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# Anticancer activity, UV visible and FTIR analysis of *Herbal formulation*

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## ABSTRACT

The present study was carried out to characterize the bioactive constituents present in seed extracts of *Herbal formulation* using UV visible and FTIR analysis and *in vitro* anticancer activity of methanolic extract of *Herbal formulation* exhibited significant anti hepatocanceractivity against a Human colon cancer HCT116 cell linesby MTT assay. The results of the UV visible and FTIR analysis analysis provide different peaks determining the presence of 20 phytochemical compounds with different therapeutic activities. The From the results, it was observed that presence of 1º,2º amines, alkynes, aromatic amines, Aliphatic amines, Alkanes, Alkyl halides, Amine and Halo compound were the major components in the extract and these compound having the properties of UV visible and FTIR analysis chromatogram of the extract of *Herbal formulation* extract possess potent anticancer activities.

**Keywords:** *Herbal formulation*, Human colon cancer HCT116 cell lines, UV visible and FTIR analysis, Anticancer, MTT assayetc

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# INTRODUCTION

Cancer is one of the most important causes of death in the world. According to the World Cancer Report 2014, approximately 14 million new cancer cases and 8.2 million cancer-related deaths were reported in 2012. Among the different types of cancer, lung cancer is associated with the greatest mortality (1.5 million deaths), followed by liver (745 000 deaths), stomach (723 000 deaths), colorectal (694 000 deaths), breast (521 000 deaths), and esophageal cancer (400 000 deaths)(WHO,2014). The number of new cancer cases is expected to increase by 70%, from 14 million to 22 million, in the next 2 decades (WHO,2014). The populations of South America, Asia and Africa represent 70% of cancer deaths and 60% of the total new cancer cases annually in worldwide.

Obesity was public health problems with health and economic consequences that have raised global concern. Cost-effective mitigation strategies rather than containment are, therefore, of paramount importance for the prevention and treatment of these diseases. As a result, a plethora of lifestyle modification, diets, pills, and weight loss regimens have been recommended. Several epidemiological studies have linked the consumption of barley with the reduction of obesity (Benkeblia and Thondre.,2014). Barley phytochemicals that may have a role in protecting against diabetes and obesity include various phenolic acids, flavonoids, phytosterols, and tocols. These compounds function as strong antioxidants. However, total phenolic acids are considered as the major components responsible for the antioxidative benefits of cereals including barley (Gamel*et al*.,2012).

## Flaxseed

Flaxseed (*Linumusitatissimum* L.), one of the oldest cultivated crops, continues to be widely grown for oil, fiber, and food. The worldwide flaxseed production between 2007 and 2011 was averagely 1,862,449 tonnes (FAO, 2011). Flaxseed oil is an excellent source of the omega-3 fatty acid linolenic acid with typical levels of 55% in the oil making it ideal for paints, varnishes, and inks due to its fast polymerization properties. Increasing demand for edible oil sources with significant percentages of omega-3 fatty acids is resulting in consumption of flaxseed as a functional food. Flaxseed is also added to animal feed to improve animal reproductive performance and health (Turner *et al*., 2014).

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Based on the complexity of the flaxseed fractions used for much of the research discussed above it is not possible to attribute the health benefits of flaxseed consumption to a sole bioactive component present in flaxseed. The exploration of the biological roles of flaxseed polyunsaturated fatty acids and lignan has been substantial in contrast to the modest efforts made on CLs and other components. Others have recently reviewed much of this research (Lane *et al*., 2014).Whole flaxseed is widely accepted as a healthy food that has anticancer activity. Controlled experimental diets have demonstrated numerous beneficial effects of flaxseed consumption (Jenkins *et al*., 1999). Dietary flaxseed flour reduces epithelial cell proliferation and nuclear aberrations in mammary glands of female rat. This finding indicates that flaxseed may reduce the growth rate of mammary cancer. Theflaxseed lignan reduces tumor growth in mammary glands at the later stages of carcinogenesis (Thompson, Seidl, Rickard, Orcheson, & Fong, 1996). Flaxseed oil(14%) and flaxseed meal(20%) are reported to reduce the occurrence of azoxymethane-induced aberrant crypt foci formation in Fisher 344 male rats (Williams *et al*., 2007). Similarly, it has been revealed that the replacement of corn meal with flaxseed meal (15%) or corn oil with flaxseed oil (15%) in a basal diet, significantly decreased tumor proliferation and size in the small intestine and colon of Fisher 344 male rats. From the study, it was concluded that flaxseed meal and oil are effective chemo-preventive agents (Bommareddy *et al.*, 2009).

**Finger millet**

The nutraceutical properties of finger millet is due to the presence of high content of calcium (0.38%), protein (6%–13%), dietary fiber (18%), carbohydrates (65%–75%), minerals (2.5%–3.5%), tannins (0.61%), phenolic compounds (0.3–3%) and trypsin inhibitory factors, and is recognized for its health beneficial effects, such as anti-diabetic, antitumerogenic, anti-diarrheal, antiulcer, anti-inflammatory, atherosclerogenic effects, antioxidant and antimicrobial properties (Devi *et al*., 2014; Sripriya*et al*.,1996;Chethan and Malleshi, 2007). Earlier it was thought that the presence of phytates, tannins, polyphenols and dietary fiber contents of finger millet act as anti-nutrients because of their metal chelating and enzyme inhibition activities but now it has been confirmed that these constituents can contribute to antioxidant activity, which is an important factor in resisting aging and metabolic diseases (Thompson, 1993) .

