Biologically Active Fraction Of Flemingia Wightiana's Ethanolic Leaf Extract And Its Cytotoxic Properties

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Abstract: Upon purification, various anti-cancer drugs derived from plant materials were tested on cells, including various cancer cell lines and laboratory animals and then sent to clinical trials. In recent years, the number of newly discovered natural compounds has increased dynamically.Such compounds have cytotoxic properties with a range of action mechanisms such as tumor cell growth inhibition, apoptosis induction, DNA disruption, topoisomerase inhibition I and II, apoptosis induction, and others.Our present study deals with extraction and isolation of different fractions of Flemingia wightiana's ethanolic leaf extract by using column chromatography and thin layer chromatography [TLC]. This purification results in three different fractions where these three were subjected to identify their cytotoxicity against HCT-116 cancer cell lines.MTT reports make it clear that fraction 1 is the active fraction of all, and this is further continued to find the exact bioactive compounds through GC-MS.

Keywords: Flemingia wightiana; Ethanolic leaf extract; Fractions; Bioactive compounds; Cytotoxicity; HCT-116 cancer cell lines; Anti-cancer.

I. INTRODUCTION

Natural products have been used for the treatment of human diseases for decades, resulting in the creation of a large proportion of current drugs from natural molecules in modern medicine [1,2]. The search for new biologically active natural products remains an intense research field [3]. Historically, plants have proven their value as a rich source of therapeutic molecules and many major current drugs are compounds derived from natural products [4]. In addition, most epidemiological studies suggest that populations eating high levels of plant-derived foods have low incidence rates of different types of cancer [5,6].It should be noted here that the search for natural phytochemicals derived from plants expressing anticancer properties is not limited to food plants [7]. Currently, pharmaceutical companies are isolating and analyzing the anticancer ability of various phytochemicals from all regions of the world to identify more effective drugs for different types of cancer [8].It has long been known that naturally occurring phenolic compounds in plants have a wide spectrum of health-promoting properties arising from their biological activity [9]. Such properties definitely include the effects of antioxidants, anti-inflammatory and anti-cancer [10].
To discover new bioactive compounds from plant sources that could become new leads or new drugs, it is important to test extracts simultaneously through chemical testing and various biological or pharmacological targets [11].Chemical screening using hyphenated methods provides detailed structural information quickly; leading to compound recognition in many cases [12]. This enables researchers to distinguish directly from crude plant extracts between known compounds and new molecules [8]. The tedious isolation of known compounds can therefore be avoided and a targeted isolation of constituents with novel or unusual spectroscopic characteristics can be undertaken [13]. At the same time, extracts are also subjected to different bioassays that should be simple, reproducible and quick [14]. Advances in our understanding of multi-stage carcinogenesis molecular mechanisms have resulted in the development of a promising "chemoprevention" cancer control strategy [15].Cancer chemoprevention refers to the use of exogenous chemical agents to suppress or reverse the carcinogenesis process [16]. A wide variety of compounds for potential chemical-preventive action have been tested [10,14,17].These include components of the diet, micronutrients, trace elements, and certain pharmaceuticals. Much attention has recently been focused on identifying phytochemicals, especially those in our diet that have the ability to interfere with carcinogenic and mutagenic processes [18,19].The aim of this research is therefore to separate the biologically active fraction from the leaves of F. wightiana ethanolic extract to be determined by cytotoxicity testing and further analysis of the active compounds from that fraction.

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II. MATERIALS AND METHODS

Plant Materials

Leaves of F. wightiana were collected from the hills of Tirumala, India. For collecting the leaves, no special permits are necessary and the plant is not a protected plant species. Fresh leaves were washed twice with tap water and rinsed with distilled water.

Chemical and Reagents

All chemicals and other reagents were analytical grade and used without further modification directly.

Preliminary Phytochemical Study

A preliminary phytochemical study was carried out for the whole leaf extract obtained from the F. wightiana by using standard methods with suitable reagents and solvents [20-23].

Extraction, isolation and purification

The air-dried whole plant leaves [1.5 kg] of F. wightiana are finely powdered and were used for n-hexane and ethanol extraction. The concentrated extract of ethanol [73 g] was separated with Soxhlet apparatus into fractions soluble in

hexane and soluble in ethyl acetate.The condensed hexane-soluble fractions under reduced pressure resulted in a dark yellow mass and further hexane-soluble fraction work did not yield any crystalline concept.

