

Phytochemical, Anticancer and Antioxidant Evaluation of Potential Chemical Constituents of *Calliandria Surinamensis*

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Abstract

Some chemical constituents (β -Sitosterol, β -Sitosterol glucoside, β -amyirin, xanthone glycoside, and a flavanol glycoside) previously isolated and characterized from the stem bark of *Calliandria surinamensis* were subjected to in vitro free radical scavenging and anticancer activities using DPPH free radical scavenging method and lung cancer cell lines.

Evaluation of the antioxidant and anticancer activities of the compounds revealed significant in-vitro antioxidant activity of the flavanol and xanthone glycosides. The xanthone glycoside was active on the cancer cell line used for the study.

The work reports for the first time the in-vitro antioxidant and anticancer profile of the isolated chemical compounds from the medicinal plant.

Key words: anticancer, antioxidant, *C. surinamensis*, phytochemical investigation, plant extract

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Introduction

Current research has shown that food rich in antioxidants plays a vital role in the prevention of several diseases which possess serious threat to man's existence such as cardiovascular diseases (Blois 1958; Emmanuel et al. 2007; Butler, 2008). Some synthetic antioxidants, such as 2- and 3-tert-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA), 2,6-di-tert-butyl-4-methylphenol (i.e. butylated hydroxytoluene, BHT) and tert-butylhydroquinone (TBHQ) are widely used as antioxidants in foodstuffs and drugs but, because of toxicity issues, their use is being questioned (Falodun and Irabor, 2008). Reactive oxygenic species in the form of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO) are natural by-products of our body's metabolism. These chemical substances are dangerous when present in excess, and can attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with degenerative diseases (Harborne, 1973;

Huisman et al. 2004). Cancer is a dreadful disease caused by abnormal and uncontrolled cell division. About 6 million new incidences of cancer are reported yearly worldwide. Nature has given man a variety of useful sources of remedies to cure a number of diseases. About 1500, natural products have played a significant role in drug discovery and development, especially agents active against cancer and infectious diseases (Ioannis, 2008). More than 70 per cent of all cancer deaths occurred in low- and middle-income countries. The WHO noted that tobacco use, alcohol use, low fruit and vegetable intake, and chronic infections from hepatitis B (HBV), hepatitis C virus (HCV) and some types of HPV are leading risk factors for cancer in low- and middle-income countries. Deaths from cancer worldwide are projected to continue rising, however, with an estimated 12 million deaths in 2030 (Lefer and Grandner, 2000). The most frequent types of cancer worldwide in order of the number of global deaths are; among men – lung, stomach,

liver, colorectal, oesophagus and prostate; and among women – breast, lung, stomach, colorectal and cervical. The need to screen medicinal plants for anticancer and antioxidant properties cannot be overemphasized.

Calliandra surinamensis is a perennial tree distributed in Nigeria, East and South Africa. The flower pigment is used as dye pigment for the manufacture of some drugs of pharmaceutical importance. The plant is known in the tropics for its ornamental/horticultural purposes. *Calliandra surinamensis* is a large multi-trunked shrub with detailed descriptive properties. The stem bark is used in folk medicine for the treatment of various disease and infections such as cough, wound healing, inflammations. Scanty information exists in the literature on the phytochemical and biological investigation of the plant. We have also reported the antioxidant activity, free radical, and proximate analysis of the flower pigment using DPPH assay procedure and in comparison with standard antioxidants (Liu and Wang 2008). The flower extract have also been shown to possess antibacterial properties (McCune and Johns (2005). The pharmacological importance of this plant motivated us to explore this species for its chemical constituents.

Therefore, the search for natural products represents an area of great research interest in which the plant kingdom has been documented the most important source to provide many antioxidant and cancer chemopreventive agents with novel chemical structure (McCune and Johns (2005).

Materials and Methods

Plant Material: Fresh stem bark of *Calliandra surinamensis* was collected from the Ugbowo Campus of the University of Benin, Benin City, Nigeria in April, 2009. Botanical identification and authentication was done by a Taxonomist Prof. M. Idu, Faculty of life Sciences, University of Benin, Benin City, Nigeria.

Phytochemical screening: The crude plant powdered material was subjected to phytochemical screening to test for the

presence of some secondary metabolites such as alkaloids, tannins, saponins, flavonoids and anthraquinones. Standard procedures were used in the determination of these metabolites (Sofowora, 1984; Trease et al. 1989; Siddhuraju et al. 2002).

Extraction and preparation of plant material: The fresh stem bark of *C. surinamensis* was dried at temperature of 30°C and reduced to a fine powder using a mechanical mill. The powdered sample (1Kg) was extracted with methanol (4 X 25 L) by maceration for 48 hours at room temperature. The combined methanol extracts were evaporated to dryness using a rotary evaporator at reduced pressure.

