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CHEMICAL PROFILING OF *Adenium obesum* (Forssk.) Roem. & Schult. STEM BARK EXTRACT AS A POTENTIAL ANTIOXIDANT COMPOUND FOR THE MANAGEMENT OF CHRONIC NON-COMMUNICABLE DISEASES

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ABSTRACT
Plants contain compounds with medicinal properties that partake in the healing process and curing of human diseases due to the presence of phytochemical constituents. This study was conducted to investigate the phytochemical, antioxidant and HPLC characterization of Adenium obesum (Forssk.) stem bark. A. obesum stem bark was obtained, identified, fractionated and subjected to maceration method for the phytochemical screening, antioxidant assay and HPLC characterization of flavonoid and phenolic compounds in the A. obesum stem bark. Preliminary phytochemical screening methods, DPPH free radical method and HPLC method were used for the analysis. The qualitative phytochemical analysis showed presence of flavonoid, tannin, cardiac glycoside, phenolics and saponins in the stem bark, ethanol extract and ethyl acetate fraction. The quantitative screening of total phenol, total flavonoid, saponin and tannin was carried out. Hence, ethyl acetate fraction has the highest quantity of total phenol (72.2±7.1) and total flavonoid content (108±2.50). The result of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) antioxidant activity revealed that ethyl acetate fraction has the highest antioxidant activity (IC_{50} 94.6±20.7) when compared with hexane, butanol and water fractions of the ethanolic extract. The result of HPLC characterization of the ethyl acetate fraction revealed the presence and concentrations of different flavonoid and phenolic compounds. Thus, this study concluded that A. obesum contain substances which make it useful for human health.

INTRODUCTION

Adenium obesum (A. obesum) (*Forssk.*) is commonly known as desert rose it belongs to the family Apocynaceae found all over Africa and Southern Africa and it is reported as a rich source of cardiac glycosides, pregnanes, triterpenes, steroids, flavonoids and carbohydrate (Khatoon *et al.*, 2018). *Adenium obesum* is an ornamental plant, cultivated worldwide because of its characteristically pink "showy" flowers that gives it the name "Desert rose". The plant is locally known as "Kariya" amongst the Hausa ethnic groups of northern Nigeria and "Akpalataa" amongst the Igbo ethnic groups of south-eastern Nigeria (Samson *et al.,* 2014). The most widely studied phytochemicals are the polyphenolics (flavonoids and other phenolic compounds) from medicinal plants which have a wide range of biological activities, including antioxidant, anti-inflammatory, anti-apoptotic, and anti-cancer effects (Jelena *et al.*, 2020). Natural antioxidants are very potent antioxidants agents that have an important role in preventing many diseases and promotion of health (Dehshahri *et al.*, 2012).

The good source of new therapeutic agents are the natural products and herbal medicines and are used for the development of complementary and alternative medicines over traditional drug regimens (Paul *et al.,* 2015). They can either be used directly or after they have been subjected to particular chemical modification processes. Plants which are medicinal in nature, contain physiological active principles, which over the years have been used in ayurvedic medicines for the treatment of various ailments (Ojiako, 2014).

Thus, this study was aimed at assessing the chemical profile of *A. obesum* stem bark ethanol extract as a potential antioxidant compound for the management of chronic non-communicable diseases.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Adenium obesum stem bark was collected from the open fields of Dala Girls Secondary School, Dala LGA, Kano State, Nigeria. The plant was identified in the Herbarium Section of the Department of Plant Biology, Bayero University Kano, Nigeria, and the sample was given Herbarium Accession Number BUKHAN 504. The plant sample was shade-dried at room temperature (29 – 31°C) for 3-4 weeks under shade. The stem was ground into fine powder using pestle and mortar and stored in an airtight container before analysis.

