Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2023.v11.i03.A16

COMPARATIVE STUDY OF LIPOLYTIC EFFICACY AND ANTI-INFLAMMATORY ACTIVITY OF NON-CAFFEINATED TEA PREPARED FROM *ROSA INDICA*, *CLITORIA TERNATEA* AND *CINNAMOMUM TAMALA* USING NEW SMART PHONE BASED DIGITAL COLORIMETRIC METHOD

Banani De*[1] and Haimanti Roy[1]

[1*]Department of Chemistry, Bijoy Krishna Girls' College, University of Calcutta, West Bengal, India.

ABSTRACT

The purpose of this study was to evaluate and compare the anti-inflammatory activity and lipolytic potential of aqueous extract of *Rosa Indica* (rose), *Clitoria Ternatea* (butterfly pea) and *Cinnamomum Tamala* (Indian bay leaf). Anti-inflammatory activity was evaluated usingprotein (egg) denaturation method taking BSA as standard reference. A smartphone application (Photo Metrix PRO app) based on partial least squares regression with the histograms of the red-green-blue images was used to determine the concentration of protein. Lipolytic activity was assessed using milk as dietary substrate and change in acid value post addition of above mentioned extracts in three dilutions (100%, 50%, 10%) were measured titrimetrically. *Rosa Indica* recorded higher anti-inflammatory activity (at 100cg/ml concentration) followed by *Cinnamomum Tamala.* Highest acid value was recorded on addition of rose extract followed by butterfly pea and Indian bay leaf in order.

KEYWORDS

Lipolytic activity, Anti-inflammatory activity, *Rosa indica*, *Clitoria ternatea, Cinnamomum tamala* and Photo metrix PRO.

Author for Correspondence:

Banani De,
Department of Chemistry,
Bijoy Krishna Girls' College,
University of Calcutta, West Bengal, India.

Email: bosebanani@gmail.com

INTRODUCTON

Lipolysis is a catabolic process which involves hydrolysis of lipids (triacylglycerols) into free fatty acids and glycerol. Lipolysis generally takes place in the adipose tissue[1]. Lipolysis caused by lipase activities is indirectly responsible for obesity, diabetes 2, non-alcoholic fatty liver disease, cancer, heart diseaseetc[2]. During pasteurization milk lipase is destroyed to prevent hydrolysis during storage[3]. The extent of lipolysis in milk and dairy products is usually evaluated by measuring free fatty acid content.

This study focuses on lipolytic activity exhibited by rose, butterfly pea, bay leaf tea on a dietary substrate, milk.

Inflammation is a complex biological response of body tissues to pathogens, damaged cells or irritants[4]. Inflammation is identified by swelling or tumour, redness, heat and pain[5]. A cell injury is repaired by inflammation and it cleanses damaged necrotic cells and tissues[6]. Popular folk medicine used in the treatment of inflammation are leaves of myrtle (Myrtus), bark of willow tree (Salix) , poplar (Populus), and meadow sweet (Spirea) etc[7]. Some commonly used non-selective non-steroidal anti-inflammatory drugs are- salicylic acid derivatives such as acetylsalicylic acid (aspirin) and sulfasalazine; para-aminophenol derivatives such as acetaminophen; fenamates such as mefenamic acid; propionic acid derivatives such as ibuprofen, naproxen, dexibuprofen, ketoprofen, flurbiprofen; enolic acid (oxicam) derivatives such as piroxicam, meloxicam[8].

More work is done with different tea especially green tea in this regard. This work deals with different types of non-caffeinated herbal tea. In this study the assorted products chosen are – (A) Rose (scientific name: *Rosa Indica*), (B) Butterfly pea (scientific name: *Clitoria Ternatea*), (C) Indian bay leaf (scientific name: *Cinnamomum Tamala*).

Rose (*Rosa Indica*)

Rose plant is grown in the garden and sometimes indoor and is used for ornamental purpose as well as industrial (rose water, perfume), medicinal purposes[9]. There are more than 100 species of roses. It is a woody perennial plant of genus Rosa and family Rosaceae that can be erect shrubs, climbing with stems which are armed with sharp prickles[10]. Rose flowers have anti-depressant, anti-spasmodic, aphrodisiac, astringent, increases bile production, cleansing, anti-bacterial and anti-septic properties. Rose hip tea is used to cure diarrhea and rose petals are used as sedative, anti-inflammatory and anti-parasitic. Rose is used as a good supportive tonic for heart, and also used for lowering cholesterol. The anti-inflammatory properties of rose petals can cure sore throats or ulcers. They can stimulate the liver and the rose petal extract is used as eye wash in burning sensation of eyes[9]. The effective and nutritional components of rose mainly contains flavonoids, triterpenes, tannins, phenolic acids, polysaccharides, fatty acids, organic acids, carotenes and vitamins[11]. Rose petals have anti-oxidant activity. Rose petal extracts are rich in phenolic compounds like *p*-coumaric acid and chlorogenic acid[12].

