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RESEARCH PAPER

Hormetic response of blackgram (*Vigna mungo*) seeds treated with organic extract of seaweeds

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Abstract

Seaweed extract has gained popularity in agriculture due to global trend of promoting sustainable agricultural development. Seaweed contains phytohormones such as IAA and gibberellin and minerals that induce seeds germination. While conventional extraction of seaweeds has been carried out with different organic solvents such as ethanol and methanol, the present study uses natural and commonly available organic liquids i.e., water, vinegar, fermented buttermilk, and cow urine as extracting agents to make them suitable for regenerative agriculture. The organic solvents used in this study have a known nutritional composition and facilitate the enhancement of plant defense systems. Three seaweed species, *Caulerpa racemosa*, *Gracilaria edulis*, and *Sargassum wightii*, were extracted with different solvents at various concentrations and compared. The mineral analysis and FTIR characterization of different seaweeds and their extracts showed variation in composition and functional groups. Seaweed species, however, showed no variation in seed germination. Fermented buttermilk and water extract improved the germination properties of blackgram between 0.1–5% concentrations and led to higher increase in shoot and root length and biomass yield. The hormetic response by blackgram to the seaweed extract was observed starting at an average of 2% concentration with toxicity at 5% concentration and above for water and fermented buttermilk extract. However, vinegar and cow urine extracted seaweed showed toxicity symptoms even at lower concentrations (0.1%) inhibiting germination. Therefore, water and buttermilk extract may be recommended at 0.5–1% for enhanced germination.

Keywords

Seaweed, organic, extraction, black gram, hormesis, germination

Introduction

Food security is becoming an enormous challenge due to the rapid population growth that is occurring worldwide. Food productivity must be raised, and as a result of pressure, chemical pesticides and fertilizers are being used more frequently. However, using chemicals in agriculture has limitations of its own, including damaging the qualities of the soil and making food unfit for human consumption. It also contaminates the environment through release of these chemicals in the soil and then to the ecosystem. Moreover, there is growing number of diseases as an outcome of chemical use in food production such as cancer, reduction in immunity and reproduction of human (Rather et al. 2017). To combat the negative impact of using chemicals in agriculture, organic agriculture is a crucial approach. It can assist in resolving issues by improving soil structure, reduction of harmful chemical in food. It is also more sustainable and environmentally friendly. Organic food is becoming more and more popular every day as people's concern about eating healthy grows. Seaweed is one of such which can contribute due to its high composition in minerals and its extract can be used for improving food production through organic agriculture.

Historically, seaweed was utilized as food, fertilizers, condiments, and a source of phycocolloids (Pena-Rodriguez et al. 2011; Yaich et al. 2011). Now, seaweed is used as animal feed and its extract for plants biostimulant as it contain high nutrients, growth hormones, amino acids, etc. (Wells et al. 2017). Seaweeds were grouped into three main groups depending on its phylum viz. green (Chlorophyta), red (Rhodophyta) and brown (Phaeophyta) and the classification depends on their chemical and nutritional composition (Gupta and Abu-Ghannam 2011). Seaweed composition varies with different groups, different species, geographical location and even with season (Terme et al. 2020).

Due to their significant nutritional value to billions of people worldwide, pulses are the second most important class of crops (Singh 2017). Blackgram (*Vigna mungo*) is one of the most desirable crop among pulses because of its high protein content and short duration of the crop (Vanaja et al. 2006). But it also face complications as it is susceptible to acidic, saline and alkaline soil conditions (Swaminathan et al. 2023). Moreover, the crop also has early establishment for which it require nutrient from early stages. Supplementing nutrients at such young stages may be beneficial to promote better crop establishment and crop growth. In light of these, an experiment was conducted to determine how three seaweeds that were extracted using organic solvents at different concentrations influenced the germination characteristics of blackgram seeds.

Materials and methods

Seaweed collection and processing

Three seaweed species *Caulerpa racemosa* (Green seaweed), *Gracilaria edulis* (Red Seaweed) and *Sargassum wightii* (Brown seaweed) were collected in dry form on March 2023 from Rameshwaram, Tamil Nadu, India. Collected seaweed samples were cleaned and further dried in hot air over at 60 °C until constant weight was obtained. Dried seaweed samples were ground with Willy Mill and stored at room temperature 30±1 °C for further extraction and analysis. Fig. 1 shows the seaweeds used for the experiments.

Extraction of seaweeds with organic liquids

All the three species of seaweeds were extracted using deionised water, vinegar, fermented buttermilk and fresh cow urine as extractants. While 5% solution of vinegar (acetic acid AR grade) was used as extractant. To obtain fermented buttermilk, fresh cow milk was obtained from Dairy Unit, TNAU. Milk was fermented using commercial yeast (30 g/liter) by allowing 24 hour fermentation. Then this fresh curd was whisked thoroughly to achieve a smooth consistency without any lumps at room temperature and left to ferment for 48 hours. After which it was diluted with water in a 1:5 ratio and stirred well to create the buttermilk. The detailed methodology of extraction of seaweeds compositions in liquid form using above organic liquids as solvents are shown in Fig. 2. All extracts were filtered after stipulated time of extraction and centrifuged. The supernatant was collected as the main stock solution constituting 100% seaweed extracts of each solvent. The prime stock solution was stored at 4 °C until further use. The working

