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Total Phenolic and Flavonoid Contents, Antioxidant Activity and Phytochemical Screening of *Calotropis Procera* Stem Bark Extracts

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Abstract In continuation of the need to search for phytochemicals in parts of some rare and native plants of Nigeria origin. This study was designed to carry out phytochemical screening, antioxidant properties and determination of total phenolics and flavonoid contents in *Calotropis procera* Stem. The phytochemical screening of stem bark of *C. procera* using aqueous and methanol extracts revealed the presence of tannins, phenols and flavonoid. The aqueous extract was also found to contain saponins while methanol extract also has steroids. Steroids was the only metabolite present in hexane extract. The anti-oxidant activity, total phenolic and flavonoid contents of aqueous and methanolic extracts of stem bark of *C. procera* were evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, Folin-Ciocalteau and aluminium chloride colorimetric assays. From the results obtained, the methanolic extract was observed to have demonstrated a significant concentration of phenolic (81.65±0.92 mg GAE/g), and flavonoid (46.08±0.71 mg RE/g) than the aqueous extract (66.07±0.43 mg GAE/g, 31.34±0.39 mg RE/g). The aqueous and methanol extracts showed maximum activities of 28.16±0.64% and 81.65±0.71% at 1 mg/ml respectively. However, the ascorbic acid exhibited 83.12±1.02% in the DPPH assay. The results of the present study, shows that both aqueous and methanolic extracts could serve as a valuable source of natural

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Key Words: *Calotropis procera*, phytochemicals screening, total phenolic and flavonoids concentrations

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1.0 Introduction

Studies on chemical composition of plants have been concentrated on the proximate, vitamins, elemental and toxicant constituents (Aletan, 2008; Eddy and Ekop, 2005). However, apart from nutritional values that can be deduced from the listed parameters, information on medicinal and other unique properties of plants can best be accessed through phytochemical composition (Bassiony *et al*., 2015). Phytochemicals are the nds found in various parts of plants including phenolic acid, flavonoids, anthocyanins, carotenoids, alkaloids, and so on (Altemimi *et al*., 2017). Phytochemical analysis can be conducted in two stages involving preliminary screening and assay for the actual identity and concentration of the phytochemicals. Among all phytochemicals, polyphenols have been found to be one of the most useful. Polyphenols are useful pro-oxidants that can be grouped into flavonoids, phenolic acid, polyphe -

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nollic amides, and others. Studies have shown that polyphenols can enhance digestion, brain function, blood sugar levels and offer protection against blood clothing, heart diseases and some forms of cancer (Hazafa *et al.,* 2019). In view of their prevailing applications, researches into plants leaves, roots and stems are majorly channelled toward the discovery of phytochemicals, especially flavonoids and phenolics (Zhou *et al*., 2016).

Calotropis belongs to the *Asclepiadaecae* family, which is reported to have over180 genera and 2200 species that are widely distributed in the tropical and subtropical region of the world. *C. procera* shows its indigenous presence in Afro-Asian monsoonal regions, subtropical America, Mascarene Island and drier parts of Australia as wasteland weeds (Orwa *et al*., 2011). It is a spreading shrub or medium size tree that can grow up to 6 m in height and it is commonly known as 'Madar' in Hindi, 'Swallow wort' in English and 'Tumpapiya' among the Hausa people of Nigeria (Quazi *et al.,* 2013. Upadhyay, 2004). *C. procera* is used in the treatment of leprosy, ulcers, tumors, piles, boils, infected wounds, rushed skin and diseases of spleen, liver and abdomen (Khare, 2007). Similarly, in Nigerian traditional medicinal system, extracts of *C. procera* stem bark in combination with (or without) extract of *Capnophyllum peregrinum* (L.) Lange leaves for the treatment of fever, leprosy, rheumatism, indigestion, cold, eczema and diarrhoea (Attah, 2012). According to Oudhia and Tripathi, (1997) latex from the stem of *C. procera* is used in treating vertigo, baldness, hair fall, rheumatoid/joints swellings and paralysis. Gupta *et al*. (2010) also stated that latex from the plant leaf is applied on fresh cuts to stop bleeding and to remove thorn from the body. The decoction of it leaves is used to relax the muscles of uterus or to increase uterine contractility in mothers to facilitate safe childbirth or to induce abortion in women (Attah, 2012; Rai *et al*. 2012). The latex extracted from the leaf and flowers of *C. procera* is processed and used in the commercial preparation of eye tonic (Vohra, 2004). The smoke from the burning dry leaves is inhaled for the cure of asthma, cough and headache. And the leaf juice is applied to skin diseases while its decoction is used for extracting guinea-worms, dropsy and enlargement of the abdominal viscera (Upadhyay, 2004).

