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Demonstration Of Phenolics Content From *Garcinia Cambogia* Extract And Evaluation Of Its Cytotoxic Activity

Amani M.D. El-Mesallamy [a], Ahmed K.H. Alahwany[a], Mohamed I.M. El-Zaidy[a]*, Sahar A.M Hussein[b]

[a]*Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt*
[b]*Department of Phytochemistry and Plant Systematics, Pharmaceutical Industries institute, National Research Center,* 33 *El-Buhouth st., Dokki P.O. 12622, Giza, Egypt.*
***Corresponding author**: moh.elzainy@gmail.com*

***Abstract: Garcinia Cambogia* traditionally usually termed kokum butter is used as a medicinal plant. The current study sought to determine the alcoholic extract of *Garcinia cambogia* leaves'and flavonoid content and total phenolic as well as evaluate the plant's cytotoxic potential.The presence of several sorts of phenolics, flavonoids, tannins, carbohydrates and glycosides were shown by phytochemical screening of the ethanolic extract. The ethanolic extract total phenolic content (TPC) in terms of gallic acid equivalent were (0.48±0.014 mg GAE /g of extract powder), while the total flavonoid content (TFC) in terms of quercetin equivalent were (0.35±0.016 mg QE/g of extract powder). Since phenols and flavonoids are potentially active, we assessed the cytotoxic activity of in*vitro* on various human cell lines, including colon (HCT-116), liver (HEPG-2) and breast (MCF-7) cancer cell lines using doxorubicin as the traditional anticancer drug as reference. The outcomes this show that the ethanol extract of *G. cambogia* leaves was more effect on breast (MCF-7) cell line than liver (HEPG-2) and colon (HCT-116) cell lines becuase presence of high flavonoids and phenolics content.**

Keywords: Phytochemicals; *G. cambogia* leaves; Total phenolics; Total flavonoids; in*vitro* cytotoxic activity

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I. INTRODUCTION

The *G. cambogia* plant (Clusiaceae family) was originally grown in Asia before being introduced to Africa. This plant contains several organic acids, including benzophenones, xanthones, and hydroxycitric acid, which are used to help people lose weight. *G.cambogia* extract is used to cure a variety of ailments in Indian medicine, including leukemia, hemorrhoids, diarrhea, and dysentery.Seeds have been the subject of preliminary investigations, which shown their antifungal, anticancer, antihistaminic, antiulcerogenic, antibacterial, antiviral, and vasodilatory properties[1].

The study of medicinal plants can yield important information for the synthesis of phytotherapeutic agents and pharmaceuticals derived from plants. Gaining criteria for the quality requirements of herbs requires an understanding of their chemical compositions, which can be achieved through both analytical and phytochemical determinations. Therefore, before being commercialized, Product quality requirements need to be regularly checked, utilizing all available chemical and analyses of plants, for products intended for medicinal use [2]. Carbohydrates, minerals, proteins, vitamins and metabolites like phenolic acids and flavonoids can all be used to evaluate the nutraceutical qualities of medicinal plants. Their therapeutic effects also depend heavily on secondary metabolites like flavonoids and phenols. Fruits can potentially have therapeutic properties; the main secondary metabolites found in fruits are flavonoids and phenols [3]. Remarkable are the 600 species and 14 genera of Clusiaceae and the approximately 140 genera and 1200 species of the former Guttiferae family of medicinal plants [4], which was split into multiple families.

The plant genus *Garcinia* (=Rheedia) of Clusiaceae family and is found in tropical regions of Brazil, New Caledonia, Africa, Asia, and Polynesia. The various *Garcinia* species contain abundant and valuable amounts of bioactive chemicals that have important medicinal qualities, like painkilling and anti-inflammatory effects [5–7]. Clusiaceae species have yielded a wide range of chemicals, primarily polyisoprenyl benzophenones, flavonoids, and xanthones [8,9]. Thus, *Garcinia* species have proven to be rich suppliers of chemicals with therapeutical characteristics [11-13]. There are 400 different species of *Garcinia* species, which are evergreen polygamous trees and bushes that grow in both the tropical and subtropical hemispheres forests. seventeen of the thirty-five garcinia speciesare reported to be from India's Western Ghats. whereas *G. cambogia* has anti-cancer properties against a number of cancer forms, including colon and adenocarcinoma cervix and colorectal cancer have been documented.

Despite the assertions and little usage is known about the use of *G. cambogia* to treat different malignancies known and proven. Thus, the current study is intended to investigate the bioactive compounds present in *G. cambogia* leaves and evaluate the in*vitro* cytotoxic activity on various human cell lines.

