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FORMULATION AND EVALUATION OF ANTIDIABETIC CHOCOLATE BY USING HERBAL EXTRACT OF TRIDAX PROCUMBEN

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ABSTRACT

The acceptance criteria for Tridax Procumben as medicine is very low due to its bitter taste, but it possesses lots of therapeutic properties such as anti-diabetic, anti-inflammatory, anti- septic, insecticidal, parasiticidal, hepatoprotective, antidiabetic activity immunomodulating property, anticancer, antihypertensive etc. The chocolate formulation was this research is based on the herbal based chocolate formulation for a diabetic person. Further, prepared medicated chocolate is evaluated for texture, taste, bloom test, stability etc.

KEYWORDS: The chocolate formulation was this research is based on the herbal based chocolate formulation for a diabetic person.

INTRODUCTION

Tridax procumbens L. belongs to the family Asteraceae (Com-positae). It is a green perennial plant with a woody base that sometimes has roots at the nodes and grows wild in many tropical countries.[1] Commonly called as ‘Jayanti-veda’ in Sanskrit, Tikki- kasa/'Ghamra' in Hindi and ‘Wild daisy’, ‘Mexican daisy’.[2] It is also known as ‘coat buttons’ in English due to the appearance of

flowers has been extensively used in Ayurvadic system of medicine for various ailments and is dispensed for "Bhringraj" by some of the practitioners of Ayurveda which is well known medicine for liver disorders.[3] Now a days the considerable attention has been paid to utilize eco-friendly and bio- friendly plant based products for the prevention and cure of different human diseases and it is documented that most of the World's population has taken in traditional medicine, particularly plant drug for the primary health care. Herbal medicine is known as botanical medicine or phytomedicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal use. Long practiced outside of traditional medicine, herbalism is becoming more mainstream as up-to-date analysis and research show their value in the treatment and prevention of disease. The Plants had been used for medicinal purposes long before recorded history.[4] The Indian flora offers a different variety of plants having medicinal properties. These plants can be utilized to find out effective alternative to synthetic drugs. Plants play a vital role in curing various ailments of the man and herbal remedies are getting increasing patient compliance as they are devoid of typical side effects of allopathic medicines.[5] In ancient times, this species has been used in Ayurveda in India .Various substances such as oils, teas and skin poultices, among others, have been manufactured using this species. T. procumbens shows different pharmacological properties like: immunomodulatory, anti-oxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities. The versatility of the species is mostly due to the plant's defense mechanisms, secondary metabolites such as flavonoids, alkaloids, tannins, carotenoids and saponins. The connection of the traditional and scientific knowledge is important for future studies.[6]

The evaluation of these drugs is mostly based on,

- Pharmacognostical investigation
- Phytochemical investigation
- Pharmacological investigation.[7]

Pharmacological activity Prolongation of clotting time

Extract of T. procumbens 200 mg/µg IP injected into experimental rabbits, reduced normal heparin-induced prolongation of clotting time.[8] Wound is a physical trauma where the skin is cut, torn or punctured. On exposure to air, microorganisms pass in the wound which leads to wound contamination and finally development of infection.[9] In the expurgation of wound healing process, and the extract of T. procumbens shows indirect corticotropic effects. This

rises the tensile strength of collagen fibers and rate of epithelialization. The leaf extract is also reported to stop bleeding when it applied topically The leaf extract of T. procumbens is commonly used in Indian traditional medicine and topically applied on open wound to stop bleeding and enhance the healing process. It enhances wound healing by interacting with epidermal cells, dermal cells, ECM, soluble proteins and angiogenesis processes co-ordinated by an array of cytokines and growth factors.[2] The Aqueous extract of whole part of plant Tridax procumbens L. has capacity to set the normal and immune compromised wound healing in rats.[10] Wound healing process involves a cascade of events characterized by completion of biological processes in a certain order and a certain time frame. These events characterize the restructuring of the damaged tissue in an attempt to restore as normal a condition as is possible. The natural response of a living organism is to repair the wounds in the shortest time possible and to re-establish the normal anatomy of the skin.[9]

Fig no 1: Tridax procumbens L.

