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Development of energy drink containing *Aegle marmelos*, *Rubia cordifolia*, *Phyllanthus emblica* and *Beta vulgaris* and its phytochemical, nutritive and antimicrobial analysis

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Abstract

Energy drink was prepared from the combination of *Aegle marmelos*, *Rubia cordifolia*, *Phyllanthus emblica* and *Beta vulgaris*. The ingredients used in the energy drink have very good nutrition as well as pharmacological use. All assays were carried out in the methanolic extract of the sample. Total phenolic, flavanoids and tannin content of the sample were found as 42.25 µg GAE/ mg of sample, 101.33 µg CE/ mg of sample, 0.5774 µg TAE / mg of the sample. Alkaloid content of the sample was found to be 6.18%. The product has good antioxidant activity. DPPH assay % inhibition was found to be 63.88% & FRAP value was found to be 268.58 µg BE /mg of the sample. GC/MS screening of sample revealed presence of hexadecanoic acid and octadecanoic acid. Antibacterial activity was done against six bacterial strains, i.e. *Salmonella enterica*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*. Nutritional analysis of the sample revealed moisture content 11.33%, dietary fibre 14.57%, protein 4.59%, total reducing sugar 8.77%, fat 0.17%. Carbohydrate content was 69.34 g/100 g and energy content was 266.46 kcal. Heavy metals and trace metals were detected by ICP-OES. Calcium was 75.31 ppm, phosphorus 81.25 ppm, magnesium 56.25 ppm and iron 7.03 ppm. Copper and chromium were present in trace amounts. Physico-chemical analysis revealed pH of the sample was 4.9, acidity was found to be 0.104% and TSS was found to be 26.2° Brix. Energy drink prepared were subjected to sensory analysis using 9 point hedonic scale and the results showed that it was acceptable to consumers in terms of colour, flavour, taste, aroma, mouth feel and solubility.

Keywords: Nutritional, Nutraceutical, Phytochemical, Antibacterial, Antioxidant, GC/MS, ICP-OES.

1. Introduction

Due to modern lifestyle and work pressure people don't get sufficient time for their meal and for their daily workout. Due to busy schedules and fast running life people are not aware that they are consuming more and more junk foods and following bad health routine. So they are more prone to disease like constipation, cardiovascular, cancer, diabetes, hypertension, obesity, depression etc.

Now a days bioactive compounds present in fruits and vegetables gaining importance as they have nutritional value as well as prevent chronic diseases, such as various types of cancers, diabetes, neurological diseases and cardiovascular disease^[1]. So people are inclining more towards natural herbs, fruit and vegetables for preventing diseases rather on medicines for curing disease as well as maintaining their health. Keeping this in mind a herbal energy drink is prepared containing *Aegles marmelos*, *Rubia cordifolia*, *Phyllanthus emblica* and *Beta vulgaris* as its main constituents. All the constituents used in the preparation of drink have proven medicinal as well as nutritional values. So drink prepared can be categorized as a functional food. India is a treasure of aromatic and medicinal plants. According to trends prevailing in the society regarding health of the people, food as well as pharmaceutical companies are giving more preferences to the discovery and production of Nutraceuticals over pharmaceuticals.

Aegle marmelos commonly known as Bael. It belongs to family Rutaceae. It is a deciduous tree having cosmopolitan distribution present throughout the deciduous forests of India. This plant has been used since ancient times for its therapeutic values.

Its therapeutic values were described by almost all the ancient Ayurvedic treatises like *Unani*,

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Siddha, Sushruta Samhita and *Charaka Samhita* etc [2]. Bael tree is a medium sized deciduous tree with unusual branches having aromatic trifoliolate leaves, sweet scented and greenish-white flowers [3]. The peel of the fruit is hard woody shell vary green to brown in colour. Colour and taste of fruit depends on the ripening stage. The edible pulp is orange to yellow which have pleasant fragrance, flavoured, slightly sweet taste and terpene-like aroma. Seeds are present in pulp and surrounded by slimy transparent mucilage [4]. *Aegle marmelos* fruit pulp contains important bioactive compounds such as alkaloids, antioxidants, carotenoids, terpenoids, phenolics, flavonoids, tannins, coumarins and pectins which helps in protecting against various chronic diseases [5, 6, 7]. Marmelin, alloimperatorin, o-methyl halfordinol, furocoumarins, aegeline, marmelosin, psoralen, Aegeline and o-isopentenyl halfordinol are bioactive compounds present in fruit pulp. It also contains linoleic acid, flavon-3-ols, leucoanthocyanins, tartaric acid, anthocyanins, phlobatannins, flavonoid and glycosides [8]. The ripe fruit is used as a mild stimulant to the intestinal mucous membrane. It has cooling and laxative property. It also stops diarrhea [9]. Quantitative analysis estimated that the fruit is rich in carbohydrates, water, fiber, minerals, vitamins and proteins [10].

