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Research Article

Cytotoxic Activity of Bioactive Compound from *Caesalpinia ferrea* Martius, Fabaceae

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ABSTRACT
The aim of the present study is to evaluate Cytotoxic Activity of aqueous ethanol extract and pure isolated bioactive compounds from *Caesalpinia Ferrea* Martius, Fabaceae (Caesalpiniaceae) Leaves against five human cancer cell lines (liver HepG2, breast MCF-7, colon HCT-116, larynx Hep2 and prostate PC3) using sulforhodamine B (SRB) assay method .Aqueous ethanol extract gave cytotoxic activity against liver HepG2 With IC_{50} 19.3µg/ml, In addition isolation of twelve pure polyphenols 1 – 12 from the extract. The structure of compounds were elucidated by conventional methods, spectroscopic analysis, including 1D and 2D NMR. The most potent cytotoxic activity of pure bioactive compound is *2''-O*-galloyl vitexin (6) against liver HepG2 with IC_{50} 18.5µg/ml exhibited. conclusion, the results suggested that ethanolic extract and pure phenolic compound can be used as good candidate for novel therapeutic strategies for cancer possessed significant anticancer activity.

Keywords: *Caesalpinia ferrea Martius*, Fabaceae, Phenolics, Cytotoxic activity, Human Cancer cell line.

INTRODUCTION

Caesalpinia is a genus of plants belonging to the family Fabaceae (Caesalpiniaceae), which include genera that embrace phenolic rich species, Fabaceae **(**Caesalpiniaceae) occupy a distinguishable situation. This is due to the fact that many of its plants are capable of synthesizing and accumulating high percentage of phenolics. However, with approximately 500 species of this genus occurring worldwide, less than 30 of these have been already studied with respect to their phytochemicals and pharmacological activities. Numerous studies conducted with species of the genus *Caesalpinia* have corroborated their effectiveness as a natural source of new chemical entities and new therapeutical applications, revealed their structural diversity, shown useful properties for the development of traditional medicines, and produced scientific guidance for the use of herbal medicines. *Caesalpinia* species are mostly woody species occurring in tropical and subtropical zones. Several classes of chemical compounds, such as flavonoids, diterpenes, and steroids, have been isolated from various species of the genus *Caesalpinia*[1], *Caesalpinia ferrea* Mart. (Caesalpiniaceae) grows throughout Brazil where it is commonly known as Juca or Pau-ferro, primarily in Pernambuco and Cearfi[2] that has traditionally been used for many medicinal purposes, such as bronchitis, diabetes, and the treatment of wounds[3]. *Caesalpinia ferrea* Martius are widely used as an antimicrobial and healing medicine in many situations including oral infections[4]. Among the metabolites described, including the predominant phenolic derivatives[5], steroids, triterpenoids, and especially the cassane diterpenes, many of these exhibited antiulcer, anticancer, antidiabetic, anti-inflammatory, antirheumatic, antimicrobial, antibacterial, and cytotoxic activities[6]. Therefore, the results available in the literature to date, when associated with the diversity of metabolites, clearly indicate that chemical pharmacological research of species belonging to the genus *Caesalpinia* could afford new drug prototypes. The species of *Caesalpinia* that did not have yet been studied may bring valuable benefits to the relentless search for bioactive molecules, which have medicinal action and therapeutic feasibility, thereby allowing the discovery and development of more efficacious drugs that are safer and more affordable. It was therefore, found interesting to subject the leaf extract of this plant to an intensive biological and phytochemical investigations of its phenolic constituents. In our own studies we isolated three di-*O*-glycosyl-*C*-glucosyl flavones from the leaves of this species. In addition, Twelve phenolics known compounds, (**1** – **12**) were isolated and identified from the aqueous ethanol extract of the *Caesalpinia ferrea Martius* leaves,

MATERIAL AND METHODS

Plant material

Fresh leaves of *Caesalpinia ferrea Martius*, family Fabaceae (Caesalpiniaceae) were collected in the flowering stage from a cultivated tree in the Zoological Garden, Giza, Egypt, in March 2015. The plant was identified by Prof.Dr. Salwa Kawashty, department of

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phytochemistry and plant systematic, National Research Center (NRC), Cairo. Egypt. A voucher specimen (C 253) has been deposited at the herbarium of the National Research Center, Cairo. Egypt.

