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Anticancer Activity of Flavone of *Excoecaria agallocha* in Cytotoxicity Studies Using Hela Cells

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In this study, the compound flavone of mangrove plant *Excoecaria agallocha* was subjected to cytotoxicity studies using Hela cells was produced from ATCC, stock cells was cultured in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10 % inactivated fetal bovine serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in an humidified atmosphere of 5 % CO_2 at 37 ºC until confluent. The cell was dissociated with trypsin phosphate versene glucose (TPVG) solution (0.2 % trypsin, 0.02% EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further 50,000 cells/well was seeded in a 96 well plate and incubated for 24 h. At 37 ºC, 5 % CO_2 incubator, it was observed that while concentration increases, the inhibition increases. Finally, the compound showing good activity in Hela cells. Docetaxel was used as standard drug.

Keywords: *Excoecaria agallocha*, Flavone, Anticancer activity, Hela cells.

INTRODUCTION

The boundless tropical variety Excoecaria is known for the generation of dangerous metabolites [1]. It is appropriated on sea-shores and edge mangroves once in a while developed for wind and ocean softens up tropical Africa and east Asia. The leaves and smooth liquid acquired from harmed branches have been utilized as a fish harm in New Caledonia and in Okinawa, the resinous wood including latex, the supposed "Okinawa-Jinko", has likewise been utilized as a substitute for the incense of agalwood [2,3]. The bark and wood of this tree have been utilized as a part of conventional solutions for tooting in Thailand [4,5]. Anjaneyulu and Rao [6,7] revealed countless diterpenoids from *n*-hexane remove and from ethyl acetic acid derivation solubles of CH_3OH:CH_2Cl_2 (1:1) concentrate of the foundations of Indian mangrove plant *Excoecaria agallocha* L. (family: Euphorbiaceae). The piscicidal constituent of the twigs and bark of *E. agallocha* local to Okinawa has been portrayed as daphnanediterpene ester excoecariatoxin. This diterpene ester and some related mixtures have likewise been gotten from the latex of *E. agallocha* in Thailand [8-10].

Naturally, *in vitro* assurance of toxic effects of unknown compounds has been performed by counting viable cells after staining with a vital dye. Different methods used are measurement of radioisotope adding as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT (3-[4,5-dimethylthiazo-2-yl]-2,5-diphenyl tetrazolium bromide) system is a means of grading the activity of living cells *via* mitochondrial dehydrogenases [11-15]. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4,5-dimethylthiazo-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT), is a water dissolved in tetrazolium salt yielding a yellowish solution when processed in media or salt solutions lacking phenol red [16]. Dissolved MTT is replaced to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, illustrating the degree of cytotoxicity caused by the test material.

The objective of the current study is to find the sensitivity of Hela cell lines against flavone as observed inhibitory activity of flavone on the proliferation of both the cells.

EXPERIMENTAL

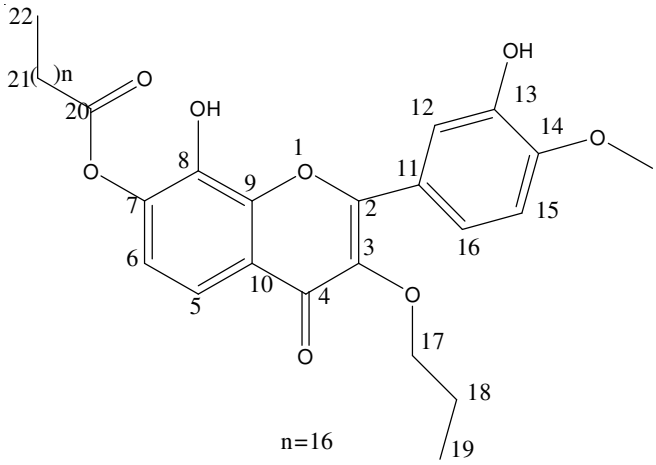
Preparation of test solutions: The MTT powder solution is filtered through a 0.2 µm filter and stored at 2-8 ºC for use. For cytotoxicity studies, each test molecule was weighed and

mixed to obtain the desired concentration and soluble in distilled DMSO and volume was made up with Dulbecco's Modified Eagle medium (DMEM) supplemented with 2 % inactivated fetal bovine serum (FBS) to obtain a stock solution of 1 mg/mL concentration and sterilized by filtration. Serial two fold dilutions (0-320 µg/mL) were prepared from this for carrying out the cytotoxic studies.