Table 1: List of various studies done on pharmacological activity of plant Tridax procumbens.[2,10,11]

Plant part	Pharmacological activity
Whole plant	Antimicrobial activity against gram-positive and gram-negative bacteria, wound healing activity (aq. extract), Antifungal, Antioxidant.
Flowers, Leaves, aerial part of leaves	Anti-inflammatory, Anti septic, Insecticidal, Parasiticidal, Hepatoprotective, Antidiabetic Activity Immunomodulating property, Anticancer, Antihypertensive.

Antidiabetic Activity

The practical usage of juices from various plants achieved the reducing of blood glucose by 10-20%. Diabetes mellitus occurs throughout the world; however, it is more common in more developed countries. Diabetes is in the top 10, possibly in the top 5, of the most significant diseases in the developed world and is still gaining significance. Therefore, it is recommended

to allow such remedial measures as supplements to other modes of therapy.[12] Diabetes is a chronic disease, and when it is not treated properly, generates serious complications that reduce patients' quality of life and raises the cost of their care. Its prevalence is increasing gradually illness.[13] Antidiabetic Properties of tridax procumbens effect were performed that are comparable to the drug Glibenclamide in rats by the phytoconstituent Saponins which is an Ethanolic extract of whole plants, pet ether, methanol, and chloroform extracts. The study showed that the extract has an antidiabetic activity that is analogous to the drug Glibenclamide used to treat diabetes mellitus type 2. The drug works by increasing the amount of insulin produced by the pancreas.[6] The knowledge of diabetes mellitus, as history reveals, existed with the Indians since from prehistoric age. Madhumeha is another name for diabetes in which a patient passes sweet urine and exhibits sweetness all over the body in the form of sugar, i.e., in sweat, mucus, urine blood, etc. from ancient times various herbs were practically used for lowering blood glucose level as such or in juices form. Aqueous and alcoholic extract of leaves of Tridax showed a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats.[3] Plant-based drugs have been used against several diseases for a long time. The Primitive man used herbs as therapeutic agents and medicament, which they were capable to procure easily.[12] Tridax procumbens may be useful in treating diabetes mellitus with no visible signs or symptoms of toxicity in normal rats showing a high margin of safety. The methanolic extracts of Tridax procumbens have shown a high level of anti-diabetic activity. The extracts exhibited anti-hyperglycemic activity comparable to that of a standard anti- diabetic drug, glibenclamide.[14] There are various types of diabetes that are Type l, Type 2, and Gestational Diabetes. Type 1 diabetes is an autoimmune disease, Type 2 diabetes occurs when the body becomes resistant to insulin, and sugar builds up in the blood. Gestational diabetes is high blood sugar during pregnancy. Insulin-blocking hormones produced by the placenta cause this type of diabetes.[15]

On the basis of etiology, two main categories of diabetes are recognized, viz. Primary diabetes Secondary diabetes

(1) Primary diabetes: It is divided into two types:

a) Juvenile onset diabetes: In Juvenile onset diabetes there is a profound decrease in the number of β cells in the islet of Langerhans and thus there is an absolute deficiency of insulin. The main treatment for this type is insulin.

b) Maturity onset diabetes: The patients are frequently obese and the treatment is usually dietary, though supplementary oral hypoglycemic drugs. It is diagnosed by blood or

urinary glucose measurement. Insulin resistance as well as loss of insulin secretion contribute to the onset of disease.

