*Section A-Research paper*
*ISSN 2063-5346*

# STUDIES OF AYURVEDIC POLYHERBAL FORMULATIONS AND ITS SPECTRAL CHARACTERISTICS

**Shanmugarathinam Alagarsamy,** Department of Pharmaceutical Technology, University College of Engineering, Bharathidasan Institute of Technology, Anna University, Tiruchirappalli, Tamilnadu.
Email: shanmugarathinam@gmail.com
**Shri Devi. S.D.K,** Sri Sarada College for Women (Autonomous), Salem, Tamilnadu
Email: saishri28@gmail.com
**Srinivasan P,** Department of Microbiology, Rathinam College of Arts and Science, Pollachi Road, Eachanari, Coimbatore, Tamilnadu
Email: srini2k8@gmail.com
**Prakash Gadipelli,** Department of Chemistry, Aditya College of Engineering & Technology, Surampalem, Kakinada, Andhra Pradesh.
Email: Jamesprakash68669@gmail.com
**M.Anchana,** PG and Research Department of Physics, Marudhar Kesari Jain College for women, Vaniyambadi, Tamilnadu
Email: selvi282amma@gmail.com
**Sheela Thangaraj,** Dept of Biotechnology, Dhanalakshmi Srinivasan College of Arts & Science for women (Autonomous), Perambalur, Tamilnadu
Email: sheela.t@dscollege.ac.in
**DOI: 10.48047/ecb/2023.12.si4.1737**

**Abstract**
As natural resources become more difficult to come by, herbal combinations gain significance. The active phytochemicals present in polyherbal formulations may contribute to their efficacy through the synergistic interaction of active compounds derived from many plants. An essential Ayurvedic polyherbal composition, Ayurgreen Natura Pain Gel is made from the dried and fresh aloe vera plant components, as well as frankincense, myrrh, ferula, and asafetida. Liquid chromatography-mass spectrometry was used to analyze the phytochemistry of Ayurgreen Natura Pain Gel, while Fourier transform infrared spectroscopy and ultraviolet-visible spectroscopy were used to identify the gel's bioactive functional groups.
**Keywords:** Fourier transform infrared spectroscopy, ultraviolet-visible spectroscopy, and apolyherbal formulation.

## Introduction

In spectroscopy, we measure how electromagnetic radiation interacts with stuff. It was discovered through careful analysis of prism-divided light of known wavelength. It has to do with how atoms and molecules interact with electromagnetic radiation, whether by absorbing it, emitting it, or dispersing it. Researchers are pondering analytical discoveries as

*Section A-Research paper*
*ISSN 2063-5346*

a result of this kind of contact [1]. UV-Vis The concentration of an analyte can be determined by measuring the amount of light absorbed by the sample as it travels through a spectrometer. The field of infrared spectroscopy has widespread application in industry as well as academia. This method is conservative and trustworthy, making it ideal for measurements, quality assurance, and dynamic analysis [2]. It is used to identify molecular functional groupings. In general, a higher stretching frequency might be expected from stronger bonds and lighter atoms. Nuclear magnetic resonance spectroscopy is widely employed in modern medical imaging techniques like magnetic resonance imaging (MRI) [3] to determine the structure of organic molecules in solution. The analytical method of atomic absorption spectrometry allows for the precise measurement of component concentrations. It's so sensitive that it can detect changes in mass that amount to one billionth of a gram. This method uses light of a certain wavelength, which is absorbed only by the element of interest. Analyte concentration can be determined by flame emission spectroscopy by quantitatively measuring the optical emission from excited atoms. These high-temperature atomization sources are powerful enough to propel the atoms to their maximum potential energies. Radiation is released, causing the atoms to disintegrate back to their original state [4]. When many molecules in a sample need to have their mass-to-charge ratio (m/z) estimated, mass spectrometry is a useful analytical tool [5].