Butterfly pea (*ClitoriaTernatea*)

Clitoria Ternatea is also an ornamental herbaceous perennial climber plant[13]. *Clitoria Ternatea* flower is a tropical flower which belongs to kingdom Plantae, phylum Tracheophyta, class of Magnoliopsida and family Fabaceae[14]. This flower is rich in blue anthocyanin and its bioactive compounds are responsible as antidiabetic, anti-cholesterol, anti-depressant, anticonvulsant, memory enhancing, anti-inflammatory and antioxidant activities[15,16]. Anthocyanin is a water-soluble flavonoid with a diphenylpropane skeleton ($C_6C_3C_6$) and it can be used as natural food additives[17]. The flower can be found single or paired, with color variation from white, mauve, light blue to dark blue[14]. Since ancient time, *Clitoria Ternatea* is used as a natural cure for many diseases and natural food additive. This flower contains 2.5% at, 2.2% carbohydrate, 2.1% fiber and 0.32% protein and 92.4% moisture content. These flowers also contain calcium (3.09mg/g), magnesium (2.23mg/g), potassium (1.25mg/g), zinc (0.59mg/g), sodium (0.14mg/g) and iron (0.14mg/g)[18]. Major constituents of this flower are alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids and phenols[19]. Malonylated flavonol glycosides were also present in the petal of *Clitoria Ternatea* flower[20]. Some fatty acids and various phytosterol are present in this flower. Polyphenols such as flavonol glycosides, myricetin, quercetin, phenolic acids and kaempferol are present inthe flowers[14,21,22].

Indian Bay Leaf (*Cinnamomum Tamala)*

Historically it is one of the oldest known spices. These are the dried aromatic leaves which are used as major ingredient of Indian cooking. It has a taste like clove and odor like faint pepper. These are kept in clothes and chewed to disguise bad mouth odor[23]. About 350

species of genus *Cinnamomum* are there in the world. This plant grows in the tropical and subtropical Himalayas, the Khasi hills, the Nilgiri hill and at the foot of the Sikkim Himalayas[24]. It is an Indian bay leaf which is commonly known as *tejpata*. These leaves have antidiarrheic, antitumor, anti-arthritic, antiparasitic, gastro urinary, antioxidant, chemopreventive and gastroprotective properties. It belongs to the Lauraceae family of plants. Sudarshanchoorna and Chandraprabhavati were two formulations made from this plant in ancient day. These leaves exhibit good anti-inflammatory activity[25]. Furanosesquiterpenoids are the main constituents in the leaf essential oils of *Cinnamomum Tamala* leaves[23]. Furanogermenone (59.5%) wasrecognized to be the amplest compound in the essential oil of *Cinnamomum Tamala* followed by caryophyllene (6.6%), sabinene (4.8%), germacrene D (4.6%) and curcumenol (2.3%). The leaf oil had an innumerable amount of sesquiterpenoids (96.8%), prevailed by furanosesquiterpenoids (79.3%), namely furanodienone (46.6%), curzerenone (17.6%), furanodiene (1.8%), and curzerene (1.2%)[25].

Studies have been executed *in vivo* and *in vitro* but more information is still needed. Hence in this research a comparative study was performed with *RosaIndica*, *Clitoria Ternatea*, *Cinnamomum Tamala* with aqueous extract at different concentration:

100% sample solution

50% sample solution

10% sample solution

A smart phone application Photo Metrix PRO newly developed in Brazil performs image acquisition and treatment of data obtained in the device itself. With this smart phone application, based on RGB system, it is possible to prepare the calibration and determine the analyte concentration of interest in the sample[26]. Generally, the digital images are based on the red-green-blue (RGB) color system, where each color channel has an 8-bit scale. Thus, each pixel can assume one of 2^8 possibilities of intensity values (0-255)[27]. All other colors generated due to mixing of these three colors can be seen within a visible spectrum region. Color histograms are used as a source of information on RGB images. It narrates the statistical distribution of the pixels as a function of the color component[28]. The feature of color histograms is that it can be used as input data for the multivariate analysis since it has a one-dimensional data structure similar to a spectrum.