Rubia cordifolia Linn. (Rubiaceae), commonly known as Manjistha in Ayurveda. It is a climbing plant grow in the North-West Himalayas, Nilgiris and other hilly districts of India [11]. It is also known as Indian Madder and widely used herb in Ayurvedic system of medicine. It has a various uses such as blood purifier, anti-platelet activation, anti-inflammatory and immunomodulator [12]. It contains triterpenes which has anticonvulsant activity [13]. A chemical compound Rubiadin which is a dihydroxy anthraquinone, possesses potent antioxidant property [14]. Roots of *Rubia cordifolia* are used to cure jaundice, paralytic affections, urinary troubles, amenorrhea and to shrink and clean mother's uterus after childbirth [15]. The major compounds present in this plant are alizarin, anthraquinones and purpurin and their derivatives like lucidin, rubiadin, quinizarin, ruberythric acid (alizerin-primeveroside), pseudopurpurin, primeveroside, munjistin and 1,8-dihydroxyanthraquinone [16]. Plant possess various pharmacological actions like blood purifying activity, anticancer, antidiarrhetic, antiseptic, antirheumatic, deobstruent properties, astringent and hepatoprotective activity [17].

Beta vulgaris also known as Beet root. It contains valuable water-soluble nitrogenous pigments called betalains, which comprise two main groups, the yellow betaxanthins and the red betacyanins. They scavenge free radicals and thus prevent active oxygen-induced and free radical-mediated oxidation of biological molecules. Betalains are found as natural dyes in various food products like baked goods, yogurt, candies, processed meat and ice cream. Various *in vitro* studies have proved that betalains found in beet root have potent antioxidant activity [19, 20]. Apart from betalains, small amounts of hydroxycinnamic acids such as gallic, syringic, caffeic acids and flavonoids have been identified [21]. Betanins found in beet root are water-soluble pigments which have antioxidant, anti-inflammatory, hepatoprotective, anti-cancer properties. The main sugar present in beetroot is sucrose with only small amounts of fructose and glucose [22]. In food industry betalains are used as natural colouring agent, but they have also received increasing attention due to their anti-inflammatory and antioxidant activities [23]. It also inhibits lipid peroxidation [24]

and increased resistance to the oxidation of low-density lipoproteins [25]. It also has chemo-preventive effects [26].

Phyllanthus emblica also known as Indian gooseberry, is a medicinal plant used abundantly in traditional system of medicine like Ayurveda, Unani, siddha etc. It belongs to the family Euphorbiaceae [27]. *Phyllanthus emblica* is rich in nutrients and is one of the richest sources of vitamin-C, amino acids and minerals [28]. It contains several phytochemicals like phenols, tannins and alkaloids [29]. Almost all parts of this plant possess medicinal properties but fruit has very high importance in Ayurveda and in customary medicine for the treatment of jaundice, diarrhea, inflammation and several other ailments [30]. Pharmacological studies on *Phyllanthus emblica* reveals its anti-tussive [31], analgesic [32], cardio [33], nephron [34], anticancer [35], gastro [36], neuro [37] protective, anti-atherogenic [38], and adaptogenic [39], free radical scavenging [40], chemopreventive [41], immunomodulatory [42], anti-inflammatory [43], radio [44], chemo [45], antioxidant [46] and anti-mutagenic activities. It is also useful in curing conditions of tridosha, asthma, cough, diabetes, bronchitis, cephalalgia, dyspepsia, ophthalmopathy, colic and peptic ulcer, flatulence, hyperacidity, skin diseases, jaundice, haematogenesis, inflammations, intermittent fevers, hepatopathy, anemia, leprosy, diarrhea, dysentery, leucorrhoea, menorrhagia, haemorrhages, cardiac disorders, emaciation and premature greying of hair.

