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FORMULATION AND EVALUATION OF MULTIVITAMIN & ANTIOXIDANT HERBAL CHOCOLATE

Ms. Geetanjali N. Badak Miss. Anuja V. Nathbone Mr.Anurag Y. Gaikwad Rajgad Dnyanpeeth's College of pharmacy, Bhor

ABSTRACT

was The aim of this study develop chocolate, which is most loved food of children, is the disliked substance. Due to its health benefits, dark chocolate has been a popular while medicine food for several decades. It is also considered a functional food due to its anti-aging qualities. It is well-known for its role in weight management and altering a lipid profile to a healthy direction. Betalains one of the chemical component of beetroot have been proven to eliminate oxidative and nitrative stress by scavenging DPPH, preventing DNA damage, and reducing LDL. Moringa contains a range of antioxidant and anti-inflammatory agents, such as quercetin, which may help protect cardiovascular health. Thyme powder helps to alleviate cough and boost immunity and is a rich source of vitamins like Vit A, Vit C and Magnesium. Cinnamon also contains traces of vitamins B and K and the antioxidants choline, and beta-carotene. The noteworthy levels of nourishing components in elaichi make it a superior nutritional food. containing remarkable quantities of vitamin B6, vitamin B3, vitamin C, zinc, calcium, magnesium and potassium. Tulsi has proved to be highly rich in vitamin C and zinc. Tulsi is rich in antioxidants and has anti-inflammatory properties, It is also believed to boost the immune system, improve digestion, and reduce stress levels. Multivitamins are utilized to provide vitamins that are not absorbed through the diet and to address vitamin deficiencies resulting from illness. Further, prepared medicated chocolate is evaluated for general appearance, dimension, hardness, blooming test, drug content determination, physical stability etc.

(Key Words: Beetroot, Brahmi, Tulsi, Multivitamins, Antioxidants.)

INTRODUCTION

✓ The Food and Nutrition Board (FNB) at the National Academies of Sciences, Engineering, and Medicine establishes RDAs and AIs. RDAs are the average daily level of intake of essential nutrients sufficient to meet the requirements of nearly all (97–98%) healthy individuals. These values vary by age, sex, and nutrient. The FNB establishes AIs for nutrients when evidence is insufficient to develop an RDA; intakes at this level are assumed to ensure nutritional adequacy. The U.S. Food and Drug Administration (FDA) develops DVs to help consumers compare the nutrient contents of foods and dietary supplements within the context of a total diet.

 \checkmark Consumer demand for healthier foods with improved taste and convenience has urged the food industry to develop functional foods added with bioactive ingredients that can supplement basic nutrition (food supplement) or exert a pharmacological effect (nutraceuticals). Chocolate could be used as an ideal carrier to deliver bioactive ingredients, mainly due to its high acceptability by consumers. The physicochemical properties and sensory

acceptability of the functional chocolates presented are also highlighted. Finally, future perspectives, such as the use of nanotechnology to improve bioaccessibility and bioavailability of active ingredients, as well as the need for clinical trials to validate the pharmacological effect of functional chocolates.

 \checkmark Using the term "antioxidant" to refer to substances is misleading. It is really a chemical property, namely, the ability to act as an electron donor. Some substances that act as antioxidants in one situation may be prooxidants—electron grabbers—in a different situation. Another big misconception is that antioxidants are interchangeable. They aren't. Each one has unique chemical behaviors and biological properties. They almost certainly evolved as parts of elaborate networks, with each different substance (or family of substances) playing slightly different roles. This means that no single substance can do the work of the whole crowd . Randomized placebo-controlled trials, which can provide the strongest evidence, offer little support that taking vitamin C, vitamin E, beta-carotene, or other single antioxidants provides substantial protection against heart disease, cancer, or other chronic conditions. The results of the largest trials have been mostly negative.

Antioxidants:-

 \checkmark A substance that protects cells from the damage caused by free radicals (unstablemolecules made by the process of oxidation during normal metabolism). Free radicalsmay play a part in cancer, heart disease, stroke, and other diseases of aging. Antioxidants include beta-carotene, lycopene, vitamins A, C, and E, and other natural and manufactured substances.