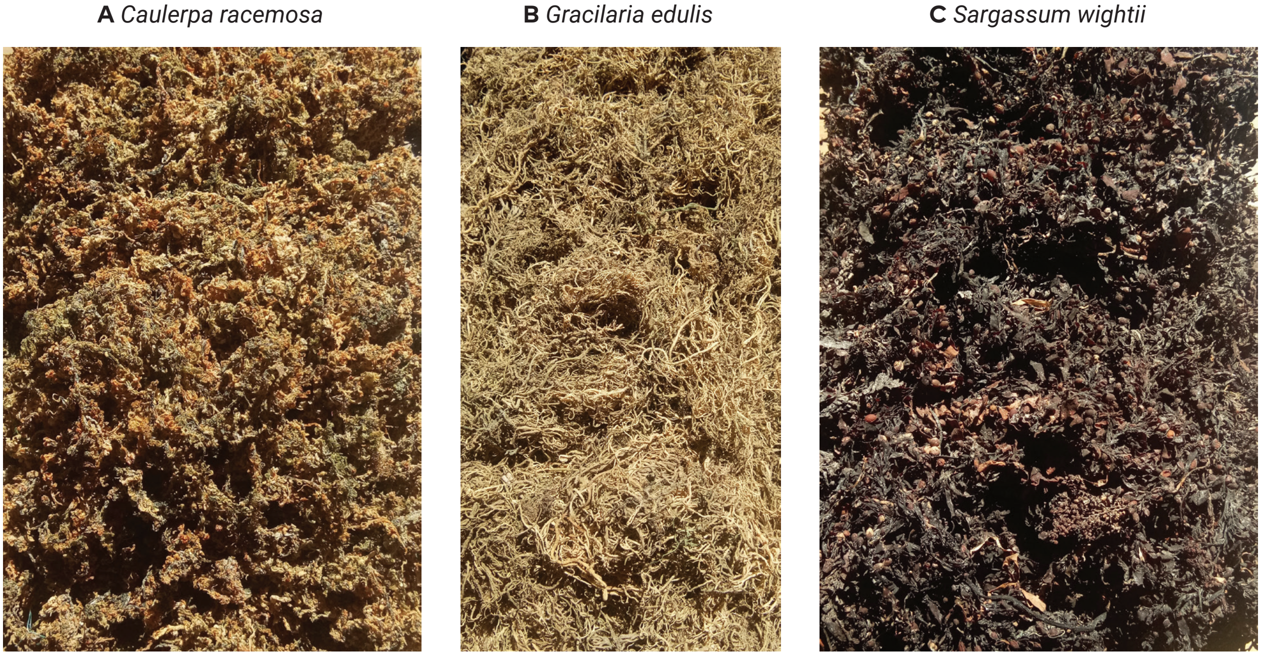

Figure 1. Seaweeds used for the experiment.

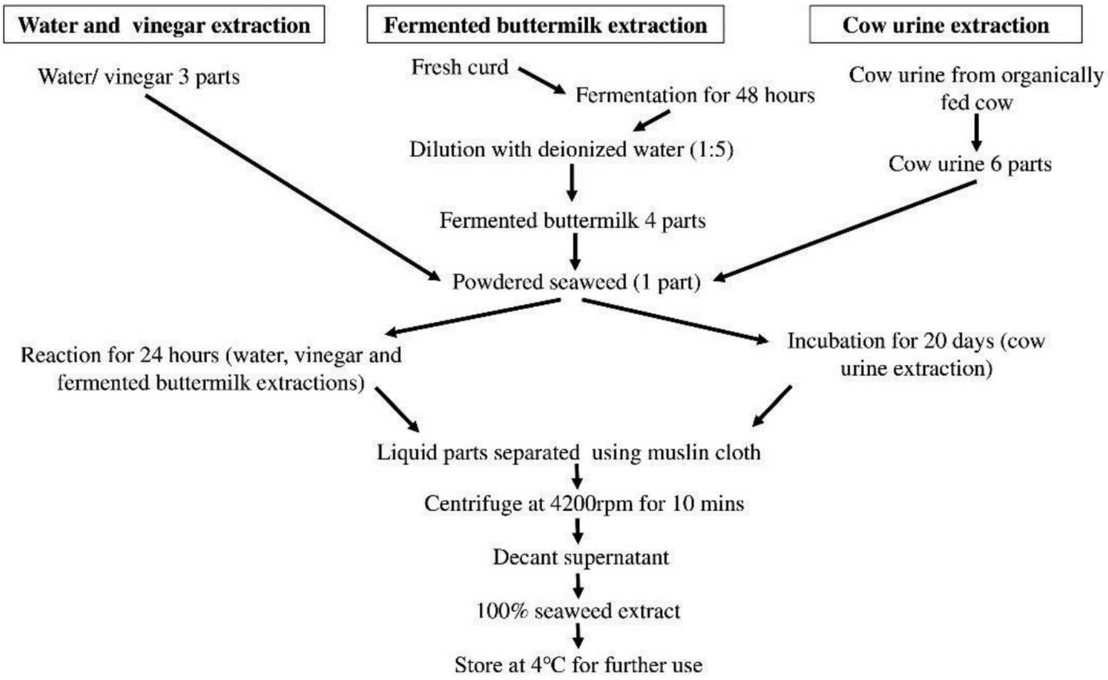

Figure 2. Procedure for organic extraction of seaweed.

concentrations from 0.1 to 15 percent was prepared by diluting the stock solution with deionized water and then utilized for characterization and growth evaluation studies.

