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Research Article

# PHYTOCHEMICAL INVESTIGATION AND CONTRIBUTION OF PERILLA FRUTESCENCE AS SPICES IN TRADITIONAL HEALTH CARE SYSTEM

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**Abstract**

Perilla frutescence is a common traditional Indian Garhwal Himalaya plant used to treat a variety of diseases. The use of spices in the traditional health care system has been a practice from time immemorial. The adaptive properties of spices to reduce food borne diseases, and the preservative properties, against food spoilage. Often nutritional value of the spices is overlooked. Although, they have low calorific value and posses high percent of minerals and secondary metabolites, which are more useful in the age of obesity. The petroleum ether, acidic & basic and ethanolic extracts from different parts of P. frutenscens was evaluated for their fatty acid, nutritional profile and phytochemical screening. Ash value (total ash 2.2%), moisture 8.0%, crude fat 42.87% and crude fiber 23.5% Extractive values were studied fresh weight. Ash content analysis was showed total crude fiber content Preliminary phytochemical analysis test showed the presence of carbohydrates and glycosides, alkaloid, flavonoid, saponins, tannins, unsaturatedtriterpenoids and sterol, resin.

**Key Words:** Perilla Frutescens, Nutritional value, Fatty acid and Phytochemical screening.

## Introduction:

Perilla Frutescens is an edible plant frequently used in Indian such as Uttarakhand. The stem of the plant is traditionally used as an analgesic and anti-abortive agent. The leaves are said to helpful for asthma, colds and flu's, and regulate stomach function. Considerable attention has been given to the anti-inflammatory, anti-allergic and

anti-tumor promoting substance contained in P. F. plant. Such plant is highly potential with medicinal value and Nutritional value due to the presence of bio-actives, and fatty acid constituents. These plants are consumed by local inhabitants to play a significant role as supplementary food and oil. Humans do not synthesize two of the fatty acids essential for the health i.e., linoleic and linolenic acids. Therefore, these essential fatty acids must be obtained with diet. Alpha linoleic acid (ALA, omega-3) is one of them which has been reported from Actinidia chinensis, Salvia hispanica, Mathiola incana in more than 60.0 % of total fatty acids, however, Perilla frutescence contained only 56.8 %. Dietary lipids and fatty acid composition of our diet plays an important role in health and disease prevention [1-3]. The n-3 PUFAs are beneficial for improving ovulation, embryo quality and cardiovascular protective effects [4-6]. The ratio of n-6/n-3 PUFAs is directly involve when considering the prevention of cancers, heart disease, hypertension and auto-immune disorder [7, 8] The present investigation has been undertaken in view of the importance of omega-3 fatty acids which are beneficial in preventing many health problems including heart diseases, rheumatoid arthritis and cancer [15, 1, 16, 17] taking into account the altitudinal, cultivation parameters and ratio of n-6/n-3 PUFAs. The food substances used as Nutraceuticals contain antioxidants, minerals, vitamins, perbiotics, probiotics, polyunsaturated fatty acids certain phytochemicals and dietary fibers. Instead of focusing on different plants, we have decided to attend the medicinal properties, nutritional value and fatty acid of Perilla frutescens.

**Material and Methods:**

The fresh seed of Perilla Frutesceae was collected from adjoining area of Ghat city (Dist- Chamoli, Uttarakhand) in the month of August-September. The seed was authenticated by botanist Dr. R. D. Guar, Department of Botany; H. N. B. G. U. Srinagar Garhwal The authenticated material was dried under shade and powdered by the help of mechanical process. The coarse powder of seed was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried. After the effective

extraction the solvent was distilled off and the extract was concentrated under vacuum. The various concentrated extracts were stored in air tight container for further studies.

**Preparation of plant Extract**:

The plant material was separated into its selected parts seed air dried ground to moderately fine powder and Soxhlet extracted with increasing polarity solvent (Petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water) [18]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of fruit bark and root was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 100gm). The various concentrated extracts were stored in air tight container for further studies.

