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## NATURAL HERBAL SUPPLEMENTS- A STUDY ON THEIR NUTRITIONAL VALUE AND THEIR PHYTOCHEMICAL CONSTITUENTS

Kruti Pandya\*, Bhavna Solanki, Kunal Maniar, Nilesh Gurav and Surendra Bhatt

Vasu Research Centre, a Division of Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India

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### Correspondence to Author:

**Kruti Pandya**

Vasu Research Centre, a Division of  
Vasu Healthcare Pvt Ltd, Vadodara,  
Gujarat, India

### ABSTRACT

Nutritional deficiency is almost impossible to avoid in these modern times, thus natural 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 calculated. The actives of Garcinia Capsule, Ginger Capsule and Holy Basil Capsule were confirmed by the HPLC method.

**INTRODUCTION:** Nutritional deficiency is almost impossible to avoid in these modern times, thus natural 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.

### Garcinia Capsule:

Botanical Name: *Garcinia cambogia*

English Name: Brindle berry, Malabar tamarind

Family Name: Clusiaceae

Habitat: In India and parts of Asia

Chemical Composition: It is extracted from its fruit and its rind is popular in many natural weight loss products. The extract is hydroxyl citric acid (HCA), claimed to suppress appetite and enhance fat-burning.

Mode of Action: The theory behind *Garcinia cambogia* is that HCA inhibits an enzyme called citrate lyase that helps turns excess carbohydrates into fat. By inhibiting this enzyme, the body boosts carbohydrate oxidation. It reduced the body's ability to form adipose.

Potential Side Effects of *Garcinia cambogia*: There are no known side effects for using this herb, however, it is not recommended for people diagnosed with diabetics or people suffering with any kind of dementia syndrome, including Alzheimer's; and in pregnant and lactating women. *Garcinia cambogia* does have contraindications with certain medications. *Garcinia cambogia* fills the glycogen stores in the liver and other tissues, thereby reducing appetite while increasing energy levels. It lowers the production of triglycerides and cholesterol and may also increase thermogenesis, the burning of calories. Unlike chemical stimulants commonly used in weight loss products, and does not act on the central nervous

system. This means that they will not cause insomnia, nervousness, changes in blood pressure or heart rate and its effectiveness will not diminish with time. *Garcinia* also contains good amount of Vitamin C and has also been used as a heart tonic traditionally. Its prime indications include:

- 1) Potent weight management herb
- 2) Useful agent against lipids and body fat
- 3) Suppresses the appetite
- 4) Lowers the formulation of LDL and triglycerides

It also tones up the gastro intestinal activity and the appetite is suppressed by promoting the syntheses of glycogen. Glycogen is the form of glucose that has been stored in the body and is one of the body's primary sources of energy.

**Phytochemistry:** The plant contains tartaric acid, citric acid and phosphoric acid. The latex of *Garcinia cambogia* contains two poly isoprenylated benzophenone derivatives, camboginol (I) and cambogin (II). As a chemical constituent of *Garcinia cambogia*, a new xanthone, garbogiol was isolated from the root; a known xanthone (rheediaxanthone A) and two known benzophenones (garcinol and isogarcinol) were obtained from the bark. However the structures were established by spectral analysis<sup>1</sup>. The major organic acid in *Garcinia cambogia* has been found to be (-) Hydroxycitric acid, present in concentrations of 16-18 %, using HPLC with 10 mM sulphuric acid as the eluent. Citric acid and Mallic acids are present in minor quantities<sup>2</sup>.

This principal acid has been found to suppress the fatty acid synthesis, lipogenesis, food intake, and promotes glycogenesis, gluconeogenesis and induced weight loss. Crystals of (-) Hydroxycitric acid were prepared from water extract of *Garcinia cambogia* by precipitation as calcium or barium salt and desalting on cation exchange resin. Water is removed by distillation with immiscible solvent, followed by recrystallization of (-) Hydroxycitric acid lactone in ether. Purity of the preparation was confirmed by spectroscopic and chemical studies<sup>3</sup>. The major organic acid in leaves and rinds has been found to be (-) Hydroxycitric acid

present to the extent of 4.1 – 4.6 and 10.3 – 12.7 % respectively by isocratic elution with 8 mM sulphuric acid.

**Traditional medicinal Uses:** Ayurvedic preparation made from *Garcinia* are used in the treatment of certain ailments.

1. An extract of the fruit is used for rheumatic pains and bowel complaints.
2. The rind is used for biliousness.
3. The leaves and fruits are used to treat ulcers, inflammation, haemorrhoids, diarrhea and dysentery.

### Garlic Capsule

**Botanical Name:** *Allium sativum*

**English Name:** Garlic

**Family Name:** Alliaceae

**Habitat:** Widely distributed

It is a species of the Onion family Alliaceae, and closely resembles it<sup>4, 5</sup>. The bulb of the garlic is used widely. The bulb is divided into numerous fleshy sections called cloves. The cloves are used for consumption (raw or cooked), or for medicinal purposes, and have a characteristic pungent, spicy flavor<sup>6</sup>.

**Medicinal Uses:** Garlic has been found to have antibacterial, antiviral, and antifungal activity. However, these actions are less clear in humans. Garlic is also claimed to help prevent heart disease (including atherosclerosis, high cholesterol, and high blood pressure) and cancer<sup>7</sup>. Garlic is used to prevent certain types of cancer, including stomach and colon cancers. In fact, countries where garlic is consumed in higher amounts, due to traditional cuisine, have been found to have a lower prevalence of cancer<sup>8</sup>.

Animal studies, and some early investigational studies in humans, have suggested possible cardiovascular benefits of garlic. A Czech study found that garlic supplementation reduced accumulation of cholesterol on the vascular walls of animals<sup>9</sup>. The known vasodilative effect of garlic is possibly caused by catabolism of garlic derived polysulphides to hydrogen sulfide in red blood cells, a reaction that is

dependent on reduced thiols in or on the RBC membrane. Hydrogen sulfide is an endogenous cardio protective vascular cell-signaling molecule<sup>10</sup>.

