicles; rays- 0.6 mm long; disk corollas cream colored, 1 mm long (Figure 1 C).

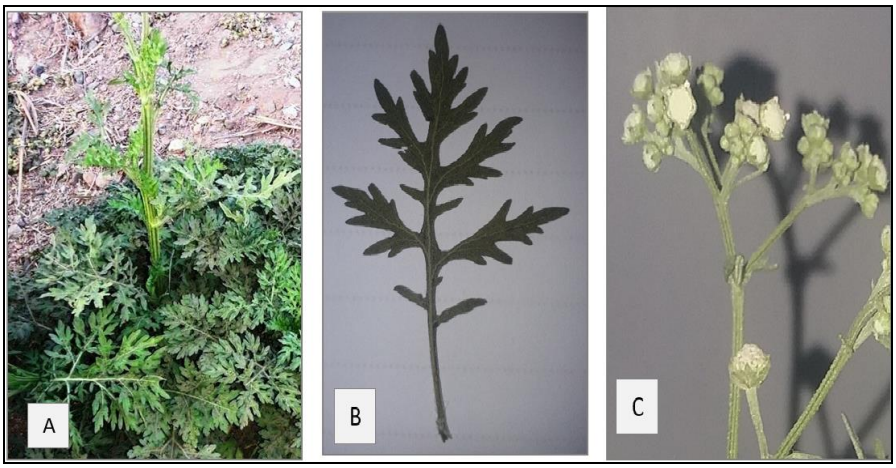

Fig 1: Aerial part of *Parthenium hysterophorus* (A), leaf (B) and flowers (C)

Microscopic studies of leaves

Surface view of Leaf

The blade, in surface view, has epidermal cells exhibiting a wavy in shape. The epidermis of both surfaces has anomocytic type of stomata (Figure 2). Trichomes are on both surfaces of epidermis. Trichomes were 3 to 6 cells**,** slightly curved, often with pointed apex and warty (Figure 3).

Transverse Section of Leaf

Epidermis was single layered and made up of parenchymatous cells; multicellular covering trichomes on both the surface were present. Under epidermis, layers of collenchyma were seen. The ground tissue of the midrib was parenchymatous**,** which contained 3 to 4 vascular bundles. Vascular bundles were bicolateral types in which phloems were present at both the side of xylems (Figure 4).

Transverse Section of stem

T.S. stem was circular in outline with shallow ridges. Epidermis was made up of single layer, wavy in shape and thick walled parenchymatous cells containing multicellular covering trichomes. Hypodermis was made up of 4 to 6 layer of collenchymatous cells. Endodermis was seen clearly on pericyclic as a single layer. Vascular bundle was bicolateral type & arranged in the form of ring. Pith was seen at the center part, which consisted of round shape parenchymatous cells (Figure 5).

The results of morphological and microscopically characters confirm that the studied plant is belonging to parthenium genus (Kohli and Rani, 1994) [16].

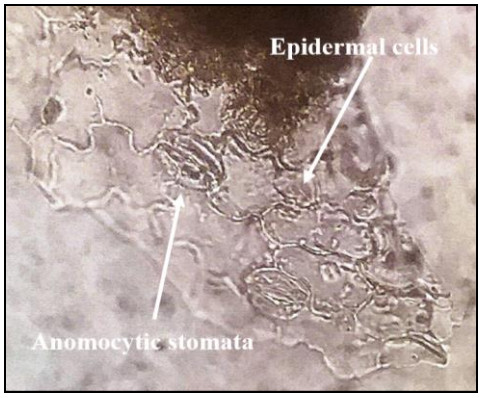

Fig 2: Surface view of upper epidermis of leaf (10x40)

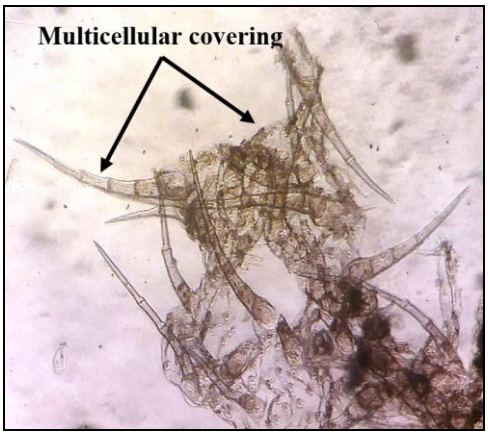

Fig 3: Surface view of upper epidermis of leaf (10x40)

