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Phytochemical and Antioxidant Assay of *Cleome gynandra* L.

*Sowunmi, I.L., Adio, A. F. and Oyedeji, O.F.
Forestry Research Institute of Nigeria, P.M.B. 5054, Jericho Hill, Ibadan, Nigeria

Abstract:- The study evaluated quantitatively the phytochemical compounds and antioxidant capacity of water and ethanol extracts of different parts of C. gynandra L. Total phenolics, flavonoids, flavonols, proanthocyanidins, tannins, saponins and alkaloids in both extracts were determined using standard procedures. 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 techniques were used to determine the antioxidant activities of C. gynandra in aqueous and ethanol extracts. The phenols, flavonoids and flavonols were found higher (P< 0.05) in both water and ethanol extracts of the leaf while the proanthocyanidins contents were seen higher in the water stem extract compared to other plant parts. The water extract showed better ABTS, reducing power and Nitric oxide radical scavenging abilities than ethanol extract. The findings of this study indicate that C. gynandra is rich in secondary metabolites and can serve as natural antioxidant. It also reveals that the water extracts exhibited significant antioxidant properties which probably accounts for its pharmacological uses.

Keywords:- *Wild vegetables, in-vitro antioxidant, secondary metabolites, Cleome gynandra.*

I. INTRODUCTION

South Africa has wide ranges of plants among which are leafy vegetables growing in the wild [1]. These wild vegetables are considered as low status foods; hence they are ignored and underutilized [2]. Despite the underutilization of wild vegetables in urban centers, the native people rely solely on their use for medicinal purposes in traditional medicine. These medicinal potentials are dependent on the presence of bioactive compounds which are stored in various plant parts like leave, stem, root, fruit, bark, rhizome and seed [3, 4]. Wild vegetables are natural radical scavengers because they exhibit antioxidant properties which play significant roles in combating the outbreak of oxidative stress related diseases [5, 6].

Cleome gynandra L. is a well-known nutraceutical plant. Besides its significance as a wild plant, its therapeutic functions include the management of inflammatory conditions, cancer and cellular ageing [7, 8].Despite the wide usage of the plant in traditional medicine, there is little or no information on the chemical constituents and *in-vitro* antioxidant activity of the species South Africa. Thus, this study sought quantitative phytochemical constituents and antioxidant capacity of various parts of *C. gynandra* in the water and ethanol extracts so as to provide scientific information on its folkloric medicinal uses**.**

II. MATERIALS AND METHODS

❖ *Plant materials and extraction*

Collection of *C. gynandra* vegetative parts were done from the University of Fort Hare Research Farm, Alice. The University lies on latitudes 32° 47' 0" South and longitudes 26° 50' 0" East. Identification of the species was done at Botany Department and a voucher specimen (LinMed 2013/01) was deposited in the University herbarium. The collected plant samples were thoroughly washed, and then dried at 40ºC in oven. This was grounded into fine powder, stored in corked bottles and then placed under refrigeration of 4ºC. 200 g of each vegetative part were scooped individually into distilled water and ethanol for 48hrs, shook with Scientific Orbital Shaker. The extracts were then filtered to obtain filtrate. The filtrate from distilled water was refrigerated at -40°C and dried for 48 h yielding 18.5 g from leaf, 17.6 g from fruit and 21.2 g from stem. The ethanol extract was allowed to dry (at 40°C) with an evaporator and yielded 16.1 g for leaf, 15.3 g for fruit and 24.2 g for stem. The filtrates were diluted with ethanol and distilled water given the dilute solutions used for the study. All the chemicals used in this study were of analytical grade.

➢ *Determination of total phenols*

The total phenolic content of the extracts was determined based on the Folin-Ciocalteu method [9].

➢ *Total flavonoids*

The determination of flavonoid content was also based on the method adopted by Otunola and Afolayan [10].

➢ *Total flavonols*

The determination of flavonol content was done using the method adopted by Olajuyigbe and Afolayan [11].

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➢ *Determination of proanthocyanidin*

The determination of proanthocyanidin was done by the procedure of Oyedemi *et al*. [12].

➢ *Tannins*

The determination of Tannin was done using the procedure of Mbaebia *et al*. [13]

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➢ *Alkaloids*

The determination of alkaloid content was carried out using Onyilagha and Islam method [14].

➢ *Saponins*

The saponin content in the plant extracts was determined by the use of Obadoni and Ochuko method [15].

➢ *Determination of ferric reducing power*

The reducing power of the plant extracts was evaluated using Wintola and Afolayan method [16].