Results from pharmacological studies of different part of *C. procera have* been reported. For example, it flowers have been reported to exhibit anthelmintic and hepatoprotective activity (Ranab *et al.,* 2002; Larhsini *et al*., 1997). The latex of the plant is reported to possess analgesic, anti-hyperglycaemic, anti-inflammatory and anti-diarrhoeal effects (Saber *et al*., 1969; Silvania, 2005; Zafar *et al*., 2005). The roots are reported to have anti-convulsing, antifertility, antitumor and hepatoprotective activities (Yoganarasimhan, 2011; Rajani and Gupta, 2009, Lal *et al*., 1985), while the whole plant has been reported to have anti-malarial and antioxidant activities (Kumar *et al*., 2005). Although *C. procera* is widely used in traditional medicine in northern part of Nigeria, there is limited literatures on the phytochemical constituents and biological activity of this plant in Nigeria. Therefore, the aim of this study is to carry out phytochemical screening on the plant stem extracts, determination of antioxidants activity and concentrations of total phenolics and flavonoid.

2.0 Materials and Methods

2.1 Sample collection and authentication

The stem bark of *C. procera* were collected at various locations within Lafia metropolis in Nasarawa State, Nigeria, West Africa. The samples were authenticated by the Botanists in the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja where a voucher specimen NIPRD/H/6893 was deposited.

2.2 Sample preparation

The stem bark was transported to the laboratory in air-tight polyethylene bags and air dried at room temperature for 21 days. The dried samples were grounded using pestle and mortar. The grounded samples were sieved, weighed, stored with proper labelling and kept away from moisture for further analyses.

2.3 Extraction of the samples

The powdered samples were successively extracted using cold extraction method with hexane as the solvent. The extraction was also repeated using methanol and water respectively. These were carried out by soaking 50 g of the sample in 200 ml of the respective solvent (i.e, hexane, methanol and water). The extraction was done at room temperature for 3 days. The solvents were removed by rotary evaporation and the concentrates were weighed and stored in the refrigerator prior to analysis.

2.4 Phytochemical Screening

The phytochemical components of the stem bark extracts of *C. procera* were screened for the presence of tannins, saponins, alkaloids, steroids, flavonoids, phenol, carbohydrates and anthraquinone derivatives using standard procedures as described by Odebiyi and Sofowora (1999) with slight modification.

2.4.1 DPPH-free radical scavenging activity assay

The analysis of the DPPH radical scavenging activity of the plant extracts was performed according to the method described by Koleva *et al.,* (2002). Stock solution was prepared by dissolving 1.0 mg of the extract in 1.0 ml of methanol and five, two fold serial dilutions was made. 0.5 ml of each of the concentrations was measured into separate test tubes and 0.3 ml of 0.5 mM DPPH was added. The reaction mixtures were vigorously shaken for 30 s in a Vortex apparatus and allowed to stand in the dark at room temperature for 30 minutes. Ascorbic acid was used as a standard for the investigation of the antiradical activity and was prepared in a similar manner. The absorbance was read using spectrophotometer at 517 nm against the blank. The blank was prepared by mixing 0.5 ml of the extract or ascorbic acid with 3.3 ml of methanol. Similarly, the control solution was prepared by mixing 3.5 mL of methanol and 0.3 ml of DPPH radical solution. The percentage of scavenging activity (X %) was calculated according to the equation 1.