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Additionally, finger millet is also useful in the management of physiological disorders such as diabetes mellitus, hypertension, vascular fragility, hypercholesterolemia, prevention of oxidation of lowdensity lipoproteins (LDLs) and also improves gastrointestinal health (Bravo, 1998) .

**Barley**

Barley is among the most ancient cereal crops grown in the world today. Archeological evidence suggests the existence of barley in Egypt along the River Nile around 17,000 years ago (Badr*et al*., 2000). Barley is considered as one of the top most cultivated crops globally (12% of total cereal cultivated), ranking fourth among cereal grains after wheat, rice, and maize (Schutle*et al*., 2009). Barley outperforms other cereals under various environmental stresses due to its winter-hardy, drought-resistant, and early maturing nature and is thus generally more economical to cultivate (Cook, 2013). Approximately 65% of cultivated barley is used for animal feed, 33% for malting, whereas only 2% is used directly for human consumption (Sullivan *et al*., 2013).

This high antioxidative activity of phytochemicals present in barley makes it a useful natural means for the prevention of diabetes and obesity development and progression. Furthermore, systemic, low-grade inflammation, especially in adipose tissue, is a trademark of obesity and diabetes. In addition to barley phytochemicals antioxidant properties, barley phytochemical compounds have potent anti-inflammatory actions and could thereby moderate diabetes and obesity risk by this mechanism (Levitan *et al*., 2008).The present study investigates the analyses TLC plates of suitable mobile phase and UV spectroscopy for solvent extracts from the herbal formulation.

## MATERIALS AND METHODS

### Collection of plant material

The fresh seed and grains were collected from Ayurvedic centre,Thiruvarur.

### Preparation of seeds and grains powder

Take 1kg of ragi grains, ½ kg of barley grains and ¼ kg of flax seeds. These 3 Components are dried in sunlight and grind well.

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**Extraction of plant material**

**Principle**

The plant material was subjected to successive extraction based on the polarity nature of solvents. In this process the substance, which is soluble in a solvent with a particular range of polarity was extracted in that solvent and remaining material further extracted with the next solvent with different polarity. The constituents which were soluble in both polar and non-polar solvents can also be extracted separately by adopting this approach. This method facilitates the withdrawal of active constituents present in the plants. These extracts were concentrated using flash evaporator under reduced pressure and controlled temperature (40-50° C).

**Materials**

| | | |
|---|---|---|
| Sample | : | The *Herbal formulation* |
| Solvents | : | Aqueous, ethanol and chloroform |
| Apparatus used | : | Soxhlet apparatus |

**Procedure**

The powder of the herbal formulation were successively extracted with different solvents *viz.*, *c*hloroform, ethanol and water by the successive solvent extraction method using a Soxhlet apparatus according to the methodology of Indian Pharmacopoeia. The extraction was carried out for 18 h with the selected solvents with a ratio 1:4 w/v, based on their polarity *viz*., chloroform, ethanol and aqueous.

**Source of chemical and reagents**

Dulbecco's Modified Eagle's Medium, streptomycin, penicillin-G, L-glutamine, phosphate buffered saline, 3-(4,5 dimethylthiozol – 2-yl)-2,5-diphenyltetrazoliumbromide, trypsin-EDTA, ethanol and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich Chemicals Pvt. Ltd (India). All other chemicals and reagents used for analytical grade are purchased from Hi media

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Laboratories Pvt. Ltd., India.

## Cell culture maintanance

Human colon cancer HCT116 cell lines were procured from the cell repository of National Centre for Cell Sciences (NCCS), Pune, India. Dulbecco`s Modified Eagle Media (DMEM) was used for maintaining the cell line, which was supplemented with 10% Fetal Bovine Serum (FBS). Bacterial contamination are prevented by adding Penicillin (100 U/ml), and streptomycin (100 µg/ml). The cell line medium was maintained in a humidified environment with 5% $CO_2$ at 37°C.

## MTT assay

The cytotoxicity of Herbal formulation on HCT116 cells was determined by the method of Mosmann, (1983).

## Principle

The yellow 3-4,5- dimethylthiozol-2-Yl)-2,5diphenyltetrazoliumbromide (MTT) is reduced by mitochondrial dehydrogenase of viable cells yielding a measurable purple formation product. Viable cells contain NAD(P) H-dependent reductase, which reduce the MTT reagent to formazon, with a deep purple colour. Formazon crystals are then dissolved using solubilizing solution and absorbance is measured at 500-600 nm by plate reader.

## Reagents

### MTT stock solution:

MTT (50 mg) dye was dissolved in 10 mL of PBS. After vortexing for 1 min, it was filtered through 0.45 micro filters. The bottle was wrapped with aluminum foil to prevent light, as MTT was light sensitive. The preparation was stored at $4^oC$.

