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NATURAL HERBAL SUPPLEMENTS – AN ASSESSMENT OF THEIR NUTRITIONAL VALUE AND THEIR PHYTOCHEMICAL CONSTITUENTS

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ABSTRACT

Nutritional deficiency is almost impossible to avoid in these modern times, thus supplements help us to overcome the nutritional deficiencies. It also helps us to boost our immune system. Nutritional supplements are also useful in getting rid of the toxins that are accumulated in our body. Thus the five natural supplements that are mentioned below are tested for the various parameters that include the basic Quality Control Parameters, the phyto chemical analysis, Microbial Analysis that includes the testing for the presence of pathogens along with the total bacterial and fungal count. It is also tested for the presence of heavy metals in them, followed by Aflatoxin and Pesticide analysis. The Nutritional Value for each of them were determined and reported in the form of mg/capsule. The actives of Licorice Capsule Mucuna Capsule and Triphala Capsule were confirmed by the HPLC method where as that of Shatavari was confirmed by the HPTLC Method.

KEY WORDS

Phytochemical analysis, Microbial analysis, Heavy Metal analysis, Nutritional Value, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography

INTRODUCTION

1. Licorice Capsule
Botanical Name: *Glycyrrhiza glabra*
Common Name: Yashtimadhu
Family Name: Leguminosae
Habitat: Widely distributed.

Phyto Chemistry: The major bio active constituent of rhizomes is a triterpenoid Saponin glycyrrhizin (which is a mixture of potassium and calcium salts of glycrhhizinic acid) the drug also contains other triterpenoid saponins like glabranin A&B, glycyrrhetol, glabrolids, isoglabrolide, isoflavones etc.[1]
Marker Constituent: Glycyrrhizin.

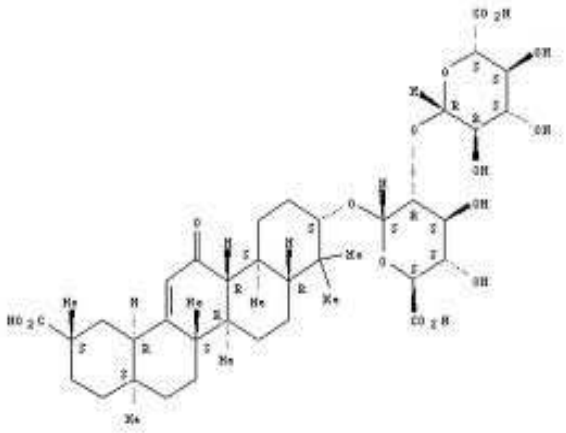


Figure 1
Structure of Glycyrrhizin.

It is generally used in herbal preparations as a tonic, expectorant, demulcent, mild laxative and for allaying cough and catarrhal affections.
In Ayurveda, it is used extensively as a demulcent, mild expectorant and anti inflammatory agent. It is also used in eye diseases, throat infections, and symptomatic relief in peptic ulcer and as an anti arthritic agent. It remains one of the most important herbs in traditional Chinese medicine. It has also been used to treat conditions ranging from diabetes and tuberculosis.
In 1760, George Dunhil of Pontefract added sugar to the extract and since then has become a popular confection. It is employed as a bakery beverage, dairy, candy and meat flavor etc. It is a common moisturizer and flavor for tobacco. [2, 3]. It has also been employed in fire extinguishers as a wetting, spreading and adhesive agent in insecticides, and textile dyes.[4]

Experimental Pharmacology: The demulcent action of the drug is one primarily to glycyrrhizin. The antitussive and expectorant properties of the drug have also been attributed to Glycyrrhizin, which accelerates trachea mucus secretion.
Glycyrrhizin reduces the toxic action of carbon tetrachloride and galactosamine-induced cyto toxicity in cultured rat hepatocytes, through its antioxidant activity. Glycyrrhizin inhibited

histamine release from rat mast cells and prevented carbon tetrachloride induced liver lesions and macrophage mediated cytotoxicity. The anti inflammatory antiallergic actions of the drug have been attributed to the corticosteroid like activity of glycyrrhizin and glycyrrhetic acid (enoxolone). These compounds act indirectly by potentiating the activity of corticosteroids.

Contraindications: Glycyrrhizae is contraindicated in patients with hypertension, cholestatic disorders or cirrhosis of the liver, hypokahemia, or chronic renal insufficiency and during pregnancy.

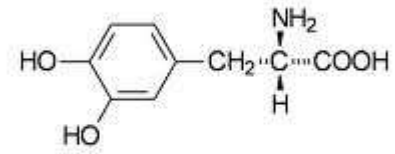
Drug Interactions: As it increases potassium loss, Glycyrrhizae should not be administered for prolonged use with thiazide and loop diuretics or cardiac glycosides. Because it reduces sodium and water excretion, and effectiveness of drugs used in the treatment of hypertension may be reduced. Glycyrrhizae should not be administered in conjunction with spironolactne or amiloride.

2. Mucuna Capsule

Botanical Name: *Mucuna pruriens*
Common Name: Deer-eye beans, Ox-eye beans or Hamburger seed
Family Name: Fabaceae
Habitat: Widely distributed

In many parts of the world *Mucuna pruriens* is used as an important forage, fallow and green manure crop.[5] Since the plant is in the legume family (peas and beans), it, with the help of nitrogen fixing bacteria, takes nitrogen gas from the air and combines it with other chemical compounds producing fertilizer and improving the soil. In history, *M. pruriens* has been used as an effective aphrodisiac.[6-7] *Mucuna pruriens* seeds have also been found to have antidepressent properties in cases of depressive neurosis when consumed.[8] The hair lining the seed pods contain 5-hydroxytryptamine (serotonin) which causes severe itching (pruritus).[9-10] The hair on the outside of the pods of *Mucuna pruriens* are a common ingredient in itching powder.[11]

Pharmacology: M*ucuna pruriens* seeds contain high concentrations of levodopa, a direct precursor of the neuro transmitter dopamine. It has long been used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease.[12-14]
The mature seeds of the plant contain about 3.1-6.1% L-DOPA, with trace amounts of 5-hydroxytryptamine (serotonin), nicotine, DMT-n-oxide, bufotenine, 5-MeO-DMT-n-oxide, and beta-carboline.[15] The leaves contain about 0.5% L-DOPA, 0.006% dimethyltryptamine (DMT), 0.0025% 5-MeO-DMT and 0.003% DMT n-oxide.[16]



Chemical structure of 3-(3',4'-dihydroxyphenyl)-L-alanine (L-DOPA).

Figure 2
Structure of L-Dopa

Uses:

1. ***Increase libido:*** According to ancient Indian medicinal science, *Mucuna* carries important properties to enhance the libido and therefore, the drug has been used as an aphrodisiac. Mucuna is still in use to increase libido in both men and women due to its dopamine inducing actions. Dopamine has a sound influence on sexual function

2. ***Nervous System and Reproductive System:*** There are many researches and clinical trials indicating Mucuna herb as a very effective nervine tonic and a mild aphrodisiac. Mucuna has also been reported assisting in normal nerve cell function. It contains active constituent alkaloids such as mucanine, pruridine, tannic acid, resin, lecithin and L-dopa.

3. ***Great Health Supplement:*** It can promote the production of human growth hormone (HCG), and extracts are commonly highlighted as body-building supplements. Mucuna has also been listed to have safer diuretic effects. It is considered to be an agent that increases tissue resiliency and hence improving coordination in a dull person.

