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Abstract

Background: Several wild vegetables have been reported for their therapeutic benefits in South Africa. Many of these plants including *Cleome gynandra* (L.) lack scientific reports on its significance in folkloric medicine. Therefore, this study was undertaken to evaluate quantitatively the compositions of phytochemicals and antioxidant properties of acetone extract of different parts of *C. gynandra*.

Materials and Method: Antioxidant activities were assessed against ferric reducing power, ABTS (2, 2'- azino-bis-3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt, DPPH (1, 1- diphenyl-2-picrylhydrazyl) and NO (nitric oxide) radical scavenging activities. Total phenolics, flavonoids, flavanols, proanthocyanidins, tannins, saponins and alkaloids were also investigated.

Results: Amongst the different plant parts, the leaf extract had the highest concentration of total phenolics (126.79 ± 0.55 mg/g), flavonoids (40.58 ± 0.06 mg/g) and flavanols (42.41 ± 0.05 mg/g) while the stem extract had the highest amount of proanthocyanidins (419.01 ± 0.67 mg/g) compared to the leaves (403.29 ± 0.89 mg/g) and fruits (107.18 ± 0.59 mg/g). The reducing power of the extracts was significantly higher than that of the standard drugs used in a concentration dependent manner. The activities of the plant extracts against ABTS, DPPH and NO radicals were dose responsive with IC_{50} value of 0.2, 0.1 and 0.03 mg/g respectively.

Conclusion: *C. gynandra* possesses high secondary metabolites which accounts for its strong antioxidant ability thus justifying its use as natural occurring antioxidants in folkloric medicine. The study encourages a regular consumption of this wild vegetable in order to avert oxidative stress related diseases.

Key words: *Cleome gynandra*, natural antioxidant, polyphenolics, antioxidant activity, phytochemical constituents.

Introduction

The production of free radicals, particularly reactive oxygen species (ROS), has been implicated to have a major impact on the human body. ROS such as superoxide anion, peroxide, hydroperoxide, hydroxyl radical and hydroxyl ion are generated during metabolic reactions in the body as they cause deterioration to lipid cells, essential proteins, DNA and immune cells (Manain et al., 2008; Mbaebie et al., 2012). They are also known to cause oxidative stress leading to the pathogenesis of several diseases in humans such as cancer, diabetes, cardiovascular diseases, atherosclerosis and chronic inflammation (Hosseinimehr et al., 2007; Raghuveer et al., 2009). Free radicals can be eliminated naturally or by synthetic antioxidant agents. Commonly used synthetic antioxidant agents include butylated hydroxytoluene, rutin, gallic acid, vitamin C and butylated hydroxyl anisole. However, these synthetic agents are associated with adverse effects on living cells (Saha et al., 2008; Wintola and Afolayan, 2011). Thus, the interest in utilizing natural antioxidants from plant derived medicines has increased in recent times. In addition, plant species with high medicinal values are believed to have high antioxidant properties (Ivanova et al., 2005; Olajuyigbe and Afolayan, 2011).

In recent times, there has been an increase in the use of medicinal plants for therapeutic antioxidant agents. More than 700 plant species are known to be traded for medicinal use throughout southern Africa (Dold and Cocks, 2001). Today, herbal remedies serve as alternative medicine, with significant role in the lives of people in South Africa (Hutchings et al., 1996; Ashafa et al., 2010). Many plant species contain substantial amounts of bioactive components which function interactively to scavenge free radicals from human body (Gurib-Fakim, 2006).

The ingestion of these natural antioxidants has been implicated in the reduction of risks in cancer, toxicity, inflammation, cardiovascular disease and diabetes (Yang et al., 2001; Sun et al., 2002).

The plant *Cleome gynandra* L. is a multi-purpose wild vegetable, widely distributed in South Africa, extending from the Limpopo, the North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State and the Northern Cape Provinces (Mishra et al., 2011). It is a multi- branched annual herb growing to a height of 1.5 m. The whole plant of *C. gynandra* has been reported as herbal medicine to treat diseases such as rheumatism, piles, malaria and tumour. The decoction of the leaves and roots are used to relieve headaches, fever and to prevent sepsis when applied on the surface of wounds (Mule et al., 2008; Bala et al., 2011).