## Procedure

Cell viability assay, HCT116 viable cells were harvested and counted using hemocytometer diluted in DMEM medium to a density of $1 \times 10^4$ cells/ml was seeded in 96 well plates for each well and incubated for 24 h to allow attachment. After HCT116 cells treated with control and the

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containing different concentrations of Herbal formulation (50 to 300 µg/ml) were applied to each well. HCT116 cells were incubated at 37°C in a humidified 95% air and 5% $CO_2$ incubator for 24 h. After incubation, the drug-containing cells wash with fresh culture medium and the MTT (5 mg/ml in PBS) dye was added to each well, followed by incubated for another 4 h at 37°C. The purple precipitated formazan formed was dissolved in 100 µl of concentrated DMSO and the cell viability was absorbance and measured 540 nm using a multi-well plate reader. The results were expressed at the percentage of stable cells with respect to the control. The half maximal inhibitory concentration ($IC_{50}$) values were calculated and the optimum doses were analyzed at different time period.

$$\text{Inhibitory of cell proliferation (\%)} = \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}} \text{ X } 10$$

The $IC_{50}$ values were determined from the various extract dose responsive curve where inhibition of 50% cytotoxicity compared to vehicle control cells. All experiments were performed at least three times in triplicate.

**Statistical analysis**

The values are expressed as mean ± SD. The statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT), using SPSS version 12.0 for windows (SPSS Inc. Chicago; http://www.spss.com). The values are considered statistically significant if the p value was less than 0.05.

**THIN LAYER CHROMATOGRAPHY**

TLC is based on the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces (**Geiss, 1987 ; Touchstone , 1992**).

**Procedure**

A thin-layered plate is prepared by spreading aqueous slurry of Silica gel G on the clean surface of a glass or rigid plastic. Calcium carbonate or starch is also added to the adsorbent to increase adhesion. The plate is then heated in an oven for about 30 mins at 105°C to activate the plate. It is then cooled inside the oven itself. Test samples (1mg/ml of extracts in respective

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solvents) were applied in the form of spots using capillary tube. The toluene and ethyl acetate solvent (Toluene : ethyl acetate 93 : 7 v/v) (Wagner and Bladt, 1996) used for caryophyllene. The solvent is poured into the chamber and closed tightly and the chamber is saturated for a few hours before running the chromatogram. The extracts were applied as spots on a stationary phase (silica-gel coated plate) about 1 cm from the base using capillary tube. The plate was then placed into a suitable solvent system (mobile phase) and covered. The solvent starts to migrates up the plate which separates different components of the mixture at different rates which appear as spots in the thin layer. After the solvent has reached almost the top edge of the plate, nearly 3/4th of the plate, the plate is removed from the tank and dried briefly at moderate temperatures 60-120°C. The presences of secondary metabolites in the extracts were detected by TLC using suitable spraying reagents. Colored substances can be seen directly but colorless substances were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The visualization of constituents on plate was achieved by spraying plate with anisaldehyde/sulphuric acid reagent (Spray with a solution of freshly prepared 0.5ml p-anisaldehyde in 50ml glacial acetic acid and 1ml of 97% sulfuric acid and heat to 105°C until maximum visualization of spots).

**Rf Value:** It is a ratio of distance travelled by the sample and distance travelled by the solvent.

$$Rf = \frac{\text{Distance of the sample (solute) from the origin}}{\text{Distance of the solvent from origin}}$$

## UV-visible spectral analysis (Omodara*et al*.,2013)

- Switch on the instrument
- Leave it for the instrument for 15 min machine calibration
- Run the software by double clicking on the icon Double BeamSpectrophotometer present on the desktop.
- Switch on the Vis and UV lamps by mouse click on the yellow and blue icons from tool bar. It takes some time period (~ 3 minutes) for UV lamp to become ready
- Fill the two cuvettes, onecuvette with appropriate background solution and the other with the sample.

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- Place the two cuvettes in appropriate sampleholders.
- After the blank solution was kept and autocorrect it to zero
- Then kept sample to read the absorbance at specific ranging from 200-900 nm with the minor unit of 250.
- The values are tabulated and therefore the graph was plotted.

**FT-IR analysis:**

Shimadzu IRTracer-100 Fourier transform infrared spectrophotometer was used for the current study. For FT-IR measurements, the Ag nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a Shimadzu FT-IR Affinity1 model in the diffuse reflectance mode operating at a resolution of 4 $cm^{-1}$ (Visweswara Rao Pasupuleti*et al.,* 2013).

