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MICROWAVE - ASSISTED EXTRACTION OF FLAVONOIDS AND PROFILING FLAVONOID CYTOTOXICITY AGAINST Hep-G2 HUMAN CANCER CELL LINE

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Keywords:

Hepatocytotoxicity, Flavonoids, Orange peel, Green tea, Rosemary, Microwave assisted extraction.

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ABSTRACT: Hepatic cancer is one of the most causes for cancer death in the world. The aim of this work was to compare and survey the cytotoxic activity of a group of differently classed flavonoids and three plant extracts known for their high antioxidant activities against Hep-G2 human cancer cell line. Also to isolate some flavonoids from the plant extracts using easier, modified methods of higher yield and lower time of extraction than conventional ones. Different flavonoidal classes were tested against Hep-G2 human cancer cell line using SBR assay. Doxorubicin was used as a positive control. Dihydroquercetin was found to be more potent even than Doxorubicin. It showed the higher activity with $IC_{50} = 4.43 \pm 0.08 \mu\text{g/ml}$ followed by quercetin and rutin with $IC_{50} = 7.88 \pm 0.89$ and $11.2 \pm 0.97 \mu\text{g/ml}$ respectively. Among the tested extracts, rosemary alcoholic extract was the potent followed by green tea alcoholic extract and orange peel alcoholic extract was the less potent. Their IC_{50} values ranged from $11.5 \pm 1.02 \mu\text{g/ml}$ to $48.2 \pm 1.8 \mu\text{g/ml}$. Diosmin and catechins showed no activity against Hep-G2 human cancer cell line. IC_{50} of Doxorubicin was calculated as $5.14 \pm 0.09 \mu\text{g/ml}$. All the tested plant extracts showed cytotoxicity especially rosemary extract which was highly potent with $IC_{50} = 11.2 \pm 0.97 \mu\text{g/ml}$.

INTRODUCTION: Primary liver cancer is the sixth most frequent cancer and the second leading cause of cancer death (World Health Organization 2014). The incidence of hepatocellular carcinoma is increasing in many countries especially in developing areas¹. Flavonoids are ubiquitous in nature and have been shown to reveal cytotoxic activity toward various human cancer cells with little or no effect on normal cells².

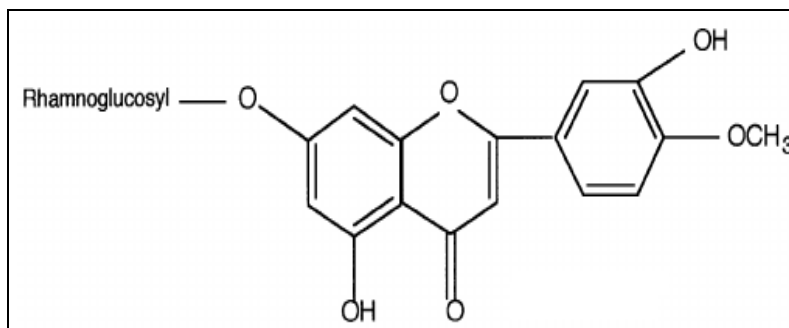
There is no survey on the edible plant flavonoids related to different classes and hepatic cancer in particular but some evidences about Hesperidin (Hesperitin-7-rhamnoglucoside), Hesperetin (5, 7, 3'-trihydroxy-4'-methoxy-flavanone), Diosmin (3', 5,7-trihydroxy - 4' - methoxyflavone 7 - rhamno glucoside) and Rutin (Quercetin 3-rutinoside), Quercetin (3,4',5,5',7-pentahydroxy-Flavone), Q), Dihydroquercetin (3, 3', 4', 5, 7 - Pentahydroxy flavanone), Morin(2', 3, 4', 5, 7-pentahydroxy flavone), \pm Catechins[2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol. (Fig. 1)

Quercetin, can inhibit the proliferation of different cancer cells^{3, 4}. Concerning hepatic cancer, it was proved that quercetin exerted its preventive effect *via* decreased oxidative stress and decreased

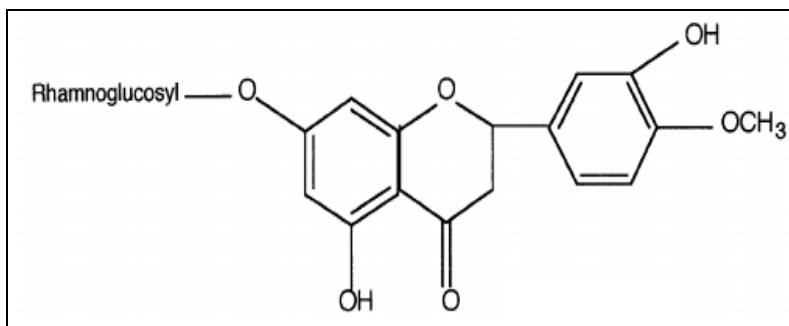
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antioxidant activity in rats ⁵. Hesperidin was found to show cytotoxic effect on MCF-7 cells with IC₅₀ of 11 μmol/L. It showed invitro synergism effect with Doxorubicin ⁶. Another study ⁷ investigated the antiproliferative effect of hesperidin on human hepatocarcinoma HepG2 cell line. Morin, the flavonol prevented acute liver damage following

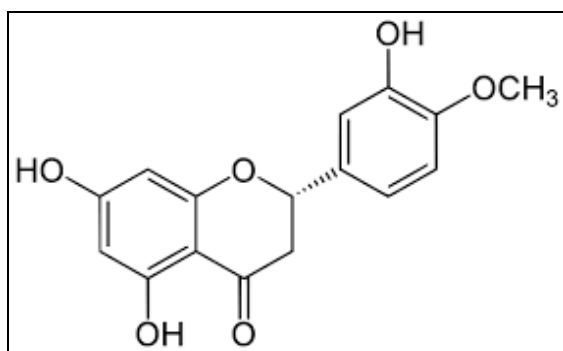
CCl₄ administration in rats by inhibiting tumor necrosis factor ⁸. Other morin cytotoxicity was reported against liver, intestine and breast tumors ⁹. Combination of diosmin and hesperidin 9:1, as in the preparation ""Detralex" increased hepato-protective effect ¹⁰.