Consequently, the qualitative screening of phytochemicals and antioxidant properties of *C. gynandra* has been reported in India and other countries (Bala et al., 2011; Aparadh et al., 2012; Meda et al., 2013; Annadurai et al., 2014). However, no scientific literature has been reported on the antioxidant and phytochemical properties of *C. gynandra* in the Eastern Cape Province of South Africa. Therefore, this study was undertaken to evaluate quantitatively the compositions of phytochemicals and antioxidant properties of acetone extract of different parts of *C. gynandra* in order to justify its ethno-medicinal importance.

Materials and methods

Collection of plant materials and preparation of extracts

Fresh leaves, fruits and stems of *C. gynandra* were collected from the University of Fort Hare Research Farm in Alice (latitudes $32^{\circ} 47' 0''$ South and longitudes $26^{\circ} 50' 0''$ East). This plant was identified at the Department of Botany; University of Fort Hare and a voucher specimen (LinMed 2013/01) were deposited in the Giffen herbarium of the University.

The plant samples were properly washed, oven dried at 30°C to a constant weight, pulverized to fine powder and stored in airtight bottles which were then kept in the refrigerator at 4°C until needed for the analysis. From the powdered samples, 200 g of each plant part was extracted separately in acetone for 48 hr under mechanical agitation (Stuart Scientific Orbital Shaker, Essex, UK). The extracts were then filtered and evaporated to dryness under reduced pressure at 40°C using a rotary evaporator.

The extraction yielded 16.1 g crude extract for leaf, 24.2 g for stem and 15.3 g for fruits. The resulting extracts were reconstituted with acetone to give the required concentrations used in the study.

Chemicals and reagents used

1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), vanillin, butylated hydroxyl toluene (BHT), rutin, potassium persulphate, sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]2\text{H}_2\text{O}$), sulfanilic acid, glacial acetic acid (CH_3COOH), gallic acid, tannic acid, ferric chloride (FeCl_2), ascorbic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminium chloride (AlCl_3), potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$), phosphate buffer, potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$], trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA). They were purchased from Merck, Gauteng, South Africa. All the chemicals used in this study were of analytical grade.

Determination of total phenols

The total phenolic content in the extracts were determined by the modified Folin-Ciocalteu method (Otang et al., 2012). An aliquot of 0.5 ml of each part of the plant extract (1 mg/ml) was mixed with 5 ml of Folin-Ciocalteu reagent which was previously diluted with distilled water (1:10 v/v) and 4 ml (75 g/l) of sodium carbonate (Na_2CO_3). The tubes containing the mixtures were vortexed for 15 sec and allowed to stand for 30 min at 40°C to develop colour. Absorbance was then read at 765 nm using the AJI- C03 UV- VIS spectrophotometer. Results were expressed as mg/g tannic acid equivalent using the equation based on the calibration curve:

$$Y = 0.1216 x$$

$$R^2 = 0.9365, \text{ where } x \text{ is the absorbance and } Y \text{ is the tannic acid equivalent.}$$

Total flavonoids

The flavonoid content was determined by the method used by Otunola et al. (2013). Briefly, 0.5 ml of 2% AlCl_3 was prepared in ethanol. This was then added to 0.5 ml of the extracts. The mixture was allowed to stand for 60 min at room temperature and the absorbance was read at 420 nm. The extracts were evaluated at a final concentration of 0.1 mg/ml. The result was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve:

$$y = 0.0255 x, R^2 = 0.9812; \text{ where } x \text{ is the absorbance and } y \text{ is the quercetin equivalent (QE).}$$

Total flavonols

The flavonol content was determined based on the method used by Olajuyigbe et al. (2011). Briefly, 2 ml of each plant extract were mixed with 2 ml of AlCl_3 prepared in ethanol. This was followed by adding 3 ml of sodium acetate solution (50 g/l). The mixture was incubated at 20°C for 2.5 hr. Absorbance was measured at 440 nm. The total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve

$$Y = 0.0255x, R^2 = 0.9812, \text{ where } x \text{ is the absorbance and } Y \text{ is the quercetin equivalent.}$$

Determination of proanthocyanidin

The total proanthocyanidin was determined by adopting the procedure of Oyedemi et al. (2010). A volume of 0.5 ml of the extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid. The resulting mixture was vortexed and allowed to stand for 15 min at room temperature. The absorbance was then measured at 500 nm. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the calibration curve equation:

$$Y = 0.5825x, R^2 = 0.9277, \text{ where } x \text{ is the absorbance and } Y \text{ is the catechin equivalent.}$$