**RESULTS AND DISCUSSION**

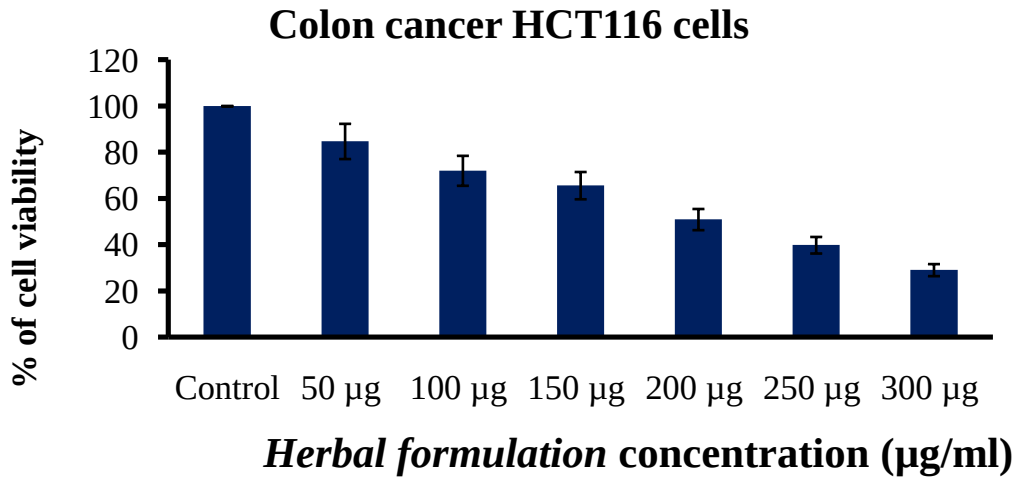


**Fig.No 1: Anti-Proliferative effects of herbal formulation on the activity of cytotoxicity in HCT116 cells**

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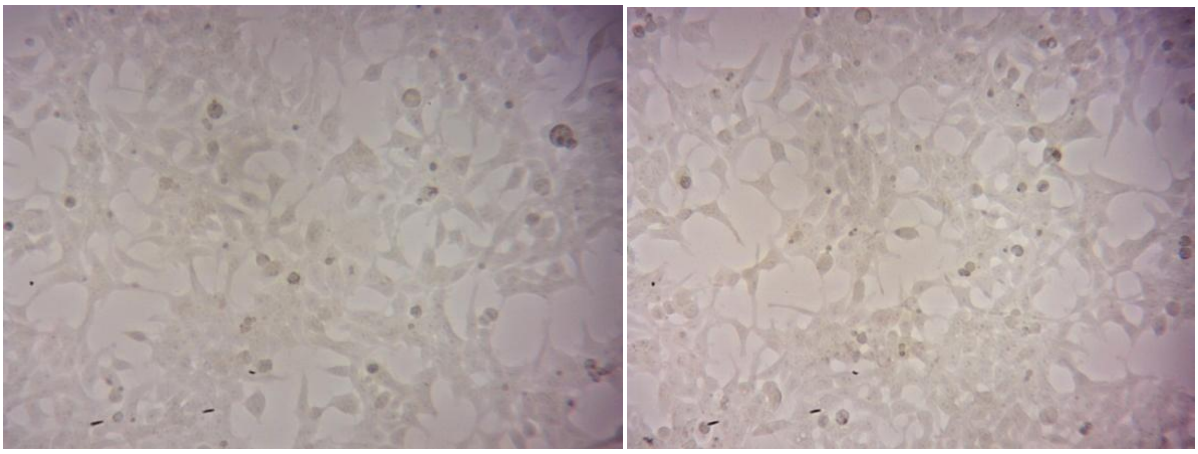
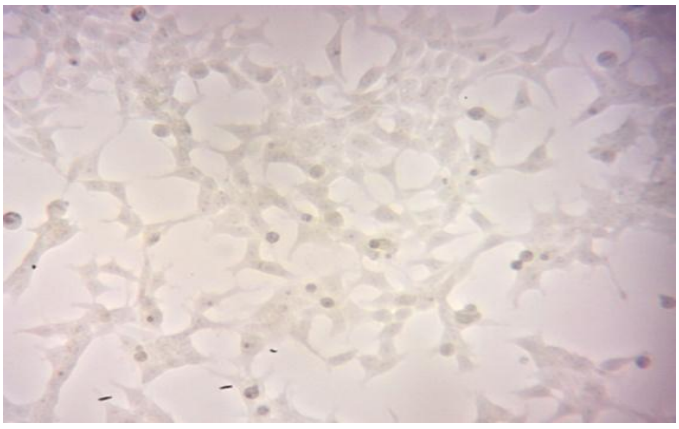
**Anti-proliferative effects of *herbal formulation*on the activity of cytotoxicity in HCT116 cells. The colon cancer cells were treated with increasing concentration of RC (50-300 µg/ml) for 24 h and the results are expressed as a percentage of the control value in presenting as a cell cytotoxicity ratio for HCT116 cells using MTT assay. Values are expressed as mean ± SD for three experiments. Values that do not share a common superscript letter are significantly different at p< 0.05 by one-way ANOVA followed by DMRT.**



**Control** **150 µg**



**200µg**

**Fig No 2: Representative Photomicrograph showing changes such as shrinkage, detachment, membrane blebbing and distorted shape in the HCT116 cancer cell lines. Treatment with**

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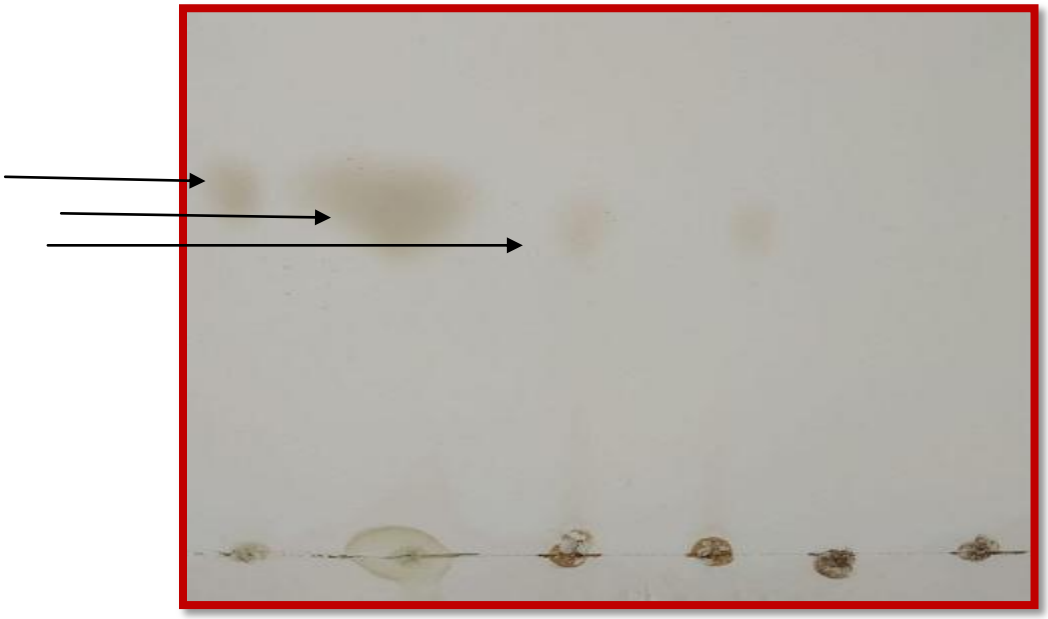
**herbal formulation is compared to intact morphologically of control HCT116 cells**