Extraction

The ethanol extract was prepared by mixing 500g of the powdered stem bark of *Adenium obesum* with 1000 ml of 80% ethanol (1litre). The mixture was then stored in appropriately labeled conical flask at room temperature (29 – 31°C) for 48h. The mixture was sieved using cheesecloth to obtain the supernatant. The supernatant was then concentrated to dryness using a rotary evaporator to obtain the ethanolic extract (Gandhi *et al*., 2003; Leila *et al*., 2007).

Fractionation

Fractionation of the extract was carried according out to Gandhi *et al*., (2003) and Leila *et al*., (2007) with some modification in the choice of primary solvent (water) and partitioning (separating) solvents (hexane, ethyl acetate and butanol). The ethanol extract residue obtained was dissolved in water (500 ml) and exhaustively extracted by consecutive liquid/liquid partition with hexane (500 ml), ethyl acetate (500 ml) and butanol (500 ml) using a separating funnel. The hexane, ethyl acetate, butanol and the remaining aqueous fractions were evaporated. The fractions obtained (hexane, ethyl acetate, butanol and the last fraction aqueous) were tested to evaluate the fraction with the highest DPPH radical scavenging activity. The most active extract fraction from the DPPH analysis was analyzed for its phytochemical constituent and also HPLC characterization of polyphenolic compounds (flavonoid and phenolic) was carried out on the DPPH active fraction.

The percentage yield of the extract was calculated as follows:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Extract (g)}}{\text{Weight of stem bark powder (g)}} \text{ X } 100$$ (Nkwocha *et al*., 2022)

Phytochemical Analysis

The phytochemical analysis was carried out on the *Adenium obesum* stem bark powder, ethanolic extract and ethyl acetate fraction using different methods described by Chimaobi *et al*., 2019 (Alkaloids, Tannins, Phenolics, Saponins, Flavonoids, Steroids, Phlobatannins, Triterpnes, Carotenoids); Borkar *et al.,* 2016 (Resins); Begum *et al.,* 2021 (Quinones); Kehinde and Abbas, 2015 (Anthraquinones); Shaikh and Patil, 2020 (Reducing sugars, Gums and mucilages, Anthocyanin, Cholesterol); Abideen *et al.,* 2020 (Terpenoids, Cardiac Glycosides); Sudha *et al.,*2020 (Phytosterol); Olaoye *et al.,* 2021 (Total Phenol Test); Roghini and Vijayalakshmi, 2018 (Total Flavonoid Content); Ezeabara *et al.,* 2014 (Total Tannin); Roland *et al.,* 2017 (Total Saponin). All tests were performed in triplicates to ensure the accurate result.

In-Vitro Antioxidant Activities

DPPH• (2, 2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity.

In Vitro antioxidant activities of the fractions (hexane, ethyl acetate, butanol and aqueous) were determined using the DPPH free radical scavenging assay described by (Nithianantham *et al*., 2011) with some modifications in concentration of the sample. This is a quick and easy method to analyze the scavenging potential of an antioxidant. DPPH in oxidized form gives a deep violet color in methanol. An antioxidant compound donates the electron to DPPH, thus causing its reduction, and in the reduced form its color changes from deep violet to yellow. DPPH solutions show a strong absorbance at 517 nm appearing as deep violet color.

Preparation of DPPH solution (0.1M)

DPPH solution (0.1M) was prepared by dissolving 0.39mg of DPPH in a volumetric flask, dissolved in methanol, and the final volume was made 100mL.

Preparation of Fraction Solutions

The stock solution of 1mg/mL were prepared by dissolving each of the fractions in methanol. From the sample stock solution, 7.8125, 15.625, 31.25, 62.5, 150, 250, 500 and 1000µg/mL solutions of each fractions were prepared.

Evaluation of DPPH Activity

To the sample solutions of concentrations, 1mL DPPH solution were added and incubated at room temperature for 30 min dark. A control was prepared by mixing 1mL methanol and 1mL DPPH solution. Finally, the absorbance of the solutions was measured by using a spectrophotometer at 517 nm. Ascorbic acid was used as the standard. Measurements were taken in triplicate Oliveira *et al*. (2008). The percentage of inhibition was calculated by using the formula:

DPPH Scavenged (%) = [(Acontrol - Atest)/Acontrol] × 100

Where Atest = Absorbance in the presence of extract or positive control and

Acontrol = Absorbance of negative control (Moyo *et al*., 2013; Ndhlala *et al*., 2013).

