

## Extraction of Medicinal Compounds and Analysing their Anticancer Activity of *Ammomum subulatum*, *Curcuma longa*, *Cinnamomum verum* and *Glycyrrhiza glabra* & In-Silico Analysis Of km23-1, A Cancer Receptor

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### Abstract:

Nature is a unique source of different high value chemical compounds, many possessing interesting biological specificities and unique medicinal properties. In the worldwide deadly conditions of variety of cancer occurring today, there is an urgent need for an intensive search for natural chemical compounds for prevention. Spices used in food preparing contains a rich wide variety of such essential compounds which on correct use can even cure these deadly diseases possessing worldwide threat. Such four spices *Ammomum subulatum*, *Cinnamomum verum*, *Glycyrrhiza glabra* and *Curcuma longa* regularly used in food preparing and home remedies are tested upon colon cancer cell lines, COLO 320DM for their effect on cancer cells the results were amazing. Black cardamom (*A.subulatum*) extracts showed interesting cancer cell growth prohibition property and other extracts showed cancer cells destroying properties. When the SDS of these extracts was done and the compounds obtained were analysed in-silico for drug designing by docking, it showed that many drugs could be designed some specific to target and some non-specific for colon cancer prevention and cure. The intake of these compounds on regular bases will definitely prove as "PREVENTION IS BETTER THAN CURE".

### Introduction:

Nature is a unique source of structure of high stereo chemical diversity, many of them possessing interesting biological activities & medicinal properties. In the context of worldwide deadly conditions such as variety of cancer (colon, breast, skin, ovary, retinal, blood, etc.) & AIDS & Hepatitis, an intensive search for lead chemical compounds for the development of novel pharmacological therapies is extremely important. These compounds being natural on one hand possess no harm to biological

system in future on the other. The fact that since past long most of the spices used in food preparing are of real importance in INDIAN AYURVEDA has led to the discovery of many novel drugs obtained from natural compounds.

### Elaichi (BLACK CARDAMOM):

Scientific name: *Amomum subulatum*: It is a seed pod, since antiquity known for its mouth refreshing smell, its culinary uses and its medicinal properties. In home remedies black cardamom is used for stomach disorders, malaria and throat infection. Many of its compounds have anti-oxidant, disease curing and health promoting properties. It also contains many essential oils like *pinene*, *sabinene*, *myrcene*, *phellandrene* etc.

### Cinnamon:

Scientific name: *Cinnamomum verum*. It has a very nice peculiar fragrance and is being used since biblical times for its medicinal and culinary properties. The pungent smell and scent is due to the presence of cinnamic aldehyde and cinnmaldehyde in the oil. It is best known for its anti-oxidant, anti-diabetic, anti-septic, local anesthetic, anti-inflammatory, rubefacient(warming and soothing), carminative anti-flatulent properties.

### Turmeric:

Scientific name: *Curcuma longa*. The roots and leaves have long been used in traditional Indian and Chinese medicines as anti-inflammatory, pain-killer, anti-oxidant and anti-cancerous. It is best known for its inhibition properties against multiplying tumor cells. The oils present in it include termerone, curhone, curumene, cineole and p-cymene, all having applications in cosmetic industries.

### Mulethi:

Scientific name: *Glycyrrhiza glabrae*. It can be used as a good remedy in treatments of viral hepatitis,

throat infection, cough, etc. having strong anti-viral effect. Glycyrrhizin from *Glycyrrhiza* root has been shown to modulate airway constriction, lung inflammation.

**Colon Cancer:**

Colon cancer includes cancerous outgrowths in the colon, rectum and appendix. With 655,000 deaths worldwide per year, it is the third most common form of cancer and second leading form of cancer related deaths in the western world. Many colorectal cancers are thought to arise from adenomatous polyps in the colon. These mushroom like outgrowths are usually benign, but some may develop into cancer overtime.

The current study focused on the protein Mn23-1, the protein that is especially responsible for colon cancer. This protein is present in all colon cancer cells. So, the study focused to design a drug against this protein to stop colon cancer cells proliferation and growth.

**Materials and Methods:**

**Preparation of Sample extracts:**

All the four samples that are black cardamom, turmeric, cinnamon, and mulethi were ground finely in pestle and mortar. All the four grounded samples were weighed 1gram each separately and the following extracts were made.

**Phosphate Buffer Saline (PBS) Solublization:**

Weighed samples were soaked completely and separately in PBS for total compound extraction.

**Extraction Protocol:**

After overnight soaking the samples were transferred to falcon tubes and centrifuged at 6000 rpm for 10 min. Supernatant was collected in all. To each sample in the appendorfs 100% ethanol in 1:1 ratio was added and again centrifuged at 10000 rpm for 2 min. Supernatant was collected in separate appendorfs. To the pellet 500uL of chloroform was added and again centrifuged at 1000 rpm for 2 min. Supernatant was collected in separate appendorfs.

**Identification of the compounds present in extracts:**

**Alkaloids:**

Alkaloid solutions produce white yellowish precipitate when few drops of mayer's reagent is added. Thus to the extract few drops of the reagent is added and studied for the colour change.