In this paper, an alternative method of detecting denaturation of protein is described based on RGB images acquired with a smart phone.

MATERIAL AND METHODS

Collection of sample and chemicals

Dried leaves of Indian bay leaf were collected from the local market of Howrah. Rose and butterfly pea flower were collected from Jagannath Ghat near Howrah station. Milk (Amul Slim 'n' Trim double toned milk) and eggs were also collected from the local market of Howrah.

BSA and all the chemicals used in this experiment were purchased from Merck Specialities Private Limited.

Chemical apparatus

A burette, a burette stand, 5ml pipette, 2ml pipette, 500ml beaker, nine 100ml beaker, 10 conical flask, 100 ml volumetric flask, few test tubes were used in this experiment.

Preparation of sample

The collected Indian bay leaves (*Cinnamomum Tamala)* were cut into small pieces. The rose petals were separated from rose flowers (*Rosa Indica*) and the blue petals of butterfly pea (*Clitoria Ternatea)* flower were separated. These three samples (10g of each sample) were added to boiled distilled water in three respective beakers (each containing 60ml) and boiled for 5 minutes. Then they were soaked for 30 minutes. Then the sample solutions are cooled in room temperature. The basic concentration was 100cg/ml. From each 100% solution three solutions were made: 1) 100% sample solution, 2) 50% sample solution, 3)10% sample solution. Hence 9 sample solutions were made.

EXPERIMENTAL PROCEDURE

Determination of free fatty acid content

By the slightly modified titration procedure of Breazeale and Bird the free fatty acid content of the fat was determined[29]. 2ml milk was added in 4ml of sample solution and then 5ml of buffer (pH 7.0) was added and together kept for 15 minutes, after then the mixture was titrated to the phenolphthalein end point with 0.01 N KOH by using a burette. The titration was performed with 9 sample solutions and the blank was water.

Determination of acid value

Acid value= $(MW_{KOH} *N*V)/W_S$

(W_S)=Sample weight = 1.82g

(N)= Normality of KOH = 0.01

(V)=Volume of KOH solution

(MW_{KOH})= Molecular weight of KOH = 56.1056g/mol

Determination of anti-inflammatory activity

In vitro anti-inflammatory activity was conducted by protein denaturation method given by Mizushima and Kobayashi (1968). The reaction mixture (5ml) contained 0.2ml of egg albumin, 2.8 ml of phosphate-buffered saline (pH 7) and 2ml of plant extract at various concentrations (10, 50, 100cg/ml). As a control, a similar volume of double distilled water is employed. Then the mixture was heated at 70°C for 5 minutes. Then it was cooled and their concentration was measured at 660nm. The concentration of protein was recorded from the Photo Metrix PROapp.

Bovine serum albumin (BSA or Fraction V) is a serum albumin protein which is derived from cows. This is used as standard protein concentration in this experiment.

The percentage of inhibition of protein denaturation was calculated by using the following formula:

Inhibition = (Cc(660nm)-Ct(660nm) ÷ Cc (660)) ×100

Cc (Concentration of control solution), Ct (Concentration of the test sample)

Statistical analysis

The experiment was performed in triplicate and data from three different experiments were subjected to analysis of variance (ANOVA) ($P < 0.05$).

RESULTS AND DISCUSSION

Lipolytic activity

From this diagram it is clear that the free fatty acid content of milk decreased significantly ($P < 0.05$) with decrease in concentration of added aqueous extract of each variety. The maximum free fatty acid content was recorded on addition of 100% *Rosa Indica* extract to milk. Whereas the minimum free fatty acid was obtained post addition of 10% of *Rosa Indica* extract. Hence the range of acid value was maximum in case of *Rosa Indica*.

Magnitude of acid value obtained on addition of 100 % *Rosa Indica* extract to milk was followed by *Clitoria Ternatea* and *Cinnamomum Tamala*. Therefore 100% *Rosa Indica* extract shows maximum effectiveness in increasing the acid value in milk and hence exhibited maximum lipolytic effect. Though 100% *Rosa Indica* extract shows higher activity than the other two, *Clitoria Ternatea* extract recorded higher lipolytic activity than other two varieties both in 50% and 10% extract.

