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ANTIBACTERIAL POTENTIAL OF SELECTED SEAWEED *SARGASSUM WIGHTII* GREVILLE EX J. AGARDH

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ABSTRACT

The seaweed *Sargassum wightii* Greville ex J. Agardh was evaluated for its antibacterial activity against different Gram-positive bacteria like *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Streptococcus mutans* (ATCC 25175), *Bacillus subtilis* (MTCC 1305) and *Micrococcus luteus* (MTCC 4821) and Gram-negative bacteria like *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella paratyphi* (MTCC 3220), *Klebsiella pneumoniae* (MTCC 3384) and *Proteus vulgaris* (MTCC 1771). The various solvent extracts tested were methanol, chloroform, diethyl ether, ethyl acetate, acetic acid and aqueous. The chloroform and methanol extract revealed better results than any other solvents. The lowest inhibition was recorded in diethyl ether and aqueous extracts. The pathogen which is highly sensitive in chloroform extract was *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Proteus vulgaris* was highly inhibited in chloroform and methanol extracts. *Escherichia coli* also showed higher inhibition zone in ethyl acetate extract. Gram-negative bacteria showed significant activity than Gram-positive bacteria.

***Keywords*:** Activity, Extracts, Inhibition, Pathogens, Solvents.

Introduction

The use of synthetic antibiotics to treat bacterial infections has emerged dramatically. Due to various side effects the use of synthetic antimicrobial drugs has certain limitations. Moreover the pathogens also develop resistance to these drugs (Fayzi *et al*., 2020). These necessitate research for the development of novel drugs based on natural resources (Kayalvizhi, 2012). Seaweeds are a group of macroscopic marine algae that form the biomass in the intertidal zone (Wong and Chung, 2002). Seaweeds are naturally renewable and contain high levels of minerals, vitamins, essential amino acids, fatty acid, dietary fiber and carbohydrates (Dawczynski *et al*., 2007). It possess natural antioxidants such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides (Vinayak *et al*., 2011). Seaweeds thus offers a rich source of potential new drugs (Bhadury and Wright, 2004; Bansemir *et al*., 2006) with various biological activities (Magesh *et al*., 2021).

Many substances isolated from red, brown and green marine seaweeds have been associated with a variety of pharmacological properties, such as antibacterial, antiviral, anti-fungal, insecticidal, anti-tumor and antioxidant activities and the bioactivity of these marine seaweed extracts and fractions have recently been the research interest in various fields (Shelar *et al*., 2012; Alves *et al*., 2016; Abirami and Kowsalya, 2017). Moreover the uses of seaweeds are inexpensive (Mahianeh *et al*., 2014). Brown algae were found to have many futuristic properties and have been used mostly in medicinal fields to treat various diseases. *Sargassum wightii* Greville ex J. Agardh, which belongs to the family Sargassaceae is commonly seen in the shorelines of India. It is a macroscopic, multicellular, photosynthetic, non-vascular, pelagic marine species rich in sulphated polysaccharides that manifest potent free radical scavenging activity (Park *et al*., 2005) and has antioxidant effects (Xue *et al*., 2001).

Several active substances isolated from *Sargassum* seaweeds, such as polysaccharides, fatty acids, proteins, phenolics, terpenes, etc., show significant antitumor, antibacterial, antiviral and anti-oxidative activities (Balboa *et al*., 2013). Hence this work is aimed to evaluate the antibacterial potentiality of *Sargassum wightii* Greville ex J. Agardh extracts from Kanniyakumari coast against some common Gram-positive and Gram-negative bacterial isolates.

Materials and Methods

Collection and Identification of Seaweeds

The fresh samples of brown algae (seaweed), *Sargassum wightii* Greville ex J. Agardh were manually collected in bulk quantity from the coastal region of Arockiapuram, Kanniyakumari district, Tamil Nadu in India. The seaweed samples were identified by the algal experts. The collected seaweeds were initially washed with sea water,

followed by running tap water and finally with sterile distilled water to remove the adhering macroscopic epiphytes, animals castings, attached debris, sand and salt particles. The algae after rinsing were shade dried under the room temperature for 15 days and then powdered into fine particles using an electric mixer. The fined powdered samples were stored in an airtight container at 4°C for further use.

Preparation of Seaweed Extracts

The dried powdered samples of *Sargassum wightii* Greville ex J. Agardh (10gm) were immersed separately in 100ml of various solvents viz., methanol, chloroform, diethyl ether, ethyl acetate, acetic acid and aqueous. The extraction was carried out using agitator system for 48 hours for the extraction of metabolites from the seaweeds. The plant extracts thus collected were filtered through Whatmann No.1 filter paper to separate the filtrate. Then the extracts were concentrated using a vacuum rotary evaporator at low temperature (40°C) and stored in a refrigerator for further use (Rebecca *et al*., 2012).

Test Pathogens

The bacterial cultures were purchased from Vivek laboratory, Nagercoil. The different solvent extracts of *Sargassum wightii* Greville ex J. Agardh were tested against the bacterial strains viz., *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Streptococcus mutans* (ATCC 25175), *Bacillus subtilis* (MTCC 1305), *Micrococcus luteus* (MTCC 4821), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella paratyphii* (MTCC 3220), *Klebsiella pneumoniae* (MTCC 3384) and *Proteus vulgaris* (MTCC 1771).

