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RESEARCH ARTICLE

Extraction of R-Phycoerythrin from *Kappaphycus alvarezii* (Doty) Doty ex Silva and analyses of its physico-chemical properties

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Abstract

The phycoerythrin (PE) is a protein acting as a photosynthetic accessory pigment in red algae (Rhodophyta). This protein has gained many biotechnological applications in food science, cosmetics and analytical processes. In this study, phycoerythrin of red macro alga *Kappaphycus alvarezii* was extracted by freezing and thawing method in sodium phosphate buffer (0.1 M). The protein was precipitated with 60% ammonium sulphate saturation and dialysed against the same buffer (10 mM) and lyophilized. The R-PE was analysed for its various physico-chemical properties and the analyses revealed that R-PE has characteristic affinity towards different metal ions, inhibitors, organic solvents, preservatives at different monochromatic irradiances.

Keywords: Phycoerythrin, red algae, *Kappaphycus alvarezii,* metal ions, inhibitors, organic solvents.

Introduction

Marine macro algae or seaweeds are one of nature's most biologically active resources as they possess a wealth of bioactive compounds. It is exploited for both human and animal health applications. Seaweeds are commonly classified into three main groups based on their pigmentation. Phaeophyta, or brown seaweeds, are predominantly brown due to the presence of the carotenoid, fucoxanthin and the primary polysaccharide present includes alginates, laminarins, fucans and cellulose (Goni *et al.,* 2002). Chlorophyta, or green seaweeds are dominated by chlorophyll a and b with ulva being the major polysaccharide component (Robic *et al.,* 2009). The principal pigments found in Rhodophyta or red seaweeds are phycoerythrin and phycocyanin and the primary polysaccharides are agars and carrageenan (Mc Hugh *et al.,* 2003). Red algae are ecologically significant as primary producers, providers of structural habitat for other marine organisms. *Kappaphycus alvarezii* is economically important red tropical seaweed which is highly demanded for its nutraceutical and pharmaceutical applications.

Phycobilisome composed of phycobillin chromophores (phycoerythrin, phycocyanin and allophycocyanin) associated with proteins. The phycobillin chromophores is a linear tetrapyrrole similar in structure to tetrapyrrole ring portion of chlorophyll molecules but linearised phycobillins do not contain a hydrophobic portion like phytol tail and therefore it is water soluble. Phycoerythrin absorbs in the green region (495-570 nm) while, phycocyanin absorb in the green yellow region (550-630 nm) and allophycocyanin in the orange red region (650-670 nm).

Visually, phycoerythrin appears red and phycocyanin range from purple (phycoerythrocyanin, R-Phycocyanin) to deep blue (C-Phycocyanin) and allophycocyanins are blue with a hint of green. Research in natural products of marine algae has made significant advances in recent years. Marine algae are known to produce a variety of compounds and some of them have been shown to possess biological activity of potential medicinal values (Moore, 1978; Konig *et al*., 1994) in the last three decades the discovery of metabolites with biological activities from seaweeds has increased significantly. Against these backdrops, this study analysed R-Phycoerythrin from *Kappaphycus alvarezii* for its various physico-chemical properties namely metal ions, inhibitors, organic solvents and preservatives at different monochromatic irradiances.

Fig. 1. *Kappaphycus alvarezii.*

Materials and methods

Collection of samples: The live and healthy specimens of red seaweed *Kappaphycus alvarezii* were collected along the coast of Mandapam (Lat. 090 17'N; Long. 790 08'E), Palk Bay, Tamil Nadu between Jan 2009 and March 2010 and brought to laboratory (Fig. 1). It was then cleaned and washed with tap water for the extraction of R-Phycoerythrin.