II. MATERIALS AND METHODS

Collection of Plant

The fresh *G. cambogia* leaves (500 gm) was collected from the Zagazig-Sharqia field at the edges of cultivation on March 2022, and identified by Professor of Plant Taxonomy, Alaaeldin Sayed Ewase, Ministry of Environment. A voucher specimen was deposited in CAIRC; the herbarium of the National Research centre, Cairo, Egypt.

Extraction

The leaves air-dried powder (100 g) was defatted with petroleum ether at 60-80o, the residue was extracted with 90% ethanol till exhaustion then evaporated under reduced pressure at 70 °C to give an aqueous ethanol extract. The remaining defatted aqueous ethanol extract (6.55g) was evaporated and kept for investigation as well.

Chemicals Reagent

Analytical-grade necessary compounds were procured from Sigma-Aldrich and Merck (Germany).

I. Chemical Studies

1. Phytochemical analysis

G. cambogia leaves ethanolic extracts were exposed to phytochemical screening for the presence of phenolic acids, flavonoids, glycosides, tannins and carbohydrates compounds according to the procedures outlined by [14] **(Table 1)**.

2. Quantitative analysis

Quantitative analysis is a useful approach for determining the amount of phytoconstituents contained in plant extract. It was done at Zagazig University in Egypt's Department of Chemistry, Faculty of Science.

2.1-Total Phenolics Content Estimation.

The total phenolic contents of ethanolic extracts of *G. cambogia* was determined using Folin-Ciocalteu reagent. [15]. The absorbance was measured at $\lambda = 750$ nm. The total phenolic content was expressed as mg gallic acid/g and calculated as follows:

The total phenolic content = Conc. $_{(gallic)} \times V \times m/ M$,

Where Conc. (gallic) is the concentration of the standard (gallic acid) established from the calibration curve, V is dilution factor, m is total extract wt (g) and M is the concentration of dry plant extract.

2.2-Total Flavonoids Content Estimation

The total flavonoid contents in the ethanolic extract of *G. cambogia* was measured using an aluminum chloride reagent following a previously reported spectrophotometric method [15]. The absorbance of the reaction mixture was read out at 430 nm. The total flavonoid content (TFC) was expressed as mg quercetin/g DW extract and calculated as follows:

TFC= Concentration $_{(quercetin)} \times V/ m$

Where Conc. (quercetin) is the standard concentration of (quercetin) constituted from the calibration curve ,V is the extract volume in mL, and m is total extract wt (g).

II. Biological Studies

In*vitro* Cytotoxic Activity

Cancer cell lines from the human colon "HCT-116", liver "HEPG-2", and breast "MCF-7" were obtained from the tissue culture unit of the National Research Centre in Giza, Egypt.
In 96-well plates, the water-soluble yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is changed into an insoluble purple formazan by mitochondrial reductase as part of the MTT test utilising the sub-culturing and culturing techniques established by (Alley et al., 1988) and a laminar air-flow cabinet [16].

Analytical Statistics

Version 23 of the Statistical Package for the Social Sciences (SPSS) was used to statistically analyze the data. (Created using IBM SPSS software, which is based in the US.) An average standard error of mean (SEM) was used to display the data.

III. RESULTS AND DISCUSSION

1-Preliminary Phytochemical Screening:

G. cambogia leaves ethanolic extract Phytochemical screening revealed the presence of several phytoconstituents such as flavonoids, phenolic acids, tannins, glycosides and carbohydrates compounds.

One of the most numerous and common types of plant metabolites is phenolic chemicals. In addition to their free radical terminator activity or antioxidant, flavonoids and other plant phenolics have been shown in the literature to have a number of other biological effects [17].

our previous research reported that, the major compounds in *G. cambogia* leaves extract; guanosine, prephenic acid, tetrahydroxy-cholanic acid, limocitrin, 6,7-dihydroxycoumarin-6-glucoside and hydroxy citric acid were shown **(Fig.1)** [18].

