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Prospecting Antioxidant and Antibacterial potential of Marine Brown Algae *Sargassum tenerrimum J. Agardh* and *Sargassum swartzii C. Agardh.*

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Abstract: ***Marine brown algae produce a range of bioactive phytochemicals which can be used as novel antioxidant and antibacterial compounds. The current study aimed to explore marine brown algal species Sargassum tenerrimum and Sargassum swartzii extracts for their bioactivity. Antioxidant activity was evaluated by TLC Bioautography which showed separation of antioxidant compounds and In DPPH assay, S. tenerrimum methanolic extract showed strong antioxidant activity with IC50 value of 80µg/mL and moderate antioxidant activity in S. swartzii extracts with IC50 value of 130µg/mL. The antibacterial assay was carried out against four microbial strains Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus and Bacillus subtilis by disc diffusion method in which highest zone of inhibition was seen against S.typhi in S.tenerrimum extract (14.66±3.055mm) and S.swartzii extract (12.33±2.309) and In Minimum Inhibitory concentration (MIC) method for the same microbial strains, five concentrations (20, 40, 60, 80, and 100 µg/mL) were used. S. typhi was more susceptible to both the algal extracts with MIC value 20 µg/mL and 40 µg/mL for S. aureus. MIC value of 40 µg/mL was shown by S. tenerrimum extracts for K. pneumoniae, and B. subtilis as compared to S. swartzii extract having MIC value of 60 µg/mL.***
Keywords: Sargassum tenerrimum, Sargassum swartzii, Antioxidant, Antibacterial, TLC Bioautography, DPPH assay, Minimum Inhibitory concentration, Disc diffusion.

I. INTRODUCTION

Exploring new natural products with benefits to human health through marine biodiversity exploration is a promising new avenue for research and development. *Sargassum is* a marine brown macroalgae found in both tropical and temperate waters. This genus is the most diverse among class Phaeophyta in India, with approximately 38 species [1]. Brown algae contain secondary metabolites which are capable of being commercially exploited. They produce a wide range of phenolic compounds and polysaccharides with significant biological activity, and are more effective antioxidants compared to green and red algae. Marine algae's harsh environment encourages the synthesis of oxidizing agents and secondary metabolites, which are responsible for their biological activity. The presence of biologically active metabolites in the body helps contribute in defense against other organisms [2]. The irreversible damage to life-sustaining macromolecules due to oxidative stress causes a number of health problems in humans. Antioxidants are commonly employed as ingredients in nutritional supplements and have been studied for the prevention of diseases, including potentially fatal disorders such as coronary heart disease and cancer [3]. The spread of antibiotic resistance in harmful microorganisms has reached pandemic proportions worldwide. Algal-derived compounds have a wide range of biological activities, including antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic, and antimitotic properties, which could be further investigated in light of the current need to find novel and efficient medications against resistant pathogenic strains [4].

II. METHODOLOGY

A. Collection and Sample preparation

In this investigation, *Sargassum tenerrimum* and *Sargassum swartzii* were collected from Kunkeshwar coast, Devgad, Maharashtra, India. The samples were authenticated by the Botanical Survey of India, Coimbatore. The collected seaweeds were thoroughly rinsed, blotted on blotting paper and the shade dried seaweeds are pulverized into a fine powder using a tissue blender. The powdered sample was then stored for further analysis.

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B. Antioxidant activity by DPPH method:

The DPPH Scavenging assay was used to study the antioxidant potential of methanol extract using standard method [5] with some modifications. 1mg/ml solution of plant extract in methanol was prepared. DPPH was used as control (Blank). One milliliter of the methanol extracts of the plants (140, 130, 120,110, 100, 90, 80,70,60,50, 40, 30, 20, 10 µg/mL) was added to 2 mL of a solution of DPPH radicals in methanol (0.004%). The test tubes were kept in the dark for 30mins. OD was taken at 517nm and absorbance was noted. Ascorbic acid was taken as standard. The result was calculated using the formula for 50% activity IC50.

$$\% \text{ DPPH Radical scavenging activity} = \frac{(\text{Control} - \text{Sample OD}) \times 100}{\text{Control OD}}$$