Extraction and estimation of R-Phycoerythrin: The red alga *K. alvarezii* was taken in a sterile container and kept in sodium phosphate buffer (pH 7.2; 0.1 M) for repeated freeze and thawing (Fig. 2). Later the biomass was separated by centrifugation at 8000 rpm for 15 min. The proteins in the supernatant were precipitated with 60% ammonium sulphate saturation and the mixture was stirred overnight. The phycoerythrin, phycocyanin and allophycocyanin pigments were assayed by reading the supernatant at 562, 615 and 652 nm using DU-40 Spectrophotometer (Beckman, USA). Fractions which show higher reading for R-Phycoerythrin (562 nm) were pooled. All the subsequent purification steps were carried out at 4°C. The R-PE obtained was used to analyze the physico-chemical properties.

Fig. 2. R-Phycoerythrin from *K. alvarezii.*

Physico-chemical analyses of R-Phycoerythrin

Effect of different metal ions on R-Phycoerythrin: Solutions of different metal ions such as manganese chloride ($MnCl_2$), cobalt chloride ($CoCl_2$), mercuric chloride ($HgCl_2$), copper sulphate ($CuSO_4$), calcium chloride ($CaCl_2$) were prepared in sodium phosphate buffer in different concentrations (0.1, 0.5, 1, 5 and 10 mM). One mg of the purified R-PE was added in the above metal ions and incubated for 1 h in room temperature and assayed at 562 nm using DU-40 Spectrophotometer (Beckman, USA).

Effect of different inhibitors on R-PE: Solutions of different inhibitors such as EDTA, DMSO, β-mercaptoethanol, SDS were prepared in sodium phosphate buffer in different concentrations (0.1, 0.5, 1, 5 and 10 mM). One mg of purified R-PE was added in the inhibitors and incubated for 1 h in room temperature and assayed.

Effect of different solvents on R-PE: One mg of the pigment was dissolved in 3 mL of different organic solvents namely chloroform, acetone, ethanol, methanol, ethyl acetate and incubated for 1 h in room temperature and assayed.

Effect of edible preservatives on R-PE: Phycoerythrin stability was determined by adding different preservatives such as sucrose (0.1 g), calcium chloride (0.1 g), sodium chloride (0.1 g) and citric acid (0.1 g) was added in 25 mL phycoerythrin solution (5 mg of R-PE was dissolved in 25 mL of sodium phosphate buffer (0.1 M, pH 7.2) and kept in two different conditions, one at 4ºC in the refrigerator and the other at room temperature 35°C. The absorbance of phycoerythrin solution containing preservative along with the control without preservative was measured after regular interval up to 30 d on DU-40 Spectrophotometer at 562 nm for R- Phycoerythrin.

Effect of different monochromatic irradiances on R-PE: Purified phycoerythrin (10 mg) was dissolved in 25 mL of sodium phosphate buffer kept under different monochromatic irradiances *viz.*, red (650-750 nm), blue (470-500 nm), green (500-560 nm) and control (white light). These monochromatic irradiances were provided by wrapping the experimental conical flask with the respective cellophane colour transparent papers. All the experiments were carried out in triplicate; the values presented in the graphs are those of the mean of three independent experiments and the error bars indicate standard deviation.

Results

Effect of different metal ions on the activity of R-PE: $MgCl_2$ showed a consistent increase in the activity with increase in concentrations. The similar kind of observation was obtained for $HgCl_2$, $CuSO_4$ and NaCl. $CaCl_2$ showed consistent effect on the activity at all concentrations except at 10 mM concentration. The decrease in activity when the concentration increased was observed in $AlSO_4$ and $FeSO_4$. In KI and $ZnSO_4$ it was observed that the activity remains same in all the concentrations (Figs. 3-11).

Fig. 3. Effect of $MnCl_2$ on R-PE activity.