Apparatus

NMR spectra were carried out using Bruker 400-MHz. Standard pulse sequence and parameters were used to obtain 1D, 2D, ^{1}H and ^{13}C- NMR,Chemical shifts (DMSO-d_6) $\delta_{(ppm)}$ were measured in ppm,^{1}H NMR chemical shifts relative to tetramethylsilane (TMS) and ^{13}C- NMR chemical shifts to acetone-d_6 and were converted to the TMS scale by adding 29.8. High resolution ESI mass spectra were measured using a Finnigan LTQFT Ultra mass spectrometer (Thermo Fisher Scientific, Bremen, Germany. UV spectra were recorded on a Shimadzu UV-Visible-1601 spectrophotometer (Kyoto, Japan). HPLC analysis. Both the mobile phase and the dissolved materials were filtered by a Millex-HX Nylon syringe filter (0.45 um, 25 mm; Millipore, Bedford, MA). The materials are subjected to chromatographic analysis with High-Performance liquid Chromatography (HPLC). Reverse phase with the following specifications; Shimadzu SCL-10Avp System controller. Dual pump Shimadzu Liquid Chromatography (LC-10Avp), shimadzu degasser (DGU-14A), Shimadzu UV-Vis detector (SPD- 10Avp) and column: Phenomenex RP-18 (UK; 250 x 4.00 mm, 5 micron). The compounds were detected at 340 and 280 nm, respectively. Elution with solvent A: water–acetic acid (97:3, (v/v)] and solvent B (methanol) injection volume: 5 µl, and the flow rate was 1 ml/min.Paper chromatography (PC) was carried out on What man paper No. 1and 3 MM using the following solvent system: H_2O; 15 vol. % HOAc; 6 vol.% HOAc; BAW [n-BuOH: HOAc:H_2O (4: 1: 5, v/v/v), upper layer].

Extraction and Isolation

Fresh leaves (2kg) of *Caesalpinia ferrea* Mart. (*Caesalpinia*ceae) were cleaned, dried in the shade and pulverized in a mechanical grinder, powdered dried samples were successively extracted with aqueous ethanol (70 %) (v/v) under reflux three times, each extraction for 8 h with 2L. The solvent was removed under reduced pressure at □ 45º C. The process yielded finally 200 g of a sticky dark brown material extract. The received extract was subjected to a series of polyamide column (Macherey Nagel, Düren, Germany) initiated with H_2O and the MeOH content was gradually increased in 10 % steps. Further fractionation was performed on a sephadex column with gradient elution with H_2O/MeOH. The flavonoid fractions was isolated by application of preparative PC using Whatman paper No. 3 MM and BAW as solvent to isolate pure bioactive compounds **1** – **12**.

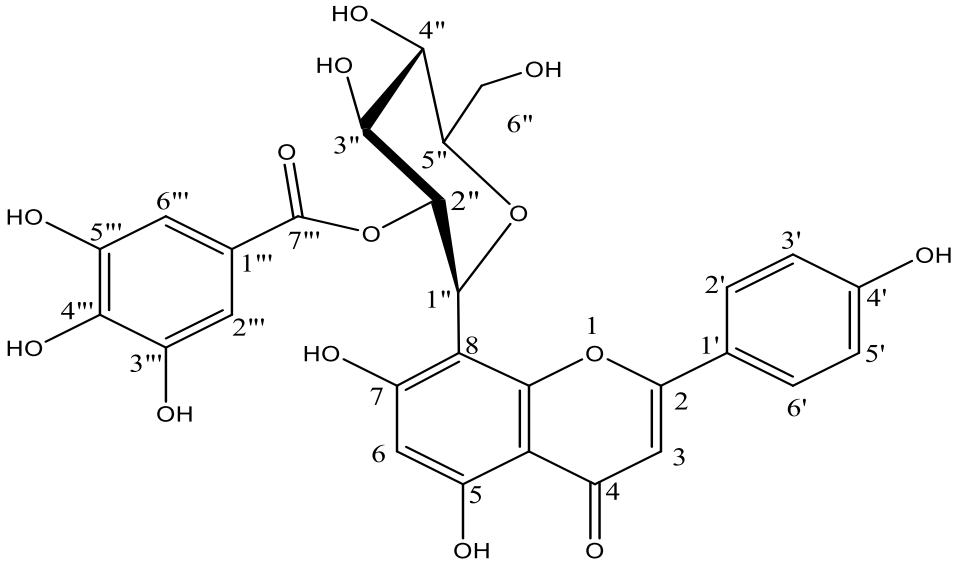
Biological activities

Cytotoxic assay

The sulforhodamine B (SRB) assay was used to measure the potential cytotoxicity[7], cells were plated in 96-well microtiter plate (10^4 cell / well) for 24h before treatment with the extract to allow attachment of the cells to the wall of the plate. Different concentrations of the extract under test were added to the cell monolayer, triplicate wells were prepared for each individual dose, the monolayer cells were then incubated with the extract for 48h at 37˚C and in atmosphere of 5% CO_2, cells were then fixed, washed and stained with Sulforhodamine B stain, excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader, the relation between surviving fraction and extract concentration is plotted to get the survival curve of each tumor cell line after application of different concentrations of the extract.

