Original Article

Anticancer and antioxidant activities of *Guiera senegalensis*

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ABSTRACT

Background: Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments.

Objectives: The ethanolic extract of *Guiera senegalensis* was tested *in vitro* as anticancer and antioxidant agent as well as for its phenolic and flavonoidal contents.

Methods: The trypan blue technique was used for the anticancer activity against Ehrlich Ascites Carcinoma Cells (EACC) while the antioxidant activity of the plant extract was determined by 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assay. The total phenolic and flavonoidal contents were estimated using colorimetric methods.

Results: The ethanolic extract at a concentration of 13 µg/ml caused 100% cytotoxic effect against EACC. Moreover, it possessed a considerable antioxidant activity against DPPH radical. *Guiera senegalensis* extract was found to contain appreciable amounts of phenolic and flavonoidal compounds.

Conclusion: It can be concluded that *Guiera senegalensis* possesses sufficient *in vitro* anticancer and antioxidant activities to warrant further detailed study of its pharmacology and phytochemistry.

Key words: Anticancer, antioxidant, phenolic content, flavonoidal content, *Guiera senegalensis*

Cancer occurs due to some molecular changes within the cell [1]. Every year, millions of people are diagnosed with cancer, leading to death. According to the American Cancer Society, deaths arising from cancer constitute 2%–3% of the annual deaths recorded worldwide. Thus cancer kills about 3.5 million people annually all over the world. Several antitumor agents are used to treat cancer, but they cause toxicity that limits their usage[2, 3].

Functional foods and dietary supplements could augment the therapeutic effect and reduce the serious side effects of discovery of new anticancer agents from natural product sources[4].

Plants have been used for treating various diseases of human beings and animals since time immemorial. More than 50% of all modern drugs in clinical use are of natural product origins, many of which have the ability to control cancer cells and according to World Health Organization (WHO) estimates, more than 80% of people in developing countries depend on traditional medicine for their primary health needs [5].

Antioxidants are a group of substances that are useful for fighting cancer and other processes that potentially lead to diseases such as atherosclerosis, Alzheimer's, Parkinson's, diabetes and heart disease[6]. Oxidants such as reactive oxygen species (ROS) that include the superoxide radical ($O_2^{\cdot}$), hydroxyl radical ($^{\cdot}OH$), hydroperoxyl radical ($ROO^{\cdot}$) and reactive nitrogen species (RNS) such as

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peroxynitrite (.ONOO$^-$) and nitric oxide (NO$^\cdot$) damage macromolecules, including proteins, lipids, enzymes and deoxyribonucleic acid (DNA)[7] . Catalase, superoxide dismutase and peroxidase enzymes found in living organisms or nonenzymatic molecules, such as glutathione, cysteine, ascorbic acid, flavonoids and vitamin K are used by them to combat free radicals[7].

Guiera senegalensis (Combretaceae) is a shrub of savannah region of West and Central Africa. Its leaves are commonly used in traditional medicine in gastrointestinal disorders, respiratory infections and malaria[8].

The aim of this study is to investigate the anticancer and antioxidant activities as well as to estimate the phenolic and flavonoidal contents of *Guiera senegalensis* leaf extract.

MATERIALS AND METHODS

Plant material:

Guiera senegalensis was collected from west Sudan. The plant material was taxonomically identified and authenticated by taxonomy expert at Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Khartoum, Sudan where the voucher specimen has been deposited.

Extraction of plant material:

The plant sample was washed and dried at room temperature during one week. The dry sample was ground and extracted by maceration using ethanol (70%) in a conical flask for 48 hours, filtered and dried at room temperature and kept in a refrigerator until use.

Anticancer activity of *Guiera senegalensis* ethanolic extract:

Trypan blue assay was used to investigate the anticancer activity of the ethanolic extracts of *Guiera senegalensis* against EACC. A line of Ehrlich Ascites Carcinoma from National Cancer Institute (NCI) Cairo, Egypt has been used. The tumor line is maintained in female Swiss albino mice by weekly intraperitoneal (I.P) transplantation of 2.5×10^6 cells. The cells were taken from tumor transplanted animals after ≈ 7 days of transplantation. The cells were centrifuged at 1000 rpm for 5 min, washed with saline then the needed number of cells was prepared by suspending the cells in the appropriate volume of saline according to the tests used. Transplantation in animals for cell line, the appropriate volume of ascites can be used directly.

The viability percentages of tumor cells after incubation with the ethanolic extract as well as saline as control were measured by the modified cytotoxic trypan blue-exclusion technique[9]. Two ml of media containing EACC (2×10^4 cells) were transferred into a set of tubes each, then 13 µg/ml from the extract were added into the appropriate tube as well as saline. The tubes were incubated at 37°C for 2 h then centrifuged at 1000 rpm for 5 min and the separated cells were suspended in 2 ml saline. For each examined plant material and control, a new clean dry small test tube was used and 10 µl of cell suspension, 80 µl saline and 10 µl trypan blue (0.4%) were added and mixed, then the number of viable cells (non-stained) was calculated from the equation below using a homocytometer slide by microscope (Nikon, TMS).

$$\% \text{ viable cells} = \frac{\text{Number of viable cells}}{\text{Total number of cells}}$$

Antioxidant activity of *Guiera senegalensis* ethanolic extract:

Sample stock solution (1 mg/ml) was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in ethanol. One ml of a 0.3 mM 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in ethanol solution was added to a 2.5 ml solution of the different concentrations of the extract and allowed to react at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula below:

$$AA\% = \frac{\text{(Absorbance of control – Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Ethanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus ethanol (2.5 ml) was used as control. Stock solution (1 mg/ml) of quercetin was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in ethanol used as positive control[10].

A freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance at 518 nm. The purple colour disappears when an antioxidant is present in the medium. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of test compound to act as free radical scavenger. Furthermore, the "efficient concentration" or EC_{50} value (the concentration of antioxidant that causes 50% loss of the DPPH activity (colour) was also used to assess the antioxidant activity of the plant extract compared to the standard drug. The higher the antioxidant activity, the lower is the value of EC_{50}[11].

Determination of total phenolic content:

The total phenol content in the ethanolic extract of *Guiera senegalensis* was determined with Folin Ciocalteau reagent .The crude extract (50 mg) was mixed with Folin Ciocalteau reagent (1ml) and deionized water (7.5 ml). The mixture was kept at room temperature for 5 minutes and then 10 ml of 7% sodium carbonate were added to the mixture and then incubated for 90 minutes at room temperature. After incubation the absorbance against the reagent blank was determined at 760 nm using UV/visible spectrophotometer. The total phenolic content of the plants was expressed as mg/g Gallic acid equivalent. All samples were analyzed in triplicates[12].

Estimation of total flavonoidal content in *Guiera senegalensis* ethanolic extract:

Extract of plant material (1 ml containing 100 µg/ml was diluted with 4 ml water in 10 ml volumetric flask. Initially 5% Sodium nitrite (0.3ml) was added and after 5 minutes 10% Aluminum chloride was added followed by addition of 2 ml 1 M Sodium hydroxide after 6 minutes. Water (2.4 ml) was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm using UV/visible spectrophotometer. The total flavonoids content of the plant was expressed as mg/g Quercetin equivalent. All samples were analyzed in triplicates[12].

RESULTS

Anticancer activity:

The extract at a concentration of 13 µg/ml caused 100% inhibition of cancer cell growth (Table 1).

Antioxidant activity:

The antioxidant activity of ethanolic extract of *Guiera senegalensis* was displayed in Tables 2. In this quantitative assay the extract exhibited a notable dose dependent inhibition of the DPPH activity. At a concentration of 250 µg/ml, *Guiera senegalensis* extract scavenged 72.5% of DPPH radicals, whereas that of 125 and 50 µg/ml caused 63.5% and 50.5% DPPH inhibition respectively and very mild inhibition was produced at a concentration of 5µg/ml. The EC 50 of the extract was found to be 7.74. Meanwhile, the standard antioxidant agent quercetin showed a fairly constant inhibition.

Phenolic and flavonoidal contents:

As shown in Table 3, the phenolic and flavonoid contents were found to be 58 mg gallic acid/g of dry material and 36 mg quercetin /g of dry material respectively.

DISCUSSION

The anticancer activity produced by *Guiera senegalensis* leaf extract may be ascribed to the presence of a naphthyl butanone compound guieranone A[8, 13].

Much information is available on the inhibitory effects of specific plant dietary phenolic compounds on mutagenesis and carcinogenesis that make them promising candidates for cancer prevention [14-16].

The antioxidant activity of *Guiera senegalensis* could be attributed to certain phytoconstituents such as alkaloids[17], guirenone and other polyphenols[18]. In fact, research workers[19]

isolated 5-methylflavesperone and rhamnetin from *Guiera senegalensis* and demonstrated that while rhamnetin strongly inhibited peroxidation of phospholipids liposomes, the former possessed little or no such activity. Similarly, using a combination of techniques such as chromatography and spectroscopy, the presence of catechin, myrecitrin, rutin and quercetin in *Guiera senegalensis* have been described by other researchers[20]. That these phytochemicals are potent antioxidant compounds has been well demonstrated[21].

Furthermore, the present study revealed appreciable amounts of phenolic and flavonoidal compounds which may contribute to the anticancer and antioxidant activities of this plant.

Table 1: Anticancer activity of *Guiera senegalensis* ethanolic extract

Preparation	Concentration	Anticancer activity
Guiera senegalensis	13 µg/ml	100%

Table 2: DPPH scavenging activity of *Guiera senegalensis* ethanolic extract.

	Concentration				
Preparation	250 µg/ml	125 µg/ml	50 µg/ml	10 µg/ml	5 µg/ml
Guiera senegalensis	72.5%	63.5%	50.5%	45.5%	31.3%
Standard (quercetin)	89.7%	85.8%	62.1%	55.5%	45%

Table 3: Phenolic and flavonoidal contents of *Guiera senegalensis* leaf extract

Plant	Phenolic content (mg gallic acid /g)	Flavonoidal content (mg quercetin/g)
Guiera senegalensis	58 mg	36 mg

CONCLUSION

Based on the results obtained in the present study, it may be concluded that *Guiera senegalensis* possesses sufficient *in vitro* anticancer and antioxidant activities to warrant further detailed study of its pharmacology and phytochemistry.

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