(2) **Secondary Diabetes:** The signs or symptoms result from the following Pancreatic dysfunction (pancreatitis, pancreatectomy). Hormonal imbalance (eg: Acromegaly, pheochromocytoma, Cushing's syndrome, glucagonoma). Drugs or chemical- induced reactions (eg: glucocorticoids, anticancer agents, streptozotocin or diazoxide, thiazide, and some psychoactive agents).[16]

Chocolate

Chocolate is an adaptable food that can be combined to create completely different taste and texture sensation. It is best drug delivery system specifically for children and youngers. There are five basic human taste qualities i.e. sweet, bitter, our, salty, savory. Sweet taste is one of the most pleasurable tastes. The goal of the sweet taste is to detect the highly calorific saccharides for injection.[17, 18] Herbal formulation means a dosage form consisting of one or more herb or proceed herbs in specified quantities to provide specific nutritional, cosmetic benefits meant for use to diagnose, treat, mitigate.[19,20] In many ways, chocolate is an excellent vehicle for delivering active chemicals. The aim of the present study to prepare study to prepare herbal chocolate for antioxidant, anti-cancer and anti-aging. Chocolate is a cocoa-based product that is high in flavonoids and antioxidant chemicals. Chocolate is a sophisticated and infinitely adaptable food that can be mixed and matched to generate a variety of taste and texture sensations.

The goal of this research is to create and develop nutritious chocolate. Chocolate drug delivery is another name for it. The main goal of this study was to develop and test a natural healthy chocolate and nutritional supplement. Chocolate is a term used to describe a variety of items made from cocoa beans. The medicated chocolate formula is frequently used to treat health conditions of patient and improve patient compliances. Organoleptic characteristics, pH, blooming test, preliminary phytochemical screening, and hardness of the resultant chocolate mixture were all assessed. The purpose of the stability study was to examine if there were any major changes in the physical properties of chocolate.[21,22] Chocolates are solid suspensions of tiny solid particles made from sugar and cocoa (and, depending on the variety, milk), with a total fat content of roughly 70%. Cocoa solids are extracted from beans harvested from the fruit of Theobroma cacao, with Forastero kinds, which are tiny, flattish, and purple beans, dominating world output. Criollo, a disease-resistant hybrid of Criollo and

Forastero, is considered as a flavor bean; Trinitario, a disease-resistant combination of Criollo and Forastero, is now scarce in production. Dark, milk, and white chocolate are the three main types, with different amounts of cocoa solids, milk fat, and cocoa butter. The result is a mixture of carbohydrate, fat, and protein proportions. Chocolate production methods varies owing to differences in customer tastes and business operations around the globe. Dark chocolate is powerful source of antioxidants which contain 70% or high coca, helps balance blood glucose, improve blood flow and blood pressure, reduce heart disease, improve brain function. It also reduces risk of diabetes for long term.[15]

Types of Chocolate

1. **Milk chocolate:** It's best to mix Ecuadorian beans with mostly medium-roasted West African beans. This combination would provide a clean, nutty, somewhat fruity chocolate. It's worth noting that the inclusion of the more acidic Brazilian and Malaysian beans might clash with the intended creamy overtones.
2. **Light milk chocolate:** This product might be prepared from gently roasted Java beans, which have a light colour and a mild flavor with pronounced nutty overtones. Because the coating would be many shades lighter than a 100 percent West African bean, this would aid in achieving an acceptable standard of identification for milk chocolate.
3. **High-quality semisweet chocolate:** For its cocoa flavour and somewhat nutty undertones (light to medium roast), use mostly West African stock to heighten desired notes and reduce burnt/bitter notes (light to medium roast). When combined with Caracas and Trinidad beans, this blend creates a balanced yet distinct profile with floral and slightly spicy notes.
4. **Bittersweet chocolate:** Because it forms bitter coatings, this product is best used over particularly sweet and intensely flavoured cream centers.
5. **Semisweet cookie drops:** To have a good cocoa impact, utilize the dominating West African beans in this product. The Brazilian and Sanchez components' strong characteristics compliment and contrast the West African.[3]