Tannins

Tannin determination was done according to the procedure of Mbaebia et al. (2012) with some modifications. 0.20 g of plant sample was added to 20 ml of 50% methanol. This was mixed thoroughly and placed in a water bath at 80°C for 60 min. The extract was filtered into a 100 ml volumetric flask; 20 ml of distilled water was added, followed by 2.5 ml of Folin-Ciocalteu reagent and 10 ml of 17% Na_2CO_3 . This was thoroughly mixed together and made up to 100 ml using distilled water. The mixture was allowed to stand for 20 min. A bluish-green colour developed and the mixture of different concentrations ranged from 0-10 ppm. Absorbance of the tannic acid standard solutions and plant samples were measured after color development at 760 nm using the AJI-C03 UV-VIS spectrophotometer. Results were expressed as mg/g of tannic acid equivalent using the calibration curve:

$$Y = 0.0593x - 0.0485, R^2 = 0.9826, \text{ where } x \text{ is the absorbance and } Y \text{ is tannic acid equivalent.}$$

Alkaloids

The alkaloid content was determined according to the method of Onyilagha and Islam (2009). Briefly, 5 g powdered plant sample was weighed, Two hundred milliliters of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hr. This was filtered and the filtrate was concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added in

drops to the extract until precipitation was completed. Following this, the whole solution was allowed to settle and the collected precipitates were washed with dilute ammonium hydroxide and then filtered again. The residue collected was dried and weighed.

The alkaloid content was determined using this formula:

$$\% \text{ alkaloid} = \text{final weight of sample} / \text{initial weight of extract} \times 100$$

Saponins

The saponin content in the plant extracts was done using the method of Obadoni and Ochuko (2001). Briefly, 20 g of the powdered sample was mixed in 200 ml of 20% ethanol. This was placed on a shaker for 30 min. Following this, the plant sample was heated in a water bath at 55°C for 4 hr with continuous stirring. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over the water bath at 90°C. The concentrated solution obtained was then transferred into a 250 ml separating funnel and extracted twice using 20 ml diethyl ether. The ether layer was discarded, while the aqueous layer was retained and 60 ml *n*-butanol was added. The *n*-butanol extracts were washed twice with 10 ml of 5 % sodium chloride (NaCl₂). The remaining solution was heated in a water bath to evaporate and the samples were oven dried at 40°C to a constant weight. The percentage saponin content was calculated using the formula below:

$$\% \text{ saponin} = \text{final weight of sample} / \text{initial weight of sample} \times 100$$

Determination of ferric reducing power

The reducing power of the extracts was evaluated according to the method of Wintola and Afolayan (2011). 1 ml of the extract and standards were prepared in distilled water (0.025-0.5 mg/ml) and mixed thoroughly with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of K₃Fe(CN)₆ (1% w/v). The resulting mixture was incubated at 50°C for 20 min, followed by adding 2.5 ml of TCA (10% w/v), the mixture was then centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1% w/v). The absorbance was read at 700 nm against a blank sample. Increased absorbance of the mixture indicated higher reducing power of the plant extract.

Nitric oxide scavenging activity

The method of Oyedemi et al. (2010) was used to determine the scavenging activity of different parts of *C. gynandra*. A volume of 2 ml of 10Mm of sodium nitroprusside was prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of the extract, vitamin C and rutin individually at various concentrations of 0.025 - 0.5 mg/ml. The mixture was incubated at 25°C for 150 min. An aliquot of 0.5 ml of the solution was mixed with 0.5 ml of Griess reagents [1.0 ml of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid) for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min and absorbance was measured at 540 nm. The amount of the nitric oxide radicals inhibited by the extract was calculated using the equation:

NO radical scavenging activity (%) = [(Abs control- Abs sample) / (Abs control)] × 100, where Abs control is the absorbance of nitric oxide radicals + methanol; Abs sample is the absorbance of nitric oxide radical + sample extract or standard.