*Allium sativum* has been found to reduce platelet aggregation and hyperlipidemia<sup>11-16</sup>. Garlic is also alleged to help regulate blood sugar levels. Regular and prolonged use of therapeutic amounts of aged garlic extracts lower blood homocysteine levels and has shown to prevent some complications of diabetes mellitus<sup>17-18</sup>. In 1858, Louis Pasteur observed garlic's antibacterial activity, and it was used as an antiseptic to prevent gangrene during World War I and World War II<sup>19</sup>.

More recently, it has been found from a clinical trial that a mouthwash containing 2.5% fresh garlic shows good antimicrobial activity, although the majority of the participants reported an unpleasant taste and halitosis<sup>20</sup>. Garlic cloves are used as a remedy for infections (especially chest problems), digestive disorders, and fungal infections such as thrush<sup>21, 22</sup>. Garlic has been found to enhance thiamin absorption and therefore reduce the likelihood for developing thiamin deficiency beriberi<sup>23</sup>.

In the year 1924 it was found that garlic is an effective way to prevent scurvy, due to its high vitamin C content. Garlic has been used reasonably successfully in AIDS patients to treat cryptosporidium in an uncontrolled study in China. It has also been used by at least one AIDS patient to treat toxoplasmosis, another protozoal disease<sup>24, 25</sup>.

**Phytochemistry:** When crushed, garlic yields allicin, a powerful antibiotic<sup>26</sup>. It also contains the sulfur containing compounds alliin, ajoene, diallylsulfide, dithiin, S-allylcysteine, and enzymes, vitamin B, proteins, minerals, saponins, flavonoids, and maillard reaction products, which are non-sulfur containing compounds. Furthermore a phytoalexin called allixin (3-hydroxy-5-methoxy-6-methyl-2-pentyl-4H-pyran-4-one) was found, a non-sulfur compound with a γ-pyrone skeleton structure with anti-oxidative effects, anti-microbial effects<sup>27</sup>.

The composition of the bulbs is approximately 84.09% water, 13.38% organic matter, and 1.53% inorganic

matter, while the leaves are 87.14% water, 11.27% organic matter, and 1.59% inorganic matter<sup>28-30</sup>.

**Benefits of Garlic:**

1. **Pre-eclampsia:** This is a condition when blood pressure increases, and there is excessive protein retained in the urine. Consumption of garlic during pregnancy cuts the risk of developing pre-eclampsia.
2. **Boosts the baby's weight:** Taking garlic boosts the weight of babies in the womb.
3. **Reduces cholesterol:** Garlic plays an important role in reducing the 'bad' cholesterol in the body.
4. **Anti Carcinogen:** Garlic also helps in reducing the risk of cancer, due to its anti-carcinogenic properties. It helps prevent cancerous compounds from forming and developing into tumors. It also inhibits the growth of tumors.
5. **Blood Clots:** Garlic helps prevent blood clots, and it's extremely beneficial. Garlic also helps regulate blood sugar. It is a natural antibiotic, and it has anti-fungal and anti-bacterial properties.

Garlic has been used for centuries and is a part of popular medicine in many cultures. New data have increased the interest in garlic and its role in normalization and treatment of cardiovascular disease risk factors. Recent studies have shown the complex composition of garlic containing many compounds that present potential positive effects in the field of health. Extracts of *Allium sativum* showed anti viral anti bacterial, anti fungal and anti cancer activities<sup>31-37</sup>.

Garlic supplementation to cholesterol rich diet suppressed the development of atherosclerosis<sup>38, 39</sup>. Garlic produced molluscidal<sup>40</sup> nematocidal<sup>41</sup> effects and showed estrogenic<sup>42</sup> anti-inflammatory<sup>43</sup>, antipyretic<sup>44</sup>, antiarthritic<sup>45</sup> activities and improved heat and cold tolerance<sup>46, 47</sup>. Garlic inhibited clastogenic<sup>48</sup> and cytotoxic effects of arsenic<sup>49</sup>. It prevented mutagenesis<sup>50</sup>, cataractogenesis<sup>51</sup> radiation injuries<sup>52, 53</sup>, dyspepsia<sup>54</sup> and depressed blood clotting time<sup>55</sup>. It also showed anti oxidant<sup>56</sup> hepatoprotective<sup>57</sup> and antiarrhythmic<sup>58</sup> properties.

Garlic juice protected myocardium from ischaemic necrosis<sup>59, 60</sup>. Nitric oxide synthase activation appeared as unique mechanism of garlic action<sup>61</sup>.

### Ginger Capsule:

Botanical Name: *Zingiber officinale*

English Name: Ginger

Family Name: Zingiberaceae

Habitat: Widely distributed

Ginger is the rhizome of the plant *Zingiber officinale*, consumed whole as a delicacy, medicine, or spice.

Chemistry: The characteristic odour and flavor of ginger is caused by a mixture of zingerone, shogaols and gingerols, volatile oils that compose 1-3 % of the weight of fresh ginger. 6-gingerol (1-[4'-Hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) is the major pungent principle of ginger. Gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative, antipyretic and antibacterial properties.<sup>62</sup> Ginger oil has been shown to prevent skin cancer in mice.<sup>63</sup>

Ginger contains up to 3 % of a fragrant essential oil whose main constituents are sesquiterpenoids, with (-) - zingiberene as the main component. Smaller amounts of other sesquiterpenoids ( $\beta$ -sesquiphellandrene, bisabolene and farnesene) and a small monoterpenoid fraction ( $\beta$ -phelladrene, cineol, and citral) have also been identified. The pungent taste of ginger is due to nonvolatile phenyl propanoid-derived compounds, particularly gingerols and shogaols, which form from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process; this compound is less pungent and has a spicy-sweet aroma<sup>64</sup>.

