**In-vitro** antioxidant and selective cytotoxicity of *Garcinia cambogia* and *Garcinia indica* leaf extracts on human kidney cancer cell line

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**ABSTRACT**
Kidney cancer is one among the top 10 cancers. Renal cell carcinoma (RCC) is commonly known kidney cancer. Almost all clinically targeted drugs used in treating RCC have many aftereffects. To overcome this problem with herbal products, the present study is aimed to investigate the preliminary phytochemicals, antioxidants and selective cytotoxicity of aqueous leaves extract of *Garcinia cambogia* (GC) and *Garcinia indica* (GI) on HEK-293 human embryonic kidney cells and (A498) human renal carcinoma cells. The phytochemical analyses were done using standard protocols. **In-vitro** antioxidant activity was carried out using DPPH, FRAP, and Phosphomolybdenum assay. Anticancer activity on A498 kidney cancer cell line was evaluated by MTT assay and the selectivity index was calculated. Preliminary phytochemical analysis of GC and GI leaf extract divulged the presence of various phyto-constituents. GI extract revealed higher phenolic content while flavonoid content was more in GC extract compared to alkaloids and saponins. Both plant extracts exhibited higher antioxidant capacities based on the test performed. GC and GI leaf extract was selectively cytotoxic **in-vitro** to (A498) human renal carcinoma cells and can be safely used against kidney cancer at 500 µM (GC extract) and 300 µM (GI extract). Selective index for GC and GI extract was 15.9 and 2.4 respectively. The results indicate that GC and GI extracts are a favourable antioxidant and anti-cancer agents for A498 human renal carcinoma cells. However, further studies to isolate the bioactive compounds responsible for these activities are underway and to explore their molecular mechanism.

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**INTRODUCTION**
Cancer holds the top second rank when it comes to deadly disease of today’s human population in entire Europe (Liu et al., 2019; Rutz et al., 2019). Among the 10 common death-causing cancers, kidney cancer is one of the debilitating health problems world wide bannering RCC as the most commonly known renal malignancy (Xu et al., 2020; Swiatek et al., 2020). Couple of years ago, 1.8 crore (18 million) new cancer cases were diagnosed globally. RCC constituted around 2% of them; although it is relatively rare compared to other cancers, both incidence and mortality are raising in an alarming rate of 3% per decade. Recent studies report about 3% of adult cancer patients suffer from malignant kidney tumour (Rutz et al., 2019). The diagnosing techniques for renal cancer have advanced in different methods over the past decades. Surgery is the best therapy for early-stage kidney cancer, but sur-
vival rate was hardly 1 year. Specific therapies pre-
vailed for advanced kidney cancer in the clinic since
the disease is unsusceptible to chemotherapy (Wang
et al., 2019). Researchers all over the world are
effectively working on exploring a cost-effective
anticancer drug with little or no side effects. In
the last fifteen years, more than 1,100 anticancer drugs
were developed. Out of which, only few drugs are in
favourable state to get the approval from Food and
Drug Administration, however, most of them are
in clinical trials (Liu et al., 2019; Millimouno et al.,
2014) reported that anticancer drugs target growth
factors, tumour suppressor proteins, apoptotic pro-
teins, and transcription factors. These drugs avail-
able in the market inhibits the cancer development
effectively; However, the chances of second can-
cer or the side effects caused by these drugs are
unavoidable. This affects the life style of the cancer-
 surviving patients. Therefore, it is essential to dis-
cover a plant-based drug, which is a potent anti-
cancer drug with low cost, eco-friendly and less after
effect.