2. Materials and methods

2.1. Raw material

Raw material like *Aegle marmelos*, *Beta vulgaris*, *Phyllanthus emblica* & Black salt were purchased from the local market of Dwarka, New Delhi. *Rubia cordifolia* Extracts were purchased from Plantae Extracts Pvt. Ltd, Sector-5, Vaishali, Ghaziabad, U.P.

2.2. Chemicals

Aluminium chloride, Acetic Acid, catechin, Ferric chloride, Folin Ciocalteu's phenol reagent, 2, 2 - diphenyl -1 picrylhydrazyl (DPPH), sodium nitrite, Gallic acid, Ascorbic acid, Sodium carbonate, Sodium acetate, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Hydrochloric acid, n-butanol, Potassium Ferrocyanide, Sodium Hydro-oxide, Methanol, Tannic Acid, Conc. liquor ammonia, Ethanol, Petroleum ether, Diethyl Ether, BHT.

2.3. Physico-chemical analysis

Physico-chemical analysis was done to determine pH, acidity and total soluble solid (TSS) of the product.

2.4. Proximate analysis of energy drink

Energy drink prepared was analyzed for moisture, ash, total dietary fiber, protein, fat, total reducing sugar, carbohydrates and energy value. Moisture and ash content were determined according to AACC, 2000 method [47]. Total dietary fiber & protein was determined by AOAC method [48]. Fat estimation was done by petroleum ether. Mineral content was determined by ICP-OES. Total carbohydrates and energy content was calculated using formulae:

Total carbohydrates (% fresh weight) = 100 - moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight) and reported as total carbohydrates in % [49].

The calorific value in kilocalories (kcal) = $(3.36 \times \% \text{ protein, fresh weight}) + (3.60 \times \% \text{ total carbohydrate fresh weight}) + (8.37 \times \% \text{ fat})$ [49].

2.5. Determination of Reducing Sugars

The reducing sugar was estimated by standard protocol IFS/C/SOP/FC/010.

2.6. Determination of Fat Content

The fat content was measured by the standard protocol IFS/C/SOP/FC/012.

2.7. Minerals, Trace Elements & Heavy Metals - by ICP-OES

The analysis of the mineral content, trace elements & heavy metals was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Ref 956.52 AOAC, 2005) [50]. 1 g of dried sample was digested by 5 ml of concentrated HNO₃ on hot plate as per Ref. 956.52 (AOAC, 2005). After digestion, the sample was cooled and volume made up to 25 ml with distilled water. Set plasma conditions for analysis here : Argon on 151/ min, auxiliary 0.21/min, nebulizer flow at 0.851/min, RF power of 1300 W and chilled at a 15 °C set of standards ("ICP multi-element standard IV" (Merck, Darmstadt, Germany) were run and then samples were analyzed against the standards.

2.8. Estimation of Phenolic Content

Phenolic content was estimated by Folin-Ciocalteu reagent according to method of Slinkard and Singleton [51]. Sample extracts were oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The results were expressed as Gallic acid equivalents ($\mu\text{g GAE/mg}$ of extract).

2.9. Estimation of Tannin Content

Tannins were determined by Folin-Denis method [52] using tannic acid as a standard. 500ml Distilled water added to 5gm of the sample. After incubation of 1hr at 30 °C & 140 rpm 5ml filtrate is taken and 0.1M Iron (III) Chloride, 0.1N Hydrochloric Acid & 0.008M Potassium Ferrocyanide added. OD was taken at 605nm after incubation for 10 min. Results were expressed as Tannic acid equivalent ($\mu\text{g TAE/mg}$ of extract).

2.10. Estimation of Flavonoid Content

Estimation of flavonoid was done by using aluminium chloride colorimetric method [53]. 4.5ml Distilled water & 0.3ml of 5% Sodium Nitrite added to 250 μl sample. Incubated for 5min. 0.3ml of 10% Aluminium chloride added. Again incubated for 6 min. After incubation, 2ml of 1M Sodium Hydroxide added. Final volume adjusted to 10ml. Vortex and OD was taken at 510nm. Results were expressed in terms of catechin equivalents ($\mu\text{g CE/mg}$ sample).