Column chromatography

The concentrated fraction of soluble ethylacetate [80 g] was subjected to column chromatography [100–200mesh, 400 g] over silica gel using chloroform and ethanol as eluents in a phase gradient manner, resulting in a total of 5 fractions of 100mL each, collected by slow elution.

MTT Cell Proliferation Assay

MTT colorimetric assay was used to evaluate the antiproliferative activity of different fractions of F.wightiana. In MTT assay mitochondrial enzyme reduce soluble MTT into an insoluble colour formazan product in viable tumour cells, which may be measured spectrophotometrically. Briefly, 200 µl of cells [1×104 cells/ml] were seeded in 96 well plates and kept for 24 h [37°, 5 % CO2]. After 24 h, prepared concentrations of every sample [25-500 µg/ml] was added. Plant samples were dissolved in DMSO and control cells contained DMSO at the equivalent concentration [0.5 % v/v] of treated cells. After 24 h of incubation, 20 µl of MTT solution [5 mg/ml in phosphate buffer solution] was added and kept the plate for another 4 h. To dissolve formazan crystals formed, medium containing MTT were gently replaced by DMSO. Absorbance was measured at 560 nm using an ELISA plate reader [Bio-Rad]. Three independent assays were performed to calculate the results. Then 50 % cell viability of F.wightiana was calculated using the Eqn., cytotoxicity [%] = OD of control sample–OD of treated sample/OD control sample×100.

GC-MS analysis

GC-MS analysis of the ethanolic leaf extract was performed using the equipment Agilent Technologies 7890B GCMS Triple quad 7000C. The equipment has a HP MS UI 30mtr, 0.25mm, 0.25mm. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on MS library Agilent mass hunter qualitative analyses [NIST]

III. RESULTS

Ethanol extract of F.wightiana leaves was subjected to preliminary phytochemical analysis that disclosed the presence of numerous secondary metabolites like Alkaloids, flavonoids, carbohydrates, steroids ,glycosides, protein, tannins, phenols, saponins [Table 1]. The crude ethanol extract of F.wightiana was further fractioned in different solvent concentrations and the fractions obtained were studied to identify the most cytotoxic fraction using the MTT assay. Cytotoxicity activities of five major fractions were carried out against HCT-116 cell line at different concentrations to determine the IC50 [50% growth inhibition]. Results of different concentrations of F. wightiana L.[FW-1] including 3.125, 6.25, 12.5, 25 and 50 mg/ml are tabulated in Table 2, and graphically represented in Figure 1. FW-1 shows significant effect on HCT-116 cells and IC50 value of this assay was 28.8484µg/ml.As this fraction showed interesting results through its cytotoxicity results, it was further analysed by GC-MS [Figure-2] to know the major compounds present in this major fraction. From the results of this analysis 2 Methoxy 4 vinyl phenol is the major compound occupied peak area of 26% and eluted at retention time of 6.260.Major bioactive compounds identified in FW-1 are summarized in Table 3.

TABLE 1

PRELIMINARY PHOTOCHEMICAL SCREENING OF FWEE

S.No	Phytochemical constituents	Chemical test	FWEE
1	Alkaloids	Dragendroff's test	+
		Mayer's test	-
		Wagner's test	+
2	Flavonoids	10% HCl & 5% NaOH test	+
		Alkaline test	+
3	Carbohydrates	Molisch's test:	+
4	Steroids	Libermann - Burchard's test	+
5	Glycosides	Liebermann's test.	+
6	Triterpenoids	Libermann - Burchard's test	-
		Salkowski's test	-
7	Proteins	Biuret's test:	+
8	Tannins and Phenols	5% $FeCl_3$ test	+
9	Saponins	Foam test	+

TABLE 2

CYTOTOXICITY OF TESTED PLANT COMPOUNDS TOWARDS CANCER CELL LINE [HCT-116] DETERMINED BY THE MTT ASSAY

S. No	Plant compounds	IC_{50}[µg/ml]
1	FW-1	28.84
2	FW-2	>400
3	FW-3	>400
4	FW-4	>1000
5	FW-5	>1000

TABLE 3

MAJOR BIOACTIVE COMPOUNDS IDENTIFIED IN FW-1

S. No	Rt	Name of the compound	Molecular formula	M.W	Peak area [%]
1	4.483	3 Methylbutyl formate	$C_6H_{12}O_2$	116	16
2	6.260	2 Methoxy 4 vinyl phenol	$C_9H_{10}O_2$	150	26
3	27.521	Dioctyl adipate	$C_{22}H_{42}O_4$	370	4
4	29.452	Pthalic acid isopropyl octyl ester	$C_{19}H_{28}O_4$	320	4
5	31.113	1-[2-acetoxyethyl]-3 6 diazahomoadamant an-9- one oxime	$C_{13}H_{21}N_3O_3$	267	3