Since ancient times, natural compounds are used as
a source in developing new drugs (Caparica et al.,
2020). Cancer, diabetes, rheumatoid arthritis, stroke
and coronary heart diseases are some of the fatal
diseases, which are generally caused by ROS such as
hydroxyl radical, super oxide anion and other exogenous factors. However, medicinal plants possessing antioxidant activity could be a
potential lead for curing the above mentioned dis-
esases (Geetha et al., 2020). Research into medi-
cinal plants also provides essential knowledge about
nutraceuticals and herbal medicines due to enriched
amount of secondary metabolites produced by
them. Among many nutraceutical plants, Garcinia
species are known to possess nutraceutical prop-
teins and proved to be rich sources of compounds
with relevant therapeutical properties (Santo et al.,
2020). Garcinia species are evergreen polygamous
trees and shrubs covering a total of 400 species
which covers both the hemispheres of tropical
forests. Seventeen out of thirty-five garcinia species
are reported from Western ghats, India (Seethapathy et al., 2018). Among which, GC and GI are
widely seen in the southern parts of Western Ghats,
and are natively marketed as Kodampulior Gummi
Gutta and Punarpuli or Kokum, respectively. The
bioactive compounds present in GC and GI leaves
were reported in our previous report (Jayakar et al.,
2020). Based on the phytochemicals present, the
current study was carried out in order to evaluate the
cytotoxicity using MTT assay. Literature reveals
that anti-cancer activity of G. indica against various
types of cancers such as gall bladder (Duan et al.,
2018), human breast cancer (Ahmad et al., 2010),
prostate, colon, pancreatic, and leukemia (Saadat
and Gupta, 2012), whereas anti-cancer activity of G.
cambogia against various types of cancer like colon,
adenocarcinoma (Banu and Ramakrishnaiah, 2018)
colorectal, cervical cancer (Hart and Cock, 2016)
have been reported. Despite the claims and the use
of GC and GI for treating various cancers, little is
known and documented. Hence, the present study
is aimed to explore the in vitro antioxidant activ-
ity and to analyse preliminary phytochemicals along
with their cytotoxic effects on Kidney cell lines. This
is the first report, as per our knowledge, in evalu-
ating the possible beneficial interaction of aque-
ous extracts on viability of the human renal cancer
cell line (A498) and human embryonic renal cell line
(HEK 293) by using MTT assay as an in vitro tech-
nique as well as determining its selective cytotoxic-
ity.

MATERIALS AND METHODS

Authentication of selected plants
The leaves of G. cambogia (GC) and G. indica
(GI) were collected from the Central Horticultural
Experiment Station, Chettalli, Kodagu district of
Karnataka, India in the month of September 2020.
Both species of Garcinia were identified and authen-
ticated by Principal Scientist at PND Herbarium,
Mangalore, Karnataka, India vide letter no. SKPND:
CR: 113: Herbarium Collection/19-20. The herbar-
ium is kept at PND Herbarium, Mangalore as G.
indica (accession no. 2286) and G. cambogia (acces-
sion no. 9743) for further reference.

Preparation of plant extracts
Fresh plant leaves of GC and GI were cleansed
in running tap water followed by deionised water
and shade dried. The air-dried leaf samples were
crushed into a coarse powder using mixer grinder.
The powdered sample was stored in airtight brown
bottle at 4°C till further use. 50 grams of shade-dried
leaves were crudely powdered before mixing with
500ml of double distilled water and kept in a shaker
incubator for 24 hrs. Temperature was set to 37°C
and the incubator was set to 150 rpm. Muslin cloth
was used to filter the extract and then by Whatman
no. 1 filter paper. The filtrate was evaporated in
hot air oven at 50°C till dryness and residue was
scraped and stored at 4°C until further use.

Phytochemical screening
The aqueous crude extracts of GC and GI leaves were
separated using water to ensure obtaining bioac-
tive constituents, which were qualitatively, screened
for secondary metabolites like phenols, alkaloids,
saponins, tannins, flavonoids and glycosides using standard procedures (Lokapur et al., 2020).

**Total alkaloids content assay**

The total alkaloid content present in GC and GI leaves extract was determined using standard protocol (Lokapur et al., 2020). The alkaloid content was expressed as mg/100 g.

**Total saponin content assay**

Saponins present in the extracts were quantitatively determined using (Nahapetian and Bassiri, 1975). The saponin content was calculated in percentage.

**Total phenolic content assay**

The amount of total phenol content (TPC) present in both plant extracts was quantitatively determined using Folin-Ciocalteu’s colorimetric method (Noreen et al., 2017). Gallic acid was used as a standard while TPC was expressed as mg/g Gallic acid equivalent (GAE).

**Total flavonoids content assay**

The quantity of flavonoids present in the extracts were quantified using the aluminium chloride assay (Iqbal et al., 2015). The total flavonoid content is expressed as mg/g quercetin equivalents of the extract.

**In vitro methods to determine antioxidant activity**

**Ferric ion reducing antioxidant power (FRAP) assay**

FRAP assay was used to determine the total antioxidant power of the extracts. The FRAP assay was performed according to Benzie and Strain (1996) with slight modifications. Aqueous plant extract of GC and GI in varied concentrations ranging from 100μg to 500μg/ml were mixed with 2.5 mL of 0.2 mM phosphate buffer (pH 7.4) and 2.5 mL of potassium ferriyanide [1% weight/volume (W/V)]. Temperature was set to 50°C and the resulting solution was incubated for 20 minutes. Later 2.5 mL of TCA (10% W/V) was added and centrifuged for 10 minutes (3000 rpm). Then, 2.5 mL of deionised water was added followed by 0.5 mL of ferrous chloride (0.1% W/V). Finally, the optical density was measured at 700 nm. A positive reference standard, ascorbic acid, was used to compare the antioxidant property of GC and GI extracts.