Bioassay studies

The bioassay experiment was conducted with the seeds of blackgram (*Vigna mungo*) var Vamban 11 following the procedure described by Pannacci et al. 2022 with slight modifications. The seeds were washed previously with water to assess the stimulatory effect of different prepared extractants at different concentrations. Germination paper was placed in petri dish (150 × 15 mm) on which 50 seeds was placed. All the four organic liquid extracts of all the three seaweeds were applied at eight different levels of concentrations (0.1, 0.5, 1, 2, 5, 7.5, 10 and 15%) along with control (no application of extracts according to factorial complete randomized design). Each treatment was replicated thrice and the petri dish were closed, sealed and placed in growth chamber under controlled conditions maintaining the temperature at 25±1 °C and relative humidity at 65±1%. Germination percentage was counted on the 3rd day and other observation were recorded on the 8th day. From each petri dish, ten seedlings were selected randomly to measure the shoot and root length. Germination percentage, shoot length, root length, fresh weight and dry weight of selected seedlings from each treatment were converted into percentage against control.

Assessing growth changes and hormesis

Change in germination percentage and seedling growth compared to control due to imposed seaweed extracts at different concentrations was subjected to non-linear regression log-logistic model as described by Streibig et al. (1993).

$$\Upsilon = c + \frac{d-c}{1+exp\,\{b\{log(x)-log(a)\}\}} \tag{1}$$

Where, ϒ is the response of the treated seedlings as a function of organic seaweed extract *x*, *d* is the upper asymptote of the maximal response, *c* is the lower asymptote of the minimal response, *b* is the slope of the parameter and *a*, is the concentration that gives a response halfway between the upper and lower asymptotes.

But in the case of hormesis, there is stimulatory response in lower concentration, hence, the following peaked regression model was used as described by (Brain and Cousens 1989).

$$\Upsilon = c + \frac{d-c+fx}{1+exp\,\{b\{log(x)-log(a)\}\}} \tag{2}$$

Where, *f* is the additional parameter affecting the steepness of the curve.

Mineral composition analysis

The raw seaweed powder and their extracts were analysed for ultimate composition following the methods described by Association of Official Analytical Chemists (AOAC 1990). The nitrogen (N) concentration was analysed by micro-kjeldahl method using the Kelplus Classic- DX VA instrument. The total phosphorus (P) concentration was analysed using Double beam Shimadzhu (Model 1800) UV-VIS Spectrophotometer. Sodium concentration was analysed with flame photometer (Elico). Potassium (K), secondary (Ca, Mg, S), micro nutrients (Mn, Fe, Zn and Cu) and heavy metals (Ni, Cd, Pd and Cr) were analysed using Thermo Fischer ICP-OES 7000.

Fourier transform infrared spectroscopy analysis

The powdered raw seaweeds and their extracts with different extractants were subjected to FTIR analysis for functional groups identification. The analysis was performed using Bruker 3000 Hyperion FTIR spectrometer (Germany) available in Department of Chemistry, National Institute of Technology, Silchar, India. Spectra were recorded in the wave number range of 400 to 4000 nm^{-1} setting the operational voltage and current at 40 kV and 100 mA, respectively.

Statistical analysis

The data collected were subjected to statistical analysis adopting FCRD design using MS Excel 2013 (Windows 10). While the FTIR graphs were generated in OriginPro 2023 (https://www.originlab.com/index.aspx?go=Products/Origin) and the dose response curve was drawn using R statistical software (version 4.3.2.https://posit.co/download/rstudio-desktop/).

Results

Hormetic response of blackgram to seaweed extracts

Data on the germination, shoot length, root length, fresh weight and dry weight of black gram seedlings treated with organically extracted seaweed solutions and the related nonlinear regression according to equation 1 and 2 were shown in Fig. 3. Estimated EC_{50} values from the regression were also given in Table 1.

Germination characteristics of blackgram seeds treated with organic extracts of seaweeds revealed that there was no significant difference among the seaweed species. However, the extractants used and the levels of concentration applied shows significant effect. In all the extracts, significantly higher percentage of germination was recorded at 0.1% concentration (98–100% germination) and 0.5% concentration (96–100% germination) except for vinegar extract. Reduction in germination with increased concentration was observed. While the complete inhibition of germination was observed for vinegar and cow urine extracts, respectively at 1 and 7.5% concentration onwards, treatment with the water and fermented buttermilk extract recorded >80% germination up to 10% concentration. Similar results were observed with respect to black gram seedlings shoot and root length, and the fresh and dry biomass. Seedling shoot length, root length and dry weight was highest with fermented buttermilk extraction at 1% with 27.37, 21.55 and 24.04% increase compared to control, respectively. Fresh weight of blackgram seedling was highest with water extraction of seaweed at 1% with 23.33% increase from control. Details on germination percentage and seedlings growth parameters were given in Suppl. material 1: tables S3–S7. Results showed hormetic response of blackgram seed to the seaweed extracts with higher concentrations inhibiting germination and seedling growth and lower concentrations.

