Evaluation of C. Ternatea Extract and Incorporation into Hard Candy

Caroline .A[1, *], Padmavati .R[1, 2]

[1]*Dept. of Food Process Engineering, SRM University, Chennai, India.*
[2]*Dept. of Food Process Engineering, SRM University, Chennai, India.*

Abstract: **Hard candies with natural colorant and high medicinal property are rare to be found. *C. ternatea* is a well known ayurvedic plant with high health benefits. These are commonly found ornamental plants with flowers in blue and white color. *C. ternatea* was a blue colored -flower with high amount of polyphenol compound. These compounds act as source of free radical scavenging. Quantitative analysis shows the presence of phytoconstituents such as flavanoids, phlobatannins, reducing sugars, proteins and carbohydrates. The free radical scavenging property of *C. ternatea* flower extract showed the highest level of 59.3 % .The microbial activity of *C. ternatea* extract was carried out against common food pathogens which include E.coli, Bacillus cereus and Salmonella species by measuring their ZOI (zone of inhibition).Blue colored-hard candies were prepared using the *C. ternatea* extract with and without preservative for shelf life study. The physicochemical characteristics of the extract and extract based candies were carried which includes analysis of carbohydrates, proteins, fats and moisture contents. Sensory evaluation of the extract based candy was based on taste, color, flavor and appearance of the product. The shelf life of the candy with preservative was carried on till the 57th day while the shelf life of candy without preservative was carried on till the 30th day. Studies based on shelf life were evaluated based on room temperature. All the parameters of candy were compared with the level of *C. ternatea* extract.**

Keywords: C. ternatea flower, Anti-oxidant property, Nutritional analysis, hard candy.

I. INTRODUCTION

C. ternatea, commonly known as Butterfly Pea or Blue Pea, is a perennial climber belonging to the family Fabaceae. It originates in Southeast Asia and is known to accumulate ternatins, a group of (poly) acylated anthocyanins, in its petals. Blue dye aqueous extract from the petal of butterfly pea was traditionally used used as a confectionary coloring in the food industry (**20).** *C. ternatea* has been evaluated for its medicinal properties and shows promising effects as having antioxidant, antidiabetic and hepatoprotective activities **(11).**The major phytoconstituents analyzed in *C. ternatea* extract are flavonoids, phlobtannins, reducing sugars and carbohydrates. Nowadays antioxidant enriched food products are many but candies with free radical scavenging activity are rare to be found. The primary objective of the study was to determine the physico-chemical properties, anti-microbial activity, and antioxidant property of extract. The extract was further incorporated into hard candy which gives color naturally with high antioxidant property.

II. MATERIALS AND METHODS

2.1. Extraction of C. ternatea (CT):

Fresh flowers were collected from koyemedu market, Chennai,India and the aqueous extraction of CT was carried according to Kuntapas Kungsuwan ***et al.,*** procedure **(13)**.

2.2. Qualitative analysis of phytochemicals:

2.2.1. Test for Flavanoids: For 1mL of the extract, a few drops of dilute NaOH were added. Appearance of yellow color turns colorless on addition of dilute acid.

2.2.2. Test for Carbohydrates:

Molischs test:

Filtrate (flower extracts) were treated with 2-3 drops of 1% alcoholic alpha naphthol solution and 2 ml of Conc Sulphuric acid was added along the sides of the test tube. Brown ring formation separating two junctions.

2.2.3. Test for Phlobatannins: Few drops of extracts were reacted with 1% aqueous Hcl. Red precipitate confirms the presence.

2.2.4. Reducing sugars-Fehling's test: To a few drops of extract, A and B solution of Fehling's reagent was added in equal amounts and heated for 30 minutes. Appearance of red brick color precipitate indicated the presence.

2.3. Physico-chemical estimation:

2.3.1. Estimation of proteins:

The proteins content in the samples was estimated according to the dye binding method of Bradford method **(22).**

2.3.2. Total lipid estimation:

Total lipid was estimated using the method of Van Handel **(23).**

2.3.3. Determination of total Carbohydrates by Anthrone method: The total content of carbohydrates was analyzed by

standard Anthrone method.