**Phosphomolybdenum (PM) assay**

PM assay was used to estimate the total antioxidant activity using the standard procedure (Ghagane et al., 2017). Aqueous leaf extract of GC and Glin different concentrations ranging from 100μg to 500μg/ml were added to each test tube individually containing 3 mL of distilled water and 1 mL of molybdate reagent solution. These tubes were kept incubated at 95°C for 90 minutes. After incubation, they were maintained at room temperature for 20-30 minutes and the optical density was measured at 695 nm. Ascorbic acid was used as the positive standard reference.

**2, 2-Diphenyl-1-picrylhydrazyl radical scavenging ability (DPPH) assay**

Free radical scavenging effect of aqueous plant extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) with slight modifications put forward by (Brand-Williams et al., 1995). In brief, the concentrations (100- 500μg/ml) of extracts were prepared. 1 mL of DPPH solution (0.004% prepared in ethanol) was treated with 1 mL of aqueous leaf extracts and standard ascorbic acid solution separately. The mixture was left for incubation in the dark under room temperature for 30 minutes and the optical density was measured at 517 nm. The extent of DPPH-purple decolourization to DPPH yellow confirmed the scavenging efficiency of the extract. Higher antioxidant activity was observed as the optical density of the reaction mixture was decreased. Scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity } (\%) = \frac{A_c - A_t}{A_c} \times 100$$

$A_c$ - the absorbance of the control reaction (1 ml of ethanol with 1 ml of DPPH solution); $A_t$ - the absorbance of the test sample.

The results were analyzed in triplicates. The IC$\text{_{50}}$ value indicates the required sample concentration to inhibit 50% of the DPPH free radical.

**In-vitro cytotoxicity assay**

**Culturing of cell lines**

The human renal carcinoma cells (A498) and Human embryonic kidney cells (HEK 293) were acquired from the NCCS, Pune, India. Cell lines were maintained using Dulbecco’s Modified Eagle Media (DMEM, Invitrogen, USA) with low glucose and supplemented with 10% Foetal Bovine Serum (FBS, Invitrogen, USA). Antimycotic 100X solution were added to the medium to prevent bacterial contamination. The medium with cell lines was maintained in a humidified environment with 5% CO$_2$ at 37°C. Cells were detached by treatment with trypsin-EDTA after reaching 80% confluency, and reseeded in fresh media.

**Treatment groups**
The cytotoxicity activity of the aqueous extracts of GC and GI was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT) assay by following Tangjitjaroenkun et al. (2021) with slight modifications. Briefly, A498 and HEK293 cells were seeded at a density of $1 \times 10^4$ cells per well in 96-well flat-bottom micro plate and controlled at 37°C in 95% humidity and 5% CO$_2$ humidified atmosphere for overnight before the treatment. These cells were treated with a varied concentration ranges of GC and GI extracts (100-500 $\mu$g/mL) followed by incubation for 48 hours respectively, which was the standard treatment time of the extracts in each of the cell lines. Cisplatin was used as a standard drug to compare the effects induced for HEK293 human embryonic kidney cell and A498 human kidney cancer cell lines. The current study was carried out with the treatment groups in the following set up. Negative control: only cells. Positive control: cells + cisplatin. Test groups: cells+ aqueous GC extract; and cells+ aqueous GI extract; same procedure was followed for human embryonic kidney cell (HEK-293) line.

MTT cell viability assays

After 48h incubation, the images were captured using phase contrast microscopy. During successive follow up, cells which were attached to the well were removed by trypsinization and the wells were washed two times with phosphate buffered saline (pH 7.4) without serum and 20 $\mu$L of the MTT staining solution was added to each well and further incubated at 37°C for next 4 hrs. Formazan crystals were dissolved by adding DMSO (100 $\mu$L) to each well, and optical density was quantified spectrophotometrically at 570 nm against the control using micro plate reader (Bio-Rad, California, USA). Experiment was carried out in triplicates and the percent-

\[
\text{Viable cells (\%)} = \left( \frac{\text{Mean OD of test compound}}{\text{Mean OD of Negative control}} \right) \times 100
\]

Selectivity index (SI)

The A498 and HEK293 cells were used to measure the SI. The SI, which represents the cytotoxic selectivity (i.e. drug safety) for both plant extracts was calculated using the following formula (Ogbole et al., 2017).

\[
\text{SI} = \frac{\text{IC}_{50} \text{ calculated for normal}}{\text{IC}_{50} \text{ calculated for cancer}}
\]

The SI values > 2 is considered as high selectivity (Machana et al., 2011; Prayong et al., 2008).