Medicinal Uses: Ginger is most commonly known for its effectiveness as a digestive aid as it increasing the production of digestive fluids and saliva, it helps relieve indigestion, gas pains, diarrhea and stomach cramping. Ginger root is also used to treat nausea related to both motion sickness and morning sickness. Therapeutic properties effectively stimulate the circulation of blood, removing toxins from the body, cleansing the bowels and kidneys, and nourishing the skin. Other uses of the root include the treatment of asthma,

bronchitis and other respiratory problems by loosening and expelling phlegm from the lungs. They may also be used to help break fevers by warming the body and increasing the perspiration.

Pharmacology: The exact mechanism of action of ginger in relation to its antiemetic properties is unclear, although it appears to inhibit serotonin receptors and to exert antiemetic effects at the level of the gastrointestinal system and in the central nervous system<sup>65</sup>. In relation to its potential anti-inflammatory properties, ginger extract has been shown to inhibit the activation of tumor necrosis factor  $\alpha$  and cyclooxygenase-2 expression during in vitro studies of human synoviocytes<sup>66</sup>.

Ginger has been studied extensively in animal and in vitro models, leading to speculation for its use as an antioxidant, antimicrobial, antifungal, antineoplastic, and antihypertensive agent. However, none of these potential uses have been studied in humans.

Adverse Effects and Interactions: Adverse effects after ingestion of ginger are uncommon, but they can include mild gastrointestinal effects such as heartburn, diarrhea, and irritation of the mouth<sup>67</sup>.

Main Functions: Positive effect: pregnancy-induced and postoperative nausea and vomiting. No consistent effect: osteoarthritis, rheumatoid arthritis, motion sickness<sup>68-77</sup>.

Interactions: There is insufficient data on drug interactions; may be prudent to exercise caution when taken in high doses with anticoagulants such as warfarin (Coumadin)

Traditional Uses: In western herbal medicine, ginger has been used for dyspepsia, flatulent colic, alcoholic gastritis and diarrhea from relaxed bowel where there is no inflammation. As a circulatory stimulant, hot infusion of ginger was said to be beneficial for amenorrhoea due to cold. It was also used as a rubifacient<sup>78, 79</sup>. The eclectics used ginger particularly as a stimulating tonic, stomachic, carminative and antispasmodic. It was used to treat nausea, gastro intestinal cramping, loss of appetite and cold extrointestinal cramping. The hot infusion was used to break up colds and to relieve painful menstruation<sup>80</sup>.

Pharmacokinetics: After injection, 90 % of 6 gingerol was found to be serum protein and elimination was mainly via the liver<sup>81</sup>, oral or intraperitoneal dosage, zingerone resulted in the urinary excretion of metabolites within 24 hours, mainly as glucuronide and/or sulphate conjugates<sup>82</sup>.

### **Gokhshurak Capsule:**

Botanical Name: *Tribulus terrestris*

English Name: Gokhshurak

Family Name: Zygophyllaceae

Habitat: Generally found throughout India on sandy soils.

The fruits of Gokhru are credited with diuretic, cooling, tonic, demulcent and aphrodisiac properties. They are used in Ayurveda in the treatment of kidney stones, painful urination, and other genito urinary disorders (in the form of an infusion). They are also prescribed for the treatment of breathing difficulties, diabetes, rheumatism, piles, dropsy, heart diseases and impotency. The root is considered to have aperient and tonic properties. It is mainly a constituent of the ayurvedic drug Dasamula and Amritha prasa grihita. The fruits of this plant are used in various major compound Ayurvedic preparations.

Gokhru Herbs promote the flow of urine, soothes the membrane of the urinary tract and hinders the production of oxalates. These are widely used in Ayurveda for enhancing hormone production in men and women, urinary tract problems, itchy skin and blood purification.

It also shows that *Tribulus terrestris* elevates the testosterone levels by increasing gonadotropin-releasing hormone (GnRH) which eventually, in turn stimulates the production of LH and follicle-stimulating hormone (FSH).

Testosterone, besides its role in body building and increasing fertility, the herb is also known to have a positive effect on bone marrow activity producing healthy red blood cell and the immune system.

*Tribulus terrestris* has been studied in China and found to decrease the frequency of angina pectoris. Laboratory studies have revealed Tribulus to have

antibiotic property and anti-tumor potentiality. It can also lower cholesterol levels in bloodstream, and, in addition they have potential antioxidant properties. They can also lower down the blood pressure in rats with hypertension and also possesses blood sugar lowering effect. In some study in laboratory, cells found in saponins from *Tribulus terrestris* inhibited the growth of a particular type of liver cancer cell line.

In Ayurveda, it is called as 'rasayana' meaning rejuvenating the health. This improves the longevity of a person by offering immunity and strength the body organs and muscles. With the combination of other rasayana drugs, this makes an excellent rejuvenating formula. The drug can also be used as single herb. Ancient science of Ayurveda also describes that the herb has excellent positive effect on urinary tract.

### **Guduchi Capsule:**

Botanical Name: *Tinospora cordifolia*

English Name: Guduchi

Family Name: Menispermaceae

Habitat: Tropical areas of India, Myanmar and Sri Lanka.

Guduchi is described as 'the one who protects the body against diseases'. It is one of the most versatile rejuvenating herbs.

Constituents: It contains Alkaloid & Glucoside: giloin, tinosporin, protoberberine alkaloids, tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid furanolactone tinosporidine, columbin and  $\beta$ -sitosterol.

Pharmacological Action: Cholagogue, detoxicant, immune modulator, anti-inflammatory, diuretic, antihelmentic, nervine tonic. The aqueous extract of guduchi stem has shown the presence of arabinogalactan that showed immunological activity<sup>83</sup>. The stem is used in dyspepsia, fevers and urinary diseases. The bitter principle present shows antiperiodic, antispasmodic, anti-inflammatory and antipyretic properties<sup>84-86</sup>.