C. TLC Bioautography for Antioxidant Screening.

Evaluation of separated compounds on precoated silica gel TLC plate for antioxidant activity was carried out by TLC Bioautography method ([6], [7]). Preparation of extract: 1gm of dried powder soaked in 10ml methanol kept for 24 hrs. and filtered through muslin cloth. Mobile phase used Toluene: Ethyl acetate in ratio (7:3). Mobile phase was saturated for 20 mins, extract was spotted 1cm above baseline. The developed air-dried plate was sprayed with a methanolic solution of 2.54 mM DPPH antioxidant reagent and the plates were air-dried after spraying. Yellow bands were seen on a purple background indicating antioxidant activity. Rf value was calculated by the formula:

$$\text{Retention factor} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

D. Screening of Antibacterial activity by Disc Diffusion:

The antibacterial assay was carried out by disc diffusion method using standard method [8]. Preparation of the plant extract: 1gm of algae powder soaked overnight in 10ml methanol filter using muslin cloth, evaporate the extract on a sand bath and reconstitute using 1ml of DMSO reagent. The microbial strains used were *Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus and Bacillus subtilis.* The bacterial isolates were cultured on nutrient agar and incubated at 37ºC for 24 hrs. and this culture of each was inoculated in the nutrient agar plates with sterile cotton swabs in sterile conditions. 10µg/disc of both algal extracts were impregnated in sterile condition with positive control Streptomycin10µg/disc and DMSO (10µg/disc) as Negative control. After incubation at 37ºC for 24 hrs. The antibacterial activity was noted by measuring by diameter of the zone of inhibition in mm.

E. Determination Antibacterial Activity by Minimum Inhibitory Concentration Method:

For preparation of extracts 5g of algae powder soaked overnight in 50mL methanol filter using muslin cloth, evaporate the extract on a sand bath and reconstitute using 5mL of DMSO reagent. Five concentrations (20, 40, 60, 80, 100µg/mL) concentrations of both algal extracts were used for MIC. Four selected bacterial strains *Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus and Bacillus subtilis* were activated on nutrient agar by incubating for 24 hrs. The optical density of the bacterial strains was adjusted to 0.1 by saline. The test tubes with various concentrations of plant extract were made separately in triplicates. The final volume of each test tube was made to 5ml with Nutrient broth. Each of these test tubes was inoculated with 0.1 mL of bacterial inoculum and 7.5 µL of 1% TTC. A positive control tube was made with 5 mL nutrient broth, 0.1 mL bacterial inoculum and 7.5 µL of 1% TTC. The negative control tube was with 5mL Nutrient broth and 7.5 µL of 1% TTC. All test tubes were then incubated at 37°C for 24 hrs. MIC was determined based on the development of pink colour in the test tube [9].

III. RESULTS AND DISCUSSION

A. Determination of Antioxidant activity by DPPH assay:

In DPPH assay, *S. tenerrimum* methanolic extract showed strong antioxidant activity with IC50 value of 80µg/mL and moderate antioxidant activity in *S. swartzii* extracts with IC50 value of 130µg/mL while ascorbic acid standard showed very strong activity of 1 µg/mL The antioxidant activity strength can be indicated as very strong (IC50 < 50 µg/mL), strong (IC50: 50–100 µg/mL), moderate (IC50: 101–150 µg/mL), and weak (IC50: 250–500 µg/mL) ([10],[11]) reported high antioxidant activity was exhibited by *Sargassum swartzii* extracts. Similar work by [12] also confirmed antioxidant properties of fucoidan fractions from *Sargassum tenerrimum.*

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B. TLC Bioautography for Antioxidant Screening.

The presence of antioxidant compounds was qualitatively analyzed by thin layer chromatography. DPPH was used as a spraying agent, bands with the antioxidant capacity were observed as two yellow bands on purple background seen in both *S. tenerrimum* at Rf values 0.64 and 0.45 and for *S. swartzii* at 0.75 and 0.5. Similar work performed by [6] utilized TLC- bio-autographic method to screen and purify the bioactive compounds from *H. elongata* seaweed.