Statistical analysis

The study was carried out in triplicates and results were expressed as mean ± SD. Mean, standard deviation, variation and level of statistical signifi-
Table 1: Preliminary phytochemical analysis of aqueous extracts of GC and GI

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>GC</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*: positive; : negative

Table 2: Quantitative analysis of aqueous extracts of GC and GI

<table>
<thead>
<tr>
<th>Secondary Metabolites</th>
<th>GC</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total alkaloid (mg/g)</td>
<td>0.243±0.002</td>
<td>0.059±0.003</td>
</tr>
<tr>
<td>Total saponin (mg/g)</td>
<td>1.323±0.025</td>
<td>1.423±0.030</td>
</tr>
<tr>
<td>Total Phenol (mg GAE/g extract)</td>
<td>1.05±0.49</td>
<td>3.0±0.47</td>
</tr>
<tr>
<td>Total Flavonoid (mg QE/g extract)</td>
<td>2.35±0.49</td>
<td>2.21±0.33</td>
</tr>
</tbody>
</table>

Values are mean of triplicate determination (n=3) ± standard deviation; GAE–Gallic acid equivalents; QE-Quercetin equivalents.

Table 3: Percentage inhibition of DPPH radical scavenging activity of GC and GI

<table>
<thead>
<tr>
<th>Concentration (mg)</th>
<th>G. cambogia extract</th>
<th>Percentage of Inhibition</th>
<th>Standard Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G. indica extract</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>35.52±0.43</td>
<td>20.78±0.78</td>
<td>64.90±0.50</td>
</tr>
<tr>
<td>200</td>
<td>39.61±0.35</td>
<td>25.68±1.57</td>
<td>80.31±1.52</td>
</tr>
<tr>
<td>300</td>
<td>43.08±0.57</td>
<td>38.18±2.07</td>
<td>89.03±2</td>
</tr>
<tr>
<td>400</td>
<td>48.16±0.50</td>
<td>46.31±0.29</td>
<td>95.15±1</td>
</tr>
<tr>
<td>500</td>
<td>51.60±0.21</td>
<td>51.49±0.14</td>
<td>98.78±1</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value</td>
<td>493.7</td>
<td>485.1</td>
<td>68.68</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD, n = 3. Results were analyzed using descriptive statistics.

Table 4: IC<sub>50</sub> and R<sup>2</sup> values of aqueous leaf extracts of GC, GI and Cisplatin on two renal cell lines by MTT

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>HEK293</th>
<th>A498</th>
<th>Selectivity [SI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous G. cambogia extract</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</td>
<td>ND</td>
<td>291.2</td>
<td>15.93</td>
</tr>
<tr>
<td></td>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.9661</td>
<td>0.9938</td>
<td></td>
</tr>
<tr>
<td>Aqueous G. indica extract</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</td>
<td>628.2</td>
<td>255.9</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.9618</td>
<td>0.8939</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</td>
<td>6.676</td>
<td>2.832</td>
<td>2.36</td>
</tr>
<tr>
<td></td>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.9272</td>
<td>0.9322</td>
<td></td>
</tr>
</tbody>
</table>

HEK293- Human embryonic kidney cells
A498- Human kidney cancer cell
SI values > 2 was considered as high selectivity.
ND - IC<sub>50</sub> > 1000 µg/mL is considered as not determined (ND).
cance between groups was analyzed using descriptive statistics. Percent inhibition of cell growth was analyzed and $P < 0.05$ and $P < 0.01$ was considered statistically significant.