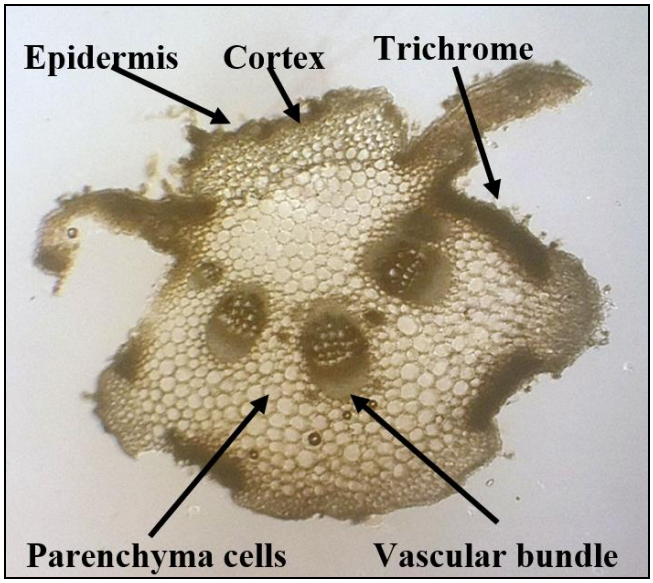

Fig 4: Transverse section of leaf with midrib (10x10)

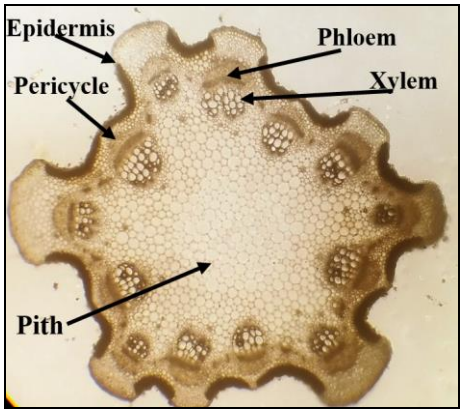

Fig 5: Transverse section of stem (10x10)

Physicochemical parameters

The determination of physicochemical parameters is important in determination of adulterants, quality and purity of powdered drug. The physicochemical parameters of the aerial part of *Parthenium hysterophorus* are in Table 1.

Table 1: Physicochemical parameter of *Parthenium hysterophorus* L.

Physicochemical parameters (%w/w)	Physicochemical parameters (%w/w)
Ash values:	
1. Total ash	13.80±0.05
2. Acid insoluble ash	1.10±0.03
3. Water soluble ash	6.60±0.05
Extractive value:	
1. Water soluble	32.00 ±0.04
2. Ethanol soluble	15.00±0.05
Moisture content Loss on drying at 110ºC	12.6 ±0.04

Phytochemical analysis

Qualitative phytochemical analysis

The percentage yield of petroleum ether, 80% methanol and water extracts of the aerial parts of *Parthenium hysterophorus* L. were 1.51 %, 35.70 % and 14.01 % respectively. Phytochemicals such as carbohydrates, amino acid, protein, saponins, triterpenes, sterols, polyphenols, flavonoids and alkaloids are identified in the studied extracts (Table 2). The secondary metabolites of plants such as phenolic acids and flavonoids possess diverse biological activities, for example, as antiulcer, anti- inflammatory, antioxidant, cytotoxic, antispasmodic, vascular activity and antitumor activity (Silva *et al.*, 2007; Ghasemzadeh *et al.*, 2010; Matkowski *et al.*, 2009; Cowan, 1999) [28, 10, 21, 7].