✓ Why antioxidants are necessary ?

 \checkmark Oxygen is absolutely essential for the life of aerobic organism but it may become toxic if supplied at higher concentrations. Dioxygen in its ground state is relatively unreactive; its partial Reduction gives rise to active oxygen species (AOS) such assinglet oxygen, super oxide radical Anion, hydrogen peroxide etc. '

 \checkmark This is partly due to the oxidative stress that is basically the adverse effect of oxidant on physiological function. The generation of reactive oxygen species (ROS) and otherfree radicals (R) during metabolism is a necessary and normal process that ideally is compensated for by an elaborate endogenous antioxidant system.

 \checkmark However, due to many environmental, lifestyle, and pathological situations, excess radicals can accumulate, resulting in oxidative stress. Free oxygen radicals plays cardinal role in the etiology of several diseases like arthritis, cancer, atherosclerosis etc. The oxidative damage to DNA may play vital role in aging and the presence of intracellular oxygen also can be Responsible to initiate a chain of inadvertent reaction at the cellular level and these reaction cause Damage to critical cell biomolecules. These radicals are highly toxic and thus generate oxidative Stress in plants. Plants and other organism have in built wide range of mechanism to combat with These Free Radical problems.

 \checkmark Free radicals are an atom or molecule that bears an unpaired Electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals. In plants and animals these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of Oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of plat materials act as radical scavengers, and convert the radicals to less reactive species.

 \checkmark Spices and herbs in food as medicine is a current hot trend that is capturing everyone'sImagination with images of a new magic bullet or fountain of youth. The intake of antioxidant Compounds present in food is an important health-protecting factor. Natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects. Because extensive and Expensive testing of food additives is required to meet safety standards, synthetic antioxidants have generally been eliminated from many food applications. The increasing interest in the Search for natural replacements for synthetic antioxidants has led to the antioxidant evaluation of a number of plant sources.



Antioxidant cycle

 \checkmark Natural antioxidants occur in all parts of plants. These antioxidants include carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites. Plant derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists. The most current research on antioxidant action focuses on phenolic compounds such as flavonoids. Fruits and Vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and Carotenoids, whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents Present in food of plant origin are also potential antioxidants.

These components include: -

• Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherol and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and Lipoic acid.

• Antioxidant enzymes, e.g., super oxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.

• Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.

• Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

Vitamins as a antioxidants:-

Vitamins are groups of complex organic compounds found in foodstuffs and essential for a healthy metabolism. Their deficiency can cause disorders, whereas resupply of these nutrients can alleviate deficiency symptoms. Vitamins are different from other food nutrients due to their distinct organic nature, and their classification depends on their chemical nature and function. Growth, development, health, and reproduction require minute amounts of vitamins. Some vitamins synthesised from other sources in the body deviate from the usual definition of Vitamins. For instance, Animals integrateascorbic acid, tryptophan an essential amino acid produce niacin, while UV radiation from sunlight synthesises vitamin D. Generally, classification of Vitamins is into two groups:

- 1) Water-soluble vitamins
- 2) Fat-soluble vitamins

Example of water-soluble vitamins is:- Vitamins B complex and C and that of fat-soluble vitamins include: A, D, E, And K. Fat-soluble vitamins are associated with fats and are absorbed with dietary fats. The absorption of fat-soluble Vitamins is like the absorption of fats. Water-soluble vitamins are not associated with fats and are unaffected by Alterations in fat absorption .

