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Phytochemical Investigation of the DCM Extract of Vanda tessellata and Effect of Fraction 3 and 6 on Cell Cycle Distribution of Adenocarcinoma Cell Lines

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Abstract: Colorectal adenocarcinomas account for the third leading cause of cancer deaths, especially in the Asian countries. The objective of the study is dichoromethane extract of Vanda tessellate was then subjected to qualitative phytochemical analysis to identify the phytoconstituents. Various fractions from the active extract were isolated using the HPTLC technique. High-Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated method used to characterize its advanced separation efficiencies and detection limits. The dichloromethane extract of Vanda tessellata was further investigated for its mechanism of cell growth. The extract was found to contain steroidal molecule/s and glycoside/s. This indicates that the constituents were partitioned into distinct fractions according to their chemical properties using the HPTLC method. Some fractions were cytotoxic to the adenocarcinoma cell lines. It caused an arrest in the cell cycle in the G0/G1 phase of cell growth in both the adenocarcinoma cell lines. Although the study's findings are encouraging, additional research is required to ascertain the precise mechanism by which the plant exerts its anticancer properties and to assess its viability as a therapeutic intervention.
Keywords: *Vanda Tessellate; Cell cycle; High-Performance Thin Layer Chromatography; Adenocarcinoma.*

INTRODUCTION

Adenocarcinomas of the colorectal gland originate in either the colon or the rectum. They constitute more than 95% of all cases of colorectal cancer and are the most prevalent [1]. Cancer develops when healthy cells undergo a multistage transformation into malignant tumour cells, encompassing the progression from a precancerous lesion to the final stage of tumour development. Adenocarcinomas originate from cells that produce mucus, which is utilized to coat the colon and rectum. The malignancy originates as a polyp, which is a growth located on the inner membrane of the colon or rectum [2]. Although certain forms of polyps have the potential to progress to malignancy, not all polyps do so. Whether an adenoma develops into cancer is contingent upon its specific type. Adenomatous polyps are the most prevalent form of polyp that has the potential to grow into malignancy. There is no correlation seen between genetic predisposition and the incidences recorded which implies that non-hereditary causes of colorectal cancers seem to be responsible for the large numbers of people suffering from this cancer [3].

Vanda tessellata is a kind of orchid that may be found from the Indian subcontinent to Indochina. It is also known as the "Grey orchid". It is a plant used for medicine [4]. The plant produces an alkaloid, a glycoside, tannins, beta-sitosterol, beta-sitosterol, and a long-chain aliphatic molecule, in addition to fatty oils, resins, and coloring compounds. Roots contain tetracosylferrulate and -sitosterol-D-glucoside [5].

Natural and semi-synthetic equivalents of anticancer medications each have separate, measurable methods by which they affect cancer cells6. For instance, the DNA topoisomerase I and II enzyme are blocked by topoisomerase inhibitors (such as podophyllotoxin, topotecan, and etoposide), which disrupt transcription, DNA synthesis, and mitosis [7]. Contrarily, vinca alkaloids and taxanes, such as vincristine and paclitaxel, prevent the polymerization and depolymerization, respectively, of microtubules, interfering with crucial steps in cell division like the organization of the mitotic spindle and, consequently, the mitotic arrangement of chromosomes [8].

Natural products offer a means for the discovery of novel chemical moieties with specific reactivity [9]. If explored systematically, new chemical moieties from plants can be isolated and may be used for the treatment of such disorders [10]. This is especially more significant in cancer treatment since cancer cells are known to develop drug resistance quickly 11. The aim of this work involved phytochemical screening of Vanda tessellate and its fractionization by HPTLC and the

use of the fractions against colorectal adenocarcinoma cell lines.

MATERIAL AND METHOD

The plant extracts of Vanda tessellata were prepared through the process of refluxing the desiccated pulverised material in a three-necked flask containing the chosen solvents. The solvent was eliminated through the utilisation of a rotary evaporator. For future use, the crude extracts were freeze-dried.

Phytochemical findings pertaining to the DCM extract of the plant. For the identification of alkaloids, saponins, tannins, flavonoids, steroid hormones cardiac glycosides, phenolic compounds, phlobatannins, coumarins, and terpenoids, methanol/DCM (1:1) and aqueous extracts from the chosen plant species were analyzed. With minimal adjustments, standard procedures for preliminary phytochemical evaluation were utilized.

