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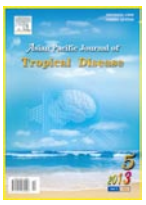

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Infrared spectroscopic analysis of skin tumor of mice treated with several medicinal plants

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PEER REVIEW

Peer reviewer

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Comments

This is a good preliminary study on exploring the chemopreventive agent for skin tumor which facilitates by a simple but sensitive analytical approach. The findings are interesting in which the active compounds used were able to kill the cancer cells and showed a differences in the IR spectrum.
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ABSTRACT

Objective: To evaluate the differences between cancerous tissue, drug treated tissue and its corresponding normal tissue by infrared spectroscopic analysis.
Methods: Methanolic extracts of *Azadirachta indica*, *Ocimum sanctum*, *Aloe barbandesis*, *Tinospora cordifolia and Triticum aestivum* were assessed for the isolation and purification of active compound. After that, combine crude and combine isolated samples were prepared. Skin tumor was induced by topical application of 7, 12-dimethyl benz (a) anthracene and promoted by croton oil in Swiss albino mice. To assess the chemopreventive potential of different drugs, it was administered at a concentration of 400 mg/kg body weight daily up to 16 weeks. Fourier transform infrared spectroscopy analysis was used to differentiate the drug treated tissues with the normal and cancerous tissue. In the present study, spectra of different tissues were recorded in the range of 400-4 000 cm^{-1}.
Results: The results of the present study have shown that the remarkable difference exists between the IR spectra of normal, drugs treated and cancerous tissue in terms of frequencies and intensities of prominent bands of cellular biomolecules.
Conclusions: Fourier transform infrared spectroscopy analysis suggests the chemopreventive effect of above treated drugs and the best result was observed in combine crude sample and in combine isolated sample or synergistic effect of individual crude and isolated extract in 7, 12-dimethyl benz (a) anthracene croton oil induced skin carcinogenesis in Swiss albino mice.

KEYWORDS
Skin carcinoma, Fourier transform infrared spectroscopy, Medicinal plants

1. Introduction

Cancer is currently one of the most frequently occurring diseases. Generic term of cancer is a malignant neoplasm. Cancer is a group of diseases occurring in all human and animal populations and arising in all tissues composed of potentially dividing cells[1]. The basic characteristic of cancer is the transmissible abnormality of cells that is manifested by reducing control over growth and function leading to serious adverse effects on the host through invasive growth and metastases. Several diagnostic methods are currently in use. The most common method used by pathologists is the microscopic inspection of tissue in order to obtain morphological data[2,3]. This effect is caused by biochemical changes to the metabolism. There is also a clear response by the immune system. Therefore, changes in biochemical composition like new proteins could be used as a marker for a disease.

Fourier transform infrared spectroscopy (FTIR) is being extensively used as a promising method in diagnostic, medical and biological studies. Both clinical and biological studies would benefit from the spectroscopic technique that could quantify the biochemical composition of specimens non destructively and with minimal sample preparation and handling[4]. It can also be used to measure the vibrational modes of the functional groups of molecules and is sensitive to molecular structure, conformation and the environment[5].

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2. Materials and methods

2.1. Collection of plant materials

Fresh leaves of *Azadirachta indica* (*A. indica*), *Ocimum sanctum* (*O. sanctum*), *Aloe barbandesis* (*A. barbandesis*), stem of *Tinospora cordifolia* (*T. cordifolia*), and whole grass of *Triticum aestivum* (*T. aestivum*) were collected from their proper origin. Herbariums was prepared and submitted for authentication at Safia College of Science, Bhopal. Plants were authenticated by Dr. Zia-Ul-Hassan.

2.2. Extraction of plant materials

The plant materials (leaves of *A. indica*, *O. sanctum*, *A. barbandesis*, stem of *T. cordifolia*, and whole grass of *T. aestivum*) were air-dried at room temperature (26 °C) for 2 weeks, after which it was grinded into a uniform powder. The mixture of acetone and methanol (30:70) extracts were prepared by using 75 g of each dry powdered plant materials in the Soxhelet apparatus at 40 °C for 48 h. The extracts were filtered after 48 h, and then were concentrated using a rotary evaporator with the water bath set at 40 °C. The percentage yield of extracts ranged from 7%-17% (w/w).

