Development and Evaluation of Herbal Formulation for Obesity

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ABSTRACT
Obesity is one of the most prevalent health concerns among all age groups and populations worldwide, resulting in a significant increase in mortality and morbidity related to metabolic disorders. The present study involves formulation development and evaluation of effervescent granules prepared by using herbal extract mixture (hydro-alcoholic extract of fruits of Garcinia indica, seeds of Achyranthes aspera & raw beans of Coffea arabica L.) along with its in-vitro anti-obesity activity. Hydro-alcoholic extract of three plant materials were prepared by soxhlet extraction method. Effervescent granules were prepared & their evaluation was done for physical properties like angle of repose, bulk density, tap density, car’s index, hausner’s ratio & effervescent cessation time. All the batches showed acceptable physical properties. Furthermore, In-vitro lipase inhibitory and α-amylase inhibitory activities were studied for herbal extract mixture at different concentration (50, 100, 150, 200, 250, 300) µg/ml. Herbal extract mixture at concentration of 300 µg/ml showed highest lipase and α amylase inhibitory action, i.e., 83.32± 10.47 and 69.14± 7.22 respectively. This study is possibly advantageous as the bottom line for further formulation for herbal extract based effervescent products.

Keywords: Herbal extract mixture, Effervescent granules, α amylase, Pancreatic lipase.

INTRODUCTION
Obesity is considered as a principal public health concern and ranked as the fifth foremost reason for death globally.1 Medically, obesity is a condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems.2 Overweight and obesity can be considered as a cosmetic problem associated with various other lifestyle disorders, like diabetes, dyslipidaemia, hypertension, cardiovascular diseases, musculoskeletal disorders, cancer, etc.3

Achyranthes aspera is an important perennial medicinal herb found as a weed belonging to the family Amaranthaceae. In traditional system of medicines roots, seeds & shoots of this plant are identified for their medicinal properties.4 Wide numbers of isolated phytochemical constituents are identified for pharmacological activities like diuretic, laxative, purgative, hepatoprotective, anti-asthmatic, anti-allergic properties. Traditionally, the plant is used in treatment of diarrhea, dysentery, asthma, cough, dropsy, ulcers, piles, arthritis, scabies snake bite and other skin diseases.5 Seeds contain Saponin A and B. Saponin A was identified as D-Glucuronic Acid and saponins B was identified as β-D-galactopyranosyl ester of D Glucuronic Acid.6

Garcinia indica (dried rind known as ‘kokum’), a tropical fruit, can be viewed as a wonder berry that has a pleasant, tangy-sweet taste and a myriad of health benefits. Traditionally, kokum is used in herbal medicines to treat diarrhoea, inflammatory ailments, bowel problems, rheumatic pains and to prevent hyper perspiration. Kokum juice from the rind is used against piles, colic problems, dysentery and diarrhoea.8,9 Kokum fruit is a potential source of hydroxy citric acid, the much valued anti-obesity agent. Other constituents found in fruits are garcinol, isogarcinol, citric acid, oxalic acid, xanthochymol, isoxanthochymol.10 Recently, hydroxy citric acid has been found to be used as a potent metabolic regulator of obesity and lipid abnormalities in mammalian system.11 One of the common traditional forms of coffee is green coffee (Coffea arabica L) extract (GCE) that prepared from green or raw (unroasted) coffee bean.12 Green coffee contains chlorogenic, caffeine, theophylline, trigonelline and theobromine melanoids, protein, lipids and minerals. Chlorogenic acid together with caffeine in green coffee are thought to have many health benefits including antiobesity, anti-tumour, anti-diabetic, anti-hypertensive, anti-inflammatory and anti-microbial effects.13,14

Oral route of administration is considered the most suitable route for drug delivery with highest patient’s compliance.15 Effervescent granules mainly contains the medicinal agent in a dry mixture usually composed

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of sodium bicarbonate, tartaric acid & citric acid. When added to water, the acids and the base react to liberate carbon dioxide, resulting in effervescence. The resulting carbonated solution normally masks the undesirable taste of the medicinal agent. Since the decoction product is not palatable enough, the addition of an effervescent vehicle to the granule formulation may help to improve the taste and palatability of the solution formed due to its property of rapidly dissolving granules in solution. This product contains sweetener and available in several flavours which is prospective to elevate the rates of patient’s compliance in taking the medication, especially for the pregnant women.