ABTS scavenging activity

The scavenging activities of the different plant extracts against ABTS radical were determined using the method described by Adedapo et al. (2008). First the working solutions were prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal amounts and allowed to stand for 12 h at room temperature in the dark. The resulting solution was then diluted by mixing 1ml ABTS⁺ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm after 7 min using the spectrophotometer. The percentage inhibition of ABTS⁺ by the various plant extracts were calculated using the formula:

$$\% \text{ inhibition} = [(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100$$

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging ability

The effect of acetone extract using different plant parts on the DPPH free radical was determined using the method adopted by Wintola and Afolayan (2011). Briefly, 1ml of DPPH prepared in methanol (0.135 mM) was mixed with 1 ml of different concentrations ranging from 0.0025-0.5 mg/ml of various plant extracts of *C. gynandra*. The mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using the spectrophotometer. The scavenging ability of the plant extract on DPPH was calculated using the equation:

$$\text{DPPH scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100,$$

Where Abs control is the absorbance of DPPH + methanol;

Abs sample is the absorbance of DPPH radical + sample (extract/standard)

Statistical analysis

All experiments were done in triplicates and where applicable, the data were subjected to one way analysis of variance (ANOVA). The differences between plant parts were determined by Duncan's Multiple Range test using the Minitab program (version 12 for windows). P< 0.05 was considered significant.

Results and discussion

Phytochemical analysis

The findings of our study revealed that the leaf extract had the highest concentration of total phenolics (126.79 ± 0.55), flavonoids (40.58 ± 0.06) and flavanols (42.41 ± 0.05) when compared with the stem and fruit extracts (Figure 1). The result also showed a different trend in the total proanthocyanidin content in which a higher concentration was observed in the stem extract compared to that of the leaf and fruit extract. The leaf extract had a considerable amount of proanthocyanidin content. The tannin, alkaloid and saponin contents were relatively low in the plant extracts; however, there was no statistical difference among the plant parts ($P < 0.05$).

Generally, the polyphenolic contents of the various parts of the plant were significantly high; this could be accountable for the strong antioxidant activity of *C. gynandra* extracts. The high concentrations of flavonoids, proanthocyanidin, flavonols, and phenols acts as the biological substances that inhibit diseases and thus serves as defence mechanism in humans; this includes antimicrobial, antioxidants and cytotoxicity activities (Harborne and Williams, 2000; Lawal et al., 2015). These compounds are also very essential because of their ability to scavenge their hydroxyl groups, quench free radicals and decompose peroxides (Adedapo et al., 2008). For instance, Flavonoids and phenols acts as good scavengers of lethal radicals thus preventing oxidative cell damage. They are also known to have various pharmacological and biological activities such as anti-inflammatory, immunomodulatory and anti-allergic properties which reduce the risk of cardiovascular mortality (Olajuyigbe and Afolayan, 2011).

The amount of proanthocyanidin in this study was observed to be the highest among the other polyphenols. Proanthocyanidins are known to possess strong antioxidant property. It has been shown that proanthocyanidins reduces the risk of cardiovascular disease, cancer and improve blood circulation by strengthening the capillaries (Steinberg et al., 2003; Mbaebie et al., 2012). Thus, the strong antioxidant activity exhibited by the extracts from *C. gynandra* could be ascribed to the high polyphenols, micro and macro mineral elements present in the different plant parts. This partially validates the utilization of this plant in folklore remedies for the treatment of diseases.

Tannin content was relatively low and similar in all parts of the plant. Nevertheless, this low concentration of tannin can still contribute significantly to the body. They play a major role in the treatment of inflamed tissues as well as prevention of cancer (Okwu and Emenike, 2006). Also, the alkaloid and saponin contents were low in this species. These compounds are known to have ethno-pharmacological uses for instance; alkaloids act as agents which possess analgesic and anti-spasmodic properties while saponin acts as antifungal agents. In spite of its medicinal characteristics, saponins are noxious to humans as they cause hemolysis of blood and irritation of mucous membranes (Kar, 2007). The low concentration of saponin and alkaloid observed would suggest low toxicity in the plant.

