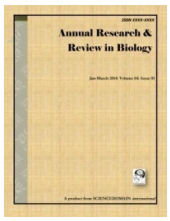

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Preliminary *In-vitro* Assessment of Some Phytochemical Constituents and Radical Scavenging Activity of Methanol Extracts of Five Flowers Varieties

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This work evaluated some phytochemical constituents and radical scavenging activity of the flower samples of *Allamanda carthica, Delonix regia, Hibiscus rosasinensis, Plumeria rubra* and *Tercoma stans*. Methanol extracts of different flower samples were used for the phytochemical analysis. The result obtained showed that *Tercoma stans* has the highest yield (14.45%) followed by *Hibiscus rosasinensis* (12.36%), *Delonix regia* (11.36%), *Allamanda Cathartica* (10.11%) and *Plumeria rubra* (8.99%) while the preliminary qualitative phytochemical screening showed that alkaloid was absent in all the extracts. The methanol extracts of the five flower samples indicated the presence of flavonoids and saponins in significant quantities. With the exception of *Allamanda Cathartica* and

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Delonix regia all other extracts dictates the presence of protein. Glycosides were not detected in *Allamanda carthica* and *Tercoma stans*, but were found in other samples. Tannins were detected in small quantities in the five flower samples. Methanol extract of *Allamanda Cathartica, Delonix regia, Hibiscus rosasinensis, Plumeria rubra* and *Tercoma stans* were prepared for the assessment of the antioxidant scavenging properties using the DPPH model and ascorbic acid as standard. The result obtained from the antioxidant activity was used to plot a graph of absorbance against time. The ascorbic acid standard showed the highest activity at 0.8 mg/dl. The methanol extracts of the flower samples showed varying degree of antioxidant activity in the decreasing order, as follows: *Plumeria rubra>Tercoma stans>Allamanda Cathartica>Hibiscus rosasinensis>Delonix regia.* The result suggests that the phytochemical and antioxidant properties of the flowers could be used for medicinal purposes since some insects and birds depend on the nectar produced from these flowers for survival.

Keywords: Allamanda cathartica; Delonix regia; Hibiscus rosasinensis; Plumeria rubra; Tercoma stans; phytochemicals; radical scavenging.

1. INTRODUCTION

Oxidative stress refers to the shift towards the pro-oxidants in the pro-oxidants/antioxidants balance, that can occur as a result of an increase in oxidative metabolism [1]. Reactive oxygen species (ROS) reactions with bio molecules such as lipid, protein and DNA, produce different types of secondary radicals like lipid radicals, sugar and base derived radicals, amino acid radicals, depending upon the nature of ROS [2]. In the presence of oxygen these radicals are converted to peroxyl radicals. Peroxyl radicals are critical in biosystems, as they often induce chain reactions. These reactions exert oxidative stress in cells, tissues and organs of the body. The biological implications of such reactions depend on several factors like site of generation, nature of the substrate, activation of repair mechanisms, redox status among many others [3,4].

Plants have been used for health and medicinal purpose since immemorial time and possess rich and important sources of medicinal potentials since human civilization. More preference of plant-based medicines has outweighed synthetic medication in the treatment and management of different diseases [5]. The medicinal value of plants lies in their chemical substances which produces a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [6].

1.1 Allamanda Cathartica

Allamanda cathartica (*Golden Trumpet*) is an ornamental plant of *Allamanda* genus in the *Apocynaceae* family, which is native from Brazil. Its large flowers are very fragrant. This South American plant is thought to blossom best in full sunshine and well drained soil. Growing the golden trumpet is a vine that requires a trellis or a fence to support it. It does not twine, nor does it have tendrils or aerial roots. This vine could also be pruned so that it grows as a shrub. If not pruned, it could rapidly grow to a height of 20 feet [7].