2.11. Estimation of Alkaloids

Concentration of the alkaloids in the sample was estimated by the method described by Harborne [54]. 2.5g of sample was taken in conical flask and 100ml 10% acetic acid in ethanol was added to the sample & incubated for 4hrs at room temp. This sample was filtered & concentrated to 1/4th of its original volume on water bath at 50-55 °C. Then concentrated liquid Ammonia was added dropwise. Filter paper was pre- weighed & using this again filter the sample. Dry the filter paper & weigh it. Alkaloid content was calculated in % as follows-

$$\% \text{Alkaloids} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

2.12. Assessment of Antioxidant Activity

The Antioxidant potential of the sample was measured by assessing their ability to reduce compound by donating an electron using the FRAP assay or their radical scavenging potential using DPPH.

2.12.1. DPPH Method (2, 2-Diphenyl-1-Picryl-hydrazyl)

DPPH radical scavenging was done as given by the method of Blois [55] with slight modification. 0.3 mM DPPH in Methanol was prepared. A 10ml extract solution of 100 ppm concentration was prepared by adding 1mg of extract in 10ml methanol. 1ml of this solution was taken in a separate test tube. To this, add 1ml methanol & 1ml of 0.3mM DPPH. Kept in dark for 10 min. OD was taken at 517nm. Methanol was used as reference. Blank was prepared without the sample. The radical scavenging activity was calculated in % as follows-

$$\% \text{inhibition} = \frac{B-A}{B} \times 100, \text{ where}$$

B: Absorbance of blank at 517nm

A: Absorbance of sample at 517nm

2.12.2. Ferric reducing antioxidant power (FRAP) Assay

The assay was based on the methodology of Benzie & Strain [56]. FRAP reagents consist of 20Mm FeCl₃, 10Mm TPTZ in 40Mm HCl, & 250 mM Sodium acetate buffer (pH3.6). FRAP reagents was freshly prepared by mixing FeCl₃ solution, TPTZ solution & Acetate buffer in ratio1:1:10. A 100 μl of extract solution contain 0.1 mg extract was mixed with 900 μl FRAP reagents and then keep the mixture at 37 °C for 4 min & absorbance is taken at 592 nm against blank solution. Results were expressed as BHT equivalents.

2.13. Determination of Antibacterial Activity

Antibacterial susceptibility was determined by using the well diffusion method given by Kirby Bauer [57]. Nutrient agar plates were prepared for analysis & incubated at 37 °C for 24 hr. The nutrient broth was inoculated with four gram-positive bacteria *Staphylococcus epidermis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* & two gram-negative bacteria namely *Escherichia coli* & *Salmonella enterica*. Then wells were made by sterile cork borer & subsequently filled with 80 μl of the sample having concentrations 200 μl and 400 μl . The plates were then incubated at 37 °C & was observed for the diameter of the zone of inhibition after 24hr & 48hrs resp. The diameter of zone of inhibition was measured in millimeters.

2.14. Product Preparation

Bael fruit was broken and fruit pulp was taken out. 50% of pulp was soaked in water for 2-3 hrs and then sieved 2 to 3 times to remove seeds and other fibrous part. To the Bael juice 5% *Phyllanthus emblica* juice, 5% *Beta vulgaris* juice and 3% *Rubia cordifolia* extract was added and homogenized for 5 minutes so that all constituent get mixed with each other in sterile conditions. Then the juice was pasteurized at 82°C for 30 minutes and then it was cooled. Mixture so obtained was converted into solid form by exposing to very low temperature and finally it was lyophilized. After lyophilization, powder

form of juice was obtained to which 4% Black salt and 20% Glucose was added under sterile condition.

2.15. Sample Preparation

100 ml of juice was taken in 200 ml of methanol in a conical flask and plugged with cotton wool and then shaken at 60 °C overnight at 150 rpm and then the supernatant was filtered using Whatman filter paper. The residue was then mixed with another 200ml of methanol and filtration process was repeated. Both the supernatants were combined and then the solvent was evaporated at 60 °C and stored at 4 °C in airtight bottles.