Antidiabetic evaluation of Mucuna pruriens:
In normal rabbits, *Mucuna Pruriens* (0.5, 1 and 2 g/kg) significantly decreased the blood glucose levels while in -diabetic rabbits only 1 and 2 g/kg body weight caused a significant fall. High levels of trace elements like manganese, zinc, and others were found in these seeds. Therefore, it is conceivable that *Mucuna pruriens* seeds contain hypoglycaemic principles, may be both organic and mineral, which seem to act indirectly by stimulating the release of insulin and/or by a direct insulin-like action.

3. Neem Capsule
Botanical Name: *Azadirachta indica*
Common Name: Indian Lilac
Family Name: Meliaceae
Habitat: Widely distributed

The Neem tree is known for its drought resistance. It generally thrives in areas with sub-arid to sub-humid conditions.

External Uses: The leaves and bark are anti microbial; it reduces burning sensation and itching. The seed oil is wound healing, antileprotic, analgesic and therefore it is used in abscess, lymphadenitis, ulcer etc. [17]

Internal Uses:
Digestive System: By its astringent and bitter property it is constipative. The leaves are antihelminthic.
The seeds are uterine stimulant.
In 1942, Indian scientists while working at the Scientific and Industrial Research Laboratory at Delhi University, British India, extracted three bitter compounds from Neem oil, which they named nimbin, nimbinin, and nimbidin respectively.[18] The seeds contain a complex secondary metabolite azadirachtin.
It is a sacred tree in India. Neem products are also used in selectively controlling pests in plants. It is considered a major component in Ayurvedic and Unani medicine and is particularly prescribed for skin disease.[19]

Eczema*:* Recent studies indicate that Neem leaf extracts (in form of soap or shampoo containing Neem oil) can easily relieve the itching and redness of eczema. Neem heals and protects us from any minor skin infections.

Effective Detoxification: It works as a blood purifier and is very helpful in eradicating toxins from the blood. It also helps making our immune system very strong and efficient to fight against any foreign invasion.

Dental care: The twig of Neem tree is largely used as a tooth brush and prevents gum problems. Neem is also used to treat bad breath, tooth decay, bleeding and sore gums and to prevent cavities.

Good for Digestion: Neem tea is an effective tonic for both indigestion and constipation

because of the content of tict rasa. Eating neem will help get rid of intestinal worms, thus performing its role as a de-worming agent. It also eliminates the problem of acidity. It is highly recommended in hyperacidity and epigastric pain.

Effective against diabetes: Neem has been shown to control blood sugar levels and prevent adrenaline as well as glucose induced hyperglycaemia.

Effective against Arthritis: The pain, inflammation, and swelling of the joints in arthritis can be greatly reduced by massaging muscle aches and joints with Neem oil. In addition to its anti-inflammatory effects there are several compounds like polysaccharides, catechin, and limonoids present in Neem that act as pain killers.

Anti cancer: The polysaccharides and limonoids found in Neem not only reduces the tumors and cancers but are also effective against lymphocytic leukemia. Another protein found in the neem leaf has been found to boost the immune response and helps to kill colon cancer cells.

Skin care: Neem leaves paste when mixed with fresh turmeric (haldi) and applied to the face, clears the face of pimples and also reduces scars. Dry skin, dandruff, itchy scalp, wrinkles, skin ulcers and other conditions that can be effectively resolved by the use of soaps, lotions, and creams, containing Neem leaf extracts and oil.

Anti bacterial: Neem oil contains powerful antiviral and antibacterial properties. Infections caused by bacteria (such as acne) or fungus (such as jock itch) are both curable by using neem oil.

Anti malaria: Neem usage boosts the body's immune system making a person less likely to contract malaria.

Hair health: Neem induces shiny, healthy hair, combats dryness, prevents premature graying and may even help with some forms of hair loss.

Contraceptive properties: Modern research suggests that neem does indeed kill sperm within seconds after contact and that the protection lasts for five hours. It is a safe and effective method of birth control, with no side effects. On top of preventing pregnancy, it may also protect from some sexually transmitted diseases.

Other Uses:

- Neem oil is used for preparing cosmetics and is useful for skin acne treatment. It has been found to be an effective mosquito repellent.
- It is known to have good anti-desertification properties and possibly as a good carbon dioxide sink and Neem seed pulp is useful for methane gas production
- A decoction prepared from Neem roots is ingested to relieve fever in traditional Indian medicine.
- The shoots and flowers are used as a vegetable in India.
- People in India also use the twigs to clean their teeth.
- People suffering from chicken pox and measles are generally advised to sleep on neem leaves.
- Neem gives out more oxygen than other trees.

Neem trees have many benefits. Growing Neem trees takes little water and their deep tap roots break through hard clay pans and mine the subsoil for nutrients. The nutrients are returned to the surface as leaf litter, for other plants to use. They are good at accumulating Calcium.

4. Shatavari Capsule

Botanical Name: *Asparagus racemosus*
Common Name: Shatavari, Wild Asparagus
Family Name: Asparagacaeae

Habitat: widely distributed but found more in Northern India.

Chemical Composition: In the fresh tubers, the water soluble ingredient is 52.5 %, fibre 33.3 % and water 9 %. The water soluble ingredients contain 7 % sugar and some mucilaginous principle.
The roots of *Asparagus racemosus* (Shatavari) are fleshy, whitish-brown in colour, slightly sweet in taste, emollient, cooling, nervine tonic and possesses rejuvenating, carminative and aphrodisiac properties. Different scientific studies have proved its efficacy in a number of physical and mental ailments.
The root *Asparagus racemosus* (Shatavari) also has proved its effectiveness as a natural sex stimulant and spermatogenic medicine in both male and female sexual and gyanecological disorders. The root is important for increasing the seminal qualities due to its ability to increase sperm count as well as improves its motility. This herb also enhances libido due to its general tonic effects. It possesses antianxiety and anti-stress properties which are of vital in the infertile male.[20-22]

Internal Uses: It is a brain tonic and a pain reliever. It provides energy to the brain and nerves. It is also used in epilepsy.
It enhances digestion, laxative and astringent.
It alleviates bleeding disorders. It is also cardiotonic. It is used as a galactagogue in humans as well as in animals.
It is also used as an eye tonic. It is useful in impaired eye sight and in many of the eye diseases.
The Asparagus genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant.[23]

Toxic Effects: In Ayurveda, *Asparagus racemosus* is describes as absolutely safe for long term use, even during pregnancy and lactation. Systemic administration of higher doses of all the extracts did not produce any abnormality in behaviour pattern of mice and rat.[24]

5. Triphala Capsule

It is an ayurvedic formulation of three different components viz, Amalaki (*Emblica officinalis*), Bibhitaki (*Terminalia bellirica*), and Haritaki (*Terminalia chebula*).[25]
The active constituents include gallic acid, chebulagic acid, and chebulinic acid. [26, 27]
There is preliminary evidence that *Triphala* contains compounds with antioxidant properties in isolated cells and rats; however this has not yet been demonstrated in people.[28-30] Because of its high vitamin content, Triphala is often used as a food supplement. In recent years, a number of research studies have found new uses for this herb, including treatment for various forms of cancer. It is also found to have high antioxidant qualities, and is even useful for treatment against noise and stress induced conditions.
Triphala combines both nutritional as well as blood and liver cleansing (detoxifying) actions. It has little function as a local demulcent but is more of a lubricating source of nourishment and also possesses some bitter anthroquinones which help stimulate bile flow and peristalsis. The nutritional aspect is partly in the form of its bioflavonoids, high vitamin C content and the presence of linoleic oil, phospholipids and other important.