1.2 Delonix Regia

Delonix regia is a species of flowering plant in the family *Fabaceae*, subfamily *Caesalpinioideae*. It is noted for its fern-like leaves and flamboyant display of flowers. In many tropical parts of the world it is grown as an ornamental tree and in English it is given the name Royal Poinciana or Flamboyant. It is also one of several trees known as Flame tree. The flowers are large, with four spreading scarlet or orange-red petals up to 8cm long and a fifth upright petal called the standard, which is slightly larger and spotted with yellow and white [8]. Also known as royal Poinciana, May flower plant or Flamboyant, is well known for its brilliant display of red-orange bloom, literally covering the tree from May to June. The *Delonix regia* will provide fullest flowering and best growth when planted in full sun location [9].

1.3 Hibiscus Rosasinensis

Hibiscus rosasinensis is an ornamental plant often planted as a fence or hedge plant. It is native in China and also grows in India and Philippines. Hibiscus is a large genus that contains herbs, shrubs and trees widely distributed in the tropical and sub-tropical region of world [10]. Plant can be propagated by cutting from mature wood of current growth. It is an

evergreen woody, glabrous, showy shrub of 5-8ft in height. Leaves are bright green, ovate, coarsely toothed above, flower are solitary, axillary, bell shaped, large 4-6 inch in diameter with pistil and stamens projecting from the centre [11].

1.4 Plumeria Rubra

Plumeria rubra grows as a spreading shrub or small tree to a height of 2–8m (20–25ft) and similar width. It has a thick succulent trunk and sausage-like blunt branches covered with a thin grey bark. They are deciduous, falling in the cooler months of the year. The flowers are terminal, appearing at the ends of branches over the summer. Often profuse and very prominent, they are strongly fragrant and have five petals. The colors range from the common pink to white with shades of yellow in the centre of the flower. Initially tubular before opening out, the flowers are 5-7.5cm (2–3 in) in diameter, and only rarely go on to produce seed–20-60 winged seeds are contained in a 17.5cm (7 in) pod [12].

1.5 Tercoma Stans

Tercoma stans is a species of flowering perennial shrubs in the trumpet vine family, Bignoniaceae, native to the Americans. Common names include; yellow trumpet bush, yellow bells yellow elder [13]. Yellow trumpetbush is an attractive plant that is cultivated as an ornamental. It has sharply-toothed, lance-shaped green leaves and bears large, showy, bright golden yellow trumpet-shaped flowers. It is drought-tolerant and grows well in warm climates. The flowers attract bees, butterflies and hummingbirds. The plant produces pods containing yellow seeds with papery wings. The plant is desirable fodder when it grows in fields grazed by livestock. Yellow Trumpetbush is a ruderal species, readily colonizing disturbed, rocky, sandy and cleared land and occasionally becoming an invasive weed [14].

The use of traditional medicine cannot fade out in the treatment and management of diseases in African continent and this could be attributed to socio-cultural and socio-economic life styles; lack of basic health care and qualified personnel [15]. Plants contain active components such as anthraquinones, flavonoids, glycosides, saponins and tannins etc, which possess medical properties that are harnessed for the treatment of different diseases [16].

Despite the availability of this plant flowers, there is no adequate information in the scientific literatures that addresses the comparative antioxidant potentials of *Allamanda cathartica*, *Delonix regia*, *Hibiscus rosasinensis*, *Plumeria rubra* and *Tercoma stans* growing in Nigeria. Thus, the present study investigated the antioxidant (*in vitro)* potentials of methanol extracts *Allamanda cathartica*, *Delonix regia*, *Hibiscus rosasinensis*, *Plumeria rubra* and *Tercoma stans* growing in Southern part of Nigeria.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Samples

The flower samples of *Allamanda carthartic*a, *Delonix regia*, *Hibiscus rosasinensis*, *Plumuria rubra*, and *Tercoma stans* were used for this study. The flower samples were sourced within the Environment of University of Nigeria Nsukka, Enugu State, Nigeria and were identified by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka.

2.2 Extraction of Plant Materials

The flowers of the various plant flower samples were shad dried and milled to coarse powder using the hammer mill. 50g each of the flower samples was macerated in 300ml of methanol for 48h. The solution was filtered using Whatman no. 4 filter paper and the filtrate was concentrated to a semi-solid residue in a rotary evaporator.