2.3.4. Moisture content analysis:

The moisture content of sample was analyzed by hot-air oven method. The result was calculated based on formula:

Moisture content (%) = [(W_2-W) / (W_1-W)]×100

Where W= weight of empty Petri dish

W1= weight of Petri dish with sample before drying and

W2= weight of Petri dish with sample after drying to constant weight.

2.3.5. Ash content:

5g of sample was added in crucible and kept in muffle furnace. Temperature was monitored till 500ºc for 5 to 10 mins .The crucible furnace was removed and weighed after it gets cooled

$$\text{Total ash content} = \frac{\text{Initial wt- final weight x 100}}{\text{Final wt}}$$

2.3.6. Crude fibre:

The crude fibre was analyzed by modified scharrer method (24) using muffle furnace.

2.4. Anti microbial analysis:

Two Gram negative *Pseudomonas aeruginosa, Escherichia coli* and three Gram positive *Staphylococcus aureus, Klebsiella pneumoniae* and one fungi *Candida albicans* were used for *in vitro* antimicrobial activity. These selected pathogenic strains were obtained from Microbiological Division (**Jayagen Biologics Analytical Laboratory, Jayagen Biologics, Chennai**).The antimicrobial activity was determined by well diffusion methods (25) method.

2.5. Antioxidant analysis:

***2.5.1.* Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrilhydrazyl):** The effect of sample extract and candy on DPPH radical scavenging was estimated according to the procedure described by Von Gadow et al. **(26)** method.

2.6. Incorporation into candy:

The CT extract of various concentrations were incorporated into hard candy and its parameters along with shelf life period were analyzed.

2.7. Sensory analysis:

Sensory evaluation offers the opportunity to obtain a complete analysis of the various properties of food as perceived by human sense. Sensory evaluation was an important and best method for evaluating new products developed which provide quality measure and production control. The sensory evaluation was done using a hedonic (9 point) score card.

III. RESULTS AND DISCUSSION

3.1. Phytochemicals test:

For the all the medicinal plants, analysis of phytochemical was the basic steps. The phytochemical constituents analysed were alkaloids and proteins. The phytochemical screening of *C. ternatea* extract gave positive results for the presence of carbohydrates, flavanoids, Phlobatannins and reducing sugars.

3.2. Physico-chemical analysis of extract and candy:

Analysis on the physicochemical characteristics of the extract and extract based candy was done in the laboratories of Food Process Technology, SRM University. Knowledge of the physicochemical properties of food is fundamental in analyzing the characteristics of food during its processing. The study of these food properties and their responses to process conditions are necessary because they influence the treatment received during the processing and also good indicators of other properties and qualities of food. In the present investigation certain physicochemical properties of the product were analyzed, to ensure the quality of products. The physicochemical properties of the extract and extract based Candy analysis include moisture content, protein content, fat/lipid content and carbohydrate content. Each analysis was repeated three times and the values reported herein are the average of them.

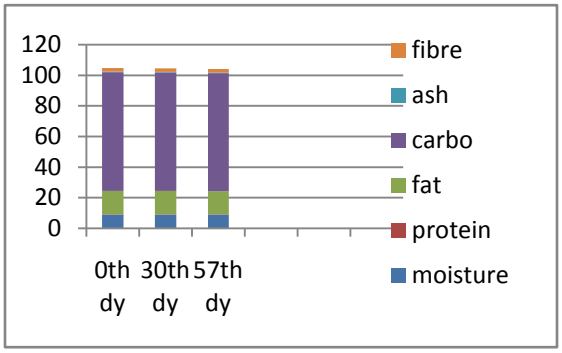
The moisture content is a good parameter for detecting the quality of any food product. Low or high moisture content compromises the quality of food and affect its efficacy. Moisture content was determined by hot air oven method. Generally, the relationship between water content and texture is such that the higher the water content, softer the candy **(27)**.

The protein estimation is a biochemical test for determining the total level of protein in the solution. The total protein concentration is exhibited by a color change of the sample solution to protein concentration, which can then be measured using colorimeteric techniques. The flowers of CT contained appreciable amount of crude fiber (2.1 ± 0.2) and fat (2.5 ± 0.1). A dietary pattern containing low-fat and high-fiber products has been associated with reduced risks of breast cancer.