Haritaki: It is a rasayana for the eyes and good for the digestive system. It helps enhance the absorption of nutrients in food. It cleanses the channels and is absorbed quickly by the body.
Amalaki: It is also a rasayana, which means that it has longevity-enhancing and disease-defying qualities. It is an excellent source of Vitamin C, and is the most concentrated and absorbable source of the vitamin. It also contains other absorbable minerals that nourish the skin, the blood and the whole body. Because of its high content of Vitamin C, it is a powerful antioxidant.
Bibhitaki: It is ideal for pacifying both Pitta and Kapha, and that it cleanses the nutritive fluid

(Rasa Dhatu), the blood, the muscle and the fat tissue.

6. *Turmeric Capsule*

Botanical Name: *Curcuma longa*
Common Name: Turmeric, Haldi
Family Name: Zingiberaceae
Habitat: Widely distributed
It belongs to the family of ginger.[31] It is generally known as the Indian Saffron.

Composition: Turmeric contains up to 5% essential oils and up to 5% curcumin, a polyphenol. Curcumin is the active substance of turmeric. It can exist at least in two tautomeric forms, keto and enol. The keto form is preferred in solid phase and the enol form in solution. Curcumin is a pH indicator. In acidic solutions (pH <7.4) it turns yellow, whereas in basic (pH > 8.6) solutions it turns bright red.

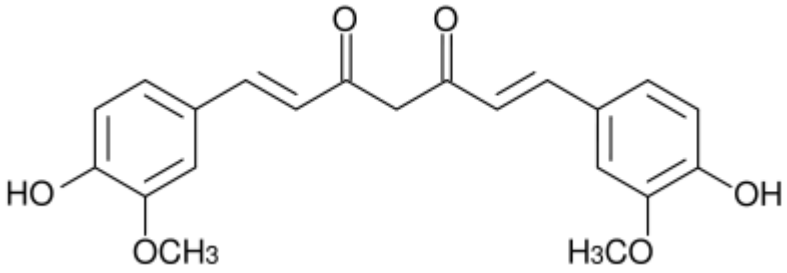


Figure 3
Structure of Curcumin

External Uses: Local application of turmeric is anti inflammatory, analgesic and complexion enhancer. It cures skin disorders and has wound healing properties. The smoke of turmeric is also known to relieve hiccups. It lowers respiration and also relieves the pain caused due to scorpion bite. Its paste is known to act as an antidote.

Digestive System:it I sbitter in taste, appetizer, laxative, cholagogue and antihelminthic, because of these properties it is used for treating loss of apetite, hepatitis, constipation and ascites.

Circulatory System:it stimulates blood formation, circulation and it is also heamostatic. It is useful in treating anemia, bleeding disorders and other blood diseases.
It is usefulas an expectorant. It is useful in several skin diseases. It is known to improve the complexion of the skin.

Latest Research

Turmeric is currently being investigated for possible benefits in Alzheimer's disease, cancer, arthritis, and other clinical disorders.[32]
Research is going on and trying to isolate tetra hydro curcuminoids (THC) from turmeric. THCs are colorless compounds that might have antioxidant and skin-lightening properties, and might be used to treat skin inflammations, making these compounds useful in cosmetics formulations.
Turmeric extract and the essential oil of Curcuma longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. In an animal study, in which guinea pigs were infected with either dermatophytes, pathogenic molds, or yeast, found that topically applied turmeric oil inhibited dermatophytes and pathogenic fungi, but neither curcumin nor turmeric oil affected the yeast isolates.[33,34]
No significant toxicity has been reported following either acute or chronic administration of turmeric extracts at standard doses. At very high doses (100 mg/kg body weight), curcumin may be ulcerogenic in animals, as evidenced by one rat study.[35] It is regarded to be very

auspicious in India and is used in all the ceremonies.

7.0 MATERIAL AND METHODS

Testing Parameters

7.1 Quality Control Parameters: The herbal supplements were tested for their basic Quality control parameters that included test like pH, Moisture by the Karl Fischer method its bulk density, disintegration time and dissolution time. These parameters are very important as they directly influence the body when consumed. The results are tabulated in Table 1

Table 1
Quality Control Parameters

Sr No	Test Parameters	Licorice Capsule	Mucuna Capsule	Neem Capsule	Shatavari Capsule	Triphala Capsule	Turmeric Capsule
1	pH 2 % Solution	5.47	4.50	5.12	4.58	4.85	5.51
2	Moisture	1.83 %	1.79 %	3.75 %	1.49 %	3.14 %	0.93 %
3	Bulk Density	0.735 g/ml	0.833 g/ml	0.609 g/ml	0.757 g/ml	0.735 g/ml	0.793 g/ml
4	Disintegration Time	15 mins 38 secs	14 mins 02 secs	11 mins 03 secs	13 mins 09 secs	20 mins 02 secs	07 mins 43 secs
5	Dissolution test	48.48	76.00 %	72.72 %	56.8 %	62.79 %	41.98 %

7.2 Phytochemical Analysis

The actives of each of the extract were examined and reported with + Standard error mean. The results were reported in Table 2

Table 2
Phytochemical Analysis of all the Capsules

Sr No	Name of the Sample	Name of the Assay	Result + SEM
1	Licorice Capsule	Assay of glycyrrhizin by HPLC [40]	25.44 + 0.28
2	Mucuna Capsule	Assay of L Dopa by HPLC [41]	10.41 + 0.1
3	Neem Capsule	Assay of Bitter Gravimetric [42]	5.1 + 0.14
4	Shatavari Capsule	Assay of Shatavarin by HPTLC	1.66 + 0.91
5	Triphala Capsule	Assay of Tannin [44]	48.42 + 0.60
6	Turmeric Capsule	Assay of Curcumin (UV Spectrophotometer) [39]	20.64 + 0.26

7.3 Microbial Analysis:

Microbial analysis was carried out for all the three extracts as per procedure of Indian pharmacopoeia 2007 and WHO Guidelines. It included Total Bacterial Count, Total Fungal Count, and Presence of *Escherichia coli,* Presence of *Salmonella ebony,* Presence of *Pseudomonas aeruginosa,* and Presence of *Staphylococcus aureus.* Pure culture of *Escherichia coli* (NCIM: 2065; ATCC: 8739), *Salmonella ebony* (NCIM: 2257 NCTC: 6017), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6358) were obtained from NCIM, Pune. The media used for the microbial limit test were of HiMedia Pvt. Ltd.[36-37]

The results are as tabulated in Table 3

Table 3
Microbial Analysis

Name of the Sample	TBC	TFC	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.spp*
Licorice Capsule	31 x 10^2 cfu/g	Absent	Absent	Absent	Absent	Absent
Mucuna Capsule	14 x 10^2 cfu/g	Absent	Absent	Absent	Absent	Absent
Neem Capsule	72 x 10^2 cfu/g	Absent	Absent	Absent	Absent	Absent
Shatavari Capsule	24 x 10^2 cfu/g	Absent	Absent	Absent	Absent	Absent
Triphala Capsule	72 x 10^2 cfu/g	Absent	Absent	Absent	Absent	Absent
Turmeric Capsule	57 x 10^2 cfu/g	Absent	Absent	Absent	Absent	Absent