**TABLE NO:1 THIN LAYER CROMOTOGRAPHY OF *HERBAL FORMULATION***

| S.No | Extracts | Spot | Rf Value |
|---|---|---|---|
| **1** | Crude water | Brown Spot | 0.50 |
| **2** | Crude alcohol | Green Spot | 0.83 |



**FigNo :3 TLC**

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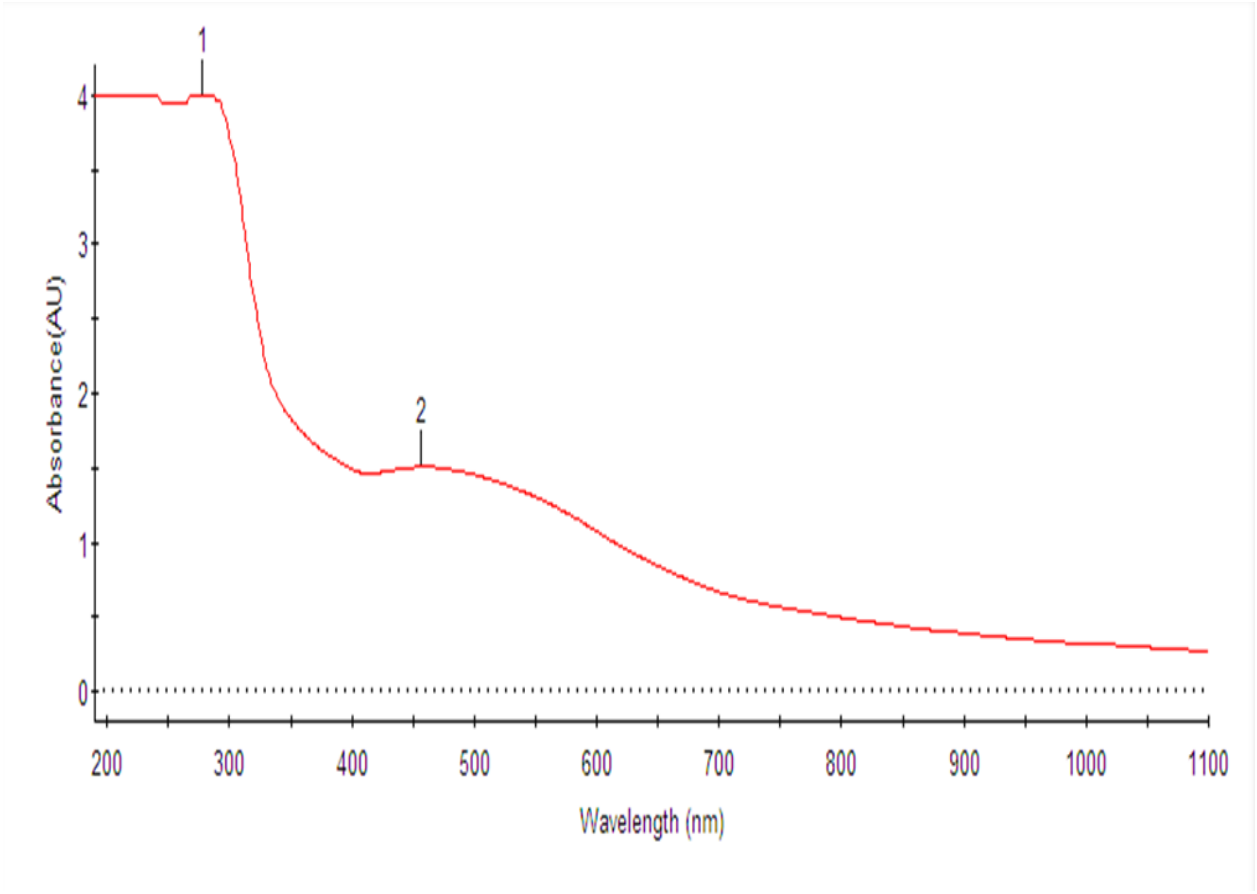


Name No. Peak(nm) Peak(AU) No. Valley(nm) Valley(AU)

**Fig No :4 UV-VIS Analysis of *Herbal formulation L***

| S.NO | Wave Length | Absorbance |
|---|---|---|
| 1 | 278.00 | 4.0000 |
| 2 | 457.10 | 1.5088 |