Anti-inflammatory activity

In vitro anti-inflammatory experiment was conducted by the method of protein denaturation using egg albumin. BSA was used as the reference. Concentration of protein in terms of BSA equivalent recorded at 660nm is shown in the bar diagram (Figure No.12). With dilution of added extract concentration in egg albumin, protein concentration was found to be increasing significantly ($P < 0.05$) and on increasing concentration of herbal extract, the concentration of protein was noted to be decreasing in value. Thus higher value of protein concentration indicates higher coagulation. Hence higher concentration of herbal extract added to albumin causes less coagulation. *Rosa Indica* shows the highest anti-inflammatory activity (at 100cg/ml concentration) and hence higher percentage of inhibition. *Cinnamomum Tamala* also exhibited a considerable percentage of inhibition to coagulation of albumin. Only *Clitoria Ternatea* recorded a significantly very low anti-inflammatory activity for all the concentrations.

Table No.1: Free fatty acid content of sample solution

S.No	Concentration of extract	*Rosa Indica* extract	*Clitoria Ternatea* extract	*Cinnamomum Tamala* extract	Water
1	100% conc	11.43±0.112	10.75±0.106	10.17±0.103	7.64±0.077
2	50% conc	9.27±0.093	9.55±0.096	9.12±0.092	
3	10% conc	7.79±0.078	8.07±0.081	7.89±0.079	

Table No.2: Reading from the photo metrix PRO app at 660nm

S.No		*Rosa Indica* extract		*Clitoria Ternatea* extract		*Cinnamomum Tamala* extract	
	Conc. of herbal extract	Conc. of protein (BSA equivalent)	% of inhibition	Conc. of protein(BSA equivalent)	% of inhibition	Conc. of protein (BSA equivalent)	% of inhibition
1	100%	131.846	67.84243902 ± 3.367	325.155	20.69390244 ± 1.021	161.906	60.51073171 ± 3.011
2	50%	138.969	66.10512195 ± 3.291	350.975	14.39634146 ± 0.735	210.142	48.74585366 ± 2.461
3	10%	256.649	37.40268293 ± 1.886	396.655	3.254878049 ± 0.151	230.511	43.77780488 ± 3.845