Mineral composition of seaweed extracts

Mineral composition of seaweeds showed that N content was highest in *C. racemosa* (0.252%) followed by *G. edulis* (0.196%). However, P content was almost similar in all the seaweeds ranging from 0.095–0.096%, and K content was significantly high in *G. edulis* (10.29%). Detail on mineral composition of three raw seaweeds *Caulerpa racemosa*, *Gracilaria edulis* and *Sargassum wightii* were given in Table 2. Mineral composition of solvents showed that water and vinegar contains no minerals that contributes in extraction of the seaweeds. However, fermented buttermilk and cow urine contributes largely on the minerals composition of the seaweed extract on top of the minerals contributed by the seaweed. Except for phosphorus, calcium and magnesium, all minerals contents were highest in cow urine than any other solvents (Table 3).

Depending on the extractant employed, the amount of nutrients extracted from the seaweed varies. The major nutrients viz., nitrogen, phosphorus and potassium were highest in *G. edulis*. The N content of the organic extract of seaweeds ranges from 0.791–0.028%, P content ranges from 0.121–0.002%, and K content ranges from 7.04–0.33%. The Ca content was the highest in *C. racemosa* extracted with vinegar 3073.10 mg L^{-1} and Mg was the highest in cow urine extract of *S. wightii* (2003.30 mg L^{-1}). However, concentration of sodium was highest in cow urine extraction of *C. racemosa* (3.94%). Micro nutrient composition of seaweed extract varies with extractant type and seaweed species. Similarly, heavy metal content

Table 1. Estimated EC_{50} values of organic extract of seaweed on blackgram seedlings.

Extract type	EC_{50} for enhanced germination (%)	EC_{50} for enhanced shoot length (%)	EC_{50} for enhanced root length (%)	EC_{50} for enhanced seedling fresh weight (%)	EC_{50} for enhanced seedling dry weight (%)
Water	23.86	5.71	18.06	8.76	7.82
Vinegar	0.56	0.61	0.54	0.57	0.62
Fermented buttermilk	5.62	5.62	5.63	5.62	5.62
Cow urine	5.48	5.18	5.23	2.16	2.15

*EC_{50} = Effective concentration at 50%.