Absorption of dietary triglycerides in small intestine involves their hydrolysis into free fatty acids by pancreatic lipase enzyme. On the other side, absorption of carbohydrates in small intestine involves their hydrolysis into simple sugars by amylase enzymes. Inhibition of these enzymes could be beneficial in weight control and weight loss treatments.

**MATERIALS AND METHODS**

**Collection & identification of plant materials**

Drug samples of *Garcinia indica* fruit, *Achyranthes aspera seeds & Coffee arabica L.* beans (raw) were procured from local market of Vadodara, Gujarat, India in the month of October 2021. Drug samples were identified by comparing its morphological characters described in various standard texts. All the samples were further authenticated by botanist Dr. P K Patel, Head of Department, Sheth P. T. Arts and Science College, Godhra, Gujarat bearing voucher specimen number PPDC/COG/2021/001, PPDC/COG/2021/002 & PPDC/COG/2021/003 as *Garcinia indica fruit, Achyranthes aspera seeds & Coffee arabica L.* beans belonging to the family Clusiaceae, Amaranthaceae & Rubiaceae respectively. All the samples were dried under sunlight for 2 days to minimize moisture content & then powdered & powder sample was passed through 20 # sieve & stored in airtight container at room temperature for further use.

**Extraction Procedure**

100 gm each of powder of drug samples of *Garcinia indica fruit, Achyranthes aspera seeds & Coffee arabica L.* beans (raw) were taken in separate round bottom flasks & extracted with 500 ml of n-hexane by using soxhlet extraction method for 6 hours. After 6 hours contents were filtered & filtrate was discarded and marc from each flask was dried. Dried marc was again transferred into separate round bottom flasks & extracted with 500 ml of methanol: water (70:30) by using soxhlet extraction method for 6 hours. After 6 hours contents were filtered & filtrates were completely evaporated to obtain dried mass (Extract).

**Procedure for preparation of effervescent granules**

Sodium phosphate, tartaric acid, citric acid and sodium bicarbonate were weighed accurately according to calculation. All the ingredients were mixed in ascending order of their weights, by trituration. Porcelain dish was placed on a water bath and heated to boiling point. Powder mixture was placed in to the hot porcelain dish, which was kept on a boiling water bath. The powder mixture was stirred with the help of spatula, for 1 to 5 minutes i.e. until a damp (coherent) mass was formed. The damp mass was immediately passed through the sieve, by placing on a butter paper. Granules were dried by spreading on a sheet of paper, in hot air oven at temperature not exceeding 60°C & finally packed in air tight container.

| Table 1: Different Batches of Herbal Effervescent Granules |
|-----------------------------------------------------------|
| **Ingredients**                                           | Batch 1 | Batch 2 | Batch 3 | Batch 4 | Batch 5 | Batch 6 |
| Extract Mixture (Each 0.5 gm)                            | 1.5     | 1.5     | 1.5     | 1.5     | 1.5     | 1.5     |
| Citric acid (gm)                                         | 2       | 2.5     | 2.5     | 2       | 2       | 2.5     |
| Tartaric acid (gm)                                       | 2.5     | 2.5     | 3.5     | 3.5     | 3       | 2       |
| Sodium Bicarbonate (gm)                                  | 5       | 4       | 3.5     | 4       | 4.5     | 5       |
| Aspartame (gm)                                           | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     |
| PEG 600 (ml)                                             | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     | 0.5     |
| Lemon oil (ml)                                           | 0.5     | -       | 0.5     | -       | 0.5     | -       |
| Peppermint oil (ml)                                      | -       | 0.5     | -       | 0.5     | -       | 0.5     |

**Evaluation of Formulated Herbal Effervescent Granules**

Prepared herbal granules were evaluated for various evaluation parameters.

