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Research Article

Comparative Study Of Vitamin B12, Iron, Zinc And Magnesium In Herbs Of Maharashtra

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ABSTRACT

Medicinal plants are the spine of traditional medicine. These therapeutic plants are rich sources of ingredients which can be utilized as a part of medication, synthesis and development. Here a comparative study has been performed for vitamin B12,zinc, iron and magnesium in the herbs of Maharashtra namely Black Mustard, Chick pea and Beet root for treating various vitamin B12 deficiency disorders. The vitamin B12 deficiency is mostly caused by anaemia, impaired intestinal absorption, malnutrition etc. In this comparative study an analytical method called HPTLC is used for quantification of vitamin B12 and Atomic Absorption Spectroscopy was performed for quantification of iron, zinc and magnesium. The extract of each plant is Phytochemically evaluated with various Phytochemical screening for Alkaloids, Glycosides, Tannins, Carbohydrates etc. The High Performance Thin layer Chromatography is performed on the alcoholic extract of these plant materials. The mobile phase is used for HPTLC is Methanol: Ammonia: 2-Propanol: Formic acid: Water (1:1:7:0.5:2) and stationary phase used is Pre-coated silica G gel 60F 254. By the study of High Performance Thin Layer Chromatography we have determined the amount of vitamin B12 present in each alcoholic plant extract. These herbal plants can be used for treatment of vitamin B12 deficiency disorders. In atomic absorption spectroscopy all the sample solutions were run by hallow cathode lamps.

INTRODUCTION

The term therapeutic plants are incorporates a different sorts of plants which are utilized as a part of herbalism and some of the plants have their own medicinal activity. These therapeutic plants are rich source of ingredients which can be utilized as a part of medication synthesis and

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development.(1) World Health Organization defines that the Traditional herbal medicines are naturally occurring, plant derived substances with minimum or no industrial processing which have been used for treating illness within local or regional healing practices. These medicines initially took the crude form of drugs like tinctures, teas, poultices, powders, and other herbal formulations. (2) Vitamin B12 is essential for DNA synthesis and for cellular energy production. Vitamin B12 deficiency is common, Mainly due to limited dietary intake of animal foods or mal absorption of the vitamin. Vitamin B12 also known as cobalamin, comprises a number of forms including cyano-methyl-deoxy adenosyl- and hydroxy-cobalamin. The cyano form, which is used in supplements, is found in trace amounts in food. Vitamin B12 absorption has been measured by a number of methods including whole Body counting of radiolabeled vitamin B12, metabolic balance studies or controlled feeding studies in vitamin B12-depleted individuals. (3) Mild deficiency manifests as fatigue and anaemia, with indices suggesting B12 deficiency. Moderate deficiency may include an obvious macrocytic anaemia with, for example, glossitis and some mild or subtle neurological features, such as distal sensory impairment. Severe deficiency shows evidence of bone marrow suppression, clear evidence of neurological features, and risk of cardiomyopathy. (4) Vitamin B12 play vital role in helping your body to produce red blood Cells. Studies show that a fetus brain and nervous system requires B12 from mother to develop properly. Vitamin plays a vital role in synthesizing and metabolizing serotonin. Therefore, vitamin B12 deficiency may lead to decreased serotonin production, which can cause a depressed mood. Vitamin B12 is involved in energy production in your body. Taking a supplement may improve your energy level, but only if you're deficient in this vitamin. (5) Metal ions are required for keeping human body healthy because several types of biological functions in human depends on presence or absence of these metal ions.some metal ions like mercury and lead can be dangerous because of their toxic effects. Metal that are essential for biological function in human body are sodium (Na), zinc (Zn), iron (Fe), magnesium (Mg), cobalt (Co), nickel (Ni) etc. the deficiency of Fe and Co leads to anemia, while due to copper leads to brain and heart diseases. (6) Metal ions are the fundamental elements for maintaining the lifespan of plants, animals and humans. They contribute to proper functioning of nerve cells, the brain and heart, muscle cells, transport of oxygen etc. (7) The main function of zinc is to help a plant to produce chlorophyll. Deficiency of zinc in plant leads to discoloration of plant leaves which is called as chlorosis. Iron is main constituent of cell redox systems like heme proteins and sulphur protiens. In plant photosynthesis it play important role for chloroplant development and chlorophyll biosynthesis. (8)