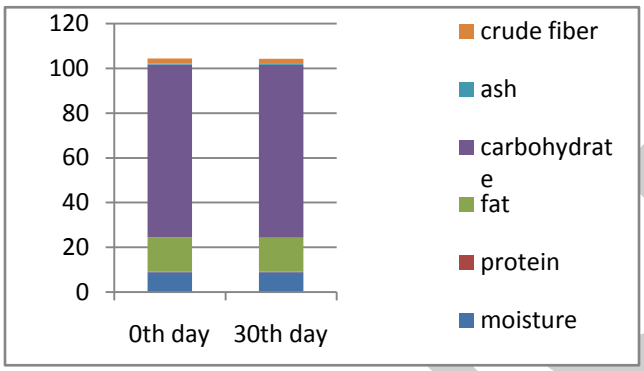
According to **Neda *et al.*,(4)** analysis of Clitoria flower shows that it contains a moisture level of 92.4±0.1protein 0.32 ±0.03 %, carbohydrate 2.23 ±0.3 %, crude fibre 2.1±0.2 %, ash 0.45±0.15 and fat 2.5±0.1.In this study, the extract contains moisture level between 91.4 to 92.5 %, protein 0.32 to 0.36 %, Fat 2.49 to 2.53%, carbohydrate 2.23 to 2.25 % crude fibre 2.19 to 2.3 % and ash contain varies between 0.39 to 0.45 %. Though there is variation in levels they correlate with the literature study mentioned above. The variation may be due various parameters which includes climatic condition, soil condition and pH of the soil.

As per USDA nutritional fact hard candy contains high amount of carbohydrate, 0% protein and 0% fat. The nutritional content of candy contains 77.1 to 77.48 % of carbohydrate and there is not much of a change in other parameters. The analysis was carried at interval of 0^{th} day, 30^{th} day and 57^{th} day. There is no

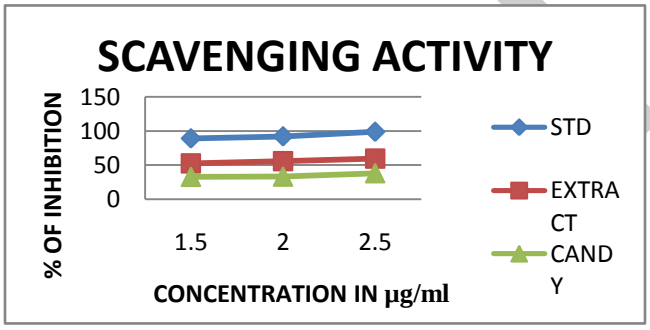
higher amount of variations in the end products. Candy was preserved with citric acid and found to be good till 57th day of analysis while candy without preservative was found to be good till 30th day of analysis. It's given in "fig 1 (a) & (b)"



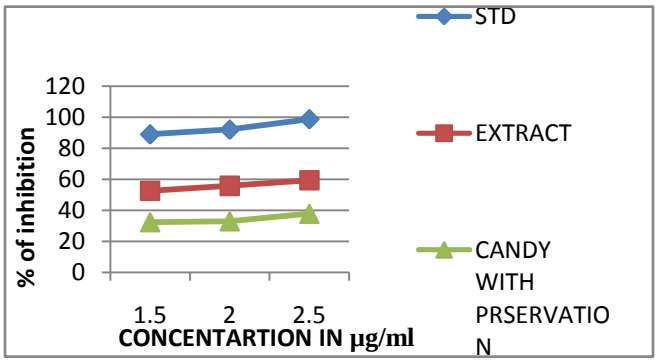
(a)Nutritional analysis of candy with preservatives



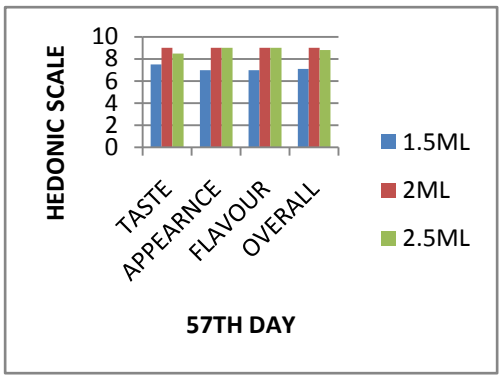
(b) Nutritional analysis of candy without preservatives