Preparation of Inoculum

Muller Hinton Agar Medium (1 L): The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30 ml/plate) while still molten (NCCLS, 1993).

Antibacterial Activity Assay

Antibacterial activity of the extracts was determined by agar well diffusion technique. Petriplates containing 20 ml Muller Hinton Agar Medium were seeded with bacterial culture of *Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphii, Klebsiella pneumoniae* and *Proteus vulgaris*. Wells of approximately 10mm was bored using a well cutter and 1000 µg of samples were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

Results and Discussion

The solvent extracts of *Sargassum wightii* Greville ex J. Agardh were screened for its antibacterial activity against human bacterial pathogens like *Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphii, Klebsiella pneumoniae* and *Proteus vulgaris* (Table 1 and Figure 1). Antibacterial activity of the seaweed extracts was determined by agar-well diffusion method on Muller Hinton Agar (MHA) medium. Six different extracts were evaluated. Most of them had a significant activity against both Gram-positive and Gram-negative bacteria. The presence of different antibacterial substances in the organic solvent extracts may be the reason for the antibacterial activity as reported by Lustigman & Brown (1991). Nowadays unique compounds from *Sargassum* species with different biological activities have been identified and some of them are under examination and are being used to improve novel pharmaceuticals (Garcia-Rios *et al*., 2012; Michalak and Chojnacka, 2015). The maximum activity (21±0.50 mm) was recorded from the chloroform extracts of *Sargassum wightii* Greville ex J. Agardh against *Klebsiella pneumoniae* and the minimum activity (11±0.10 mm) by the diethyl ether against *Micrococcus luteus, Salmonella paratyphi* and *Klebsiella pneumoniae* and also in aqueous extract against *Bacillus subtilis* (11±0.96 mm) and *Micrococcus luteus* (11±0.81 mm). The potency of the activity could also depend on solvents being used as well as the type of seaweeds (Salem *et al*., 2011).

The methanol and chloroform extracts showed good antibacterial activity against all the tested bacterial pathogens except *Staphylococcus aureus*. In the methanolic extract, the maximum zone of inhibition was recorded against *Pseudomonas aeruginosa* (18±0.99 mm) and *Proteus vulgaris* (18±0.94 mm) and minimum zone of inhibition (13±0.51 mm) was noticed against *Micrococcus luteus*. Similar results in methanol extract were also reported by Kumar *et al*. (2008). It was reported that methanol extracts of seaweeds contain phenolics, alkaloids and amino acid which may be the reason for its high activity (Devi *et al*., 2008; Srivastava *et al*., 2010). Cox *et al*. (2010) also revealed that methanol was a good solvent for extraction from brown seaweeds. The chloroform extract of *Sargassum wightii* Greville ex J. Agardh showed maximum highest activity against the growth of *Klebsiella pneumoniae* (21±0.50 mm). Similar activity was also reported by Moorthi and Balasubramaniam (2015) and the lowest activity with the zone of inhibition (12±0.33 mm) was noticed against *Micrococcus luteus.*

No inhibitory effect was noticed from the seaweed extracts of *Sargassum wightii* Greville ex J. Agardh in diethyl ether against *Staphyloccus aureus, Enterococcus faecalis, Streptococcus mutans* and *Proteus vulgaris*. However, diethyl ether extract of *Sargassum wightii* Greville ex J. Agardh also showed moderate to lowest activity against six strains among ten bacterial strains. The ethyl acetate extract strongly inhibited the growth of *Escherichia coli* (19±0.51 mm) and the lowest activity with the zone of inhibition was noticed against *Micrococcus luteus* (13±1.96 mm) and *Klebsiella pneumonia* (13±1.23 mm) Moderate to high activity was seen in acetic acid extract against *Pseudomonas aeruginosa* (19±0.36 mm), *Escherichia coli* (18±0.93 mm), *Enterococcus faecalis* (15±0.60 mm), *Salmonella paratyphi* (14±0.80 mm) and lowest activity with zone of inhibition was seen in *Bacillus subtilis* (12±0.10 mm) and *Micrococcus luteus* (12±1.56 mm), however *Staphylococcus aureus* and *Streptococcus mutans* was more resistant to this extract. This difference in inhibition may be due to the extraction method, solvents used, seasonal

variation and drying of the seaweeds. That can alter the results by the elimination of active ingredients (Choi *et al*., 2012).

Aqueous and diethyl ether extracts showed less activity when compared to any other solvent extracts. This is in accordance with Kausalya and Rao (2015) where the activity of aqueous marine algal extracts against pathogens was significantly lower when compared to other solvent extracts. The Gram-negative bacteria exhibited higher activity than Gram-positive bacteria. In Gram-negative bacteria outer membrane acts as a barrier to many environmental substances including antibiotics (Tortora *et al*., 2001). This high inhibition gives an indication of developing a potent drug from marine resources. However some of the pathogens are resistant to some of these extracts. Some of the plant extracts were unable to exhibit antibacterial activity against some tested pathogens as suggested by Schwarz and Noble (1999) because these bacterial strains may have some kind of resistant mechanism. The results revealed that solvent extracts are always better than aqueous extracts. The present results paved way for further research and can be explored for its phytochemical constituents.

