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FORMULATION OF NUTRITIONAL DEFICIENCY SUPPLEMENT WITH ANTIBACTERIAL AND ANTI-INFLAMMATORY POTENTIAL

Priyanka. S*, Dr. M. Thenmozhi Ph.D **

*(Department of biotechnology, Prathyusha Engineering College, Thiruvallur, Tamil Nadu, India
Email: priyankas0427@gmail.com)

** (Department of biotechnology, Prathyusha Engineering College, Thiruvallur, Tamil Nadu, India)

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Abstract:

Nutritional deficiency turning up when our body doesn't get required nutrient from food. Nutritional supplement is taken to overcome the nutritional deficiency. The current study was carried out to explore the nutritional activity of a methanol extract. The extract is combination mixture of *Fragaria ananassa*, *Annona Squamosa L*, *Oryza punctata* and *Salvia hispanica* extract in equal ratio. A series of test on the phytochemical, anti-oxidant, anti-inflammatory and anti-bacterial are performed. The quantitative phytochemical analysis of this species exhibited the presence of alkaloids, terpenoids, flavonoids, phenolic compounds and saponins. Total phenolic and flavonoids compound present in extract was 219±0.17 µg/mg and 38.478±0.17 µg/mg. The extract was investigated for *in vitro* antibacterial activity against microorganism including Gram-positive bacteria and Gram-negative bacteria. Anti-inflammatory assay showed the IC_{50} was 72.99 µg/mL concentration. $IC_{50.}$ Fe^{3+} Reducing assay and $DPPH^{\cdot}$ radical scavenging activity IC_{50} was found to be 59.63 µg/mL and 138.95 µg/mL Using the above combination of extract nutritional food supplement is formulated. Evaluated under physical parameters and nutritional value.

Keywords **—** Nutritional deficiency, *Fragaria ananassa*, *Annona Squamosa L*, *Oryza punctata*, *Salvia hispanica***.**

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I. INTRODUCTION

Nutritional deficiency is very commonly by depressing immune system. The very common nutritional deficiencies among children are calcium, fiber, iron, folate, potassium, vitamin D and vitamin E. our needs macronutrients like vitamins and minerals in very less quantity but their impact on body health is critical and deficiency in any severe and threatening life's condition. If deficiency is caused by hampering our body ability to absorb nutrition, then treatment will focus on treating the condition and along with providing supplements needs to bring number up [1]. *Fragaria ananassa* are rich in several nutritive and non-nutritive compounds and also have more benefits such as inflammatory disorder and oxidation property [2]. *Annona Squamosa L.* vitamins like A and C, other than nutrients like Vitamin A, Vitamin C, iron, potassium, magnesium, and copper and magnesium. Extract of *Annona Squamosa L.* have their biological activities like anticancer, antidiabetic, antioxidant, antimicrobial and antiobesity [3]. *Oryza punctata* is also loaded with fibre, vitamin B1 and B2, calcium, and iron content. The red rice gets its colour from an antioxidant called anthocyanin which is a compound found in several other red-coloured vegetables. *Oryza punctata* also considered to reduce inflammation in the body, controls cholesterol levels and lowers blood pressure [4]. Therapeutic effects of *Salvia hispanica* in the control of diabetes, dyslipidaemia, hypertension, as anti-inflammatory, antioxidant, anti-blood clotting, laxative, antidepressant,

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antianxiety, analgesic, vision and immune improver is scientifically established. Plant-based foods have long been associated with a reduced risk of many adverse health condition including obesity, diabetes, heart disease, and overall mortality [5]. Gummies are used to treat nutritional deficiencies by preventing health which can cure any resulting health condition tending arise with those deficiencies [6].

II. MATERIALS AND METHODS

A. PREPARATION OF CRUDE METHANOL EXTRACT

The strawberry, custard apple, red rice and chia seed were collected from the ponnu super market, perambur, Chennai, Tamilnadu, India, (13.1210° N, 80.2326° E). Pretreatmenting fresh Strawberries by washing and cutting and fresh Custard apple by removing the seeds. *Fragaria ananassa*, *Annona Squamosa L*, *Oryza punctata*, *Salvia hispanica* were taken 15g and crushed with mortal and pestle. 15ml of methanol is added to crushed source and covered with aluminium foil. Incubate at room temperature for 72hrs. Each source methanolic extract where taken in 1:1:1:1 ratio [7].

