

***In vitro* antioxidant potential of methanol extracts of *Allamanda schottii* Pohl and *Thevetia peruviana* (pers.) K. (Apocyanaceae)**

Adedokun Oluwasegun¹, Abdulkadir Nasiru² and Efosa John²

¹ Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin-city, Nigeria

² Department of Science Laboratory Technology, Federal Polytechnic Auchi, Nigeria

ABSTRACT

Antioxidants are known for their potential in improving health and lowering the risk for certain metabolic diseases such as hypertension, cancer and heart disease. The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods. Phytochemical screening of both methanolic extract of *Allamanda schottii* and *Thevetia peruviana* methanol extract, as well as *in vitro* antioxidant activity of the aforementioned plants using Ferric Reducing Power Assay and DPPH-Radical Scavenging Assay were done in this research. Phytochemical screening result showed the presence of saponin, terpenoids, flavonoid, tannin and alkaloid in both plants, but the presence of cardiac glycoside was observed in *T. peruviana* only. The total antioxidant effect was found to be concentration dependent in both plants, lowest and highest activity were observed at 20 and 100 µg/ml respectively for both plants, with *A. schottii* having the highest activity. A similar activity was noted in ferric reducing power assay, where no significant difference in activity of both plants $P < 0.5$ was observed. The study indicates that both *A. schottii* and *T. peruviana* have radical scavenging activity and possibly may be relevant in preventing some diseases induced by oxidative stress.

Keywords: Antioxidant, DPPH, Ferric Reducing Power Assay, *Allamanda schottii*, *Thevetia peruviana*.

INTRODUCTION

Antioxidant simply implies "against oxidation". Antioxidants are effective because, they are willing to give up their own electrons to free radicals. As a result they no longer need to attack the cells and the chain reaction of oxidation is broken. After electron donation, an antioxidant becomes a free radical by definition. Antioxidants, in this state, are not dangerous because they can remain unreactive despite their ability to accommodate change in electrons [1]. The human body has an enormous antioxidant defence system. Antioxidants can be synthesis within the body and likewise from exogenous means such as fruits, vegetables, seeds, nuts, meats, and oil [1].

Antioxidants are known for their potential in promoting health and lowering the risk for cancer, hypertension, heart disease and other cardiovascular diseases [2, 3]. The uses of natural antioxidants from plant extracts have experience growing interest due to health professionals and consumer's concern about the safety of synthetic antioxidants [4, 5]. Antioxidants may be enzymatic or non-enzymatic. Superoxide dismutase, Glutathione peroxidase, and Catalase are some examples of enzymatic antioxidants. In the non-enzymatic category, some of the known and documented antioxidants are Vitamin C, Vitamin E, Vitamin A, Carotenoids, Uric acid, Ubiquinone and synthetic compounds like Melatonin and Dehydroepiandrosterone (DHEA). Antioxidant activity has been reported for several triterpenes among other related compounds [6, 7]. The plant family Apocyanaceae has been suggested to contain plants with antioxidant properties, and for this reason, two common plants, *Allamanda schottii* and *Thevetia peruviana* were picked from this family.

Allamanda schottii Pohl is an evergreen tropical shrub with trumpet-shaped flowers, orange-red throat stripes and leathery, elliptic to obovate, dull green leaves (about 5-10 cm long) that appears in whorls of 3-5 along the stem.

Rounded, prickly, bur-like fruits will form when spent flowers are not deadheaded. Commonly known as Bush Allamanda, it is commonly used as an ornamental [8].

Thevetia peruviana (Pers.) K. Schum. is a tropical shrub or small tree with long funnel-shaped, sometimes fragrant, yellow (less commonly apricot, sometimes white) flowers in few-flowered terminal clusters. Its leaves are willow-like, linear-lanceolate and glossy green in colour, covered in waxy coating. Its stem is green turning silver/gray as it ages. Due to its toxicity, it is mostly used as an ornamental. It is commonly known as the Yellow Oleander [9, 10, 11].

The aim of this study is to evaluate the *in vitro* antioxidant activity of the aforementioned plants using Ferric Reducing Power Assay and DPPH-Radical Scavenging Assay.

MATERIALS AND METHODS

Collection of plant materials

A. schottii and *T. peruviana* leaves were collected from Ewu community of Esan-Central Local Government Area of Edo state, Nigeria and authenticated by Mr. Immanuel Okanufa (Professor J.C Okafor herbarium, Ewu, Nigeria); Voucher specimen was deposited in the same herbarium.

Preparation of powder

Both plants were air dried at room temperature in the laboratory. The dried leaves were powdered by pounding using the wooden mortar and pestle until very fine. Both powdered plant leaves were stored separately in double cellophane wrappers tightly sealed.

Preparation of extracts

Extraction from the powdered materials was by Soxhlet extraction method using methanol. The extracts were concentrated to dryness using the evaporating dish.