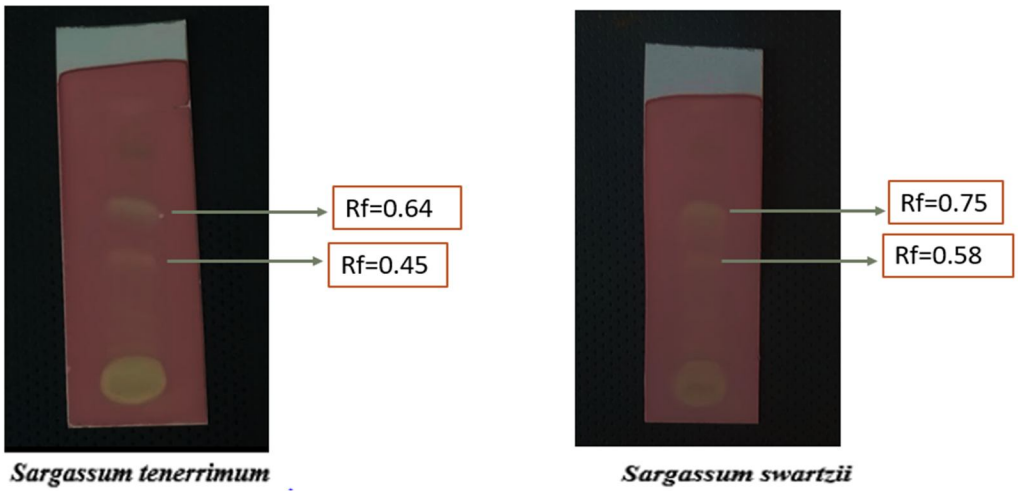


Figure1: Separation of antioxidant compounds in *S. tenerrimum* and *S. swartzii* extracts by TLC Bioautography method.

C. Antibacterial activity by Disc Diffusion

In this investigation, Antibacterial activity of *Sargassum tenerrimum* extracts and *Sargassum swartzii* extracts were found to be active against all tested bacterial strains. Highest zone of inhibition in each algal extract was seen against *S. typhi* which is (14.66±3.055mm) in *S. tenerrimum* and (12.33±2.309mm) in *S. swartzii* extracts. In a similar study of antibacterial activity of *Sargassum tenerrimum.* Methanolic extract of *Sargassum tenerrimum* was found to have significant antibacterial activity against all the tested pathogens. The maximum antibacterial activity was observed against *K. pneumoniae* (12.1 mm) followed by *S. aureus* (11.9 mm), *P. aeruginosa* (11.8 mm), *V. cholerae* (11.7), *E. coli* (11.6 mm) and *S. typhi* (11.5 mm) [13]. Antibacterial properties of a sulfated polysaccharide from the brown marine algae *Sargassum swartzii* against ten human pathogenic strains were tested, *E. coli* was the most sensitive [14]. Antibacterial activity of both algal extracts with negative and positive control is shown in the following table. (Table: 1)

Observation table 1: Antibacterial activity by Disc Diffusion method

	Zone of Inhibition in mm			
Bacterial isolates	DMS0	Streptomycin	*S. tenerrimum* Extracts	*S. swartzii* Extracts
S. typhi	No Zone of Inhibition	26.66 ± 2.886	14.66 ± 3.055	12.33 ± 2.309
B. subtilis	No Zone of Inhibition	30.33 ± 0.577	10.33 ± 0.577	10.66 ± 1.154
S. aureus	No Zone of Inhibition	31.66 ± 2.886	11.66 ± 1.154	12 ± 1.732
K. pneumoniae	No Zone of Inhibition	30.33 ± 0.577	11.66 ± 1.527	10.33 ± 0.577