B. PHYTOCHEMICAL ASSAY OF PREPARED CRUDE EXTRACT

QUALITATIVE ANALYSIS

Test for alkaloids a few drops of Wagner's reagent is added to few ml of crude extract along the sides of test tube [8]. Test for Terpenoids (Salkowski test) add 2 ml of extract was mixed with 2 ml of acetic anhydride. Few drops of concentrated sulfuric acid were then added to this solution [9]. Test for saponins 2 ml of extract was taken in a test tube and 6 ml of distilled water was added to it and mixture was then shaken vigorously [9]. Test for flavonoids 2 ml of extract was treated with few drops of 1N sodium hydroxide solution and add of dilute hydrochloric acid [10]. Test for phenolic compounds few drops of the extract were treated with 5% aqueous ferric chloride [10].

QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL

The total flavonoid content of crude extract was determined using aluminium chloride colorimetric method. One mL of extract was mixed with 0.5 mL of 5% sodium nitrite solution and incubated for 5 min at 37°c. Then add 0.5 mL of 10% aluminium chloride solution was added and incubated for 5 min at 37°c and followed by 1 mL of 1 M NaOH solution was added. The total volume was made up to 5 mL using distilled water. Absorbance at 510 nm using spectrophotometer. The result was expressed in term of quercetin equivalent [11].

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds. 100 µL of crude extract was mixed with 900 µL of distilled water and 1 mL of Folin Ciocalteau reagent. After 5 min, 1 mL of Na_2CO_3 (20%) was added. The mixture was then allowed to stand for 30 min incubation in dark at 37°c. The absorbance at 765 nm by spectrophotometer. The total phenolic content was expressed in form of gallic acid [12].

C. ANTIMICROBIAL STUDIES OF PREPARED CRUDE EXTRACT

The microorganisms of Gram-positive strains such as *Bacillus subtilis* and *Staphylococcus aureus* as well as Gram negative strains such as *Escherichia coli* were used for the evaluation of antibacterial activity. Tetracycline was chosen as the standard reference. Nutrient broth agar medium was prepared according to the standard methods (peptone-5 g, yeast-3 g, NaCl-5 g, distilled water- 1000 mL, agar-20 g) and was suspended in 200 mL of distilled water in a 500 mL conical flask, stirred, boiled to dissolve and then autoclaved at 15 lbs and at 121˚C for 15 minutes. The hot medium was poured in sterile petriplates and allowed to solidify for 15 min. Antibacterial activity of extract was carried out using agar well diffusion method. The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs which is previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Four wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The crude extract was then poured into each well containing 250,

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375 and 500 µg/mL concentrations. All the plates with extract loaded wells were incubated at 37ºC for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline (25 µg) was taken as positive control [13].

D. ANTI-INFAMMATORY STUDIES OF PREPARED CRUDE EXTRACT

The Blood was collected from healthy human volunteer who has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tube. The tubes were centrifuged at 3000 rpm for 10 min and discard the pale-yellow plasma. Then, about 10 mL of PBS was poured into centrifuge tube, centrifuged at 3000 rpm for 5 min, discard and discard the supernatant and repeat the washing process once again. The dissolved red blood pellets obtained was measured and made over again with a 40% v/v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The reconstituted red blood cells (resuspended supernatant) were used as such normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline. The reaction mixture (2 mL) consisted of 1 mL of different concentrations of extract (20-120 µg/mL) and 1 mL of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30 min. At the end of the incubation the test tubes were cooled using water. The mixture was centrifuged at 3000 rpm for 5 min and the supernatants were measured at absorbance at 560 nm. Aspirin was used as a standard drug [14]. Calculate the percentage inhibition of haemolysis using the follows:

% Inhibition of haemolysis = [(Absorbance (control) – Absorbance (Sample)/Absorbance (Control)] x 100

E. ANTIOXIDANT STUDIES OF PREPARED CRUDE EXTRACT

Fe^{3+} REDUCING ASSAY

The reducing of extract was determined by slightly modified method. One mL of crude extract of different concentrations (20 - 120 µg/mL) was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1 % (w/v) potassium ferricyanide [$K_3Fe(CN)_6$]. The reaction mixtures were incubated at 50°C for 20 min. One mL of 10%(w/v) trichloroacetic acid was added to each mixture. Then to the 1 mL mixture of 0.1%(w/v) $FeCl_3$ was added and the absorbance was measured at 700 nm using Spectrophotometer [15]. Formula for percentage of inhibition:

