Screening of Marine Algae associated Potential Bacteria producing Antagonistic Bioactive Compounds against Human Pathogens

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Abstract
Serious consequences can occur if a urinary tract infection spreads to kidneys. Multidrug resistant bacteria raise a serious lineal problem for treating infectious diseases worldwide. Different seaweed algae including Chlorophyta, Phaeophyta and Rhodophyta were investigated as natural sources for antibacterial and antifungal compounds. A total of 35 marine algae were collected from the coast of Gujarat and Tamilnadu. Different solvents methanol, acetone and distilled water were used. Shaker method was to extract the antibacterial substances from these seaweeds and were examined against urinary tract pathogens. The present study investigates Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli., Pseudomonas aeruginosa and a fungus Candida albicans.

Keywords: *Laurencia karachiana*, *Sargassum wightii*, Nosocomial infection, Seaweeds, Antibacterial activity, Antifungal activity.

Introduction

Worldwide, urinary tract infections are among the most common infectious diseases. About 150 million people are diagnosed with urinary tract infection every year[15]. These are organisms that are too small to be seen without a microscope. Most urinary tract infections are caused by bacteria but some are caused by fungi and in rare cases by viruses. Urinary tract infections are among the most common infections in humans. Urinary tract consists of kidneys, ureters, bladder, urethra. Urinary tract infection only involves the urethra and bladder in the lower tract. Urinary tract infection can involve the ureters and kidneys in the upper tract. *Escherichia coli.* remains the predominant uropathogen in acute community acquired uncomplicated urinary tract infections. Patients with a history of previous urological procedures recent or long term catherisation, recent or long term antibiotics and a recent hospitalization tend to present with complicated urinary tract infection. *E. coli, Proteus, Klebsiella, Pseudomonas, Serratia* and *Enterococci* genus are the usual strains found.

Material and Methods

Sample Collection: Collect the urine sample in sterile container. Urine sample streak on cystine lactose electrolyte deficient agar with bromothymol blue. Place one drop of urine sample on the medium and wait for 10 minutes and place inside the incubator in normal position. The positive colonies only perform biochemical test. The confirmative organisms perform biochemical test and antibiotic sensitivity test. Hospital borne pathogens were received from Galaxy hospitals, Tirunelveli and Q lab, Tirunelveli.

Collection of the Seaweed: Nine species of green algae, ten species of red algae, eight species of brown algae and one microalgae were collected in different seasons. All of the seaweeds were collected manually in the intertidal zone region of the Gujarat coast and preserved on ice. Voucher specimens were frozen at -20°C for identification and future reference. The seaweeds were washed thoroughly with sea water to remove epiphytic and extraneous materials and brought to the laboratory in plastic bags. Then samples were shade dried for one month, ground in an electric mixer or using mortar and pestle and stored in refrigerator at 4°C.

Biochemical Test: Biochemical test has been performed for all the selected pathogens.

Column Chromatography: About 10g of silica gel is packed in a glass column over which acetone solvent is layered. To this, algal extracts are added and when it tends to dry, add the algal extract. Collect the fractions and the antibacterial activity was proceeded for all the algal extracts.

Results

Nosocomial and community acquired urinary tract infection common in most of the patients. Lower urinary tract symptoms complicated by recurrent or persistent urinary tract infections retention or renal impairment were suspected to be caused by lower urinary tract dysfunction and suspected urological cancer.

Synthetic antibiotics, by its own, constitute many harmful side effects. In discriminate use of antibiotics to treat such infectious diseases, increased the bacterial pathogenicity and resistance to these medications creating multidrug resistant strains[26]. Urinary tract infections diseases have become one of the leading causes of death worldwide especially when improperly treated. Therefore, seaweeds were rediscovered as cheap and safe sources in many medicinal and pharmacologial applications[1].

The antifungal activity was studied from the acetone extracts and methanol extract of algal extract against *Candida albicans*. The result obtained with methanol and acetone

extract of whole plant exhibited significant antifungal activity. Seaweeds provide natural bioactive compounds with anti-microbial activity such as phlorotannins and other phenolic compounds. These secondary metabolites have a molecular weight ranging from 10 to 100KDA due to the high variability that these molecules can present in the structural bonds between phloroglucinal and the hydroxyl groups. In this context, phlorotannins can be categorised into six categories: Fucols (aryl-aryl bonds), Phloretols(aryl ether bonds), Eckols di benzo-1,4 dioxin bonds, Fucophloretols ether or phenyl lineage and Carmalotsdibenzoidioxin moiety. Fuhalols (orthgo/para) avenged ether bridges containing an additional hydroxyl group on one unit.