Vitamins functions and deficiencies:-

| Vitamin | VitaminType | Functions | Deficiency |
|---------|---------------|--|---|
| Vit A | Fat Soluble | Vision, Reproductionand Immunity | Night-blindness |
| Vit B | Water Soluble | Growth, development and cellular agility | Beri-beri |
| Vit C | Water Soluble | For bone health andstability | Scurvy |
| Vit D | Fat Soluble | maintaining strengthand integrity of your bones | Rickets in children and osteomalacia in Adults |
| Vit E | Fat soluble | Antioxidant, | Cell Membrane damage especially red blood cells |
| Vit K | Fat Soluble | Formation of clottingfactors in the blood | Haemorrhage |

Material and Profile

| Sr.no | Ingredient | Role Of Ingredient |
|-------|-----------------|--|
| | Moringa Powder | Antioxidant |
| 2 | Beetroot Powder | Natural health-boosting supplement |
| 3 | Thyme Powder | Antioxidant |
| 4 | Tulsi Powder | Improves Immunity |
| 5 | Cinnamon Powder | Powerhouse of Antioxidants |
| 6 | Cardamon Powder | Improves blood circulation |
| 7 | Chocolate | Contains anti-oxidants that can protectcells |
| 8 | Jaggery | Detox the liver and blood |

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<u>Moringa</u>



Fig : Moringa Powder<u>Botanical name-</u> Moringa oleifera Lam

Common name-

Drumstick tree, Horseradish tree, Ben tree, Subhanjana, Saguna, Sainjana ,Shevga

Chemical constituents-

• Moringa is rich in nutrition owing to the presence of a variety of essential phytochemicals present in its leaves, pods and seeds. In fact, moringa is said to provide7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach.

• Moringa is rich in phytosterols like stigmasterol, sitosterol and kampesterol which are precursors for hormones. These compounds increase the estrogen production, which in turn stimulates the proliferation of the mammary gland ducts to produce milk. It is used to treat malnutrition in children younger than 3 years .

• The leaves of M. Oleifera are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper

• Vitamins like beta-carotene of vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E also present in M. Oleifera.

• Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar present along with anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9- octadecanoate.

• Moringa leaves also have a low calorific value and can be used in the diet of the obese. The pods are fibrous and are valuable to treat digestive problems and thwart colon cancer.

• Immature pods contain around 46.78% fiber and around 20.66% protein content. Pods have 30% of amino acid content, the leaves have 44% and flowers have 31%. The immature pods and flowers showed similar amounts of palmitic, linolenic, linoleic and oleic acids.

Beetroot Powder



Fig : Beetroot Powder

Synonyms:-

Chukandar, Sugar beets, Mangel, Spinach beet Biological source: It consists offresh root of Beta vulgaris.

Chemical Constituents:

1. **Betalains:** Beetroot owes its vibrant red color to a group of antioxidants called betalains. These compounds, including betanin and vulgaxanthin, have been shown to have potent antioxidant and anti-inflammatory effects.

2. **Vitamin C:** Beetroot is a good source of vitamin C, which is a powerful antioxidant that helps neutralize free radicals and protect cells from damage. Vitamin C also playsa crucial role in collagen synthesis and supports immune function.

3. **Manganese:** Beetroot contains manganese, a mineral that acts as a cofactor for several antioxidant enzymes, such as superoxide dismutase, which helps protect cells from oxidative damage.

4. **Vitamin A:** While not as abundant in beetroot as in some other vegetables, it does contain a small amount of vitamin A, which has antioxidant properties and helps protect cells from damage caused by free radicals.

5. **Flavonoids:** Beetroot contains various flavonoids, including quercetin, kaempferol, and rutin, which are potent antioxidants. Flavonoids have been associated with numerous health benefits, including reduced inflammation and protection against chronic diseases.

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Thyme



Fig : Thyme Powder

Svnonvms:-

Indian Pennywort, Mangosteen

Chemical Constituents:-

The drug contains triterpenoid saponin glycosides, indocen-telloside, brahmoside, brahminoside, asiaticosides, thankuni-side and isothankuniside. The corresponding trirerpene acids obtained on hydrolysis of the glycosides are indocentoic, brahmic, asiatic, thankunic and isothankunic acids. These acids, except the last two, are also present in free form in the plant from isobrahmic and betulic acids.

| Component | Am <mark>ount(/100gm)</mark> |
|----------------|------------------------------|
| Protein | 2.1 gm |
| Fat | 0.6 gm |
| Cabohydrates | 5.9 gm |
| Crude fiber | 1.05 gm |
| Calcium | 202.0 gm |
| Phosphorus | 16.0 gm |
| Ascorbic acid | 63.0 |
| Nicotinic acid | 0.3 |

<u>Tulsi Powder</u>



Fig: Tulsi Powder

Synonyms:-

Sacred basil, Holy basil.

