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Evaluation of some bioactive effect of phenolic compounds in *Costus speciosus* rhizome extract

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Abstract

This work was evaluated the antioxidant, antibacterial and cytotoxic activity of *Costus speciosus* rhizomes methanol extract. The FLC analysis showed the presence of five compounds in the methanol extract of *C. speciosus* rhizomes. These compounds were Quercetin (5.2mg/ml), Rutin (6.02mg/ml), Luteolin, (18.3mg/ml), Kaempherol, (11.34mg/ml) and Coumarin (1.41mg/ml). The maximum antioxidant activity of the extract was at concentration 1000 µg/ml with free radical scavenging activity approximately 67.5%. It was less than standard ascorbic acid 85.5% and Gallic acid 90% with significant difference ($p \leq 0.05$), with no significant difference in comparison with standard TBA 68.5%. The IC50 of extract was 3093µg/ml, while the IC50 of ascorbic acid, Gallic acid and TBA were (277.2, 364.5 and 601.3 µg/ml) respectively. The extract revealed influential growth inhibition for all bacteria used in this experiment. The extract was moderately effective at concentration 400 µg/ml of extract with inhibitory activity 50.7 % on MCF-7 cell line and IC50 139.1 µg/ml.

Keywords: *Costus speciosus*, antioxidant, phenolic compounds, antibacterial, cytotoxicity.

تقييم تاثير بعض الفعاليات الحيوية للمركبات الفينولية في مستخلص جذور نبات القسط الهندي

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الخلاصة

قيم هذا العمل تأثير المستخلص الميثانولي لجذور نبات القسط الهندي في فعاليته كمضاد للاكسدة وعلى البكتريا المرضية المختارة للدراسة وفعاليته السميه تجاه نوع من الخلايا السرطانية . تحليل FLC اظهر وجدود خمس مركبات ، وهي الكوارستين بتركيز 5,2 ملغم/مل والروتين بتركيز 6,02 ملغم/ مل واللوتين بتركيز 18,3 ملغم/مل والكامفيرول بتركيز 11,34 ملغم/مل والكومارين بتركيز 1,41 ملغم/مل. اعلى فعالية كمضاد للاكسدة كانت عند تركيز 1000 مايكروغرام / مل ، وبلغ نشاط الجذور الحرة حوالي 67,5% والتي كانت اقل من العقار القياسي حامض الاسكوربك 85,5% وحامض الكاليك 90% مع اختلاف معنوي عند ($P \leq 0,05$) بينما لم يكن هناك فرق معنوي عند المقارنة مع TBA 68,5%. وكان IC50 للمستخلص 3093 مايكروغرام / مل ، بينما كان لحامض الاسكوربك ، وحامض الكاليك، و TBA (277,2 ، 364,5 و 601,3 مايكروغرام/ مل) على التوالي.. اظهر المستخلص تأثيرا واضحا في تثبيط نمو كل انواع البكتريا المستخدمة في التجربة .كما اظهرالمستخلص فعالية سمية متوسطة وعند تركيز 400 مايكروغرام/مل وبفعالية تثبيطية 50,7% على خط الخلايا السرطانية MCF-7 ، و IC50 139,1 مايكروغرام / مل.

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Introduction

From early years, there is a virtual increasing interest in formation of drugs derived from herbes which can be used as alternative therapy. Also herbal products are safer, less expensive and infrequently have side effects in comparison with synthetic drugs [1]. Among various herbals valued, *C. speciosus* is an erect herbaceous pharmacologically important one [2]. *Costus speciosus* is pharmaceutical and decorative plant cultivated in India crepe ginger belongs to family Costaceae (Zingiberaceae)[3]. *Costus speciosus* have traditional uses such as food and medicine [4]. Recently the juice of rhizome therapeutically implemented as cooling and relief from head-ache, powder of leaves implemented as antipyretic, the extract acquired by boiling stem part of this plant is used against dysentery and fever, and many cures against diarrhea [5] cough, cuts, burning sensation, scabies, arthritis [6], wounds, constipation, leprosy, for abortion, asthma, inflammations, anemia [7] intestinal pains, nose pain , rash, worm infection, to stop vomiting [8] spermatorrhoea [9] used as antivermin and skin diseases [10]. Earlier studies paid attention on antioxidant, cytotoxicity and antimicrobial effects of plant extracts. On the other hand the diagnosis of compounds also had extensive studies to estimate the effect of these compounds [11]. Hence the aimes of the present study were to determine *in vitro* antioxidant, antibacterial and cytotoxic activity of phenolic compounds in methanol extract of *C. speciosus* rhizomes.

