WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.041
Volume 5, Issue 6, 1328-1337 Research Article ISSN 2278 – 4357

PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL POTENTIAL OF *KAPPAPHYCUS ALVAREZII* METHANOL EXTRACT AGAINST CLINICAL ISOLATED BACTERIA

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Article Received on
27 March 2016,

Revised on 18 April 2016,
Accepted on 09 May 2016

DOI: 10.20959/wjpps20166-6900

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ABSTRACT

The methanol extract of *Kappaphycus alvarezii* was evaluated for antibacterial activity and against the human clinical isolated pathogens of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomons aeroginosa*, *Escherichia coli*, *Proteus vulgaris* and *Bacillus subtilis*. The phytochemical analysis showed the presence of alkaloids, saponin, phenols, terpenoids, coumarins, protein, carbohydrates, flavonoids, tannins and absence of steroids, glycosides and anthraquinone. *Kappaphycus alvarezii* possesses significant antibacterial activity and in future the methanol extract may pave the way for designing the new drugs for controlling bacterial diseases.

KEYWORDS: *Kappaphycus alvarezii*, antibacterial, phytochemical and disc diffusion method.

INTRODUCTION

Seaweeds have long been used in food diets, as well as traditional remedies. In Asian countries such as in China, Japan and Korea, seaweeds serve as an important source of bioactive natural substances. Many metabolites isolated from marine algae possess bioactive effects. The discovery of metabolites with biological activities, from macro algae, has increased significantly in the past three decades; on the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants. Marine organisms are a rich source of structurally novel and biologically active metabolites [25].

Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Some seaweed has the valuable

medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. In the marine ecosystems seaweeds are directly exposed and are susceptible to ambient microorganisms such as bacteria, fungi and viruses [4].

Antioxidants are effective in protecting the body against damage by reactive oxygen species. In Japan, various kinds of edible seaweeds have been traditionally consumed as additives and seasonings with different food stuffs. They have multiple therapeutic benefits such as suppression against some types of cancer. However, natural antioxidants are not limited to terrestrial sources. Some of the seaweeds are consider being a rich source of antioxidants. Examples include, chlorophylls, carotenoids, tocopherol derivates such as vitamin E and related isoprenoided that are structurally related to plant – derived antioxidant were found in some marine organisms. During the course of antioxidant activity screening of seaweeds commonly found in various seasons [1].

On the other hand, *K. alvarezii* is known for its ability to produce carrageenan [5]. The carrageenan is the hydrophilic colloid that can be obtained by aqueous extraction of *K. alvarezii* or other carrageenan seaweed species [6]. Which were used as food thickener, agar and stabilizer in cosmetics creams [7]. The *K. alvarezii* also have been reported to demonstrate antibacterial properties against plant bacteria [8], possess antioxidant properties [9] and has the ability to bind to mutagenic amines [10] [2].

In order to investigate the antibacterial activity of seaweeds extracts, antimicrobial susceptibility testing (AST) was carried out. The disc diffusion method is one of the methods of choice to screen the potential of antibacterial properties [11] of particularly new agent such as from plant extracts, including the seaweeds. In addition, the disc diffusion method has been the mainstay for AST in most clinical microbiological laboratories since Bauer, Kirby first described this technique in the 1960s [12]. It is a well-established method that explained the appearance of zones of inhibition in determining the susceptibility of antibiotics or antimicrobial agents against a particular tested bacterium. The zones of inhibition are the area on the agar plate where the growth of bacterial lawn around the disc is prevented by antibacterial agent that was placed on the disc, on top of the agar [12] [2].

The purpose of this research was to screen the antibacterial potential of *K. alvarezii* methanol extracts against *E. coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *B. substilis*, *B. cereus* and *S. aureus* using disc diffusion method.

MATERIALS AND METHODS

Preparation of seaweed extracts

K. alvarezii algae were obtained from in and around the areas of Pulicat lake and they were washed with running tap water and again thoroughly washed with double distilled water to remove the debris sand and unwanted particles after washing then were shade dried at room temperature for a week. Dried seaweeds were well powdered using kitchen blender and 10 g of coarse powder were rinsed in 100 ml of methanol for 72 hrs and then filtered through Whatman No. 1 filter paper and then filtrates were dried in hot air oven for 37ºC and finally the semi solid extract were used for the further analysis.