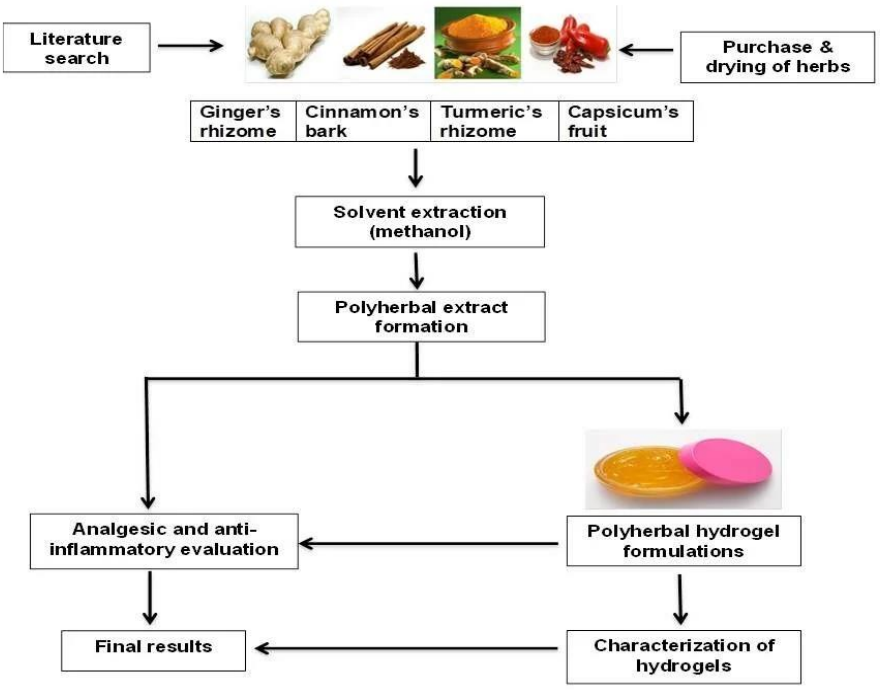


**Figure 1: Biological activities of polyherbal extracts.**

Applications of fluorescence spectroscopy include the detection and quantification of chemical molecules. Surface quality and cleanliness testing have industrial uses. Exciting lasers to generate fluorophores in the proposed constituents that are emitted following relaxation within a reach of nanoseconds [6] is possible using laser-induced fluorescence spectroscopy. Electron spin resonance spectroscopy measures the splitting of an unpaired electron's energy when subjected to a powerful magnetic field through the absorption of

microwave radiation. The fields of geology, environmental science, material science, engineering, and biology all rely heavily on the results of X-ray powder diffraction experiments because of the critical role they play in identifying and characterizing unknown crystalline materials. The purpose of this review is to provide a centralized location for information regarding all of the many approaches to spectroscopic analysis. As a result, this analysis emphasizes the most crucial aspects of spectroscopy.