$$X\ \% = \frac{Absorbance\ of\ sample - Absorban\quad of\ Blank}{Absorbance\ of\ control} \times \frac{100}{1} \quad (1)$$

2.4.2 Total phenolic content assay

The total phenolic content of each extract was determined using the Folin-Ciocalteau method as described by Ghasemi *et al*. (2009). 1 mg of the extract was dissolved in 1 ml of the extraction solvent. To obtain a standard curve using gallic acid, stock solution of gallic acid prepared by dissolving 1 mg of gallic acid in 100 µl of absolute ethanol was used in preparing varying concentrations of the compounds by serial dilutions to obtained 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/ml. 50 % of methanol solution was used as the blank. 10 µl of of the samples required for analysis was introduced into the cuvette in triplicates, 790 µl of distil H_2O and 50 µl of Folin-Ciocalteau reagent were added to each of the sample, mixed and incubated at room temperature for 8 minutes. This was succeeded by addition of 150 µl of Na_2CO_3 (20% w/v) and further incubated at room temperature for 2 hours. After the incubation, absorbance was read at a wavelength of 750 nm using the AT1 UNICAM UV/VIS spectrometer (UV4 coupled to Vision V3.40 computer software). From the measured absorbance, the total phenolic content of each extract was calculated through extrapolation of the calibration curve and expressed as gallic acid equivalents (GAE).

2.4.3 Determination of flavonoid content

The level of flavonoid content in the aqueous and methanol extracts of the sample were determined spectrophotometrically using rutin as standard (Odonotez *et al.,* 2006). In this evaluation, 1 mg of the extract was dissolved in 1 ml of methanol. To obtain a standard curve using rutin, 1mg of rutin was dissolved in 1 ml of methanol and seven, two-fold serial dilutions was made to obtain a concentration of 0.015625 to 1 mg/ml. Then, 100 µl of the extract and rutin standard concentrations were aliquoted into a cuvette and an equal volume of 2% aluminium chloride ($AlCl_3$) was subsequently added. The mixtures were incubated at room temperature for 1 hour. The absorbance was measured at 415 nm, using the AT1 UNICAM UV/VIS spectrometer UV4 coupled to Vision V3.40 computer software. The sample, blank (methanol) and standards were prepared in triplicate for each analysis and the mean value of absorbance was obtained. From the absorbance readings, the total flavonoid content of each extract was calculated from the regression equation of the rutin standard curve and expressed as rutin equivalents.

3.0 Results and Discussion

Phytochemicals identified in the preliminary screening are presented in Table 1 for the different solvents. .

Table 1: Result of the phytochemical screening of *Calotropis procera* stem extracts

Phytochemicals	Hexane extract	Methanol extract	Water extract
Alkaloids	-	-	-
Tannins	-	+	+
Steroids	+	+	-
Flavonoids	-	+	+
Phenols	-	+	+
Saponins	-	-	+
Anthraquinones	-	-	-
Carbohydrates	-	-	-

**** + = Presence, - = Absence**

The phytochemical screening of stem bark of *C. procera* using aqueous and methanol reveal the presence of tannins, phenols and flavonoid as presented in Table 1. The aqueous extract has in addition saponins while methanol extract has steroids. Steroids is the only metabolite present in hexane extract. This study contradict the reports of other researchers (Mbinda and Musangi, 2019; Morsy *et al.,* 2001) that reported the presence of alkaloid and the absence of tannins in the methanol extract of the stem bark. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwu and Josiah, 2006). Plant rich in tannins are used in the treatment of diarrhoea, inflammations of mouth and throat because tannins form water stable complexes with proteins that kills bacteria by directly damaging their cell walls (Baskaran *et al*., 2012; Idris *et al*., 2009).