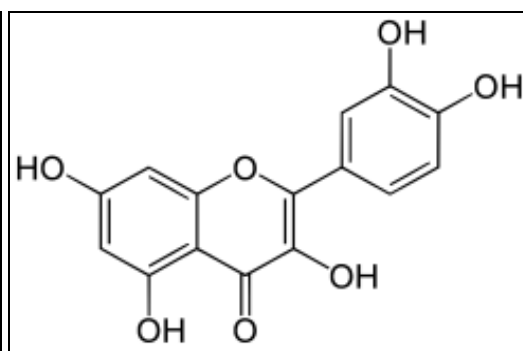
DIOSMIN



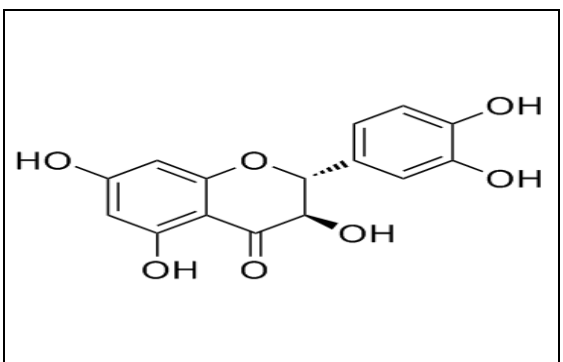
HESPERIDIN



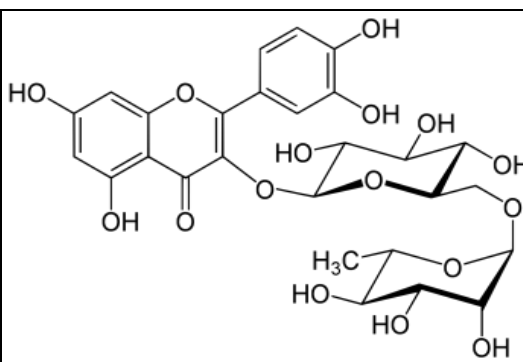
HESPERETIN



QUERCETIN



DIHYDROQUERCETIN



RUTIN

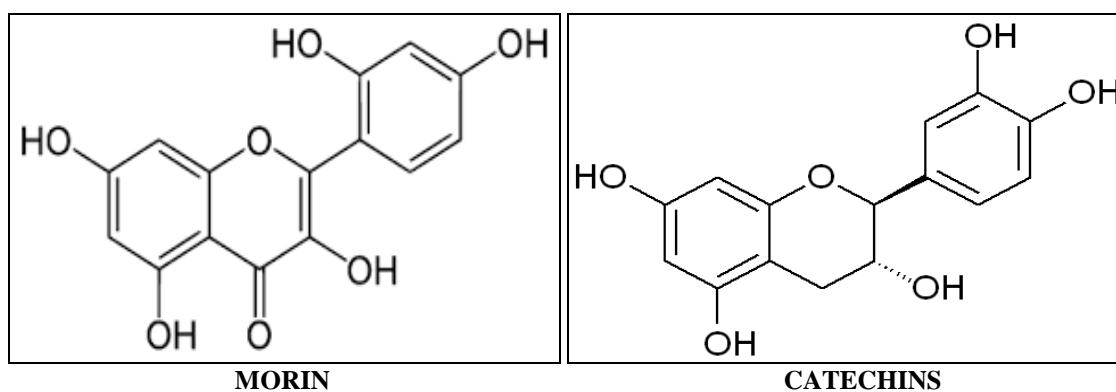


FIG. 1: FLAVONOIDAL STRUCTURES

Water extracts of sweet orange (*Citrus sinensis*) peel and its flavonoidal glycoside, hesperidin exhibited a significant hepatoprotective effect by attenuating oxidative stress *in-vivo*¹¹. Rosemary extracts have exhibited potential cytostatic and cytotoxic effects in colon human cancer cell lines¹². Oral administration of 1.5% green tea extract produced significant protection against tamoxifen - induced liver damage in rats¹³. Application of microwave assisted extraction process for isolation and extraction of phytoconstituents from plant material has gained an increasing importance¹⁴.

Plant Material: Ripen and freshly harvested orange (Baladi) fruits (season October 2015) were obtained from a local farm in Egypt. Voucher specimen was kept in herbarium of Faculty of Pharmacy, Damanhour University as 15Or.

Green tea and rosemary leaves were purchased from a local herb-market and voucher specimens were kept in herbarium of Faculty of Pharmacy, Damanhour University as 118G and 96R for green tea and rosemary respectively. The plants were confirmed by Professor Samir El-Kady, Faculty of Agriculture, Kafr El-Shiekh University.

Chemicals and Apparatus: All solvents and Chemicals were purchased from Sigma Chemical Company (St., Louis, USA). Flavonoids (quercetin, dihydroquercetin, morin, diosmin, hesperetin, catechin and rutin (purity>98%) were purchased from Sigma - Aldrich. Solvent evaporation and concentration was performed on Rotavapor Heidolphv 2000l and determination of flavonoidal content using Spectrophotometer (Optima SP-300, Japan). TLC analysis was done on precoated silica gel 60 F254 plates (Merck). Sephadex LH20 (Pharmazie). Polyamide (Merck) for TLC plates.