Cell line and culture: A-549 (human lung adenocarcinoma epithelial cell line) cell culture was obtained from Biomedicine Research and Development Centre of Jinan University (Guangzhou, China). The cell line was cultured in growth medium (RPMI-1640 medium, pH 7.4), supplemented with 10 % fetal bovine serum (FBS) and antibiotics [penicillin (100 units/ml) and streptomycin sulfate (100 µg/ml)]. The cell lines were grown in 50 cm² tissue culture flasks (Corning, NY, USA) and used for cytotoxicity assay.

Cytotoxicity assay: The cytotoxicity assay of the plant samples were determined using the method Zhao et al. (2007) MTT (3-(4, 5-dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium bromide) assay. In brief, human cancer cells were plated at 2×10^4 cells per well in 96 well microtiter plates (Costar 3599, Corning, NY, USA) with 100 µl RPMI-1640 growth medium and incubated for 24 h at 37°C, with 5 % CO₂ in a humidified atmosphere (Incu-Safe, Sanyo, Japan), during which period a partial monolayer was formed. Later, the medium was removed and fresh growth medium containing different concentrations (100, 50, 25, 12.5, 6.5, and 3.125 µg/ml) of the tested compound was added. After 2 days of incubation at 37°C, with 5 % CO₂, the growth medium was removed and MTT reagent (0.1 mg/ml) was added. After incubating at 37 °C for 4 h, the MTT reagent was removed and DMSO (100 µl) was added to each well and then shaken for another 15 min. The absorbance was then determined

by an ELISA reader (Bio-Rad, USA) at a wavelength of 492 nm. Control wells received only the media without the tested compound. The conventional anticancer drug, cisplatin, was used as a positive control in this study. The inhibition of cellular growth by the tested sample was calculated as the percent inhibitory activity and expressed as the IC₅₀ value (concentration of the tested sample to inhibit 50 % growth of the cells).

Quantitative test: The DPPH radical scavenging of compounds from *Calliandra surinamensis* extract (CSE) activity was measured by the method of Blois with minor modifications (Wang et al. 2007). The compounds were each dissolved in 10 ml of distilled water to a final concentration of 100 g/ml. Two milliliters of 0.2 mM DPPH in methanol was added to 1 ml of the test compound solution. The absorbance was measured at 517 nm after 20 min of incubation at 25°C. Quercetin was used as the positive control. The scavenging activity of DPPH radicals by the sample was calculated according to the following equation:

The radical scavenging activity was calculated using the formulae:

$$\% \text{ inhibition} = \left\{ \frac{Ab - Aa}{Ab} \right\} \times 100$$

Statistical analysis: Data were expressed as means ± standard deviations (SD) of three replicate determinations and then analyzed by SPSS V.13 (SPSS Inc., Chicago, USA). One way analysis of variance (ANOVA) and the Duncan's New Multiple-range test were used to determine the differences among these means. *P* values < 0.05 were regarded to be significant.

Results and Discussion

The results of the phytochemical screening as shown in Table 1 revealed the presence of alkaloids, tannins, flavonoids and saponins. There was complete absence of anthraquinones in the plant. The chemical principles previously isolated from the plant, and their chemical structures elucidated and

determined by spectroscopic techniques are shown in figure 1.

In Table 2, the results of the antioxidant property of the compounds 1- 6 revealed that compounds 1, 2 and 4 did not show antioxidant activity against DPPH radical at concentrations of 10, 25 and 50 µg/ml. The compounds therefore lacked DPPH antioxidant property. There was high absorbance and antioxidant activity of 5 and 6 using DPPH radical as control. Flavonoids have been shown to possess antioxidant activity (Zhao et al. 2007). The class of compounds called xanthenes is potential anti radical agents in medicinal plants.

The result of the anticancer activity presented in Table 3 showed that the compounds (except compound 5) were not active against Human lung cancer cell line A549 at the concentration investigated. Compound 5 gave an IC₅₀ of 106.35. Compounds 1 and 2 did not exhibit any anticancer activity at the concentration used. The inhibitory activity of the compounds against the cancer cell lines is an indication of the potential anticancer benefit of the compounds of *Calliandra surinamensis* stem bark extract. The flower of *Calliandra surinamensis* have been known to show significant antioxidant activity in a concentration dependent level^[8]. The presence of copious amount of flavonoids and xanthenes may probably be responsible for the marked antioxidant activity. These chemical constituents have been known to cause free radical scavenging property. The anticancer activity of some compounds isolated from the plant stem bark could be due to the potent antioxidant activity.

Table 1: Phytochemical components of *Calliandra surinamensis* stem bark

| Chemical components | Presence of components |
|---------------------|------------------------|
| Alkaloids | + |
| Saponins | + |
| Flavonoids | + |
| Tannins | + |
| Anthraquinones | - |

Conclusion

The study demonstrates the biological evaluation of the secondary metabolites from the plant. The significant antioxidant activities of the flavanol and xanthone glycosides lend support to the antioxidant property of the plant, for which the plant is

known and used for. The xanthone glycoside growth inhibitory effect on the Human lung cancer cell line A549 and antiradical scavenging activity indicates that the compounds has great potential to prevent disease caused by the overproduction of radical constituents.