**Terpenoids and Steroids:**

To the extract 0.5 ml acetic acid and 0.5 ml chloroform was added. Then conc. Sulphuric acid was added and sample was observed. Appearance of red-violet color suggests the presence of terpenoid and greenish-blue steroids.

**Flavanoids:**

4 ml Sample solution was treated with 1.5 ml 50% methanol the solution is warmed and a small piece of magnesium metal was added. To this solution 5-6 drops of conc. HCl was added. Appearance of red color indicates the presence of flavanoids and orange color indicates flavones.

**Purification of the Extracts Obtained:**

The 12 extracts obtained from 4 samples are subjected for purification. Initially Ammonium salt precipitation is performed followed by Dialysis.

**Testing the Efficacy and Anticancer Property of the Extracts on the colon cancer cell lines COLO 320DM obtained from MTCC:**

The colon cancer cell lines COLO 320DM were obtained from MTCC. Colon cancer cell lines were poured in thirteen test tubes 1 ml each. One of these test tubes was marked as BLANK. In other twelve tubes 100uL of each of sample was added and kept at 4°C overnight to allow the compounds to exhibit their activity on the cell lines. After the incubation time the tubes were brought to 37°C and re incubated at room temperature for 1.5 hrs. The OD of the samples was measured at 240nm in a spectrophotometer. The protocol was repeated and the OD was taken at an interval of 48hrs and 96hrs.

**Insilico Analysis**

**Selection of the Protein Receptor responsible for colon cancer:**

Gene cards data base was used to identify the causative proteins responsible for colon cancer. Among the list of protein the **km 23-1** protein has been selected due to its major role. The protein sequence was also downloaded from the same database.

**HH Pred for the 3D Structure Prediction:**

In order to collect the 3D structure of the selected protein HHpred tool was selected that works on the principle of homology modelling. The pdb id that can represent the best structure of the selected proteins can be selected from the out put and the structure is downloaded from the RCSB PDB data bank. This

structure can be further visualized using Rasmol software.

**Collection of the Chemical compounds:**

All the compounds whose anti cancer activity has to be tested are collected from Pubchem. The chemical structures were also downloaded from this page.

**Docking:**

The chemical compounds collected earlier were than used to dock with the km 23-1 receptor using Hex

software. 8 docking pairs were generated and the data is analyzed.

**Results and Discussion:**

**Identification of the compounds present in the selected samples:**

All the 12 extracts of the samples were subjected for various tests to identify the chemical compounds present in them the following results were obtained.

**Table 1: The table showing the total compounds present in the 12 extracts of the Samples**

COMPOUND	EXTRACT	ALKALOIDS	TERPENOIDS & STEROIDS	FLAVONOIDS
ELAICHI	Crude	Negative	Negative	Negative
	Ethanol	Positive	Terpenoid positive	Negative
	Chloroform	Negative	Steroid positive	Negative
MULETHI	Crude	Negative	Terpenoid	Negative
	Ethanol	Positive	Terpenoid	Negative
	Chloroform	Positive	Steroid	Negative
TURMERIC	Crude	Negative	Terpenoid	Negative
	Ethanol	Positive	Terpenoid	Negative
	Chloroform	Negative	Steroid	Negative
CINNAMON	Crude	Negative	Terpenoid	Both positive
	Ethanol	Positive	Terpenoid	Flavones
	Chloroform	Negative	Steroid	Negative

**Selection of the Major Compounds present in the Extracts:**

The results of the major compounds identified in the samples of Cardamom, Turmeric, Cinnamon and Liquorice by SDS were the following:

**Cardamom:** Azoxymethane, Cineole

**Turmeric :** Curcumin, Piperine

**Cinnamon :** Cinnamaldehyde

**Mulethi (Liquorice):**

Glabridin, Galbrene, Glabrone, Shinpterocarpin, Licoisoflavones A and B, Formononetin, Glyzarin, Kumatakenin, Liquiritin, Isoliquiritin (acholacone), Liquiritigenin, Isoliquiritigenin (cholacone form), Formononetin, Glabrin, Glabral, glycerol, Glubridin, Glysarin, 3-hydroxyglabrol, Glycyrrhisoflavonic,  $\beta$ -Amyrin, Glycyrrhizin, Glycyrrhetol, Galabrolide, Licoric acid, Liquiritic Acidz

**Table 2:** Screening for the Anticancer activity of the test samples against the Colon cell lines COLO 320DM

SAMPLE	24 HOURS		48 HOURS		96 HOURS	
	OD	TRANSMITTANCE	OD	TRANSMITTANCE	OD	TRANSMITTANCE
C.CRUDE	0.00	100%	3.00	-1.0%	3.00	-1.0%
C.ETHANOL	0.00	100%	0.00	100%	0.00	100%
C.CHLOROFORM	0.00	100%	0.00	100%	0.00	100%
E.CRUDE	0.00	100%	0.00	100%	0.00	100%
E.ETHANOL	0.00	100%	0.00	100%	0.00	100%
E.CHLOROFORM	0.00	100%	0.00	100%	0.00	100%
M.CRUDE	0.00	100%	3.00	-1.0%	3.00	-1.0%
M.ETHANOL	0.00	100%	0.00	100%	0.00	100%
M.CHLOROFORM	0.00	100%	0.00	100%	0.00	100%
T.CRUDE	0.00	100%	3.00	-1.0%	3.00	-1.0%
T.ETHANOL	0.00	100%	0.00	100%	0.00	100%
T.CHLOROFORM	0.00	100%	0.00	100%	0.00	100%