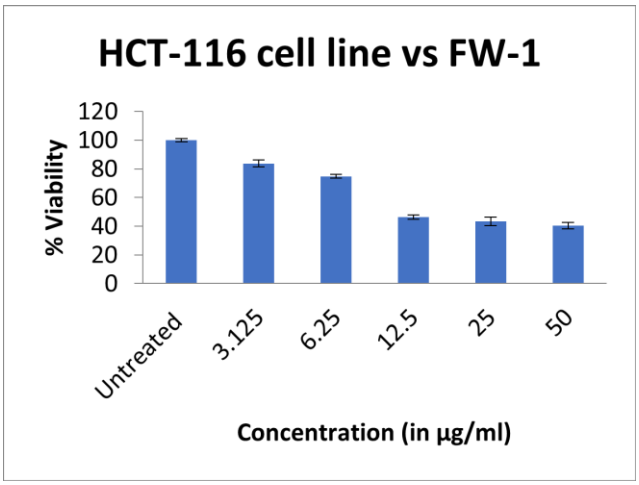

Fig:1 *Cytotoxicity of Flemingia wightiana FW-1 against HT-116*

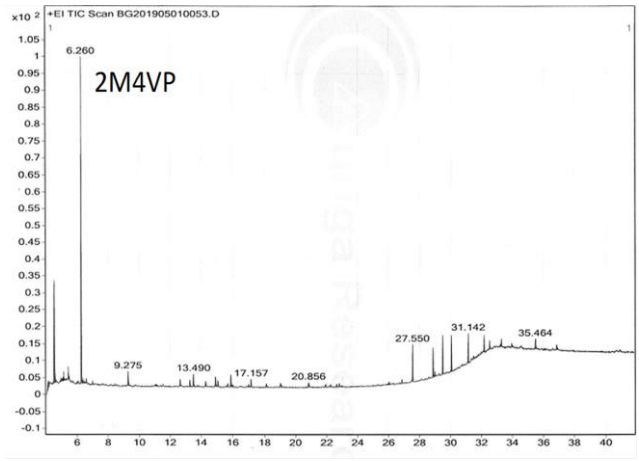

Fig. 2 *GC-MS analysis Flemingia wightiana FW-1 fraction*

IV. DISCUSSION

The investigation of the plant has opened up a new biopharmaceutical study. Natural extracts plays a key role in the healing process of infection acceleration however there is no scientific evidence of their efficacy [24,25]. Therefore, in medical research, attempts to classify the bioactive compounds of medicinally essential herbal extracts and their mechanism of action have always been of considerable importance [12,24]. In this study, the preliminary phytochemical study of F. wightiana leaves indicated the existence of various secondary metabolites such as alkaloids, flavonoids, carbohydrates, steroids, glycosides, proteins, tannins, phenols and saponins.The extract of crude ethanol from F. wightiana was further fractionated in different concentrations of solvents and the fractions obtained were analyzed using the MTT assay to determine the most cytotoxic fraction.Five major fractions of cytotoxicity activities against HCT-116 cell line were performed at different concentrations to evaluate the IC50.FW-1 has substantial effect on HCT-116 cells and this assay's IC50 value was 28.8484µg / ml. Given that this fraction showed interesting results through its tests of cytotoxicity, GC-MS further analyzed the major compounds present in this major fraction. 2 Methoxy 4 vinyl phenol is the largest compound occupied peak area of 26 percent and eluted at retention time of 6,260 from the results of this study.The findings of this study have revealed that F. wightiana leaf extracts could be used as a potential alternative for bioactive lead development in cancer treatment.

V. CONCLUSION

The current study results that the different fractions of FWEE isolated by column chromatography and their cytotoxicity, which was confirmed by MTT assay.The active fraction is selected and studied through GC-MS and found five major compounds in the active fraction. Among five, major peak area is occupied by 2-Methoxy4-vinyl phenol which is responsible for cytototoxicity. The obtained data in this study, suggest that F. wightiana leaf extracts has possible anti cancer properties and can be aplausible source for the extraction of natural healing compounds.

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