**Table No :2 UV-VIS Analysis of *Herbal formulation***

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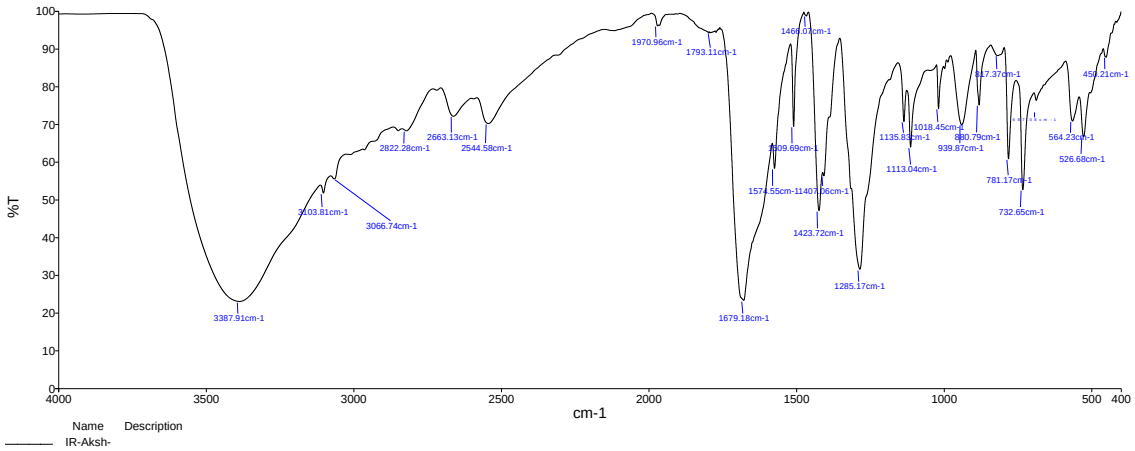


**Fig No : 5FTIR ANALYSIS OF *Herbal formulation L* EXTRACT**

| S.NO | FREQUENCY RANGE | TYPE OF BOND | TYPE AND GROUP |
|---|---|---|---|
| 1. | 3387 | N–H stretch | 1º,2º amines, |
| 2. | 3066 | –C ≡ C– stretch | Alkynes |
| 3. | 2822 | –C=C– stretch | Alkenes |
| 4. | 2544 | C–N stretch | aromatic amines |
| 6. | 1970 | C–N stretch | Aliphatic amines |
| 7. | 1679 | =C–H bend | Alkanes |
| 8. | 1423 | C–Cl stretch | Alkyl halides |
| 9. | 1285 | C–Br stretch | Alkyl halides |
| 10 | 1113 | C-N Stretching | Amine |
| 11 | 781 | C-Br Stretching | Halo compound |

**Table No:3 FTIR ANALYSIS OF *Herbal formulation L* EXTRACTS**

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Epidemiological data indicate that diet/lifestyle is responsible for approximately 20–80% human cancer mortality. Dietary factors, especially those that reduce the impact of reactive oxygen species, can protect against DNA damage and stimulate the immune system, thus lowering cancer risks. Barley and its products have bioactive compounds with antioxidative and immunomodulatory activities that are associated with cancer moderation. Most studies regarding the chemoprevention of carcinogenesis by barley have been *in vitro* and have mainly involved the effect of barley fiber, especially β-glucan, and the moderation of this disease.. GBF also contains phytochemicals, especially phenolic acids, present in free or bound forms which contribute to its health benefits (Floridi*et al*., 2003).

The flax seed oil was proven to be a very good alternative for treating different types of oral cancer. This has proven to be a good cytotoxic agent in this study as well as a very good nutritious food to be added in our diet (Austria *et al*.,2008). The fatty acids found in the flax seed oil was seen to prevent different types of cancers like breast cancer and the oral cancers (Faintuch*et al*., 2011). From the correlation from various investigations of flax seed oil, it is dissected that the plant item is sheltered to use in treatment of oral disease (Ashwini, 2017).

Chemopreventive is accordingly, ahead extensive attention as a promising and alternative approach for cancer control (Patel *et al*., 2007). Cancer cell line derived from human tissue not only provides a fundamental platform to understand molecular biology of neoplasia, also served as a basis for the investigation of specific therapeutic strategies towards cancer types (Clement *et al*., 1998). In vitro schemes allow both more precise dosing of drug and length of exposure in a uniform, chemically and experimentally defined medium.

Anti-proliferative effects of *herbal formulation* on the activity of cytotoxicity in HCT116 cells. The colon cancer cells were treated with increasing concentration of RC (50-300 µg/ml) for 24 h and the results are expressed as a percentage of the control value in presenting as a cell cytotoxicity ratio for HCT116 cells using MTT assay. Representative photomicrographs showing morphological changes such as shrinkage, detachment, membrane blebbing and distorted shape in the HCT116 cancer cell lines treatment with *herbal formulation* is compared to intact morphologically of control HCT116 cells dose dependent manner.

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TLC analysis indicates the presence of different kinds of phytochemicals in leaves extract. Thin layer chromatography was performed on plant extracts using different solvent systems ethanol : Water : chloroform (5:1:4) .

TLC of plant extract in choloroform reports three spots for various phytochemicals. The reported spots are separated with enough space and having various $R_f$ values showing the presence of atleast three phytochemicals in ethanol extracts. In our study, the most suitable TLC system for analysis was shown to be ethanol : Water : chloroform (5:1:4) with the largest discriminating power. Three bands found in this method and its $R_f$ values were 0.50 and 0.83. This values indicate the presence of phenolic compound(Table 1)

The qualitative UV-VIS profile of ethanolic extract of *herbal formulation* was taken at the wavelength of 300 nm to 800nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 278 and 457nm with the absorption 4.000, and1.5088 respectively. Figure 4 shows the absorption spectrum of *herbal formulation* and these are almost transparent in the wavelength region of 300-800 nm.