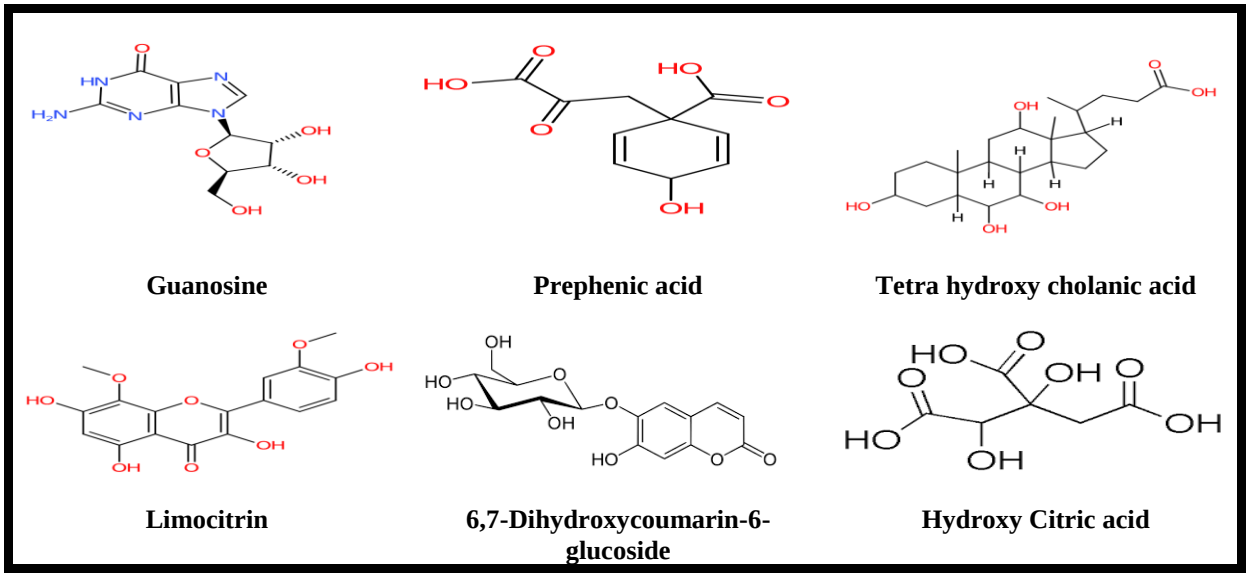

Fig. (1): Chemical structures of bioactive compounds in *G. cambogia* ethanolic extract.

The findings of this study show that the identified phytochemical substances may be the bioactive ingredients, and these plants are proving to be an increasingly useful reservoir of bioactive chemicals with significant medical value.

2-Estimation of Total Phenolic and Flavonoids Content

In terms of gallic acid equivalent, the total phenolic acid contents of the ethanol extracts of *G. cambogia* leaves were 0.48±0.014 mg GAE /g of extract powder, while the total flavonoids contents in terms of quercetin equivalent were 0.35±0.016 mg QE/g of extract powder, as shown in (**Table 1**).

Table 1: Total Phenolic And Flavonoids Content Of Ethanolic Extract Of *G. Cambogia*

Estimation	*G.cambogia* leaves ethanolic extract
Total Phenolics (mg GAE/g)	0.48±0.014
Total Flavanoids (mg QE/g)	0.35±0.016

Each value represents the average of three analyses ± standard deviation.

Because of their antioxidant characteristics, large quantities of phenolic acids and flavonoids improve the plant's ability to defend itself against insects, bacteria, fungi, and viruses [19].

Assessment of biological activity

In*vitro* cytotoxic activity

The in *vitro* cytotoxic activity of *G.cambogia* ethanolic extract on diverse human cell lines, including colon (HCT-116), liver (HEPG-2), and breast (MCF-7) cancer cell lines, was examined in contrast to conventional anticancer medication (doxorubicin). According to the findings in (**Table 2**), the ethanolic extract displayed moderate antiproliferative activity against breast (MCF-7) and liver (HEPG-2), while lower against colon (HCT-116).

The ethanolic extract of *G. cambogia* demonstrated a moderate cytotoxicity effect against breast (MCF-7) and liver (HEPG2)cancer cell lines **(Fig. 2)**, as evidenced by the low IC_{50} values (48.23 ± 9.31 and 59.3 ±10.07µg/mL, respectively) , whilst the cytotoxicity effect on HCT-116 was considered weak activity with IC_{50} values (84.6 ± 12.2 µg/mL), in comparison to doxorubicin drug is demonstrated by values (2.7 ± 0.06 , 5.03 ± 0.7and 4.8 ± 0.6) against human cancer cell lines (HEPG-2, MCF-7 and HCT-116), respectively.

Table 2: In*vitro* cytotoxic activity *G. cambogia* ethanolic extract towards liver cancer (HEPG-2), human colon (HCT-116) and Breast (MCF-7) cell lines.