Antibacterial activity

Test Microorganisms

The test microorganisms used for antibacterial analyses were clinical isolates of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Bacillus subtilis*. The bacterial strains were maintained in the Nutrient Agar (NA) at the Department of Bacteriology, King Institute of Preventive Medicine, at Guindy, Chennai and were identified using Gram staining to confirm their nature [23] [28].

Antibacterial assay

The antibacterial efficacy was assayed by following the methods of Kirby Bauer [24] disc diffusion method against the bacterial pathogens. The bacterial suspension (10^6 CFU/ml) swabbed on the MHA (Muller Hinton Agar) plates using sterile cotton swab. The sterile disc of 6mm dimension was impregnated with the methanol extract treated with different concentrations *i.e.*, 10 µg/disc (10µg/ml), 15 µg/ disc (15µg/ml), 20 µg/ disc (20µg/ml), 25 µg/ disc (25µg/ml) 30 µg/ discs (30µg/ml) respectively. The discs with Ampicillin (20 µg/ disc) were placed on the Muller Hinton Agar plates maintained as positive control. The discs were gently pressed and incubated in inverted position for an hour at 37°C. After the incubation period, the susceptibility of the test organisms was determined by measuring the diameter of the zone of inhibition using antibiotic zone scale and the results obtained were tabulated for evaluation.

Phytochemical screening

Phytochemical screening of aqueous extract was carried out according to the standard methods as described by Trease and Evans, (1989) [22].

RESULTS AND DISCUSSION

Seaweeds are primitive non flowering plants without root, stem and leaves. They contain different vitamins, minerals, trace elements, protein, iodine, bromine and bioactive substances [3]. Infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a global growing problem nowadays. Thus, there is an urgent need to discover new antimicrobial compounds from plants with diverse chemical structures and novel mechanisms of action for new and emerging infectious diseases [13].

The antibacterial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plants, experimental methods etc. But variation in antibacterial activity may be due to the method of extraction and solvent used in the extraction preparation [14].

The antibacterial activity of *Kappaphycus alvarezii* were illustrated: table 1and the plate photos were showed in figure 1. Highest zone of inhibition was observed in *E. coli*, *Staphylococcus aureus* when compared to other species of bacteria such as *B. substilis*, *Proteous vulgarius* and *Pseudomonas aeruginosa*.

Variations were noted in occurred each one of the experimental results of antibacterial activity that could be due to the different bioactive substances and their concentration would have present in the respective extract. These bacterial strains may have some kind of resistance mechanisms *e.g*. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation or the concentration of the compound used may not be sufficient [15].

Previous reports have shown that gram positive bacteria were effectively controlled by the marine algal extracts as compared to gram negative bacteria [16, 17]. In this present study, it was observed that the extracted by methanol showed significant activity against all the test pathogen. It is also necessary for the separation and purification of the biological active compounds to develop new drugs to control the deadly diseases. This research finding gives

further scope to screen the chemical constituents of the extracts which will be very useful to combat the various diseases caused by pathogenic bacteria [3].

Phytochemical screening of *K. alvarezii* extract revealed that the extract contains significant quantity alkaloids, saponin, phenols, Steroids, protein, phytosterols, aminoacids, flavonoids, steroids, tannins and absence of terpenoids, sugars and anthraquinone (Table 2).

Algae are considered very popular sea vegetables, and many people consume this vegetable as a health food in China, Japan, and South Korea. Recent studies have examined the biological and pharmacological activities of marine algae and it was shown to be potentially prolific source of highly bioactive secondary metabolites and could be used for the identification of novel pharmaceutical agents [18].

Several bioactive compounds such as phlorotannins, diterpenes, polysaccharides, phytosterols, and phytopigments have been isolated from algae and many of these compounds have been demonstrated to possess numerous biological activities, including antioxidant, cytotoxic [20], hepatoprotective [19], antiviral [21], antifungal [26] and antidiabetic properties [27].