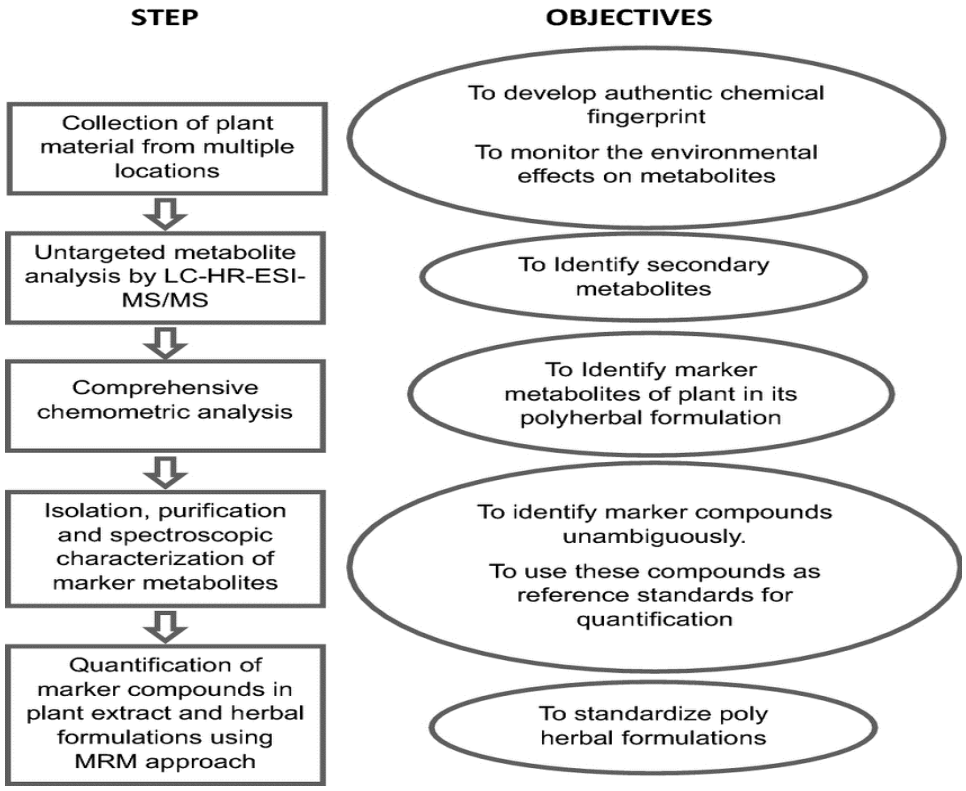


**Figure 2: Formulation standardization for polyherbal medicines: a five-step process**

This research presents a comprehensive discussion of the chemical composition, physical properties, and biological effectiveness of an innovative herbal preparation. For the purpose of identifying bioactive chemicals, a chemical profile has been performed using LC-MS/GC-MS, a combination of liquid chromatography and mass spectrometry. Different spectroscopic methods, such as Fourier Transform Infrared Spectroscopy (FT-IR) and UV-Visible spectroscopy, and differential scanning calorimetry (DSC), were used to perform physical characterisation. Additionally, the radical scavenging activity of superoxide dismutase (SOD) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was studied to get insight into the synergistic antioxidant capability. In RAW - cells, the ability to scavenge nitric oxide radical was measured to determine anti-inflammatory efficacy. Finally, mouse cancer cell lines (Ehrlich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLA)) were used to test the herbal formulation for its anticancer potential.

## MATERIALS AND METHODS

### Chemicals

### Ingredients Quantity (in gm)

- Frankincense 800gm
- Dried aloe vera 875gm
- Myrrh 125gm
- Magnesium silicate 300gm
- Ferula asafetida 100gm
- Fuller's earth 375gm
- Aloe vera 1250gm

### Preparation of Ayurgreen Natura Pain Gel

A melt is made by boiling together frankincense and dried aloe vera with aloe vera juice. In order to ensure a uniform mixture, the remaining materials were ground into a fine powder before being added to the melt. For the next three days, without fermentation or contamination, the stirring will continue.

### LC-MS analysis

Agilent 6520 accurate mass MS Q-TOF coupled with Agilent LC 1200 was used for all LC-MS/MS investigations. Dual AJS ESI ion sources were used for the MS analysis, one in positive mode and one in negative mode. The Agilent molecular ion extraction technique was used to decipher the Maas spectral data. Mass spectrometry was performed with the following parameters: capillary voltage 3500 V; nebulizer pressure 35 psig; drying gas temperature 250$^{o}$C; fragmentor volt 750 V; Oct RF Vpp. For ESI ionization mode, the mobile phase comprised a gradient of water (95%) and acetonitrile (5%) flowing at a constant rate of 0.3 ml/min. For ESI ionization mode, a gradient of acidified methanol (A) and water (B) was chosen as the mobile phase. At a pressure of 1200.00 bar and a flow rate of 0.9 ml/min, a gradient elution was carried out.

### UV-Vis Spectroscopy

The Jasco UV-Visible Spectrophotometer model V-550 was used to take UV-Vis spectra of a polyherbal mixture in ethanol. Before analysis, the baseline was adjusted with ethanol solvent.

### F T-IR Spectroscopy

The polyherbal formulation's FTIR spectra were taken at room temperature using a JASCO FTIR-4100 spectrometer. The KBr pellet was used to take the readings, which were taken between 400 and 4000 cm-1.

## RESULT AND DISCUSSION

Positive and negative ESI ionization modes were used for the LC-MS analysis of the polyherbal formulation, and Agilent Mass Hunter was used to obtain data on the presence of chemical substances. Based on the number of ions further fragmented in auto ms/ ms analysis at varied collision energies, 25 molecular ions were found in negative mode, and 15 molecular ions peaks were displayed in positive mode. The auto ms/ms analysis served as the foundation for the targeted ms/ms analysis, which used a constant collision energy to verify the pieces' consistency. Figure 3 shows the chromatograms in both the positive and negative modes.