Uses:

**Liver:** Liver damage, viral hepatitis or toxicity from alcohol, chemicals and medicinal drugs. It is also useful in repairing fibrosis and regenerating liver tissue.

**Immunity:** All auto-immune diseases causing inflammation. Applicable in degenerative diseases such as cancer, AIDS and arthritis as it boosts the immune system. Use to offset the ulcerative and toxic effects of chemo-radiotherapy.

**Skin:** Suppurative and inflammatory skin conditions such as eczema, psoriasis, Systemic Lupus Erythmatosus

**Gastro Intestinal Tract:** It heals a bowel affected with constipation, intestinal bleeding, haemorrhoids or dysentery. It is useful at redressing intestinal floral imbalance with candida-like symptoms (krimi, grahani) such as bloating, flatulence and malabsorption.

**Metabolic:** It regulates blood sugar levels via its direct effect on rakta and medas-dhatu thus benefiting diabetes and hypoglycaemia. Guduchi is very calming to vata and the nervous system via its unctuous nature soothing nervous irritation. **Reproductive:** Its ability to clear heat is applied when sexual dysfunction is caused by a hyper-heat condition. It is often used for male sexual dysfunction.

### Holy Basil Capsule

Botanical Name: *Oscimum sanctum*

English Name: Basil, Tulasi

Family Name: Lamiaceae

Habitat: Widely distributed

It is often referred to as the "Queen of herbs" and is regarded as the most sacred herb of India. Tulsi has been used for thousands of years in Ayurveda for its diverse healing properties. It is mentioned by Charaka in the Charaka Samhita<sup>87</sup>. Tulsi is considered to be an adaptogen<sup>88</sup>, balancing different processes in the body, and helpful for adapting to stress<sup>89</sup>. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity<sup>90</sup>.

Tulsi extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders,

inflammation, heart disease, various forms of poisoning, and malaria. Essential oil extracted from Karpoora Tulsi is mostly used for medicinal purposes and in herbal cosmetics, and is widely used in skin preparations due to its anti-bacterial activity.

Modern scientific research showed evidence that Tulsi reduces stress, enhances stamina, relieves inflammation, lowers cholesterol, eliminates toxins, protects against radiation, prevents gastric ulcers, lowers fevers, improves digestion and provides a rich supply of antioxidants and other nutrients. Tulsi is especially effective in supporting the heart, blood vessels, liver and lungs and also regulates blood pressure and blood sugar.

**Phyto Constituents:** The unique chemistry of Tulsi is highly complex. Tulsi contains hundreds of beneficial compounds known as phyto-chemicals. Working together, these compounds possess strong antioxidant, antibacterial, antiviral, adaptogenic, and immune-enhancing properties that promote general health and support the body's natural defense against stress and diseases. The essential oils in the leaves of Tulsi that contribute to the fragrance and refreshing flavor of Tulsi Tea, are a particularly rich source of valuable phyto-chemicals.

**Antimicrobial effects:** Essential oil of Tulsi has antibacterial, antifungal and antiviral properties. It inhibits the growth of *E coli*, *B.anthraxis*, *M. tuberculosis* etc. Its antitubercular activity is one-tenth the potency of streptomycin and one-fourth that of isoniazid.

**Antimalarial effects:** Essential oil of Tulsi has been reported to possess 100% larvicidal activity against the *Culex* mosquitoes.

**Antifertility effect:** One of the major constituents of the leaves, ursolic acid has been reported to possess antifertility activity in rats and mice, this effect has been attributed to its anti-estrogenic effect which may be responsible for arrest of spermatogenesis in males and inhibitory effect on implantation of ovum in females.

**Others:** The juice of the leaves is given in catarrh and bronchitis in children. The plant is said to have

carminative, diaphoretic and stimulant properties. A decoction of the plant is used for cough and also as mouth washes for relieving tooth ache. It is good for headache, convulsions, cramps, fevers and cholera. The drinking of Tulsi tea keeps one free from cough and colds and other ailments associated with 'Kapha' dosha in the body. This Tulsi tea is an instant pick-me-up (energy drink).

## MATERIALS AND METHODS:

### Testing Parameters:

**TABLE 1: QUALITY CONTROL PARAMETERS**

Test Parameters	Garcinia Capsule	Garlic Capsule	Ginger Capsule	Gokhshurak Capsule	Guduchi Capsule	Holy Basil Capsule
pH 2 % Solution	4.40	4.52	8.53	5.18	4.45	5.31
Moisture	1.0 %	1.12 %	0.35 %	2.41 %	1.81 %	1.17 %
Bulk Density	0.847 g/ml	0.862 g/ml	0.609 g/ml	0.800 g/ml	0.694 g/ml	0.735 g/ml
Disintegration Time	8 mins and 50 secs	12 mins 37 secs	11 mins and 05 secs	8 mins and 1 sec	19 mins 25 secs	12 mins 38 secs
Dissolution test	98.17 %	91.39 %	95.65 %	59.32 %	98.42 %	66.41 %

**TABLE 2: PHYTOCHEMICAL ANALYSIS OF ALL THE EXTRACTS**

Name of the Sample	Name of the Assay	Result $\pm$ SEM
Garcinia Capsule	Assay of Hydroxy citric acid – calcium salt by HPLC	33.62 $\pm$ 0.30
Garlic Capsule	-----	-----
Ginger Capsule	Assay of 6 – Gingerol by HPLC	5.43 $\pm$ 0.20
Gokhshurak Capsule	Assay of Saponin by gravimetric method	28.95 $\pm$ 0.36
Guduchi Capsule	Assay of Bitter gravimetric method	5.27 $\pm$ 0.05
Holy Basil Capsule	Assay of (sum of ursolic acid & Oleanolic acid) by HPLC	6.24 $\pm$ 0.05

**Microbial Analysis:** Microbial analysis was carried out as per procedure of Indian pharmacopoeia 2007 and WHO Guidelines. It included the test of Total Bacterial Count, Total Fungal Count, and presence of pathogens like *Escherichia coli*, *Salmonella ebony*, *Pseudomonas aeruginosa*, and *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.,<sup>59-60</sup>. The results are as tabulated in **Table 3**.