2.3. Preparation of combine sample of crude and isolated extracts of plant materials

Above extracts of each plant material were mixed in equal proportion and obtained a combine sample of crude extracts. Isolation of active compound from the individual plant extract was done according to the different methods which were described elsewhere. Nimbolide, 3,3′,4′,5,7-pentahydroxyflavone, 10-glucopyranosyl-1, 8-dihydroxy-3-(hydroxylmethyl)-9, (10H)-anthracenone, 2,3,9,10-Tetramethoxy-5,6-dihydroisoquinolino[2,1-b] isoquinolin-7-ium chloride, and quercetin-3-O-α-L-rhamnopyranosyl-(1-6) β-D-glucopyranoside were identified and isolated from extracts of *A. indica*, *O. sanctum*, *A. barbandesis*, *T. cordifolia* and *T. aestivum* according to a method of Gunasekaran *et al*., Meena *et al*., Bhayadiya *et al*., Satya *et al*., and Machado *et al*., respectively[6-10]. After that, active components of particular plant extracts were mixed in equal proportion and obtained a combine sample of isolated extracts.

2.4. Chemicals

7, 12, Dimethyl benzanthracene (DMBA) and croton oil were purchased from Sigma Aldrich.

2.5. Animals

Swiss albino mice of either sex were selected at random from animal house of the Pinnacle Biomedical Research Institute (PBRI), Bhopal. Animals were housed in polypropylene cages with sterile husk and provided standard pellet (Golden feeds, New Delhi) and water *ad libitum* as they were feeded throughout the experiment. The animals were maintained with a 12-hour light-dark cycle at (22± 2) °C at controlled condition. All animal experiments were performed with prior permission of the Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Reg No. 1283/c/09/ CPCSEA).

2.6. Experimental procedure

Eight groups of six animals each of swiss albino mice were used for the study. Animals were dorsally shaved with hair clipper. Animals of all groups were treated with a single dose of DMBA (100 μg/100 μL of acetone) over the shaven area of the skin of the mice afterwards 1 % croton oil was applied to skin three times a week up to 16 weeks. After the single dose of DMBA, animals of Group I (control group) were treated with water, while Group II, III, IV, V, VI, VII and VIII were treated with the extract of *A. indica*, *O. sanctum*, *A. barbandesis*, *T. cordifolia* and *T. aestivum*, combine crude sample and combine isolated sample respectively at a concentration of 400 mg/kg orally each day till completion of experiment and 1% croton oil was applied on skin after 1 h of drugs administration three times a week till 16 weeks.

2.7. Tissue sampling

On the last day of the experiment, animals of all the groups were sacrificed by cervical dislocation. The animals were immediately dissected to remove their skins which were washed in ice-cold saline (0.9% NaCl) and the extraneous material was removed. It was then weighed and blotted dry.

2.8. Spectral measurements

The normal and different skin tumor tissue samples were lyophilized. The lyophilized samples were mixed with potassium bromide uniformly and made into standard pellets for IR measurements. Shimadzu Corp. 0141 FTIR spectrophotometer was used to record the spectra. FTIR measurements of the samples were recorded in the transmit mode. The tissue samples were scanned in the mid IR region 400-4 000 cm^{-1} with the resolution of 4 cm^{-1}. Sixty four scans were coded for each spectrum and the spectra were rated against the background spectrum.

3. Results

The spectra of the normal skin and the malignant skin tissues were recorded in the frequency range 400 cm^{-1} to 4000 cm^{-1}. The spectra of the normal and the malignant tissues of different groups were compared. The malignant tissue of Group I showed appreciable biochemical deviations from their normal forebear. The spectra of the normal tissues showed well defined spectral features, while the spectra of the malignant tissues appeared to be more complicated. In some easily identifiable regions, the differences between the normal and the carcinomatous tissues were highly marked.

The IR spectra for tissues mainly consisted of proteins, peptides and nucleic acid and so on. Since N-H and C=O

stretches were major absorption peaks along with C-H stretch which appears at frequency of: N-H stretch appears at 3250 cm^{-1}; C=O stretch appears at 1740 cm^{-1}; C-H stretch appears at 2924cm^{-1}.

Spectra of Group I showed bands for N-H, C=O and C-H stretch appears at 2924 cm^{-1}. Intensity of transmittance for C-H stretch was lower in malignant tissue of Group I (control group). Spectra of normal group also showed bands for N-H, C=O and for C-H stretch. Intensity of transmittance for C-H stretch was higher in normal group in comparison with malignant tissue of Group I. The spectral features were indicative of increased intensity in the normal group (Figure 1).

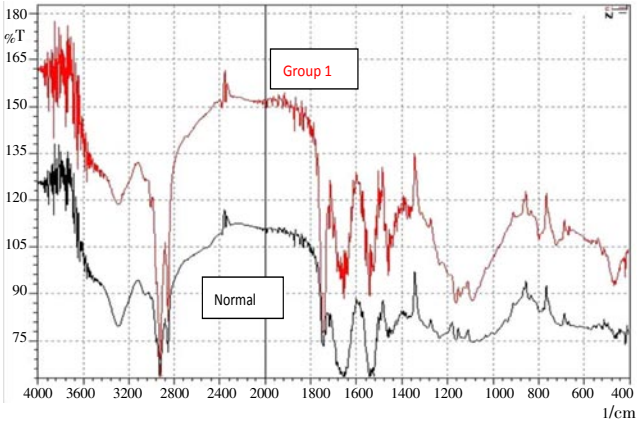

Figure 1. Infrared spectra of normal and malignant Group I skin tissues of mice.

Spectra of Group II also showed bands for N-H, C=O and C-H stretch. Spectra of group III showed that the intensity of transmittance for C-H was reduced and N-H, C=O bands were weak. Spectra of Group IV and Group V showed reduced intensity of all bands. Spectra of Group VI showed N-H and C=O bands with reduced intensity (Figure 2).

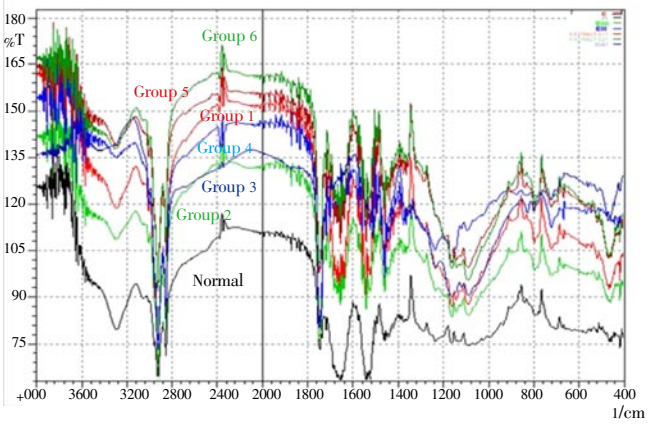

Figure 2. Infrared spectra of normal and malignant Group I, Group II, Group III, Group IV, Group V and Group VI skin tissues of mice.

Spectra of Group VII (crude combine sample) showed that the intensity for C-H stretch at 1085 cm^{-1} was increased and some other bands also appeared in the spectra (Figure 3). The spectra of Group VIII (Isolated combine sample) showed that the intensity for C-H stretch at 1087 cm^{-1} was also increased and C-N stretch occurred at 1085 cm^{-1}. Even C-O stretch bands appeared at 1180 cm^{-1} with better intensity.

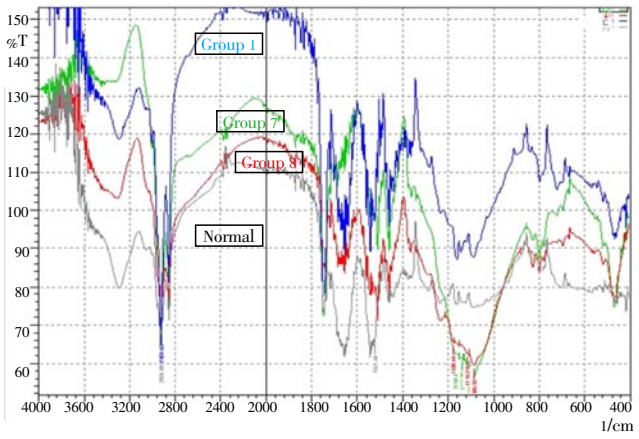

Figure 3. Infrared spectra of normal and malignant Group I, Group VII and Group VIII skin tissues of mice.