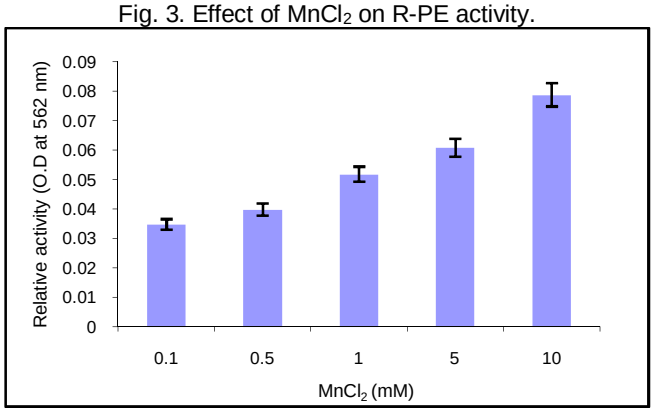


Fig. 4. Effect of $HgCl_2$ on R-PE activity.

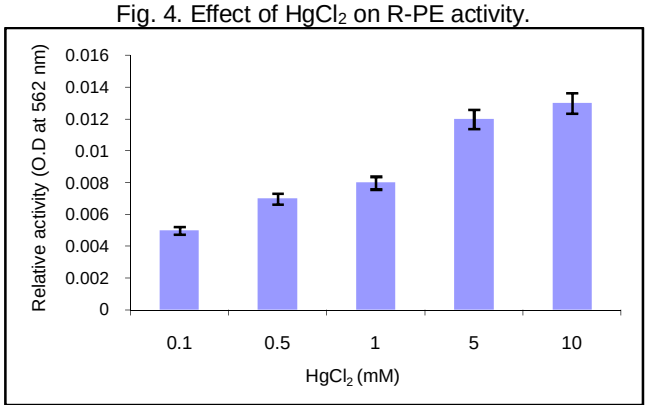


Fig. 5. Effect of $CuSO_4$ on R-PE activity.

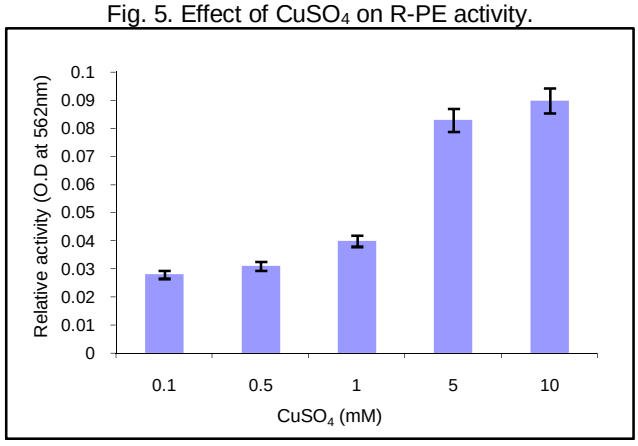


Fig. 6. Effect of $CaCl_2$ on R-PE activity.

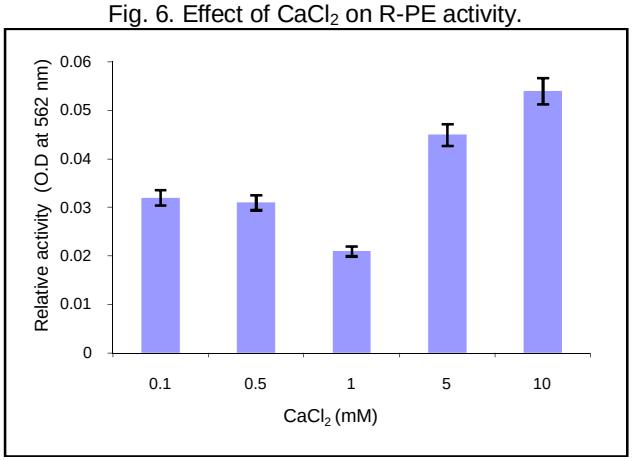


Fig. 7. Effect of $AlSO_4$ on R-PE activity.