**Heavy Metal Analysis:** Accurately weigh 2 g of the sample in a Kjeldahl flask. An acid mixture of

**TABLE 3: MICROBIAL ANALYSIS REPORT**

**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**.

**Phytochemical Analysis:** The actives of each of the extract were examined and reported with  $\pm$  Standard error mean. The results were reported in **Table 2**.

$\text{HNO}_3:\text{HClO}_4$  (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 samples were analyzed for the presence of Pb, Cd, As, and Hg using atomic absorbance spectrophotometer (AAS) 6300 (by SHIMADZU). The results are as tabulated in **Table 4**.

Name of the Sample	TBC	TFC	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S.spp</i>
Garcinia Capsule	12 x 10 <sup>2</sup> cfu/g	Absent	Absent	Absent	Absent	Absent
Garlic Capsule	25 x 10 <sup>2</sup> cfu/g	Absent	Absent	Absent	Absent	Absent
Ginger Capsule	11 x 10 <sup>2</sup> cfu/g	Absent	Absent	Absent	Absent	Absent
Gokhshurak Capsule	17 x 10 <sup>2</sup> cfu/g	Absent	Absent	Absent	Absent	Absent
Guduchi Capsule	11 x 10 <sup>2</sup> cfu/g	Absent	Absent	Absent	Absent	Absent
Holy Basil Capsule	14 x 10 <sup>2</sup> cfu/g	Absent	Absent	Absent	Absent	Absent

TBC: Total Bacterial Count; TFC: Total Fungal Count; *E.coli*: *Escherichia coli*; *P.a*: *Pseudomonas aeruginosa*; *S.a*: *Staphylococcus aureus*

**TABLE 4: HEAVY METAL ANALYSIS REPORT**

Name of the Sample	Lead (10 ppm)	Cadmium (0.3 ppm)	Arsenic (10 ppm)	Mercury (1 ppm)
Garcinia Capsule	3.599	0.127	0.082	0.030
Garlic Capsule	ND	0.108	0.173	0.030
Ginger Capsule	1.562	0.021	0.075	0.070
Gokhshurak Capsule	1.148	0.091	0.131	0.050
Guduchi Capsule	1.375	0.084	0.174	0.030
Holy Basil Capsule	1.574	0.048	0.188	0.030

**Test for the presence of Aflatoxin and Pesticide:** 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. Tabulated format of aflatoxin and pesticide is given in **table 5**.

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 °C

Oven Temp: 70°C and held for 5 minutes

Rate: 10 °C/min upto 290°C and held for 30 minutes

El Source Temp: 250 °C

Scan range: 30-650 amu

**TABLE 5: TABULATED FORMAT OF AFLATOXIN AND PESTICIDE**

Name of the Sample	Aflatoxin	Pesticide
Garcinia Capsule	Not Detected	Not Detected
Garlic Capsule	Not Detected	Not Detected
Ginger Capsule	Not Detected	Not Detected
Gokhshurak Capsule	Not Detected	Not Detected
Guduchi Capsule	Not Detected	Not Detected
Holy Basil Capsule	Not Detected	Not Detected

**Nutritional Value:** When one has to take herbal supplement as a part of their daily diets 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.

**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<sub>2</sub>CO<sub>3</sub> 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.

**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 0 $\mu$ g - 250 $\mu$ g concentration (0 $\mu$ g 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 aliquots 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 tartrate and 0.5 % CuSO<sub>4</sub>.)

**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 =

$$\frac{\text{Burette reading} \times \text{Factor} \times \text{Actual Normality of EDTA} \times 100}{\text{Weight of the sample} \times \text{normality of EDTA}}$$

Where Factor =0.005004

**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 0 $\mu$ g - 250 $\mu$ g concentration (0 $\mu$ g 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.

**Niacin Estimation:** The sample was accurately weighed and dissolved in 30 ml of 4 N H<sub>2</sub>SO<sub>4</sub>. 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 H<sub>2</sub>SO<sub>4</sub> 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<sub>4</sub> 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 0 $\mu$ g - 250 $\mu$ g concentration (0 $\mu$ g 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.

**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<sup>3+</sup> is used as standard for preparation of standard graph which ranges from 0 $\mu$ g - 250 $\mu$ g concentration (0 $\mu$ g 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 0.1%  $\alpha$  -  $\alpha$  di pyridine and incubate at room temperature for 30 minutes and take the reading at 540 nm on a UV Spectrophotometer.

**Estimation of (Vitamin B2) Riboflavin:** Accurately weigh the sample and dissolve it in 100 ml of 0.1 N H<sub>2</sub>SO<sub>4</sub>. 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 0 $\mu$ g - 250 $\mu$ g concentration (0 $\mu$ g 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<sub>4</sub>. 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.

**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.

**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<sub>3</sub> –acetic acid and add 2 ml of Conc. H<sub>2</sub>SO<sub>4</sub>. Mix well and incubate at room temperature for 10 min and take the reading at 540 nm on a UV Spectrophotometer.

**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<sub>2</sub> 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 nutritional values are as tabulated below and are represented as mg/capsule in **table 6**.