RESULTS AND DISCUSSION

In previous research, The following known compounds have been isolated from the aqueous ethanol extract of *C.ferrea*: gallic acid (1)[8], brevifolin carboxylic acid[9] (2), brevifolin[9] (3), ellagic acid[9] (4), tellimagrandin-I[10] (5) ,2”-*O*-galloylvitexin[11] (6), vitexin[11] (7), 2”-*O*-galloylorientin[11] (8), orientin (9), isovitexin 2''-O-β-[xylopyranosyl-(1'''' --- 2''')-*O*-β- xylopyranosyl][12] (10), Isovitexin[13] (11), orientin



2''-*O*-galloyl vitexin

Table 1: Cytotoxic activity of the *Caesalpinia ferrea* leaves methanol extract

Conc. of the extract, µg/ml	Survival fractions of the cell lines tested				
	HepG-2	Hep2	HCT-116	MCF-7	PC3
0.00	1.000	1.000	1.000	1.000	1.000
5.0	0.861	0.749	1.167	0.963	1.000
12.5	0.754	0.677	0.763	0.780	0.930
25.0	0.292	0.381	0.495	0.408	0.659
50.0	0.388	0.291	0.488	0.319	0.512

liver HepG-2 , larynx Hep2, colon HCT-116, breast MCF-7 and prostate PC3 ,human cell line

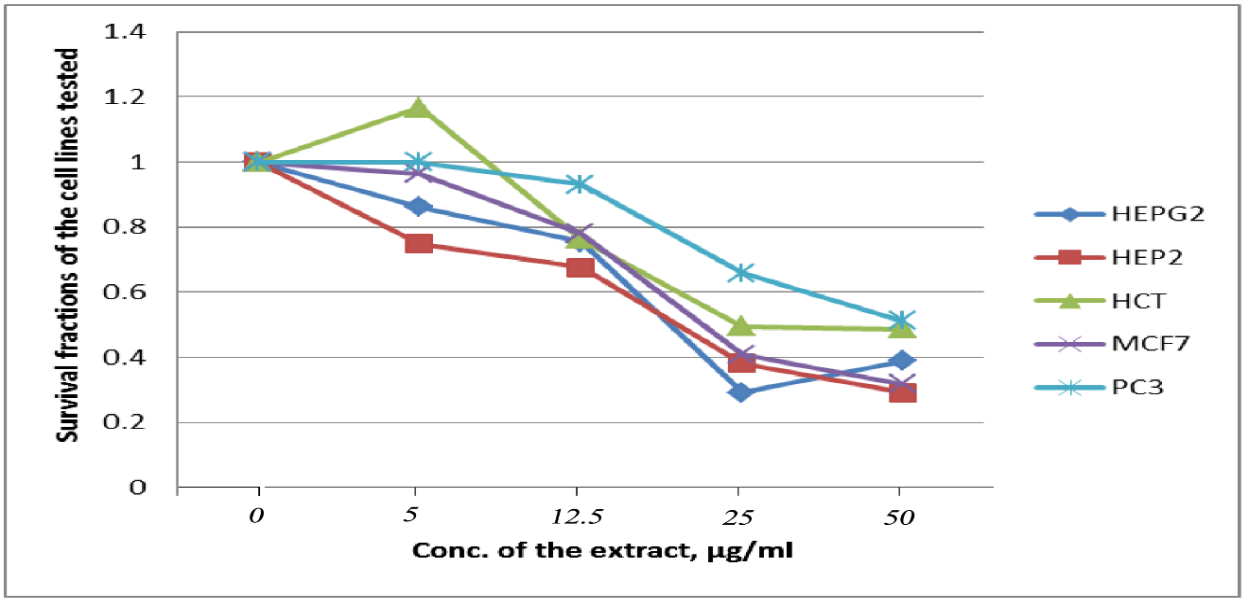


Figure 1: % of survival fractions of tumor cells against concentration (µg/ml) of *Caesalpinia ferrea* leaves extract