Antioxidant Assay

The potential of *C. gynandra* extracts in reducing Fe^{3+} to Fe^{2+} shows the reductive capability of the plant. The reducing ability of acetone extract of different parts of this species was significantly higher than those of rutin and vitamin C (Figure 2). The different parts of the plant exhibited a higher ferric reducing ability when compared with the standards. At the lowest concentration, the stem extract exhibited the highest ferric reducing ability while the fruit extract showed a different trend at a higher concentration. In effect, an increase in absorbance indicates decrease in the reductive ability of the plant. This implies that the lowest concentration dose of the plant extract was very active. The reducing capability of the extract followed this trend: stem > fruit > leaf > rutin > vitamin C. The reductive potential of the extracts decreased with increase in concentration. The extract from the various plant parts exhibited the reductive ability at a lower concentration. Judging by this, it is imperative to know that the lower concentration of the crude extract is very potent in reducing ferricyanide to ferrous form. In addition, the plant extracts was significantly higher than that of the standards. The observed reductive potential of the extracts is as a result of the presence of hydrophilic polyphenolic compounds (Mohammed et al., 2008; Omoruyi et al., 2012). The finding is in full agreement with other reports which showed that the reductive potential of plant extracts corresponds with the amount of phenolic constituents present (Amin et al., 2006).

The scavenging activity of the different parts of *C. gynandra* extracts against ABTS radical was concentration dependent (Figure 3). Vitamin C activity was significantly higher than rutin and the extracts. The stem extract exhibited the highest scavenging activity at 0.5mg /ml while the fruit extract showed the least percentage inhibition. The activities of the extracts from the stem, leaf and fruit against $ABTS^+$ were significantly ($P < 0.05$) different from each other. On the other hand, the stem extract was not significantly different from vitamin C at the concentration of 0.5 mg / ml. ABTS is a bluish green radical that reacts with potassium persulphate which is incubated and kept in the dark. The ABTS assay is based on the inhibition of the absorbance of the radical cation. When the ABTS radical reacts with the plant extract, the radical cation dis-colourizes the bluish green chromophore. Decolourization of ABTS radical reveals the ability of the plant extract to donate electrons which will inactivate the radical cation in a concentration dependent order. The scavenging activity of the various plant extracts against ABTS radical was found to be higher in the leaf than the stem and fruit. Although all plant parts exhibited their highest inhibition at a concentration of 0.5 mg / ml. This could imply that the scavenging activity of ABTS radical by the plant extracts is dose responsive and could be helpful in treating radically related diseases especially at higher concentrations (Veerapur et al., 2009; Wintola and Afolayan, 2011).

The trend of the inhibition of DPPH radical by the extract was concentration dependent (Figure 4). With respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend: vitamin C < rutin < acetone leaf < acetone stem < acetone fruit (Table1). It is interesting to note that the lower the IC_{50} value, the higher the scavenging activity of the plant extract. The results of the DPPH also showed that the standard drugs were not significantly different ($p < 0.05$) from each other. On the other hand, the fruit extract showed the least scavenging activity amongst the plant extracts. The DPPH assay is based on the principle that the antioxidant compounds will scavenge the DPPH radicals in order to form constant reduced DPPH molecules. When these molecules are formed, the absorbance decreases and the DPPH solution decolourises from purple to yellow. The degree of discolouration is an indication that the plant extract has the potential to scavenge free radicals as a result of its ability of hydrogen donation. More yellowish colour of DPPH is an indicator of stronger antioxidant activity of the extracts (Moein et al., 2008).

The DPPH scavenging activity of the various plant extracts and standards were dose responsive. It was observed that vitamin C had the highest activity, followed by rutin, acetone stem, leaf and fruit respectively. Although the DPPH radical scavenging ability of the extracts was significantly lower than that of rutin and vitamin C. The result obtained from this study corroborates with the findings of Odeyemi et al. 2011 who

reported the scavenging activity of *Leonotis leonurus* to be lower than that of the standard drugs. It was evident that the plant extracts showed proton-donating potential which could serve as free radical inhibitors.