**TABLE 6: THE NUTRITIONAL VALUES ARE AS TABULATED BELOW AND ARE REPRESENTED AS mg/capsule**

	Garcinia Capsule	Garlic Capsule	Ginger Capsule	Gokhshurak Capsule	Guduchi Capsule	Holy Basil Capsule
Carbohydrate Content	26.4	12.59	220.82	47.79	90.64	40.65
Protein Content	ND	6.48	2.135	834.21	5.939	9.36
Assay of Calcium	10.82	3.76	1.90	3.50	2.94	2.85
Thiamine Estimation	4.005	29.78	0.664	13.0	7.75	17.65
Estimation of Niacin	0.034	0.109	ND	0.022	0.014	ND
Iron Estimation	0.232	1.82	0.182	2.004	1.125	0.925
Riboflavin Estimation	0.531	2.17	0.070	1.37	0.808	2.79
Total Fat Content	ND	ND	ND	ND	ND	ND
Cholesterol Content	74.92	133.72	75.27	ND	13.71	--
Vitamin C Estimation	26.1	156.0	15.86	40.47	48.41	45.42

**High Performance Liquid Chromatography:** 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. The results are shown in **figures 1-7**.

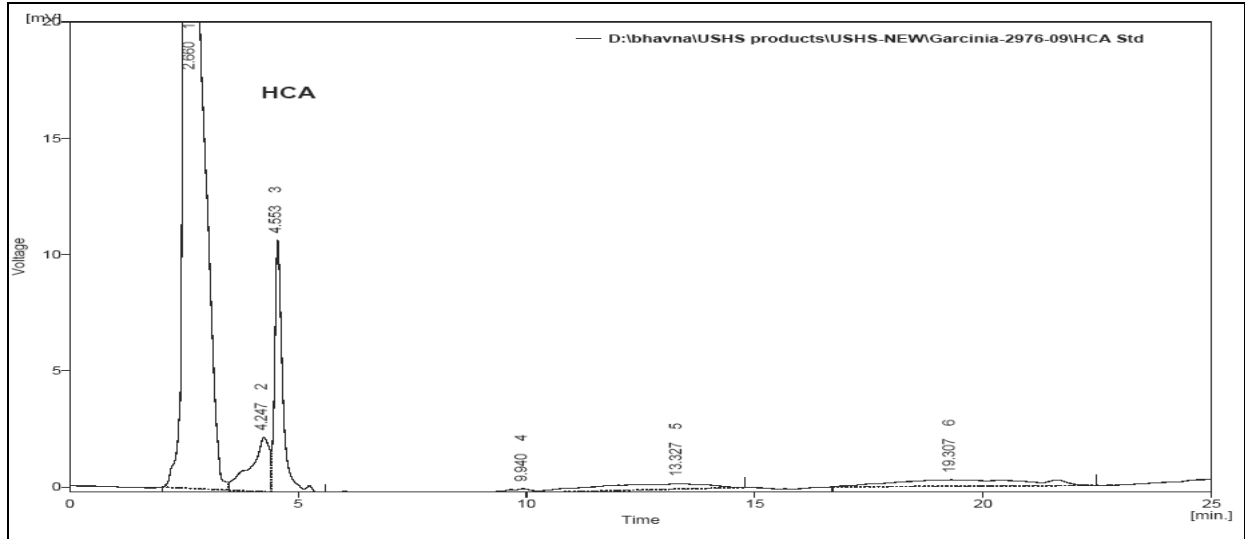


FIG. 1: REFERENCE STANDARD OF GARCINIA

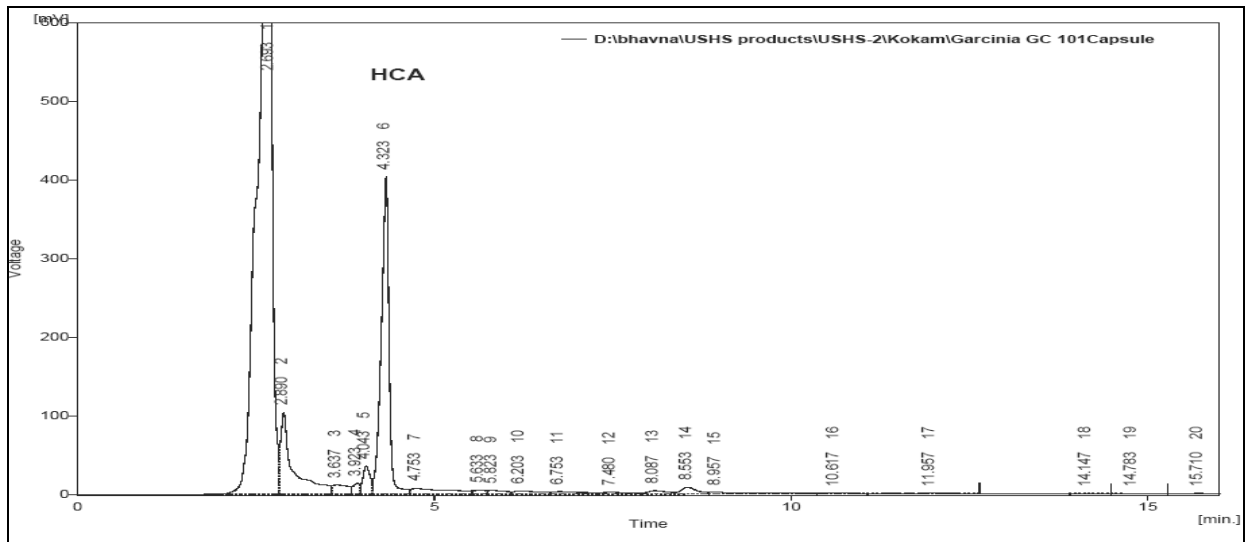


FIG. 2: GARCINIA CAPSULE