7.4 Heavy Metal Analysis

Accurately weigh 2 g of the sample in a kjeldahl flask. An acid mixture of HNO_3:$HClO_4$ (4:1) was added in the flask and heated continuously till the solution becomes colorless. The sample was then transferred to a 25 ml volumetric flask and volume was made up with distilled water. A reagent blank was synchronously prepared accordingly to the above procedure. The standard of Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) were prepared as per the protocol in the manual and calibration curve developed for each of them. The sample were analyzed for the presence of Pb, Cd, As, and Hg using atomic absorbance spectrophotometer (AAS) 6300 (by SHIMADZU) [38]

The results are as tabulated in Table 4

Table 4
Heavy Metal Analysis

Sr No	Name of the Sample	Lead (10 ppm)	Cadmium (0.3 ppm)	Arsenic (10 ppm)	Mercury (1 ppm)
1	Licorice Capsule	1.465	0.023	0.132	0.05
2	Mucuna Capsule	0.965	0.029	0.247	0.02
3	Neem Capsule	1.133	0.112	0.147	0.03
4	Shatavari Capsule	ND	0.046	0.052	0.02
5	Triphala Capsule	1.026	0.072	0.153	0.02
6	Turmeric Capsul	0.125	0.045	0.022	0.06

ND: *Not Detected*

7.5 Aflatoxin and Pesticide Analysis

The analysis was also carried out as below
Sample Preparation: 500 mg of the sample was dissolved in 10 ml of Methanol. It was then concentrated on water bath to approximately 7-8 ml. This is then used as the test solution.
Analytical parameters:
Analysis done on a: GC-MS
Model: Auto system XL with Turbo mass
Make: Perkin Elmer
Column used for analysis: PE-5MS (30 meters capillary column)
Carrier gas: Helium
Flow: 1ml / min
Injection Temp: 250 $^{\circ}$C
Oven Temp: 70^{0}C and held for 5 minutes
Rate: 10° C/min upto 290°C and held for 30 minutes
EI Source Temp: 250° C
Scan range: 30-650 amu
The results are as tabulated in table 5

Table 5
Aflatoxin and Pesticide Analysis

Sr No	Name of the Sample	Aflatoxin	Pesticide
1	Licorice Capsule	Not Detected	Not Detected
2	Mucuna Capsule	Not Detected	Not Detected
3	Neem Capsule	Not Detected	Not Detected
4	Shatavari Capsule	Not Detected	Not Detected
5	Triphala Capsule	Not Detected	Not Detected
6	Turmeric Capsule	Not Detected	Not Detected

ND: *Not Detected*

7.6 Nutritional Value: [39]

When one has to take herbal supplement as a part of their daily diet it is very important to estimate the amount of nutrition present per capsule. Thus they were tested for 10 important parameters that included Total Carbohydrate Content, Total Protein Content, Assay of Calcium, Thiamine Estimation, Estimation of Niacin Content, Iron Estimation, Riboflavin Estimation, Total Fat Content, Cholesterol Content, and Vitamin C Estimation.

7.61 Total Carbohydrate Content

The standard Anthrone method was used to check for the amount of Total Carbohydrate in the given sample. The sample to be tested was accurately weighed in boiling tubes and dissolved in 10 ml of 2.5 N HCl solution. It was then hydrolyzed by keeping it in a boiling water bath for 3 hours and cooled down to room temperature. It was then neutralized with solid Na_2CO_3 until the effervescence ceases and made up the volume to 100 ml with distilled water. It is then centrifuged and the supernatant was collected and two different aliquots were prepared. Glucose was used as a standard for the preparation of the standard graph with ranges of 0µg - 200µg concentration (0µg served as a blank). Make up the volume to 2 ml with Distilled water then add 4 ml of anthrone reagent to all the tubes and heat for 10 minutes on a boiling water bath. Cool the tubes and their absorbance was read at 630 nm on a UV Spectrophotometer.

7.62 Total Protein Content

The protein estimation was carried out by the Lowry's method. Bovine serum albumin (BSA) was used as a standard for preparation of the standard graph with ranges from 0µg - 250µg concentration (0µg served as a blank). The sample to be tested was weighed accurately and dissolved in distilled water and filter and use as the sample. Take different aliquotes and make up the volume with distilled water and add reagent C (Alkaline copper solution: Mix 50 ml of Reagent A and 1 ml of Reagent B) and incubate at room temperature for 10

minutes, after which 0.5 ml of Folin- Ciocalteau reagent was added and incubated at dark for 20 minutes and the absorbance was read at 660 nm on a UV Spectrophotometer.
(Note: Reagent A- 2 % Sodium carbonate in 0.1 N NaOH, Reagent B- 0.1 % Na-K tarterate and 0.5 % $CuSO_4$.)

7.63 Estimation of Calcium

Accurately weigh the sample and dissolve it in a 150 ml conical flask containing 3 ml dilute HCl and 10 ml distill water. Boil for 10 minutes to dissolve the sample and cool down to room temperature. Dilute it with 50 ml of Distilled water. Titrate against 0.05 N disodium EDTA solution nearing the end point and then add 8 ml of 20 % NaOH solution with the addition of 0.1 g calcon mixture which acts as an indicator. Continue the titration till the end point is achieved. The percentage of Calcium is then calculated according to the formula below.
% of Calcium **=** Burette reading x Factor x Actual Normality of EDTA x 100
Weight of the sample x normality of EDTA
Where Factor =0.005004

7.64 Thiamine (Vitamin B12) Estimation

Accurately weigh the sample and dissolve it in 100 ml of 0.1 N HCl and mix well. Incubate it overnight. Filter the solution and the filtrate is used as the sample for further analysis. Thiamine is used as standard for preparation of the standard graph which ranges from 0µg - 250µg concentration (0µg served as a blank). Make up the volume to 5 ml with Distilled water. Add 2.5 ml of Buffer solution having a pH 6.6 followed by 2.5 ml 4% Cyanogen bromide solution and shake well. Incubate for 30 minutes at room temperature and take the reading at 366 nm on a UV Spectrophotometer.

7.65 Niacin Estimation:

The sample was accurately weighed and dissolved in 30 ml of 4 N H_2SO_4. It was boiled for 30 minutes, cooled down to room temperature and the volume was made up to 50 ml with distilled water. 60 % lead acetate was added and the pH was adjusted to 9.0 and centrifuged. 2 ml of concentrated H2SO4 was added to the supernatant and incubated at room temperature for 1hour. It is then centrifuged again and the supernatant was collected and 5 ml of 40 % $ZnSO_4$ was added and the pH was adjusted to 8.4 and centrifuged again and the supernatant was collected again and pH is now adjusted to 7.0 and then is used as the sample. Niacin was used as the standard for preparation of the standard graph which ranges from 0µg - 250µg concentration (0µg served as a blank). The volume was made up to 5 ml with distilled water after which 5 ml of 1 % Cyanogen bromide was added and set aside at room temperature for 10 minutes. 1 ml of 4 % aniline is added to all the tubes and then the absorbance was read at 420 nm on a UV Spectrophotometer.

7.66 Estimation of Iron

Accurately weigh the sample and dissolve it in 50 ml of distilled water and mix well. Filter the solution and the filtrate is used as the sample for further analysis. Fe^{3+} is used as standard for preparation of standard graph which ranges from 0µg - 250µg concentration (0µg served as a blank). Make up the volume to 2.5 ml with Distilled water. Add 0.5 ml of Hydroquinone solution followed by 2.5 ml of Acetate buffer having a pH of 5.0. Then add 0.5 ml of ά0.1% ά - ά di pyridine and incubate at room temperature for 30 minutes and take the reading at 540 nm on a UV Spectrophotometer.