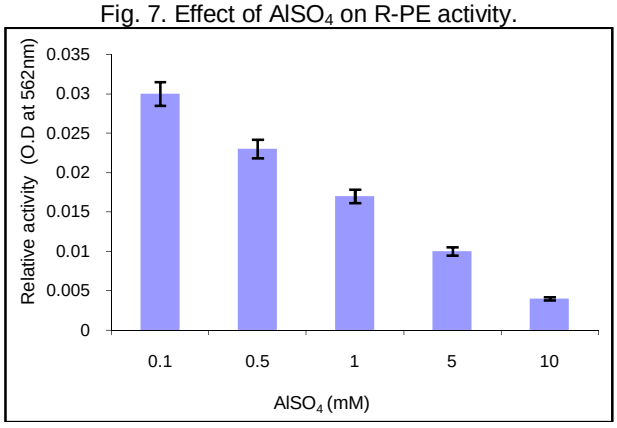


Fig. 8. Effect of KI on R-PE activity.

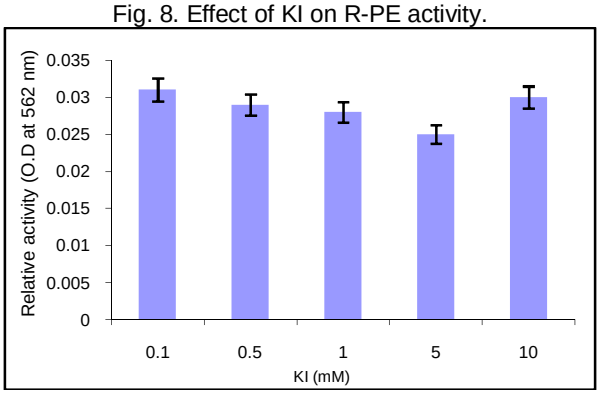


Fig. 9. Effect of NaCl on R-PE activity.

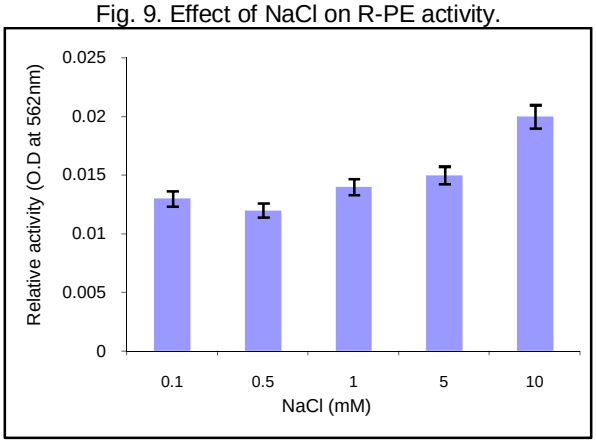


Fig. 10. Effect of $ZnSO_4$ on R-PE activity.

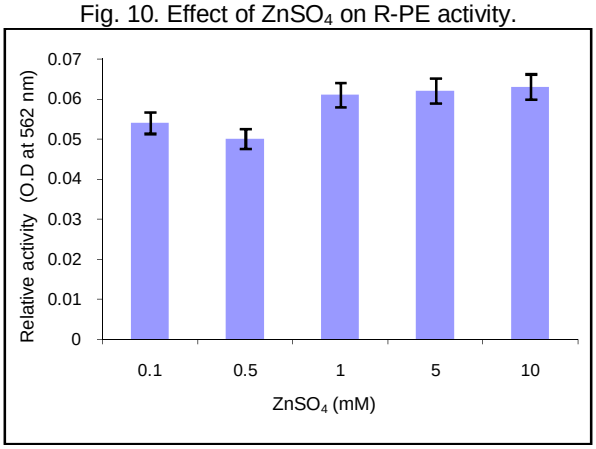


Fig. 11. Effect of $FeSO_4$ on R-PE activity.