**Chemicals:**

Sodium hydroxide, Alcohol, Hydrochloric acid, and Sulphuric acid, Silica crucible, Distilled water etc solvents (Methanol HPLC grade) and chemicals analytical grade were purchased from Merk India Ltd. all the chemicals and reagents used were of analytical grade and obtained from sigma chemical co. (U.S.A), Aldrich or merk.

**Tran's esterification:**

The oils from wild as well as cultivated seeds were trans esterifies by Bureau of Indian Standard (BIS) method [19, 20] with some modification. Hexane fraction was reflex with 0.5 N methanolic NaOH and extracted with diethyl ether, 5 ml water containing 1 ml concentrated HCL was added in remaining part and extracted with petroleum ether. Solvent was evaporated under reduced pressure and reflex with methanol containing two drops of $H_2SO_4$, diluted with water and extracted with petroleum ether.

**GC and GC-MS analysis of methyl esters:**

Methyl esters were analyzed by using Agilent 6890N gas chromatograph equipped with FID and data handling system. Analytical conditions were Perkin Elmer® (Precisely) Cat # N 9316013 Phase Elite-I (Crossbond 100 % dimethyl Polysiloxane) Capillary Column (60 m × 0.25 mm, film thickness 0.25µm), injector and detector

temperatures were 210 $^0$C and 280 $^0$C respectively while nitrogen was used as carrier gas. Oven temperature was held for 5 minutes at 50 $^0$C, with 10 min solvent delay then Programmed at 3 $^0$C / min up to 230 $^0$C and then held isothermal at 230 $^0$C for 20 min. Identification of constituents was based on the comparison of their retention times with those of standard samples. GC-MS was done by Perkin Elmer make Clarus 500 GC-MS equipped with data handling system. Analytical condition and temperature programming was the same as described above, helium used as carrier gas and GC-MS operating in EI mode at 70 eV. Identification of the constituents was based the comparison of their retention times with those of standard samples, and by comparison of their mass spectral fragmentation patterns matching against Commercial Library mass spectra (Nist, Pfleger, Wiley etc.). The fatty acid chemical constituents are listed.

**Nutritional & Mineral assay:**

The edible portion of seed was analyzed for moisture, ash, fat and Fiber as per method [21]. Total nitrogen was analyzed by micro-kjeldhal method [22] and for crude protein the value was multiplied by 6.25. Total carbohydrates were obtained by subtracting the value moisture, crude protein, crude fat crude fiber and ash from 100% [23]. The total energy value equal to addition of fat, protein and sugars calorie, each gram of fat give 9 kcal, protein and sugar give 4 kcal energy. The minerals analyzed were Potassium using atomic absorption spectrophotometer, Calcium and Phosphorus by flame photometer. Ascorbic acid in fruits was estimated [24].

**Phytochemical analysis**:

The qualitative phytochemical properties of the dried powdered sample were determined using standard methods [25].

**Result and Discussion:**

The results of Nutritional Value, fatty acid and also phytochemical studies were tabulated in Table -1, 2, 3, And 4. The phytochemical study revealed the presence of steroids; phytochemical tests are helpful in finding chemical

constituents in the plant materials flavonoids, alkaloids, Saponins, coumarins, unsaturated sterol and triterpenoids, tannins and carbohydrate.

**Nutritional value:**

The level of nutrients such as crude protein, carbohydrates, crude fiber, and ash content (5.12%, 18.53%, 23.28% and 2.2 %) and minerals as calcium, magnesium, potassium and phosphorus (0.238, 0.325, 0.5004 and 0.2124 mg/gm) respectively.

**Fatty acid**:

The fatty acid profile revealed octadecatrienoic (ALA, omega-3) acid as the major component of these oils ranging from 65.96-74.29 %, However, omega-3 fatty acid was reported in Perilla. It is noteworthy to mention here that all the cultivated samples showed ratio of n-6/n-3 as recommended by WHO/FAO. The total polyunsaturated fatty acids (pufa) ranged from 82.26-84.49% in seed oils from wild environment while 83.99-87.09% in cultivated form. In the present study we observed octadecenoic acid in the range of 0.8-2.3 % as against the reported values. From the present study we may conclude that Perilla should be cultivated for the oil vis-à-vis omega-3 fatty acid.