7.67 Estimation of (Vitamin B2) Riboflavin

Accurately weigh the sample and dissolve it in 100 ml of 0.1 N H_2SO_4. Boil the sample for 30 minutes and allow it to cool down to room temperature and then add 5 ml of 2.5 M sodium acetate and incubate it at room temperature for 1 hour. Filter the solution and the filtrate is used as the sample for further analysis. Riboflavin is used as a standard for the preparation of standard graph which ranges from 0µg - 250µg concentration (0µg served as a blank). Make up the volume to 2 ml with Distilled water. Add 1 ml of Glacial

acetic acid followed by 0.5 ml 4% $KMnO_4$. Incubate for 2 seconds and add 0.5 ml 30 % Hydrogen peroxide. Shake well and read the absorbance at 366 nm on a UV Spectrophotometer.

7.68 Total Fat Content

The sample is accurately weighed and dissolved in 250 ml of hexane and kept in a thimble of soxhlet apparatus. Then add about 250 ml hexane and keep it for the extraction of fat. Switch on the heating mantle and adjust the temperature at 70°C and cool the solution after 5 hours. Evaporate the solution in a previously weighed evaporating dish and calculate the percentage of fat present in the given sample.

7.69 Total Cholesterol Content

Accurately weigh the sample and dissolve it in 50 ml of Isopropyl alcohol and mix well. Filter the solution and the filtrate is used as the sample for further analysis. Cholesterol is used as standard for preparation of the standard graph which ranges from 0µg - 250µg concentration (0µg served as a blank). Make up the volume to 2 ml with Isopropyl alcohol. Add 1 ml of $Fecl_3$ – acetic acid and add 2 ml of Conc. H_2So_4. Mix well and incubate at room temperature for 10 min and take the reading at 540 nm on a UV Spectrophotometer.

7.70 Estimation of Vitamin C (Ascorbic acid)

Accurately weigh the sample and dissolve it in 75 ml of m- Phosphoric acid (m-PA) taken in $SnCl_2$ solution and mix well. Filter the solution and the filtrate is used as the sample for further analysis. Ascorbic acid is used as the standard for preparation of standard graph which ranges from 0µg - 250µg concentration (0µg served as a blank). Make up the volume to 2.5 ml with m-PA. Add 0.5 ml of 2% Dinitrophenyl hydrazine (DNPH). Incubate it for 1 hr at 50°C. Then add 2.5 ml of 85% sulphuric acid and take the reading at 540 nm on a UV Spectrophotometer.

The results are as tabulated in Table 6

Table 6

The nutritional values are as tabulated below and are represented as mg/capsule

	Licorice Capsule	**Mucuna Capsule**	**Neem Capsule**	**Shatavari Capsule**	**Triphala Capsule**	**Turmeric Capsule**
Carbohydrate Content	33.79	326.72	19.54	143.96	47.73	138.33
Protein Content	10.34	15.84	10.81	2.53	37.47	0.072
Assay of Calcium	4.35	6.38	3.61	2.49	2.95	2.58
Thiamine Estimation	7.32	9.66	11.36	8.96	23.79	0.871
Estimation of Niacin	ND	ND	ND	0.125	ND	ND
Iron Estimation	2.24	38.22	0.674	1.81	2.53	ND
Riboflavin Estimation	1.8	1.83	1.48	0.886	5.81	0.086
Total Fat Content	ND	ND	ND	ND	ND	ND
Cholesterol Content	18.86	41.21	ND	85.58	53.59	170.41
Vitamin C Estimation	21.25	16.40	95.88	181.82	22.39	2.96

ND: *Not Detected*

8. RESULTS

9. *High Performance Liquid Chromatography HPLC*

It is a chromatographic technique that is used to separate a mixture of compounds. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector that provides a characteristic retention time for the analyte. The pump provides the higher pressure required to propel the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography.

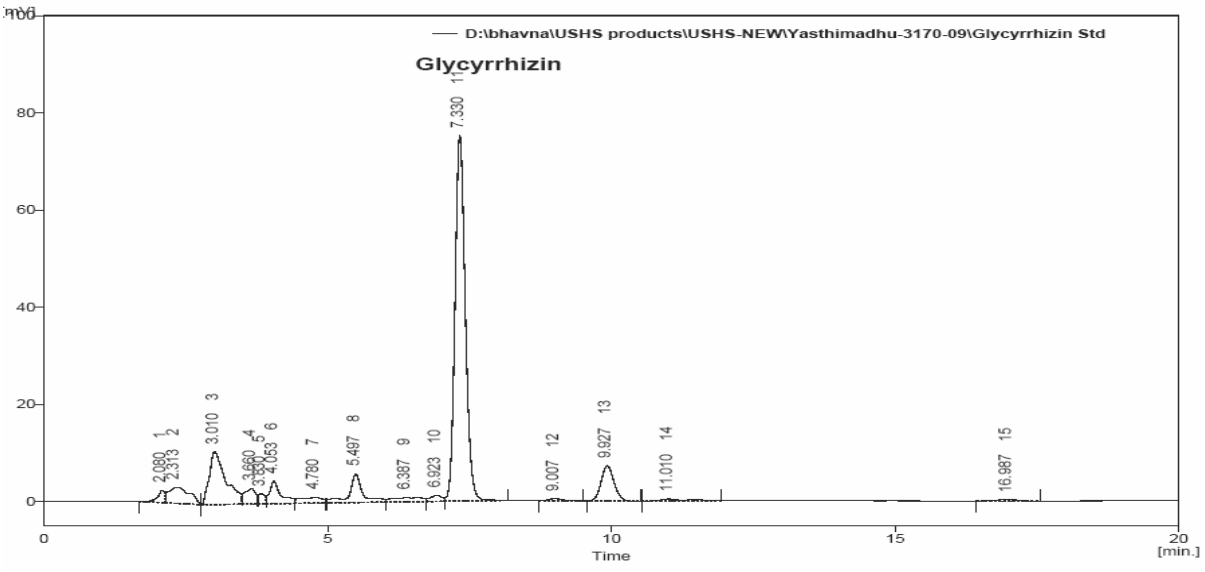


Figure 4
Reference Standard of Licorice (Glycyrrhizin)