For the isolation of compounds from the DCM extract was fractionated onto precoated silica gel 60 GF254 plates utilising a mobile phase consisting of toluene: ethyl acetate: 90% formic acid (15:4.5:1.5). Solvents containing DCM (dichloromethane) are frequently employed to extract plant compounds. Plant compounds can be extracted from DCM extracts using a variety of techniques, such as chromatography and crystallization.

The fraction 3 and 6 underwent HPLC analysis. The analysis employed a gradient phase consisting of acetonitrile and 0.1% trifluoroacetic acid over a C18 column. The analytical method of HPLC profiling is utilized to distinguish, quantify, and identify every constituent present in a mixture.

Further optimization of the HPLC profiling procedure can be achieved through the execution of method scouting, method optimization, robustness testing, and method validation.

In the context of cancer research, the impact of Dichloromethane (DCM) extract on the cell cycle has been investigated. G0/G1 and G2/M phase cell cycle arrest were induced in MCF-7 cells by DCM-DS at low concentrations and high concentrations, respectively, according to the study.

The statistical methods employed in this investigation include post-hoc analysis, one-way ANOVA, the t-test, and Pearson product moment correlation. SPSS was utilised to conduct the analyses, and the interpretation procedure was derived from version 21 of the SPSS Survival Manual. In situations involving the comparison of means across multiple groups or populations, and assuming there is a single independent variable, one-way ANOVA is the suitable statistical method. The bivariate statistical method, in which only one independent variable is considered, is denoted as one-way ANOVA.

RESULT AND DISCUSSION

Phytochemical Screening

The findings of phytochemical investigation of the Dichloromethane (DCM) extract of Vanda tessellata are shown in table no. 1. The DCM extract of the plant showed the presence of steroids and glycosides.

Tab. 1. Phytochemical evaluation of various extracts of Vanda tessellata

Phytoconstituents	Petroleum ether	Dichloromethane	Ethyl acetate	Methanol
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	-
Flavonoids	-	-	-	-
Proteins/Amino acids	-	-	-	-
Steroids	-	+	-	-

Saponins	-	-	-	-
Phenolic compounds/ Tannins	-	-	-	-
Glycosides	-	+	-	-

- stands for negative, + stands for positive

Isolation of compounds from the DCM Extract

Using a mobile phase of toluene: ethyl acetate: 90% formic acid (15:4.5:1.5), the DCM extract was fractionated on precoated silica gel 60 GF254 plates. Figure no. 1a depicts the images of the DCM extract at 366 nm, 1b indicates the results of LB test and 1c, its HPTLC finger-print respectively. The Rf values are reported in Table 2.

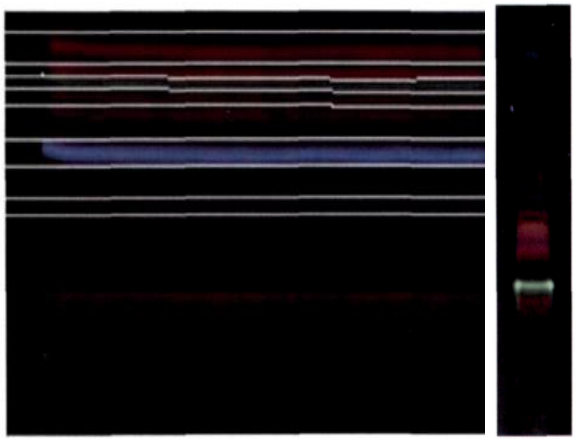

Fig. 1 (a). Photo documentation of DCM extract **(b)** Photo image of LB test for steroidal nucleus

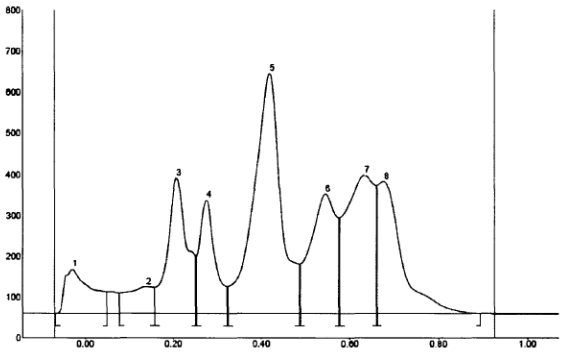

Fig. 1 (c). HPTLC finger-print of DCM extract.