Phytochemical Screening

Screening for the presence of alkaloids, flavonoids, saponins, tannins, and cardiac glycoside were performed in accordance with the standard method

Determination of Antioxidant activity

The radical scavenging activities of the plant extract against 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described by [12, 13]. 10-100µg/ml of crude extract and vitamin c were prepared in methanol (Analar grade). 1ml of the extract was placed in a test tube, and followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following

Formula:

$$\% \text{ Inhibition} = \{ [A_b - A_a] / A_b \} \times 100 \dots\dots\dots (1)$$

Where A_b is the absorption of the blank sample and A_a is the absorption of the extract.

Ferric Reducing Power Assay

Ferric reducing power was determined by mixing various concentrations of plant extract and standard ascorbic acid solution (viz. 20, 40, 60, 80 and 100µg/ml) in 1ml of methanol with phosphate buffer (2.5ml, 0.2 M at pH 6.6) and Potassium ferricyanide [$K_3Fe(CN)_6$] (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5ml) of 10% Trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged at 3000g (rpm) for 10 min at room temperature. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferric chloride ($FeCl_3$) (0.5 ml, 0.1%), and the absorbance of the reaction mixture was measured at 700 nm as indicative of increased reducing power. All the tests were performed in triplicate and a graph was plotted for the average of three observations [14, 15].

Statistical Analysis

Data obtained with three replicates of each sample were used for statistical analysis. Statistical processing of the original data was performed using one-way analysis of variance (ANOVA) and differences at $P < 0.05$ were considered significant

RESULTS

Phytochemical screening of both methanol extract of *Allamanda schottii* and *Thevetia peruviana* showed the presence of saponin, terpenoids, flavonoid, tannin and alkaloid. Although, the presence of cardiac glycoside was observed in *T. peruviana* only as shown in Table 1.

Table 1. Phytochemical screening of both methanol extract of *A. Schottii* and *T. peruviana*

Parameters	<i>A. schottii</i>	<i>T. peruviana</i>
Saponins	++	++
Terpenoids	++	+++
Hydrolysable tannins	-	-
Condensed tannins	+++	+++
Flavonoids	+	+
Phlobatannins	-	-
Cardiac glycosides	-	+++
Alkaloids	+++	+

The percentage of DPPH radical scavenging activity of *Allamanda schottii* Pohl & *Thevetia peruviana* (Pers.) K. Schum extracts are presented in Figure 2. The antioxidant effects are found to be concentration dependant. Higher dose has showed higher activity compared to the other doses. Both plants showed presence of an average free radical scavenging activity in DPPH assay. The effect was statistically highly significant in all the doses tested in comparison to control group and *Allamanda schottii* showed better result.

For determining the reducing ability, the $Fe^{3+} - Fe^{2+}$ transformation was investigated in the presence of the both extracts. Figure 1 shows the reducing power of the both extracts, which proved to be concentration-dependent. This behavior indicates that the both extracts consist of hydrophilic polyphenolic compounds that account for the reducing power. As is known, antioxidants chelate and deactivate transition metals thereby preventing these metals from participating in the initiation of lipid peroxidation (LPO) and oxidative stress through metal catalyzed reactions. As indicated in Table 1, there is no significant difference in metal chelating rate of *Allamanda schottii* Pohl & *Thevetia peruviana* (Pers.) K. Schum and over all activity is relatively low as compared to Vitamin C. Table 3, showed the IC50 of the two plants where *T. peruviana* showed an improved activity, although statistically significant from Vitamin C.

