SELECTION OF SEAWEEDS FOR FEED PELLET PREPARATION

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***Abstract*—SEAWEEDS were found to contain lot of nutrients when consumed by coastal cattles gives them immense immune power. This feature of seaweeds was used for the preparation of feed pellet for cattle consumption. The collected seaweeds (*Gracilaria edulis, Chaetomorpha linum, Enteromorpha intestinalis*) were tested for the presence of phytochemicals as well as the quantitiy of protein and reducing sugars. It was observed that the presence of alkaloids, tannin, steroids, cardiac glycoside, Phenol, Terpenoid, carbohydrate and Flavanoid were present in almost all the extracts. Among the 3 seaweeds *Gracilaria edulis* showed maximum number of active constituents. Also *G. edulis* showed maximum quantity of protein and carbohydrate when compared to other 2 species. The antioxidant activity showed concentration dependent pattern. The *G. edulis* obtained same antioxidant effect as that of Ascorbic acid at a concentration of 2.5 mg/mL. Future research shall be focused on replacement of active ingredients in the marketed feed formulation for cattle with one of seaweeds to get a better nutrient content.**

***Keywords*—Seaweeds; phytochemical; Biuret method; antioxidant; DNSA method**

I. INTRODUCTION

The seaweeds are large and diverse group of marine macro algae. Like land plants, they also use photosynthesis to convert inorganic nutrients into organic biomass. They are simple in their structural composition because they take up nutrients into their blades directly from seawater, which take up nutrients through the roots.[1]

They constitute a vital part of marine ecosystems. It was studied that about 90% of the species of marine plants are algae and about 50% of the photosynthesis is globally contributed from algae. These seaweeds are reservoirs of pigments, polyphenols, enzymes, carotenoids, diverse functional polysaccharides. [(1)(2)] They have good source of vitamin A, B_1, B_{12}, D, C and E. These seaweeds are potential reservoirs of bioactive compounds, which might be a potential source of nutrients. These naturally produced seaweeds are known as secondary metabolites, which possess a broad range of biological activity.[7] From the study, it is well known that seaweeds have antioxidant, antibacterial, antiviral, antifungal, cytotoxic and potential characteristics. The uses of seaweeds at present are as human foods, cosmetics, fertilizers, and for the extraction of industrial gums and chemicals.[9] They are potentially used as a source of long-chain and short-chain chemicals with medicinal uses.[2]

The comparative study of three different seaweeds consisting its nutrient supplements including proteins, carbohydrates, reducing sugars, etc. was made. The feed pellet for animals prepared from these three seaweeds was found to be cost effective with predominant yield and as a quality product.

II. MATERIALS AND METHODS

A. MATERIALS REQUIRED

Biuret reagent, DNSA, Alcoholic sodium hydroxide, distilled water, concentrated sulphuric acid, 1% aqueous iron chloride, acetic anhydride, sulphuric acid, Maeyer's and Dragendeuff's reagent, glacial acetic acid, chloroform, sodium hydroxide, hydrochloric acid, ammonia, aqueous ferric chloride, Fehling's reagent.

B. COLLECTION OF SEAWEED

Three types of seaweeds namely *Chaetomorpha linum*, *Enteromorpha intestinalis* and *Gracilaria edulis* were collected from Pulicat Lake, Tamil nadu.

Chaetomorpha linum

Kingdom	:	*Plantae*
Order	:	*Cladophorales*
Family	:	*Cladophoraceae*
Genus	:	*Chaetomorpha*
Species	:	*C. linum*

Chaetomorpha is a genus of green algae. It was fully grown of significant size and a chunk of the algae was thrown out to another aquarist, taking up the nutrients and it was absorbed out of the system.[13]

Enteromorpha intestinalis

Kingdom	:	*Plantae*
Order	:	*Ulvales*

Family : *Ulvaceae*
Genus : *Enteromorpha*
Species : *E. intestinalis*

The fronds have branches and are completely tubular, expanded till mid-thallus, reaching 15 cm long or more. The species may be 10–30 cm (3.9–11.8 in) long and 6–18 mm (0.24–0.71 in) wide. [6]

Gracilaria edulis

Kingdom : *Gracilariaceae*
Order : *Gracilariales*
Family : *Gracilariaceae*
Genus : *Gracilaria*
Species : G. *edulis*

Gracilaria is a genus of red algae and it is notable for its economic importance as an agarophyte; it is consumed as food for humans and various species of shell fish.[14]

These selected seaweeds were dried under shade for 10 days and crushed into fine powder. An ethanolic extract was prepared, filtered and stored.