% Of inhibition = [(Absorbance(sample) – Absorbance (control)/Absorbance (sample)] x 100

DPPH- RADICAL SCAVENGING ASSAY

The antioxidant activity of extract was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (20-120 µg/mL) of crude extract. Incubated the reaction mixture in dark for 30 min at room temperature. The absorbance was measured using Spectrophotometer at 517 nm [16]. Formula the percentage of inhibition:

RSA % = [(Absorbance(control) –Absorbance (Sample)/Abs orbance (Control)] x 100

F. FORMULATION OF FOOD SUPPLEMENTATION

3g of Gelatin was solubilised in 30ml of distilled water for 15 mins, at 60°c. Add sugar syrup (25%) and *Fragaria ananassa*, *Annona Squamosa L*, *Oryza punctata* and *Salvia hispanica* each were taken 25g and crushed finely and added, gel stabilised and flavour enhanced. Moulding the final soluble content and let cool and settled for 30mins [17].

III. RESULTS

A. PHYTOCHEMICAL ASSAY OF CRUDE COMBINATION EXTRACT

QUALITATIVE ANALYSIS :

Preliminary phytochemical analysis for alkaloids, terpenoids, flavonoids, phenolic compounds and saponins were made by following

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standard procedures. Yellow colour precipetate appears indicates present of alkaloids. A reddish-brown ring coloration of the interface indicates the presence of terpenoids. A yellow colour formation within short period is positive for flavonoids. Dark brown colour indicates the presence of phenol. No persistence of foam observed indicates the absence of saponins.

QUANTITATIVE ANALYSIS

The phenol and flavonoid compounds quantified in the of Composition of the methanol extract (Table.1) seemed to be responsible for the antioxidant activity. The total phenol content was 291±0.17 µg/mg of GAE (Table 1) and the total flavonoid content was 38.478±0.17 µg/mg (Table 1) of QE in the extract.

Table.1

Quantitative estimations of combination extract

S. No	Phytochemicals	Amount (µg/mg)
1.	Phenols	291±0.17
2.	Flavonoids	38.478±0.17

B ANTIMICROBIAL ACTIVITIES OF CRUDE COMBINATION EXTRACT

The extract was investigated for *in vitro* antibacterial activity against microorganism including Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). Evaluation of antibacterial activity of this extract was recorded in table 2 and illustration in fig 1. The maximum zone of inhibition of crude combination extract against *Bacillus subtilis* was 15mm in 500µg/ml, *Staphylococcus aureus* was 16mm in 500µg/ml, and *Escherichia coli* was 22mm in 500µg/ml. The antibacterial activities of crude extract were found to be independent of the habitat factor.

Table 2:

Antimicrobial activity of crude extract

Organism	Zone of inhibition (mm in diameter)			
	std	**250µg/ml**	**375µg/ml**	**500µg/ml**
Bacillus subtilis	16mm	12mm	13mm	15mm
Staphylococcus aureus	17mm	14mm	15mm	16mm
Escherichia coil	29mm	18mm	20mm	22mm

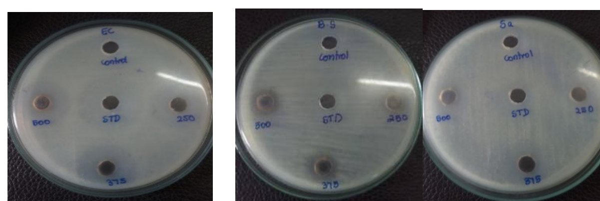

Fig. 1 Antimicrobial activity of crude extract for *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*

C. ANTI-INFLAMMATORY ACTIVITY OF CRUDE COMBINATION EXTRACT

The result indicated that extract at various concentrations has significant anti-inflammatory property. The maximum hemolysis inhibition was 75.67±0.06 at 120 µg/mL concentration (Fig 2) and the IC_{50} was 72.99 µg/mL concentration.