Cell lines and culture medium: Hela Cells was produced from ATCC, stock cells was cultured in DMEM medium supplemented with 10 % inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/mL) in an humidified atmosphere of 5 % CO_2 at 37 ºC until confluent. The cell was dissociated with trypsin phosphate versene glucose (TPVG) solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells/well was seeded in a 96 well plate and incubated for 24 h at 37 ºC at 5 % CO_2 incubator.

RESULTS AND DISCUSSION

8-Hydroxy-2-(3-hydroxy-4-methoxy phenyl)-4-oxo-3-propoxy-4*H*-chromen-7yl-propionate (**1**): m.p. 110 ° C, m.f. $C_{38}H_{54}O_8$ [17]. UV ($CHCl_3$) λ_{max} 270 nm indicates the presennce of conjugated-enone. IR (KBr, ν_{max}, cm^{-1}): 3534 (-OH), 2918, 2850 (-C-H),1710 (>C=O),1513,1464,1267 (-Asr group), 1174, 755 (aliphatic cyclic chain). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.6 (d, 1H, *J* = 16Hz), 7.1 (dd,1H,J=3.6 Hz), 7.0 (dd, 1H, J=1.6 Hz), 6.9 (d, 1H, *J* = 8 Hz), 6.3 (d, 1H, *J* = 16 Hz), 4.2 (t, 2H, *J* = 6.8 Hz), 3.9 (s, 3H), 2.4-2.3 (t, 3H, 7.6 Hz), 1.7-1.5 (m), 1.4-1.2 (m), 0.9 (t, 9H, 6.4 Hz). ^{13}C NMR (400 MHz, $CDCl_3$) : δ 178.0, 167.4, 148, 146.8, 144.6, 127.1, 123, 115.8, 114.7, 109.4, 77.3, 77, 76.7, 64.6, 63.1, 56, 33, 32, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.8, 26, 25.7, 24.7. M+1 = 639.



Structure of 8-hydroxy-2(3-hydroxy-4-methoxy phenyl) 4-oxo-3-propoxy-4H-chromen-7yl-propionate (**1**)

The monolayer cell culture was trypsin zed and cell count was adjusted to 1.0×10^5 cells/mL using DMEM containing 10 % fetal bovine serum (FBS). To each well of 96 well micro-titer plate, 100 µL of the diluted cell suspension (50,000 cells/well) was added. After 24 h a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of opposed test concentrations of test drugs were added on to the limited monolayer in microtiter plates. The plates were then incubated at 37 ºC for 3 days in 5 % CO_2 atmosphere, further microscopic observations were noted for every 24 h. After 72 h, the test solutions in the wells were discarded and 50 µL of MTT (5 mg/10 mL of MTT in PBS) was added to each well. The plates were gently shaken and incubated for 4 h at 37 ºC at 5 % CO_2 atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was consistent using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of drug desired to inhibit cell growth by 50 % (IC_{50}) values is generated from the dose-response curves for each cell line.

$$\text{Inhibition (\%)} = \frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

The compound **1** has shown good activity in Hela cells at different concentrations as shown in the following Table-1. The IC_{50} found to be 123.2 mg/mL, it has been observed that the inhibition increases with the concentration increases. Similarly, compound **1** has shown 28 and 34 % acts as 160 and 320 mg/mL concentration, respectively. Hence, the compound seems to be more active in Hela cells.

TABLE-1
HELA-CELLS

Compound name	Conc. (µg/mL)	OD at 590 nm	Inhibition (%)	IC_{50}
Flavone	Control	0.6312	0.00	
Flavone	10	0.5993	5.05	
Flavone	20	0.5667	10.22	
Flavone	40	0.4923	22.01	123.2
Flavone	80	0.4085	35.28	
Flavone	160	0.2906	53.96	
Flavone	320	0.2003	68.27	

Conclusion

The anticancer screening of flavone isolated from the root of *Excoecaria agallocha* using Hela cells is counducted. It is found that the flavone compound shows a good activity in Hela cells.

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