MATERIALS AND METHODS:

Collection of Plant materials –

Collection of plant material was done from local market of Amravati. Chickpea – Seeds Beetroot – Roots Black mustard – Seeds. Authentication of plant material of Black mustard and Mango was done by Dr. P. A. Gawande in Botany department of Sant Gadge Baba Amravati University.

Extraction of Plant materials - (9)

In this method finely ground powder of 10gm of each sample is placed in a porous bag or "thimble" made of strong filter paper. This thimble is placed in a chamber of soxhlet apparatus. Extraction solvent an ethanol of 50ml is heated in the bottom flask, vaporizes into the sample thimble, condense in a condenser and drip back. When liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued for 3-4 cycles.

High Performance Thin Layer Chromatography-

HPTLC is a sophisticated and automated form of thin layer chromatography with better and advanced separation and detection limits. HPTLC also known as High Pressure Thin Layer Chromatography or Planar chromatography or Flat-bed Chromatography.

Steps involved in HPTLC –

1. Selection of chromatographic layer
2. Layer Pre-wash
3. Pre-conditioning
4. Sample and standard preparation
5. Application of sample and standard
6. Chromatographic development
7. Detection of spot
8. Scanning and documentation of chromatoplate.(10)

HPTLC was performed on 20×10 aluminum packed HPTLC plates which is coated with silica gel 60F254 (Merck) . before application of spots the plates were prewashed with methanol and air dried on hot plate. For HPTLC Analysis the volume having 4µl, 6µl, 8µl, 10µl and 12µl of standard vitamin B12 solution were applied on silica gel plate of 2mm bands, 8mm from lower edge by Camag linomate 5 applicator. The plate were placed in developing chamber of mobile phase methanol: ammonia: 2-propanol: formic acid : water (1:1:7:0.5:2) for running of standard vitamin B12 and sample solution. After development, the plates were air dried and scanned by camag scanner 4 with vision cats software using deuterium lamp. (11,12)

Table 1- Chromatographic Conditions.

Application Mode	Camag Linomate, Hamilton Syringe
Development Chamber	Camag Twin Trough Chamber (20×10)
Stationary Phase	Precoated Silica G Gel 60F 254
Mobile Phase	Methanol: Ammonia: 2-Propanol: Formic Acid: Water. (1:1:7:0.5:2)
Detection Wavelength	254 And 366nm
Chamber Saturation	30min
Development Distance	8cm
Development Time	30min
Scanner	Camag Scanner
Source	Deuterium Lamp
Data System	Vision Cats Software

Atomic Absorption Spectroscopy -

Standard preparation – The standard solutions of zinc, iron and magnesium were prepared in 3-5 different concentration to obtain calibration curve by diluting stock standard solution of concentration of 1000ppm. Analysis method – Samples under study were digested by wet digestion method. About 0.2gm of samples were taken in volumetric flask and 4ml HNO3 added in solution and it is stand for few hours then it is heated over water bath till red fumes comes out of flask. Flask was allowed to cool at room temperature and then add 4ml perchloric acid was added in flask heared again on water bath to evaporate till portion which which was than filter through whattman filter paper and made volume upto 100ml with distilled water. Instrumentation – The metal determination was done using atomic absorption spectroscopy model No. A Analyst 300 Perkin Elmer. All the sample and solutions of zinc, iron and magnesium were run by using the hollow cathode lamps.(13,14)

RESULT:

Table 2-Track assignment on HPTLC-

Track	Vial ID	Description	Volume	Type
1	1	Vitamin B12	4.0 µl	Reference
2	1	Vitamin B12	6.0 µl	Reference
3	1	Vitamin B12	8.0 µl	Reference
4	1	Vitamin B12	10.0 µl	Reference
5	1	Vitamin B12	12.0 µl	Reference
6	2	CP	6.0 µl	Sample
7	2	CP	8.0 µl	Sample
8	3	BM	6.0 µl	Sample
9	3	BM	8.0 µl	Sample
10	4	BR	6.0 µl	Sample
11	4	BR	8.0 µl	Sample

TLC Plate

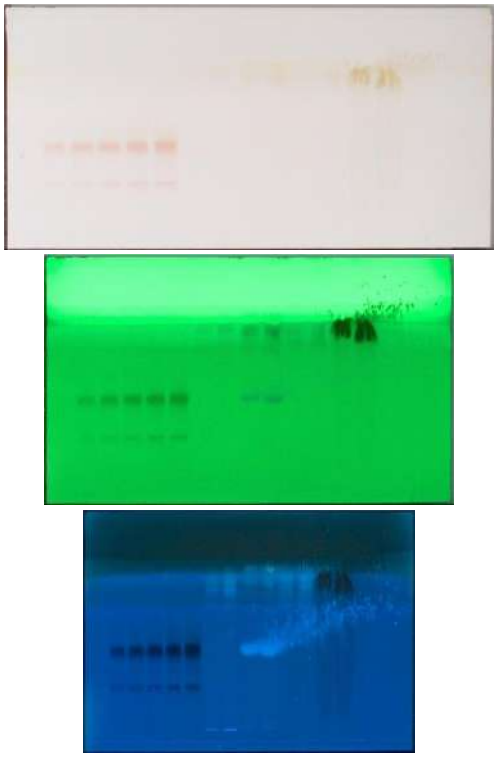
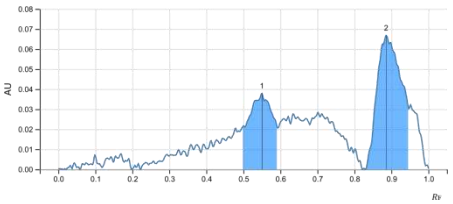
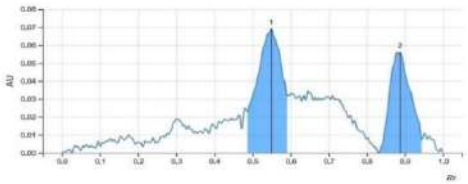


Fig.1. TLC of Standard Vitamin B12 and Samples.

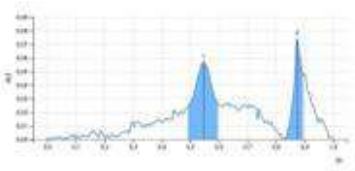
Evaluation of each track -



Track 1



Track 2



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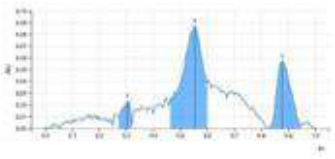

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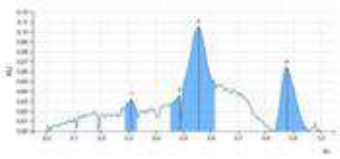

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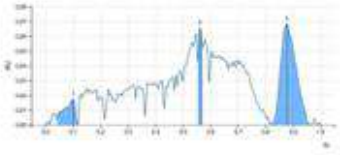

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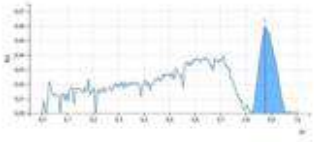

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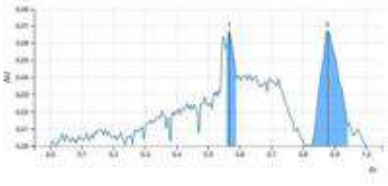