The above table shows the OD values obtained for the test samples of Cell lines incubated with the sample extracts so as to check the anticancer activity, The results were obtained using a spectrophotometer

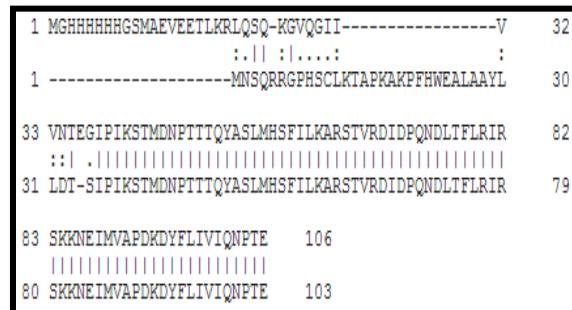
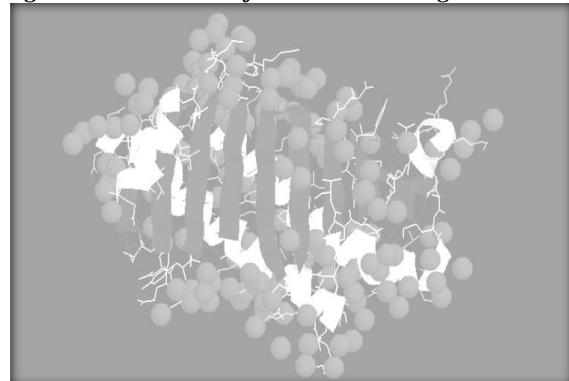
From the above results it can be inferred that only Cardamom showed best anti-cancer activity by controlling the proliferation and uncontrolled growth of cancer cells even on the 4<sup>th</sup> day of incubation. While other three compounds (Cinnamon, Liquorice and Turmeric), crude form just killed the cells suggesting that these compounds can also kill the normal cells of the body which is not required.

#### ***km23-1 Cancer Receptor Analysis using Insilico tools :***

The sequence of the one of the cancer receptor proteins km23-1 was retrieved from NCBI software. The sequence is used for the further analysis. The Protparam tool of Expsy server is employed for the physicochemical characterization of the protein in detail. The results of the tool indicate that the protein is stable with its Iso electric point of 9.41 and a molecular weight of 11790.6.

#### ***The 3D Structure Prediction using HH Pred:***

Using the homology identification tool HH Pred the PDB id of the protein was retrieved that could represent the structure of the given sequence with maximum homology. The PDB id selected was **2hz5**. The structure of the protein was retrieved from Protein Data Bank.

**Fig 1:** Showing the structural alignment of km23-1 with the most identical protein id (pdb id) 2hz5 is:**Fig 2: Visualization of the Protein using Rasmol**

**Collection of the Chemical structures from the databases:**

Pubchem data base has been used to collect the structures of the compounds like Cineole, Curcumin, Cinnamaldehyde, Azoxymethane, Piperine, Shinpterocarpin, Glabridin, Liquiritin which are later used to dock to the selected receptor km23-1.

**Screening for the Chemicals:**

The compounds selected were downloaded from the Pubchem database and were optimized in the Argus lab. Once the chemicals are optimized they are used for docking with the selected receptor km23-1.

**Docking using Hex Software:**

The compounds were later used to dock to the selected receptor using the software Hex followed by the analysis of their docking energies.

The figure 3 below shows the results of the 8 docking results performed using HEX software. From the results of the docking it can be found that the dockings between the km 23-1 and **Curcumin (-261) / Liquitin (-256)**, are energetically much favourable when compared to the other compounds.

**Conclusion:**

From the above works it can be concluded that black cardamom can be best used as the treatment remedy for cancer control and even prevention. Cardamom extract was efficient in preventing the uncontrolled cell proliferation without killing the cells. The extract has posed a barrier on the cell division which was one of the very good effects seen. On the other hand, turmeric, mulethi and liquorice killed the cancer cells at certain interval of time, proving that they can be used as targeted drugs for cancer treatment rather as a basic drug for cancer prevention because an overdose can also be fatal for normal body cells and the normal surrounding cells.

The study suggests that the regular use of cardamom may prove as a good prevention for cancer. Similarly the spices such as mulethi and liquorice can also be employed for the same. The compounds obtained from all the four spices after docking, suggests that drugs can be made by their combinations or even solely against cancer and more specifically for colon cancer. The drug will really prove best as a cure in cancer treatment.

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