2.16. Sensory Evaluation

Product so formed were presented to the panelist for sensory evaluation. The sensory panel consisted of 20 persons comprising 8 males and 12 females. The sensory attributes taken for consideration were colour, flavour, texture, taste, solubility and overall acceptability. The product was sensory evaluated based on 9 point hedonic scale. The nine point hedonic scale was as 9-like extremely, 8-like very much, 7-like moderate, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely.

2.17. Determination of secondary metabolite composition

The Gas chromatography-Mass spectrometry (GC-MS) analysis was carried out for the methanolic extract of the product. 1 µl of injection was performed by split injection (1:20) at 280 °C. The oven was programmed from 65 °C (5min) at 15 °C/min to 180 °C (10 min), at 5 °C/min to 280 °C (15 min). Helium was used as carrier gas. An Agilent 5975B mass spectrometric detector (MSD) was used in scan mode (m/z 35-1050) for all samples. Screening of volatiles and semi volatiles were performed using automatic RTL screener software in combination with the Agilent NIST'05 library. Gas saver option was turned off, transfer line temperature was set to 300 °C, solvent delay was 3 min, ion source and quadrupole temperature were 230 °C and 150 °C resp.

3. Results and Discussion

3.1. Physico-chemical Analysis

pH value tells us about the acidic or basic property of the product. pH of the product was found to be 4.9. Acidity of the product was found to be 0.104%. TSS of the bael juice was found to be 26.2° Brix.

3.2. Nutritional Composition

Table 1: Nutritional composition of the product

Composition	Content
Moisture (%)	11.33
Ash (%)	3
Protein (%)	4.59
Dietary fibre (%)	14.57
Fat (%)	nil
Total reducing sugar	8.77
Carbohydrates (%)	69.34
Energy value (kcal/100g)	266.46

3.3. Mineral Analysis

Table 2: Mineral content of the product

Analyte	Concentration (Mg/100g)
Calcium	75.31
Phosphorus	81.25
Magnesium	56.25
Iron	7.03
Chromium	0.94
Copper	0.47

Mineral analysis showed the presence of calcium, phosphorus, magnesium and iron in high amount while chromium and copper in less amount. The important role of calcium in prevention and treatment of osteoporosis [58], colorectal cancer [59], kidney stones [60, 61]. Magnesium is important for prevention or treatment of hypertension and heart diseases [62], diabetes [63], osteoporosis [64, 65], migraine headaches [66] and asthma [67]. Another important mineral was phosphorous, which include many health benefits such as bone formation, proper digestion, regulated excretion, formation of protein, improved energy extraction, hormonal balance, cellular repair and also helps in constipation, diarrhea, healthy bowel movements. Phosphorus aids in the transmission of nerve impulses & helps in the treatment of cancer. Iron is a very important element for the formation of haemoglobin, brain function, body metabolism, anemia, immunity, insomnia, muscle activity and the regulation of body temperature.

3.4. Phytochemical Analysis

Phytochemicals are non-nutritive bioactive compounds found in plants. They are categorized as plant secondary metabolite but recent research revealed that they can also protect humans against various diseases. Phytochemicals are also responsible for colour and organoleptic properties of fruits, flowers and vegetables. Phytochemicals possess various pharmacological properties such as antioxidant properties, stimulate enzymes, protect DNA from carcinogens, regulate hormonal action, decrease platelet aggregation and modulate hormone metabolism and antibacterial properties. The Best way to get phytochemicals in the body is to eat more and more fruit and vegetables. Phenolics are a diverse group of phytochemicals. The three most important groups of dietary phenolics are phenolic acids, flavonoids, and polyphenols. Tannins are heterogeneous high molecular weight polyphenolic compounds have capacity to form reversible and irreversible complexes with protein polysaccharides, alkaloids, nucleic acids and minerals etc [69, 70, 71]. Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial activity, anticancer actions. [72] Some alkaloids such as caffeine and nicotine have stimulant property whereas quinine has the antimalarial property and morphine has analgesic property [73]. Flavanoids have antioxidant property which protect biological macromolecules such as carbohydrates, proteins, lipids and DNA against harmful effect of oxidative processes [68].

Table 3: Phytochemical Analysis

Analyte	Total Phenolic content (µg GAE / mg sample)	Total Flavonoid content (µg CE / mg sample)	Tannin content (µg TAE / mg sample)	Alkaloid (%)
sample	42.25	101.33	0.574	6.18

3.5. Determination of Antioxidant Activity

Antioxidants Are the bioactive compounds which prevent oxidation catalysed free radical [74]. Antioxidant activity in the sample might be due to the presence of phenolic compounds such as Phenolic acids, flavonoids [75] and phenolic diterpene [76]. Antioxidants prevent reactive oxygen species (ROS) construction by the disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack [77].