RESULTS AND DISCUSSION

Qualitative and quantitative phytochemical analysis

Results of preliminary phytochemical analysis obtained from aqueous extracts of GC and GI revealed the presence of diversity of secondary metabolites. Steroids were present only in aqueous GC extract while tannin and resin were absent in both extracts. Contrary to this, alkaloids, flavonoids, saponins, glycosides, cardiac glycosides, terpenoids and polyphenols were present in both extracts (Tables 1 and 2). Existence or lack of bioactive compounds may be due to the solvent medium used for extraction. These bioactive components are responsible for biological activities (Hemshekhar et al., 2011). Both GC and GI showed highest quantity of flavonoid and phenolic compounds compared to saponins, and alkaloids. However, GC extract contains highest flavonoids ($2.35\pm0.49$) and saponin as second highest level followed by phenolic compounds and alkaloids whereas GI contain highest phenolic compounds ($3.0\pm0.47$) followed by flavonoids, saponins and alkaloids. Alkaloids were found to be the least in both plant extracts (Table 2).

Garcinia species are widely used for their phenolic compounds such as flavonoids, phenolic acids, xanthones, biflavonoids and benzophenones (Han et al., 2007). Flavonoids were known to possess many biological properties like anti-inflammatory, antimicrobial, enzyme inhibition, anti-allergic, antioxidant and anti-tumour activity (Harborne and Williams, 2000). Even though both the Garcinia species showed least alkaloid content, its presence in plants can be used in medicine as aesthetic agents (Hong and Wrolstad, 1990). Saponins are traditionally used as detergents, and pesticides as well as advantageous health effects apart from their industrial applications as foaming and surface-active agents (Shi et al., 2004).

Antioxidant activity of plants

As health-related risks are increasing at an alarming rate, evaluation of plant-derived antioxidants is of immense importance in today’s context. Many bioactive components like phenols and flavonoids acts as sources of antioxidants and perform scavenging activity (Diplock, 1997). In order to obtain the complete potential of antioxidants from any source, it is wise to utilise different assays while estimating the total antioxidant activity (Sethi et al., 2020). In the present study, FRAP, PM and DPPH were used to evaluate in vitro antioxidant capacity of aqueous extracts of GC and GI.

FRAP assay

In the current study, the antioxidants present in the extracts would result in the reduction of ferri cyanide $\text{Fe}^{3+}$ to ferro cyanide $\text{Fe}^{2+}$ by contributing an electron, which was measured spectrophotometrically at 700 nm. Standard ascorbic acid and aqueous leaf extracts of GC and GI were subjected to FRAP assay. The experimental data revealed that the aqueous crude extract of GI showed stronger FRAP activity compared to GC (Figure 1). In this
Figure 5: Morphological changes showing inhibition of A498 and HEK-293 cell line for 48 h. CS: Cellular shrinkage; BL: Membrane blebbing (Magnification for A498 was 40X and HEK-293 was 20X).

PM assay

Phosphomolybdenum method was selected to analyse the total antioxidant activity of the sample. It is a colorimetric method, which helps in measuring the reduction of Phosphate-Mo (VI) to Phosphate-Mo (V) by the sample and eventually development of a bluish green coloured Phosphate-Mo (V) complex (Prieto et al., 1999). In the current study, Phosphomolybdenum assay showed better result in aqueous crude extract of GI extract compared to GC (Figure 2). In this Data is expressed as mean ± SEM (n=3). Statistical significance was assessed using one way ANOVA (* p <0.05) as compared to standard group. For FRAP and PM assay, both the plants exhibited higher activity with increasing concentration compared to standard ascorbic acid.

DPPH assay
In the current study, the varied concentrations of GC and GI leaf extracts were subjected to 2-diphenyl-1picrylhydrazyl free radical scavenging assay. Ascorbic acid was used as a standard drug to compare the antioxidant capacity of the extracts. Aqueous crude extract of GC showed stronger antioxidant activity than GI (Figure 3). In this Each value is expressed as means ± standard deviation. Concentration (µg/ml) take non x-axis and percentage inhibition taken on y-axis. When the antioxidant activities of both the plants were compared, GC showed the IC50 value of 493.7 µg/mL and GI showed IC50 value of 485.1 µg/mL with R² value of 0.9430 and 0.9536 respectively (Table 3). Even though both the crude extracts of Garcinia species showed good antioxidant activity when compared to standard, GC showed slightly better result with consistency at different concentrations while GI gradually increased along with the concentration. The difference in the IC50 value and potential DPPH radical scavenging activity observed in this study may be due to the phytochemical components present in the extracts. Our observation is in agreement with the studies of (Izuegbuna et al., 2019).