HPLC Characterization of Phenol and Flavonoid Compounds in Ethyl acetate Fraction of *Adenium obesum*

The HPLC Characterization analysis was carried out using an already existing method described by Mahendra *et al.*, 2012

Statistical Analysis

All results were expressed as Mean ± Standard deviation (SD). The data from Quantitative phytochemistry result was statistically analyzed by one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test, using INSTAT version 3.05 software. The data from the DPPH result was statistically analyzed by one-way ANOVA followed by Dunnets multiple comparison test, using GraphPad prism version 3.7 software. The *p-value* <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1 showed phytochemical constituent present in the plant. The qualitative secondary metabolite studies of the powdered stem bark, ethanolic extract and ethyl acetate fraction of *A. obesum* showed the presence of flavonoid, tannin, cardiac glycoside, phenolics and saponins. The presence of terpenoids, phytosterols, resins, gums and mucilages were found to be present in the powdered stem bark and ethanolic extract. While triterpenoids were found only in the powdered stem bark and cholesterol was found to be present only in the ethanolic extract. And Anthraquinone was only found to be present in the ethyl acetate fraction.

Table 2 showed result for total phenolic content, total flavonoid, total tannin content and total saponin content of the powder, ethanol extract and ethyl acetate fraction of *Adenium obesum* stembark. The total phenolic content has no significant difference ($p<0.05$) with each other. Total flavonoid showed significant difference ($p<0.05$) among the mean values for powdered and ethyl acetate fraction. Significant difference was observed for total tannin content among the means of various samples used and there was no significant difference observed for total saponin content in all the samples. The total phenolics shows highest in this order, ethyl acetate fraction > powder > ethanol extract; total flavonoid in the highest order as follows ethyl acetate fraction > ethanol extract > powder; total tannin highest order as follows powder > ethanol extract >ethyl acetate fraction. And finally, total saponin highest order is as follows, ethanol extract > powder > ethyl acetate fraction.

Table 1: Qualitative Phytochemical Constituents of *A. obesum* Stem Bark

Phytochemical	Powder	Ethanol Extract	Ethyl-Acetate Fraction
Alkaloid	+ -	-	-
Tannin	+	+	+
Phenolics	+	+	+
Saponin	+ +	+ +	+ +
Flavonoid	+ +	+ +	+ +
Steroid	-	-	-
Phlobatannin	-	-	-
Triterpenes	+	-	-
Carotenoid	-	-	-
Anthraquinone	-	-	+
Reducing sugars	-	-	-
Terpenoid	+	+	+
Cardiac glycosides	+	+	+
Phytosterol	+	+	-
Resins	-	-	-
Gums and mucilages	+	+	+
Quinone	-	-	-
Anthocyanin	-	-	-
Cholesterol	-	+	-

+ = Present; - = Absent

Table 2: Quantitative Phytochemical of *A. obesum* Stem bark Powder, Ethanolic Extract and Ethyl Acetate Fraction

Sample I.D	TPC (mg of GAE/g)	TFC (mg of QE/g)	Total Tannin (mg of TE/g)	Total Saponin (mg of SE/g)
Powder	58.9±7.80	103±1.60[a]	73.2±1.06[a]	20.0±1.90
Ethanol Extract	57.7±1.90	107±0.50	42.1±9.90[a]	25.5±7.80
Ethyl-Acetate Fraction	72.2±7.1	108±2.50[a]	31.4±9.1[a]	17.1±5.60

Values are Mean ± SD, n=3. Values with the same superscript lowercase "a" along columns shows a significant difference in the mean values (at $p<0.05$) by one-way ANOVA followed by Turkey multiple comparison test. **Key:** TPC = Total Phenolic Content; TFC = Total Flavonoid Content