➢ *Nitric oxide scavenging activity*

Nitric oxide scavenging activity of *C. gynandra* was determined by the method of Oyedemi *et al*. [12].

➢ *ABTS scavenging activity*

To determine the scavenging activities of various plant parts, the method of Adedapo *et al*. [17] was adopted.

❖ *2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging ability*

The influence of water and ethanolic extracts on the DPPH free radicals was done using Wintola and Afolayan method [16].

➢ *Statistical analysis*

This study was in triplicates and data collected were subjected to one way analysis of variance (ANOVA) and differences among plant parts of *C. gynandra* were separated by Duncan's Multiple Range Test using Minitab program. P value < 0.05 was regarded as significant.

III. RESULTS

The phytochemical composition of the ethanol extract of the different plant parts are shown in Figure 1. The phenolic (168.86 mg/g), total flavonoids (40.47 mg/g), flavonols (40.25 mg/g) and proanthocyanidin (209.7 mg/g) contents was higher in leaf extract compared to those in the stem and fruit. The alkaloid and saponin contents were lower in all the parts tested in comparison with the other compositions and their values did not differ significantly among the plant parts.

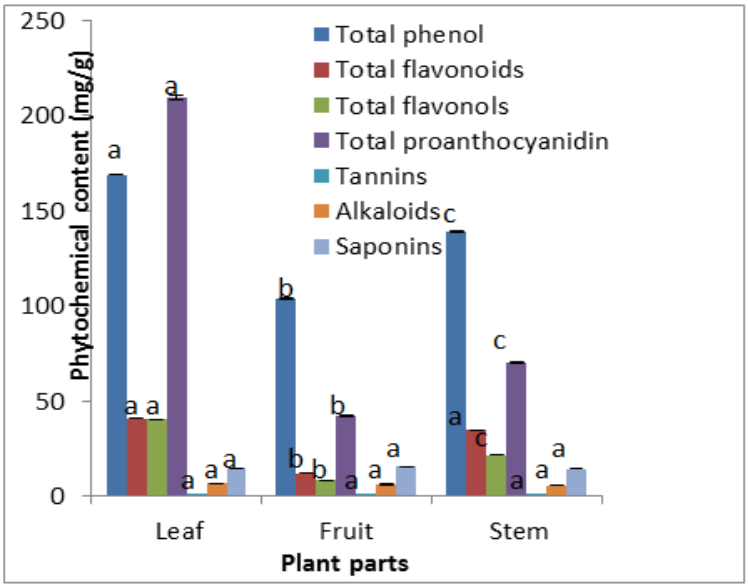

Fig 1:- Phytochemical constituents of ethanol extracts of *C. gynandra*. Data presented means of three replicates. Phytochemicals in the different plant parts having the same alphabet are not significantly different from each other.

The aqueous leaf extract had the highest concentration of phenols (183.77 mg / g), flavonoids (13.56 mg / g) and flavonols (11.77 mg / g) when compared to that of the stem and fruit extracts (Figure 2). The proanthocyanidin content in the stem extract (275.33 mg / g) of *C. gynandra* was found higher than that of the leaf and fruit. The tannin content was lower and same trend was observed in all plant parts. A similar trend was also observed in the alkaloid content. The saponin content of the fruit extract (22.35 mg / g) was higher compared to the other plant parts.

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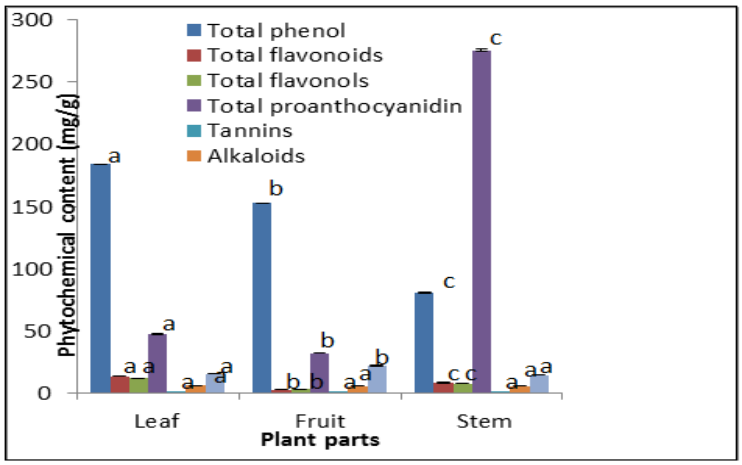

Fig 2:- Phytochemical constituents of aqueous extracts of *C. gynandra*. Data presented means of three replicates. Phytochemicals in the different plant parts having the same alphabet are not significantly different from each other.