Mechanism

Alpha amylase and alpha glucosidase are responsible for the hydrolysis of poly and oligosaccharides into monomers or cleavage of bonds between sugars and non- carbohydrate aglycone. Thus, this enzyme is involved in a number of important biological processes, such as digestion of carbohydrate into glucose or processing of the oligosaccharide moieties of glycoprotein. There is now a great deal of interest in amylase inhibitors, because these are

important biochemical tools for studying the mechanism of enzymes. The search for amylase inhibitors has yielded a number of chemically distinct inhibitors from plants. The results clearly showed that methanolic extract has potential to reduce postprandial glucose levels via α amylase inhibitory action.[12] Cocoa has been claimed to be a source of natural antioxidants, which are free radical scavengers that preserve cell membranes, protect DNA, and inhibit the oxidation of LDL cholesterol, which leads to atherosclerosis and plaque formation in artery walls. The procyanidins and their monomeric precursors, epicatechin and catechin, which block LDL oxidation, have been credited with cocoa's antioxidant potential. Dark chocolate and cocoa reduce LDL oxidation while increasing HDL cholesterol levels. Catechins are phytochemical substances that are abundant in a wide range of plant- based meals and beverages. Dark chocolate has a catechin concentration of 12mg/100 gm. Dark chocolate has 41.5 milligrams of epicatechin per 100 gm. Catechin intake has been linked to a number of health benefits, including enhanced plasma antioxidant activity, bronchial artery dilatation, fat oxidation, and LDL oxidation resistance. Epicatechin appears to be a key bioactive component of cocoa and other foods and beverages high in flavanols. In both animals and people, it has been found to increase endothelium function. Epicatechin reduces blood pressure and end-organ damage in salt-sensitive animal models of hypertension. Both hypertension and endothelial dysfunction are thought to be protected by nitric oxide. Dark chocolate has an antioxidant content of 13.1/100 g.

Therapeutic benefits of chocolate

1. **Diuretic characteristics:** Theo bromine has diuretic qualities. This is an efficient treatment for fluid build-up in tissues (oedema), the elimination of excess water and salts from the body through urine, and the decrease of high blood pressure caused by increased water content in the fluid connective tissues.
2. **Vasodilation** - Theo bromine aids in greater blood vessel dilation. This aids in the improvement of blood pressure and hence contributes to the decrease of blood pressure.
3. **Improving cardiac function** - Theo bromine has long been known for its ability to improve heart function. Theo bromine, according to the American Journal of Clinical Nutrition, can be used to treat a variety of heart issues including arteriosclerosis, vascular diseases, angina, and hypertension.
4. **Preventing tooth decay** - Theo bromine has been discovered to be particularly helpful in preventing tooth decay by working against the microorganisms that create Muscle relaxation: Theobromine is a muscle relaxant. It causes the smooth muscles of the heart

tissues to relax.

5. **Anti-cancer and anti-inflammatory effects –** Antioxidants such as flavonoids and polyphenols are abundant in dark chocolate. By suppressing excessive and unregulated cell division and lowering inflammation by neutralizing the formation of free radicals, these chemicals have anti-cancer and anti-inflammatory capabilities.

6. **heart disease prevention -** Chocolate is high in minerals like iron, copper, magnesium, zinc, and others. All of these factors are important in preventing coronary heart disease (CHD).

7. **Food cravings are reduced** - Dark chocolate has a bitter taste and a high calorie and fat content. As a result, dark chocolate intake has been shown to lessen cravings for a variety of sweet, salty, and fatty meals.

8. **Antidepressant qualities –** Chocolate consumption increases endorphin generation. The creation of these neurotransmitters causes a positive mood in the individual, which helps to combat depression. Chocolates are also high in serotonin, another neurotransmitter that has antidepressant qualities.