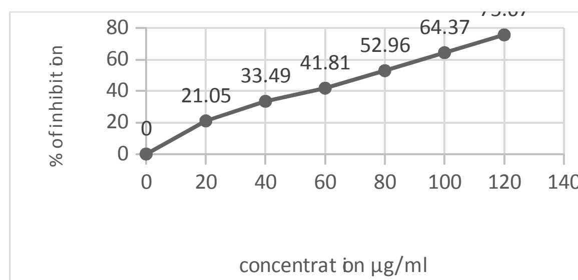

Fig 2: Anti-inflammatory activity of crude extract graph

D. ANTIOXIDANT ASSSAY OF CRUDE COMBINATION EXTRACT

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The maximum Fe^{3+} reduction was 93.16±0.7% at 120 µg/mL concentration (Fig 3) and the RC_{50} was 59.63 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50} = 7.72 µg/mL concentration). The maximum $DPPH^{\cdot}$ radical scavenging activity was 41.33±0.11% at 120 µg/mL concentration (Fig 4). Extract demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC_{50} was found to be 138.95 µg/mL concentration and was compared with standard (Ascorbic acid, IC_{50} = 11.98 µg/mL concentration).

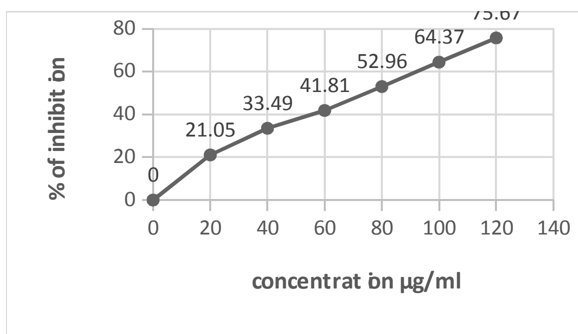

Fig 3: Fe^{3+} reducing assay of crude extract graph

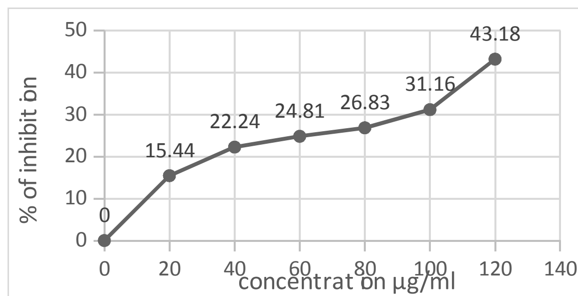

Fig 4 DPPH- radical scavenging assay of crude extract graph

E. Evaluation of food product under various parameter

Evaluation of formulated product (Fig 5) based on physical parameter (table 3) are red in colour, soft jelly appearance, fruity flavored taste, semi solid gumminess, sugar is added for preservation, shelf life of product is 7 days and ph of product is 3.7±0.05. Nutrition present in formulated product is protein 7.5g, carbohydrate 35.75g, total fat 0.25g, energy 250kcal, sodium 2.1mg, potassium 25.27mg, calcium 28.3mg, magnesium 8.72mg and phosphate 33.64mg (table 4).

Table 3

Physical parameter of formulated product

PARAMETERS	EVALUATION
Colour	Red
Appearance	Soft jelly
Taste	Fruity flavor
Gumminess	Semi-solid
Preservative	Sugar
Shelf life	7 days
pH	3.7±0.05

Table 4

Nutritional value of formulated product

Parameter	Unit	Result
Protein	g/100g	7.5
Carbohydrate	g/100g	35.75
Total fat	g/100g	0.25
Energy	Kcal/100g	250
Sodium	mg/100g	2.1
Potassium	mg/100g	25.27
Calcium	mg/100g	28.3
Magnesium	mg/100g	4.72
Phosphate	mg/100g	33.64

Fig 7 Formulated product

IV. CONCLUSIONS

Sources like *Fragaria ananassa*, *Annona Squamosa L*, *Oryza punctata*, *Salvia hispanica* has more constituent's nutrition for nutritional deficiency. Thus, in this present study methanolic extracts were evaluated. Various tests like phytochemical analysis, antioxidant assay confirmed antioxidant properties, anti-

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inflammatory, antibacterial and presence of alkaloids, flavonoids, phenol and other components. Present study spotlights activity against nutritional supplement in modern supplement. This has provided an insight into phytochemical and pharmacological activity of these source. Custard apple and Strawberry syrup, chia seed and red rice was used to preparation of gummy jellies.

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