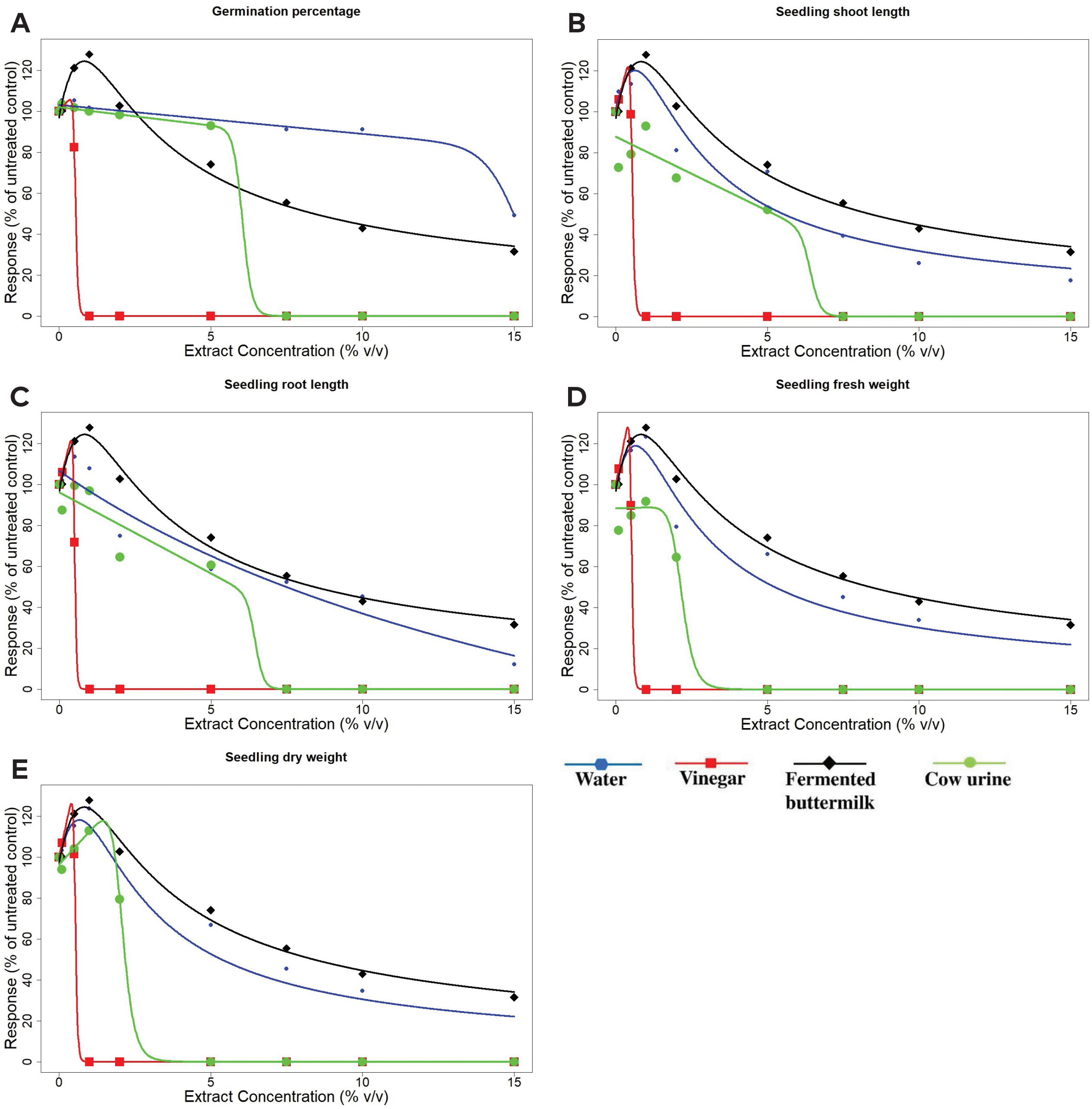

Figure 3. Dose-response curve of blackgram seedling on germination percentage, shoot length, root length, fresh weight and dry weight treated with different organic extract of seaweed. Extraction solvent being water, vinegar, fermented buttermilk and cow urine. Symbols show observed data in bioassay experiment and lines show fitted curves according to log-logistics model 1 and 2.

also varied with seaweed and extractants used, however, total heavy metal contents were higher in *S. wightii* than other seaweeds with all the extractant. Details on mineral composition of seaweed extracts were given in Tables 4–6 and Suppl. material 1: fig. S1 shows the effectiveness of solvents on extracting minerals from dry seaweeds.

Variation in mineral composition of seaweed extract was observed depending on solvents used for extraction and among seaweed species. This could be attributed to differential mineral content of the seaweed and the solvents contributing towards variation in the content of the extract. Synergic and antagonistic effect of the solvent seaweed interaction could also play role in the mineral extracted.

Functional groups in seaweed extract

The raw seaweeds powder and extracts were analyzed in FTIR to identify the functional groups (Fig. 4). Irrespective of seaweeds and the extractant type, a strong band was seen at 3342.17 to 3366.89 cm^{-1} corresponding to O-H and N-H stretching vibrations of amino acids and polysaccharides. Similarly the moderate band appeared at 1615.45 to 1636.05 cm^{-1} corresponding to C=O stretching and N=O asymmetric stretching of esters and pectin complexes. In all the raw seaweeds, vinegar and cow urine extractions a weak peak was observed around 1421.76 cm^{-1} and 1275.46 cm^{-1} corresponds to O-H bends/ C=C stretch and C-O stretch respectively. Specifically the red seaweed alone was identified with a peak at 1102.38 attributes to C=O (of ethers). Weak peak was observed between 591.37 to 653.18 cm^{-1} attributing C-S (of sulfates) stretching. Details of the peaks observed in FTIR analysis were given in Suppl. material 1: table S1, S2.

Table 2. Mineral composition of seaweeds *Caulerpa racemosa*, *Gracilaria edulis* and *Sargassum wightii*.

Mineral composition	*Caulerpa racemosa*	*Gracilaria edulis*	*Sargassum wightii*
N (%)	0.252±0.12	0.196±0.06	0.112±0.04
P (%)	0.095±0	0.096±0.005	0.095±0.003
K (%)	1.60±0.144	10.29±0.634	4.66±0.042
Ca (mg kg^{-1})	7077.20±198.56	3378.40±219.34	3089.75±232.28
Mg (mg kg^{-1})	2110.20±114.98	1066.10±126.85	2235.55±89.31
S (%)	0.191±0.012	0.221±0.00	0.191±0.001
Na (%)	18.31±0.78	3.08±0.11	4.69±0.04
Fe (mg kg^{-1})	945.05±9.83	6513.3±374.34	789.95±81.39
Zn (mg kg^{-1})	14.9±0.28	32.25±2.05	9.6±1.56
Mn (mg kg^{-1})	27.3±0.85	653.55±31.32	31.7±1.70
Cu (mg kg^{-1})	8.2±0.14	56.65±1.77	15.8±0.28
Ni (mg kg^{-1})	2.9±0.28	8.6±0.85	6.25±0.21
Cr (mg kg^{-1})	18.85±2.05	34.6±1.27	29.5±1.70
Pb (mg kg^{-1})	3.5±0.71	4.65±1.06	2.95±0.35
Cd (mg kg^{-1})	0.4±0.00	1±0.00	1.45±0.07

Table 3. Mineral composition of solvent used for extractions.

Mineral composition	Water	Vinegar	Fermented buttermilk	Cow urine
N (%)	0±0	0±0	0.189±0.14	0.649±0.07
P (%)	0±0	0±0	0.094±0.01	0.039±0.001
K (%)	0±0	0±0	0.002±0.001	2.59±0.06
Ca (mg L^{-1})	0±0	0±0	1349.30±283.55	28.40±3.18
Mg (mg L^{-1})	0±0	0±0	540.50±38.47	522.08±4.91
S (%)	0±0	0±0	0.05±0.02	1.03±0.35
Na (%)	0±0	0±0	0.01±0.001	2.11±0.19
Fe (mg L^{-1})	0±0	0±0	19.25±2.33	32.85±1.48
Zn (mg L^{-1})	0±0	0±0	1.55±0.29	4.95±0.49
Mn (mg L^{-1})	0±0	0±0	0±0	0±0
Cu (mg L^{-1})	0±0	0±0	0.75±0.07	1±0
Ni (mg L^{-1})	0±0	0±0	0±0	0±0
Cr (mg L^{-1})	0±0	0±0	1.50±0	9.25±0.63
Pb (mg L^{-1})	0±0	0±0	0±0	0.15±0.07
Cd (mg L^{-1})	0±0	0±0	0±0	0±0

Table 4. Ultimate mineral composition of green seaweed (*Caulerpa racemosa*) extracted with different organic solvents.