Lyophilizer (Hosokawa, England) and Domestic microwave oven (600 W, Daewoo, China). Cytotoxicity assays were measured in an ELISA reader spectrophotometer (Tecan Group Ltd. Sunrise), Germany. Melting points were determined using Fisher- Johns melting point apparatus.

Preparation of Alcoholic Extract of Orange Peel:

Fruits of *Citrus sinensis* (Balady) were chipped to thin, small slices then dried at room temperature to give 50 g of whitish orange powder. Powder is then extracted with 70% alcohol three times each of 100 ml till exhaustion. The total alcoholic extract is then collected and evaporated on rotary vapor under reduced pressure at 50 °C. The brownish residue (7.8 g) had a characteristic odor.

Preparation of Alcoholic Extract of Green Tea and Rosemary:

The air dried powdered plant material (50g) of each plant was extracted separately and successively with 70% alcohol at room temperature for 3 times. The resultant extracts were separately concentrated under reduced pressure at 30 °C. *Camellia sinensis* residue was waxy, dark brownish solid residue weighed 2.4g while that of *Rosmarium officinale* was powdered yellowish to light brown residue, of characteristic odor that weigh 3.5g. TLC analysis of *Camellia sinensis* residue on silica gel F percolated plates showed five spots using ether: CHCl₃ (8:2) as solvent system and anisaldehyde/H₂SO₄ as spray reagent. R_f of these spots were: 0.13, 0.35, 0.75, 0.83 and 0.92. TLC analysis of *Rosmarium officinale* residue on silica gel F percolated plates showed six spots using ether: CHCl₃ (8:2) as solvent system and anisaldehyde/

H₂SO₄ as spray reagent. R_f of these spots were: 0.21, 0.31, 0.52, 0.63, 0.89 and 0.96. Both extracts were kept as residue for cytotoxic activity test.

Isolation of Hesperidin from Orange Peel: Fruits were peeled off sharply and then chipped to thin, small slices then dried at room temperature to give 250 g of whitish orange powder. Powder was defatted with light petroleum then mixed with 600 ml of 10% calcium hydroxide, pH was adjusted to 11. Solution was then microwaved in 600w microwave at 90 °C for 3min initially then off for 2 min then microwaved again for another 2 min. Microwaving on and off was repeated for three times. The solution was cooled and then acidified by dilute HCl till pH = 5 was reached and centrifuged for 3 min. The solution was kept in refrigerator for 8 hours. Crude hesperidin (10.8 g) was separated that was filtered through Buchner funnel then dissolved in dimethylsulfoxide, stirred and heated to 60 - 80 °C the water is added slowly whilst stirring.

On cooling, the hesperidine precipitates. The purity of the isolated crystals (7.1 g) was tested by giving one spot on TLC precoated silica gel F₂₅₄ plates in three different mobile phase systems as following: n-butanol : acetic acid : water = 4:1:5 R_f = 0.6, CHCl₃:CH₃OH (1:1) R_f = 0.51 and in MeOH R_f = 0.77.

Microwaved Assisted Hydrolysis of Compound H1 to compound H2: Compound H1 (5g) was dissolved in 300 ml of 0.25M H₂SO₄:C₂H₅OH (2:8) mixed well then the hydrolysis reaction was done in 600 w microwave at 120 °C a total time of 10 min. Procedure was carried out as following: the solution was microwaved for 3 min initially then off for 2 min, microwaved again for another 3 min then off for 2 min followed by on for 4 min. Sample interval was taken after 10 min and examined by TLC using solvent system. Complete hydrolysis achieved after 20 min and recognized by the appearance of nearly one major spot on TLC plate. The resulting solution was concentrated and then extracted with ethyl acetate (100 ml x3). The collected ethyl acetate layer was concentrated under reduced pressure till residue. Crude crystals were purified by dissolving residue in minimum of acetone, and the resulting solution was added to a mixture of water and acetic acid in an ice bath.

Pure yellow powder of compound H2 (2.8 g) was obtained. TLC of the isolated compound on silica gel F₂₅₄ using methanol/ chloroform (9.5:0.5) as solvent system and iodine vapour for detection gave one spot R_f=0.72.

Isolation of Compound C from Green Tea: The powdered plant (250 g) was macerated with 500 ml distilled water then microwaved at 600w at 90 °C for 4 min. The solution was then filtered.

The filtrate was centrifuged at 800 rpm. The supernatant was lyophilized to give dark greenish residue (17.5g). One gram was kept for chromatographic test and cytotoxic activity. The bulk residue (16.5g) was partitioned between ethyl acetate: water (1:1) for three times. The combined ethyl acetate layers were concentrated to small volume and decaffeinate by the addition citric acid solution¹⁵ followed by extraction with ethyl acetate. The collected ethyl acetate fractions were then concentrated under reduced pressure to give 11.74 g of buff powder.

Recrystallization from hot water was repeated several times to improve the yield. Purification of the isolated compound was done on Sephadex LH20 column to give 8.4 g buff- white powder.

TLC of the isolated compound C was done on polyamide plate using n-butanol: acetone: acetic acid (5:5:3 v/v) and detected by ethanolic FeCl₃ as spray reagent (R_f =0.90). TLC on silica gel F₂₅₄ plates using toluene: ethyl acetate: formic acid (10:8:1) and detected by ethanolic FeCl₃ as spray reagent (R_f = 0.86).