9. **Improving memory** - Hot chocolate consumption improves neurovascular coupling (the response of blood flow to brain activity) in the white matter of the brain, as well as cognitive functioning. This beneficial impact of chocolate consumption on brain function might aid in the prevention of brain degenerative illnesses like Alzheimer's disease. In many ways, chocolate is an excellent vehicle for delivering active chemicals. Chocolate's organoleptic properties, for example, are good for disguising disagreeable Flavors associated with some active agents and providing a smooth and creamy texture to otherwise undesirable granular active agent formulations. Saturated fat, polyphenols, sterols, di and triterpenes, aliphatic alcohols, and methylxanthines are all prevalent in chocolate. Chocolate is also an anhydrous media, which means it's resistant to microbial growth and water-sensitive active agent hydrolysis. Chocolate is an excellent medium for delivering active substances in a variety of ways. Chocolate is one of the most popular foods, with a creamy texture, sweet flavor, and a brown hue. It helps you feel happy for a short time. It's created from roasted and ground Theobromine cacao beans, and it comes in liquid or paste form. Cocoa seeds have a bitter flavour and are used as a flavouring element in a variety of cuisines. Chocolate production is the culmination of years of research and development.[23, 24, 25,26]

MATERIAL AND METHOD

Material

Materials: whole plant of tridax procumbens powder, Cocca butter, Dark chocolate, coffee, stevia sugar, methanol, Distilled water.

Equipment's: Measuring cylinder, Beaker, Mortar pestle, Conical flask, Funnel, Chocolate mold, Petri dish.

Instruments: Refrigerator, water bath, sieves, grinder.

METHOD

Preparation of Chocolate

1. Collect Tridax procumbent plant and wash it with water to remove dirt.
2. Shield dried it naturally for 4-5 days.
3. Reduce size into powder form by using grinder.
4. Pass prepared powder through sieves
5. Take Dark chocolate and Cocca butter melt it on water bath then add plant extract and other ingredients and mix well.
6. Transfer mixture into chocolate mold and and freeze it 8 to 10 hours.[15]

Table 2: Formulation Table.

Sr no.	Ingredients	Quantity given	Quantity Taken	use
1	Plant Extract	100gm	10gm	Antidiabetic
2	Dark chocolate	1000gm	100gm	Antidiabetic
3	Butter	50gm	5gm	Shine
4	vanilla essence	1-2drops	1-2drops	Flavoring agent

Experimental Work

Preparation of methanolic extract

- The plant material was collected locally, identified, authenticated and dried in shade and further grinded and passed through sieve number 80 to form a coarse powder for extraction.
- 100gm of powdered were extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus.
- The extract was cooled and filtered and were kept in vacuum dryer until used.[5,27]

RESULT AND DISCUSSION

Fig no 2: Herbal Chocolate.

1. **Texture:** Evaluate the texture of the chocolate by checking its softness and brittleness. Place chocolate in mouth and feel it.
2. **Taste of chocolate:** Taste the chocolate.

3. Blooming test

1. **Fat Bloom** - When the thin layer of fat crystals was form on the surface of chocolate formulation. This will cause the chocolate to lose its brightness and a soft white layer will appears, giving the finished article an unattractive look. Fat bloom is caused by the recrystallization of fat and/or an immigration of a filling fat to the chocolate layer. Storage at a constant temperature will delay the appearance of fat bloom.

2. **Sugar Bloom –** Sugar bloom is rough and irregular layer on top of chocolate formulation. This is caused by condensation (when chocolate is taken out of the refrigerator). This dampness or moisture will dissolve the sugar in the chocolate. When the water vaporizes, sugar recrystallizes into rough, irregular crystals on surface. This results into unpleasant look

4. Physical stability: To check the physical stability, sample of chocolate was kept in closed container for 1 month at 28°C After 1month interval, Test sample of chocolate was observed to check the physical appearance and drug degradation.

5. **Melting Point:** Heat the residual of the chocolate to melt it. Do not depend on appearance alone when microwaving chocolate. The only way to know if it is completely melted is to gently stir it and use an instant-read thermometer to check the chocolate's temperature while melting. Use a rubber spatula to stir the melting chocolate.