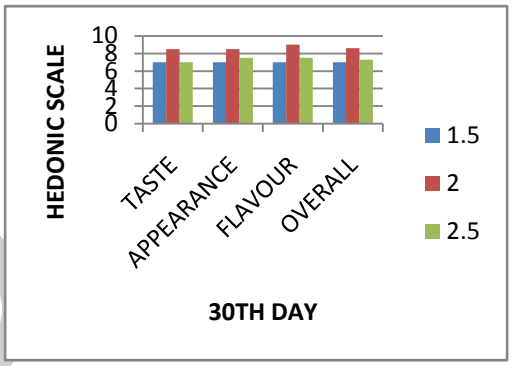
(c) Antioxidant property of candy without preservative



(d) Antioxidant property of candy with preservative



(e) Sensory evaluation of candy with preservative



(f) Sensory evaluation of candy without preservative

Figure 1.Physico-chemical, antioxidant characteristics and sensory evaluation of candy.

3.3 .Anti-microbial Activity:

According to the literature study Clitoria flower was analyzed against micro-organism *Staphylococcus aureus* and the zone if inhibition was found to be 13mm (50µl/well) and 17 mm (100µl/well) **(28).** In this study the extract and extract based candy was analyzed against four micro-organisms. Zone of inhibition (mm) of extract against *E.coli* ranges between 10 to 16 mm and *Pseudomonas aeruginosa* ranges between 8 to 15 mm. The highest inhibition activity was shown against *Staphylococcus aureus* which ranges between 11 to 22 mm. Klebsiella and Candida showed the least inhibitor effect. Antimicrobial activity of candies (with and without preservative) was also analyzed against the same microbes. Candy (with preservative) showed slight higher inhibition effect compared with candy (without preservative). The highest ZOI shown by candy (with and without preservative) is against *Staphylococcus aureus* ranging between 21 to 24 mm for candy with preservative and 15 to 23 mm. The test material was highly active against all test pathogen at higher concentration. The microbial activity of candies is slightly higher than the extract due to the presence of sugar molecules. The given samples were capable to kill all the tested pathogens ranging from 10 mm to 21 mm of zone of inhibition. The test material was highly active against all test pathogen at higher concentration.

3.4. Anti-oxidant Activity:

In most of the studies *Clitoria ternatea* leaves are analyzed for antioxidant property and the result showed activity of 74 % **(29)** whereas in this study flowers are used for analyzing their antioxidant property using DPPH method. The result showed free radical scavenging activity of 59.3. This study proves that antioxidant property of flower is less when compared with leaves activity. Hard candies formulated using extract at various concentration are also analyzed for anti-oxidant activity. Candies (with preservative) showed higher antioxidant activity at 2.5 ml concentration with 38.05 % antioxidant property and candy (without preservative) contains 37.79% of property.

3.5. Sensory Analysis:

The extract based candies were prepared and stored in aluminum foil by standard technique. The samples were evaluated organoleptically. The sensory attributes of the developed product were graded by numerical scoring, on a nine point scale. The results of organoleptic evaluation were reported. The organoleptic evaluation shows same overall acceptability even after 57 days of storage. There is no change in color, flavor and taste. But there is slight difference in the appearance due to moisture absorption. Hence maximum storage period of 57 days in room temperature was analyzed for candy with preservative. For candy with preservative 2.5ml concentration was overall accepted which was in purple-red color while for candy without preservative ,2ml concentration was overall accepted with highest hedonic scale throughout the study .The color of candy without preservative was blue in color with very slight flavor of flower. Sensory evaluation for candy prepared without preservative was done and 30 days of shelf life was found. Though there was reduction in the value but there were no significant changes in any of the sample, over the entire period. Sensory has been done in terms of taste, texture, flavor and overall acceptability on nine point hedonic scale.