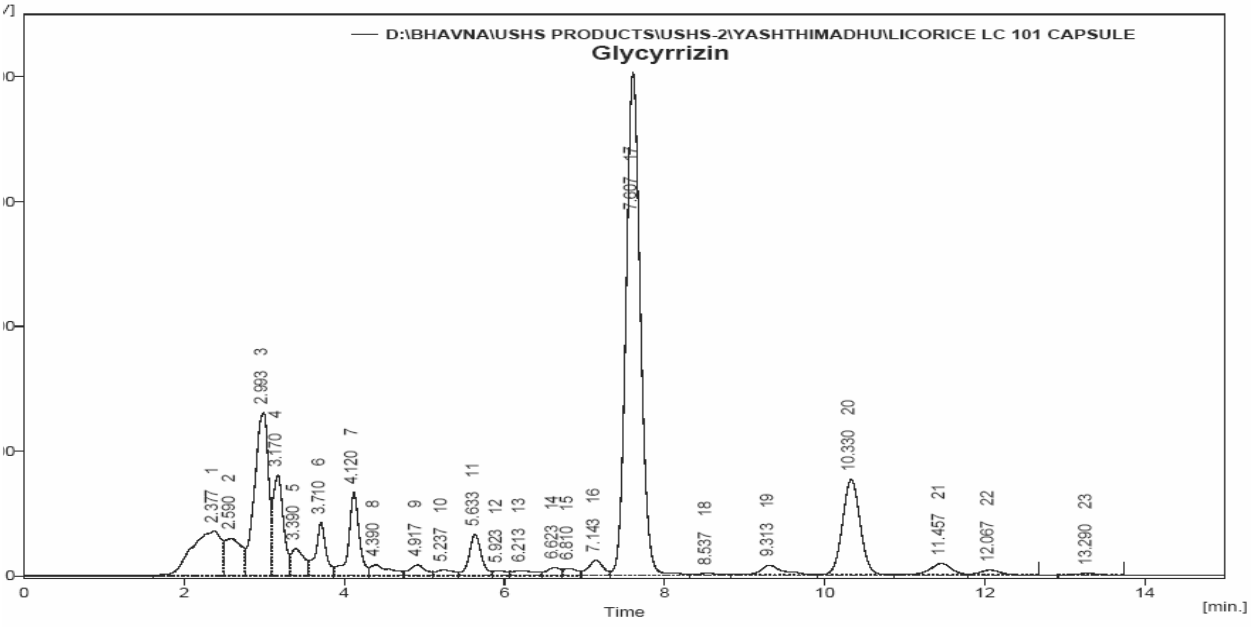


Figure 5
Licorice capsule (Yastimadhu)