Calculation of percentage yield

$$\% \text{ yield} = \frac{\text{Sample extract (g)}}{\text{Dried flower (g)}} \times 100$$

2.3 Qualitative Phytochemical Analysis of the Various Plant Flower Samples

The preliminary phytochemical analysis of the methanol extracts of *Allamanda carthartic*a, *Delonix regia*, *Hibiscus rosasinensis*, *Plumuria rubra* and *Tercoma stans* was carried according to the method of Harborne [17]; Trease and Evans [18], as outlined in Edeoga et al. [5].

2.4 Test for Alkaloids

20ml of 3% sulphuric acid in 50% ethanol were added to 2g of the extract and heated on a boiling water bath for 10 minutes, cooled and filtered. The filtrate (2ml) was tested with a few drops of Mayer's reagent, Dragendorff's reagent, Wagner's reagent and picric acid solution (1%). The remaining filtrate was placed in 100ml separatory funnel and made alkaline with diluted ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portions of diluted sulphuric acid. The extract was tested with a few drops of Mayer's, Wagner's, Dragendorff's reagents and picric acid solution. Alkaloids gave milky precipitate with few drops of Mayer's reagent; reddish brown precipitate with few drops of Wagner's reagent; yellowish precipitate with few drops of picric acid and brick red precipitate with few drops of Dragendorff's reagent.

2.5 Test for Flavonoids

0.2g of the sample was heated with 10ml ethyl acetate in boiling water for 3 minutes. The mixture was filtered and the filtrate was used for the the Aluminium chloride test–4ml of the filtrate was shaken with 1ml of 1% aluminium chloride solution and observed for light yellow coloration that indicates the presence of flavonoids.

2.6 Test for Glycosides

2.0g of the sample was mixed with 30ml distilled water and 15ml of diluted sulphuric acid respectively and heated in a water bath for 5 minutes. The mixtures were filtered and the filtrates used for the following test:

(i) to 5ml of each of the filtrates, 0.3ml of Fehling's solutions A and B were added until it turned alkaline (tested with litmus paper) and heated on a water bath for 2 minutes. A brick-red precipitate indicated the presence of glycosides.

2.7 Test for Saponins

0.1g of the sample was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for the following tests:

(i) The emulsion test: 1ml of the filtrate was added to two drops of olive oil. The mixture was shaken and observed for the formation of emulsion.
(ii) The frothing test: 1ml of the filtrate was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

2.8 Test for Tannins

2g of the sample was boiled with 5ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was treated with the following solutions:

(i) Lead sub acetate solution-to 1ml of the filtrate, 3 drops of lead sub acetate solution were added. A gelatinous precipitate indicates the presence of tannins.
(ii) Bromine water-to 1ml of the filtrate was added 0.5ml of bromine water and then observed for a pale brown precipitate.
(iii) Ferric chloride solution-1ml of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution were added. A transient greenish to black colour indicates the presence of tannins.

2.9 Test for Reducing Sugar

5ml of a mixture containing equal parts of Fehling's solution A and B were added to 5ml aqueous extract and then heated in a water bath for 5 minutes. Brick red precipitate showed the presence of reducing sugars.

2.10 DPPH Antioxidant Scavenging Activity

This was determined by the method described by [19], as adopted by [20]. 1mg/ml of the plant flower extracts were prepared by dissolving 0.1g of the extracts in 100ml methanol. Serial dilutions of the 1mg/ml the plant flower extract were diluted with methanol to give various concentrations: 1mg/ml, 0.8mg/ml, 0.6mg/ml, 0.4mg/ml, 0.2mg/ml and 0.1mg/ml. To each test tube, 0.3ml of DPPH was added. Absorbance (517nm) was read at time = 0 minutes and time = 30 minutes. The experiment was done in triplicate. A control was prepared by adding 1ml of methanol to 0.3ml of DPPH and absorbance was measured at time = 0 minute and time =30 minutes. The percentage inhibition was calculated using the formula:

Antioxidant Activity = 100 – (Increase in absorbance of sample/ Increase in absorbance

of control). A graph of absorbance against concentration was plotted.