➢ *Antioxidant activities*

The antioxidant potential of *C. gynandra* extracts was evaluated on the scavenge ability of ABTS radical (Figure 3). The fruit and leaf aqueous extracts exhibited a higher radical scavenging ability (73.9% and 72.9% respectively) than that of the stem (55.8 %). The aqueous extracts consistently showed stronger inhibition against the ABTS radical compared to the ethanol extract. The scavenging activities of both standards were found to be higher than the plant extracts.

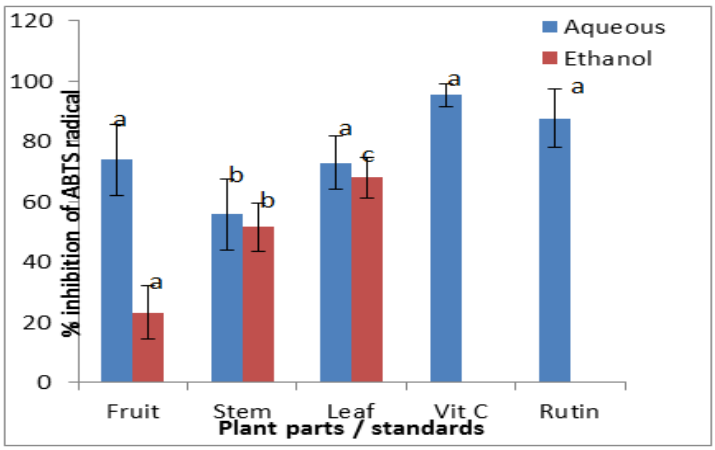

Fig 3:- ABTS radical scavenging activities of water and ethanol extracts of *C. gynandra* fruits, stems and leaves. Results are means of 3 replicates. Antioxidants in the different plant parts having the same letter are not significantly different from each other.

The water fruit extract and leaf had IC_{50} values of 0.1 and 0.03 mg/ml followed by aqueous stem, ethanol leaf and ethanol stem at 0.2, 0.23, 0.2 mg/ml respectively (Table 1).

Sample	Reducing Power		ABTS		DPPH		Nitric oxide	
	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2
Aqueous fruit	0.1	98.8	0.1	32.3	0.2	78.5	0.3	22.1
Ethanol fruit	0.2	91.7	0.4	92.3	0.05	14.3	0.3	82.2
Aqueous leaf	0.3	98.4	0.03	6.8	0.3	22.4	0.3	92
Ethanol leaf	0.3	99.5	0.23	55.3	0.2	74.4	0.3	13.9
Aqueous stem	0.05	10.1	0.2	78.7	0.2	69.7	0.1	57.4
Ethanol stem	0.3	99.2	0.2	85.7	0.1	68.4	0.3	97.7
Rutin	0.3	91.3	0.3	98.1	0.5	1.3	0.2	17.4
Vitamin C	0.2	72.6	0.5	13.9	0.2	47.8	0.2	91.2

Table 1:- shows Scavenging activities of water and ethanol extracts of *C. gynandra* fruits, stem and leaves.

A: *IC_{50} = concentration (mg/ml) capable to attain 50% of maximum scavenging ability.*
B: *R^2 = coefficient of determination; values obtainable from regression lines at 95% probability level.*

Assessment of the scavenging activity of *C. gynandra* was done using DPPH radical (Figure 4). The ethanol extract of the stem had maximum 41.8 % inhibition of the DPPH radicals. This was followed by the leaf with 32.4 %. The least activity was found in the fruit extract (19.2 %). The ethanol extracts of the stem and leaf had higher DPPH radical scavenging ability compared to the aqueous extract. Both standards showed higher scavenging activity than all extracts.

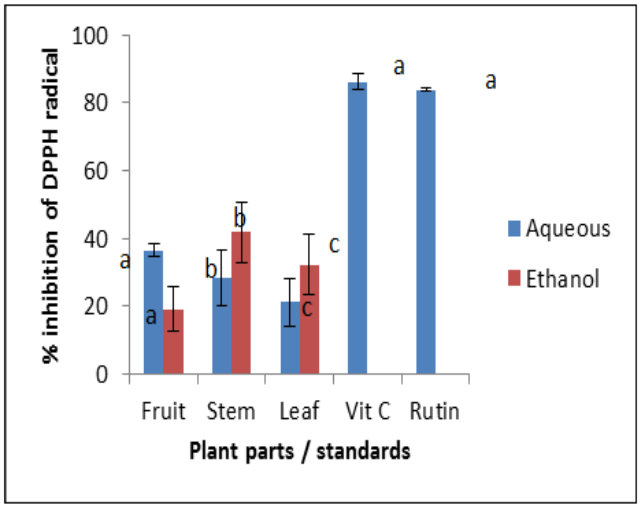

Fig 4:- DPPH activities of aqueous and ethanol extracts of *C. gynandra* fruits, stems and leaves. Results are means of 3 replicates. Antioxidants in the different plant parts having the same letter are not significantly different from each other.