Chemical Constituents:

Vitamin C: Tulsi leaves are a good source of vitamin C, which is known for its immune-boosting properties. Vitamin C supports the immune system, aids in collagen production, and acts as an antioxidant to protect cells from damage.

Vitamin A: Tulsi leaves also contain vitamin A, which is essential for maintaining healthy vision, supporting the immune system, and promoting skin health. Vitamin A is known for its role in promoting good eyesight and overall eye health.

Vitamin K: Tulsi contains vitamin K, which is necessary for proper blood clotting and bone health. Vitamin K plays a crucial role in maintaining healthy bones and ensuring proper blood coagulation.

B-vitamins: Tulsi contains various B-vitamins, including thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), and folate (B9). B-vitamins are essential for energy production, nerve function, metabolism, and red blood cell production.

Vitamin E: Tulsi leaves contain vitamin E, which is a powerful antioxidant that helps protect cells from damage caused by free radicals. Vitamin E also plays a role in maintaining healthy skin and supporting the immune system

Cinnamon Powder



Fig: Cinnamon Powder

<u>Synonym:-</u>

Camphorina cinnamomum (L.) Farw. Cinnamomum alexei Kosterm. Cinnamomum aromaticum J.Graham, Dalchini

Chemical constituents:

• Cinnamon consists of a variety of resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and numerous essential oils .

• the spicy taste and fragrance are due to the presence of cinnamaldehyde and occur due to the absorption of oxygen.

• As cinnamon ages, it darkens in color, improving the resinous compounds.

• The presence of a wide range of essential oils, such as trans-cinnamaldehyde, cinnamyl acetate, eugenol,

L-borneol, caryophyllene oxide, b-caryophyllene, L- bornyl acetate, E-nerolidol, α -cubebene, α -terpineol, terpinolene, and α -thujene, has been observed.

Cardamom



Fig : Cardamom Powder <u>Svnonvms:-</u>

Cardamom fruit, Cardamon seed, Ilachi,

Chemical Compositions of Cardamon:-

Dried fruit of cardamom contains steamvolatile oil, fixed (fatty) oil, pigments, proteins, cellulose, pentosans, sugars, starch, silica, calcium oxalate and minerals.

The major constituent of the seed is starch (up to 50 per cent) while in the fruit husk it is crude fibre (up to 31 per cent).

Volatile oil is the most functionally important constituent of cardamom. The volatileoil content of seeds varies from 6.5 to 10.5% for the two types of cardamom (Malabar and Mysore) grown in India.

In immature capsules, obtained in small quantities in all harvests (and more particularly in the last harvest), the volatile oil content is low, on the order of 4 to 5% [13].

Cardamom contains 2.8–6.2% volatile oil, 10% protein, 1–10% fixed oil and up to 50% starch.

The aroma and flavor of cardamom are obtained from the essential oils which is composed of mainly α -terpinyl acetate (20–55%) and 1,8-cineole (20–60%) which are responsible for specific flavor to the cardamom.

Jaggery Powder



Fig: Jaggery Powder

• Jaggery is a common product in Asia and Africa. It is made from the juices of palm tree or sugarcane and is growing in popularity as a replacement for white sugar. It is a staple in India, where people call it gur.

• Jaggery contains some vitamins and minerals, making it comparatively healthier than white sugar. However, it is still a type of sugar, and consuming too much of it can have a negative impact on a person's health.

Compositions of Jaggery:-

| sucrose: 65–85 g | fructose and glucose: 10–15 g |
|--|--|
| protein: 280 milligrams (mg), or 5.6% | potassiu <mark>m: 1056mg, or</mark> 22.5% DV |
| daily value (DV) | |
| magnesium: 70–90 mg, orapproximately 19% | calcium: 40–100 mg, orapproximately 5% DV |
| DV | |
| manganese: 0.2–0.5 mg, orapproximately 15% | phosphorus : 20-90 mg, or approximately 5% |
| DV | DV |
| | |

Dark Chocolate



Fig : Dark Chocolate

 \checkmark Chocolate is a food made from roasted and ground cacao seed kernels that is available as a liquid, solid, or paste, either on its own or as a flavoring agent in other foods. Cacao has been consumed in some form since at least the Olmec civilization (19th-11th century BCE), and the majority of Mesoamerican people including the Maya and Aztecs made chocolate beverages.