IV. CONCLUSION

This study mainly aims in replacing the synthetic colorants which are widely used in food products like cake toppings, ice cream, candies...etc. Natural candies with high antioxidant property and medicinal properties are not commercially available at low cost. Candy equally attracts children and adult age people.

C. ternatea plant can adapt any climatic condition and commonly grown in southern parts of India as ornamental plant. Nowadays activity of food pathogens was very high. This medicinal flower has high level of antimicrobial activity against the common food pathogens such as E.coli, Pseudomonas species. This plant was a traditional herb with many medicinal properties such as diabetic controller, immune booster, improves blood circulation and cures Psoriasis. Research was going on based on its anti cancer activity. In countries like china, Malaysia *C. ternatea* flower was used for making blue colored tea and blue rice. According to India's statistical analysis food products with natural colorants are very less compared other countries. This overall study concluded that the edible-natural colorant can be used in any food compounds replacing synthetic colorants in terms of color, taste and cost economy

ACKNOWLEDGEMENTS

My sincere thanks to and Mr.Krishnaraj,Managing Director of Jayagen Biologic laboratory, Chennai.

REFERENCES

[1] Neelmani Chauhan*, Sourabh Rajvaidhya and B. K. Dubey (2012).“pharmacognostical, phytochemical and pharmacological review on *C. ternatea* for antiasthmatic activity”. IJPSR Vol. 3(2): 398-404.

[2] Varsha jadhav, Swati deshmukh and shivprasad mahadkar (2013) .evaluation of antioxidant potential of C. ternatea ternatea. International Journal of Pharmacy and Pharmaceutical Sciences ISSN-0975-1491 Vol 5, Suppl 2.

[3] *ŠMÍDOVÁ,ČOPÍKOVÁ, MARYŠKA and A. COIMBRA(2003).* Crystals in Hard Candies. *Czech J. Food Sci. Vol. 21, No. 5: 185–191*..

[4] Neda, G. D., Rabeta, M. S. and Ong, M. T. (2013). Chemical composition and anti-proliferative properties of flowers of *C. ternatea Ternatea.* International Food Research Journal 20(3): 1229-1234.

[5] Obasi, n. E., okorocha, c. And orisakwe, o. F.(2013).production and evaluation of velvet tamarind (*dialium guineese wild)* candy. European Journal of Food Science and Technology Vol.1, No.1, pp.1-8.

[6] *Prashant R. Verma, Prakash R. Itankar*, Sumit K. Arora, (2013).*Evaluation of antidiabetic antihyperlipidemic and pancreatic regeneration, potential of aerial parts of *C. ternatea ternatea.*Rev Bras Farmacogn 23:819-829.

[7] Rabeta, M. S. and An Nabil, Z ((2013).Total phenolic compounds and scavenging activity in *C. ternatea* and *Vitex negundo* linn. International Food Research Journal 20(1): 495-500 .

[8] Sarumathy.k, .Dhana Rajan.MS, Vijay,J.Jayakanthi.T. (2011).Evaluation of phytoconstituents, nephro-protective and antioxidant activities of *C. ternatea ternatea*. Journal of Applied Pharmaceutical Science 01 (05); 164-172.

[9] Subramanian.M.S and P.Prathyusha(1987).Pharmaco Phytochemical Characterization of C. ternatea Linn. International Journal of Pharm-Tech Research CODEN (USA): IJPRIF ISSN : 0974-4304 Vol. 3, No.1, pp 606-612740–741.

[10] Manalwasha Deka, A.K.Medhi2, J.C.Kalita, K.K.Sarm and Leena Deka (2013).Proximate Analysis of Primary Metabolites in Different Parts of *C. ternatea* L. A Comparative Study. IAAST; Vol 4 [3] September: 62-67.

[11] Manju Lata Zingare1, *, Prasanna Lata Zingare1, Ashwash Ku Dubey2, Md. Aslam Ansari(2013).C. ternatea (APARAJITA): A REVIEW OF THE ANTIOXIDANT, ANTIDIABETIC AND HEPATOPROTECTIVE POTENTIALS. IJPBS |Volume 3| Issue 1 .