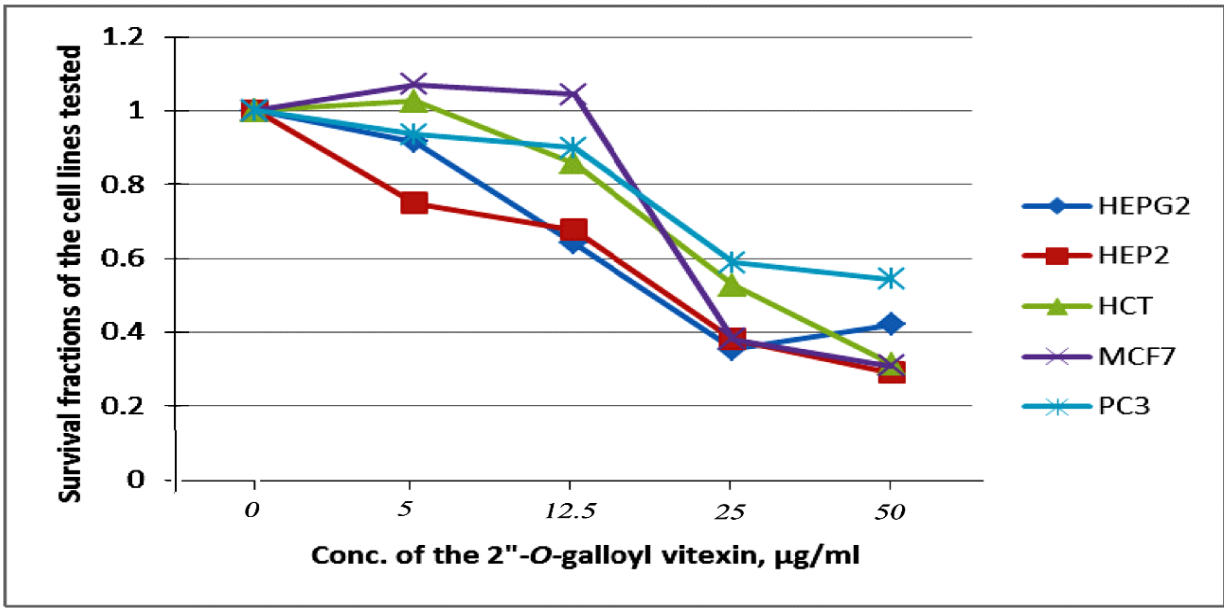


Figure 2: % of survival fractions of tumor cells against concentration (µg/ml) of 2"-*O*-galloyl vitexin isolated from *Caesalpinia ferrea* leaves extract

Table 2: IC_{50} of the *Caesalpinia ferrea* leaves extract against the cell lines tested

Human cell lines	HepG-2	Hep2	MCF-7	HCT-116	PC3
IC_{50}	19.3µg/ml	20µg /ml	21.8µg/ ml	24.47µg/ml	-----

IC_{50}: Concentration of the extract which causes the death of 50% of the tumor cells

2''-*O*-β-[xylopyranosyl-(1''' --- 2''') -*O*-β- xylopyranosyl][12] (12). Direct comparison with authentic sample (Co-TLC) and literature data confirmed the structures. Further support for the structures was obtained from 1D and 2D-NMR measurement. This compound has the similar NMR data with those reported in the literature.

*Identification of bioactive compound (**6**)*

Pure yellow amorphous powder of this compound (89 mg) appeared on PC, under UV light as y. spot, which gave d. blue colour with cold aqu. $NaNO_2$ /glacial CH_3COOH and a purple colour with aniline/xylose, thus proving its nature as flavanoid compounds. The UV spectra of compound (6) was almost identical [λ_{max} (nm) (MeOH)=268, 330 nm] and reminiscent to that of an apigenin derivative It showed a *Mr* of 584 in its negative ESI-MS analysis $[M-1]^-$ at m/z = 583. ^{1}H- NMR (DMSO-d6) δ(ppm):6.69(s, H-3), 6.047(s, H-6), 8.06(d, J=8 Hz, H-2' and H-6'), 6.97(d, J= 8 Hz, H-3' and H-5'), 4.98(d, J=8, H-1''), 5.41 (t, H-2''), 3.3-3-9 (m, sugar proton), 6.887(s, H-2''' and H-6''') Apigenin moiety: 166.6(C-2), 102.7(C-3), 183.1 (C-4), 161.7(C-5), 100.2(C-6), 163.3(C-7), 104.6(C-8), 157.0(C-9), 102.7(C-10),122.0(C-1'), 129.1(C-2'), 115.9(C-3'), 161.7(C-4'), 115.9(C-5'), 129.1(C-6') Glucosyl moiety: 71.4(C-1''), 73.0(C-2''), 77.(C-3''), 71.5(C-4''), 82.2(C-5''), 61.8(C-6'') Galloyl moiety: 119.9(C-1'''), 109.2(C-2'''), 145.2(C-3'''), 138.6(C-4'''), 145.2(C-5'''), 109.2(C-6'''), 165.7(C-7'''). From ^{1}H and ^{13}C NMR analyses were employed. The ^{1}H NMR spectrum revealed the distinct pattern of proton resonances belonging to 2''-*O*- galloyl vitexin[6]