Table 2: Results of phytochemical screenings of petroleum ether, 80% methanol and water extracts of *Parthenium hysterophorus*

Phytochemical Screening		Petroleum Ether	Methanol 80% extract	Water
Alkaloids	Wagner's test	-	+++	-
	Mayer's test	-	+++	-
	Dragendroff's reagent	-	+++	-
Polyphenols	Ferric chloride test	-	++	++
Flavonoids	Shinoda test	-	++	++
	NaOH Test	-	++	++
	Lead acetate test	-	++	++
	Aluminium solution test	-	++	++
Saponins	Foam test	-	+++	+
Sterols/ Triterpenes	Salkowski test	+++	++	++
	Liebermann-Burchard test	+++	++	++
Carbohydrates	Molisch's test	-	++	+
	Fehling's test	-	++	+
Amino acid/ Protein	Ninhhydrin test	-	+	+

+++ = Most intense, ++ = moderately intense, + = Least intense, - = absent.

Spectrophotometric measurement of total phenolic content (TPC)

The total phenolic content (TPC) of the 80% methanol and water extracts of the aerial parts of *Parthenium hysterophorus* L. was measured according the Folin-Ciocalteau method. The content of the phenolic compounds was determined from the regression equation of the calibration curve (y=0.0008x−0.0086, R2 = 0.9222) of gallic acid (40–100 µg/mL) and expressed in mg gallic acid equivalent (GAE) per gram dry extract. The result was 121.00, 95.30 mg/g in 80% methanol and water extracts

respectively. The standard calibration curve of gallic acid is shown in Figure 6.

Spectrophotometric measurement of total flavonoids (TFC)

The total flavonoid content of 80% methanol and water extracts of the plant was determined from regression equation of calibration curve (y=0.001x+0.0248, R2= 0.9933) of quercetin (20-100 µg/mL) and expressed in mg quercetin equivalent (QE) per gram dry extract. The result of total flavonoid content (TFC) was 81.85, 23.00 mg/g in methanol and water extracts respectively. The standard calibration curve of quercetin is shown in Figure 7.