Materials and methods

Plant material

The rhizome of *C. speciosus* was obtained from herbalists market in Baghdad region and was identified by the Faculty of Agriculture, University of Baghdad.

Plant extraction

The sundried rhizomes were powdered with grinder and then passed through sieve with 40 meshes. About 25g of dried rhizomes powder subjected to Soxhlet apparatus and extracted with 250ml.of methanol 80%, the extract solution filtered through Whatman No. 4 filter paper and then evaporated to remove solvent by rotary evaporator at (45-50˚C). The dried collected extract was stored under freezing condition at -18˚C until used for further experiments.

Active compounds detection

The detection of phenolic compounds in methanol extract of *C. speciosus* was done by Fast Liquid Chromatography (FLC) with separation conditions: column C-18(3µm particle size 50×2.0 mm ID), volume injection sample 20µL, flow rate 1.4 ml/min, mobile phase was 0.1acetic acid in deionized water: acetonitrile (20:80V/V) at temperature 30˚C and detected with UV spectrophotometer at 280nm. Quercetin, Rutin, Luteolin, Kaempherol and Coumarin Standard compounds from sigma Aldrich were used in the analysis. The concentration of compound was calculated as follow:

$$compound(\mathrm{mg/ml}) = \frac{peak\ area\ of extract}{peak\ area\ of\ standard} \times standard\ soluion\ concentration \times total\ volume\ of\ extract$$

The experiment was carried out in the laboratories of ministry of science and technology [12].

Antioxidant Activity assay

In Vitro antioxidant activity of different concentration of *C.speciosus* methanol extract was measured by DPPH assay [13]. 0.5 mL of 1 mM DPPH fresh solution was added to 3 mL of various concentrations of the extract (1000,500,250,125,26.5 µg/ml). Ascorbic acid, Gallic acid and thiobarbituric acid (TBA) were used as positive control at the same concentrations. After 30 min of incubation at room temperature and dark condition, the absorbance was estimated at 517 nm. IC50 value represents the concentration of sample which is needed to scavenge 50% of DPPH free radicals. The values of radical scavenging activity were measured by the following equation

% Radical scavenging activity = (A0 - AT / A0) ×100

A0 is the absorbance of the control sample (all reagents without test sample) and AT is the absorbance of the test samples

IC50 was evaluated from equation of line acquired by plotting a graph of concentration µg/ml opposite to % inhibition.

Antibacterial activity test

C. speciosus extract was evaluated for antibacterial activity by agar well diffusion method against nominated pathogenic bacteria obtained from biotechnology research center / Al-nahrain university, *Proteus volgaris* and *Klebsiella peneumonia* (Gram negative), *Staphylococcus aureus* and *Bacillus*

cereus (Gram positive). Each bacterium subcultured on Muller Hinton Agar (MHA) and spreaded onto plates individually by sterile cotton swabs. 50 µL of extract solution poured onto each well with diameter of 8 mm. the tested plates were incubated at 37 ˚C for 24 h, and then the zone of inhibition was measured [14].