**Angle of repose**

Angle of repose has been used to characterize the flow properties of solids. Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles. The angle of repose is the
constant, three dimensional angle assumed by a cone like pile of material formed. Angle of repose was determined by funnel method. The blend was poured through a funnel that can be raised vertically until a maximum cone height (h) was obtained. Radius of the heap was measured and the angle of repose was calculated. It is the angle produced between the heap of the pile and base.

\[ \tan \theta = \frac{h}{r} \]

Where, \( h \) = height \( r \) = radius \( \theta \) = angle of repose

**Bulk density**

When particles are loosely packed, there are lots of gaps between particles. Hence bulk volume increases making powder light. Powders are classified as “light” and “heavy” based on bulk volume. Smaller particles silt between the larger particles, so powder assumes low bulk volume. Such powders are called heavy powders. The bulk density depends on particle size distribution, shape and cohesiveness of particles. Bulk density was determined by pouring the blend of granules in graduated measuring cylinder of bulk density apparatus. Initial volume occupied by the granule is measured. This is the bulk volume. Bulk density is calculated by following equation.

\[ \text{Bulk density (Pb)} = \frac{\text{wt. of powder}}{\text{bulk volume}} \]

**True density**

The density is dependent on the type of atoms in a molecule, arrangement of atoms in a molecule and the arrangement of the molecules in the sample. Apart from true density, powder is also characterized by bulk density. Volume occupied by voids (inter-particle spaces) and intra-particles pores are not included in the measurement. The true density is measured by helium or nitrogen displacement, liquid displacement and by bulk density apparatus. The measuring cylinder containing known amount of blend was tapped in bulk density apparatus. After around 100 tapings, the volume occupied by the granule is noted. This gives the true volume. True (tapped) density was calculated by following equation.

\[ \text{True density (Pt)} = \frac{\text{wt. of powder}}{\text{true volume}} \]

**Compressibility Index (Carr’s Index)**

It is directly related to the relative flow rate, cohesiveness and particle size. It is simple, fast and popular method for predicting powder flow characteristics. Compressibility Index is a measure of the potential strength that a powder can build up in a hopper and also the ease with which such an arch could be broken

\[ \text{Compressibility Index} = \left( \frac{\text{Pt-Pb}}{\text{Pt}} \right) \times 100 \]

Where, Pb = bulk density Pt = true density

**Hausner’s ratio**

Hausner’s ratio is an indirect index of ease of powder flow.

\[ \text{Hausner’s ratio} = \frac{\text{Pt}}{\text{Pb}} \]

Where, Pt = tapped density Pb = bulk density

**Effervescence Cessation Time**

100ml of distilled water was taken in 250ml beaker, one dose of effervescent granules was poured in to the beaker, effervescence cessation time and effervescent production was observed.

**In-vitro Anti-Obesity Activity of Herbal Extract Mixture**

**In-vitro Lipase Inhibitory Activity**

Lipase inhibitory activity of prepared extracts mixture was determined by using a method described by Etoundi CB et al. The rate of release of oleic acid from triolein was determined for measuring lipase inhibitory action. A suspension containing 1% (v/v) of triolein, and 1% (v/v) tween 40 in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. Porcine pancreatic lipase (0.5 gm) was dissolved in 15 ml 0.1 M phosphate buffer (pH 8). 800 µL of the triolein emulsion was added to 200 µL of porcine pancreatic lipase and to those different concentrations of extract mixture (50, 100, 150, and 200 µg/mL) were added. Orlistat, a potent pancreatic lipase inhibitor was taken as reference standard drug. Immediately after mixing the contents the absorbance was measured at 450 nm and designated as T1. The test tubes were incubated at 37°C for 30 minutes, and at the end of the incubation, the absorbance at 450 nm was recorded and designated as T2. The variation in absorbance = \( [\Delta A450 \text{ (T1)} - \Delta A450 \text{ (T2)}] \) was calculated for both control and the treatment, and the % inhibition was calculated using the formula:

\[ \% \text{ inhibition} = \left( \frac{[\Delta A450 \text{ control} - \Delta A450 \text{ extract}] \times 100}{\Delta A450 \text{ control}} \right) \times 100 \]