The preliminary phytochemical analysis of powdered stem bark, ethanolic extract and ethyl acetate fraction of *A.obesum* revealed the presence of various phytochemicals. This is similar to the reports of Suleiman and Brima (2020) who reported the absence of anthraquinones but presence of alkaloids, glycosides, saponin, triterpenes, sterols, tannin, phenols and flavonoids only in the bark of *A. obesum* ethanolic extract. This difference can be as the result of the chronological age of the plant, percentage humidity of the harvested material, the situation and time of harvest and whether the method of extraction was a possible source of variation for the chemical composition (Roland *et al*., 2017). Medicinal plants have shown that the presence of saponins, terpenoids, tannins, steroids, flavonoids, cardiac glycosides, and phenol are of great importance in the field of drug research (Roland *et al*., 2017). The medicinal power of these plants lies in the phytochemical constituents that cause definite pharmacological actions on the human body. Phytochemical, a natural compound that occurs in plants, works with nutrients and fibers to act against diseases or more specifically to protect against diseases (Roland *et al*., 2017). The plant phenols and flavonoids are highly effective free radical scavengers that can protect against damage caused by free radicals in humans (Akhtar *et al*., 2017). Consumption of leafy vegetables was associated with reducing the risk of diseases due to the presence of their antioxidant properties. Studies have shown that phytochemicals in leafy vegetables and fruits contain biologic effects including scavenging of oxidative agents. The antioxidants work to achieve two main goals: reduce the harmful effects of free radicals either by preventing their formation or by scavenging and inactivating them or boost the natural defense systems by inducing the activities of antioxidant enzymes and regenerating other proteins involved in antioxidant pathways (Oyenihi *et al*., 2015).

Table 3 is the in-vitro antioxidant activity as determined by DPPH radical scavenging assay revealed the ethanolic extract and the solvent fractions showed dose-dependent radical scavenging activity in the following orders. Ascorbic acid > ethyl acetate> aqueous> n-butanol> n-hexane. The ethyl acetate solvent fraction showed significant high radical scavenging activity.

Table 3: DPPH· Radical Scavenging Activity of Various Fractions of Ethanolic Extract of *Adenium obesum* Stem Bark

Concentrations (µg/mL)	Ascorbic acid %Inhibition	Aqueous %Inhibition	Butanol %Inhibition	Ethyl acetate %Inhibition	Hexane %Inhibition
1000	98.7±0.50[a]	90.5±1.50	63.5±5.90[a]	91.5±3.70	53.2±1.67[a]
500	95.8±2.89	89.3±1.26[a]	52.7±2.41[a]	88.4±2.6	52.2±6.21[a]
250	91.2±2.93[a]	78.4±7.95[a]	49.4±1.56[a]	70.4±9.55[a]	49.2±0.6[a]
125	72.8±3.4[a]	64.9±7.60[a]	44.9±4.91[a]	56.8±7.50[a]	44.2±1.96[a]
62.5	57.0±5.41[a]	48.6±4.60[a]	43.9±2.74[a]	50.3±6.44[a]	42.5±1.53[a]
31.25	42.9±4.24	39.3±2.72	38.4±2.91[a]	47.6±6.09[a]	42.1±1.06
15.625	36.3±3.10	29.9±7.21[a]	36.57±5.80[a]	44.4±5.42[a]	38.7±3.07
7.8125	28.2±3.16	30.5±4.19	29.8±8.98	43.5±5.60[a]	32.9±4.59[a]
IC_{50}	11.1±2.68	96.9±19.8	395±20.4[a]	94.6±20.7	562±43.3[a]

Values are Mean ± SD, n=3. Values with the same superscript superscript "a" along rows shows a significant difference in the mean values ($p<0.05$) when compared with control Ascorbic acid by one-way ANOVA followed by Dunnets multiple comparison test.