3.5.1. DPPH method is very sensitive and simple. It measures radical scavengers compounds. The antioxidant effect is proportional to the disappearance of DPPH in test samples. The colour of DPPH turns from purple to yellow and finally colourless. Change in colour of DPPH is a sign of presence of antioxidant in the sample.

Table 4: % inhibition results by DPPH assay

sample	% inhibition
Extract	63.88

3.5.2. Ferric ion reducing antioxidant power (FRAP)

It measures antioxidant compounds present in the sample by reducing ferric ion into ferrous complex.

Table 5: BHT equivalents ($\mu\text{g BE/mg sample}$) for Extract.

Sample	BE equivalents ($\mu\text{g BE/ mg sample}$)
Extract	268.58

3.6. Antimicrobial Test

Antimicrobial test was done against six pathogenic bacteria i.e. *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteric*, *Staphylococcus aureus*, *Staphylococcus epidermis* and *Bacillus cereus*. A zone of inhibition was observed in 200 mg/ml extract concentration against *Staphylococcus epidermis*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella enterica* indicating that antibacterial activity is present. No

zone of inhibition was observed for *Bacillus subtilis* and *Escherichia coli*.

Table 6: Zone of inhibition of various test pathogens in Antimicrobial Test.

Bacteria	Type	Zone of inhibition (mm)
<i>Bacillus subtilis</i>	Gram positive	No zone of inhibition
<i>Salmonella enterica</i>	Gram negative	2.1mm
<i>Staphylococcus aureus</i>	Gram positive	1.5mm
<i>Staphylococcus epidermis</i>	Gram positive	1.9mm
<i>Bacillus cereus</i>	Gram positive	1.8mm
<i>Escherichia coli</i>	Gram negative	No zone of inhibition

3.7. Sensory Analysis

Product qualified sensory test in term of taste, colour, flavour, texture and overall acceptability. Out of 20 people 12-like very much, 4 like moderate, 2 like slightly and 2-like.

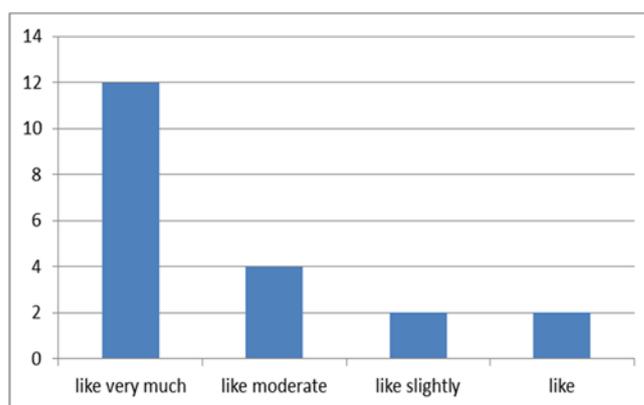


Fig 1: Sensory Analysis

3.8. Characterization of GC-MS analysis

Various secondary metabolites were found in the methanolic extract of the sample which are listed in the table.

Table 7: GC-MS profiling of methanolic extract of the sample.

Compound Name	CAS#	RT	% AREA
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	028564-83-2	9.895	4.18
n-Hexadecanoic acid	000057-10-3	20.246	3.16
9,12-Octadecadienoic acid (Z,Z)	000060-33-3	21.929	3.49
2(1H)-Naphthalenone, octahydro-4a- methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-	054594-42-2	22.702	0.51
Pyridine-3-carboxamide, oxime, 2-trifluoromethylphenyl)	288246-53-7	27.581	1.07