Cytotoxic activity of Caesalpinia ferrea leaves extract against human tumor cell lines

All *Caesalpinia ferrea* leaves extract and isolated compounds were subjected to cytotoxic activity screening against human tumor cell lines, Potential cytotoxicity of the aqueous ethanolic extract of *Caesalpinia ferrea* leaves was tested using the SRB assay[7] based on the protein staining using sulforodamine. Five different human cell lines were used. The relationship between the surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line (Table1). The cytotoxic activity of the extract was determined by SRB method and proved that it possesses potent cytotoxic activity against the human cell lines tested[15-17], with IC_{50} ranging from19.3μg/ml to 24.47μg/ml (table 2). The relationship between the surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line[18] (Figure 1).

From the results, aqueous ethanol extract gave cytotoxic activity against five Human cancer cell line**,** the most potent cytotoxic activity which causes the death of 50% of the tumor cells with IC_{50} 19.3μg/ml, is liver HepG-2, followed by cytotoxic activity against larynx Hep2 with IC_{50} 20μg /ml also the cytotoxic activity against breast MCF-7 with IC_{50} 21.8μg / ml and cytotoxic activity against HCT-116 with IC_{50} 24.47μg/ml , negative activity against prostate PC3. In conclusion, the results suggested that ethanolic extract can be used as good candidate for novel therapeutic strategies for cancer possessed significant anticancer activity.

Cytotoxic activity of isolated pure bioactive phenolic compound (2''-O-galloyl vitexin):

The cytotoxic activity of all isolated phenolic compounds were determined against five cell line. It was found that (2''-*O*-galloyl vitexin) exhibited most potent cytotoxic activity (Table 3) with IC_{50} ranging from 18.5μg/ml to 28.4μg/ml (table 4). The relationship between the surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line (Figure 2).

From the previous results, It was found that the most bioactive pure compound (2''-*O*-galloyl vitexin) is the most potent cytotoxic activity against liver HepG-2 exhibited with IC_{50} is 18.5μg/ml, which is more active than the extract, followed by colon HCT-116 with IC_{50} 22.6μg/ml, also cytotoxic activity against larynx Hep2 with IC_{50} 24.2μg/ml, beside it gave activity against breast MCF-7 with IC_{50} 28.4μg/ml, with negative activity against Prostate PC3.

CONCLUSION

The results in this work demonstrate that the extracts of the selected medicinal plant *Caesalpinia Ferrea* Martius, family Fabaceae (Caesalpiniaceae) contain a considerable amount of phenols, flavonoids and antioxidant potentials. The plant showed significant cytotoxic activity against the liver (HepG-2) and the isolated bioactive compound *2''-O*-galloyl vitexin showed high activity against liver (HepG-

Table 3: Cytotoxic activity of (2''-*O*-galloyl vitexin)

Conc. of the 2''-*O*-galloyl vitexin, μg/ml	Survival fractions of the cell lines tested				
	HepG-2	Hep2	HCT-116	MCF-7	PC3
0.00	1.000	1.000	1.000	1.000	1.000
5.0	0.916	0.749	1.026	1.072	0.936
12.5	0.641	0.677	0.858	1.044	0.899
25.0	0.354	0.381	0.528	0.381	0.588
50.0	0.421	0.291	0.314	0.309	0.543

Table 4: IC_{50} of the (2''-*O*-galloyl vitexin) against the cell lines tested

Human cell lines	HepG-2	HCT-116	Hep2	MCF-7	PC3
IC_{50}	18.5μg/ml	22.6μg/ml	24.2μg/ml	28.4μg/ml	-----

IC_{50}: Concentration of 2''-*O*-galloyl vitexin which causes the death of 50% of the tumor cells.

2). The natural bioactive compounds found in plant could be used for many therapeutic purposes as they often exhibit a huge amount of medicinal properties such as antioxidant, anticarcinogenic, antitumor activities.

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CONFLICT OF INTEREST STATEMENT
We declare that we have no conflict of interest

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