Preparation of Alcoholic Extract of Rosemary: *Rosumarium officinale* was powdered yellowish to light brown residue, of characteristic odor that weigh 3.5g. TLC analysis of *Rosumarium officinale* residue on silica gel F percolated plates showed five spots using ether: CHCl₃ (8:2) as solvent system and anisaldehyde/ H₂SO₄ as spray reagent. R_f of these spots were: 0.21, 0.31, 0.52, 0.89 and 0.96. Using solvent system CH₂Cl₂: CH₃OH (6:1) and UV detection before and after spraying with AlCl₃. R_f of these spots and their color under UV lamp were: 0.08 yellow, 0.31 brownish, 0.61 yellow, 0.82 greenish yellow and 0.94 yellow.

Determination of Anti-hepatocytotoxicity of the Extracts and Flavonoids:

Human Tumor Cell Lines: Non small human hepatocellular liver carcinoma (Hep-G2), was obtained in frozen state under liquid nitrogen (-180 °C) from the American Type Culture Collection. The tumor cell lines were maintained by serial sub-culturing in the National Cancer Institute, Cairo, Egypt.

Culture Media: The cells were suspended in RPMI 1640 medium (Sigma Aldrich) supplemented with 10% fetal calf serum (Sigma, USA) in presence 1% antibiotic antimycotic mixture (10.000 U/ml K-penicillin, 10.000µg/ml streptomycin sulphate and 25 µg/ml amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium).

Assay Method for Anti-hepatocytotoxic Activity: Anti-hepatocytotoxicity against Hep-G2 cells were tested in the National Cancer Institute, according to the SRB (Sulforhodamine B) assay.

(3-(4, 5-di-methylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide) method by Skehan *et al.*,¹⁶ Adriamycin® (Doxorubicin) 10 mg vials Pharmacia, Sweden) was used as the reference drug. Briefly, cells were seeded in 96-multiwell plates at densities of 10⁴ cells/well in a fresh media and incubated under normal growth condition for approximately 24 h before treatment with the tested sample to allow attachment of cells to the wall of the plate. Then, 200 µl aliquot of serial dilution with DMSO (100%) of each of the total alcoholic extracts of orange peel, green tea and rosemary and the isolated flavonoids (hesperidin, hesperetin and ± catechin) as well as the purchased flavonoids (quercetin, dihydroquercetin, morin, diosmin, rutin (0, 1, 2.5, 5 and 10µg/ml) were added and the plates were incubated for 24, 48 and 72 h at 37 °C in a humidified incubator containing 5% CO₂ in air.

Control cells were treated with vehicle alone. Three wells were prepared for each individual dose. Following 24, 48 and 72 h treatment, cells were fixed, washed and stained with Sulforhodamine B stain (Sigma, USA). Color intensity was measured in an ELISA reader spectrophotometer (Tecan Group Ltd. Sunrise, German). The relation between the mean of surviving fraction and drug

concentration is plotted to get the survival curve of each tumor cell line after the specified plant extract. Negative control was treated with the vehicle (0.1% DMSO) used for diluting the tested drug.

Doxorubicin (1.0 µg/ml) was used as the positive control. Also, determination of IC₅₀ of Doxorubicin for all the tested cell lines was also so as done for direct comparison with the tested extracts and flavonoids. The experiment was performed triplicate with DMSO at 1% and Doxorubicin as negative and positive controls respectively.

Statistical Analysis: All the aforementioned experiments were conducted in triplicate trials. Data were expressed as mean ± standard deviation (SD). Data were analyzed by using one-way ANOVA followed by Duncan's multiple range tests using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS:

Isolation of Compound H1 from Orange Peel: Compound H1 has melting point of the isolated crystals was 260 - 262 °C. It gave positive Molisch test for glycosides.

IR of the isolated compound H1 was superimposed with an authentic sample of hesperidin.

¹H-NMR (DMSO-d₆, 400 MHz) δ 12.00 (1H, br s, 5-OH), 6.95 (1H, d, J= 2.0 Hz, H-2'), 6.85 (1H, J= 8.0 Hz, H-5'), 6.81 (1H, dd, J= 8.0, 2.0 Hz, H-6'), 6.16 (1H, d, J= 2.0 Hz, H-8), 6.12 (1H, d, J= 2.0 Hz, H-6), 5.50 (1H, dd, J= 11.0, 5.0 Hz, H-2), 4.96 (1H, d, J= 7.2 Hz, H-1''), 4.55 (1H, br s, H-1), 3.78 (3H, s, 4-OCH₃), 3.20–3.60 (6H, m, H-2'' to H-6''), 3.20–3.60 (3H, m, H-2 to H-6), 3.11 (1H, dd, J= 17.0, 11.0 Hz, H-3a), 2.78 (1H, dd, J= 17.0, 5.0 Hz, H-3b), 2.51 (1H, d, J= 6.0 Hz, H-5), 1.09 (3H, d, J= 6.0 Hz, H-6).

¹H-NMR (DMSO-d₆ isolated compound H1 was identical with that reported for hesperidin¹⁷.

Microwaved Assisted Hydrolysis of Compound H1 to compound H2: Compound H2 (2.8 g) was obtained has melting point of 220 - 221 °C. The IR spectrum as KBr disk showed a strong band of OH at 3497 cm⁻¹, CH (aliphatic) at 3120, 3039 and 2836 cm⁻¹, CH (aromatic) at 2890 - 2957 cm⁻¹,

C=C (aromatic) at 1579, 1498 cm^{-1} of C=O band at 1635 cm^{-1} , C-O at 1170 cm^{-1} . Also, IR of the isolated compound H2 was superimposed with an authentic sample of hesperetin in the fingerprint region.