2.11 Statistical Analysis

Data were reported as means ± SEM, where appropriate and Duncan multiple test range was used to compare the means obtained from each sample with that of the ascorbic acid standard. The results were represented graphically.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield Of Methanol Flower Extract of *Allamanda cathartica, Delonix regia, Plumeria rubra, Hibiscus rosasinensis, Tercoma stans*

The results obtained (Table 1) showed that *Tercoma stans* had the highest yield (14.45%) followed by *Hibiscus rosasinensis* (12.36%), *Delonix regia* (11.36%), *Allamanda cathartica* (10.11%) and *Plumeria rubra* (8.99%).

3.2 Qualitative Phytochemical Analysis of the Flower Samples

The result obtained from the qualitative phytochemical analysis of the methanol extracts indicated the absence of alkaloids, while flavonoids were present in significant quantities. With the exception of *Allamanda cathartica* and *Delonix regia,* all other extracts were positive for proteins. Glycosides were not detected in *Allamanda cathartica* and *Tercoma stans*. Tannins were detected in small quantities in all the samples.

The result of the phytochemical analysis of the flower samples of *Allamanda cathartica, Delonix regia, Plumeria rubra, Tercoma stans* and *Hibiscus rosasinensis* revealed the presence of flavonoids. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinating animals. Flavonoids are good antioxidants and therefore contribute significantly to the antioxidant scavenging properties of the plant samples [21].

Alkaloids where not detected in any of the flower samples using the crude aqueous flower extract (Table 2). However ethanol extracts, as well methanol and petroleum ether extracts of virtually all the investigated flower samples showed the presence of alkaloids [11,14,22]. This result was in agreement with the qualitative phytochemical screening of flower samples of *Allamanda cathartica* using different solvent extracts [23]. This could be the result of differences in the solvent used and it is based on the polarity of the active compounds in the extracts. *Plumeria rubra* and *Allamanda cathartica* revealed the presence of alkaloid using petroleum ether extract [24,25]. Meanwhile the ethanol extract of *Hibiscus rosasinensis*, *Tercoma stans* and *Delonix regia* all revealed the presence of alkaloids [11,14,22]. The above mentioned plant samples may have analgesic, antioxidant and anti-inflammatory activities which could be attributed to their alkaloid content [26].

Tannins were detected in moderate quantities in all the investigated flower samples. Saponins were also detected in all the samples in small quantities. Recent studies have shown that saponins and tannins were reported to have anti-inflammatory activity [5]. Glycosides were detected in *Delonix regia, Plumeria rubra* and *Hibiscus rosasinensis.* Many plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body [27]. From these findings, the extracts of the above mentioned flowers could have various medicinal values such as anti-inflammatory, analgesic, antioxidant and potential anti-diabetic activities, since they contain saponins, flavonoids, tannins and alkaloids.

Table 1. The percentage yield of methanol extracts of *Allamanda cathartica, Delonix regia, Plumeria rubra, Hibiscus rosasinensis* and *Tercoma stans*

Sample type	Dried flower sample(g)	Extract (g)	Percentage (%) yield
Allamanda carthica	50	5.055	10.11
Delonix regia	50	5.68	11.36
Plumeria rubra	50	4.49	8.99
Hibiscus rosasinensis	50	6.18	12.36
Tercoma stans	50	7.22	14.45

3.3 DPPH Antioxidant Properties of the Flower Samples

3.3.1 DPPH antioxidant property at time zero

Allamanda carthartica, *Plumeria rubra*, *Hibiscus rosasinensis* and *Tercoma stans* showed higher absorbance at 517nm compared with the ascorbic acid standard at the zero time point (Fig. 1). *Delonix regia* gave the lowest absorbance value. The absorbance of the ascorbic acid increased progressively with increasing concentration of the sample. The same trend was observed in *Delonix regia*, *Allamanda carthartica*, *Plumeria rubra*, *Hibiscus rosasinensis* and *Tercoma stans* all showed varying degree of absorbance against increasing concentration at a higher absorbance than the ascorbic acid standard.