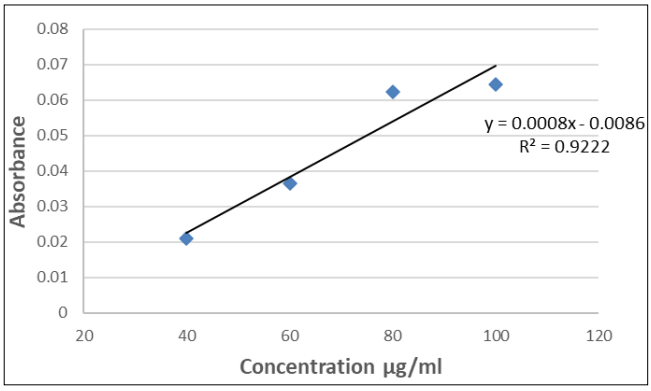

Fig 6: Calibration curve of Gallic acid

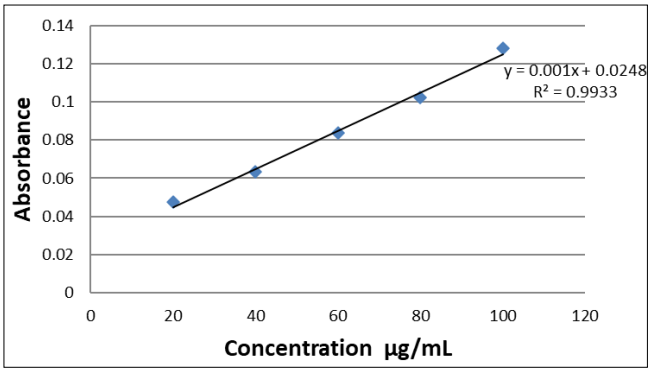

Fig 7: Calibration curve of Qurcetine

Thin layer chromatography of extracts and fractions

Thin layer chromatography was performed for petroleum ether, 80% methanol and water extracts as well as for the chloroform and ethyl acetate fractions. TLC is still the basic tool for the identification of natural compounds given in various pharmacopoeias. It is often used to provide the first characteristic fingerprints of herbs (Liang *et al.*, 2004) [20]. Various solvent systems were tested to obtain best results. Using petroleum ether–acetone (9:1), the TLC of petroleum ether extract showed 5 spots at Rf 0.41, 0.47, 0.52, 0.66 and 0.71, whereas 80% methanol extract showed 4 spots at Rf 0.17, 0.24, 0.41 and 0.66 (Figure 8-A). With n-butanol–acetic acid–water (3:1:1), the TLC of 80% methanol extract showed 12 spots at Rf 0.12, 0.15, 0.16, 0.18, 0.21, 0.32, 0.38, 0.43, 0.47, 0.56, 0.71 and 0.75, whereas water extract showed 9 spots at Rf 0.13, 0.18, 0.21, 0.32, 0.43, 0.52, 0.57, 0.71 and 0.75 (Figure 8-B).

In system n-butanol–acetic acid–water (3:1:1), TLC of chloroform fraction showed 6 spots at Rf 0.36, 0.51, 0.54, 0.71, 0.78 and 0.81, whereas ethyl acetate fraction showed 9 spots at Rf 0.32, 0.48, 0.65, 0.66, 0.78, 0.81, 0.83, 0.85 and 0.86 (Figure 8-C).

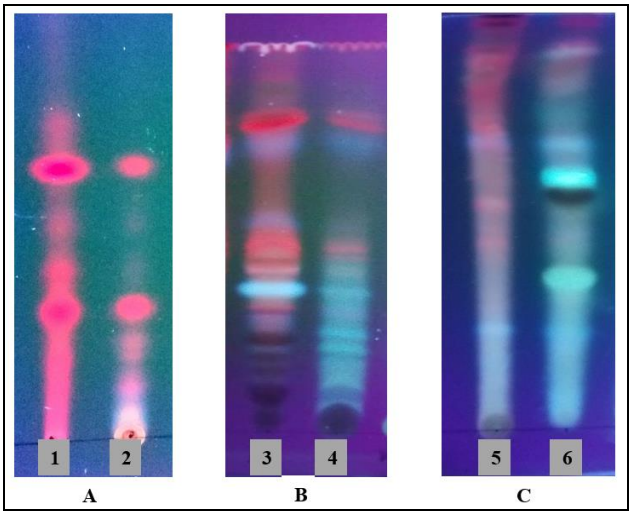

Fig 8: TLC plates under UV 365 nm: A- petroleum ether (1) and 80% methanol extracts (2) in petroleum ether–acetone (9:1); B- 80% methanol (3) and water (4) extracts in n-butanol–acetic acid–water (3:1:1); C- chloroform (5) and ethyl acetate (6) fractions obtained in n-butanol–acetic acid–water (3:1:1).

Antioxidant studies of chloroform and ethyl acetate fractions

Rapid screening of antioxidant activity by dot-blot and DPPH staining

The plant kingdom is a wide range of natural antioxidants. A great number of TLC techniques have been developed and successfully applied for qualitative and quantitative analysis of antioxidants (Jasprica *et al.*, 2007; Zhao *et al.*, 2010) [14, 36], and the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was often used as a derivatization reagent for this purpose (Kusznierewicz *et al.*, 2012) [19]. The antioxidant potential activity of chloroform and ethyl acetate fractions was determined via eye-detected semi-quantitatively via a rapid DPPH staining-TLC technique. Quercetin was used as a positive control. This method relied on inhibiting the accumulation of oxidative products, inhibiting the generation of free radicals, and masking free radicals (El-Sayed *et al.*, 2011; Soler-Rivas *et al.*, 2000) [8, 29]. The result of dot-blot assay showed yellow colored spot when stained with DPPH solution. All dots of ethyl acetate fraction at concentration 500, 250, 125, and 62.5 µg/ml showed scavenging activity. Dots of the chloroform fraction at 500 and 250 µg/ml showed medium scavenging activity, but at 125 and 62.5 µg/ml showed weak scavenging activity (Figure 9)

TLC bioautography assay

Antioxidant constituents in chloroform and ethyl acetate fractions were detected by TLC with system: toluene-ethyl formate-formic acid (5:4:1). The developed plate was sprayed with 0.05% DPPH reagent to give antioxidant constituents. In ethyl acetate fraction were identified five compounds of Rf, 0.36, 0.47 (deep yellow), 0.56, 0.70 and 0.75 (light yellow). In chloroform fraction were identified three compounds of Rf 0.47, 0.62 and 0.75 (light yellow). The eluted compounds showed yellow colour corresponding with antioxidant behavior (Figure 10).