Free radicals are produced in living organisms due to normal cellular metabolism. They contain unpaired electrons and are extremely reactive and unstable (Phaniendra *et al*., 2015). Several degenerative conditions such as diabetes, neurodegenerative diseases, cataracts, cancer, cardiovascular diseases, asthma, inflammation, rheumatoid arthritis, burns, intestinal tract diseases, progerias and ischemic and post-ischemic pathologies have been reported to be associated with free radicals (Umamaheswari and Chatterjee, 2008). These pathological conditions are cause by oxygen-containing free radicals such as oxygen singlet, nitric oxide radical, hypochlorite, superoxide anion radical, hydroxyl radical, hydrogen peroxide, and peroxynitrite radical (Gutteridge and Halliwell, 1993). Several enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic antioxidants such as metal binding proteins, glutathione, uric acid, melatonin, bilirubin and polyamines are also found within the body scavenge free radicals (Mirończuk-Chodakowska, *et al*., 2018). Although, these natural enzymatic and non-enzymatic antioxidants found within the body may be overwhelm by the exposure of cells, tissues and extracellular matrix to harmful reactive species caused by free radicals reactions resulting in chronic and degenerative conditions. It is therefore reported that phytochemicals have the ability to modify cellular metabolism of humans in a way that is beneficial for the prevention, treatment and control of these ailments (Mbinda and Musangi, 2019). Several synthetic drugs are used in the management of oxidative stress and these drugs have been reported to have adverse and irreversible side effects. To avoid these challenges, the consumption of medicinal plants such as C. procera which is rich in natural antioxidants is encouraged.

The DPPH radical scavenging ability was used as an index to evaluate the antioxidant potential of aqueous and methanolic extracts of *C. procera* stem bark. The extent of the DPPH radical scavenging at different concentrations of each extract (1, 0.5, 0.25, 0.125 and 0.0625 mg/ml) was measured, with ascorbic acid as the standard. The radical scavenging effect of these extracts were found to increase with increase in concentrations. Methanol extract showed inhibition of 81.65% as compared to standard ascorbic acid of 83.12% while aqueous extract displayed 28.16% as presented in Table 2.

Table 2: Antioxidant activities of methanol extract of *C. procera* leaves and ascorbic acid standard.

	% Inhibition in DPPH Assay		
Concentration (mg/ml)	Methanol extract	Aqueous extract	Ascorbic acid
1.0	81.65±0.71	28.16±0.64	83.12±1.02
0.5	76.05±1.07	29.52±0.21	81.05±0.59
0.25	75.08±1.22	27.27±0.41	79.39±1.01
0.125	73.28±1.15	27.67±0.62	80.02±1.02
0.0625	53.22±1.31	16.19±0.17	78.37±1.11
IC50 (mg/ml)	38.92±1.03	15.03±0.31	17.62±0.42

Values are presented as mean (SD of three replicates)

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The IC_{50} value of each plant extract was also determined, and it is defined as concentration of the extract that is required to inhibit 50% of DPPH free radical. The lower the IC_{50} value of an extract the higher its antioxidant capacity. In this study, the IC_{50} values of the extracts and standard ascorbic acid were 15.03±0.31 mg/ml aqueous, 38.92±1.03 mg/ml methanol and 17.62±0.42 mg/ml ascorbic acid. The result shows that the methanolic extract of stem bark of *C. procera* has a good DPPH radical scavenging effect.

The total phenolic content (TPC) of the crude aqueous and methanolic extract of *C. procera*

stem bark was calculated from the linear regression equation of standard curve (y = 3.4015x - 0.0787, R^2 = 0.9954) for aqueous extract and (y = 3.9248x – 0.0801, R^2 = 0.9986) obtained from the calibration curve shown in Fig. 1. The results indicated the concentration of total phenolic in the aqueous and methanolic extracts are 66.07±0.43 and 81.65±0.92 mg GAE/g respectively.