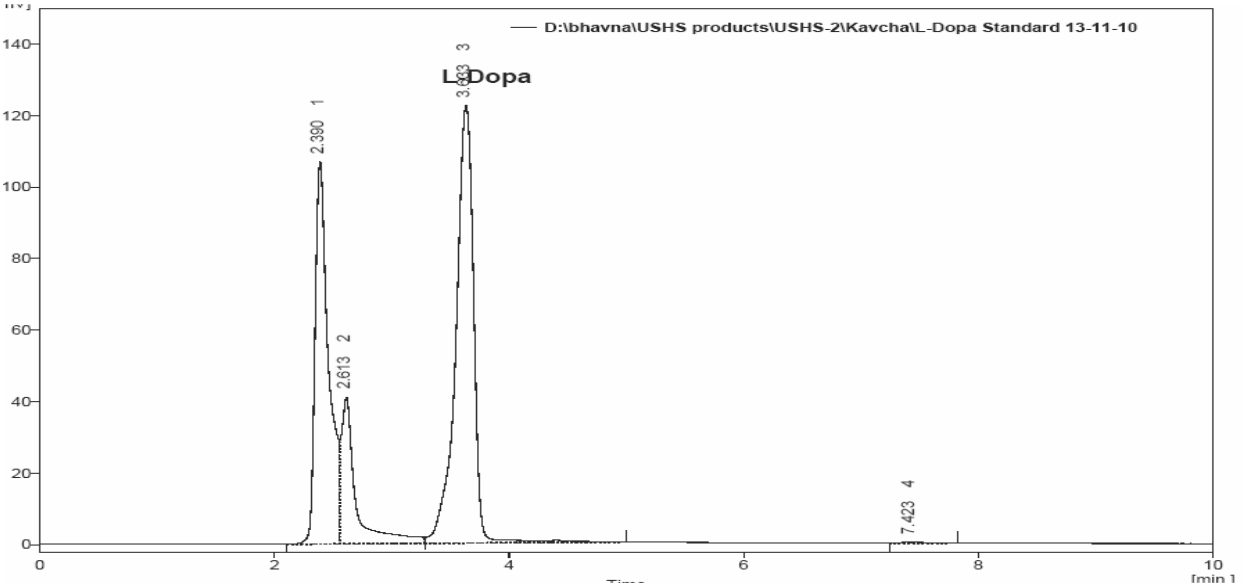


Figure 6
Standard of L-Dopa

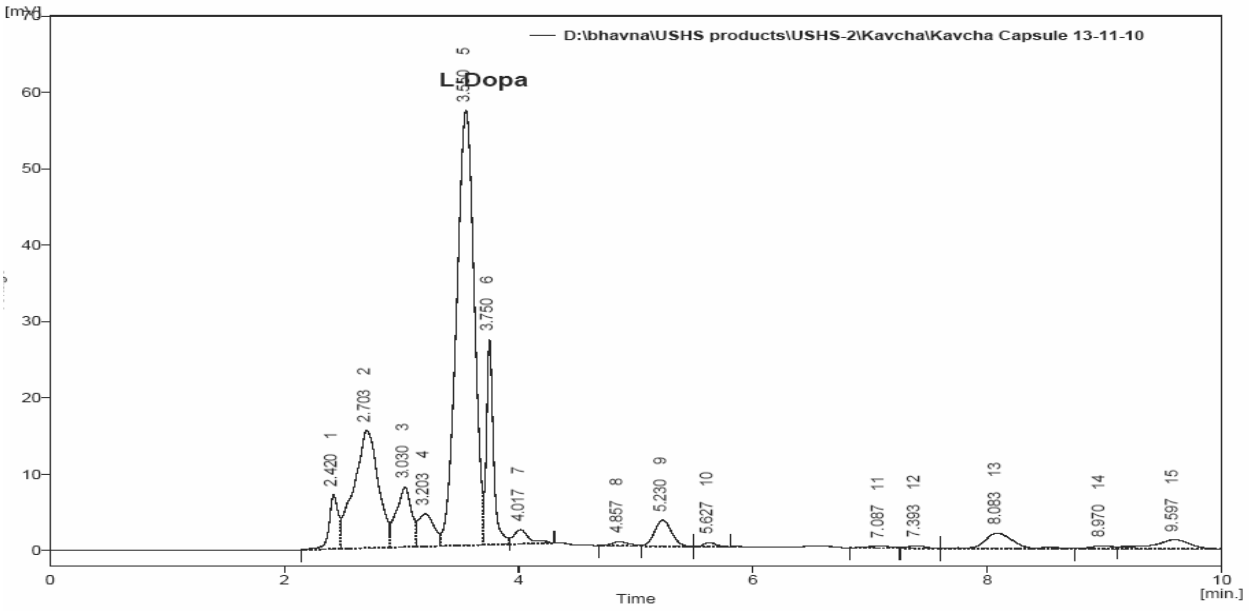


Figure 7
Mucuna Capsule

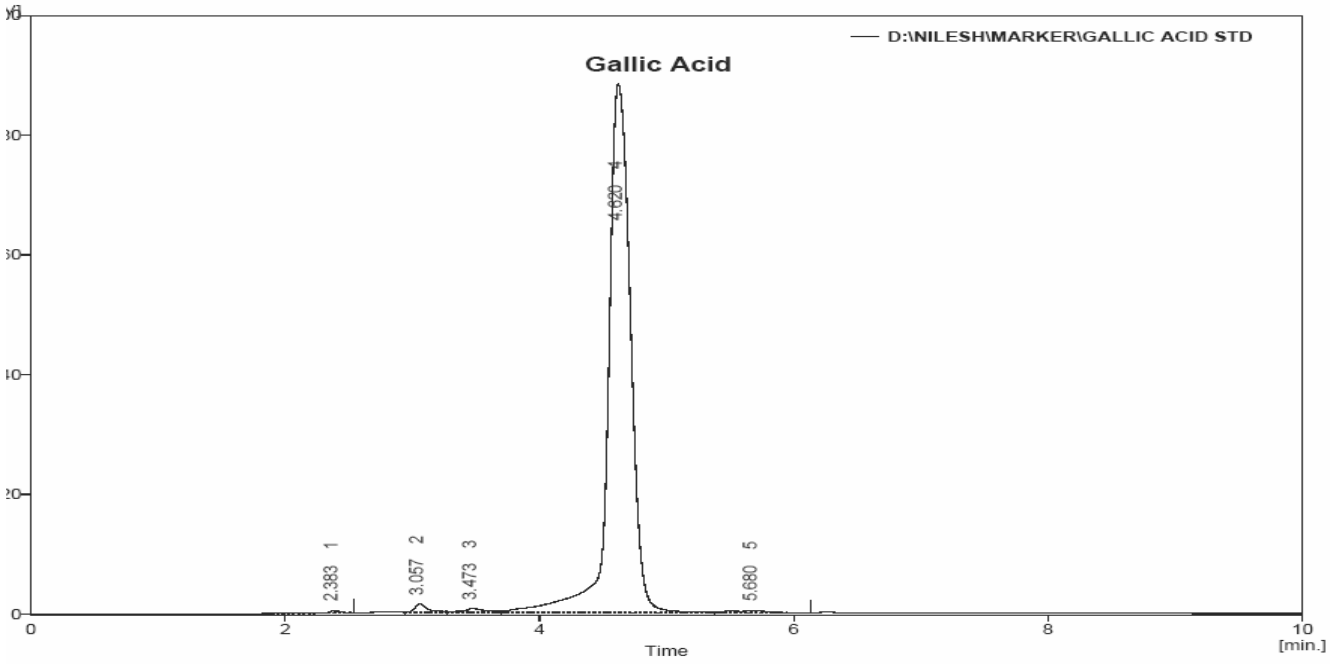


Figure 8
Reference Standard (Gallic Acid - Triphala)

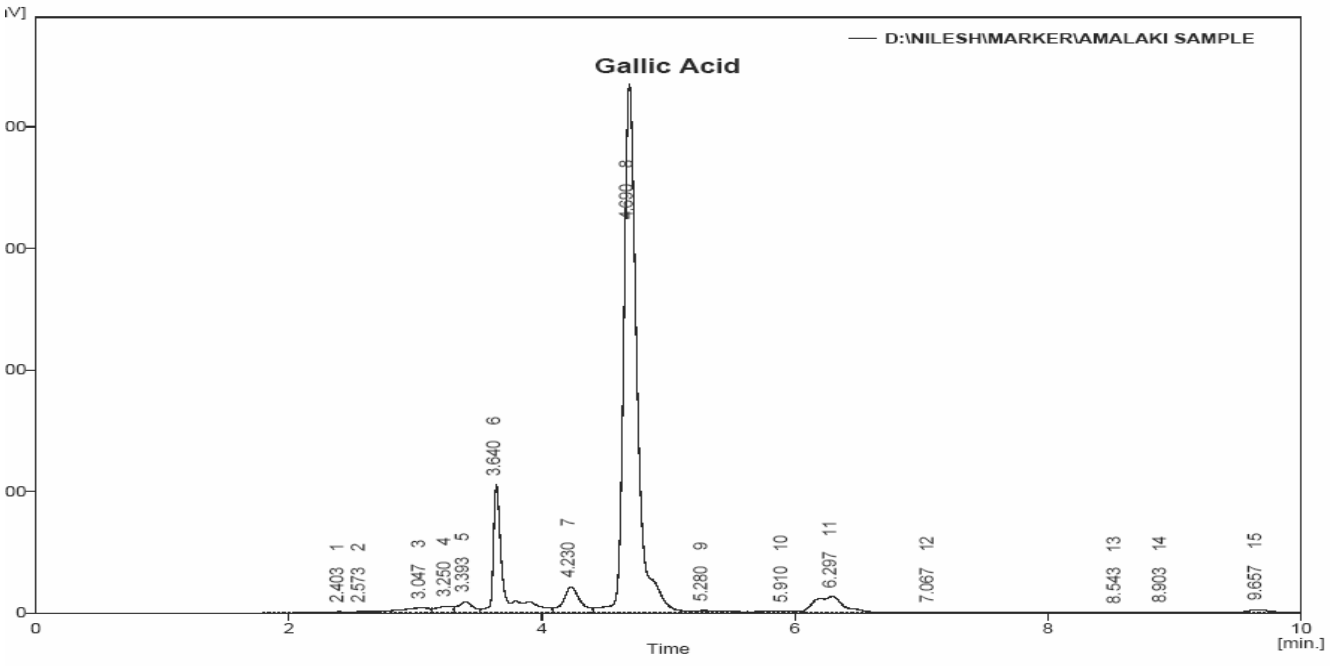


Figure 9
Amalaki (Triphala Formulation)

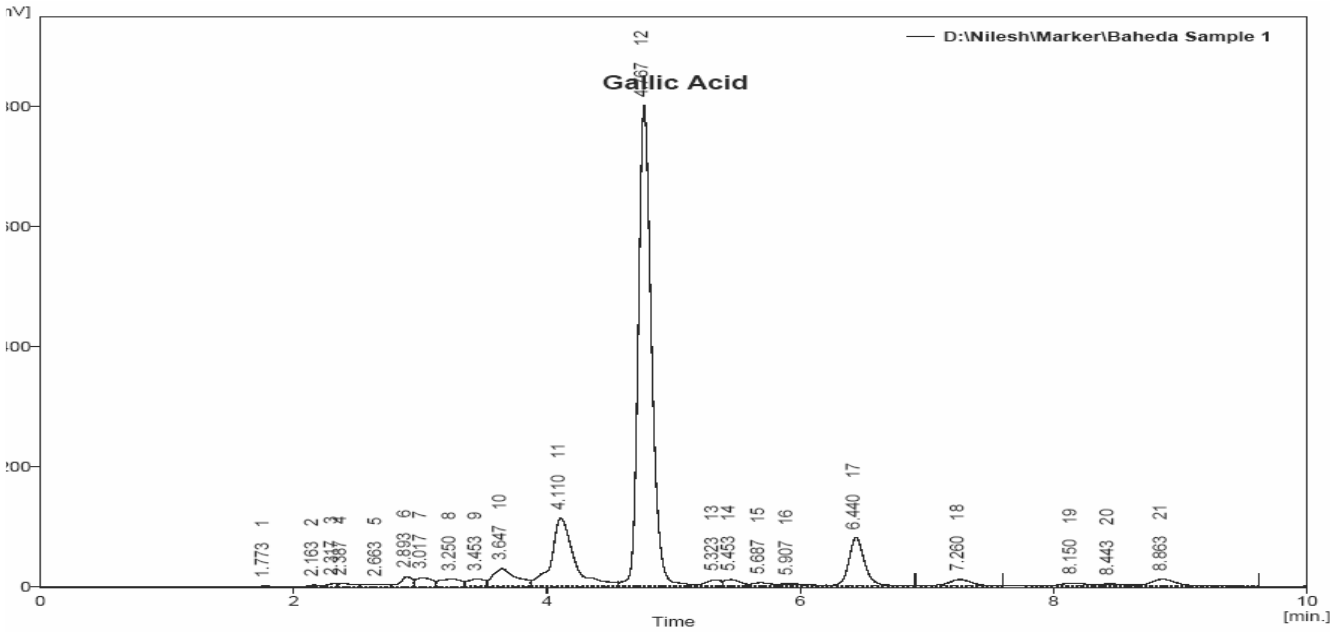


Figure 10
Bibhitaki (Triphala Formulation)