The scavenging activity of the different parts of *C. gynandra* extracts against nitric oxide radical was evaluated (Figure 5). The leaf, stem and fruit extract inhibited nitric oxide radical in a concentration dependent manner exhibited at 2.0 mg/ml. The leaf extract showed the highest inhibition followed by the stem and fruits. Rutin had the highest scavenging activity against the nitric oxide radicals at all concentrations. The trend of inhibition is as follows: rutin > vitamin C > acetone leaf > acetone stem > acetone fruit. The result also showed the minimum inhibitory concentration required to reduce the nitric oxide radicals by 50%. The leaf extract had the least IC₅₀ value (Table 1). A lower IC₅₀ value corresponds to a higher scavenging activity. Nitric oxide plays a vital role in the pathogenesis of several diseases associated with inflammation in humans (Gates et al., 2008). It is a free radical formed from sodium nitroprusside which reacts with oxygen to form nitrite. The scavenging ability of the different parts of *C. gynandra* extracts against nitric oxide radical was evaluated by its potential to inhibit the formation of nitrite. The level of nitric oxide radical was significantly reduced by the plant extracts as a result of the scavenging ability of the extracts. This could be related to the presence of flavonoids which are able to compete with oxygen thus inhibiting the anions which will be formed.

Conclusion

This study revealed that the different parts *Cleome gynandra* contains substantial amount of phenolics, proanthocyanidins and flavonoids which are of high medicinal value. These phytochemical constituents are known to be responsible for the strong antioxidant activity exhibited by this species. The antioxidant potential of the extract revealed that all parts of this plant have the ability to scavenge free radical at different concentrations providing scientific credence for its therapeutic usage in folklore medicine.

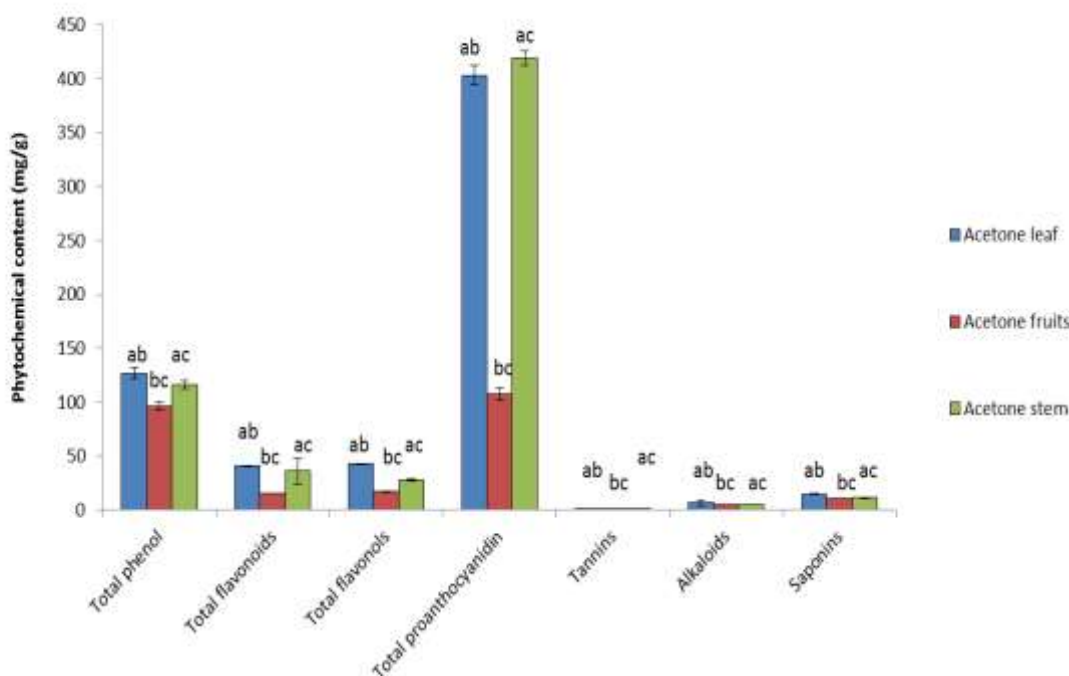


Figure 1: Phytochemical constituent in the acetone extracts of different parts of *Cleome gynandra*. Data are presented as means \pm standard deviation of three replicates with significance differences.