*Section A-Research paper*
*ISSN 2063-5346*

**Physiochemical characterization**

**LC-MS analysis**

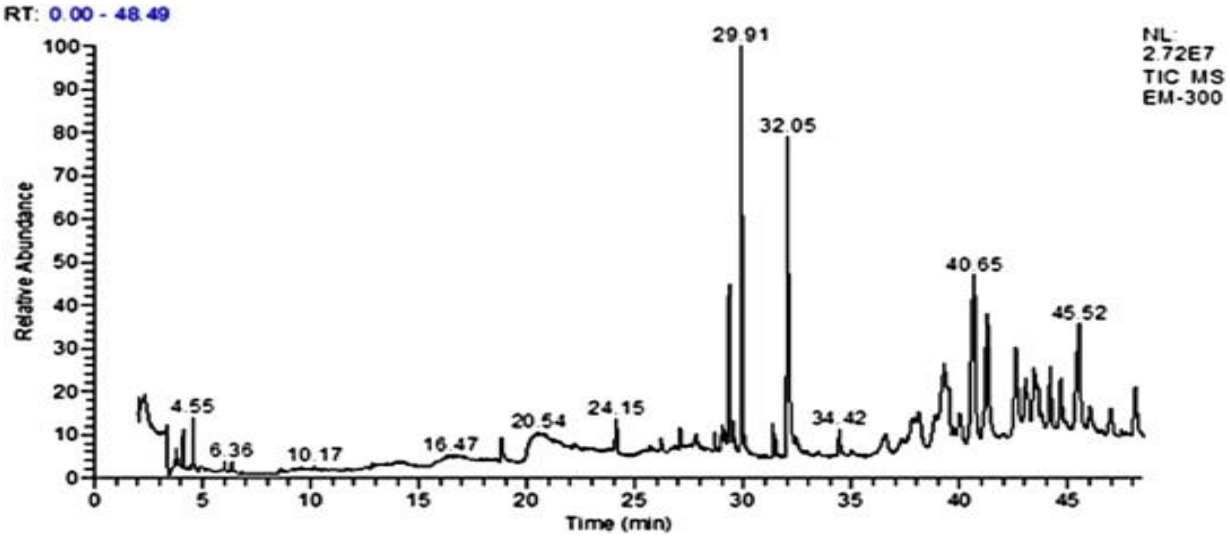


**Figure 3: LC-MS analysis Chromatogram**

Quality control in ayurveda formulations can benefit from UV-Vis spectroscopy analysis, as spectra can be thought of as the "fingerprint" of each authorized standard formulation. As a quantitative tool, it can be used to assess the presence of adulterants in a formulation relative to a set of well-defined characteristics of certified standards. The compounds found in plants and the compounds created in chemical processes during production are the basis for electronic absorption in the UV/Vis spectroscopy approach. We can examine the concentration of various compounds by looking at many peaks over a wide frequency range (from 200 to 900 nm) using total spectral analysis [8]. The primary oxidation products, such as conjugated dienes with rich orbitals primed for electronic transformation, may account for the greatest absorption peaks of lepam, which were discovered in the range 232 nm [9]. Three further absorptions at 268 nm, 293 nm, and 403 nm are seen once the graph is deconvoluted. Double bonds C=C, C=O, and N=N of aromatic or unsaturated components of humic compounds often absorb light between 260 and 280 nm [10]. The polyherbal formulation is rich in hydroxy acids, phenolics, amino acids, etc., as shown by the LCMS chemical profiling. Flavonoids from various herbs, including 6-hydroxydaidzein, 4'-glucoside glycyrrhiza isoflavone c, quercitrin, homoerio dictyol, 2"-O-trans-p-coumaroylastragalin, 7-O-methylluteone, and 7-O-methylluteone, contribute to a minor absorbance peak at 293 nm in the formulation [11]. Peroxide chemicals, possibly generated from moringa olifera, are responsible for an increase in luminosity at 403 nm [12].