C. PHYTOCHEMICAL ANALYSIS

The presence of phyto-constituents was evaluated through the following standard methods.

Test for coumarin [5]

Each test solution was treated with a few drops of sodium hydroxide. Appearance of yellow colour indicated the presence of coumarin.

Test for saponin [5]
Each extract was diluted with distilled water to 20 ml and shaken vigorously for 15 minutes. There was a formation of 1 cm layer of foam, which indicated the presence of saponin.

Test for quinones [4]
Each test solution was treated with few drops of concentrated sulphuric acid or aqueous sodium hydroxide solution. Colour formation indicated the presence of quinoid compound.

Test for tannin [3]
Each extract was added 1% aqueous Iron chloride ($FeCl_3$) solution. The appearance of dark green colour indicated the presence of tannins.

Test for steroid [5]
Each aqueous extract was added 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The change in colour from violet to blue indicated the presence of steroids.

Test for alkaloid [3]
To each extract few drops of Mayer's and Dragendorff's reagents were added. A white precipitate is formed which indicated the presence of alkaloid.

Test for cardiac gylcoside [5]
Each extract was treated with 2 ml of glacial acetic acid along with a drop of $FeCl_3$. The brown colour ring formed which indicates the presence of cardiac glycoside.

Test for terpenoid [3]
Each solvent extract was mixed in 2 ml chloroform and 3 ml of conc.H_2SO_4. A reddish brown layer was formed which indicated a positive result for the presence of terpenoids.

Test for anthocyanin [3]
Each aqueous extract was mixed with 2 ml of 2N HCl & NH_3.The appearance of pink red indicated the presence of Anthocyanin.

Test for betacyanin [4]
2ml of each extract was added to 1ml of 2N sodium hydroxide and the mixture was heated for 20mins at 100°C. Formation of yellow colour indicates the presence of betacyanin.

Test for phenol [4]
To each solvent extract, add 2ml of distilled water was added followed by the addition of few drops of 10% ferric chloride solution. Formation of blue colour indicated the presence of phenols.

Test for phlobatannin [3]
Deposition of red precipitation when aqueous extract of each sample was boiled with 1% Aqueous Hcl confirmed a positive test.

Test for flavonoid [3]
Each extract was treated with 10% NaOH solution. Formation of intense yellow colour indicated the presence of Flavonoid.

Test for reducing sugar [3]
To each extract 1 mL each of Fehling's solution A and B were added. The mixture was heated in a water bath for

10 minutes. A brick-red precipitate was formed indicating the presence of reducing sugar.

D. ESTIMATION OF PROTEIN

The protein was estimated by using biuret test method. Working standard solution was treated with biuret reagent at 37°C for 10 minutes and the absorbance value was recorded at 540 nm. The standard graph was interpreted for all the three seaweeds to estimate the protein. [9]

E. ESTIMATION OF REDUCING SUGAR

The presence of reducing sugar was identified by using DNSA test method. The working standard solution was treated with DNSA reagent and few drops of sodium potassium tartrate was added to it. The contents were mixed well and the absorbance was recorded at 575 nm. The standard graph was interpreted for all the three seaweeds. [10]

F. ANTI OXIDANT ACTIVITY BY FERRIC ION EFFECT

The anti-oxidant activity of these seaweeds was identified by the presence of ferric ion reducing on them. [11] 2 ml of each extract solution was taken and 0.5% of ferric chloride solution was added. Readings were taken after 10 minutes and 48 hours at 700 nm in UV spectrometer. Ascorbic acid was used as positive control.

III. RESULTS AND DISCUSSION

The present study of phytochemical investigation qualitatively and Isolation and Identification of various compounds from *C.linum*, *E.intestinalis*, and *G.edulis* has been presented and discussed.

A. PYHTOCHEMICAL ANALYSIS

The preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, tannin, steroids, cardiac glycoside, phenol, terpenoid, carbohydrate and flavonoid in acetone extract, ethyl acetate extract, methanol extract and predominantly in water.

In *C.linum*, Coumarin and saponin was obtained in methanol extract. The presence of Quinone was found to be in high composition in acetone, methanol and water whereas in ethyl acetate the presence was moderately less. In water extract, alkaloid presence was subsequently high. Presence of Betacyanin and phlobatannins was obtained in water extract. In food industry, betalanins is grown since they were identified in vitro methods as antioxidants, which may protect against oxidation of low-density lipoproteins. [8] By the phytochemical analysis, the presence of reducing sugars in all the extracts and their composition was found to be comparatively high.