The IC_{50} values of the extracts showed the following trend: ethanol stem < ethanol leaf < ethanol fruit < vitamin C < aqueous stem < aqueous fruit < aqueous leaf <rutin (Table 1).

The ferric reducing ability of *C. gynandra* extracts was further assessed by their ability to reduce Fe^{3+} to Fe^{2+} (Figure 5). It was observed that both aqueous and ethanol extracts of all plant parts exhibited high reducing activities when compared to the standards. The ferric reducing activities of the extracts are shown in the following order: aqueous stem > ethanol fruit > ethanol stem > aqueous leaf > aqueous fruit > ethanol leaf >rutin> vitamin C. There was no significant difference amongst the different plant extracts.

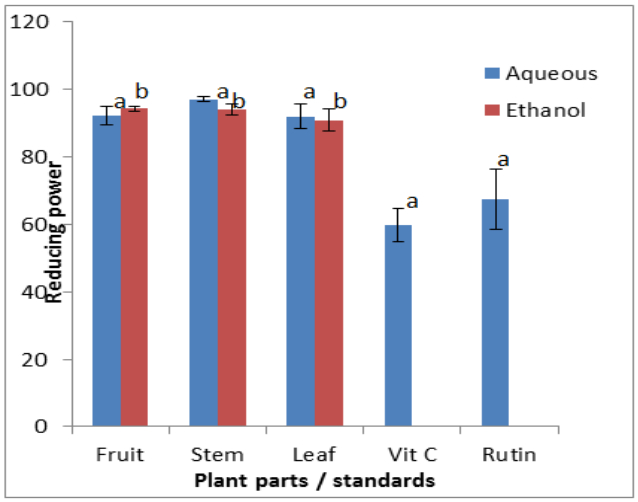

Fig 5:- Reducing power activities of aqueous and ethanol extracts of *C. gynandra*. Results are means of 3 replicates. Antioxidants in the different plant parts having the same alphabet are not significantly different.

In order to evaluate the antioxidant potency of the different extracts of *C. gynandra* against nitric oxide radical, the percentage inhibition of the radical was examined (Figure 6). The inhibition of nitric oxide by both extracts was lower when compared to the standards. Among the plant extracts, aqueous leaf (32.9 %) was significantly higher than that of the stem (25.3 %) and fruit.

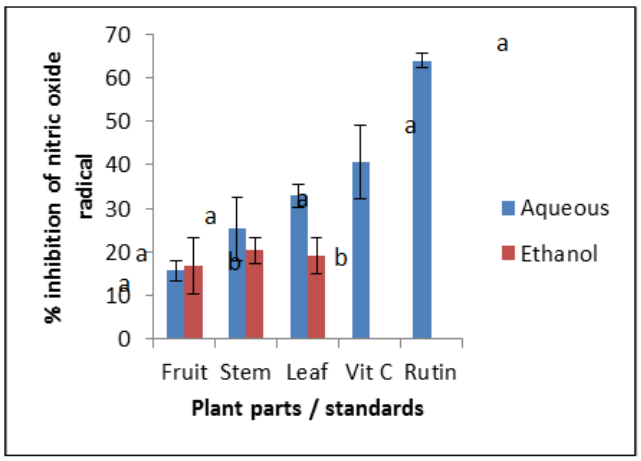

Fig 6:- Nitric oxide radical scavenging activities of aqueous and ethanol extracts of *C. gynandra* fruits, stems and leaves. Results are means of 3 replicates. Antioxidants in the different plant parts having the same letter are not significantly different from each other.

The minimum concentration required to inhibit the nitric oxide radicals by 50% were found to be 0.1, 0.2, 0.2 and 0.3 mg/ml for water stem extract, rutin, vitamin C and ethanol stem respectively, while a similar value occurred in the other plant extracts (Table 1).