Table 1 : The pathogens showing the zone of inhibition in different extracts

Test Pathogens		Zone of Inhibition (mm)					
		Methanol	**Chloroform**	**Diethyl Ether**	**Ethyl Acetate**	**Acetic Acid**	**Aqueous**
Gram-positive bacteria	*Staphylococcus aureus*	Nil	Nil	Nil	16±1.23	Nil	12±1.00
	Enterococcus faecalis	15±0.93	15±0.13	Nil	Nil	15±0.60	14±0.86
	Streptococcus mutans	15±1.03	15±0.23	Nil	18±0.93	Nil	Nil
	Bacillus subtilis	14±0.89	13±0.80	12±1.90	14±0.51	12±0.10	11±0.96
	Micrococcus luteus	13±0.51	12±0.33	11±0.10	13±1.96	12±1.56	11±0.81
Gram-negative bacteria	*Escherichia coli*	16±0.78	16±1.00	13±0.96	19±0.51	18±0.93	14±1.23
	Pseudomonas aeruginosa	18±0.99	19±0.89	12±2.06	18±0.36	19±0.36	13±0.86
	Salmonella paratyphi	15±0.89	15±1.23	11±0.10	17±0.89	14±0.80	14±1.89
	Klebsiella pneumoniae	17±0.36	21±0.50	11±0.10	13±1.23	13±0.96	Nil
	Proteus vulgaris	18±0.94	19±0.94	Nil	15±0.89	13±1.36	Nil

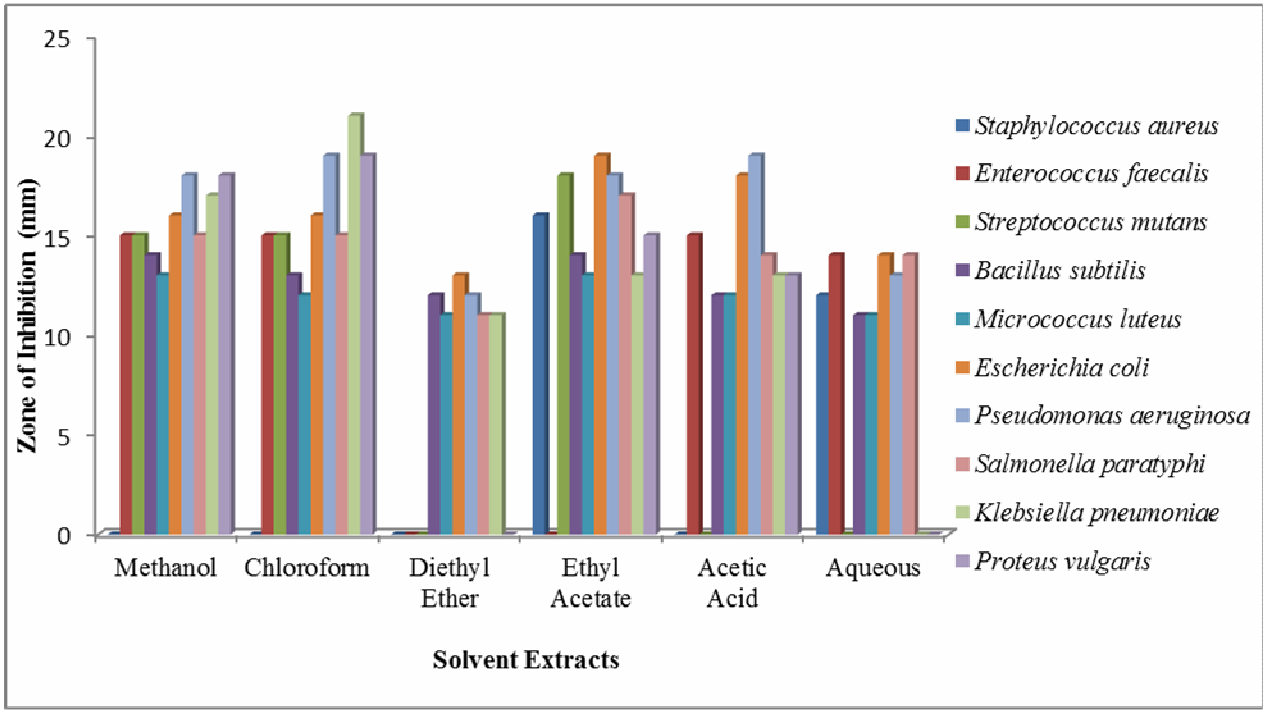

Fig. 1: Zone of inhibition recorded in various solvent extracts

Conclusion

The results of this present study on *Sargassum wightii* Greville ex J. Agardh showed significant antibacterial activity. However more research is needed to isolate, purify and to identify the active ingredients in order to understand their bio prospects.

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