	Water extract	Vinegar extract	Fermented buttermilk extract	Cow urine extract
N (%)	0.056±0.02	0.084±0.04	0.028±0.01	0.756±0.30
P (%)	0.019±0.002	0.043±0.012	0.036±0.01	0.056±0.01
K (%)	0.55±0.04	0.33±0.07	1.26±0.17	3.06±0.21
Ca (mg L^{-1})	1109.60±52.04	3073.10±115.54	489.15±10.39	516.60±3.25
Mg (mg L^{-1})	1253.20±170.41	1781.70±141.28	894.30±14.99	1447.90±126.01
S (%)	0.024±0.006	0.001±0.00	0.17±0.01	0.69±0.03
Na (%)	3.08±0.59	2.43±0.22	2.38±0.19	3.94±0.26
Fe (mg L^{-1})	20.8±4.38	55.6±9.09	15.85±2.9	14.25±2.19
Zn (mg L^{-1})	3.55±0.19	7.05±1.48	5.35±0.78	4.05±0.14
Mn (mg L^{-1})	6.25±0.49	7.1±0.28	1.75±0.21	0±0
Cu (mg L^{-1})	3.80±0.97	4.00±0.55	1.95±0.35	0.75±0.21
Ni (mg L^{-1})	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Cr (mg L^{-1})	7.10±0.42	1.15±0.21	6.75±1.06	1.20±0.28
Pb (mg L^{-1})	1.05±0.38	1.80±0.12	0.5±0.28	0.0±0.0
Cd (mg L^{-1})	0.10±0.00	0.20±0.00	0.20±0.0	0.0±0.0

Table 5. Ultimate mineral composition of red seaweed (*Gracilaria edulis*) extracted with different organic solvents.

	Water extract	Vinegar extract	Fermented buttermilk extract	Cow urine extract
N (%)	0.084±0.04	0.07±0.02	0.042±0.02	0.791±0.19
P (%)	0.022±0.005	0.051±0.014	0.043±0.02	0.121±0.07
K (%)	1.73±0.33	2.18±0.16	3.88±0.30	6.34±0.79
Ca (mg L^{-1})	372.70±62.22	447.25±33.45	273.05±16.62	18.30±3.96
Mg (mg L^{-1})	879.65±79.55	956.40±33.23	795.35±45.89	1155.90±100.69
S (%)	0.186±0.011	0.194±0.06	0.23±0.02	1.17±0.14
Na (%)	1.99±0.47	0.85±0.28	0.49±0.01	1.30±0.2
Fe (mg L^{-1})	27.75±16.90	21.85±2.76	36.3±5.94	54.55±10.82
Zn (mg L^{-1})	4.00±0.14	3.90±0.28	7.75±0.64	1.35±0.92
Mn (mg L^{-1})	12.65±2.33	65.4±0.42	47.8±2.69	0.3±0.07
Cu (mg L^{-1})	3.15±0.34	3.00±0.56	3.50±0.71	1.10±0.28
Ni (mg L^{-1})	0.1±0.01	0.0±0.0	0.0±0.0	0.3±0
Cr (mg L^{-1})	5.50±0.14	4.80±0.69	7.75±0.64	4.05±1.20
Pb (mg L^{-1})	0.40±0.14	1.30±0.28	0.60±0.42	0.0±0.0
Cd (mg L^{-1})	0.10±0.00	0.20±0.00	0.20±0.0	0.0±0.0

Table 6. Ultimate mineral composition of brown seaweed (*Sargassum wightii*) extracted with different organic solvents.

	Water extract	Vinegar extract	Fermented buttermilk extract	Cow urine extract
N (%)	0.056±0.02	0.084±0.04	0.056±0.02	0.611±0.17
P (%)	0.015±0.003	0.002±0.001	0.026±0.01	0.117±0.02
K (%)	2.13±0.97	1.15±0.05	1.93±0.57	7.04±0.13
Ca (mg L^{-1})	516.05±22.13	501.35±32.17	159.75±9.69	171.75±4.59
Mg (mg L^{-1})	1325.15±329.44	1761.15±98.78	1303.15±182.36	2003.30±186.25
S (%)	0.028±0.015	0.015±0.003	0.18±0.01	0.96±0.08
Na (%)	2.46±0.26	0.92±0.14	0.53±0.01	1.42±0.14
Fe (mg L^{-1})	28.3±7.92	26.5±5.32	38±3.82	65.2±11.17
Zn (mg L^{-1})	4.10±0.42	3.70±0.57	9.35±1.63	0.85±0.64
Mn (mg L^{-1})	0.95±0.07	4.05±1.06	2.45±0.49	2.7±0.28
Cu (mg L^{-1})	4.35±1.20	2.55±0.21	1.90±0.57	1.40±0.14
Ni (mg L^{-1})	0.0±0.0	0.15±0.02	0.0±0.0	0.0±0.0
Cr (mg L^{-1})	8.60±1.27	10.25±0.07	6.55±0.99	5.35±0.35
Pb (mg L^{-1})	2.35±0.35	2.65±0.48	0.30±0.14	0.15±0.07
Cd (mg L^{-1})	0.15±0.07	0.20±0.00	0.20±0.0	0.30±0.0