Cytotoxic activity against MCF-7 cell line

The anticancer of methanol extract was performed on human breast cancer cell line (MCF-7). The colorimetric technique via MTT assay was used to determine cell viability as described by [15]. 100µL/well (10^6 cell / ml) of MCF-7 cells were cultured in tissue culture plate which contain RPMI-1640 medium provided with 10% heat-inactivated FBS, 2 mM L-glutamine and 100 U/mL of penicillin-streptomycin, and then 100µL of different concentrations of extract (400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5, 0.78, 0.39 and 0.19µg/ml) were added to each well and incubated at 37◦C for 24h. After the incubation, 10µL of MTT solution (5mg/mL) was added to each well and incubated with condition 37◦C for 4 hrs in dark room. At the end step, 50 µL of DMSO (dimethyl sulfoxide) was added to each well and incubated for 10 min. MCF-7 cells were cultured at complete medium without treatment as positive control, and complete medium free cells and extract solution as blank. The absorbance was measured at 620 nm using an ELISA reader. The cells viability ratios were estimated according to the formula:

$$\% \text{ Viability} = \left(\frac{\text{Sample Absorbance}}{\text{Control Absorbance}}\right) \times \text{x100}$$

IC50 was evaluated from equation of line acquired by plotting a graph of concentration µg/ml opposite to % viability.

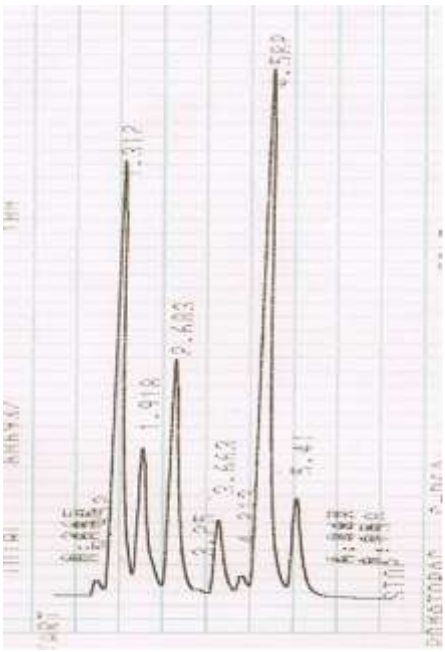
Statistical Analysis

The data were statistically expressed using 2way ANOVA with mean ± SE.

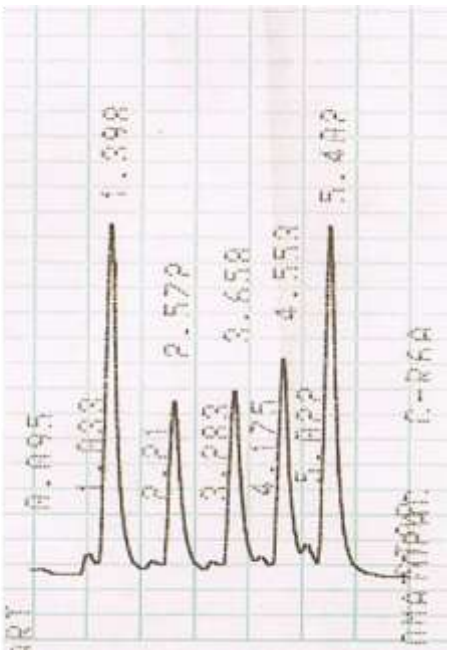
Results and discussion

Active compounds detection

According to the Table-1 and Figure-1 results of FLC analysis showed presence of five compounds in the methanolic extract of *C. speciosus*. These compounds were Quercetin with concentration (5.2mg/ml), Rutin with concentration (6.02mg/ml), Luteolin with concentration (18.3mg/ml), Kaempherol with concentration (11.34mg/ml) and Coumarin with concentration (1.41mg/ml). The presences of phenolic active compounds in the extract are very important because of their responsibility for biological activity like antioxidant ability due to owning hydroxyl groups and antimicrobial activity [16].



(a)



(b)

Figure 1- FLC analysis for methanolic extract of *Costus speciosus*: (a) represent analysis of sample (methanolic extract) and (b) represent analysis of standard compounds.

Table 1- FLC analysis for methanolic extract of *Costus speciosus*.