[12] Sudhir Sharma1*, Neelima Rathi1, Barkha Kamal2, Dipika Pundir3, Baljinder Kaur4 and Sarita Arya (2010).Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology- a review.AGRICULTURE AND BIOLOGY JOURNAL OF NORTH AMERICAWASSN Print: 2151-7517, ISSN Online: 2151-7525, doi:10.5251/abjna. 1.5.827.833.

[13] Kuntapas Kungsuwan, Kanjana Singh, Somchai Phetkao and Niramon Utama-ang(2014).Effects of pH and anthocyanin concentration on color and antioxidant activity of *C. ternatea* extract. Food and Applied Bioscience Journal, 2 (1): 31-46.

[14] L. Kamilla, a S.M. Mnsor, a S. Ramanathan,a and S. Sasidharan (2009).Antimicrobial Activity of *C. ternatea* (L.) Extracts. *Pharmacologyonline* 1: 731-738.

[15] Hiremath,J.B and Rokhade,A.K.(2012).Preparation of sapota candy. International Journal of Food, Agriculture and Veterinary Sciences. ISSN: 2277-209X; Vol. 2 (1) January-April, pp.107-112

[16] Gowd M. J. S Pratap, Kumar M. G. Manoj.(2012).Evaluation of three medicinal plants for anti-microbial activity. www.ncbi.nlm.nih.gov/pubmed/23723653

[17] Daisy.P, Kanakappan Santosh and M. Rajathi.(2009). Antihyperglycemic and antihyperlipidemic effects of *C. ternatea* Linn. in alloxan-induced diabetic rats. African Journal of Microbiology Research Vol. 3 (5) pp. 287-291.

[18] Anis Alam Siddiqui, M. H. R. Bhuiyanand M. Easdani (2003).Ginger (*Zingiber officinale*) reserve and candy development. Bangladesh research publications journal ISSN:Volume: 7, Issue: 3, Page: 283-290.

[19] S. P. Anand., A.Doss and v. Nandagopalan (2011).Antibacterial studies on leaves of C. ternatea linn. IJABPT;volume: 2: issue-3: july-sept ; Issn 0976-4550.

[20] Angkana Tantituvanont*, Pornpen Werawatganone, Pacharaporn, KasornManopakdee (2008) .Preparation and stability of butterfly pea color extract loaded in microparticles prepared by spray drying. *Thai J. Pharm. Sci. 32 59-69.*

[21] Babu uma, (2009).Phytochemical analysis and antimicrobial activity of *clitorea ternatea* linn against extended spectrum betalactamase producing enteric and urinary pathogen. Asian Journal of Pharmaceutical and Clinical Research, Vol.2 Issue 4, ISSN 0974-2441.

[22] Marion m. Bradford (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Proteins Utilizing the Principle of Proteins-Dye Binding. ANALYTICAL BIOCHEMISTRY 72, 248-254.

[23] Van Handel E. (1985): Rapid determination of total lipids in mosquitos. J. Am. Mosq. Control .Assoc. 1: 302-304

[24] Rai (1982). Indian standard method for determination of crude fibre content in food product. WAS 10226-2.

[25] Perez, C., Pauli, M. and Bazerque, P. (1990). An antibiotic assay by agar-well diffusion method. Acta Biologiae et Medicine Experimental, 15:113-115.

[26] Von Gadow, A.; Joubert E.; Hansmann C. F. 1997.Comparwason of the antioxidant activity of rooibostea (Aspalathus linearis) with green, oolong and black tea. Food Chemistry 60, 73-77.

[27] Helga Hoffmann, Andrea Felgne (2009).Determination of Water Content in Candies" AnalytiX Volume 10 Article 4-sigmaaldrich .

[28] Bhushan Pahune*, Kamlesh Niranjane, Kishor Danao, Megha Bodhe and Vijayashree Rokade (2013).Antimicrobial activity of clitoria ternatea l. Flower extract and use as a natural indicator in acid base titration. J. Nat. Prod. Plant Resour., 3 (2):48-51.

[29] Jayachithra and Krithiga. (2012).Study on antioxidant property of selected medicinal plants. Int.J.Med.Arom.Plants, Vol.2,No.3,pp.495-500.