6. Chemical Test

1) **Test for Carbohydrate (Fehling's Test):** To 1 ml of the solution, equal quantities of

Fehling's solution A and B was added and heated. The formation of brick red precipitate indicates the presence of carbohydrates.

2) Test for Protein (Biuret Test/General Test): Take 3ml of chocolate formulation; add 4% NaOH and few drops of 1% copper sulphate solution, violet color indicate presence of protein.

3) Test for amino acids (Ninhydrin test): 3ml of test solution was heated and 3 drops of 5% Ninhydrin Solution was added in boiling water and was boiled for 10 min. purple and bluish color indicates presence of amino acid.

4) Test for Saponins (Foam Formation): Place 2ml of chocolate formulation in water and this was added in test tube, shake well and stable foam is form indicate the presence of saponins.

7. pH: 2gm of prepared chocolate was dissolved in 100ml of phosphate buffer solution and pH of the resulted solution was studied by digital pH meter with glass electrode.

Table No. 3: Evaluation test results.

Sr.no.	Name of test	Test group	Control group
1	Carbohydrate	+	+
2	Protein	+	+
3	Amino acid	-	-
4	Saponin	-	-

pH: The pH of chocolate formulation was done by using pH meter and the result was found to be pH=6.4.

Blooming Test

Table No. 4: Blooming test.

Sr.no	Test	Result
1	Fat bloom	No
2	Sugar bloom	No

8. Stability: Test group selected for stability study

Table No. 5: Stability test.

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

SUMMARY AND CONCLUSION

Summary

As an outcome of the previous, the sweetening property, pH, and stability profile are all satisfactory. Further in vivo research can be done using a cognitive model or any other relevant animal model. Herbal extracts of Tridax procumben were successfully formed in chocolate formulations. Chocolates organoleptic qualities are ideal for masking unpleasant flavours associated with some active agents and providing a smooth and creamy texture to active agent compositions. As a result, chocolate formulations provide an appealing manner of administering medications via oral administration. The drug extracts used in the dose range are safe to consume and can be ingested without difficulty.

CONCLUSION

In the present study, development of Herbal Chocolate having antioxidant activity was carried out. Methanolic extract of Tridax procumben was prepared and phytochemical analysis was carried out to check the presence of desired compounds that shows the acceptable results. From above study, we concluded that the chocolate provides smooth and creamy texture to the formulation and are good for masking and gives therapeutic effect.

REFERENCES

1. Taddei A, Rosas-Romero AJ. Bioactivity studies of extracts from Tridax procumbens. Phytomedicine, 2000; 7(3): 235-238.
2. Gubbiveeranna V, Nagaraju S. Ethnomedicinal, phytochemical constituents and pharmacological activities of Tridax procumbens: a review. Int J Pharm Pharm Sci, 2016; 8(2): 1-7.
3. Beck S, Mathison H, Todorov T, Calder E, Kopp OR. A review of medicinal uses and pharmacological activities of Tridax procumbens (L.). J Plant Stud, 2018; 7(1).
4. Chhetri HP, Yogol NS, Sherchan J, Anupa KC, Mansoor S, Thapa P. Formulation and evaluation of antimicrobial herbal ointment. Kathmandu University Journal of Science, Engineering and Technology, 2010; 6(1): 102-7.
5. Jadhav VD, Talele Swati G, Bakliwal Akshada A, Chaudhari GN. Formulation and evaluation of herbal gel containing leaf extract of Tridax Procumbens. J Pharm Biosci, 2015; 3: 65-72.
6. Beck S, Mathison H, Todorov T, Calder E, Kopp OR. A review of medicinal uses and pharmacological activities of Tridax procumbens (L.). J Plant Stud, 2018; 7(1).