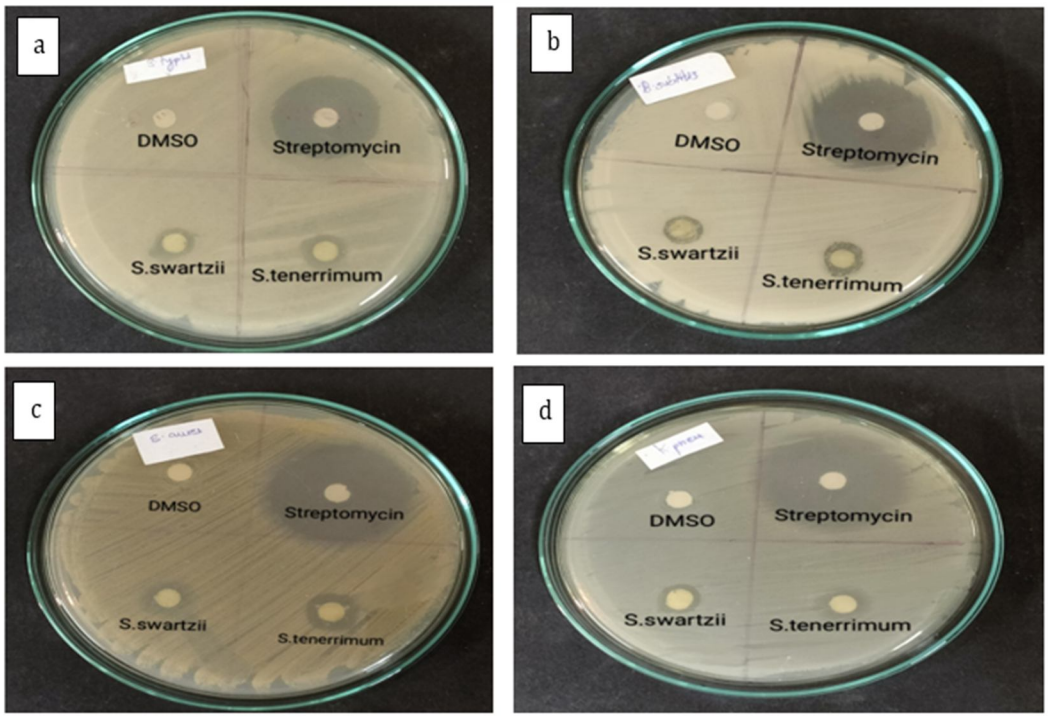
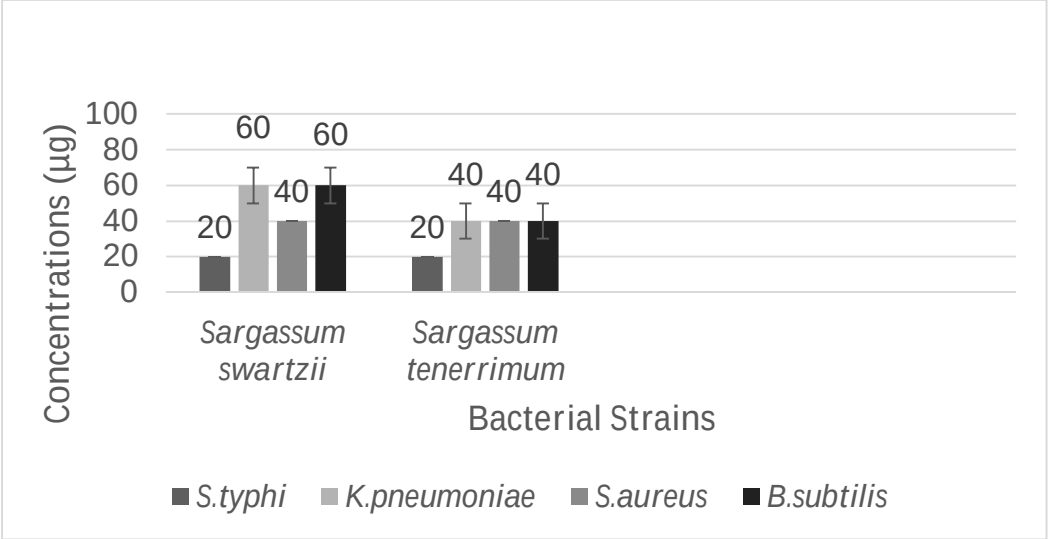


Figure 2: Antibacterial activity by Disc diffusion method, Plate a: *S. typhi*, b: *B. subtilis*, c: *S. aureus* and d: *K. pneumoniae*.

D. Determination of antibacterial activity by minimum inhibitory concentration method.

The minimum inhibitory concentration (MIC) of an antimicrobial agent is the lowest concentration that prevents bacteria from growing visibly in vitro. The broth/tube dilution test is the standard method for assessing microbial resistance to an antimicrobial agent. Serial dilutions of the test agent are prepared in a liquid microbial growth medium that has been inoculated with a predetermined number of organisms and cultured for a set period of time. The test tubes were then incubated at 37°C for a period of 24 hours. The 2,3,5-triphenyltetrazolium chloride (TTC) is a tetrazolium salt that is widely used in MIC determination. When solubilized with water, it is colourless; however, in the presence of metabolically active bacteria, it is reduced to red-colored formazan, which is directly proportional to the quantity of viable cells. Where no colour indicates no bacterial growth and pink colour indicates bacterial growth. The graph below indicates the MIC values of the four bacterial strains against *S. tenerrimum* and *S. swartzii* extracts. Similar work has been done by [15] on *Sargassum polyceratium*.



Graph 1: Minimum Inhibitory Concentration of *S. tenerrimum* and *S. swartzii* extracts.