The result of radical scavenging activity of DPPH, determines the free radical scavenging capacity or antioxidants potential of the test samples, which shows its effectiveness, prevention, interception, and repair mechanism against injury in a biological system (Oliveira *et al.*, 2008).

The HPLC characterization of polyphenolic compounds (flavonoids and phenols) in *A. obesum* stem bark ethyl acetate fraction revealed the presence of many compounds. For flavonoid, 15 compounds were identified and for phenolic 19 compounds were identified represented in table 4 and table 5 respectively.

Table 4 showed the flavonoid compounds present in the ethyl acetate fraction of *A. obesum* using HPLC, with different retention time and concentration. The flavonoid compounds detected were Gallic acid, Protocatechuric acid, Gallotechin, Epigallocatechin, Chlorogenic acid, Catechin, 4-hydroxybenzoic acid, Caffeic acid, Valleric acid, p-coumaric acid, 3,4-dimethoxybenzoic acid, Sinapic acid, Quercetin, Naphthoresorcinol and Rosmarinic acid.

Table 4: HPLC Characterization of Flavonoids in *Adenium obesum* Ethyl acetate Fraction

Peak No.	Peak ID	Ret Time	Height	Area	Conc. mg/100g
1	Gallic acid	0.732	2601.447	20134.729	0.1557
2	Protocatechuric acid	1.082	319812.156	3122317.750	24.1472
3	Gallotechin	1.307	66072.438	470501.531	3.6387
4	Unidentified	1.448	71884.234	585864.875	4.5309
5	Epigallocatechin	1.673	83925.719	2125133.750	16.4352
6	Chlorogenic acid	2.190	58024.664	1384026.000	10.7037
7	Catechin	2.698	50695.734	2253391.000	17.4271
8	4- hydroxybenzoic acid	4.173	85820.727	2956461.000	22.8645
9	Caffeic acid	5.390	835.000	1023.800	0.0079
10	Valleric acid	5.448	888.200	1180.900	0.0091
11	p-coumaric acid	5.507	853.800	1145.050	0.0089
12	3,4-dimethoxybenzoic acid	5.565	840.286	1214.000	0.0094
13	Sinapic acid	5.632	910.381	1258.117	0.0097
14	Unidentified	5.690	907.048	1284.150	0.0099
15	Quercetin	5.748	939.714	1320.583	0.0102
16	Naphthoresorcinol	5.807	1001.714	1337.450	0.0103
17	Unidentified	5.865	1000.857	1382.400	0.0107
18	Rosmarinic acid	5.923	1001.000	1395.500	0.0108