Table 2: Antioxidant Activity of compounds 1- 6 from stem bark of *Calliandra surinamensis*

| compounds | 10µg/ml | 25µg/ml | 50µg/ml | Value of significance |
|-----------|-----------------|-----------------|-----------------|-----------------------|
| 1 | 2.22 ± 0.491 % | 3.30 ± 0.00 % | 7.25 ± 0.00 % | P<0.01 |
| 2 | 4.35 ± 0.058 % | 4.35 ± 0.058 % | 9.52 ± 0.346 % | P<0.01 |
| 3 | 27.95 ± 0.00 % | 56.31 ± 0.058 % | 80.12 ± 0.00 % | P<0.01 |
| 4 | 2.56 ± 1.24 % | 7.12 ± 0.0145 % | 10.18 ± 0.00 % | P<0.01 |
| 5 | 15.25 ± 0.242 % | 46.35 ± 0.067 % | 67.12 ± 0.012 % | P<0.01 |
| 6 | 22.45 ± 0.200 % | 36.45 ± 0.018 % | 71.02 ± 0.190 % | P<0.01 |

Values are mean± SEM p <0.0001, Comparing row means.

Table 3: Growth Inhibition Assay (Human lung cancer cell line A549)

| Compounds | Concentration (µg/mL) | | | | | | IC ₅₀ (µg/mL) |
|-----------|-----------------------|-------|-------|-------|-------|-------|--------------------------|
| | 6.25 | 12.5 | 25 | 50 | 100 | 200 | |
| 3 | 4.63 | 13.84 | 16.22 | 4.75 | 13.10 | 23.13 | - |
| 4 | 22.2 | 23.38 | 24.19 | 29.30 | 35.29 | 39.06 | 1416.23 |
| 5 | 12.93 | 26.46 | 34.07 | 35.93 | 47.14 | 59.63 | 106.35 |
| 6 | 4.41 | -9.94 | 5.79 | 9.04 | 0.87 | 19.35 | - |

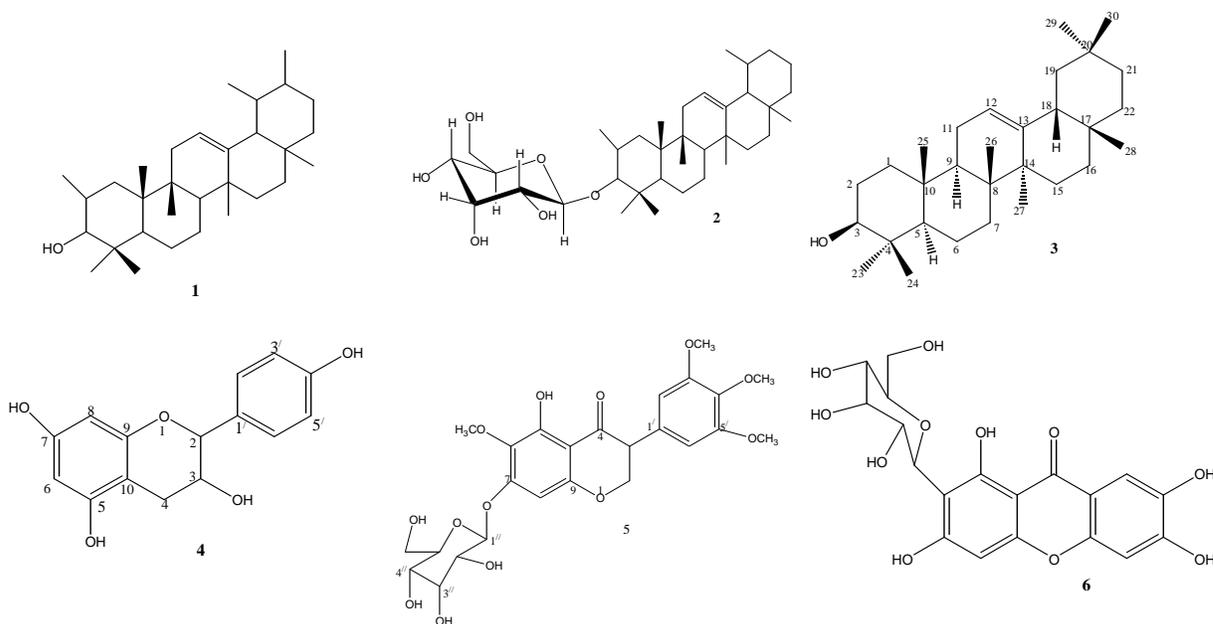


Figure 1: Chemical structures of isolated compounds of *C. surinamensis*

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