^1H NMR (DMSO- d_6 , 400 MHz) δ 12.92 (1H, br s, 5-OH), 8.04 (2H, dd, $J = 9.0, 2.0$ Hz, H-6' and H-2'), 7.15 (2H, dd, $J = 9.0, 2.0$ Hz, H-3' and H-5'), 6.93 (1H, s, H-3), 6.82 (1H, d, $J = 2.0$ Hz, H-8), 6.45 (1H, d, $J = 2.0$ Hz, H-6), 5.03 (1H, $J = 7.2$ Hz, H-1''), 4.59 (1H, br s, H-1), 3.85 (3H, s, 7-OCH₃), 3.20–3.40 (6H, m, H-2'' to H-6''), 3.50–3.60 (3H, m, H-2 to H-6), 2.51 (1H, d, $J = 6.0$ Hz, H-5), 1.12 (3H, d, $J = 6.0$ Hz, H-6).

^1H NMR of the isolated compound H2 was identical with that reported for hesperetin¹⁸.

Isolation of Compound C from Green Tea:

Isolation of compound C was done by MAE of green tea to give buff- white powder, has melting point 174 - 175 °C. The isolated compound was

confirmed by co-chromatography with an authentic sample of catechin. It gave UV λ_{max} (Methanol) 222, 277 nm. IR as KBr pellet showed bands at 2560 - 3400 (broad), 1622, 1518, 1473, 1240, 1155, 1123, 1078. The spectrum was superimposed with an authentic catechin sample in the fingerprint region.

Hepatocytotoxicity of the Three Plants Alcoholic Extracts and the Flavonoids:

The three plant extracts showed cytotoxicity against Hep-G2 human cancer cells. Rosemary extract showed to be the most potent with $\text{IC}_{50} = 11.2 \pm 0.97 \mu\text{g/ml}$. Green tea was found to be more potent than orange peel extract with IC_{50} values of $35.5 \pm 1.1 \mu\text{g/ml}$ followed by $40.1 \pm 1.45 \mu\text{g/ml}$. The results are shown in **Fig. 2**. Within the tested flavonoids, dihydroquercetin showed high potency. It showed the higher activity with $\text{IC}_{50} = 4.43 \pm 0.08 \mu\text{g/ml}$ followed by quercetin and rutin with $\text{IC}_{50} = 7.88 \pm 0.89$ and $11.2 \pm 0.97 \mu\text{g/ml}$ respectively. The results are shown in **Fig. 3**.

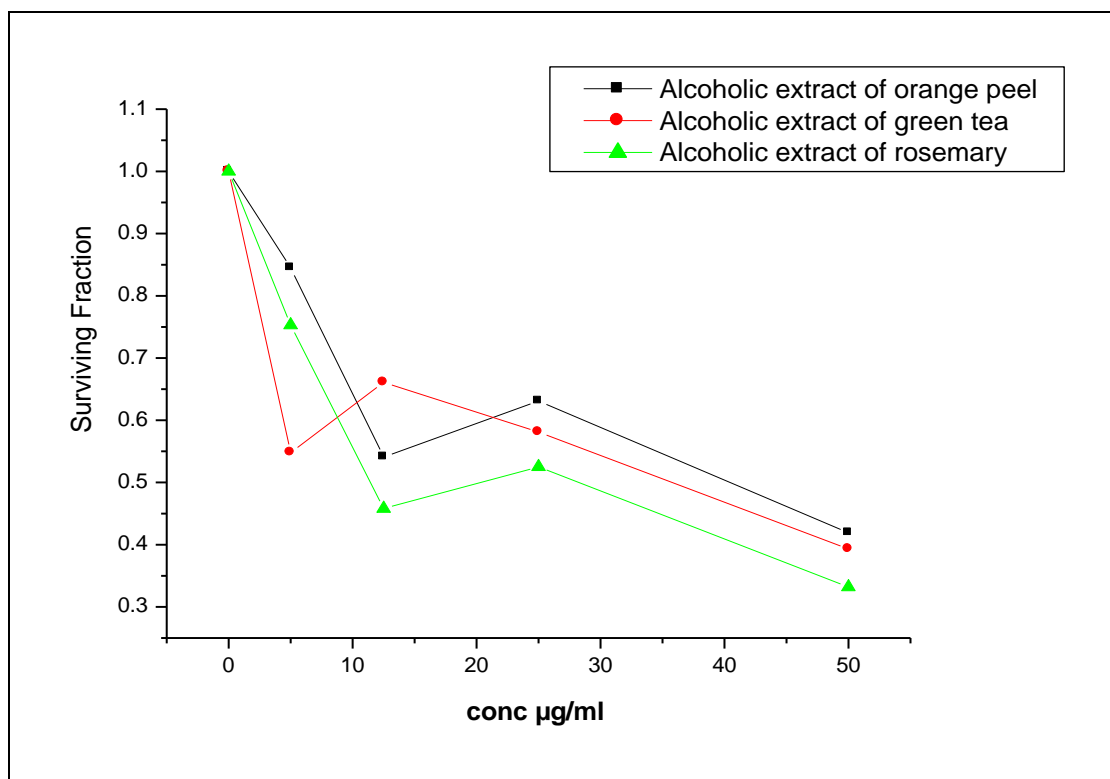


FIG. 2: CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACTS OF ORANGE PEEL, GREEN TEA AND ROSEMARY AGAINST Hep-G2 HUMAN CANCER CELL LINE

Comparison between cytotoxic activities of all the tested extracts and flavonoids together with Doxorubicin against Hep-G2 human cancer cells is

shown in **Fig. 4**. All results are tabulated in **Table 1**.