✓ The seeds of the cacao tree have an intense bitter taste and must be fermented to develop the flavor. After fermentation, the seeds are dried, cleaned, and roasted. The shell is removed to produce cocoa nibs, which are then ground to cocoa mass, unadulterated chocolate in rough form. Once the cocoa mass is liquefied by heating, it is called chocolate liquor. The liquor may also be cooled and processed into its two components: cocoa solids and cocoa butter. Baking chocolate, also called bitter chocolate, contains cocoa solids and cocoa butter in varying proportions, without any added sugar.

 \checkmark Powdered baking cocoa, which contains more fiber than cocoa butter, can be processed with alkali to produce dutch cocoa. Much of the chocolate consumed today is in the form of sweet chocolate, a combination of cocoa solids, cocoa butter or added vegetable oils, and sugar. Milk chocolate is sweet chocolate that additionally contains milk powder or condensed milk. White chocolate contains cocoa butter, sugar, and milk, but no cocoa solids.

Preparation Formula:-

| Ingredients | Quantity of Ingredients (in gm) | | |
|-----------------|---------------------------------|-----------|----------|
| | F1 Batch | F 2 Batch | F3 Batch |
| Moringa Powder | 0.5 | 1.5 | 0.8 |
| Beetroot Powder | 2 | 1 | 1 |
| Thyme Powder | 0.5 | 0.7 | 1 |

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| Tulsi Powder | 0.05 | 0.05 | 0.05 |
|-----------------|-------|-------|-------|
| Cinnamon Powder | 0.002 | 0.002 | 0.002 |
| Cardamon Powder | 0.005 | 0.005 | 0.005 |
| Dark Chocolate | 20 | 20 | 20 |
| Jaggery Powder | 7 | 7 | 7 |

METHODOLOGY

Formulation of chocolate :-

To formulate herbal multivitamin antioxidant chocolate all ingredients are weighed appropriately according to formula.

7 gm of Jaggery was weighed and 10 ml water was added in Jaggery powder.

Jaggery powder solution was melted in water bath by using double boiler method.

When the Jaggery solution was formed then weighed powdered herbal ingredients areadded in the Jaggery solution.

The powdered herbs mixed homogeneously with Jaggery solution.

Dark chocolate was weighed and melted in water bath by using double boiler method.

Then melted chocolate was added to the herbal solution and mixed it throughly till it become homogeneous.

Then the prepared chocolate containing Herbal drug extract was poured in moulds Andkept in freeze to set overnight.

Total 3 formulations were prepared by Varying the concentration of herbal drugExtract used, while the concentration of Excipients was kept constant.

EVALUATION AND CHARACTERIZATION

General appearance:-

The visual identity and overall elegance of a chocolate formulation are what determineits Overall appearance, which is important for consumer acceptability and trouble-freeManufacture.



Dimensions:-

VCE The dimension of the chocolate was evaluated while using Vernier's callipers. Weight Variation :-

Six chocolate recipes were weighed separately and collectively. The weight of all the Chocolate was used to calculate the average weight. The average weight was contrasted with the individual weights. The weight variation's percentage difference must stay within the Allowed bounds. The following formula was used to determine the percent deviation.

Individual weight-final weight×100 %Deviation = Average weight

Hardness test:-

To shatter a chocolate bar across its circumference, a certain amount of hardness isneeded. The strength of chocolate can be determined by how hard it is. Using a Monsanto Hardness Tester, the hardness was determined. Kg/cm2 was used to express he values

pH of chocolate formulation:-

Procedure for pH measurement :-

- 1. Rinse the electrode.
- 2. Electrode is calibrated by using distilled water till the pH become 7
- 3. Put the meter into measurement mode.
- 4. The electrode is inserted into the chocolate sample.
- 5. Readings are recorded



Fig : pH of Chocolate

Drug content determination:-

Drug content of medicated chocolate was determined by Thin Layer Chromatography. Here, control was taken as Aqueous moringa extract and test as melted chocolate Sample.