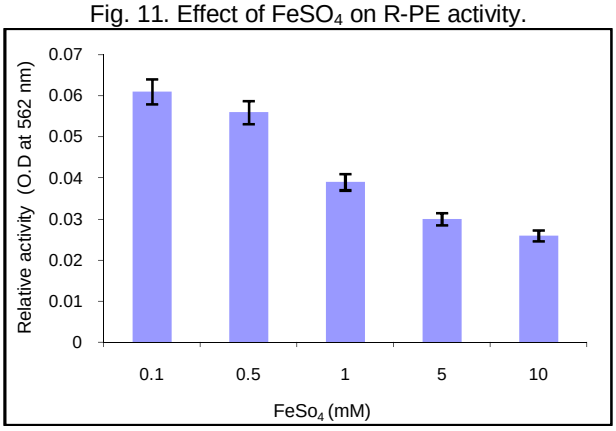


Fig. 12. Effect of EDTA on R-PE activity.

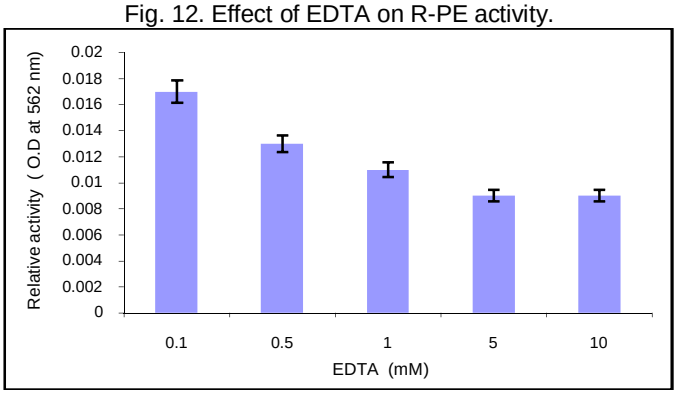


Fig. 13. Effect of SDS on R-PE activity.

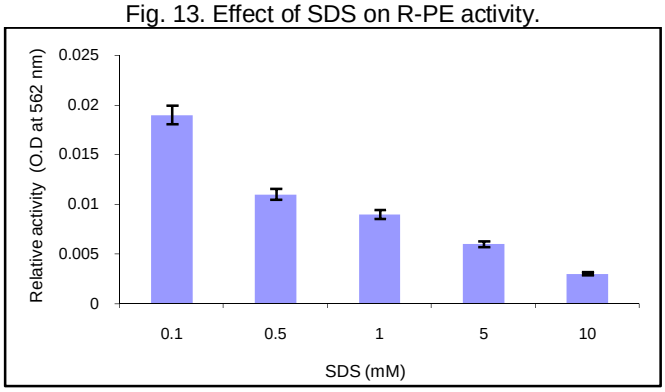


Fig. 14. Effect of DMSO on R-PE activity.

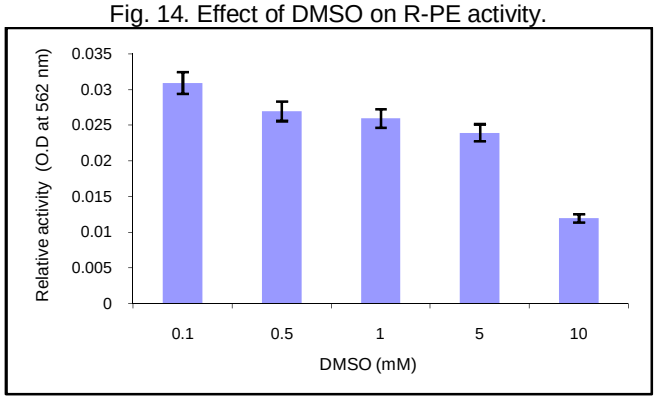
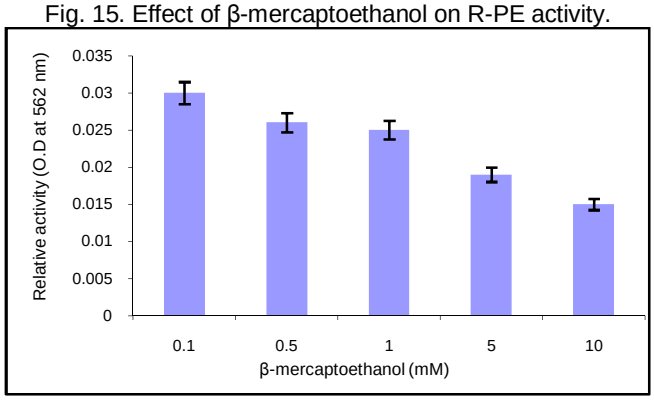