4. Discussion

It emerged from the above points that the IR spectra of different groups showed different spectral features. Spectral features of normal skin tissue were significantly different from cancerous tissue. These changes can be used for differentiating normal tissue from malignant ones.

The ethanolic leaf extracts of *Catharanthus roseus*, *Lawsonia inermis* and *Chrysanthemum odoratum* showed less activity against *Staphylococcus aureus* when used separately. Whereas, the combination of these three plants extracts exerted a higher activity[11]. The ethanolic extract of *Balanites aegyptiaca* leaves in combination with other parts (stem bark, root bark and fruits) of *Balanites aegyptiaca* exerted synergistic effect against food-borne diarrheagenic bacteria[12]. Similarly in this study, the overall intensity of various IR bands such as, C-H, N-H, C=O and C-N stretch when compared with control indicate that in Group VII and VIII, tissues remaining intact or damage were not observed. This result of IR spectrum could probably be due to the synergistic effect of the individual sample and stand out a good result. The above studies strongly support the present finding.

Throughout people's lives, the cells in their bodies are growing, dividing, and replacing themselves. Many genes produce proteins that are involved in controlling the processes of cell growth and division. An alteration (mutation) to the DNA molecule can disrupt the genes and produce faulty proteins. This causes the cell to become abnormal and lose its restraints on growth. The abnormal cell begins to divide uncontrollably and eventually forms a new growth known as a neoplasm. FTIR spectroscopy is a powerful technique for providing useful information regarding biochemical changes occurring in the tissue and hence in diagnosing cancer.

The result of the present study has shown that remarkable differences exist between the spectra of the normal and the malignant tissues in terms of spectral profiles and transmittance frequencies. The spectral differences reflect the changes in the content, conformation and composition of the nucleic acid proteins and fats in the cells. The results

are in accordance with *in vivo* study. With further studies, the technique can be envisioned as a rapid and a sensitive diagnostic tool to help the pathologist in detecting skin cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Cancer remains the most public health problems although scientist continuously focused on exploring the best anticancer agents to destroy the cancerous cells. This study explored the potential of active components from several plants as a chemo preventive agent of skin tumor and the observation was done by infrared spectroscopic.

Research frontiers

The method applied in this study is a quite new technique for cancer research and diagnosis, which provides a highly acceptable result, and recognized as a non-destructive, highly sensitive and specific analytical methods. Previously, scientist in these field used other biochemical and molecular approaches to support their finding pertaining anticancer agent activity. Those methods are time consuming and expensive. FTIR is a simple, rapid and low cost analytical technique, which should be explored to provide an interdisciplinary research finding.

Related reports

The analytical approach was first reported by Petibois and Delereis, which presented several cancer markers. Those markers might be best highlight in this manuscript, *i.e.* in the FTIR spectrum for each cancer tissues treatment to exhibit the expressions of the markers after treatment and compared with control.

Innovations & breakthroughs

Study on the synergistic effects of combined plant extracts for the treatment of the skin tumor was a good innovation since both extracts, used together exhibited better suppression against skin tumor than when it was applied solely. This research should be further with the mechanism of how the agents act on the cancerous cells, and study the effects on normal cells. If they does not kill normal cells, it means that the compounds are specific to cancerous cells and should be applied on cancer patients.

Applications

Further studies needed to be done with clinical trial on cancer patients to make sure that the potential of these plants extract could be beneficial in medical and oncology.

Peer review

This is a good preliminary study on exploring the chemopreventive agent for skin tumor which facilitates by a simple but sensitive analytical approach. The findings are interesting in which the active compounds used were able to kill the cancer cells and showed a differences in the IR spectrum.

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