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SPECTROSCOPIC ANALYSIS OF NOVEL CHEMICAL COMPOUNDS

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Abstract

Herbal compositions are becoming increasingly important in today's world of raw material scarcity. Polyherbal formulations exhibit high efficacy due to the presence of active phytochemicals which may enhance their potency due to the synergetic interaction of active ingredients of different plants. Ayurgreen Natura Pain Gel is an important Ayurvedic polyherbal formulation prepared using specified plant parts of dried aloe vera and fresh aloe vera pulp, frankincense, myrrh, ferula asafetida. The phytochemistry of Ayurgreen Natura Pain Gel has been evaluated using a liquid chromatography-mass spectrometer and their bioactive functional groups were characterized using Fourier Transform Infrared Spectroscopy and UV-Visible spectroscopy.

Keywords: Polyherbal formulation, Fourier Transform Infrared Spectroscopy and UV Visible spectroscopy.

Introduction

Spectroscopy is the evaluation of the interaction between matter and electromagnetic radiation. Reliably, it emerged through the study of visible light dispersed accord to its wavelength by a prism. It is associated with the absorption, emission or scattering of electromagnetic radiation by atoms or molecules. The consequence of such an interaction has researchers contemplating analytical findings [1]. UV-Vis Spectroscopy is accustomed to conclude analyte concentration by the absorption of light over the range through a liquid sample. Infrared spectroscopy is choicely used in industry besides in research. It is a conservative and reliable approach for measurement, quality control and dynamic measurements [2]. It is applied to determine functional groups in molecules. Generally, stronger bonds and light atoms will vibrate at a high stretching frequency. Nuclear magnetic resonance spectroscopy is the widest use to determine the structure of organic molecules in solution and used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI) [3] . Atomic absorption spectrometry is an analytical technique that quantifies the concentrations of components. It is thus sensitive that it can work through parts per billion of a gram in a test. The technique comprises wavelengths of light specifically absorbed by anelement. Flame emission spectroscopy allows quantitative measurement of the optical emission from excited atoms to examine analyte concentration. These extreme temperature atomization sources provide sufficient energy to endorse the atoms into high energy levels. The atoms crumble back to lower levels by emitting radiation [4]. Mass spectrometry is an analytical weapon beneficial for estimating the mass-to-charge ratio (m/z) of one or more molecules

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present in a sample [5]. Fluorescence spectroscopy is valuable in applications such as detecting and quantifying organic compounds. Industrial applications include testing surface quality and cleanliness. Laser-induced fluorescence spectroscopy permits exciting lasers to energies fluorophores in the proposed constituents which are emitted during relaxation within a reach of nanoseconds [6] . Electron Spin Resonance spectroscopy determines the absorption of microwave radiation resemble to the energy splitting of an unpaired electron when it is placed in a strong magnetic field. X-ray powder diffraction is a constructive tool for the identification of unknown crystalline materials and determination of solids which is decisive to studies in geology, environmental science, material science, engineering and biology[7] . From the correlation of entire spectroscopic analysis, the aim and objective of this review is to accomplish content of learning about each method in a single article. Therefore, the highlights of every important feature of spectroscopy are focused in this study.

The current study describes a detailed confab on chemical profiling, physical characterization, and biological evaluation of a novel herbal formulation. Chemical profiling has been carried out using liquid chromatography-mass spectrometry (LC-MS) coupled to gas chromatography-mass spectrometry (GC-MS) for the identification of bioactive compounds above mentioned formulation. Physical characterization was done with different spectroscopic tools including Fourier Transform Infrared Spectroscopy (FT-IR) and UV-Visible spectroscopy and the thermal stability was evaluated using differential scanning calorimetry (DSC). Further, the synergistic antioxidant capacity was investigated in terms of its radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide dismutase (SOD) radicals. The anti-inflammatory activity was evaluated towards nitric oxide scavenging radical in RAW – cells. Finally, the anticancer potential of the herbal formulation was screened against mice cancer cell lines (Ehrlich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLA).

MATERIALS AND METHODS

Chemicals

Ingredients Quantity (in gm)

1. Frankincense 800gm
2. Dried aloe vera 875gm
3. Myrrh 125gm
4. Magnesium silicate 300gm
5. Ferula asafetida 100gm
6. Fuller's earth 375gm
7. Aloe vera 1250gm

Preparation of Ayurgreen Natura Pain Gel

A mixture of frankincense and dried aloe vera is boiled with juice of aloe vera to form a melt. All other ingredients were grinded to form fine powder and added to the melt while stirring to form a homogeneous mixture. The stirring will be continued for 2 -3 days without having fermentation and contamination.

LC-MS analysis

LC–MS/MS experiments were performed on Agilent 6520 accurate mass MS Q-TOF coupled with Agilent LC 1200. The MS analysis was performed with dual AJS ESI ion source in positive and negative

mode. Maas spectral data analysis was done by Agilent molecular ion extraction algorithm. The general conditions for mass spectrometry were drying gas (nitrogen) flow 13 L/min; nebulizer pressure 35 psig; drying gas temperature 250oC; capillary voltage 3500V; fragmentor volt 750 V; Oct RF Vpp. A gradient of water (95%) and acetonitrile (5%) was used as mobile phase for ESI ionization mode at constant flow of 0.3 ml/min. The mobile phase was fixed as gradient of acidified methanol (A) and water (B) system for ESI ionization mode. Gradient elution was performed at a constant flow rate of 0.9 ml/min and 1200.00 bar pressure.

UV-Vis Spectroscopy

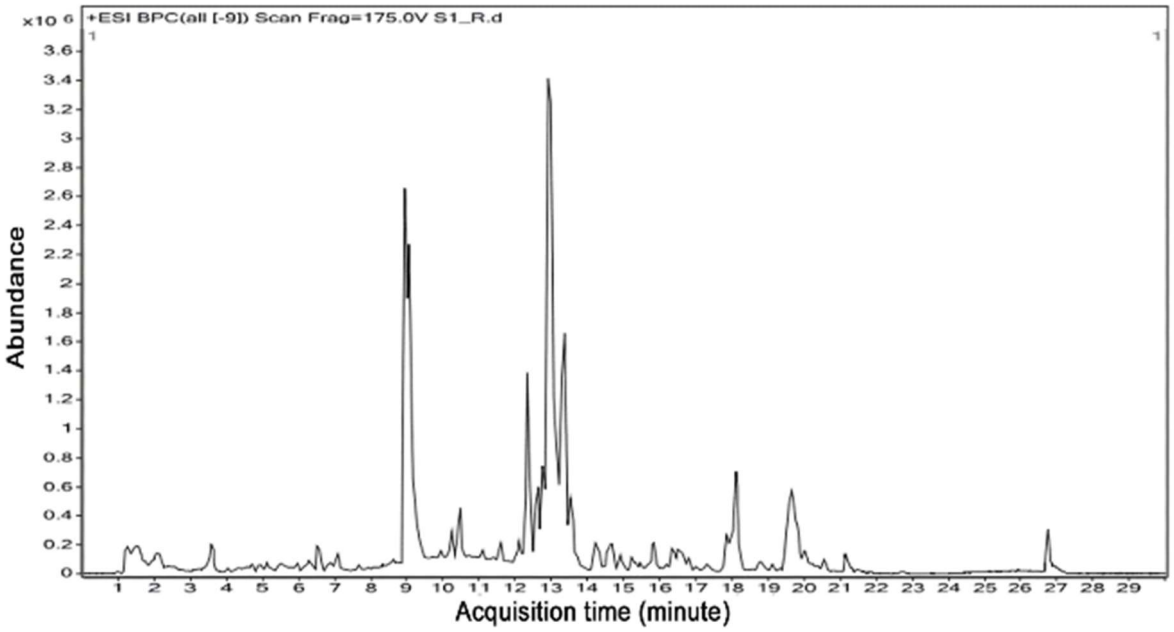
UV–Vis spectra of polyherbal formulation was recorded in ethanol on Jasco UV–Visible Spectrophotometer model V-550. The base line wascorrected using ethanol solvent before analysis.

F T-IR Spectroscopy

The FTIR spectra of polyherbal formulation was recorded by JASCO FTIR-4100 spectrometer at room temperature. The measurements were taken in the range from 400 to 4000 cm-1 using KBr pellet.

RESULT AND DISCUSSION

LC/MS analysis of polyherbal formulation was carried out with ESI ionization in both positive and negative modes and information regarding the presence of chemical compounds was extracted by Agilent Mass Hunter software. 25 molecular ions were detected in negative mode and 15 molecular ions peaks were exhibited in positive mode based on the abundance of ions were further fragmented in auto ms/ ms analysis with varying collision energy. The consistency of fragments was confirmed by targeted ms/ms analysis with fixed collision energy based on the auto ms/ms analysis. The chromatogram in positive and negative mode were depicted in Figure 2.



Physiochemical characterization

UV-Vis Spectroscopy

UV-Vis spectroscopic analysis can be used as a quality control in ayurvedic formulations, since spectra can be considered as fingerprint of each authenticated standard formulations. It can be also used for quantitative analysis for evaluating adulterants in the formulations in terms of specific markers of standard authenticated samples. UV/Vis spectroscopic method is based on electronic absorption caused by the compound present in the plants and compound formed in chemical reactions during manufacturing. The total spectral analysis help us to investigate concentration of different chemicals through multiple peaks over broad frequency range from 200 to 900 nm [8]. It was observed that maximum absorption peaks of lepam, were found in the region of 232 nm which may be ascribed to the primaryoxidation products like conjugated dienes with rich orbitals primed for electronic transition in the formulation [9]. The deconvoluted graph reveals three more absorptions at 268, 293, and 403 nm. Normally, the absorptions of radiation in between 260-280 nm are meant for double bonds C=C, C=O and N=N of the aromatic or unsaturated components of humic substances [10]. As the chemical profiling using LCMS indicated the polyherbal formulation is abundant with such hydroxy acids and phenolics, amino acids etc., A small absorbance peak at 293 nm associated to flavonoids present in the formulation from different herbs like 6-hydroxydaidzein, 4'-glucoside glycyrrhizaisoflavone c, quercitrin, homoeriodictyol, 2"-O-trans-p-coumaroylastragalin, 7-OMethylluteone, 7-O-methylluteone [11]. A light jump at 403 nm is credited to the presence of peroxide compounds, which may be derived from moringa olifera [12].

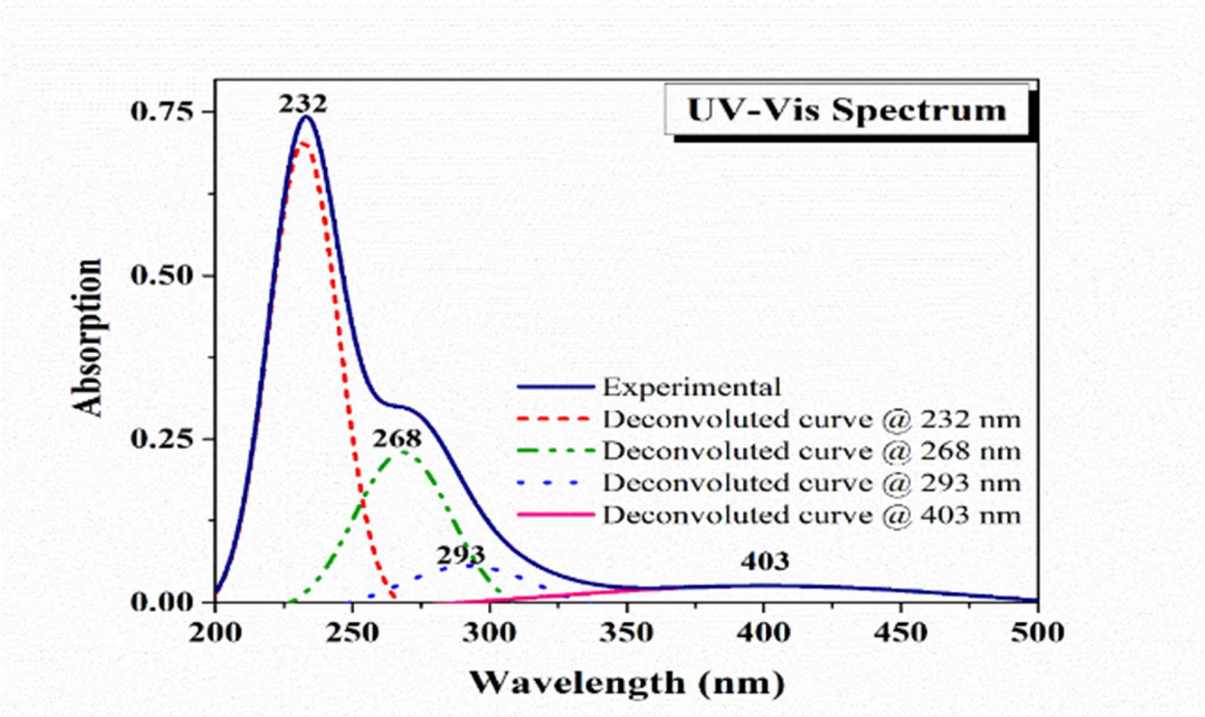


Figure 3: UV-Visible spectrum and their deconvoluted gaussian peaks of polyherbal formulation

FTIR Spectroscopy

To our knowledge from chemical profiling the polyherbal formulation has the presence of 40+ chemical constituents. For each molecules have N atoms and each will have 3N-6 normal modes of vibration. So, it is not that much easy to trace the origin of harmonic vibrations for observed wave numbers. Thus, a detailed vibrational assignment of fundamental modes along with IR intensities will be a tedious job, the only

possibility is to interpret in terms of fundamental modes of vibrations of functional groups like NH stretching, CH stretching and bending vibrations. The observed FT-IR spectra of the polyherbal formulation were shown in Figures 4 and their corresponding assignments were tabulated in table 3.

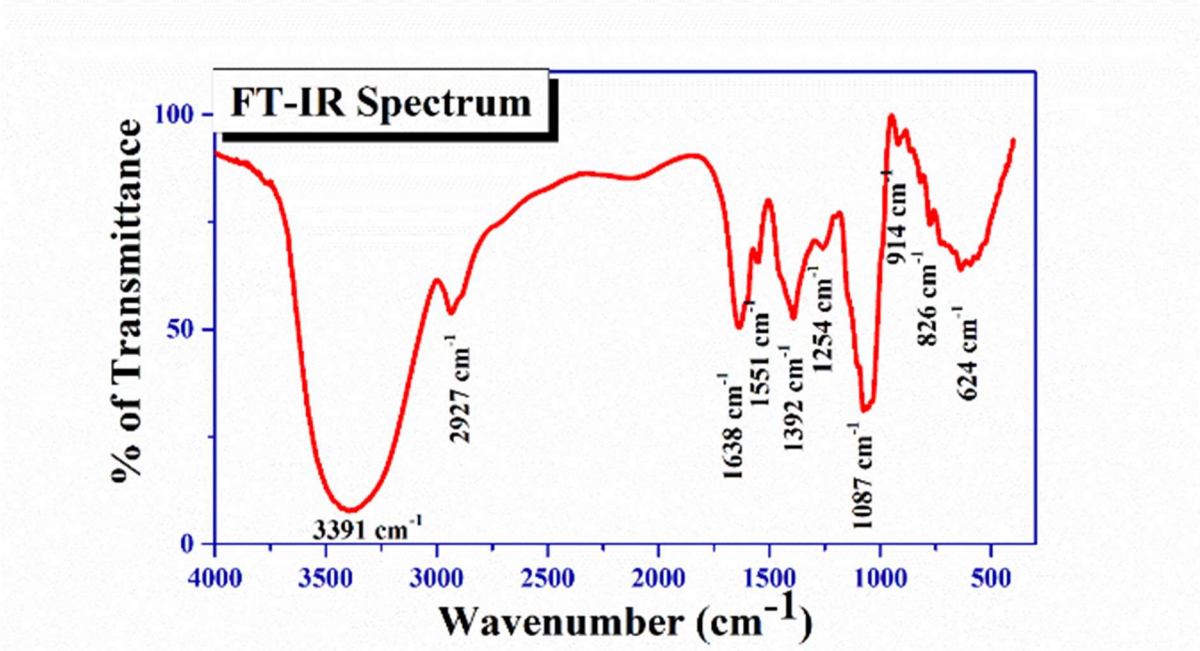


Figure 4: Infrared spectra of polyherbal formulation

CONCLUSION

A novel polyherbal formulation named Ayurgreen Natura Pain Gel were prepared using specified plant parts of dried aloe vera and fresh aloe vera pulp, frankincense, myrrh, ferula asafetida with natural binders like magnesium silicate and a clay mineral. It is found that the prepared polyherbal formulation exhibited high efficacy due to the presence of active phytochemicals which may enhance their potency due to the synergetic interaction of active ingredients of different plants. The phytochemistry of Ayurgreen Natura Pain Gel has been evaluated using a liquid chromatography-mass spectrometer and revealed the presence of 40 phytoconstituents contains a variety of chemical compounds including phenolics, flavanones, furans, gallotannin, glucoside, oligosaccharide, acids with different biological activities like anti-inflammatory, anti-bacterial, ant-fungal, anti-viral and anticancerous. The bioactive functional groups were characterized using Fourier Transform Infrared Spectroscopy and UV-Visible spectroscopy Moreover, the thermal analysis was performed using differential scanning calorimetry and revealed the presence of volatile ingredients, melting, and degradation temperature[13-15].

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