*Section A-Research paper*
*ISSN 2063-5346*

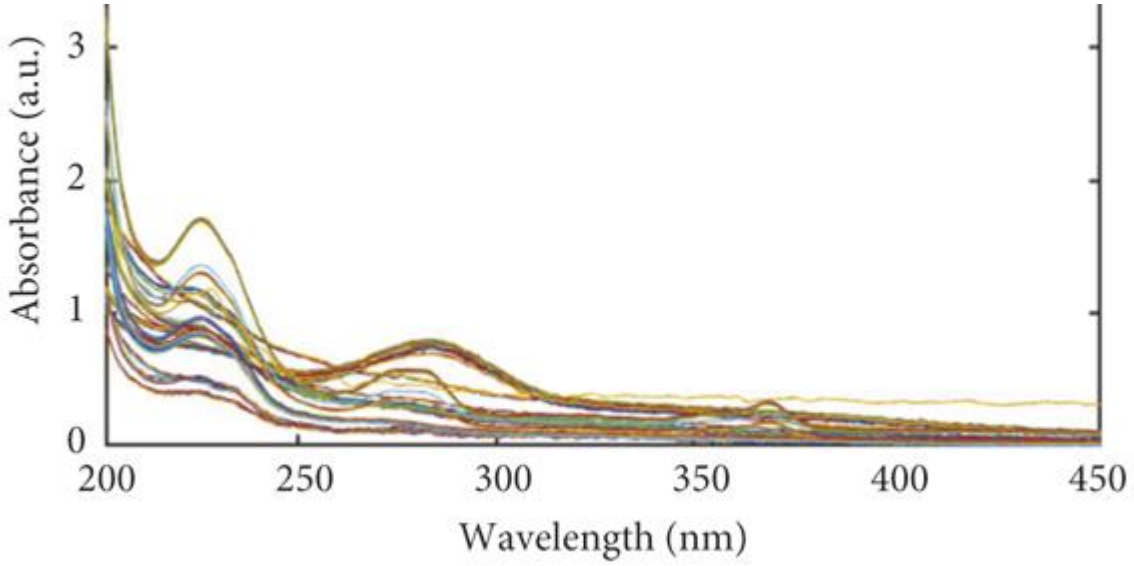


**Figure 4: UV-Visible spectrum and their deconvoluted gaussian peaks of polyherbal formulation**

FTIR Spectroscopy

Chemical profiling has revealed that the polyherbal composition contains 40 or more active chemical ingredients. Since there are N atoms in a molecule, there must be 3N6 possible vibrational modes. Finding the source of harmonic vibrations for measured wave numbers is thus not trivial. Since it would be laborious to assign vibrational fundamental modes alongside IR intensities, one possible interpretation is in terms of the vibrational fundamental modes of functional groups such as NH stretching, CH stretching, and bending. Figures 5 displays the measured FT-IR spectra of the polyherbal formulation.