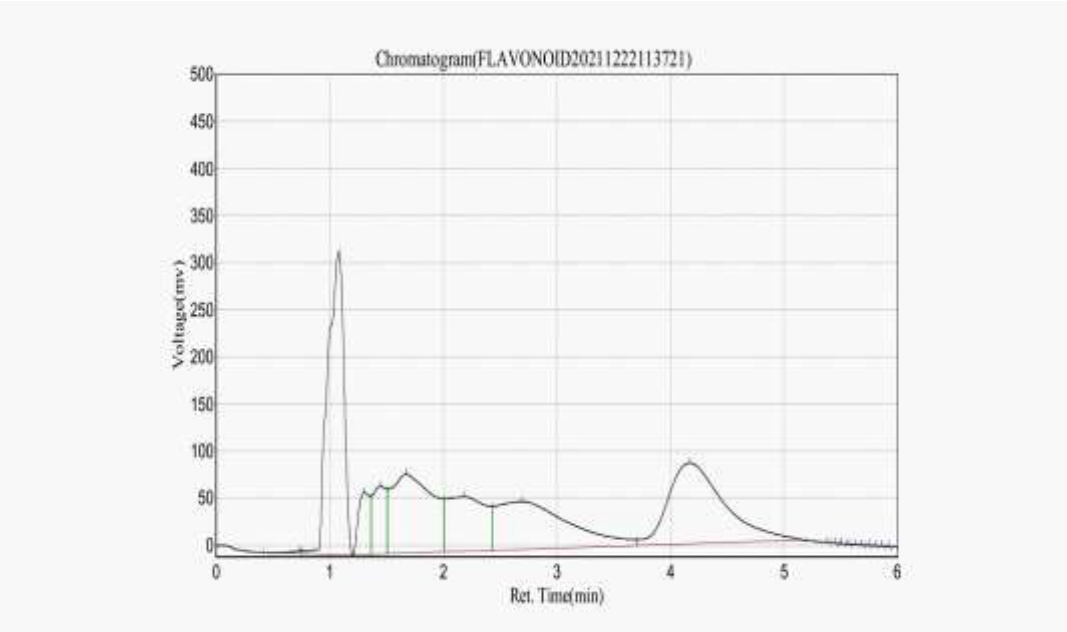

Figure 1: HPLC Chromatogram of Flavonoid Compounds in *A. obesum* Ethyl Acetate Fraction

The HPLC characterization of phenols in *Adenium obesum* ethyl acetate fraction showed presence of many phenolic compounds having different retention time and different concentration(Table 5). The phenolic compounds detected are Gallic acid, Protocatechuric acid, Epigallocatechin, Catechin, Acutumine, Cephatonine, Ferulic acid, Benzoic acid, Phenylacetic acid, Luteolin, Sinapic acid, Naringenin, 4-hydroxybenzoic acid, Valleric acid, Quercetin, Apigenin, Naphthoresorcinol, Kaempferol and Chrysin.

Table 5: HPLC Characterization of Phenols in *Adenium obesum* Ethyl acetate Fraction

Peak No.	Peak ID	Ret Time	Height	Area	Conc. (mg/100g)
1	Gallic acid	0.315	77.982	1119.100	0.0524
2	Protocatechuric acid	1.198	67.857	204.300	0.0096
3	Epigallocatechin	1.882	152723.781	1699005.625	79.6028
4	Catechin	2.540	10401.216	202372.297	9.4817
5	Acutumine	2.832	497.443	997.092	0.0467
6	Cephatonine	2.890	941.493	2675.437	0.1254
7	Ferulic acid	2.957	1642.264	5078.612	0.2379
8	Benzoic acid	3.190	7086.464	62948.195	2.9493
9	Phenylacetic acid	3.240	7734.793	137351.953	6.4353
10	Luteolin	3.773	1304.964	3614.886	0.1694
11	Sinapic acid	3.832	1097.014	2901.462	0.1359
12	Naringenin	3.890	905.064	2206.539	0.1034
13	4-hydroxybenzoic acid	3.948	675.114	1369.513	0.0642
14	Valleric acid	4.482	312.667	518.000	0.0243
15	Quercetin	4.540	509.000	1148.667	0.0538
16	Unidentified	4.598	587.667	1429.200	0.0670
17	Apigenin	4.657	662.333	1684.533	0.0789
18	Unidentified	4.715	698.000	1773.267	0.0831
19	Naphthoresorcinol	4.773	675.667	3067.834	0.1437
20	Kaempferol	4.898	449.500	755.800	0.0354
21	Chrysin	5.765	330.000	2132.800	0.0999