Cytotoxicity

Plant extracts are the best sources to evaluate the anticancer activity with least or no side effects for safe diagnosis. It helps in identifying the deep-rooted toxicity of the plant and the effects of critical overdose. It could also help in screening possible cytotoxic properties of GC and GI leaf extract (Gha-gane et al., 2017). In the current study, the MTT assay was used to evaluate the influence of aqueous extract (0–500 µM; 48 h) treatment on the cell viability of two kidney cell lines, HEK293 human embryonic renal cells and A498 human renal carcinoma cells. It is one of the most commonly used in vitro model system to evaluate the cytotoxic effects of many toxic substances and plant extracts against cancer cell lines (Sharif et al., 2017).

The viability of A498 cells decreases with increase in concentration of the aqueous extracts. Only viable cells have the ability to reduce MTT tetrazolium into a coloured formazan product. The cytotoxic activity was expressed as percentage of cell viability in HeK-293 and A-498 cell lines when compared with the control and both the plant extracts revealed more cytotoxicity towards cancer cell line A498. Concentration in the range of 100 -500 µg/ml and 100 – 500 µM for aqueous extracts of GC and GI extract and Cisplatin (control) respectively were used for the study. Both plants showed no cytotoxic effect towards non-cancerous HEK-293 cell line. To be precise, in GC extract, 500 µM did not show significant cytotoxic effects in HEK-293 cells but in A498 cells, a drastic decrease in cell viability was observed. Similarly, GI extract (upto 300 µM) did not show cytotoxicity in HEK-29. However, in A498 cells, a remarkable downfall in cell viability was observed (Figure 4a, Figure 4b). In this Data is expressed as mean ± SEM (n = 3). Statistical significance was determined using one-way ANOVA (* p < 0.05, **p < 0.01) as compared to standard and control group.

The effect produced by the extracts is comparable to that of the standard drug cisplatin, which is commonly, used in the treatment of renal carcinoma. The results exposed morphological changes and cellular shrinkage resulting to cell death caused by the extracts in the renal cancer cell lines (Figures 5 and 6). In Figure 6, CS: Cellular shrinkage; BL: Membrane blebbing (Magnification for A498 was 20X).

The survivability of cells to the leaf extract of GC, GI and Cisplatin was characterized by IC50 and R² values (Table 4). In vitro growth inhibition effects was observed in the kidney cancer cell line (A498), while there was no effect on the growth of normal cells (HEK-293). Such selective effects were incubation time and concentration dependent. All the extracts were evaluated in triplicates with respect to concentration (100, 200, 300, 400, 500µg/ml) by serial dilution. Higher concentrations, 500 µg/ml of both plant extracts were the most effective in producing growth inhibition. However, the pure standard Cisplatin drug showed significant inhibition on the cancer cell lines. The results confirmed the differential effect induced by the extracts and cisplatin in A498 and HEK-293 cell lines.

When the concentration of the extract was increased, it was observed that there was rapid decrease in cell-cell contact and cell proliferation. It indicates that the cytotoxic effect gradually
increases with increase in the concentration. SI values were also calculated for both the extracts on renal cell lines and compared to those calculated for cisplatin (Table 4). The highest SI values calculated for aqueous extract of GC was 15.93 and GI extract was 2.45. The SI values calculated for cisplatin for renal cell line was low (2.36), indicating the superiority of GC and GI extract on the cancer cell line compared to cisplatin. Based on the low IC50 value and high SI values for both extract in these cells suggest GC and GI extract as a promising therapeutic candidate in patients with renal cancers. Higher the SI value, the more selective it is and SI values less than 2 indicate general toxicity (Badisa et al., 2009). Gleaned from the results, it can be inferred that compared to cisplatin, a common chemotherapy drug, GC and GI extracts are better candidates for growth suppression of renal cell lines with SI values > 2.

CONCLUSION

It was observed that the aqueous extract of GC and GI contains a wide variety of bioactive components that possess strong antioxidant capacity based on the experiments performed which gives a scientific evidence to conduct further studies. The present in-vitro anti-cancer activity exposed the abilities of GC and GI extract as a curative agent for cancer treatment as it not only has a highly potent activity at lower concentrations but also exhibits a high degree of selectivity in kidney cancer cells. This activity may be due to the presence of bioactive compounds and antioxidants. Our results reveal that aqueous leaf extract of GC and GI displays cytotoxic effects on A498 human renal carcinoma cells at 500 μM and 300 μM respectively, it may be safely used against kidney cancer since, at this concentration; no significant effect was observed in normal renal cells. Our results suggest GC and GI extracts are attractive option for pharmaceutical companies as a potential agent for the management of human cancer. However, further studies to isolate the secondary metabolites responsible for these activities are underway and to explore their molecular mechanism.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES