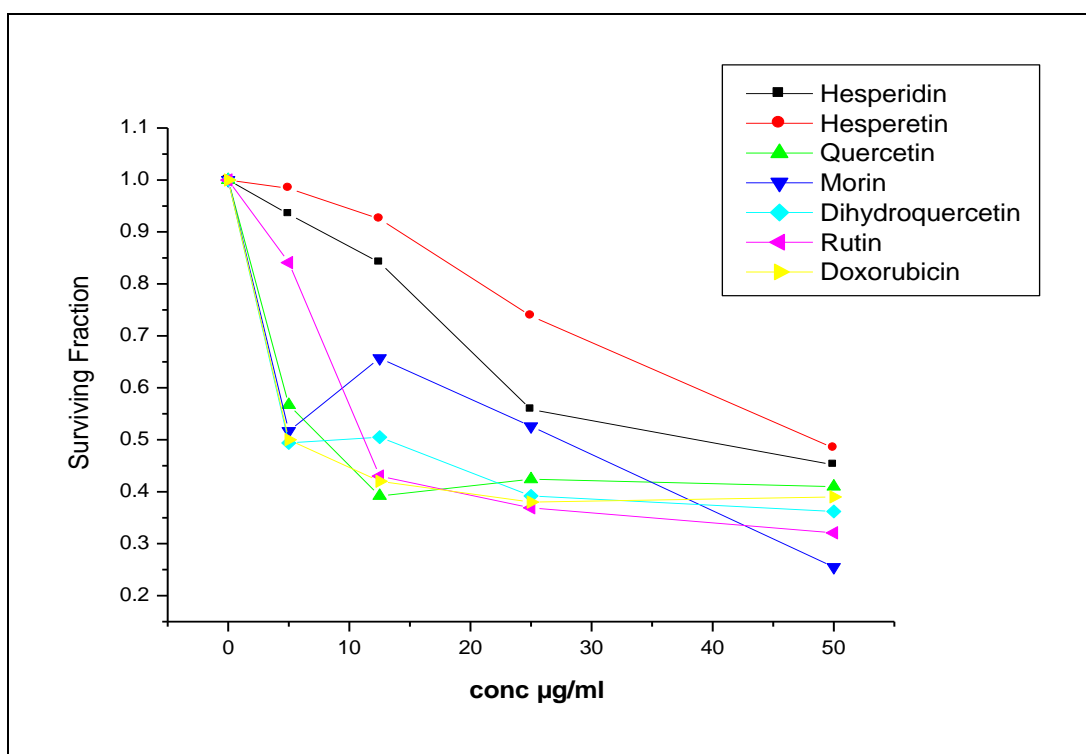


FIG. 3: CYTOTOXIC ACTIVITY OF THE TESTED FLAVONOIDS RELATED TO DIFFERENT CLASSES AND DOXORUBICIN AGAINST Hep-G2 HUMAN CANCER CELL LINE

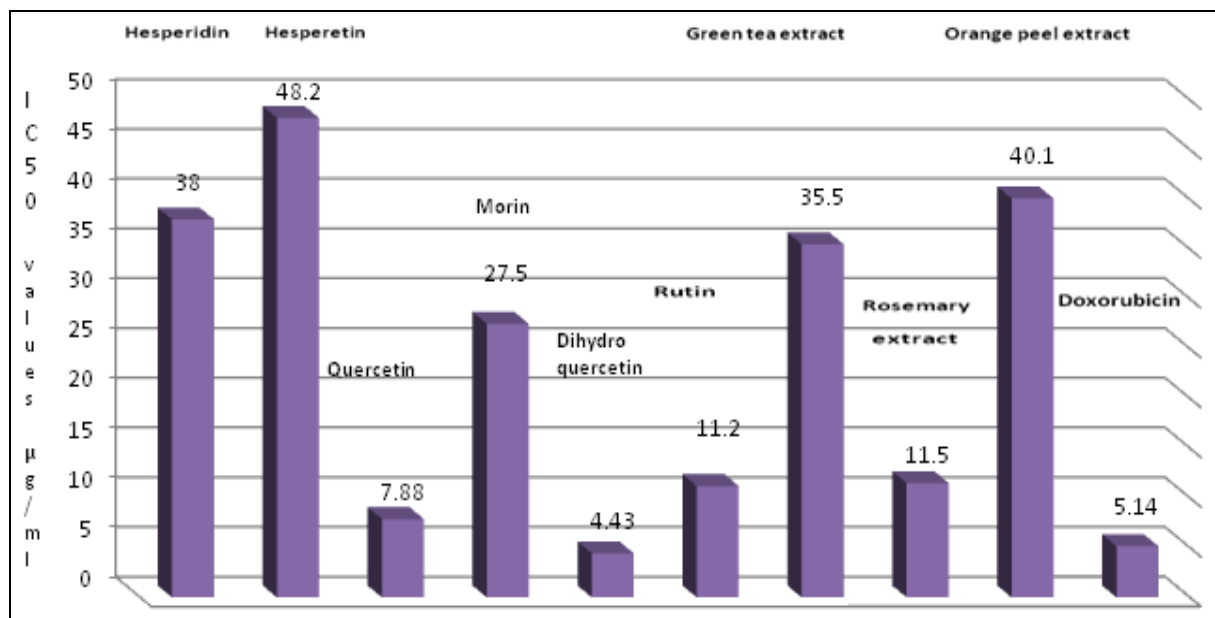


FIG. 4: COMPARISON BETWEEN CYTOTOXIC ACTIVITIES OF ALL THE TESTED EXTRACTS AND FLAVONOIDS TOGETHER WITH DOXORUBICIN AGAINST Hep-G2 HUMAN CANCER CELLS