**In vitro α-Amylase Inhibitory Activity**

Soluble starch (500 mg) was dissolved in 25 mL of 0.4 M NaOH and heated for 5 minutes at 100°C. The pH of solution was adjusted to 7 with 2 M HCl cooling in ice water, and water was added to adjust the volume to 100 ml. The substrate (40 µl) and extract mixture’ (20 µl) solutions (50, 100, 150, and 200 µg/mL) were mixed in a micro plate well, and the mixtures were pre-incubated at 37°C for 3 minutes, followed by addition of 20 µl of α-amylase solution (50 µg/ml) to each well, and incubation of plate for 15 minutes. At last 80 µl of 0.1 M HCl and 200 µl of 1 mM iodine solution were added to terminate the reaction. Acarbose, a potent α-amylase inhibitor, was selected as reference standard drug. The absorbance (Abs) was measured at 650 nm. Inhibitory activity was calculated as follows:

\[ \text{Inhibition} (%) = \left[ 1 - (\text{Abs 2} - \text{Abs 1}) / (\text{Abs 4} - \text{Abs 3}) \right] \times 100 \]

Where,

Abs 1 is the absorbance of incubated solution containing fractions, starch, and amylase; Abs 2 is the absorbance of incubated solution containing fractions and starch;
Abs 3 is the absorbance of incubated solution containing starch and amylase;
Abs 4 is the absorbance of incubated solution containing starch
RESULTS AND DISCUSSION

Effervescent granules formulation & evaluation

Oral pharmaceutical dosage form remains popular route of the drug administration regardless of the several drawbacks which need to be unraveled i.e. causing slow absorption, low acceptance due to the bitter taste and even peculiar odor (i.e. antibiotics and natural extract based-tablet), frequent compliance problem on pediatric and geriatric patients, and the delayed onset of action. On the other hand, natural extract draws massive attraction as an alternative towards conventional drugs owing to their safety and efficacy, despite of unpleasant appearance, odor, and taste. To solve this, the advanced pharmaceutical dosage form i.e. effervescent granules were successfully formulated for the selected herbal mixture corresponding to a breakthrough in oral based-herbal drug formulation giving benefits in rapid adsorption, friendly use for majority patients due to instantly dissolved in water, widely accepted by all age groups attributable to its yummy taste.

At first, six batches were prepared according to the variation of acid-base and flavoring agents. The acid component selected are the citric acid and tartaric acid with regard to the suitable granule characteristics. Indeed, lemon and peppermint flavors were selected to improve the taste of formulation because of their acceptance and popularity among Indians and was commonly used as flavoring agents. Out of all six batches batch no. 6 showed excellent flow properties was found to be best with respect to physical evaluation.

| Table 2: Physical Characteristic of Herbal Effervescent Granules |
|---------------------------------------------------------------|
| Properties           | Batch 1 | Batch 2 | Batch 3 | Batch 4 | Batch 5 | Batch 6 |
|----------------------|---------|---------|---------|---------|---------|---------|
| Bulk Density (gm/ml)| 1.10    | 1.12    | 1.06    | 1.09    | 1.16    | 1.11    |
| Tapped Density (gm/ml)| 1.43   | 1.37    | 1.28    | 1.21    | 1.31    | 1.19    |
| Carr’s Index (%)     | 23.07   | 18.24   | 17.81   | 9.91    | 11.45   | 9.24    |
| Hausner’s Ratio      | 1.30    | 1.22    | 1.20    | 1.11    | 1.12    | 1.07    |
| Angle of Repose      | 26.53   | 37.05   | 36.12   | 26.41   | 31.25   | 27.22   |
| Effervescence Cessation Time (Sec) | 137 | 145 | 108 | 124 | 96 | 80 |

In vitro pancreatic lipase inhibitory activity

Around 50-80% of the dietary fats get hydrolyzed by pancreatic lipases (PL) and produces fatty acids (FAs) and monoglycerides, which further form mixed micelles with cholesterol, bile salts, and lysophosphatidic acid. These formed micelles are absorbed into enterocytes where triglycerides (TGs) are resynthesized. Formed TGs are stored in adipocytes as their main energy source. Addition of more and more adipocytes in the form of fat pads in different parts of the body leads to obesity. One of the key targets to treat obesity is the development of lipase inhibitors.