7. Kumar S, Prasad A, Iyer SV, Vaidya S. Pharmacognostical, phytochemical and pharmacological review on Tridax procumbens Linn. International Journal of Pharmaceutical & Biological Archives, 2012; 3(4): 747-51.
8. Bhagat VC, Kondawar MS. A comprehensive review on phytochemistry and pharmacological use of Tridax procumbens Linn. Journal of Pharmacognosy and Phytochemistry, 2019; 8(4): 01-10.
9. Singh CP, Mishra PK, Gupta SP. Design and Formulation of Tridax procumbens based Polyherbal Cream for Wound Healing Potential. Pharm. Lett, 2016; 8: 15-21.
10. Gupta R, Sharma P, Pandey P, Jain AP. A Comprehensive Review on Medicinal Importance of Tridax Procumbens Linn. Journal of Biomedical and Pharmaceutical Research, 2013; 2(3): 109-13.
11. Ingle NA, Dubey HV, Kaur N, Gupta R. Tridax procumbens: A multiuseful weed a review. Journal of Advanced Oral Research, 2014 Jan; 5(1): 14-6.
12. Sonawane A, Srivastava RS, Sanghavi N, Malode Y, Chavan B. Anti-diabetic activity of Tridax procumbens. Journal of Scientific and Innovative Research, 2014; 3(2): 221-6.
13. Barrios JL, Francini F, Schinella GR. Natural products for the treatment of Type 2 Diabetes Mellitus.
14. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti-hyperglycemic potential of Tridax procumbens (Linn.). BMC complementary and alternative medicine, 2009; 9: 1-7.
15. Ramprasad GJ, Kale VR. Formulation and Evaluation of Antidiabetic Chocolate by using Guava Leaves and Mulberry Fruits. IJFMR-International Journal For Multidisciplinary Research, 5(1): 1-6.
16. Wadkar KA, Magdum CS, Patil SS, Naikwade NS. Antidiabetic potential and Indian medicinal plants. Journal of herbal medicine and toxicology, 2008; 2(1): 45- 50.
17. Bhushan MS, Rao CH, Ojha SK, Vijayakumar M, Verma A. An analytical review of plants for anti diabetic activity with their phytoconstituent & mechanism of action. Int J Pharm Sci Res, 2010; 1(1): 29-46.
18. Stephen A. Gravina, Gregory L. Yep, Mehmood Khan. Human Biology of Taste, 2013; 33(3): 217-2222013 DOI: 10.5144/0256-4947.2013.217
19. Niroumand, M. C., Heydarpour, F., & Farzaei, M. H. (2018). Pharmacological and therapeutic effects of Vitex agnuscastus L.: A review. Pharmacognosy Reviews, 12(23).
20. Pandey, G., & Madhuri, S. (2010). Pharmacological activities of Ocimum sanctum (tulsi): a review. Int JPharm Sci Rev Res, 5(1): 61-66.

21. Afoakwa E. 2010. Chocolate science and technology. Wiley-Blackwell Publication.
22. Philip KW. 2015. Chocolate in Science, Nutrition and Therapy: An Historical Perspective, in Chocolate and Health: Chemistry, Nutrition and Therapy.
23. Knight Ian. 1999. Chocolate and Cocoa: Health and Nutrition. Blackwell Publication
24. Narayan D. 2015. Therapeutic Benefits of Chocolate. [ONLINE] Available at: http: //www.biotecharticles.com/Healthcare-Article/Therapeutic Benefits-of- Chocolate 3155.html. [Accessed on 15 June 2015].
25. Vasani, C., & Shah, K. (2016). Preparation and evaluation of chocolate drug delivery system of albendazole. Research Journal of Pharmacy and Technology, 9(11): 1994-1998.
26. James F, Gerd P, Albert K. Effect of Chocolate on Acne Vulgaris. The Journal of the American Medical Association, 1969; 210(11): 2071-2074.
27. Shaikh M, Avalaskar A, Wadekar K, Shaha C, Diwate S, Shah S. Formulation and Evaluation of Topical Herbal Gel For Local Inflammation. World Journal of Pharmaceutical Research, 2018 Oct 17; 7(19): 1284-9.