**Phytochemical screening:**

The phytochemical screening for the presence of glycosides, flavonoids, phenols, resin and tannins. However, alkaloids were absent. This analysis revealed that, the seed contained higher value of fat, protein, fiber and minerals as compared to the cultivated fruits with apple and 200 gm fruits contain sufficient amount of nutrients, required per day by a person.

**Conclusion:**

In conclusion, the results of this investigation revealed that fatty acid and nutritional value against selected part of plant seed. Now our research will be directed to develop a broadly used medicine herbal formulation with this plant. Even at low concentrations, these species showed high important activity nearly equal to that of the commercial drugs used as a positive control. Further studies are needed to determine the chemical identity of the

bioactive compounds responsible for the observed chemical activity. it can be used in the treatment of infectious diseases caused by resistant microbes and other problem.

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**Table -1: Nutritional value of Perilla frutenscens seed.**

| **Nutrients** | **Value** |
|---|---|
| Moisture (%) | 8.0 ± 0.15 |
| Ash (%) | 2.2 ± 0.05 |
| Total protein (%) (Nx6.25) | 5.12 ± 0.03 |
| Crude fat (%) | 42.87 ± 0.25 |
| Crude fiber (%) | 23.28 ± 0.01 |
| Organic Matter (%) | 97.80± 0.40 |
| Carbohydrates (soluble) | 18.53± 0.24 |
| VitA | 0.90± 0.0.8 |
| Tannins Mg/100gm | 11.00± 0.10 |
| Mg/100gm Ca | 0.238 ± 0.15 |
| Mg/100gm Mg | 0.325± 0.18 |
| Mg/100gm K | 0.5004± 0.40 |
| Mg/100gm P | 0.2125 ± 0.24 |