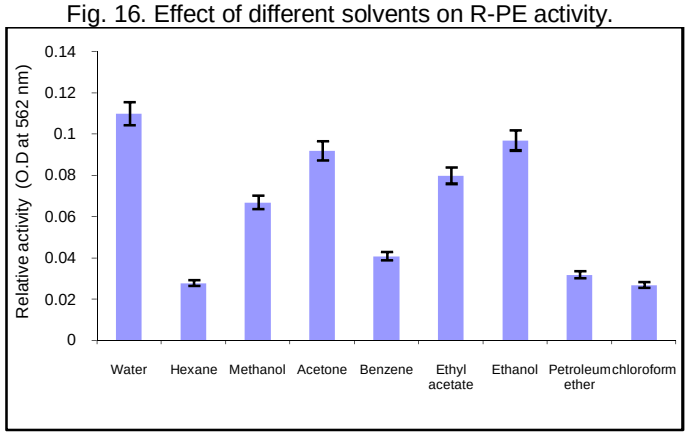
Fig. 15. Effect of β-mercaptoethanol on R-PE activity.



Effect of different inhibitors on the activity of R-PE: DMSO at lower concentrations such as 0.1, 0.5 and 1 mM enhanced the activity of R-PE whereas at 10 mm concentration it reduced the activity. There was a consistent decrease in the activity of R-PE with the increase in different concentrations of SDS. In case of β-mercaptoethanol, higher activity was observed at 0.1 mM next to 0.5 and 0.1 mM. EDTA showed higher activity at 0.1mM and it was retained same at 5 and 10 mM (Figs. 12-15).

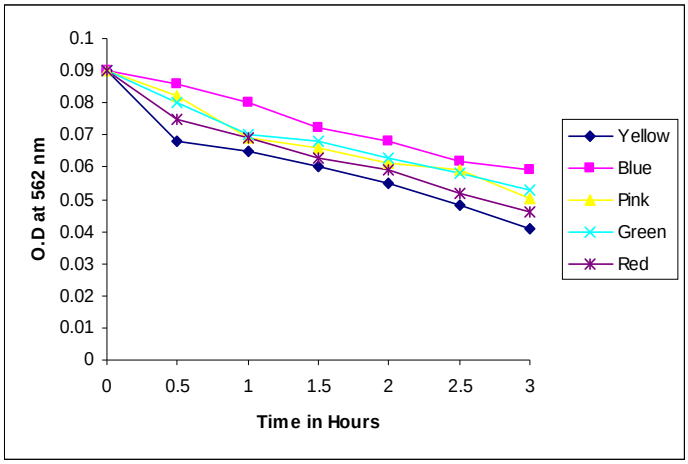
Effect of different solvents on the activity of R-PE: When different organic solvents were used for to test their effect on the activity of R-PE, it was observed that water has the best activity followed by ethanol, acetone, ethyl acetate and methanol where as hexane, benzene, petroleum ether and chloroform showed the poor activity (Fig. 16).

Fig. 16. Effect of different solvents on R-PE activity.



Effect of different monochromatic irradiances on the activity of R-PE: When R-PE was exposed to different monochromatic irradiances, the enhanced activity was observed in the blue irradiance followed by pink, green and red monochromatic irradiances, moderate activity and stability was observed in yellow irradiance (Fig. 17).

Fig. 17. Effect of monochromatic irradiances on R-PE activity.