Compound	Retention Time of Standard	Retention Time of Sample	Sample concentration (mg/ml)
Quercetin	1.39	1.31	5.2
Rutin	2.57	2.60	6.02
Luteolin	3.65	3.66	18.3
Kaempherol	4.55	4.58	11.34
Coumarin	5.40	5.41	1.41

Antioxidant Activity assay

The results revealed that the maximum antioxidant activity of methanol extract of *C. speciosus* at concentration 1000 µg/ml with free radical scavenging activity was approximately 67.5%. It was less than standard ascorbic acid 85.5% and Gallic acid 90% with significant difference ($p \leq 0.05$), while no significant difference was recorded in comparison with standard TBA 68.5% Figure-2. The IC50 of extract was 3093µg/ml, while the IC50 of ascorbic acid, Gallic acid and TBA were (277.2, 364.5 and 601.3 µg/ml) respectively. This experiment postulated that *C. speciosus* extract has an electron donating ability, and this is belong to phenolic compounds in the extract which is supposed to reduce the free radicals when they react with hydrogen donors in antioxidant concept [17].

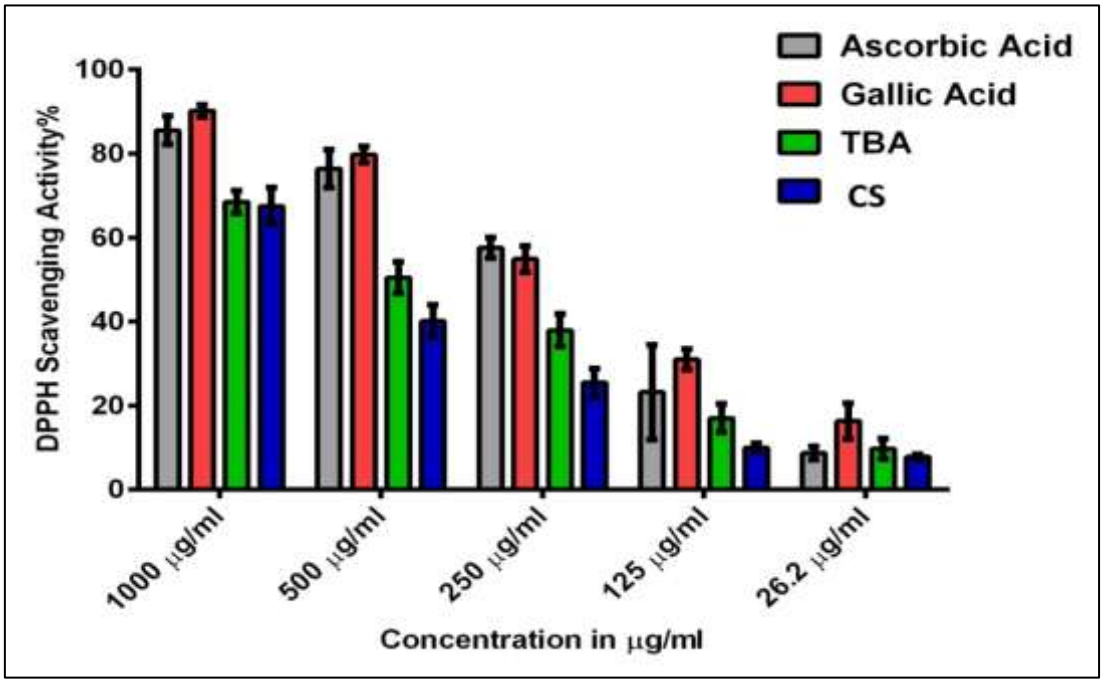


Figure 2- Comparative DPPH Scavenging activity% showed by standard antioxidant (ascorbic acid, Gallic acid and TBA) with *Costus speciosus* (CS) methanolic extract,(mean ± SE of three experiments).

Antibacterial activity test

The inhibition zones of *Costus speciosus* methanolic extract against selected bacteria in the experiment were determined. As shown in Table-2, the extract revealed significant growth inhibition for all bacteria used in this experiment. This plant has the ability to resist disease, which may be due to

existence of phenolic and alkaloid compounds. [2] Quercetin, Rutin, Luteolin, Kaempherol and Coumarin showed strong antibacterial activity in many studies [6].