3.3.2 DPPH antioxidant property at after 30 minutes

The methanol extract of *Allamanda cathartica*, *Delonix regia*, *Tercoma stans*, *Plumeria rubra* and *Hibiscus rosasinensis* all showed significant decrease in absorbance at various concentration after 30 minutes (Fig. 2). After 30 minutes, *Allamanda cathartica, Hibiscus rosasinensis* and *Delonix regia* showed the highest anti-oxidant activity at 0.6mg/ml, while *Tercoma stans* and *Plumeria rubra* showed the highest antioxidant activity at 0.8mg/ml and 0.4mg/ml respectively. The ascorbic acid standard showed the highest activity at 0.8mg/ml.

Table 2. The preliminary qualitative phytochemical analysis of methanol extracts of *Allamanda cathartica, Delonix regia, Plumeria rubra, Hibiscus rosasinensis* and *Tercoma stans*

Phytochemicals	Allamanda carthica	Delonix regia	Plumeria rubra	Hibiscus rosasinensis	Tercoma stans
Alkaloid	_	_	_	_	_
Flavonoids	+++	++	+++	+++	+++
Glycosides	_	+	+	+	_
Protein	_	_	++	++	++
Reducing sugar	+	++	+++	++	++
Saponins	++	++	++	++	++
Tannins	+	+	+	+	+

Keys: - Absent , + Present in small quantities, ++ Moderately present , +++ Abundantly present

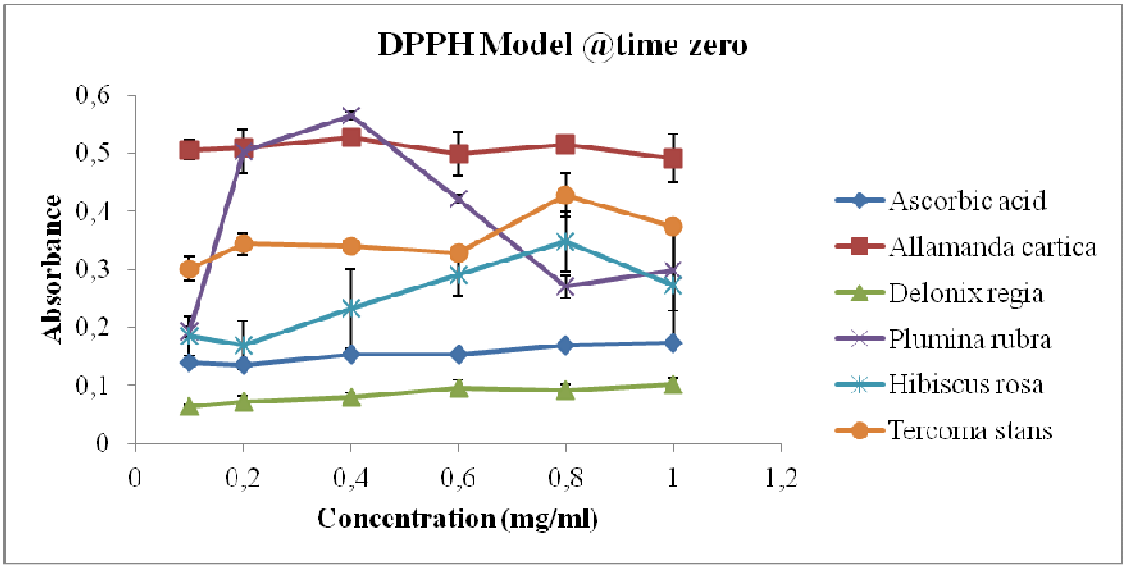

Fig. 1. Graph of absorbance against concentration at time=0 minute of *Allamanda cathartica*, *Delonix regia*, *Hibiscus rosasinensis*, *Tercoma stans* and *Plumeria rubra*