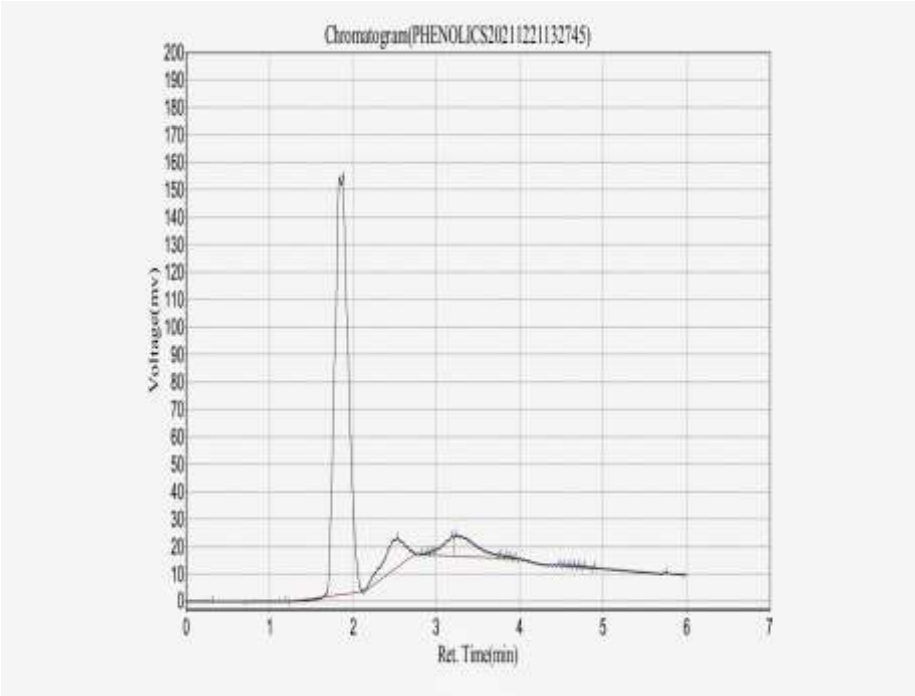

Figure 2: HPLC Chromatogram of Phenolic Compounds in *A. obesum* Ethyl Acetate Fraction

Polyphenol compounds (PCs) are naturally occurring compounds found largely in fruits, vegetables, cereals and beverages. The polyphenols contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Long term consumption of diets rich in plant polyphenols offered protection against development of diseases (Pandey and Rizvi, 2009).

Flavonoids are the most studied group of polyphenols (Pandey and Rizvi, 2009). The Phenolic acids are found abundantly in foods (Pandey and Rizvi, 2009). Outer layers of plants contain the highest levels of PCs than those located in their inner parts. There is increasing evidence that polyphenols may protect cell constituents against oxidative damage and limit the risk of various diseases associated with oxidative stress (Pandey and Rizvi, 2009). Polyphenols have received tremendous attention among nutritionists, food scientists and consumers due to their roles in human health. Polyphenols are strong antioxidants that complement and add to the functions of antioxidant vitamins and enzymes as a defense against oxidative stress caused by excess reactive oxygen species (ROS). Phytochemicals, especially polyphenols, are the contributors to the plant's total antioxidant activities. All PCs have a common mechanism of action (Rasouli *et al*., 2017). Polyphenols work by suppressing the formation of free radicals, thereby reducing the level of oxidation by inhibiting the formation of or they deactivate the active species and precursors of the free radicals (Tsao, 2010). More frequently, they act as direct radical scavengers of the lipid peroxidation chain reactions (chain breakers). Chain-breakers donate an electron to the free radical, neutralizing the radicals and themselves becoming stable (less reactive) radicals, thereby stopping the chain reactions (Tsao, 2010).

Fruits and vegetables can be used as dietary supplements of PCs, the presence of these compounds in food has improved the quality of food. Moreover, these compounds have a low toxicity effect in the human body, and can therefore be considered a safe dietary element (Rasouli *et al*., 2017).

CONCLUSION

This study showed that *Adenium obesum* stem bark ethanolic extract and ethyl acetate fraction contains different phytoconstituents, with ethyl acetate fraction having the highest DPPH radical scavenging activity and there was presence of different flavonoid and phenolic compounds in the ethyl acetate fraction as confirmed by the HPLC analysis.

The presence of the flavonoid and phenolic compounds in the ethyl acetate fraction of *A. obesum* stem bark showed that the ethyl acetate fraction has beneficial biological activity to humans, which can be used as dietary supplements. Hence, *A. obesum* can be used as a potent antioxidant compound for the management of the chronic non-communicable diseases. It is recommended to further isolate each or any of the flavonoid or phenolic compounds for the treatment of chronic diseases with oxidative stress aetiology.

CONFLICT OF INTEREST

We declare there was no conflict of interest

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