Tab. 2. HPTLC data of DCM extract at 366 nm

Peak	Maximum Rf	Maximum height (in mm)	Area (AU)
1	0.03	108.3	5475.0
2	0.14	66.4	3253.0
3	0.21	340.4	10218.5
4	0.28	274.9	8034.4
5	0.43	514.5	26513.2

6	0.55	220.8	10797.0
7	0.64	205.9	17643.6
8	0.68	323.4	14528.8

As indicated in figure no. 1 (a,b, c), the bands were scrapped so as to obtain eight fractions. The compounds were extracted from the silica using chloroform; the solvent evaporated under vacuum, and the fractions were further tested for their cytotoxic potential.

It was seen that fractions 1, 2, 3, 4, 6 and 8 exhibited good cytotoxicity on both Colo205 as well as HT-29 and % cell growth inhibition increased with increased duration of exposure. The cells, however, showed proliferative effects when exposed to fraction [5]. Exposure to fraction [7] showed dual response- at low concentrations it caused proliferation, while at higher concentrations it showed toxicity. Also, with increased exposure time, it caused increased cytotoxicity 12. These fractions were further tested on INT407 to check their toxicity potential on normal cells. • Fraction 3 seems to be more potent on the HT-29 cells as compared to Colo205. Its IC50 concentration was the same on Colo205 and INT407.

Effect of fractions 3 and 6 on cell cycle

Recently, tumour cells with defective checkpoint functions are more vulnerable to anticancer agents. Hence, compounds that interfere with cell cycle checkpoints may be more effective in anticancer therapy [11]. Effect on stages of cell growth cycle on exposure to IC50 concentration of fractions 3 and 6 for 24 and 48h respectively.

Tab. 3(a-c). Summarize the findings of the cell cycle distribution after exposure to the IC5o values of fractions 3 and 6 on the three cell lines.

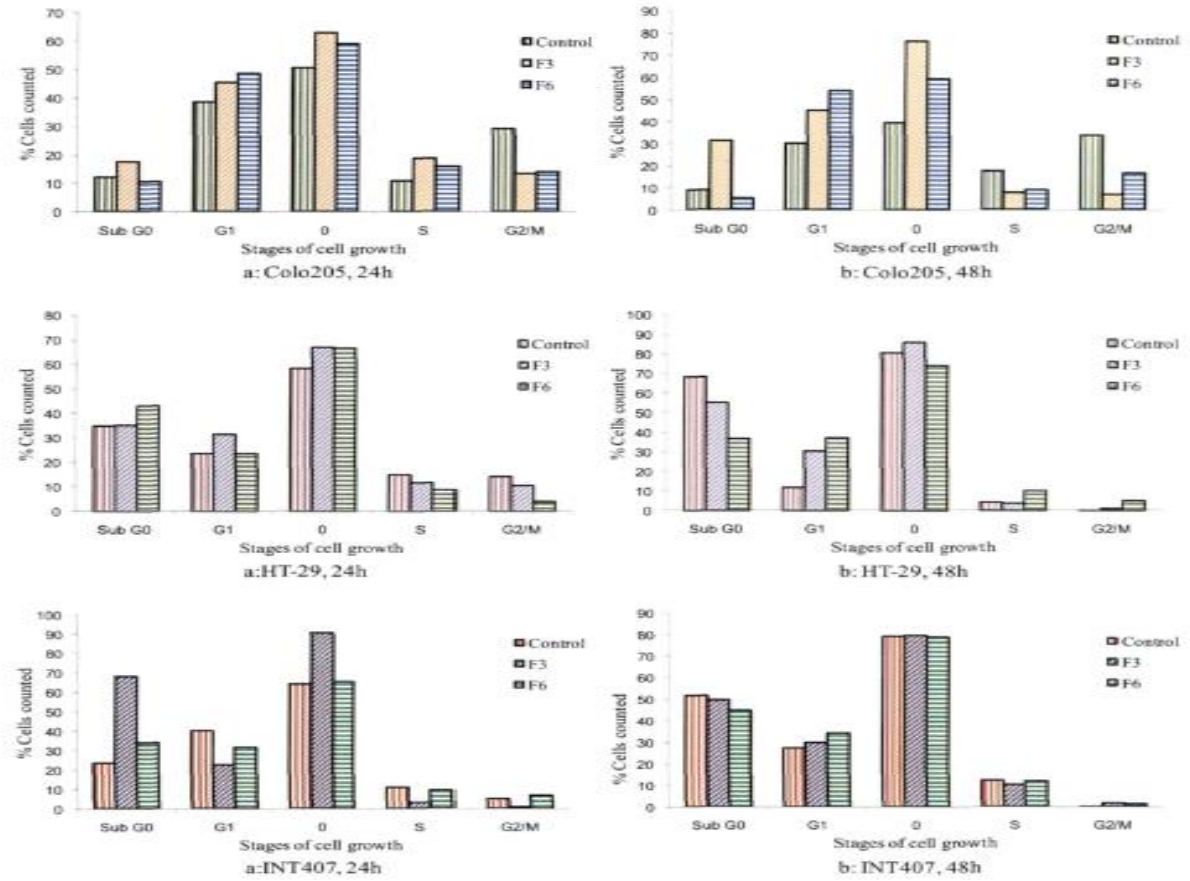

Fig. 2(a-f). Depict the effect of exposure to IC50 concentrations of fractions 3 and 6 on the three cell lines.