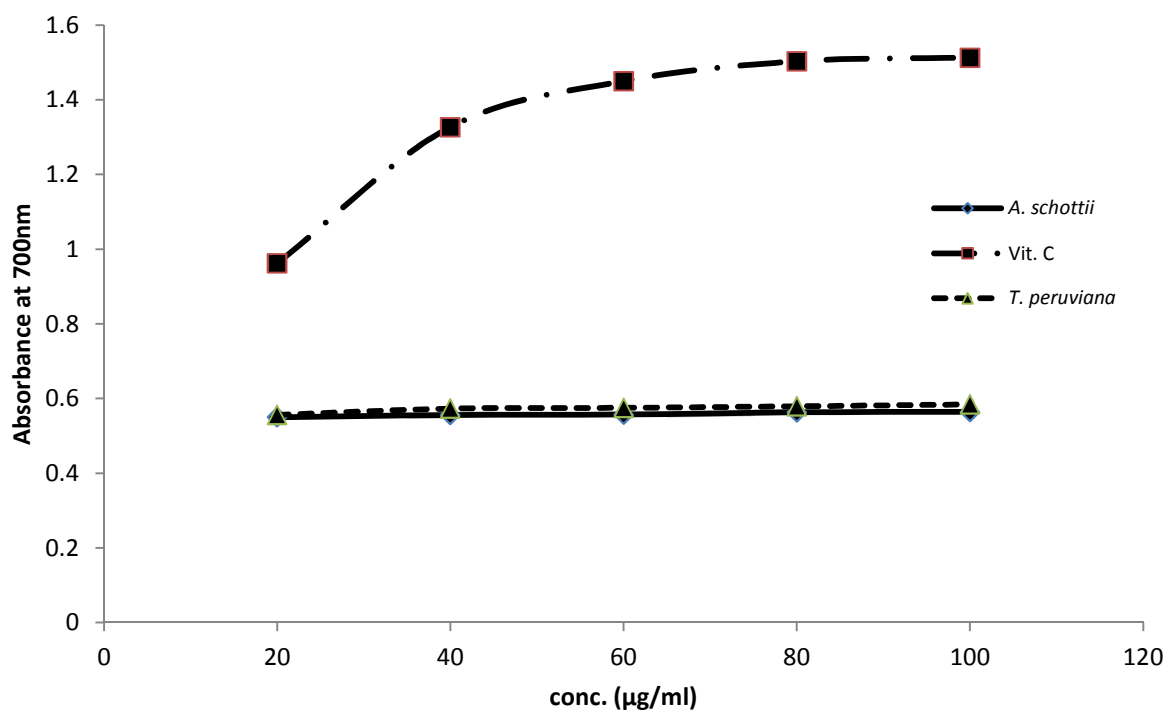


Figure 1: Ferric Reducing power of *Allamanda schottii* and *Thevetia peruviana* extract

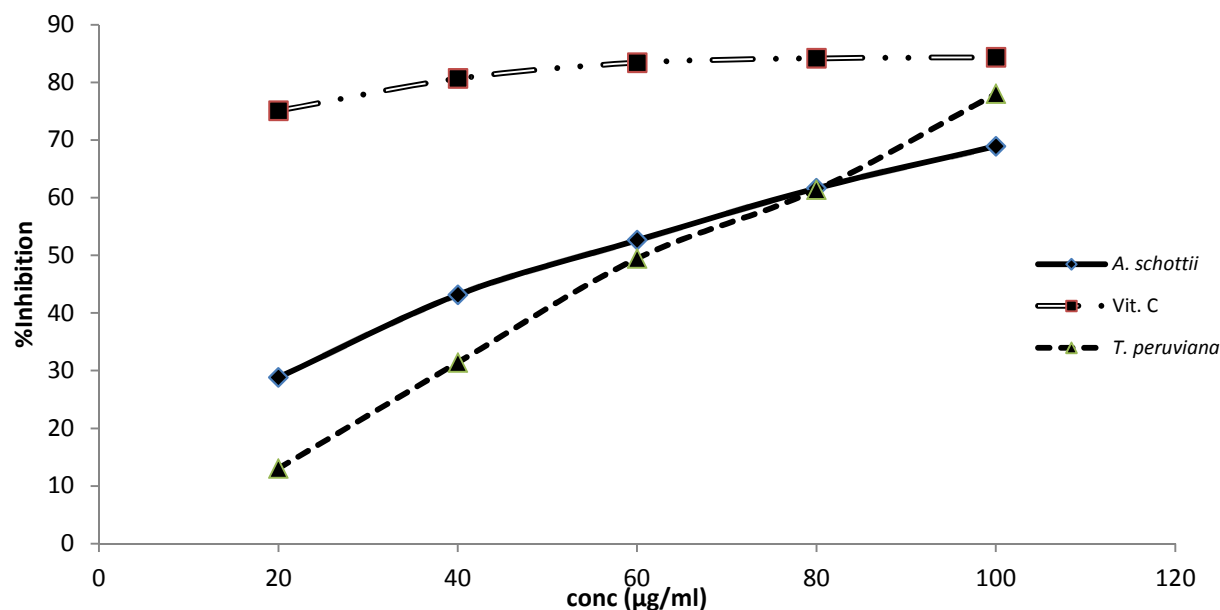


Figure 2: Total Antioxidant assay of *Allamanda schottii* and *Thevetia peruviana* extracts using DPPH method

CONCLUSION

From the entire work, it was deduced that *Allamanda schottii* and *Thevetia peruviana* possess an average total antioxidant and weak ferric reducing potential relative to Vitamin C. Also, the presence of cardiac glycoside was confirmed in *Thevetia peruviana*, as stated in literature of phytochemistry (presence of thevetose sugar, the glycone of cardiac glycoside present in *Thevetia peruviana*). However, more researches should be done in isolating the cardiac glycoside compound in *Thevetia peruviana* and Lipid peroxidation assay should be done on both plants, because of their ferric reducing activity, which has a relationship with male infertility.

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