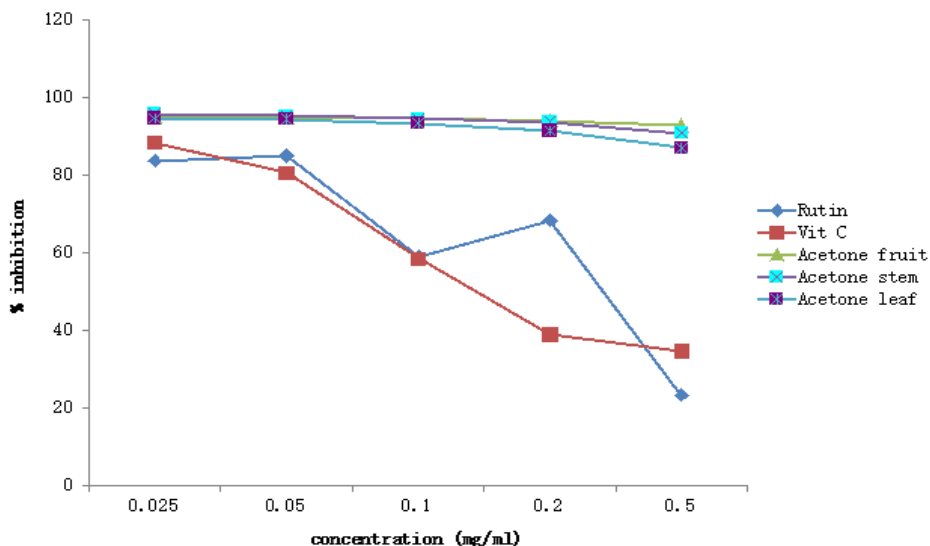


Figure 2: Reducing power activity of the acetone extracts of *C. gynandra* fruits, stems and leaves in comparison with Rutin and vitamin C. Results are means of 3 replicates.

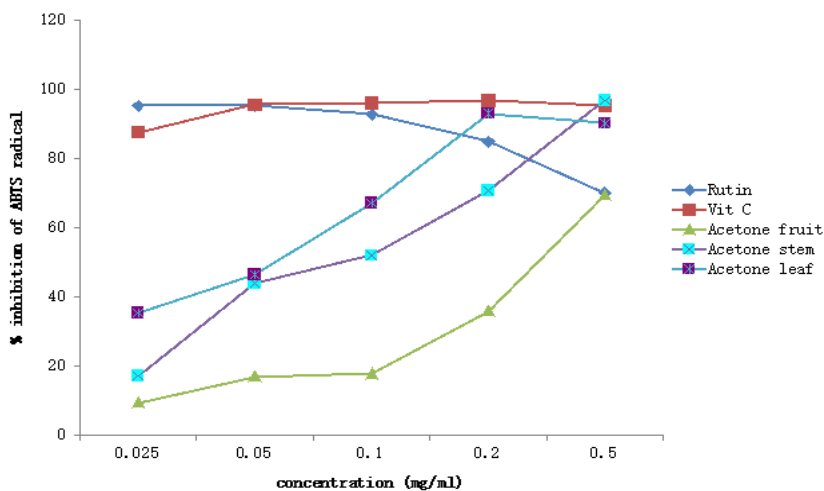


Figure 3: ABTS radical scavenging activity of the acetone extracts *C. gynandra* fruits, stems and leaves. Results are means of 3 replicates.

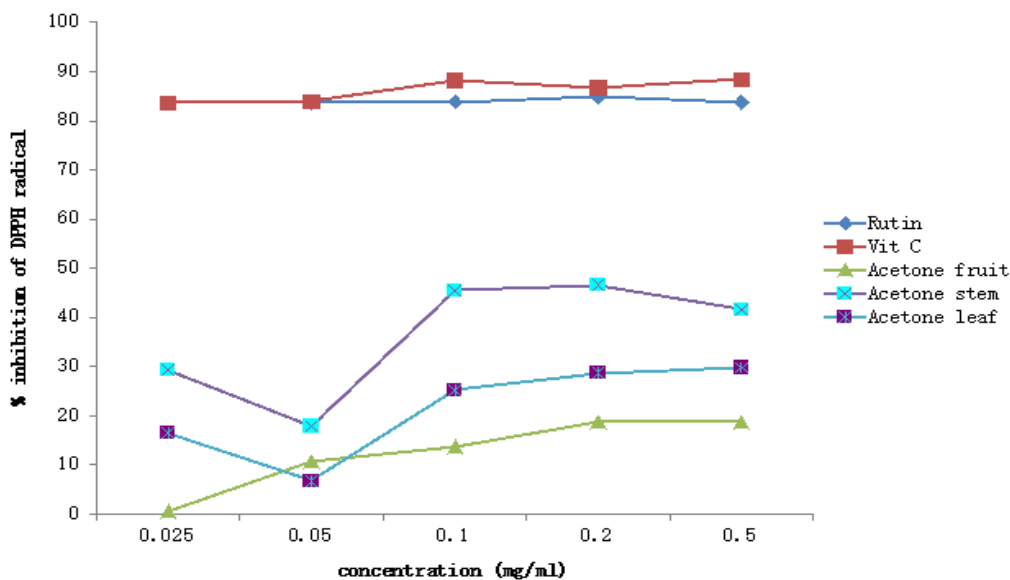


Figure 4: DPPH radical scavenging activity of the acetone extracts *C. gynandra* fruits, stems and leaves. Results are means of 3 replicates.