Absorption bands observed pertaining to *herbal formulation* plant extract are displayed in figure 2.In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O . The spectrum for *herbal formulation* extract shows two peaks at positions 278 nm, and 457 nm. This confirms the presence of organic chromophores within the *herbal formulation* extract. The applications of UV-visible spectrophotometery in the analysis of complex media is restricted by the difficulties in assigning the absorption peaks to any particular constituents in the system. Thus, UV–VIS findings must be analysed with some other technique such as GC/MS etc, to enable proper extract characterization and constituent identification.

These absorption bands formed are attributed for the presence of flavonoids and its derivatives. The flavonoids spectrum typically consist of two absorption maximum in the range of 230-285 nm (band I) and 300-350 nm (band II). The specific position and relative intensities of these maxima give valuable information on the nature of the flavonoids. This result obtained are compared with the previous literature on *Acoruscalamus* (NehaSahu, JyotiSaxena 2013)

FTIR measurement was carried out to identify the possible biomolecules responsible for antiobesity activity using *herbal formulation* extract**.** This spectrum shows lot of absorption bands (Fig.5)

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indicates the presence of active functional groups in the herbal formulation.The intensity peaks are slightly increased for the period of 3387,3066,2822,2544,1970 cm-1 as well as some intensity peaks decreased like 1679,1423 and 785cm-1. Fig 5 shows the band at 3387correspond to 1º,2º amines, amides. The peak at 3066 represents to C-H in plane bend to alkenes. The peak at 1423 corresponds to C–N stretch vibrations to aromatic amines. The weak band at 785 indicates C–Br stretch stretching vibrations and it corresponds to the presence of Alkyl halides and aliphatic amines in the plant extract (Muruganatham*et al.*, 2009).

FTIR spectrometry is used to measure the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample. By attaining IR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolites. At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites (Stehfest*et al.*, 2005)

FTIR is one of the most widely used methods to identify the chemical constituents and elucidate the compound structures to propose in medicinal purposes. Previous researchers carried out the FTIR to notice the minor changes of primary and secondary metabolites and to recognize the concrete structure of certain plant secondary metabolites (Marimuthu*et al*.,2013) The characteristics functional groups are responsible for the medicinal properties of plant are confirmed by FTIR analysis (Ashokkumar*et al*.,2014)

FTIR spectrum analysis is used to confirm the functional constituent's presence in the given parts and extract, identify the medicinal materials from the adulterate, and even evaluate the qualities of medicinal materials. Similarly, Cayratiatrifolia plant stems ethanolic extract holds more phytochemical and bioactive compounds which were confirmed using FTIR (Sundaram *et al.*,2016).

## CONCLUSION

In conclusion, our collective data suggested that Herbal formulation prevents cell growth of HT116 cell lines by dose and time dependent manner. Our data confirm the potential of Herbal formulation as an agent of chemotherapeutic and cytostatic activity in human Colon carcinogenesis.Herbal formulations have an assortment of phytochemicals with the

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potential to impact human health. These benefits are due to inherent properties present in phytochemicals, such as high antioxidative activity against different free radicals, anti-inflammatory, and immune stimulation potentials, or the ability to inhibit LDL cholesterol, while increasing HDL cholesterol levels. However, there is a need for more systemic and detailed study on Herbal formulation phytochemicals, thus establishing a chemical profile for phytochemicals in barley and possibly identifying unique compounds as have been done with avenanthramides in oat and alkylresorcinols in rye and wheat. In addition, since specific studies on health effects of phytochemicals in Herbal formulation are limited, it is worthwhile to further study the efficacy and the underlying molecular mechanisms of barley phytochemicals, thereby promoting the use of barley as a functional food.

**REFERENCES**

1. Ashokumar. R and Ramaswamy.M. 2014. Phytochemical screening by FTIRspectroscopic analysis of fruit extracts of selected Indian Medicinalplants. Int.J.Curr.Microbiol.App.Sci.3(1):395-406.
2. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic Effect of Caralluma fimbriata Against Human Colon Cancer Cells. Pharmacog J, 2017, 9(2), 204 - 207.
3. Austria, JA, Richard, MN, Chahine, MN .Bioavailabil- ity of alpha - linolenic acid in subjects after inges- tion of three different forms of flaxseed. J Am Coll Nutr, 2008, 27(2), 214 - 221.
4. Badr A, Sch R, El Rabey H, Effgen S, Ibrahim H, Pozzi C, Rohde W, Salamini F. On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol., 17 (2000), pp. 499-510
5. Bommareddy A, Zhang X, Schrader D, Kaushik RS, Zeman D, Matthees DP, *et al.* Effects of dietary flaxseed on intestinal tumorigenesis in Apc Min mouse Nutrition and Cancer, 61 (2009), pp. 276-283.
6. Bravo L, Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance Nutr. Rev., 56 (11) (1998), pp. 317-333
7. Chethan S and Malleshi NG, Finger millet polyphenols: characterization and their nutraceutical potential Am. J. Food Technol., 2 (7) (2007), pp. 582-592
8. Clement MV, Hirpara JL, Chawdhury SH, Pervaiz S, Chemopreventive agent resveratrol

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5636 - 5655
Received 25 April 2021; Accepted 08 May 2021.

a natural product derived from grapes, triggers CD 95 signallingdependant apoptosis in human tumour cells, Blood 92 (1998) 996–1002.