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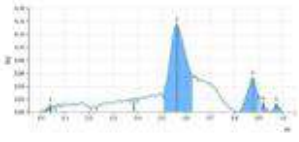

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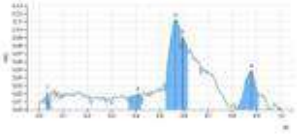

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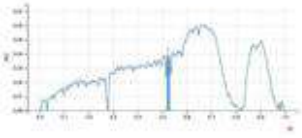
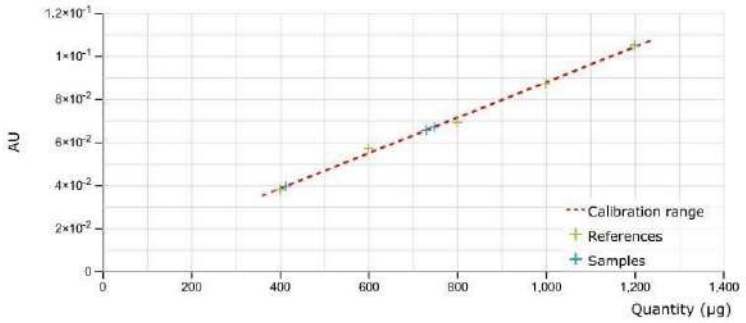


Track 11



Calibration Curve

- Calibration Function –
- Coefficient of variation – 2.05%
- Correlation Coefficient – R = 0.998030
- Sample – 300mg each

Calibration Results –

Substance name	Vial ID	Application volume (µl)	Track number	Rf	Concentration (micro)	Quantity
Vitamin B12	2 (CP)	6	6	0.56129	121757.7	730.5 µg
Vitamin B12	3 (BM)	6	8	0.566129	124729	748.4 µg
Vitamin B12	4 (BR)	8	11	0.52414	51664.93	413.3 µg

Analytical Report

Central Instrumentation Cell

Gadge Baba Amravati University

Element	CP	BR	BM	Wavelength (nm)	Slit width
Mg	19.124±0.15	8.054±0.09	21.184±0.17	285.2	0.7
Zn	5.887±0.08	3.967±0.04	5.743±0.05	213.9	0.7
Fe	12.390±0.13	15.555±0.43	8.533±0.12	248.3	0.7

DISCUSSION AND CONCLUSION

The present work concludes for the confirmation of presence of various phytochemicals like alkaloids, glycosides, tannins, carbohydrates, saponins and inorganic metals i.e. zinc, iron and magnesium etc. The thesis work comaparatively determined quantity of vitamin B12 in each plants. Vitamin B12 in vial ID -2 (application volume 6µl) gives Rf value 0.56129 and gives quantity of vitamin B12 UPTO 730.5µg/ml in chick pea, in beetroot it is observed that vitamin b12 is present in 413.3µg/ml with the Rf value 0.524194 vial ID-4 (Application volume 8µl) and in black mustard the highest quantity of vitamin B12 was observed is 748.4µg/ml with Rf of 0.566129 of vial ID 3. The Black mustard gives highest amount of vitamin B12 in his alcoholic extract but in beetroot and and chickpea the vitamin B12 is present in very less amount of quantity. It also gives comaparatively quantity of each metal in each plants in which – 1mg of drug extract was dissolved in the solution gives a) The magnesium is highly present in black mustard with the quantity of 21.184 ±0.17mg/L and in beetroot and chickpea it is present in quantities of 8.054±0.09mg/L, 16.171±0.14mg/L, 19.124±0.15mg/l. b) The zinc metal is highly present in with the quantity of 8.949±0.11mg/ml while in chickpea, beet root and black mustard it Is present in the amount of 5.887±0.08mg/l, 3.967±0.04mg/l,

5.743±0.11mg/l. c) The iron metal is highly present in beetroot with quantity of 15.555±0.43mg/l, and in chickpea, black mustard it is present in amount of 12.390±0.13mg/l, 8.533±0.12mg/l, 11.611±0.12mg/l etc.

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