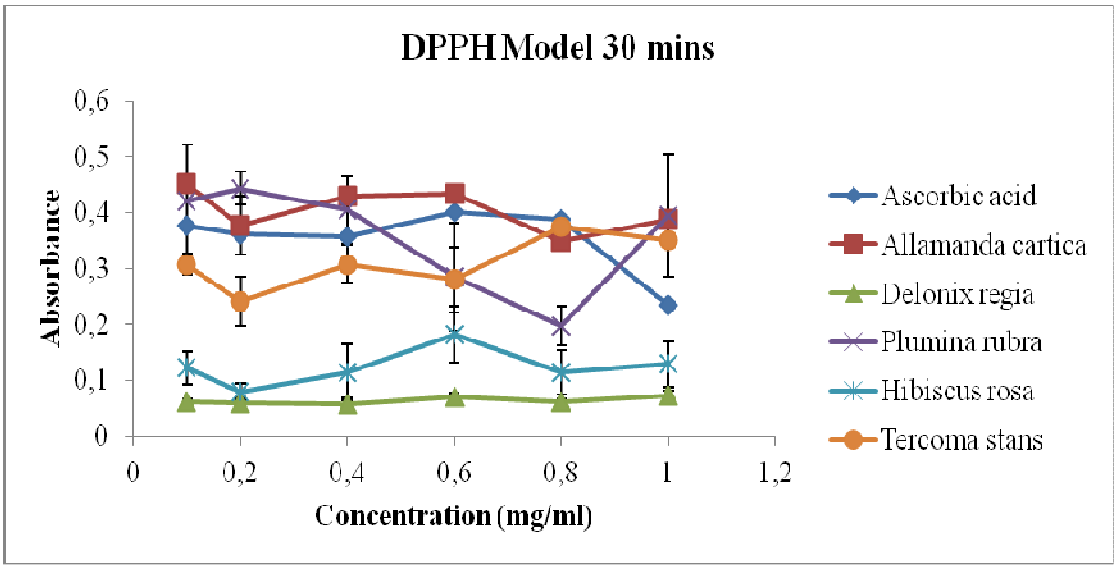

Fig. 2. Graph of absorbance against concentration after 30 minutes of *Allamanda cathartica*, *Delonix regia*, *Tercoma stans*, *Plumeria rubra* and *Hibiscus rosasinensis* together with the ascorbic acid standard

From the results of the antioxidant scavenging activity using the DPPH model which is based on the assumption that when a solution of DPPH is mixed with that of a test sample which has the tendency of donating a hydrogen atom, it gives rise to the reduced form of the DPPH with the loss of this violet colour and hence reduced absorbance reading which is an indication of the substance antioxidant property [19]. At zero minutes, increasing concentrations of *Plumeria rubra* showed increase in absorbance up to 0.4mg/ml, where there was a sharp decline in absorbance with further increases in concentration (mg/ml). This result indicated a strong antioxidant activity above 0.4mg/ml. *Hibiscus rosasinensis* showed a decrease in absorbance at concentrations above 0.8mg/ml, which ignin d antioxidant activity at the mentioned concentration. *Tercoma stans* showed increased antioxidant activity at 0.2mg/ml and 0.8mg/ml. *Allamanda cathartica* revealed a gradual increase in antioxidant activity with increasing concentration, while *Delonix regia* showed the lowest activity (Fig. 1).

After 30 minutes, *Allamanda cathartica, Hibiscus rosasinensis* and *Delonix regia* revealed the highest antioxidant activity at 0.6mg/ml, while *Tercoma stans* and *Plumeria rubra* showed the highest antioxidant activity at 0.8mg/ml and 0.4mg/ml, respectively (Fig. 2). All the flower samples showed varying degrees of antioxidant activity in the following order: *Plumeria rubra*> *Tercoma stans*> *Allamanda carthica*>*Hibiscus rosasinensis*>*Delonix regia*. *Plumeria rubra* appeared to show the highest antioxidant activity; this could be the result of high concentration of phenolics, flavonoids, tannins and alkaloids [6]. The investigated plant extracts might be therefore important in fighting disease and in promoting good health in humans.

4. CONCLUSION

This study reveals that the methanol extracts of *Allamanda cathartica, Delonix regia, Plumeria rubra, Tercoma stans* and *Hibiscus rosasinensis* flowers possess antioxidant potential and could be harnessed in the management of oxidative stress and its related complications.

5. SUGGESTION FOR FURTHER STUDIES

Further studies should be done to isolate the bioactive components of these flowers for the possible development of potent medicinal drugs and likewise, ascertain the toxicity and potency of these extracts in animal models.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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