In *E. intestinalis*, saponin was extracted in water and it is used widely for their effects on ammonia emissions in animal feeding.[7] The mode of action seems to be an inhibition of urease, which splits up excreted urea in feces into ammonia and CO_2. Consumption of this can have less damage to the respiratory tract of animals, and may help to make them less vulnerable to diseases. [8] Betacyanin is comparatively high in water than ethyl alcohol.

In *G.edulis*, almost all the biochemical compounds were found. Phytochemical analysis revealed the presence of coumarin in all the extracts. The concentration of Quinone was comparatively very high concentration in all the extracts. Alkaloid has high nutrient value and a good anti-oxidising agent in animals. It was identified that all the extracts have better results for reducing sugars.

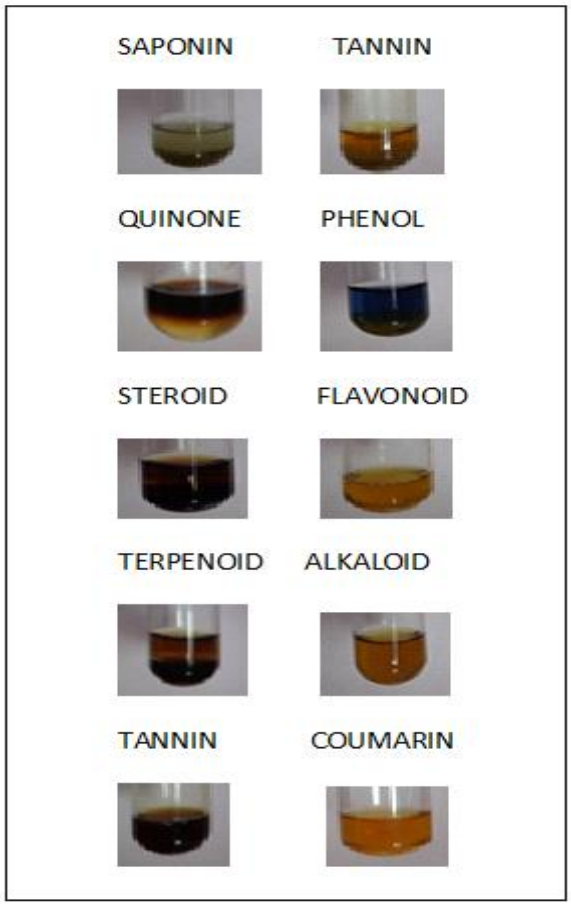

Figure 1. Results for Phytochemical Analysis.

B. ESTIMATION OF PROTEIN

The biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, copper ion forms violet-colored complex in alkaline solution. The change in colour indicated the presence of protein.[13] Cattles need relatively high concentration of protein in their diets to support muscle growth. Correct protein nutrition is important not only for animal performance, but also to minimize nitrogen excretion and reduce pollution.[16] It was estimated that the concentration of protein in *G.edulis* was about 9.09 mg/ml while in *C.linum* and *E.intestinalis* it was about 6.36 mg/ml (Fig 2).

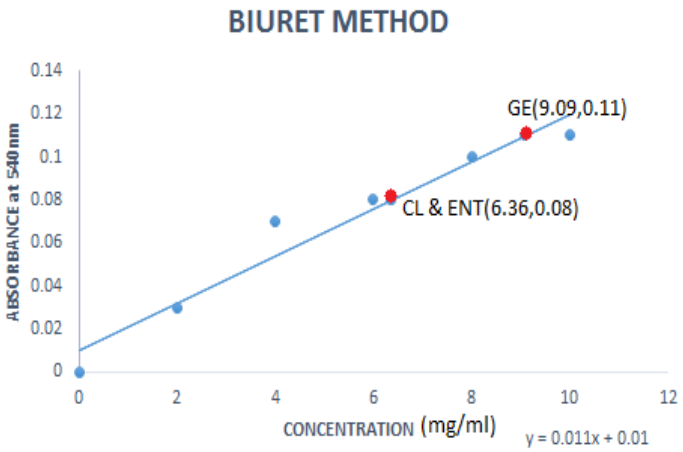

Figure 2. Estimation of Protein by Biuret Method.