**Fig 1.1: Nutritional value of Perilla frutenscens seeds.**

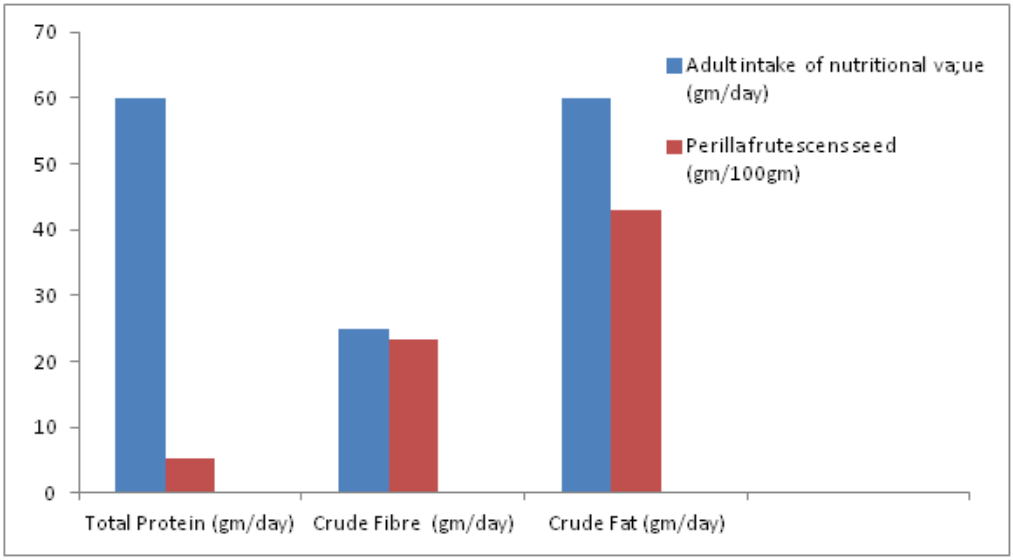



**Fig.1.2: Mineral value of Perilla frutescens seed.**

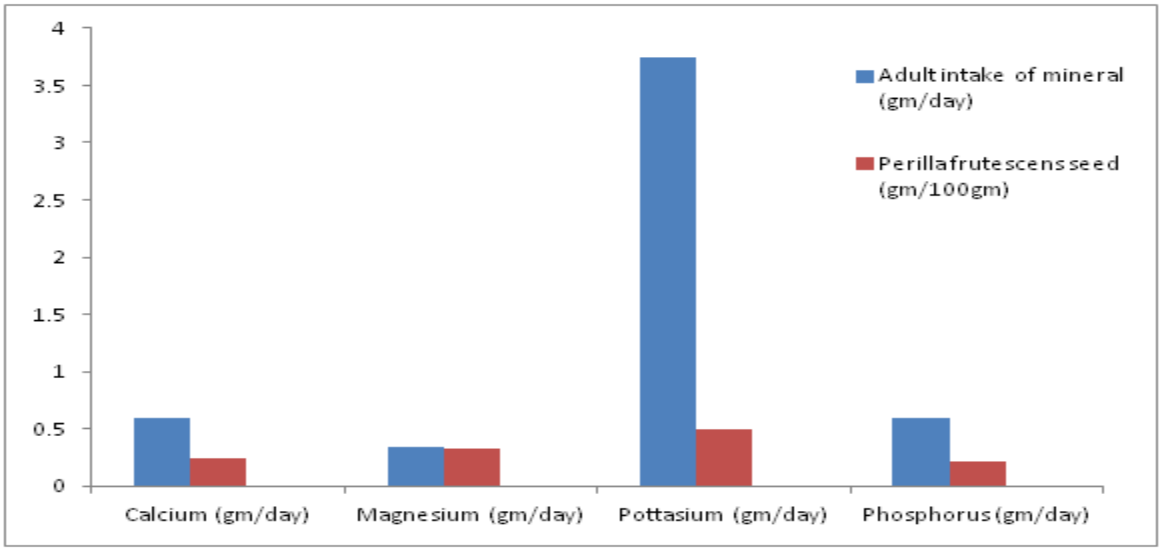


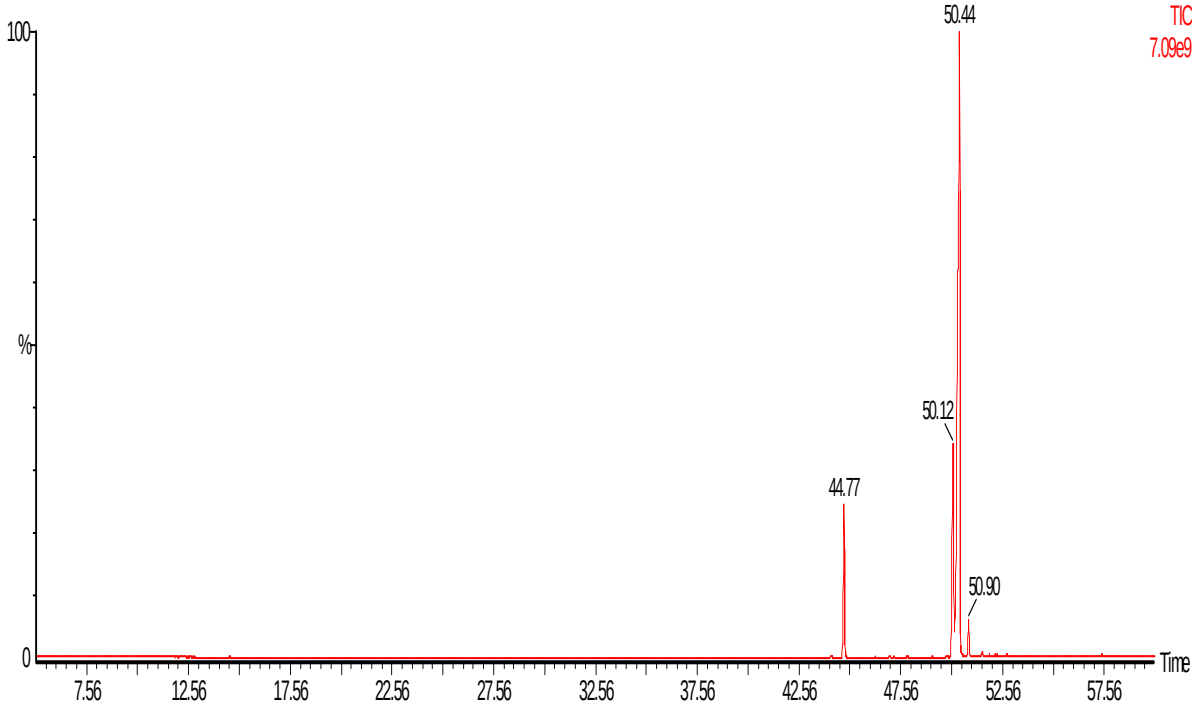

**Table-2: Fatty acid value of Perilla frutenscens seed.**

**Analysis of wild P. Frutescens which was collected from (G.C) Ghat Chamoli:**

| Oil yield (%) | | | | |
|---|---|---|---|---|
| Sample | | | Statistical analysis | |
| GC1 | GC2 | GC3 | Average | SD Value |
| 40.5 | 43.6 | 44.9 | 43.0 | ± 2.261 |
| | | | | |

**Fatty acid constituents (%)**

| Fatty acid | GC1 | GC2 | GC3 | Mean | SD Value |
|---|---|---|---|---|---|
| **Palmitic acid (16:0)** | **10.370** | **9.630** | **9.813** | **9.938** | **± 0.3854** |
| **Hexadecenoic (16:1)** | **0.132** | **0.105** | **0.099** | **0.112** | **± 0.01758** |
| **Stearic acid (18:0)** | **2.475** | **2.183** | **2.343** | **2.334** | **± 0.14622** |
| **Oleic acid (18:1)** | **0.108** | **0.130** | **0.087** | **0.108** | **± 0.02150** |
| **Linoleic acid (18:2)** | **14.366** | **14.543** | **14.619** | **14.509** | **± 0.12982** |
| **Linolenic acid (18:3)** | **66.962** | **69.581** | **69.402** | **68.648** | **± 1.46315** |
| **Arachidic acid (20:0)** | **0.110** | **0.114** | **0.102** | **0.109** | **± 0.0061** |