In present study in vitro lipase inhibitory effect of herbal extract mixture was evaluated using rate of release of oleic acid from triolein. Results of % lipase inhibitory activity of different concentrations & standard drug orlistat are shown in table 3. Extract mixture showed pancreatic lipase inhibitory activity in concentration dependent manner. Highest concentration 300 µg/ml showed 83.32±10.47 % inhibition which was comparable with highest concentration of standard drug orlistat (98.34 ± 3.06).

| Table 3: Inhibitory effects of different concentrations of Herbal extract mixture on Pancreatic lipase |
|---------------------------------------------------------------|
| Test sample | Concentration (µg/ml) | Inhibition (%) |
|---------------|-----------------------|----------------|
| Herbal Exacts Mixture | 50 | 26.11± 3.56 |
|                | 100 | 42.34 ± 7.25 |
|                | 150 | 56.82 ± 4.98 |
|                | 200 | 68.28 ± 9.33 |
|                | 250 | 79.57± 12.81 |
|                | 300 | 83.32± 10.47 |
| Orlistat | 50 | 33.56 ± 2.63 |
|            | 100 | 47.31 ± 5.11 |
|            | 150 | 61.72 ± 1.78 |
|            | 200 | 76.28 ± 2.56 |
|            | 250 | 84.27 ± 5.75 |
|            | 300 | 98.34 ± 3.06 |

(Results are expressed as Mean± SD, n=3)
**In vitro α-Amylase Inhibitory Activity**

Carbohydrates are considered as the main macronutrients for energy intake in almost all dietary patterns, which are converted into monosaccharides by two main enzymes namely α-amylase and α-glucosidase to be used by tissues and cells. The alpha-amylases are the calcium metalloenzymes which function only in the presence of calcium. There are many digestive enzymes in humans and among them the most important one is pancreatic alpha-amylase that act as a catalyst in the reaction which involves the hydrolysis of the alpha-1, 4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. Extract mixture showed α amylase inhibitory activity in concentration dependent manner. Highest concentration 300 µg/ml showed 69.14± 7.22 % inhibition which was comparable with highest concentration of standard drug acarbose (88.32±2.89). Results of % α-amylase inhibitory of extract mixture & standard drug acarbose are shown in table 4.

![Graph](image1.png)

**Figure 1**: Inhibitory effects of different concentrations of Herbal extract mixture on pancreatic lipase

![Graph](image2.png)

**Figure 2**: Inhibitory effects of different concentrations of Herbal extract mixture on α amylase

| Test sample          | Concentration (µg/ml) | Inhibition (%) |
|----------------------|-----------------------|----------------|
| Herbal Extracts Mixture | 50                    | 9.07± 0.63     |
|                      | 100                   | 15.25 ± 2.03   |
|                      | 150                   | 25.62 ± 5.28   |
|                      | 200                   | 38.17 ± 4.56   |
|                      | 250                   | 57.38 ± 9.62   |
|                      | 300                   | 69.14 ± 7.22   |
| Acarbose             | 50                    | 21.67±2.56     |
|                      | 100                   | 32.17±3.22     |
|                      | 150                   | 48.42±1.16     |
|                      | 200                   | 63.23±0.77     |
|                      | 250                   | 76.54±1.72     |
|                      | 300                   | 88.32±2.89     |

(Results are expressed as Mean± SD, n=3)
CONCLUSION
In conclusion, the effervescent granules produced from herbal extract mixture of *Garcinia indica fruit, Achyranthes aspera seeds* & *Coffee arabica L.* beans (raw) met the Pharmacopoeial quality parameters with acceptable physical characteristics. Targeting one or more enzymes involved in lipid & carbohydrate metabolism can be selective for evaluation of anti-obesity action of drug. *In vitro* studies results showed that herbal extract mixture have inhibitory effect on pancreatic lipase & α amylase and can be useful in the weight loss treatment & other metabolic disorders. The effervescent granules can be regarded as a capable vehicle for plant extracts due to their easy handling & administration, rapid disintegration and affordable cost.