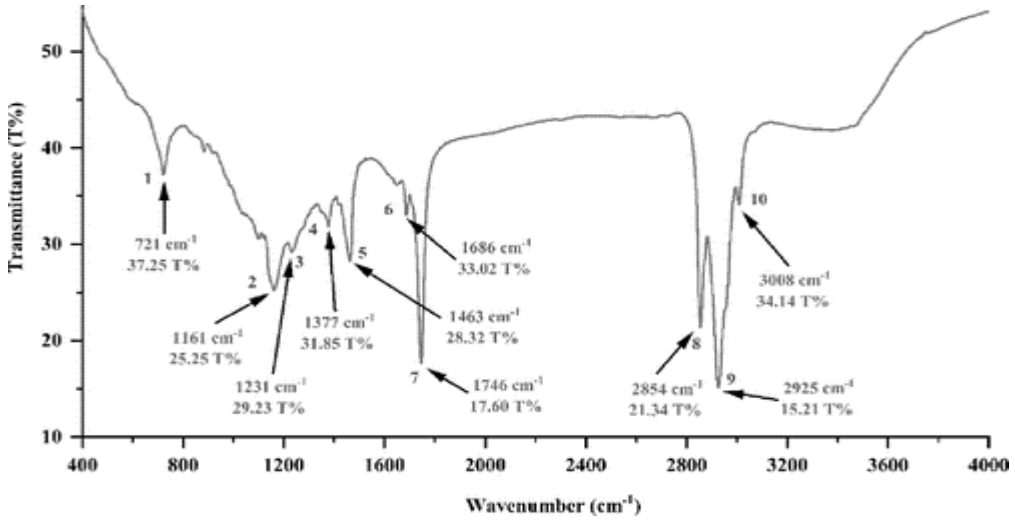


**Figure 5: Infrared spectra of polyherbal formulation**

## CONCLUSION

Natural binders like magnesium silicate and a clay mineral were combined with specific plant parts like dried aloe vera and fresh aloe vera pulp, frankincense, myrrh, and ferula asafetida to create a novel polyherbal formulation called Ayurgreen Natura Pain Gel. Due to the presence of active phytochemicals, which may increase their potency due to the synergistic interaction of active compounds of diverse plants, the developed polyherbal mixture showed high efficacy. A liquid chromatography-mass spectrometer analysis of the phytochemistry of Ayurgreen Natura Pain Gel revealed the presence of 40 phytoconstituents, including phenolics, flavanones, furans, gallotannin, glucoside, oligosaccharide, acids, all of which have different biological activities, such as anti-inflammatory, antibacterial, antifungal, antiviral, and anticarcinogenic properties. Fourier transform infrared spectroscopy and ultraviolet-visible spectroscopy were used to characterize the bioactive functional groups. Differential scanning calorimetry was used for the thermal investigation, which uncovered the existence of volatile substances as well as the melting and degradation temperatures [13-15].

## References

1. Robert D Braun. Introduction to Instrumental Analysis. Introduction to spectral methods of Analysis. Indian reprint. Pharma Book Syndicate, Hyderabad. 2006; p. 136-174.
2. Sharma YR. Elementary Organic Spectroscopy. Principles and Chemical Applications. 5th revised edition. S. Chand and Company Pvt. Ltd., New Delhi. 2013: p. 1-156, 191-308.
3. Mahinder Singh. A Text Book of Analytical Chemistry. Instrumental Techniques. Spectroscopy. Dominant Publishers and Distributors, New Delhi. 2005: p. 1-234.
4. Giridhar V. Quality control of herbal drugs through UV-Vis spectrophotometric analysis. International Journal of Ayurvedic Medicine,2015;6(1):102-9.
5. Vieira TM, Shimano MY, da Silva Lima R, de Camargo AC. Optimization of extraction of antioxidants from aromatic herbs and their synergistic effects in a lipid model system. Journal of Food Bioactives. 2019:7.
6. Yadav KD, Chaudhary AK. Characterization of Sahaj Vati using Infrared and UV/Vis Spectroscopy. Indian Journal of Pharmaceutical Sciences. 2018;80(6):1165-70.
7. Shinde KN, Dhoble SJ, Swart HC, Park K. Basic mechanisms of photoluminescence. InPhosphate Phosphors for Solid-State Lighting,2012:41-59.
8. Khavari-Nejad S, Attar F. In Vitro study of acriflavine interaction with horseradish peroxidase C. Biomacromolecular Journal. 2015;1(1):130-9.
9. Lim ZX, Cheong KY. Effects of drying temperature and ethanol concentration on bipolar switching characteristics of natural Aloe verabased memory devices. Physical Chemistry Chemical Physics, 2015;17(40):26833-53.
10. Otálora MC, Wilches-Torres A, Castaño JA. Extraction and Physicochemical Characterization of Dried Powder Mucilage from Opuntia ficus-indica Cladodes and Aloe Vera Leaves: A Comparative Study. Polymers. 2021;13(11):1689.
11. Mohani N, Ahmad M, Jahan N. Evaluation of phytoconstituents of three plants Acorus calamus linn. Artemisia absinthium Linn and Bergenia himalaica boriss by

*Section A-Research paper*
*ISSN 2063-5346*

FTIR spectroscopic analysis. Pakistan Journal of Pharmaceutical Sciences. 2014;27(6).

12. Sharma BK. Instrumental methods of Chemical Analysis. Spectroscopy. 24th edition, GOEL Publishing house, Meerut. 2005: p. 3 – 938.
13. Willard, Merritt, Dean and Settle. Instrumental methods of Analysis. An Introduction to Absorption and Emission spectroscopy. 7th edition. CBS Publishers & Distributors Pvt.Ltd., New Delhi. 1986: p. 97-116.
14. Ravi Sankar S. Text Book of Pharmaceutical Analysis. 4 th edition. Spectroscopy. Rx Publications, Tirunelveli, India. 2010: p. 1.3-8.5.
15. Galen W Ewing. Instrumental methods of Chemical Analysis. Introduction to Optical Methods. 5th edition. McGraw Hill Education (India) Private Limited, Delhi. 2013: p. 7-31.