Table 1: Antimicrobial activity (Zone of inhibition) of methanol extract of *K. alvarezii*

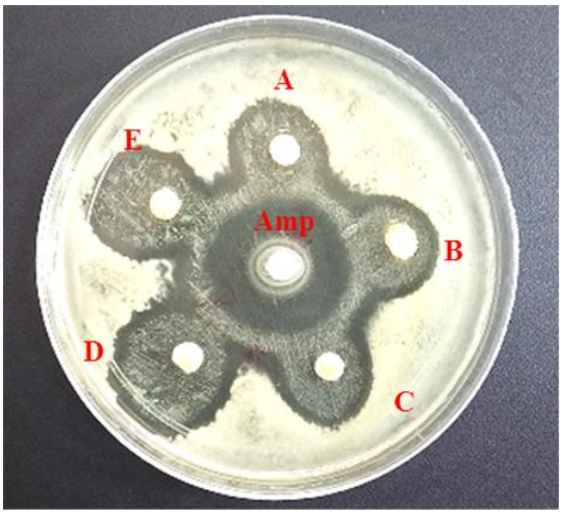
Organism	10 µg/ml	15 µg/ml	20 µg/ml	25 µg/ml	30 µg/ml	Amp 20µg/ml
S. aureus	6mm	6mm	7mm	7mm	8mm	10mm
B.cerius	1mm	1mm	1.5mm	2mm	2.2mm	11mm
E. coli	2mm	4mm	5mm	5mm	7mm	10mm
Bacillus subtilis	1mm	2mm	3mm	3mm	5mm	10mm
proteus vulgaris	1mm	2mm	2mm	3mm	3mm	8mm
P.aeruginosa	1mm	2mm	2mm	3mm	4mm	12mm

Table.2 Phytochemical analysis of ethanol extract of *K. alvarezii*

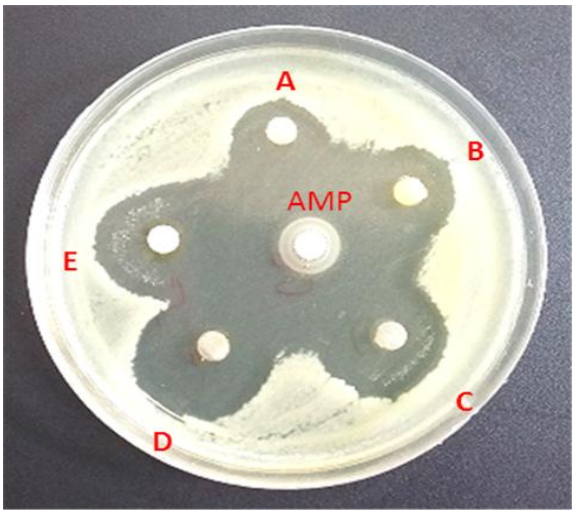
Phytochemical constituents	Observation
Alkaloids	+
Saponin	+
Tannins	+
Anthraquinone	-
Flavanoids	+
Phenol	+
Steroids	-
Terpenoids	+
coumarins	+
Proteins	+
carbohydrate	+

glycosides	-

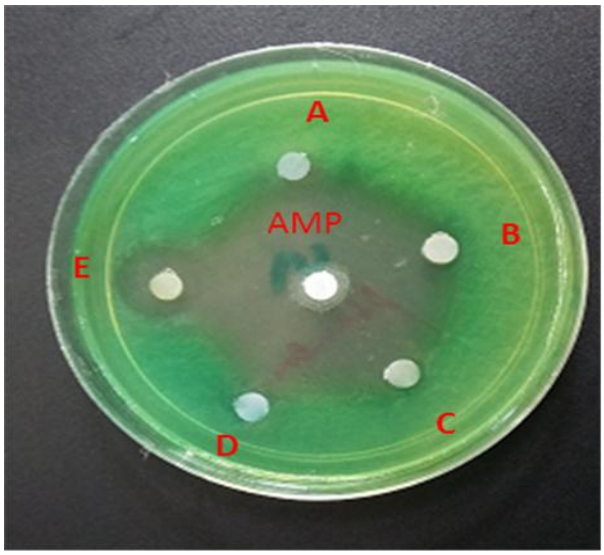
'+' = presence, '–' = absence of inhibition