TABLE 1: CYTOTOXIC ACTIVITIES OF THE TESTED FLAVONOIDS AND THE THREE PLANT ALCOHOLIC EXTRACTS AS WELL AS DOXORUBICIN AS A POSITIVE CONTROL AGAINST Hep-G2 HUMAN CANCER CELL LINE

Flavonoid / Extract	IC ₅₀ value (µg/ ml)	Flavonoid / Extract	IC ₅₀ value (µg/ml)
Hesperidin	38±0.08	Dihydroquercetin	4.45±0.08 µg/ml
Hesperetin	48.2±1.8	Rutin	11.2±0.97 µg/ml
±Catechin	No activity	Green tea alcoholic extract	35.5±1.1 µg/ml
Diosmin	No activity	Rosemary alcoholic extract	11.5±0.97µg/ml
Quercetin	7.88±0.89	Orange peel alcoholic extract	40.1±1.45µg/ml
Morin	27.5±0.05	Doxorubicin	5.14±0.06 µg/ml

DISCUSSION: In this work, hesperidin hydrolysis was achieved using microwave assisted method only in 20 min. Also, the yield was higher by this new method as yield of crude hesperidin was 4.32% and that of pure hesperidin was 2.84%. The parallel ordinary method¹⁹ gave only 3.9% crude hesperidin and 2.25% of pure hesperidin. In case of \pm catechin extraction from green tea, by the method used in this work, the yield given was 73.22mg % while that of the similar ordinary method of isolation and decaffeination¹⁵ gave only 69.447mg %.

Concerning the anticancer activities, rosemary alcoholic extract was found to show much more potency against Hep-G2 human cancer cell line. It showed $IC_{50} = 11.5 \pm 0.97 \mu\text{g/ml}$. This means that its potency is more than 3 times as potent as the highly antioxidant plant extract of green tea ($IC_{50} = 35.5 \pm 1.1 \mu\text{g/ml}$). Glycosylation of hesperetin with rutinose in C₇ resulted in increase in potency ($IC_{50} = 38 \pm 0.08$) as in hesperidin. However glycosidation of quercetin in C₃, as in rutin, resulted in decrease its anticancer activity against Hep-G2 ($IC_{50} = 11.2 \pm 0.97 \mu\text{g/ml}$).

Diosmin is a flavone constituent of both rosemary²⁰ and in orange peel¹⁵. It showed no anticancer activity against Hep-G2 human cancer cell lines in this work. This may agree with previous reported results²¹ showed that diosmin has low antioxidant properties due to its structural characteristics and showed low cytotoxicity against lung and breast cancer cell lines. Among the two tested flavonolaglycones, quercetin showed much higher (nearly 4-fold) anti-hepatocytotoxicity than morin. Reduction of C2-C3 double bond in quercetin (as in dihydroquercetin) nearly doubled its activity and gave a highly potent cytotoxic drug against Hep-G2 that is even more potent than Doxorubicin itself. Fortunately, taxifolin was reported to be not mutagenic and less toxic than quercetin²².

This work results differ from that reported against breast cancer cell lines²³ that concluded that for flavonoids to be strongly cytotoxic, they must possess the 2, 3-double bond in the C-ring.

CONCLUSION: The results of this investigation clearly prove potent anticancer activities of rosemary extract with a low IC_{50} value. Also, the

study ascertains the value of flavonoids as anticancer drugs against liver cancer and reports for the first time a potency comparison of 8 flavonoids related to different groups. It was wonderful to investigate that dihydroquercetin (taxifolin) is more potent than Doxorubicin with $IC_{50} = 4.45 \pm 0.08 \mu\text{g/ml}$. Taxifolin is one of flavonoidal constituents of different plants including the highly known hepatoprotective drug silymarin extract from the milk thistle seeds²⁴. Thus this hepatoprotective activity may be related to its content of dihydroquercetin. It acts as a potential chemopreventive agent against ovarian²⁵ and breast²⁶ cancers. Thus taxifolin is a promising flavonoid as anticancer against Hep-G2 as well as its chemopreventive activity. Its value may be increased being comparatively safe. This study also develops an effective extraction and acid hydrolysis method utilizing microwaves that results in rapid penetration of the plant tissues.

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CONFLICT OF INTEREST: The author declares no conflict of interest.

REFERENCES:

1. Montalto G, Cervello M, Giannitrapani L, Dantona F, Terranova A and Castagnetta LA: Epidemiology, risk factors, and natural history of hepatocellular carcinoma. *Ann N Y Acad Sci* 2002; 963: 13-20.
2. Plochmann K, Korte G, Koutsilieri E, Richling E, Riederer P and Rethwilm: Structure-activity relationships of flavonoid-induced cytotoxicity on human leukemia cells. *Arch Biochem Biophys*. 2007; 460: 1-9
3. Choi JA, Kim JY, Lee JY, Kang CM, Kwon HJ, Yoo YD, Kim TW, Lee YS and Lee SJ: Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *Int J. Oncol* 2001; 19: 837-844.
4. Ong CS, Tran E, Nguyen TT, Ong CK, Lee SK, Lee JJ, Ng CP, Leong C and Huynh H: Quercetin-induced growth inhibition and cell death in nasopharyngeal carcinoma cells are associated with increase in Bad and hypophosphorylated retinoblastoma expressions. *Oncol Rep* 2004; 11: 727-733.
5. Seufi AM, Ibrahim SS, Elmaghraby TK and Hafez EE: Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats *via* oxidant/antioxidant activity: molecular and histological evidences. *Journal of Experimental and Clinical Cancer Research* 2009; 28: 80.
6. Febriansah R, Putri DP, Nurulita NA and Meiyanto E: Hesperidin as a preventive resistance agent in MCF-7 breast cancer cells line resistance to doxorubicin. *Asian Pac J Trop Biomed*. 2014; 4(3): 228-233.
7. Fathy SA, Abdel-Hamid FF, Agwa SA and El-Diasty SM: The anti-proliferative effect of hesperidin on