Extract	Colon (HCT-116) IC_{50} [µg/ml]	Liver (HEPG-2) IC_{50} [µg/ml]	Breast (MCF-7) IC_{50} [µg/ml]
***G. cambogia* ethanolic extract**	84.6 ± 12.2	59.3 ±10.07	48.23 ± 9.31
Doxorubicin	4.8 ± 0.6	2.7 ± 0.06	5.03 ± 0.7
DMSO	NA	NA	NA

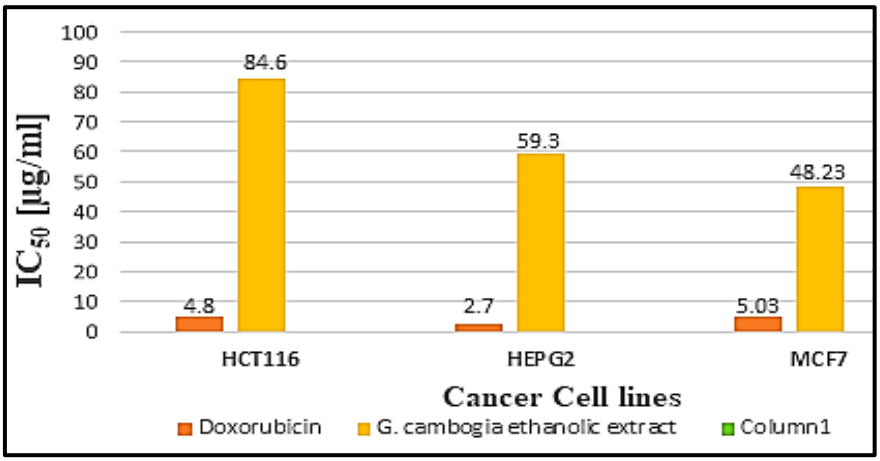

Fig. (2). IC_{50} of ethanolic extract of *G. cambogia* in comparison to Doxorubicin on the HCT-116, HEPG-2 and MCF-7 cancer cells.

Current anticancer medications are cytotoxic and have significant negative effects on numerous human organs. Because of the restrictions on the use of synthetic anticancer drugs, As alternatives that are less harmful to normal cells, have a greater therapeutic index, a different mechanism of action, and shorter treatment cycles, new medicinal plant extracts need to be assessed and generated. [20].

According to this study, *G. cambogia* crude extract displayed a cytotoxic effect on various types of cancer cell lines, stronger than typical cell lines. This medication has been used as an oral contraceptive for men and has anticancer characteristics, especially against bladder carcinoma and melanoma [21].

The flavonoids and phenolics included in *G. cambogia* ethanolic extract may be responsible for its anticancer effects. It is suggested that the high concentration of separated phenolic and flavonoid chemicals in ethanolic extract is responsible for its activity. These compounds feature various hydroxy groups in their flavonoid structure, This, when combined with a highly conjugated electron system, enables them to function as free radical scavengers through hydrogen atom or electron donation activities. Furthermore, they can stop the production of reactive oxygen species (ROS) such hydroxyl radicals by chelating redox-active transition metal ions [22]. This could lead to a more accurate cancer diagnosis by reducing things like DNA oxidative damage. The current anticancer drugs are astonishingly cytotoxic and show severe unfavorable effects on a variety of human tissues at the clinical level. These limitations on the use of synthetic anticancer compounds would necessitate the development of new medicinal plant extracts as alternatives that are less toxic to healthy cells, have a higher therapeutic index, a different mechanism of action, and require fewer treatment cycles. "*G. cambogia* extract clearly demonstrated a cytotoxic impact on cancer cell lines of various types, with greater potency than normal cell lines," according to the study's conclusions. This medication is used to treat male infertility and has anticancer characteristics. It works well for bladder cancer and melanoma [23,24]. It's unknown which biochemical and/or molecular pathways are involved in the production of such cytotoxic/antiproliferative biological effects. Given the underlying mechanisms in cancer cell death, significant work is being done to integrate this understanding into rational design and development of innovative options of treatment to boost the effectiveness of chemotherapeutic drugs [25]. The current study discovered that the ethanolic extract was cytotoxic to HEPG-2 and MCF-7 cancer cell lines, except for HCT-116, which was resistant (IC_{50} value 84.6 ± 12.2 µg/mL).

IV-CONCLUSION

The findings of this study indicate that phenolic acids, flavonoids, tannins, glycosides, and carbohydrates are present in *G. cambogia* ethanolic extract, which can be an excellent choice for biological and chemical analysis and can be further subjected for the isolation of the therapeutically active compounds with anticancer potency. As a result, the anticancer efficacy of ethanolic extract of *G. cambogia* was evaluated in*vitro* against several human cell lines, including colon (HCT-116), liver (HEPG-2) and breast (MCF-7) cancer cell lines, in comparison to Doxorubicin, the traditional anticancer drug. *G. cambogia* ethanol extract has showed a cytotoxic impact on HEPG-2 and MCF-7 cancer cell lines, except for HCT-116 due to presence of high flavonoids content and phenolics content.

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