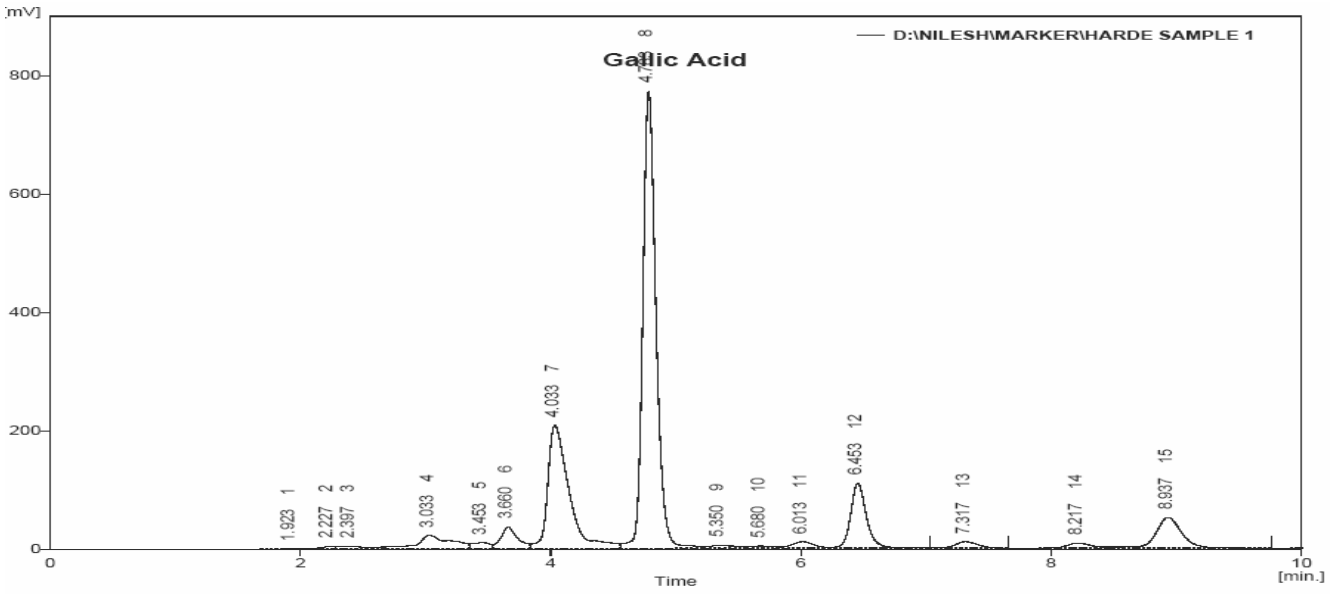


Figure 11
Haritaki (Triphala Formulation)

10. High Performance Thin Layer Chromatography – HPTLC[43]

High performance thin layer chromatography (HPTLC) is used for the quality assessment for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds present. It is a liquid chromatography which involves the separation of the compounds on the basis of their polarity.

Sample Preparation:

Shatavarin IV Standard (Purity: 98.5%): 1mg standard dissolved in 10 ml methanol. i.e.: 0.1mg/ml.

Shatavari capsule: 125 mg of the substance is refluxed with 30 ml of methanol for an hour. It is then filtered & evaporated. Dissolve the residue in 25 ml methanol (i.e.:5mg/ml) and use the solution for TLC profiling.
Solvent System: Chloroform: methanol (7:3)
Chromatographic condition:
Instrument : CAMAG HPTLC system comprising of Linomat IV Spotter, Scanner II, CAMAG CATS 3 software
TLC Plate : TLC plate, Silica gel 60 F254
Mobile Phase: Chloroform: Methanol (7:3)
Number of samples: 01
Saturation Time: 15 min.
Migration distance: 8 cm
Band length: 6 mm
Calibration range: 100 to 600 ng
Detection : At 450 nm
Visualization: Anisaldehyde sulphuric acid reagent

Table 7
Sample Volume Table

	Appl. position (mm)	Appl. volume	Units	Vial	Sample ID	Active
1	15	1	µl	1	Shatavarin Standard 1	☑
2	25	2	µl	1	Shatavarin Standard 1	☑
3	35	2	µl	2	Shatavari Extract	☑
4	45	3	µl	1	Shatavarin Standard 1	☑
5	55	4	µl	1	Shatavarin Standard 1	☑
6	65	2	µl	3	Shatavari Capsule	☑
7	75	5	µl	1	Shatavarin Standard 1	☑
8	85	6	µl	1	Shatavarin Standard 1	☑

Table 8
Densitometry Results

Substance: Shatavarin @ 450 nm Regression mode: Linear

Regression via: Area Y = 40.2393 +13.74* X r = 0.9984 sdv = 6.08%

Track	Vial	Rf	Amount / Fraction	Area	X(calc)	Sample ID /Remark
1	1	0.35	100.00 ng	1205.89		
2	1	0.35	200.00 ng	2861.23		
3	2	0.36		2359.53	167 ng	Shatavri extract
4	1	0.35	300.00 ng	4391.84		
5	1	0.35	400.00 ng	6125.36		
6	3	0.35		2260.62	160 ng	Shatavari capsule
7	1	0.35	500.00 ng	6972.36		
8	1	0.35	600.00 ng	8156.36		

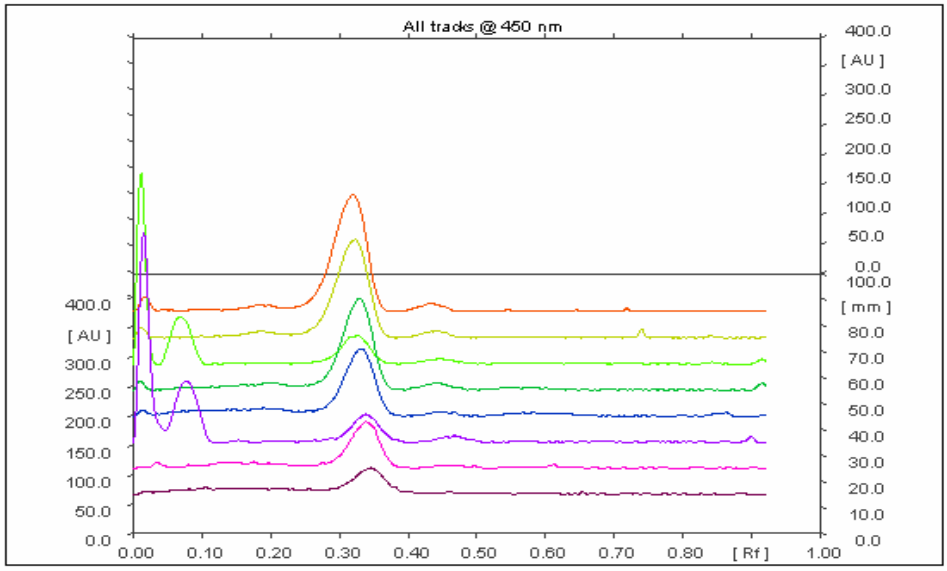


Figure 12
Overlay Chromatogram

Quantification data: Each ml of Shatavari Capsule sample contains 80.0 μg of Shatavarin
Result: Capsule contains 1.60 % Shatavarin.

11. DISCUSSION AND CONCLUSION

Nutritional deficiency is almost impossible to avoid so it is very necessary to consume natural herbal supplements in order to cope up.
These natural herbal supplements are prepared in such a way that there are no additives or excipients that have been added and hence are considered to be more nutritive. All the six supplements have good amount of actives, have no presence of any kind of contamination neither traces of heavy metal in them.

11. ACKNOWLEDGEMENT

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