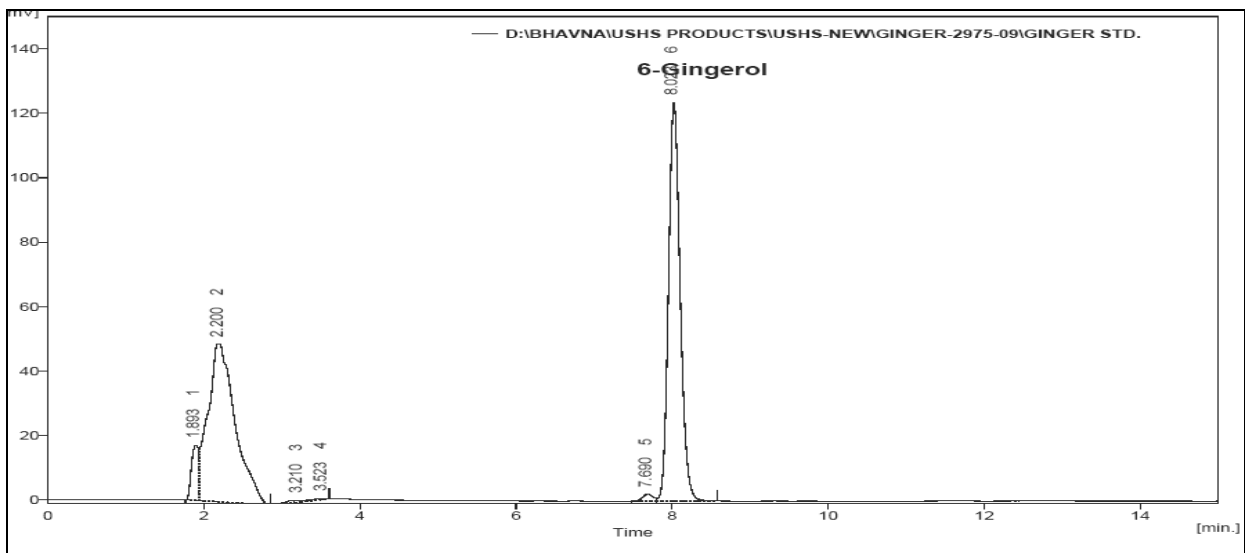


FIG. 3: REFERENCE STANDARD OF 6 GINGEROL

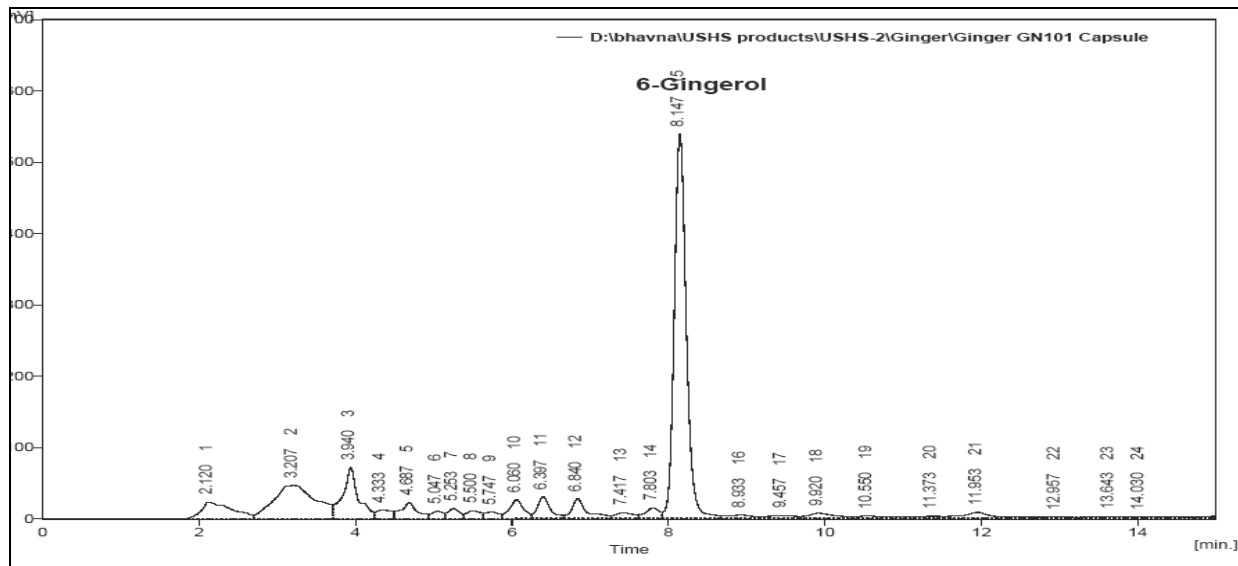


FIG. 4: CAPSULE OF ZINGIBER OFFICINALE

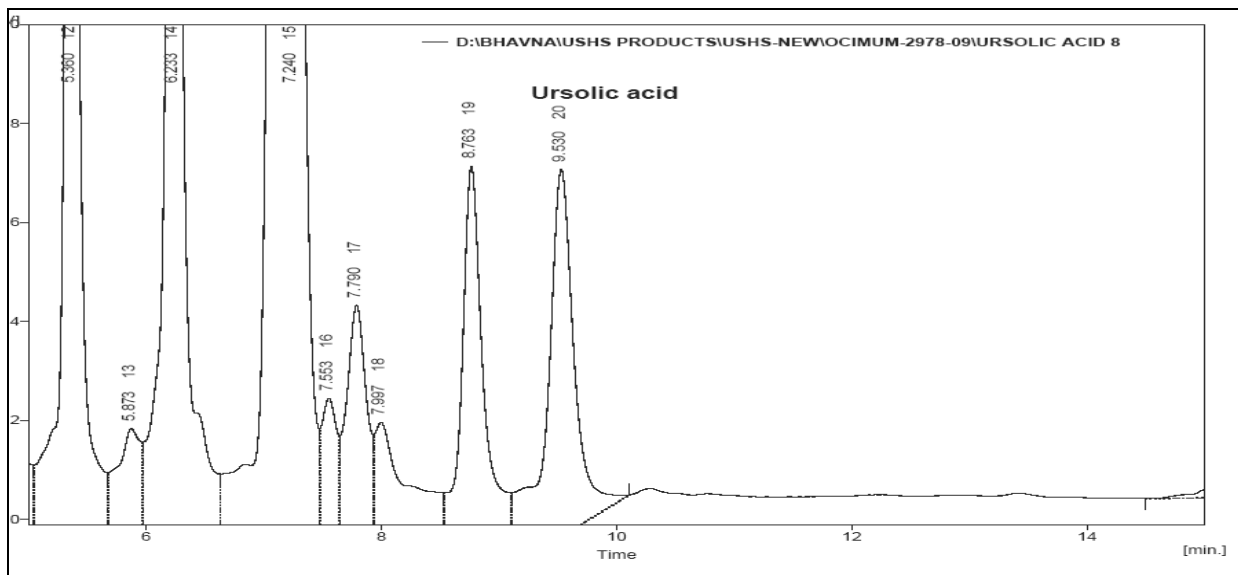


FIG. 5: REFERENCE STANDARD OF URSOLIC ACID

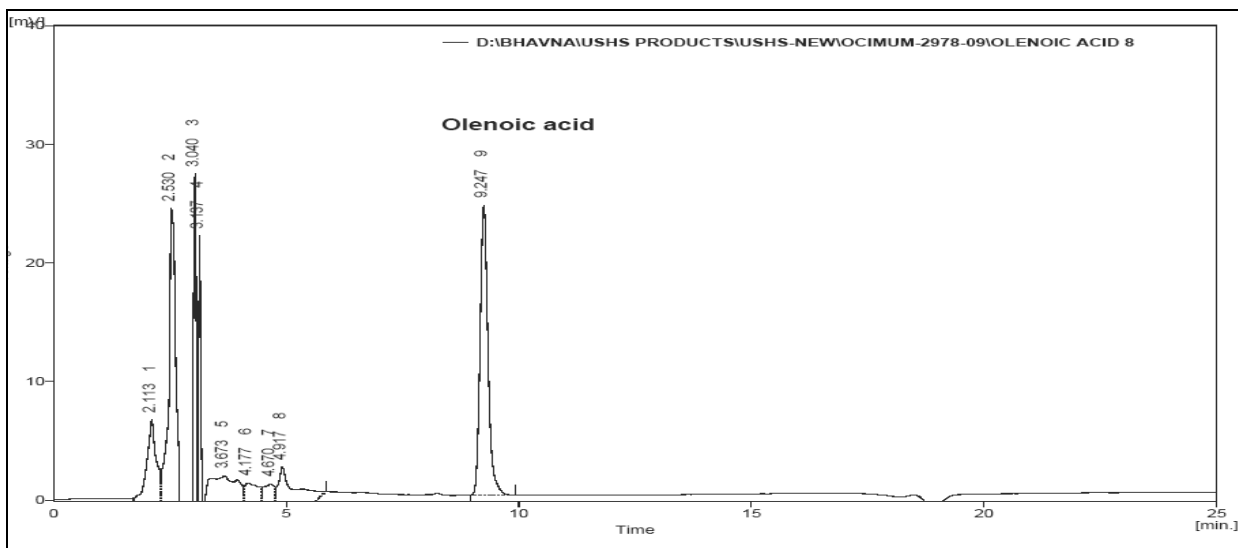


FIG. 6: REFERENCE STANDARD OLEOIC ACID

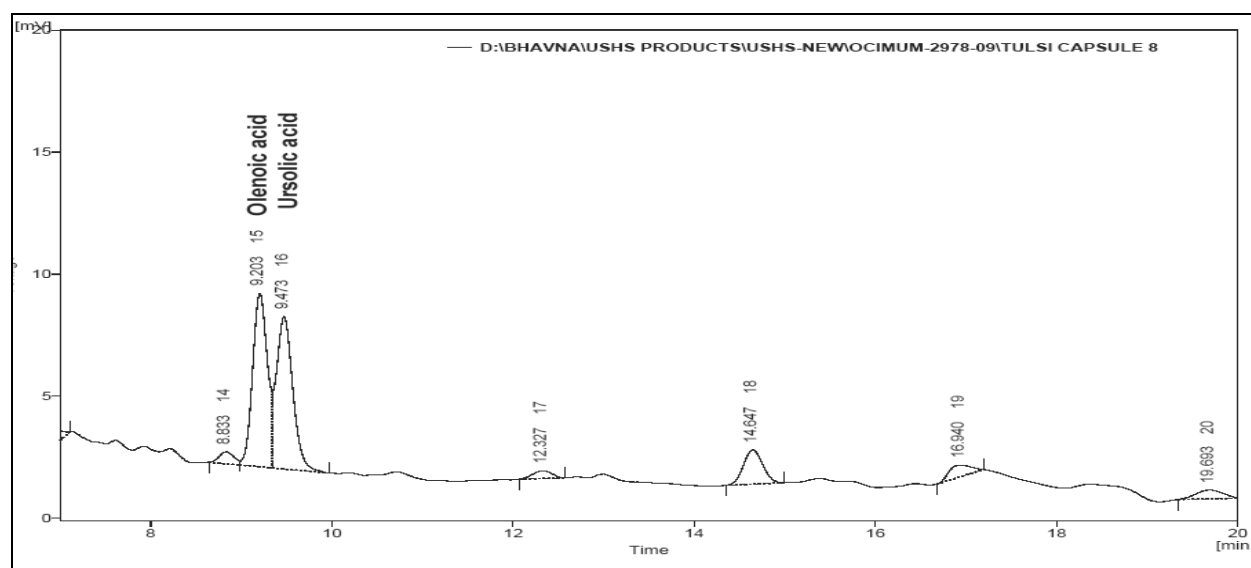


FIG. 7: HOLY BASIL CAPSULE

**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.

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