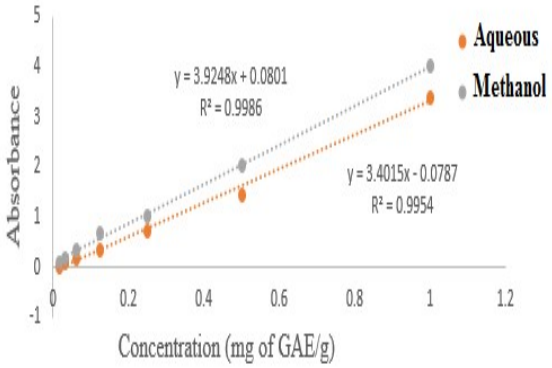

Fig. 1: Calibration curve for total phenolic contents in the aqueous and methanol leaves extracts.

The total flavonoids content (TFC) of the extracts were determined through extrapolation of the measured sample absorbance to the calibration curve (Fig. 2) which gave the following regression equations, y = 2.1663x + 0.1197; R^2 = 0.9852 and y = 2.1663x + 0.1197; R^2 = 0.9852 for aqueous and methanol extracts respectively (Fig. 2). The results obtained gave flavonoid concentrations of 31.34±0.39 and 46.08±0.71 mg RE/g for aqueous and methanolic extracts respectively.

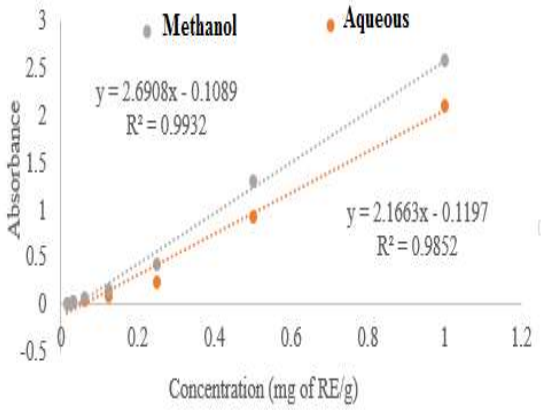

Fig 2: Calibration curve for total flavonoid content in the aqueous and methanol leaves extracts

From the results obtained, it is evidence that methanol extract of the stem bark of *C. procera has* higher antioxidant property, total phenolic and flavonoid contents than the aqueous extract. The result of this study revealed that percentage inhibition and total phenolic content of methanol extract are comparable to 90.55% and 79.80 mg GAE/g that was reported by Mbinda and Musangi, (2019) but inconsistent with 71.63 mg QE/g reported for the concentration of total flavonoids. Also, the result observed for TPC and TFC of the methanol extracts of this study is higher than 15.67 mg GAE/g and 1.62 mg QE/g reported for the methanol root bark extract of the same plant (Kumar *et al.,* 2005). Verma, (2014) reported 5.2 mg GAE/g and 7.8 mg QE/g for TPC and TFC for the methanol extract of the flowers of *C. procera*. The high phenolic content of these plant extracts suggests high antioxidant capacity because phenolic can react with active oxygen radicals such as oxygen singlet, nitric oxide radical, hypochlorite, superoxide anion radical, hydroxyl radical, hydrogen peroxide, and peroxy nitrite radical (Gutteridge and Halliwell, 1993). Studies have revealed high correlation between antioxidant capacity and phenolic content (Mako *et al*., 2012). Generally, plant extracts with high antioxidant activity are often found to have high concentrations of phenolic while those with high phenolic contents also exhibit high flavonoid content (Makepeace *et al.,* 1985). Amorati and Valgimigli, (2015) reported that Folin-Ciocalteau assay is easy and rapid to perform but the reagent react with other phyto-constituents beside phenol resulting in high amount of total phenolic content in the extract. The present study showed that the antioxidant potential in this plant extract is not only due to the presence of phenolic but also flavonoids which are known to have redox properties, which allowed them to scavenge free radicals.

4.0 Conclusion

This study assessed the total phenolic and flavonoid contents in addition to antioxidant activity of stem bark of *C. procera*. Our findings revealed that both aqueous and methanolic extract have high phenolic and flavonoid contents as well as antioxidant activities and could help to prevent the progress of different oxidative stresses. This antioxidant potential of the extracts may be due to the presence different phytochemicals present in it. Further investigation is therefore necessary to isolated and identified different phytochemical compounds present in the extracts and their biological activity must be determined.

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