Table 2- Inhibition zone size of *Costus speciosus* methanolic extract against selected bacteria.

Bacterial isolate	Zone of inhibition (mm)*
Proteus volgaris	20.7±1.9
Klebsiella peneumonia	19.8±1.4
Staphylococcus aureus	21.2±1.3
Bacillus cereus	16.4±1.2

*Zone of inhibition is mean of triplicate values

Cytotoxic activity against MCF-7 cell line

The cytotoxic activity of methanolic extract was performed on MCF-7 cell line and results are presented in Figure- 3. The extract was moderate effective and the 400 µg/ml of extract concentration showed the potent activity with inhibitory activity 50.7 % on MCF-7 cell line. The extraction having IC50 139.1 µg/ml is generally considered as moderate cytotoxic extract. Previous study indicated potency cytotoxic activity against brine shrimp lethality and concluded the presence of active compounds such us phenolic compounds responsible for revealing such biological potency [18, 17].

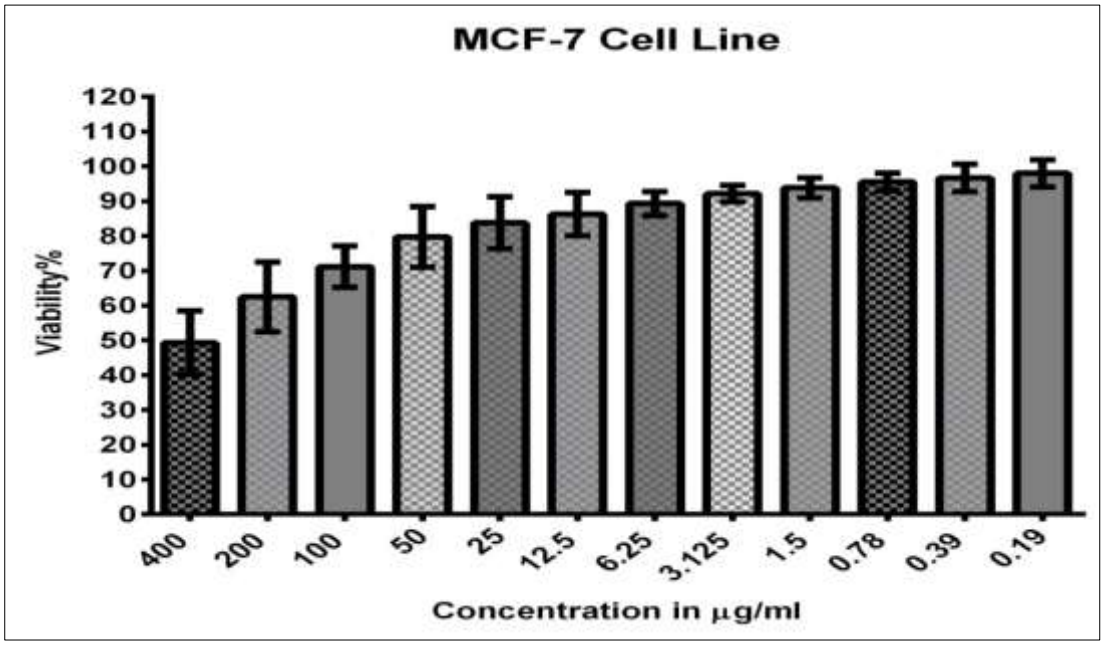


Figure 3- Cytotoxic activity of *Costus speciosus* methanolic extract on MCF-7 cell line,(mean ± SE of three experiments).

Conclusion

Costus speciosus methanolic extract showed interesting activity like antioxidant and antibacterial activity as well as moderate anticancer activity against MCF-7 cell line and these results indicate to select another extraction method to further cell line assay. According on the results it can be used as pharmaceutical product and need further study as food preservative.

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