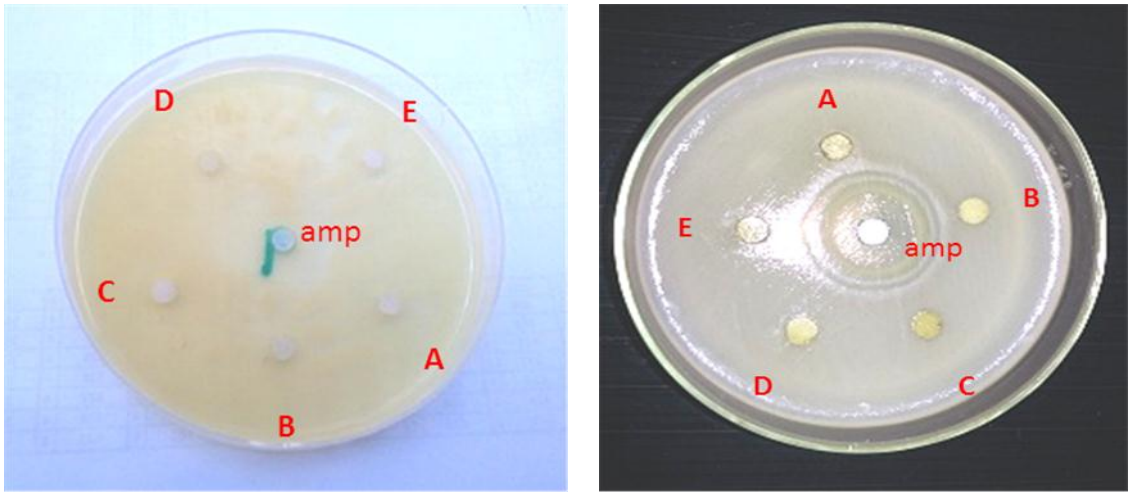
Staphylococcus aureus



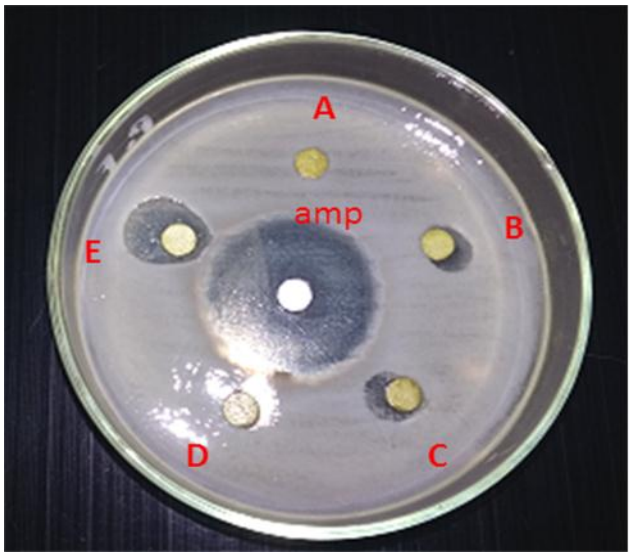
E. coli



Pseudomonas aeruginosa



proteus vulgaris ***B.cerius***



Bacillus subtilis

A-10µg/ml, B- 15 µg/ml, C-20 µg/ml, D-25 µg/ml, E-30 µg/ml

Fig.1 Effective antibacterial activity of ethanol extract of *K. alvarezii*

CONCLUSION

The results of the present study demonstrated that the *K. alvarezii* respectively possessed antibacterial activities against the clinically isolated species of bacteria. The antibacterial activities suggest the possibility of therapeutic value of seaweed against the bacterial infection. Methanol were suitable solvents for *K. alvarezii*, the methanol was a better choice.

ACKNOWLEDGMENT

Authors are grateful to the King Institute of Preventive Medicine, Guindy, Chennai for providing bacterial culture.

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