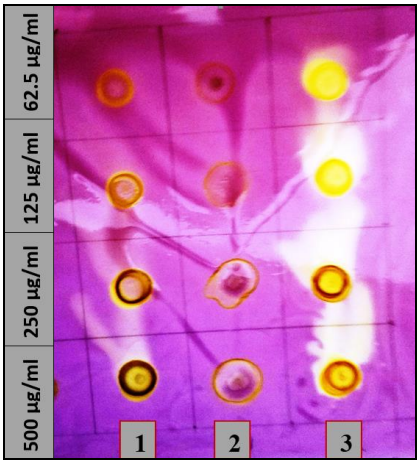

Fig 9: TLC plat of dot blot assay of the ethyl acetate fraction (1), chloroform fraction (2) and quercetin (3) on a silica sheet stained with a DPPH solution in methanol.

Fig 10: TLC plate of chloroform (1) and ethyl acetate (2) fractions obtained in toluene-ethyl formate-formic acid (5:4:1) in dally light after derivatization with 0.05% DPPH.

DPPH radical scavenging activity assay

The antioxidant activity of chloroform and ethyl acetate fractions was determined. The result of antioxidants activity ranged from 28.74±5.21 for the chloroform fraction to 78.77±4.40 for the ethyl acetate fraction. The percentage (%) scavenging of DPPH free radical was found to be concentration dependent, i.e. concentration of the extracts between 100-500 µg/ml greatly increasing the inhibitory activity. The DPPH scavenging assay revealed that the ethyl acetate fraction was more potent than chloroform because the IC50 value of ethyl acetate and chloroform were 181.56 µg/mL and 343.39 µg/mL respectively compared to that of standard quercetin with IC50 value 97.78 µg/ mL (Table 3, Figure 11).

Table 3: The DPPH free radical scavenging activity of the chloroform and ethyl acetate fractions of *Parthenium hysterophorus* and quercetin

Concentration µg/ml	Radical scavenging effect (%)		
	Quercetin	Chloroform fraction	Ethyl acetate fraction
100	50.37±4.22	28.74±5.21	45.29±4.42
200	62.5±2.24	36.032±3.23	50.29±3.52
300	70.54±4.12	45.43±4.31	57.33±5.22
400	85.32±3.32	56.36±3.24	69.13±3.41
500	95.38±4.31	63.84±4.11	78.77±4.40

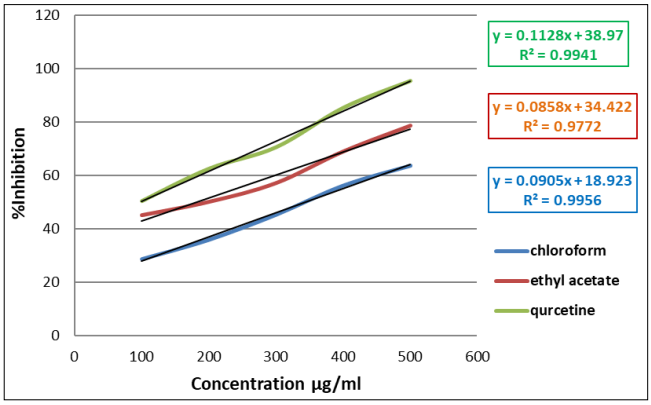

Fig 11: The DPPH free radical scavenging activity of the chloroform, ethyl acetate fractions and Quercetin

Conclusion

In this research the pharmacognostical properties, chemical properties and antioxidant activity of the aerial part of *Parthenium hysterophorus* were studied. From the results, the macroscopic, microscopic and physicochemical parameters were established and the chemical composition was analyzed qualitatively and quantitatively, and the antioxidant activity was evaluated. The information obtained can be useful for correct identification as well as standardization and quality assessment of the studied crude drug. Specific and deep phytochemical and pharmacological studies should be carried out for this plant**.**

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