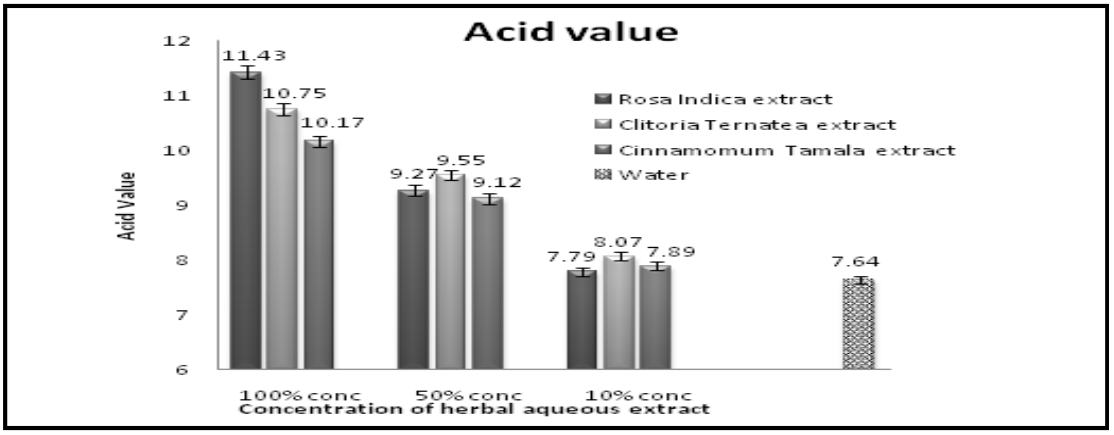

Figure No.1: Acid value bar diagram

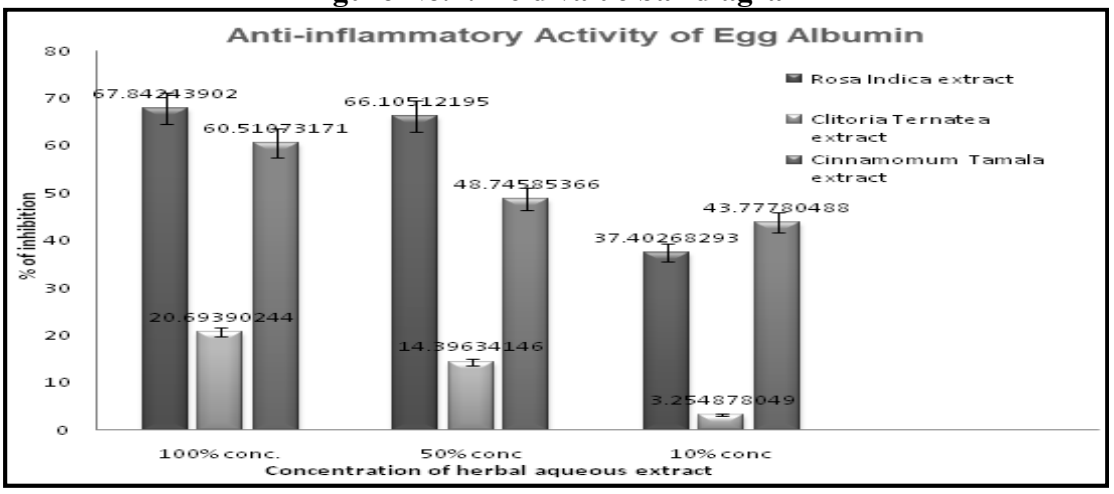

Figure No.2: Anti-inflammatory activity of egg albumin

CONCLUSION

Of all the three varieties of tea evaluated in this study *Rosa Indica* and *Clitoria Ternatea* are common flowers and *Cinnamomum Tamala* is a naturally available spice popularly used in cooking. Aqueous extracts of these herbs contain certain compounds which are responsible for lipolytic and anti-inflammatory activity. From the above comparative study of these properties it can be concluded that at 100% concentration (100cg/ml) *Rosa Indica* exhibited highest anti-inflammatory and lipolytic activity. *Clitoria Ternatea* extract though recorded significant lipolytic activity at all concentrations turned out to be least effective as an anti-inflammatory agent compared to other two. *Cinnamomum Tamala* revealed considerable lipolytic activity and a good anti-inflammatory activity though lesser than *Rosa Indica.*

These observations especially demand further study to determine the exact components responsible for these activities. Further research may help to find the dosage and proper ratio of effective formulation with other extracts to effectively function as a lipolytic agent and prevent inflammation problems.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Chemistry, Bijoy Krishna Girls' College, University of Calcutta, West Bengal, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

BIBLIOGRAPHY

1. Saponaro, Gaggini M, Carli F, Gastaldelli A. The subtle balance between lipolysis and lipogenesis: A critical point in metabolic homeostasis, *Nutrients,* 7(11), 2015, 9453-9474.
2. Grabner G F, Xie H, Schweiger M, Zechner R. Lipolysis: Cellular mechanisms for lipid mobilization from fat stores, *Nature Metabolism,* 3(11), 2021, 1445-1465.
3. Deeth H C. Lipolysis in milk and dairy products- A research journey, *Food Australia,* 54(10), 2002, 433-436.
4. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs, *Oncotarget,* 9(6), 2018, 7204-7218.
5. Hurley J V. Acute inflammation: The effect of concurrent leucocytic emigration and increased permeability on particle retention by the vascular wall, *British Journal of Experimental Pathology,* 45(6), 1964, 627-633.
6. Eming S A, Krieg T, Davidson J M. Inflammation in wound repair: Molecular and Cellular Mechanisms, *Journal of Investigative Dermatology,* 127(3), 2007, 514-525.
7. Hedner T, Everts B. The early clinical history of salicylates in rheumatology and pain, *Clinical Rheumatology,* 17(1), 1998, 17-25.
8. Wohrl S. NSAID hypersensitivity-recommendations for diagnostic work up and patient management, *Allergo Journal International,* 27(4), 2018, 114-121.
9. Chahar S. Rose: Ornamental as well as medicinal plant, *Journal of Research in Agri and Ani Sci,* 4(1), 2016, 8-10.
10. Mishra R P, Arshad M, Sami A. Antibacterial properties of *rosa indica* (l.) stem leaves, and flowers, *Journal of Pharmaceutical and Biomedical Sciences,* 12(12), 2011, 15-20.
11. Wang Y, Zhao Y, Liu X, Li J, Zhang J, Liua D. Chemical constituents and pharmacological activities of medicinal plants from rosa genus, *Chinese Herbal Medicines,* 14(2), 2022, 187-209.
12. Shameh S, Hosseini B. Phytochemical composition and antioxidant activity of petals of six rosa species from Iran, *Journal of AOAC Inter,* 101(6), 2018, 1788-1793.
13. Mukherjee P K, Kumar V, Kumar N S, Heinrich M. The ayurvedic medicine *clitoriaternatea*- from traditional use to scientific assessment, *Journal of Ethnopharmacology,* 120(3), 2008, 291-301.