Tab. 3a. Cell cycle distribution seen in Colo205 after exposure to fractions 3 and 6.

Colo205	Cell count (%), 24h			Cell count (%), 48h		
	Control	Fraction 3	Fraction 6	Control	Fraction 3	Fraction 6
GO	12.17	17.44	10.61	9.37	31.49	5.33
Gl	38.61	45.7	48.7	30.15	45.24	54.36
G0+G1	50.78	63.14	59.31	39.52	76.73	59.69
S	10.85	18.86	16.05	17.51	7.84	9.02
G2/M	29.4	13.56	13.98	34.15	7.28	16.94

Tab. 3b. Cell cycle distribution seen in HT-29 after exposure to Fractions 3 and 6

Colo205	Cell count (%), 24h			Cell count (%), 48h		
	Control	Fraction 3	Fraction 6	Control	Fraction 3	Fraction 6
GO	34.7 '	35.19	43.12	68.58	55.33	36.93
Gl	23.56	31.75	23.57	12.1	30.54	37.25
G0+G1	58.26	66.94	66.69	80.68	85.87	74.18
S	15.03	11.83	8.71	4.37	3.99	10.42
G2/M	14.26	10.84	4.24	0.68	1.05	5.19

Tab. 3c. Cell cycle distribution seen in INT407 after exposure to Fractions 3 and 6

Colo205	Cell count (%), 24h			Cell count (%), 48h		
	Control	Fraction 3	Fraction 6	Control	Fraction 3	Fraction 6
GO	23.79	68.35	34.01	51.9	49.99	45
Gl	40.39	22.7	31.84	27.4	29.85	34.19
G0+G1	64.18	91.05	65.85	79.3	79.84	79.19
S	11.04	3.54	9.78	12.51	10.25	11.92
G2/M	5.43	1.28	7.14	0.77	1.79	1.43

The above tables indicate that exposure to the IC50 concentration of fractions 3 and 6 causes an arrest of the cell growth cycle in the GO/G1 phase in all three cell lines. Fraction 3 was more capable of causing an arrest in the G0/G1 phase in all the three cell lines. Fraction 6 was found to cause a greater cell cycle arrest in HT-29 as compared to Colo205. Fraction 3 was more capable of causing apoptosis than fraction 6. Both the fractions did not cause much apoptosis in INT407. Fraction 3 seemed to cause an arrest to a greater extent in all three cell lines. Fraction 6 on the other hand, was more effective in causing an arrest in the GO/G 1 phase in HT-29 than in Colo205. The cells arrested in any one phase of the growth cycle eventually undergo apoptosis [13]. The tables represent Colo205 and HT-29 respectively. Colorectal adenocarcinomas comprise an estimated ninety percent of malignant lesions of the large intestine. Early-stage colorectal cancer is frequently characterised by the absence of symptoms; therefore, early detection via screening programmes is crucial1. The quantification of tumour necrosis factor-a or -b effects, macrophage-induced cell death and the evaluation of cytotoxic or growth-inhibiting substances, including inhibitory antibodies, are some examples in order to investigate cellular activation [2].

CONCLUSION

Malignant epithelial cells that arise from the glandular epithelial cells lining the colon and rectum are called colorectal adenocarcinoma cells. They constitute the prevailing form of gastrointestinal cancer, comprising 98% of all colonic malignancies. While performing the phytoconstituents analysis and HPTLC, it was observed that some of the isolated fractions were almost equitoxic to both, the normal intestinal cells as well as on colorectal adenocarcinoma cells (as evident

from the similar IC50 concentrations on the three cell lines). Also, the IC50 concentrations of the individual fractions were similar to or greater than the IC50 concentration of the DCM extract on Colo205 and HT-29. This may suggest that the selective cytotoxicity of the extract towards the adenocarcinoma cells could be attributed to synergistic action of more than one component present in the same. Nevertheless, future research on the potential anticancer agent content of Vanda tessellata is supported by the study's results.

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