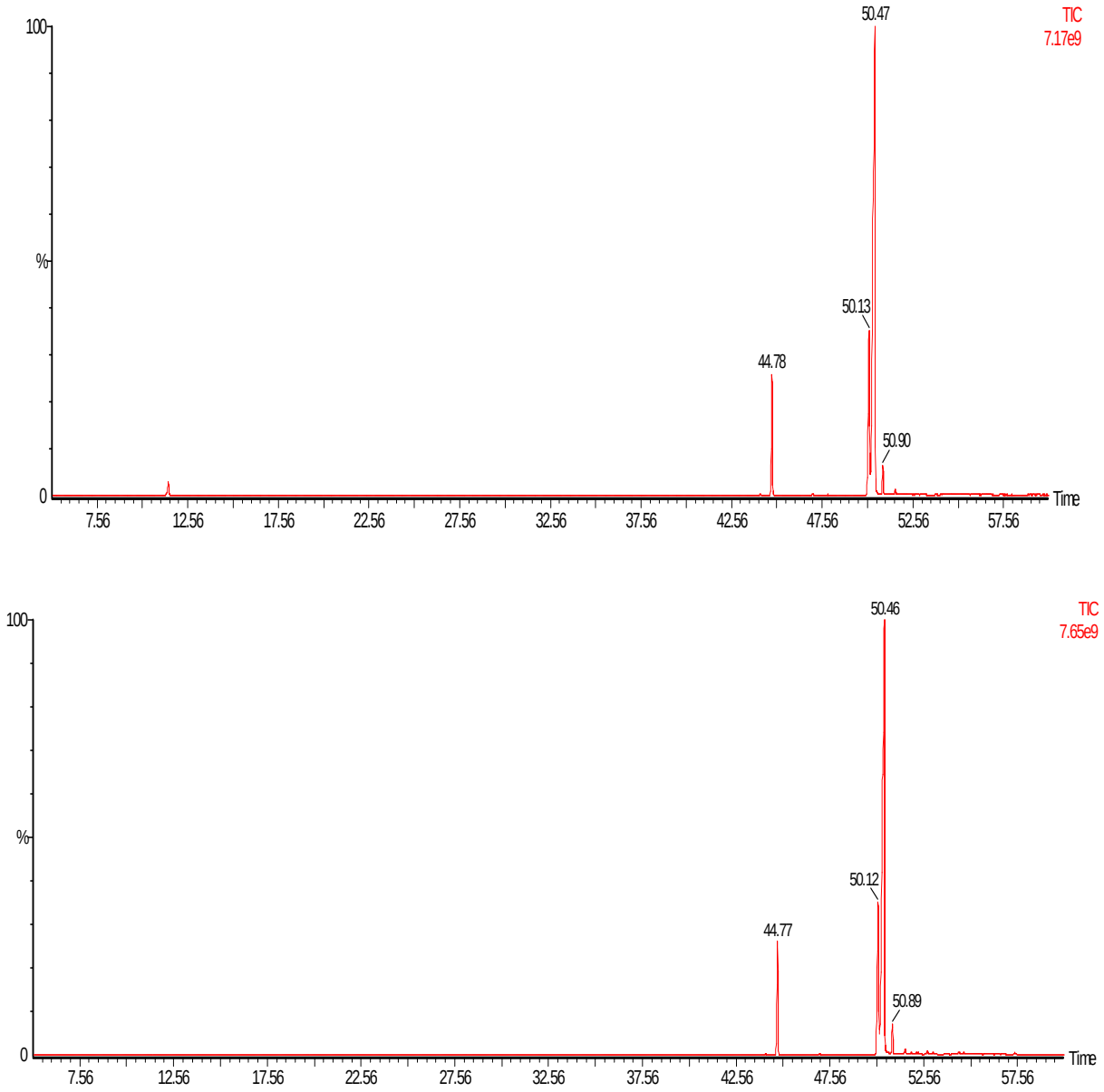



**Table-3: Qualitative estimation of perilla frutescens seed phytochemical screening.**

| Test | Perilla Frutescens Seed |
|---|---|
| Carbohydrates/ glycosides | |
| (1)Molish test | **(+)** |
| (2)Fehling test | **(+)** |
| (3)Benedict test | **(+)** |
| Alkaloid | |
| (1)Mayer's test | **(-)** |
| (2) Dragondroff test | **(-)** |

| | |
|---|---|
| Flavonoid | **(+)** |
| Saponins | **(+)** |
| Tannins<br>(1)Pyrogoll & catechol<br>(2)Gallic acid | <br>**(+)**<br>**(+)** |
| Unsaturated sterol/triterpenes<br>(1)Liebermann Burchard test<br>(2)Salkowiskis test | <br>**(+)**<br>**(+)** |
| Resin | **(-)** |

**Table 4, Qualitative estimation of Perilla Frutescens Seed amino acid screening.**

| **Amino acid test** | **Perilla Frutescens Seed** |
|---|---|
| L- Hydroxy proline | (+) |
| DL Serine | (-) |
| DL Iso-leucine | (+) |
| DL Valine | (+) |
| DL-2-Aminobutyric acid | (+) |
| L-Ornithin | (-) |
| L-Cystein hydroxyl | (+) |
| DL-Nor-leucine | (-) |
| DL-Tryptopham | (+) |
| DL-Alanine | (+) |
| L-Glutamic acid | (-) |
| Glycine | (-) |
| L –Proline | (+) |
| L- Arginine | (+) |
| DL – Aspartic acid | (+) |
| L –Cystein hydroxychloride | (+) |

| | |
|---|---|
| L- Histidine | (-) |
| L – Leucine | (+) |
| L –Lysine monochloride | (+) |
| DL – Methionine | (-) |
| DL – β-Phenyl alanine | (-) |
| DL – Threonine | (+) |
| L – Tyrosine | (+) |
| 3-C-3-4Dihydroxy phenyl | (-) |

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