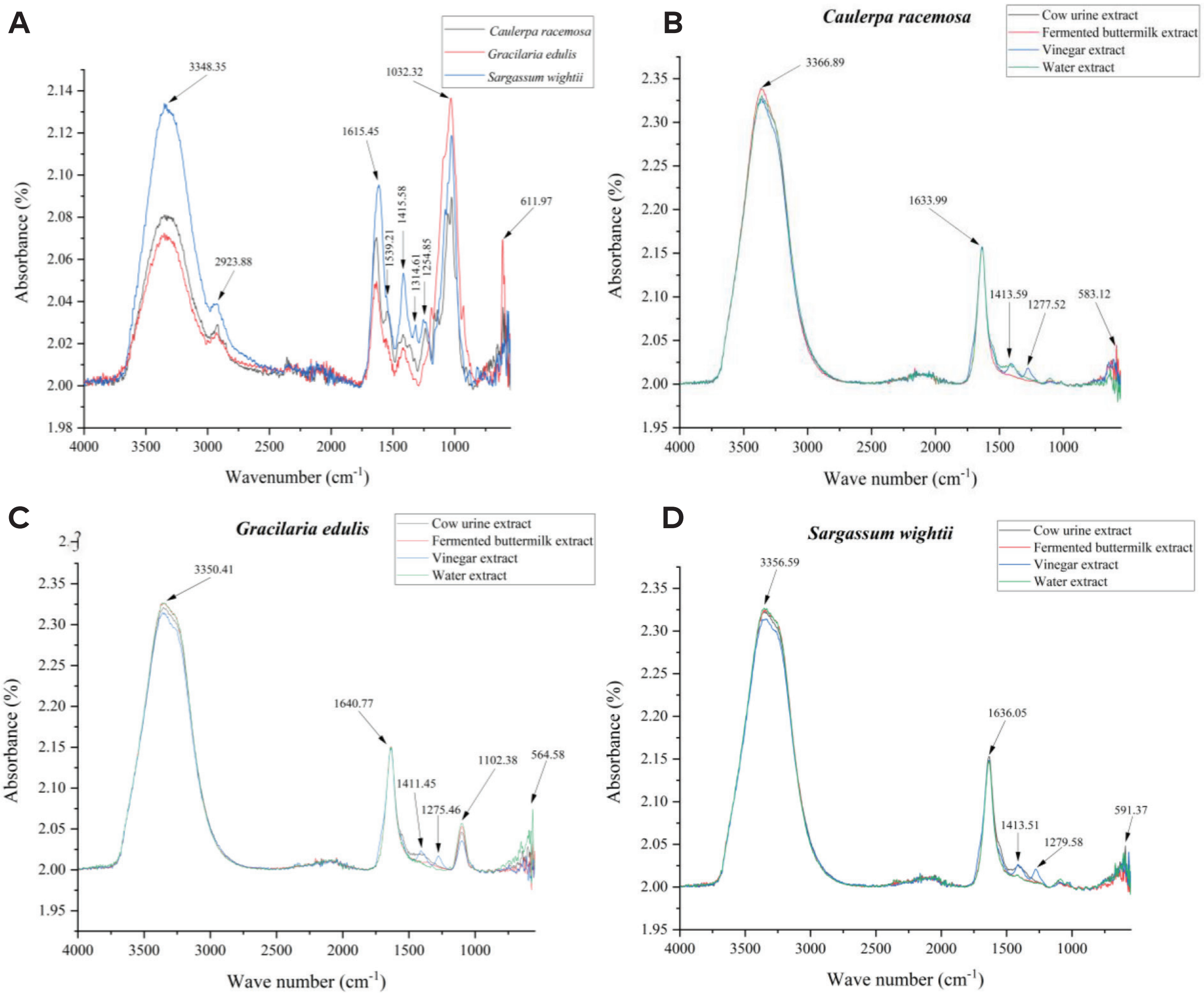

Figure 4. FTIR spectrum of three seaweed and its extract with water, vinegar, fermented buttermilk and cow urine (**a** Raw seaweeds, **b** *Caulerpa racemosa*, **c** *Gracilaria edulis*, **d** *Sargassum wightii*).

Discussion

Seaweed is a mixture of many components like mineral nutrients, organic compounds, hormones, and other metabolites. Moreover, seaweed also contain phytohormones such as IAA, IBA, ABA, kinetin and gibberellin (Spagnuolo et al. 2022). Presence of phytohormones in combination with mineral content improves seed germination (Sasikala et al. 2016). These components can show either synergetic or antagonistic effects on the stimulatory response (Agathokleous et al. 2020). Seaweeds as raw powder and as extracted or formulated products were largely used in agriculture as growth stimulant. However, seaweed formulations mostly did not focus on the availability of solvents or extractants for farmers to prepare themselves organically. Hence, in the present study, an attempt was made to extract the composition of different seaweed species using various extractants of natural (organic) origin viz., fermented buttermilk and cow urine in comparison with water and vinegar. The stimulant effect of these extracts on blackgram seed was tested at different concentrations.