Effect of different edible preservatives on the activity of R-PE: The absorption spectrum was recorded with preservative and without preservative at 25°C and 4°C. It was found that the loss of R-PE content in aqueous solution at 4°C is less than that at 25°C. R-PE in aqueous phase was found to be more stable with citric acid as preservative at 25°C as observed through the spectral analysis showed no discoloration of R-PE after 30 d both at 25°C and 4°C with citric acid as preservative. R-PE solution without any preservative that is control showed discoloration after 10 d and completely discolored after 30 d at 25°C, since it is heat sensitive at pH 7.2, while no discoloration was seen in R-PE sample even after 30 d at 4°C followed by citric acid, sodium chloride, sucrose and calcium chloride which were found as the effective preservatives (Figs. 18 and 19).

Fig. 18. Effect of preservatives on R-PE activity at 4°C.

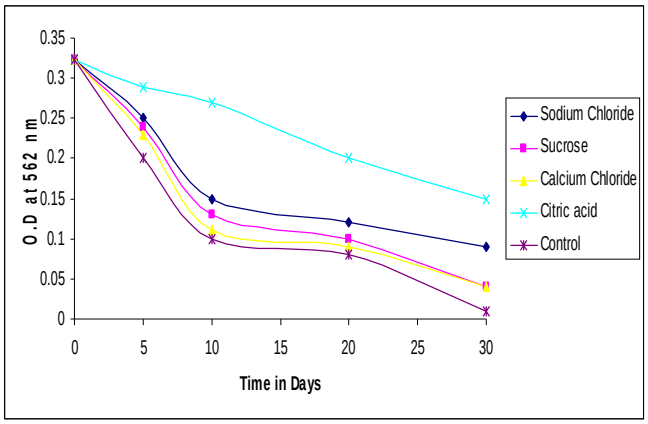
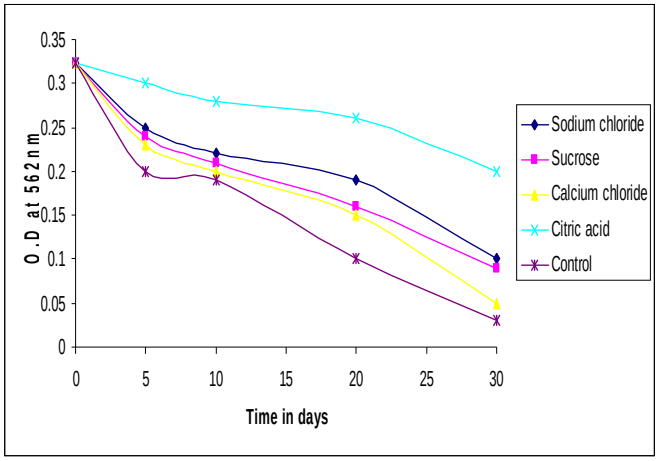


Fig. 19. Effect of preservatives on R-PE activity at 25°C.



Discussion

Many studies have demonstrated that the phycobiliproteins from red algae possess biological properties that may have potential use. To explore the potential use of seaweed, investigation on R-PE was taken in to account. The red algal seaweed *Kappaphycus alvarezii* was chosen for this investigation because the pigment protein of this has been reported to be pharmacologically active.

This alga occurs in abundance in waters near Rameshwaram and in the islands of Palk bay. This investigation was an attempt on the extraction of the R-PE from the red algal seaweed *K. alvarezii.* The possible role of inhibitors on the activity of R-PE is of great interest because of the increased use of R-PE in different areas of biomedical applications though nothing conclusively can be said of, it can be inferred that R-PE is sensitive to the different inhibitors that are normally used in the biomedical applications. The role of metal ions and solvents on the activity is very limited. In this study, $MnCl_2$ and ethanol recorded higher activity than all other metal ions and solvents whereas, other metal ions and solvents did not record any consistent data for apparent analysis.

Conclusion

Intensive investigation were made on the R-PE from the red alga *Kappaphycus alvarezii* was analyzed for phycobiliproteins R-Phycoerythrin and the effect of physico-chemical parameters on its activity was studied. The study revealed that R-PE has characteristics affinity towards different metal ions, inhibitors, organic solvents, preservatives and its effect at different monochromatic lights which may be further exploited in future for various biotechnological applications.

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