IV. CONCLUSION

The present study revealed potential antioxidant activities of the selected marine algae *S. tenerrimum and S. swartzii* and TLC bioautography method showed separation of these compounds which can be further isolated for the development of novel natural antioxidants. The antibacterial assay confirmed inhibition of gram-negative bacteria *K. pneumoniae* and *S. typhi* as well as gram-positive bacteria *S. aureus* and *B. subtilis*, indicating the significance of the antibacterial properties for development of new drugs against resistant human pathogenic diseases.

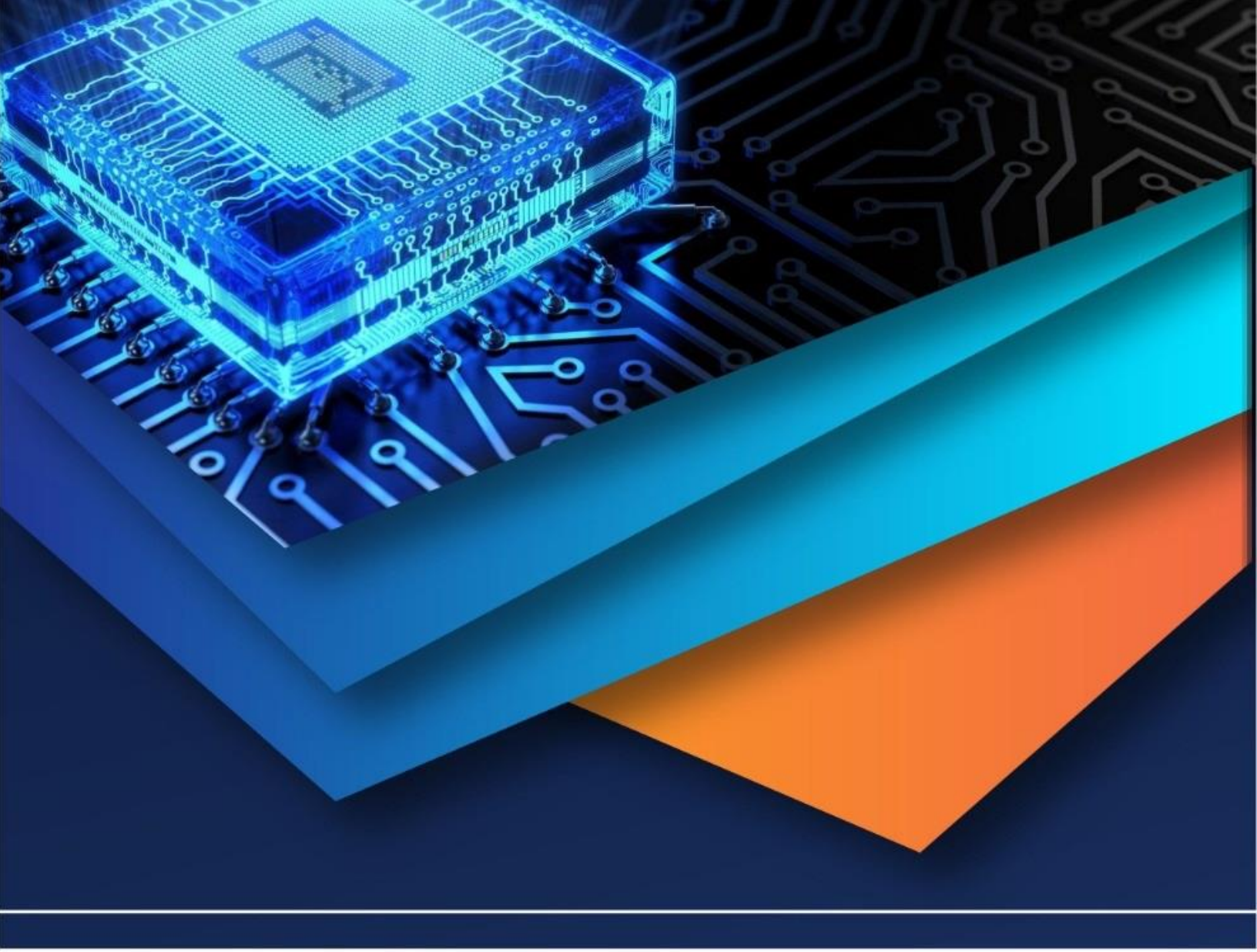
V. ACKNOWLEDGMENT

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