There has been variation in mineral composition of seaweeds. Nitrogen content in the present study when compared to nitrogen found in other studies is much lower such as 2.93% reported by Rameshkumar et al. 2012 and 2.37–2.16 by Sravani et al. 2023. However, compared to nitrogen content reported by Hernández Herrera et al. 2022 (0.007%) on *C. racemosa*, the nitrogen content in the present study (0.252%) was much higher. Many other studies also reported that K, Ca and Mg were the most abundant mineral in seaweeds which could vary depending on the seaweed species, extraction methods and solvents (Garai et al. 2021; Hassan et al. 2021). Test on heavy metal content of seaweed extracts has not been conducted to a large extent. However, seaweed extracted are known to contain heavy metals which resulted in inhibition of seedling germination (Jia et al. 2015). Heavy metals were known to show phytoxicity effect on plants and pulses is one of the plants highly affected by heavy metals (Shankar et al. 2009). Chromium which is one of the most prominent heavy metals in seaweed extract inhibited germination, shoot and root growth of seedling (Singh et al. 2013). This was also observed in the present study.

Using the bioassay data, dose-response curve was constructed to understand the hermetic effect of seaweed extracts to blackgram seed. Hormesis is commonly found natural plant biostimulants. While different seaweed species did not show significant variations, there was significant difference between different types of extractants and levels of concentrations on germination and seedling growth of blackgram.. Despite water extraction significantly enhancing germination percentage, a reduction in shoot and root length of seedlings was observed compared to fermented buttermilk extract. This may be due to the minerals nutrients contributed by the fermented buttermilk extract. Fermented buttermilk contained nutrients and other growth promoters which are beneficial for plant growth (Surya et al. 2021).

Vinegar, even though it was known to improve seeds ability to germinate, impaired seed germination when 5% or above was used for seeds treatment (Tobias et al. 2007), however, concentration above 1% completely inhibited blackgram germination in the present study. Reduction in seed germination and inhibitory effect on shoot and root development by seaweed extract at higher concentrations by seaweed extract was also reported by Arun et al. (2014).

Inhibition of seeds germination and seedling development at higher concentration might be due to phytoxicity effect of the growth substances of seaweed which might cause adversity in growth and execution of the seeds (Barreto et al. 2022). In addition to the organic compounds, some of the elements as sodium and nickel at higher concentration might also have affected the seed germination and seedling growth (Das et al. 2015; Priyadharshini et al. 2019). Moreover, other heavy metals such as cadmium and lead also inhibited seed germination (Li et al. 2005; Atta et al. 2021). This was confirmed by the presence of functional groups/compounds like sulfated polysaccharides, esters, pectin substances identified in all the three seaweeds by the FTIR. However, compounds like fucoidans was reported to be beneficial in many circumstances (Shukla et al. 2021). These compounds are responsible for antibacterial, antioxidant, and other therapeutic qualities (Palaniyappan et al. 2023). The compounds found in the seaweed extract of the present study were also documented previously by Deepika (2018) and Sravani et al. (2023).

However, there are no stress inhibitor that occur in solitude. So, it is crucial to know if hormesis is triggered by combinations of chemicals or by other substances (Agathokleous et al. 2019a). These might show significant impact on the germination and seedling growth, as dose response curve showed hormetic effect. Hormetic response was also observed in greengram treated with aqueous seaweed extract (Hernández-Herrera et al. 2022). Moreover, other plant extracts such as mugwort extracts also induced hormetic effect on six different vegetables viz., tomato, carrot, rapeseed, onion, cauliflower and lettuce (Pannacci et al. 2022). In the context of plant hormesis, biostimulants enhance health and stress tolerance (Agathokleous et al. 2019b), as well as growth and production. Through secondary metabolites and gene expression, biostimulant-induced hormetic responses enable plants to survive stress and regain equilibrium (Ahkami et al. 2017; Vargas-Hernandez et al. 2017).

Conclusion

Extraction solvents of seaweed paved way in improvement of seed germination and seedling development. Moreover, concentration level also played huge role in germinating ability of blackgram seeds. Fermented buttermilk and water extracted of seaweed promoted germination and seedling growth, however, vinegar and cow urine seem to be on the contrary. This proved the importance of solvent selection for extraction of seaweed. Functional compounds presented in the seaweed extract confirmed the fundamental role of seaweed i.e, immunity boost of the plants. Additionally, the concentration response studies supported hormetic response of seaweed extraction at different application levels. This study confirms that water and fermented buttermilk extraction of seaweed is beneficial for seed treatment and has lower phytotoxicity effect at higher concentrations. Thus, we recommended use of water and fermented buttermilk extracted seaweeds at 0.5 to 1% to improve seed germination.

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Supplementary material

Supplementary material 1
Details of FTIR analysis, obervation data and extraction efficiencies (docx. file)
Link: https://doi.org/10.3897/ejfa.2024.123151.suppl1