C. ESTIMATION OF REDUCING SUGAR

DNSA method indicates the presence of free carbonyl group, the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present. Simultaneously, three, 5-dinitrosalicylic acid (DNS) is reduced to 3-amino-5-nitrosalicylic acid under alkaline condition. Formation of Red-orange colour indicated the presence of reducing sugar. Animals get energy from carbohydrates, which are oxidized in the body. These yield heat, which maintains body temperature, furnishes energy for growth and muscle activity, and sustains vital functions. They get much more energy for growth, work, or milk production than for simple maintenance. Thus, the concentration of reducing sugar was estimated and it was about 57.57 mg/ml in *G.edulis* whereas in *C.linum* and *E.intestinalis*, it was about 38.2 mg/ml and 7.68 mg/ml respectively. (Fig.3)

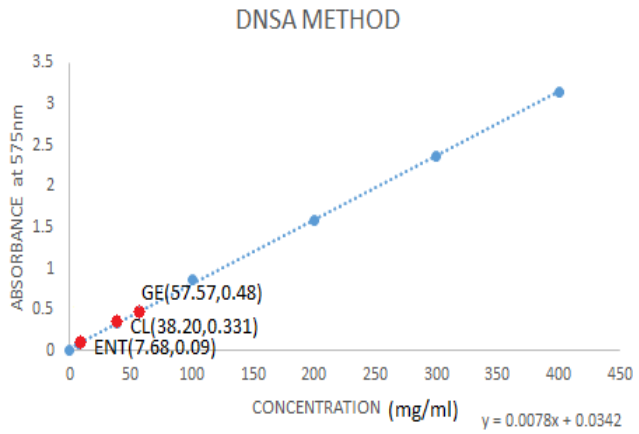

Figure 3. Estimation of Reducing Sugar by DNSA Method.

D. ANTI-OXIDANT ACTIVITY BY FERRIC ION EFFECT

The ability to reduce ferric ions indicates that the antioxidant compounds are electron donors and could reduce the oxidized intermediate of lipid peroxidation processes, thus acting as primary and secondary antioxidants. In cattles, Antioxidant supplements can reduce the occurrence of udder infections and improve the quality of their production.[16] In fishes, Antioxidants can reduce the high risk of quality loss due to oxidation.[16] *G.edulis* and *E.intestinalis* showed better results when compared to *C.linum*. All the three seaweeds have antioxidant activity. (Fig.4&5)

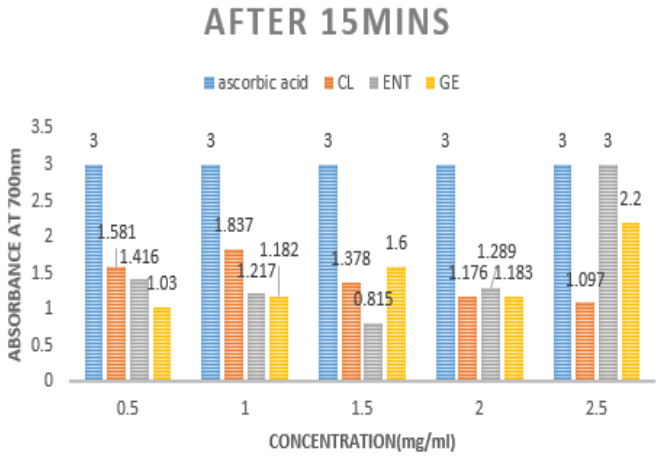

Figure 4. Graphical Representation of Anti-Oxidant Activity after 15 minutes.

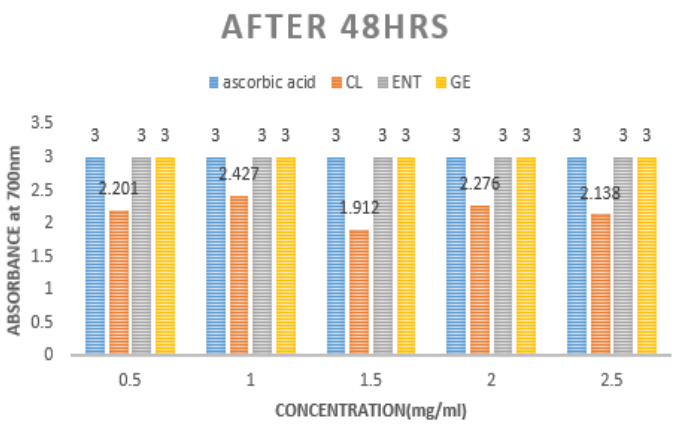

Figure 5. Graphical Representation of Anti-Oxidant Activity after 48 hours.

E. CONCLUSION

The analysis proved the presence of nutrient supplements such as proteins, carbohydrates, reducing sugars, etc. Thus it shall be concluded that an effective feed pellet can be prepared from the seaweeds such as *G.edulis*, *E.intestinalis* and *C.linum*. This feed pellet has all the constitutents of an essential soybean and it can be readily replaced as an animal feed. The prepared feed pellet can be a cost effective nutrient supplement with predominant yield. As a quality product, it has the potential to benefit health, growth and performance of an animal.

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