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REFERENCES
1. Safaei M, Sundararajan EA, Driss M, Bouilila W, Shapi'i A. A systematic literature review on obesity: Understanding the causes & consequences of obesity and reviewing various machine learning approaches used to predict obesity. Computers in biology and medicine. 2021 Sep 1; 136: 104754.
2. Premalatha K, Sneha M. Green cure to obesity-A review. Journal of Innovations in Applied Pharmaceutical Science. 2016;30:34-40.
3. Patel D, Kumar V. Protective Effects of Fagonia cretica L. Extract in Cafeteria Diet Induced Obesity in Wistar Rats. Journal of Natural Remedies. 2020 Jul 1; 20(3):185-90.
4. Srivastav S, Singh P, Mishra G, Jha KK, Khosa RL. *Achyranthes aspera*-An important medicinal plant: A review. J Nat Prod Plant Resour. 2011; 1(1):1-4.
5. Pandey NK, Sharma HP, Patnaik A, Jain P. A review on potential magic folk herbal medicinal plant: *Achyranthes aspera* L. International Journal of Medicinal Plants Photon. 2013; 105: 350-63.
6. Hariravan V, Rangaswami S. Structure of saponins A and B from the seeds of *Achyranthes aspera*. Phytochemistry. 1970 Feb 1; 9(2):409-14.
7. Rani N, Sharma SK, Vasudeva N. Assessment of antiobesity potential of *Achyranthes aspera* Linn. seeds. Evidence-Based Complementary and Alternative Medicine. 2012 Jan 1; 2012.
8. Baliga MS, Bhat HP, Pai Rj, Boloor R, Palatty PL. The chemistry and medicinal uses of the underutilized Indian fruit tree *Garcinia indica* Choisy (kokum): a review. Food Research International. 2011 Aug 1; 44(7):1790-9.
9. Watt G. Dictionary of the Economic Products of India, Vol. 4. Office of the Superintendent Government Printing; Calcutta; 1890.
10. Yamaguchi F, Saito M, Ariga T, Yoshimura Y, Nakazawa H. Free radical scavenging activity and antiluver activity of garcinol from *Garcinia indica* fruit rind. Journal of Agricultural and Food Chemistry. 2000 Jun 19; 48(6):2320-5.
11. Jagtap P, Bhise K, Prakya V. A phytopharmaceutical review on *Garcinia indica*. International Journal of Herbal Medicine. 2015; 3(4):02-7.
12. Samadi M, Mohammadshahi M, Haidari F. Green coffee bean extract as a weight loss supplement. J Nutr Disorders Ther. 2015; 5(4):1-3.
13. Bicchi CP, Binello AE, Pellegrino GM, Vanni AC. Characterization of green and roasted coffees through the chlorogenic acid fraction by HPLC-UV and principal component analysis. Journal of Agricultural and Food Chemistry. 1995 Jun; 43(6):1549-55.
14. Naidu MM, Sulochanamma G, Sampathu SR, Srinivas P. Studies on extraction and antioxidant potential of green coffee. Food Chemistry. 2008 Mar 1; 107(1):377-84.
15. Hirani JJ, Rathod DA, and Vadalia KR, Orally disintegrating tablets: A review. Trop J Pharm Res, 2009;8(2): 161-172.
16. L Allen, H.C. Ansel, Ansel’s Pharmaceutical Dosage Forms and Drug Delivery Systems, Lippincott Williams & Wilkins (2013), p. 794
17. V.S. Iyer, S.C. Srinivas, Effervescent Granular Formulations of Antiretroviral Drugs. Google Patent WO2007060682 (2007)
18. M.E. Aulton, T. Kevin, Powder Flow. Pharmaceutics. The Design and Manufacture of Medicines (fourth ed.), Churchill Livingstone, Edinburgh (2013), pp. 187-199.
19. Aslani A and Jahangiri H, Formulation, characterization and physicochemical evaluation of ranitidine effervescent tablets. Adv Pharm Bull, 2013;3(2): 315-322.
20. W. James, Pharmaceutical preformulation: the physicochemical properties of drug substances: Aulton ME. Pharmaceutics the science of dosage form design, Churchill living stone, Spain, 2006;2:113-138.
21. G.S Banker, N.R Anderson, Tablets: Lachman L, Lieberman H, The theory and practice of Industrial Pharmacy, CBS publishers, New Delhi, 2009, 293-345.
22. Etoundi CB, Kuaté D, Ngondi JL, Oben J. Anti-amylase, anti-lipase and antioxidant effects of aqueous extracts of some Cameroonian spices. J Nat Prod. 2010; 3(165):17.
23. Xiao, Zhizhuang, et al. "A quantitative starch iodine method for measuring alpha-amylase and gluco-amylase activities." Analytical Biochemistry, 2006;351(1):146-148.