14. Jamil N, Zairi M N M. Influences of environmental conditions to phytoconstituents in *clitoriaternatea* (butterfly pea flower)-A review, *Journal of Science and Technology,* 10(2), 2018, 208-228.
15. Jeyaraj E J, Lim Y Y, Choo W S. Extraction methods of butterfly pea (*clitoriaternatea*) flower and biological activities of its phytochemicals, *Journal of Food Science and Technology,* 58(6), 2021, 2054-2067.
16. Rahman A K M S, Saha R, Talukder N, Khaleque S M A, Ali H A. Bioactivity guided cytotoxic activity of *clitoria ternatea* utilizing brine shrimp lethality bioassay, *Bangladesh Journal of Physiology* and *Pharmacology,* 22(1/2), 2006, 18-21.
17. Cisowska A, Wojnicz D, Hendrich A B. Anthocyanins as antimicrobial agents of natural plant origin, *Natural Product Communications,* 6(1), 2011, 149-156.
18. Neda G D, Salleh R M. Chemical composition and anti-proliferative properties of flowers of *clitoria ternatea*, *Inter Food Res Jour,* 20(3), 2013, 1229-1234.
19. Manjula P, Ch. Mohan, Sreekanth D, Keerthi B, Prathibha Devi B. Phytochemical analysis of *clitoria ternatea* linn, a valuable medicinal plant, *The Journal of Indian Botanical Society,* 92(3/4), 2013, 173-178.
20. Kazuma K, Noda N, Suzuki M. Malonylated flavonol glycosides from the petals of *clitoriaternatea*, *Phytochemistry,* 62(2), 2003, 229-237.
21. Al-Snafi A E. Pharmacological importance of *clitoria ternatea*- A review, *IOSR Journal of Pharmacy,* 6(3), 2016, 68-83.
22. Tuan Putra T N M, Zainol K, Mohdlsa N S, Maidin N M. Chemical characterization of ethanolic extract of butterfly pea flower (*clitoria ternatea*), *Food Research,* 5(4), 2021, 127-134.
23. Shah M, Panchal M. Ethnopharmacological properties of *cinnamomum tamala* - A review, *International Journal of Pharmaceutical Sciences Review and Research,* 5(3), 2010, 141-144.
24. Jayaprakasha G K, Rao L J M, Sakariah K K. Volatile constituents from cinnamomum zeylanicum fruit stalks and their antioxidant activities, *Journal of Agricultural and Food Chemistry,* 51(15), 2003, 4344-4348.
25. Mohanan M, Sakthi G, Anusha M, Vinothini A. *In-vitro* analysis of anti-inflammatory activity of *cinnamoumumtamala* and ointment formulation, *International Journal for Research Trends and Innovation,* 7(6), 2022, 1743-1750.
26. Bock F C, Helfer G A, Magnus V S, Teichmann A, Ferrao M F, Costa A. Photo metrix: An application for univariate calibration and principal components analysis using colorimetry on mobile devices, *Journal of the Brazilian Chemical Society,* 28(2), 2016, 328-335.
27. Morais C, Silva S R B, Vieira D S, Lima K. Integrating a smart phone and molecular modeling for determining the binding constant and stoichiometry ratio of the iron (II)-phenanthroline complex: An activity for analytical and physical chemistry laboratories, *Journal of Chemical Education,* 93(10), 2016, 1760-1765.
28. Morais C, Lima K. Determination and analytical validation of creatinine content in serum using image analysis by multivariate transfer calibration procedures, *Analytical Methods,* 7(16), 2015, 6904-6910.
29. Breazeale D F, Bird E W. A study of methods for the determination of acidity in butter fat, *Journal of Dairy Science,* 21(7), 1938, 335-344.

Please cite this article in press as: Banani De and Haimanti Roy. Comparative study of lipolytic efficacy and anti-inflammatory activity of non-caffeinated tea prepared from *rosa indica, clitoria ternatea* and *cinnamomum tamala* using new smart phone based digital colorimetric method, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 11(3), 2023, 114-120.