 \checkmark TLC plates were prepared by using silica G and Plates were activated for $\frac{1}{2}$ Hr. Spotting was carried out On both plates i.e., control and test plate by using Capillary.

Run both the plates in mobile phase i.e., Chloroform :Methanol: Water having ratio 12:3:1 After Running of both plates air drying of plates was carried Out.

✓ Further, visualization of both plates was carried out By using iodine chamber.

✓ By comparing the RF value of both the plates i.e., control and test, Drug content in Medicated chocolate was determined.

<u>RESULT AND DISCUSSION</u> <u>Organoleptic properties:</u>

| Parameter | F1 | F2 | F3 |
|------------|---------------------------|---------------------------|---------------------------|
| Colour | Brown | Brown | Brown |
| Odour | Chocolaty | Chocolaty | Chocolaty |
| Taste | Sweet and slightly bitter | Sweet and slightly bitter | Sweet and slightly bitter |
| Mouth feel | Smooth & Pleasant | Smooth &Pleasant | Smooth &Pleasant |
| Appearance | Glossy | Glossy | Glossy |

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Dimensions:-

It was measured by Vernier's callipers

Avg. Width of 5 chocolate formulations:

1.85 + 1.90 + 1.84 + 1.85 + 1.86

5.9+5.8+5.6+5.7+5.9

The average width of 5 chocolate is observed to be = 1.86Weight variation determination:-

Average Weight of 5 formulations:

5

5

W<u>1+W2+W3+W4+W</u>5

Average weight calculated to be (for batch 3) =

5

Average weight calculated to be (for batch 3) = **5.78gm**

Similarly weight variations for other 2 batches was also calculated

| Parameter | F1 | F2 | F3 |
|-----------|----------------------|-------|--------|
| Avarage | 6.0 <mark>2gm</mark> | 5.82g | 5.78gm |
| weight | | m | |

Hardness test:-

To shatter a chocolate bar across its circumference, a certain amount of hardness isneeded. The strength of chocolate can be determined by how hard it is. Using a Monsanto Hardness Tester, the hardness was determined. Kg/cm2 was used to express the values.

Initial reading on hardness tester = 2.9 kg/cm

After breakage of chocolate = 8.2kg/cm

Therefore, hardness present in the chocolate formulation = 8.2 kg/cm-2.9 kg/cmHardness present in the chocolate formulation is = 5.3 kg/cm

| Parameters | Storage condition | At the time of preparation | After the one month |
|---|----------------------|--|------------------------|
| Colour, Odour, Taste, Mouth feel, Appearance | 2-8 °c | Brown, chocolaty, slightly bitter, smooth, glossy | No change |

Stability study:-

F3batch was selected for stability study.

Stability of the chocolate is evaluated by studying it with appropriate storage conditions of nearly 2-8°C at time of preparation and after 15days of storage at givenstorage conditions

<u>pH of chocolate formulation:-</u>

| Formulation | F1 | F2 | F3 |
|-------------|------|------|------|
| pH | 6.41 | 6.26 | 6.35 |

Drug content determination:-

Observation:

- 1) Distance travelled by solvent of control 4cm.
- 2) Distance travelled by solute of control -0.9cm.
- 3) Distance travelled by solvent of test -4.3 cm.
- 4) Distance travelled by solute of test -1 cm.

Formula:

RF value = Distance travelled by solute ÷ Distance Travelled by solvent

Calculation:-

Distance travelled by solute of Control - Distance travelled by solvent of control

 $= 0.9 \div 4$ = 0.22

1) RF value = Distance travelled by solute of Test ÷ Distance travelled by solvent oftest

= 1÷4.3 =**0.23**

By comparing RF value of both i.e., control and test Approximately nearby. So,we can determine drug Content.

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