IV. DISCUSSION

Phytochemical constituents like phenolic compounds are associated with biological activities such as antioxidant and scavenging potentials against free radicals. Interestingly, *C. gynandra* extracts showed that they are rich in polyphenolic compounds, thus, partially justifying this plant's used in the management of diseases cause by oxidative stress. In addition, the high concentration of proanthocyanidin in the stem extract shows that this compound is a source of bioactive components in the cancer treatment and other related ailments [13]. Tannins is an important ingredient in the treatment of inflammation, diarrhoea and cancer prevention [18]. The presence of alkaloids in *C. gynandra* extracts shows the potential of the plant to have analgesic,anti-inflammatory and adaptogenic properties [19]. This justifies the traditional use of this species in the treatment of malaria and inflammatory conditions [7, 8]. The saponins in the extracts also boost the antioxidant activities of the plant ranging from antitumor, anti-mutagenic and anti- inflammatory properties [9]. In addition, the low concentration of saponins, alkaloids and tannins observed in this species would suggest low toxicity in the plant.

ABTS is a protonated radical with characteristic absorbance maxima at 734 nm that decreases with the scavenging of the proton radicals [20]. When the plant extract reacts with the ABTS radical, the free radical decolorizes the blue-green chromophore. This decolorization shows that the extract has the ability to donate electrons freely. The scavenging activity of the plant extracts can be linked to the occurrence and structure of the hydroxyl groups which are associated with high phenolic compounds. These compounds are responsible in oxidizing the proton radical formed within the species [21].

A deep purple color is observed in DPPH scavenging radical. This is due to the presence of an odd electron [22]. When the electron becomes paired due to the presence of antioxidants in the plant extracts, the absorption disappears and the resulting discoloration from purple to yellow occurs. The degree of decolorization of the test solution is an indication of the scavenging ability of the plant extract in terms of hydrogen radical. Thus, the scavenging activity of the plant extracts against DPPH radical indicates their ability in giving out hydrogen proton to a free radical in order to eliminate odd electron that causes radical's reactivity [23].

Presence of reductants in *C. gynandra* extracts causes the conversion of Fe^{3+} to Fe^{2+}. This was confirmed by the discoloration of the test solution from yellow to green. The reducing power of the plant extracts was potently active compared to the control drugs. This observation could be ascribed to the high accumulation of flavonoids and phenolics in the plant parts, these constituents are responsible for metal reduction due to their nucleophilic nature which breaks the free radical chain through donating hydrogen atom [9]. This finding support Mbaebia *et al*. [13] findings on the water extract of *Schotia latifolia* Jacq.

Nitric oxide is a reactive free radical generated by neurons, phagocytes and endothelial cells, to form more reactive nitrogen species such as peroxy nitrite anion (ONOO-). It is generated from sodium nitroprusside in aqueous solution [21]. The scavenging activity of the extract was moderately comparable to the standards in inhibiting the formation of nitrite. This observation may be attributed to the presence of flavonoids in the plant extract. It is well known that nitric oxide has an important role in various inflammatory processes [24].

The phytochemical screening of *C. gynandra* showed that the leaf had more phytochemical constituents when compared to the stem and fruit parts. The plant could serve as an excellent source of natural antioxidants in food and medicinal industries. Hence, the inclusion of this wild vegetable in the diet is recommended in order to build the immune system against chronic diseases. The result also shows that extracts from *C. gynandra* have strong antioxidant activity. Therefore, both solvents (aqueous and ethanol) were able to extract the secondary metabolites which really help antioxidant activity of the plant. This activity explains the pharmacological use of the plant species in the management of diseases in traditional medicine.

The phytochemical screening of *C. gynandra* showed that the leaf had more phytochemical constituents when compared to the stem and fruit parts. The plant could serve as an excellent source of natural antioxidants in food and medicinal industries. Hence, the inclusion of this wild vegetable in the diet is recommended in order to build the immune system against chronic diseases. The result also shows that both the aqueous and ethanol extracts from *C. gynandra* had strong antioxidant characteristics. Therefore, both solvents extract the secondary metabolites which help the antioxidant activity of the plant. This activity explains the pharmacological use of the plant in the treatment of diseases in traditional medicine.

V. CONCLUSION

The phytochemical screening of *C. gynandra* showed that the leaf had more phytochemical constituents when compared to the stem and fruit parts. The plant is a good source of natural antioxidants in food and medicinal industries. Hence, the inclusion of this wild vegetable in the diet is recommended in order to build the immune system against chronic diseases. The result also shows that both the aqueous and ethanol extracts from *C. gynandra* had strong antioxidant character. Therefore, both solvents extract the secondary metabolites which help the antioxidant activity of the plant. This activity explains the pharmacological use of the plant in the treatment of diseases in traditional medicine.

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