9. Cook AH. Barley and malt: biology, biochemistry, technology Academic Press, New York (2013).
10. Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, PriyadarisiniVB.,Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review J. Food Sci. Technol., 51 (6) (2014), pp. 1021-1040
11. FAO (Food and Agriculture Organization of the United Nations). Flaxseed production in Canada and the world (2011).
12. Floridi S, Montanari L, Marconi O, Fantozzi P. Determination of free phenolic acids in wort and beer by coulometric array detection. J Agric Food Chem, 51 (2003), pp. 1548-1554
13. Gamel T, Abdel-Aal ESM.,Phenolic acids and antioxidant properties of barley wholegrain and pearling fractions Agric Food Sci, 21 (2012), pp. 118-131.
14. Geiss F. Fundamentals of thin layer chromatography planar chromatography. Heidelberg: A. Hüthig; 1987. Touchstone JC. Practice of thin layer chromatography. 3rd ed. New York: Wiley; 1992.
15. International Agency for Research on Cancer, "World cancer report 2014," WHO, Geneva, Switzerland.
16. Jenkins DJ, Kendall CW, Vidgen E, Agarwal S, Rao AV,Rosenberg RS, *et al.* Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and *ex vivo* androgen and progestin activity: a controlled crossover trial American Journal of Clinical Nutrition, 69 (1999), pp. 395-402.
17. Lane K, Derbyshire E, Li W, Brennan C. Bioavailability and potential uses of vegetarian sources of omega 3 fatty acids: a review of the literature Critical Reviews in Food Science and Nutrition, 54 (2014), pp. 572-579.
18. Levitan EB, Cook NR, StampfeMJr, Ridker PM, Rexrode KM, Buring,. Manson JE, Liu S. Dietary glycemic index, dietary glycemic load, blood lipids, and C-reactive protein Metabolism, 57 (2008), pp. 437-443.
19. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55-63.
20. Moten A, Schafer D, Farmer P, Kim J, and Ferrari M, "Redefining global health priorities: improving cancer care in developing settings," Journal of Global Health, vol. 4, no. 1,

Article ID 010304, 2014.

21. Muruganatham, S., Anbalagan, G., and Ramamurthy, N. 2009. FT-IR and SEM-EDS Comparative Analysis of Medicinal Plants, EcliptaalbaHassk and Ecliptaprostrata Linn. Romanian Journal of Biophysics. 19(4):285-294
22. Nehasahu and JyotiSaxena. Phytochemical Analysis of Bougainvillea GlabraChoisybyFTIRandUV-VIS Specroscopic Analysis. Int. J. Pharm. Sci. Rev. Res., 21(1), Jul – Aug 2013;n°33, 196-198.
23. Omodara NB, Amoko JS, Obijole OA, Ojo BM. Infrared and ultraviolet spectroscopic analysis of methanol extract of Phyllanthus muellerianus root. Greener J Physical Sci 2013; 3 (4): 159-164.
24. Patel R, Garg R, S.Erande S, Maru GB, Chemopreventive herbal anti-oxidants:current status and future perspectives, J. Clin. Biochem. Nutr. 40 (2007) 82–91.
25. Schulte D, Close D, Graner A, Langridge P, Matsumoto T, Muehlbauer G, Sato K Schulman AH, Waugh R, Wise RP, Stein N. The international barley sequencing consortium—at the threshold of efficient access to the barley genome Plant Physiol, 149 (2009), pp. 142-147
26. Sripriya G. Chandrasekharan K, Murty VS, Chandra TS. ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*) Food Chem., 57 (4) (1996), pp. 537-540
27. Stehfest, K., Toepel,J., Wilhelm, C., 2005. The application of micro-FTIR spectroscopyto analyse nutrient stress-related changes in biomass composition of phytoplankton algae. Plant Physiol. Biochem. 43, 717-726.
28. Sudaram S, Palanisamy CP, VelliyurKG.Chromatographic and spectrophotometricanalysis of bioactive compounds from Cayratiatrifolia(L) stem. Int J Pharm Sci 2016;8(6):56-64.
29. Sullivan P, Arendt E, Gallagher E. The increasing use of barley and barley by-products in the production of healthier baked goods Trends Food Sci Technol, 29 (2013), pp. 124-134.
30. Thompson LU. Potential health benefits and problems associated with antinutrients in foods Food Res. Int., 26 (2) (1993), pp. 131-149
31. Turner TD,Mapiye C, Aalhus JL, Beaulieu AD, Patience JF, Zijlstra RT, *et al.* Flaxseed fed pork: n-3 fatty acid enrichment and contribution to dietary recommendations Meat cience, 96 (2014), pp. 541-547

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5636 - 5655
Received 25 April 2021; Accepted 08 May 2021.

32. Visweawara Rao Pasupuleti, TNVKV Prasad, Rayees Ahmad Shiekh, Satheesh Krishna Balam, ganapathiNarasimhulu, Cirandur Suresh Reddy, Ismail AB Rahaman, Siew Hua Gan. International journal of nanomediciine 8, 3355, 2013.
33. Williams DS, Verghese M, Walker LT, Boateng J, Shackelford L, Chawan CB. Flax seed oil and flax seed meal reduce the formation of aberrant crypt foci (ACF) in azoxymethane-induced colon cancer in Fisher 344 male rats Food and Chemical Toxicology, 45 (2007), pp. 153-159
34. World Health Organization, Global Battle against Cancer Won't Be Won with Treatment Alone. Effective Prevention Measures Urgently Needed to Prevent Cancer Crisis, International Agency for Research on Cancer, London, UK, 2014.