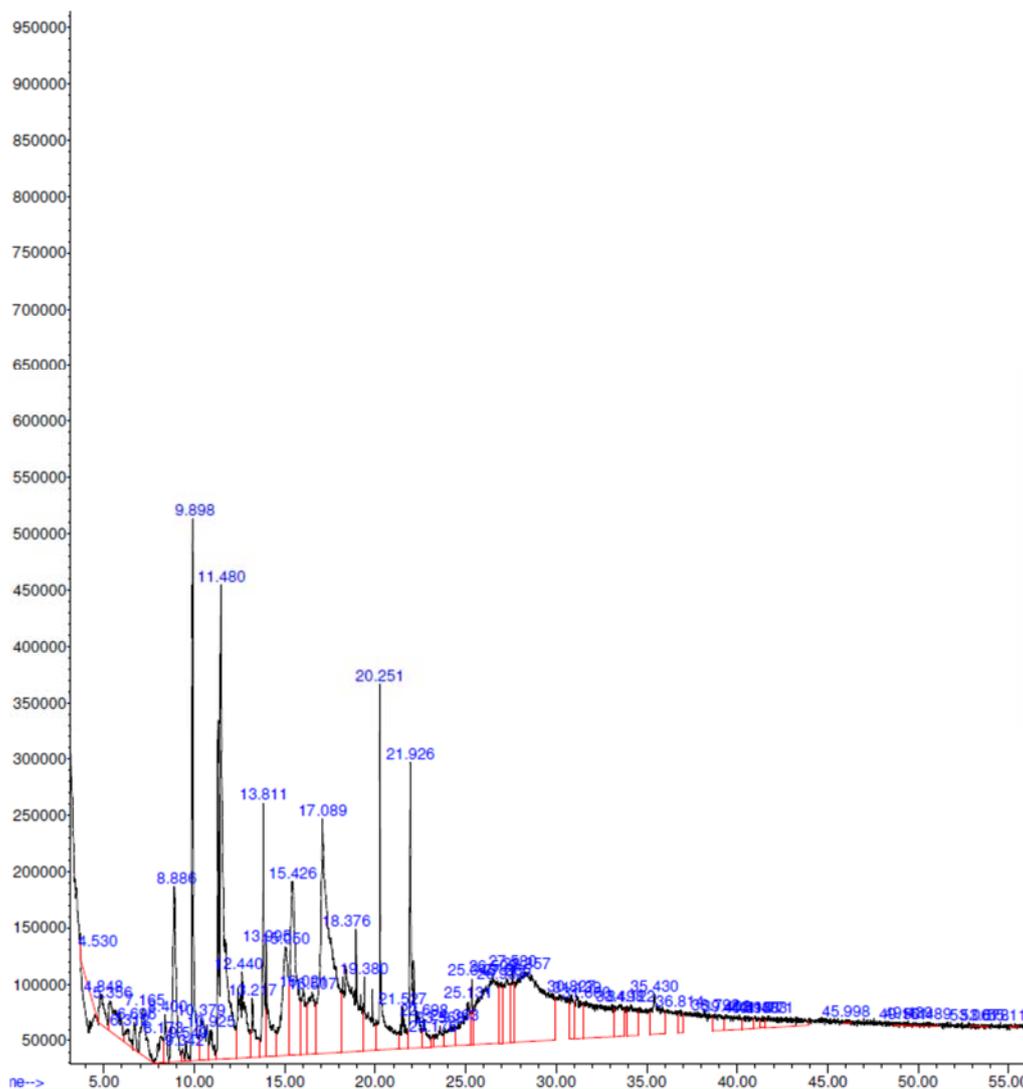


Fig 2: Chromatogram for GC-MS analysis of methanolic extract of the sample.

4. Conclusion

All the ingredient of the energy drink found to have good nutritional As well as pharmacological property. Development and utilization of such functional and nutritional product can be used to improve the nutrition status of the people and their health. Phytochemical analysis revealed that energy drink formed is highly rich in antioxidants. Nutritive analysis revealed presence of good amount of calcium, phosphorus, magnesium and iron which are required for the basic metabolic processes of the body and also for the growth. Fat content in the drink was found to be nil. So this drink can be used as a good source of energy for obese people and can be used to cure obesity. It can also be concluded from the study that it is a complete energy drink that give instant energy as well as impart nutritional and pharmacological benefit to its consumer. The energy drink has a very attractive colour and pleasant flavour. Sensory evaluation results showed overall acceptability of energy drink. So the Product developed can be categorised as functional food which provide nutrition as well as impart health benefits by preventing degenerative diseases like cancer, cardiovascular diseases & can also reduce chances of chronic diseases to its consumers.

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