- hepatocarcinoma cells HepG2. Egypt. Acad. J. Biolog. Sci. 2014; 6(2): 75-83.
8. Lee H-S, Jung K-H, Hong S-W, Park I-S, Lee C and Hang H-K: Morin Protects acute liver damage by carbon tetrachloride (CCl₄) in rat. Arch Pharm Res 2008; 3(9): 1160-1165.
 9. Yang SH, Choi HG, Lim SJ, Lee MG and Kim SH: Effects of morin on the pharmacokinetics of etoposide in 7, 12-dimethylbenz[a]anthracene-induced mammary tumors in female Sprague-Dawley rats. Oncol Rep. 2013; 29(3): 1215-23.
 10. Sergeeva EO, Dorkina EG, Dukhanina IV, Skulte IV, Andreeva OA and Sharenko OM: Study of protective action diosmin, hesperidin and "detralex". hepatotoxicity. Digital Scientific Magazine. Modern Problems of Science and Education. 2014.
 11. Chen S, Chyau and C and Chu C: Hepatoprotection using sweet orange peel and its bioactive compound, hesperidin, for CCl₄-induced liver injury *in-vivo*. Journal of Functional Foods 2013; 5(4):1591-1600.
 12. Borrás-Linares I, Pérez-Sánchez A and Lozano-Sánchez J: A bioguided identification of the active compounds that contribute to the antiproliferative/cytotoxic effects of rosemary extract on colon cancer cells. Food Chem Toxicol. 2015; 80: 215-22.
 13. El-Beshbishy HA: Hepatoprotective Effect of green tea (*Camellia sinensis*) extract against tamoxifen- induced liver injury in rats. Journal of Biochemistry and Molecular Biology 2005; 38: 563-570.
 14. Tatke P and Jaiswal P: An overview of microwave assisted extraction and its applications in herbal drug research. Research Journal of Medicinal Plants 2011; 5: 21-31.
 15. Dong J-J, Ye J-H and Lu J-L: Isolation of antioxidant catechins from green tea and its decaffeination. Food and Bioproducts Processing 2011; 89: 62-66.
 16. Skehan P, Storeng RD, Scudiero A, Monks MC, Mahon JD, Vistica JT, Warren H, Bokesch SK and Boyd MR: New colorimetric cytotoxicity assay for anticancer drug screening. J. Natl. Cancer Inst. 1990; 82: 1107-1112.
 17. Nieto JL and Gutierrez AM: ¹H NMR spectra at 360 MHz of diosmin and hesperidin in DMSO solution. Spectroscopy Letters: An International Journal for Rapid Communication 1986; 19 (9): 427-434.
 18. Belboukhari N, Cheriti A and Sekkoum K: Hesperidin and hesperitin preparation and purification from *Citrus sinensis* peels. Der Pharma Chemica 2015; 7(2): 1-4.
 19. Mauro AD, Fallico B, Passerini A, Rapisarda P and Maccarone E: Recovery of hesperidin from orange peel by concentration of extracts on styrene- divinylbenzene resin. Journal of Agriculture and Food Chemistry 1999; 47(10): 4391- 4397.
 20. Verotta L: Isolation and HPLC determination of the active principles of *Rosmarinus officinalis* and *Gentiana lutea*. Fitoterapia 1985; 56: 25-29.
 21. Naso L, Martínez VR, Lezama L, Salado C and Valcarcel M: Antioxidant, anticancer activities and mechanistic studies of the flavone glycoside diosmin and its oxidovanadium (IV) complex. Interactions with bovine serum albumin. Bioorganic and Medicinal Chemistry 2016; 24(18): 4108- 4119.
 22. Makena, PS, Pierce SC, Chung K-T and Sinclair SE: Comparative mutagenic effects of structurally similar flavonoids quercetin and taxifolin on tester strains *Salmonella typhimurium* TA102 and *Escherichia coli* WP-2 uvrA". Environmental and Molecular Mutagenesis 2009; 50 (6): 451-459.
 23. Yadegarynia S, Pham A, Ng A, Nguyen D, Lialitska T, Bortolazzo A and Sivryuk V: Profiling flavonoid cytotoxicity in human breast cancer cell lines: determination of structure-function relationships. Nat Prod Commun. 2014; 9(5): 597-606.
 24. Abou Zid SF, Chen SN and Pauli GF: Silymarin content in *Silybummarianum* populations growing in Egypt. Ind Crops Prod. 2016 (5); 83: 729-737.
 25. Haitao L, Bing-Hua J, King S and Chen Y C: "Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids". Nutrition and Cancer 2008; 60(6): 800-8009.
 26. Rogovskii VS, Matiushin AI and Shimanovskii NL: Antiproliferative and antioxidant activity of new dihydroquercetin derivatives". Eksperimental'naia i Klinicheskaia Farmakologija. 2010; 73 (9): 39-42.

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