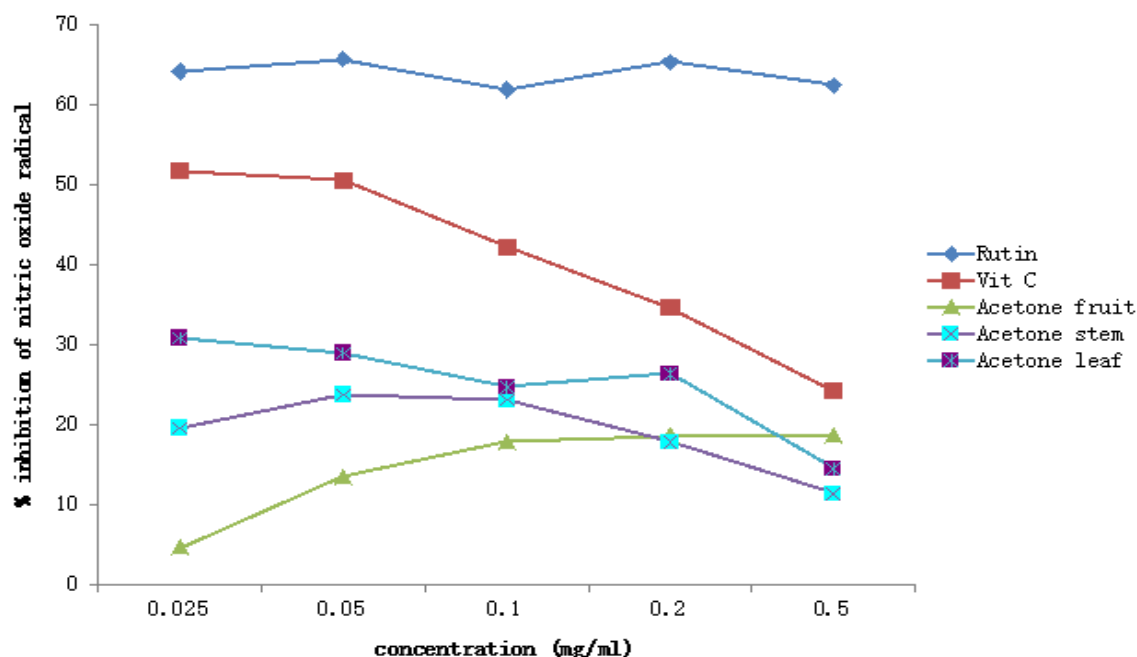


Figure 5: Nitric oxide scavenging activity of the acetone extracts of *C. gynandra* fruits, stems and leaves. Results are means of 3 replicates.

Table 1: Scavenging activities of acetone extracts of *C. gynandra* fruits, stem and leaves.

Sample	Reducing Power		ABTS		DPPH		Nitric oxide	
	IC ₅₀	R ²	IC ₅₀	R ²	IC ₅₀	R ²	IC ₅₀	R ²
Acetone Fruit	0.5	99.8	0.25	98.9	0.2	50.2	0.1	36.9
Acetone stem	0.25	99	0.2	84.9	0.17	23.8	0.2	81.9
Acetone leaf	0.3	99.3	0.2	60.9	0.1	47.8	0.025	91.9
Rutin	0.255	88.6	0.3	99.1	0.05	1.6	0.2	17.6
Vitamin C	0.2	69	0.1	14.5	0.025	49.5	0.2	91.2

IC₅₀ is defined as the concentration (mg/ml) capable to attain 50% of maximum scavenging ability.
R² is the coefficient of determination; values obtained from regression lines with 95% confidence level.

Declaration of interest

The authors declare no conflicting interest.

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