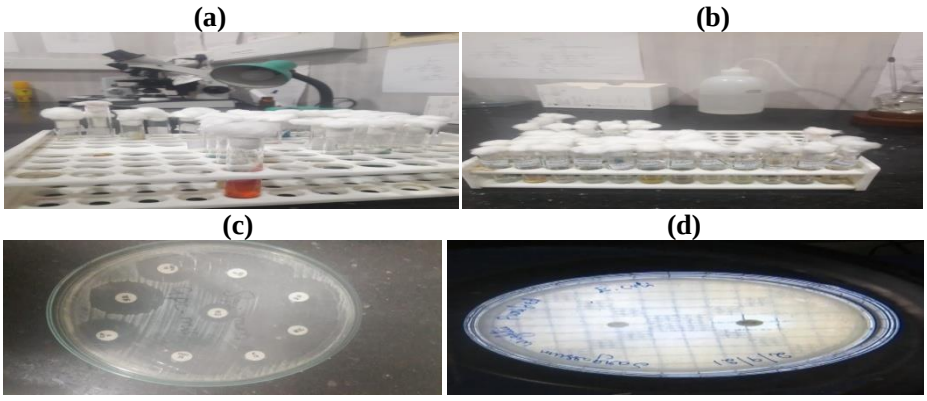


Fig. 1: (a) Manual biochemical test identification (b) Microorganism species identification (c) Antibacterial activities against synthetic drugs (d) Comparative study of Seaweed Column purified *Sargassum wightii* (1.7mm) extract against synthetic antibiotic Streptomycin (2.1mm) using disc diffusion method

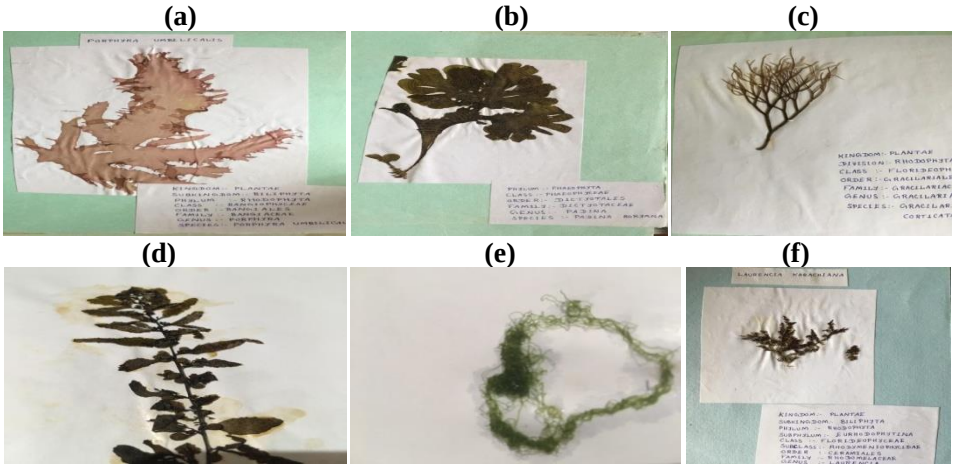


Fig. 2: Morphology of algae (a) *Porphyra umbilicalis* (b) *Padina boryana* (c) *Gracilariacorticata* (d) *Sargassum wightii* (e) *Cladophora glomerata* (f) *Laurencia karachiana*

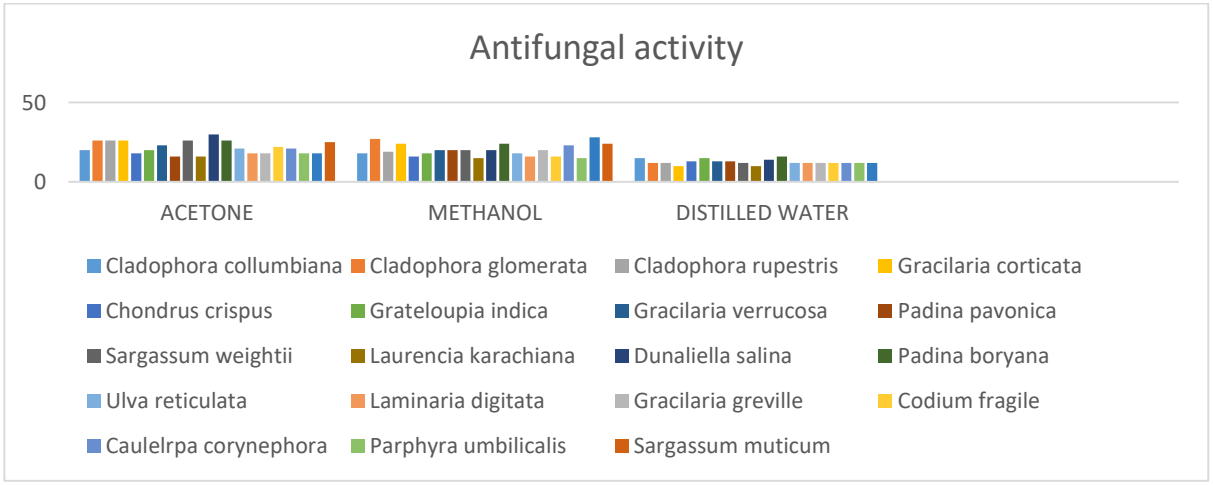


Fig. 3: Antifungal activity of seaweeds against *Candida albicans*

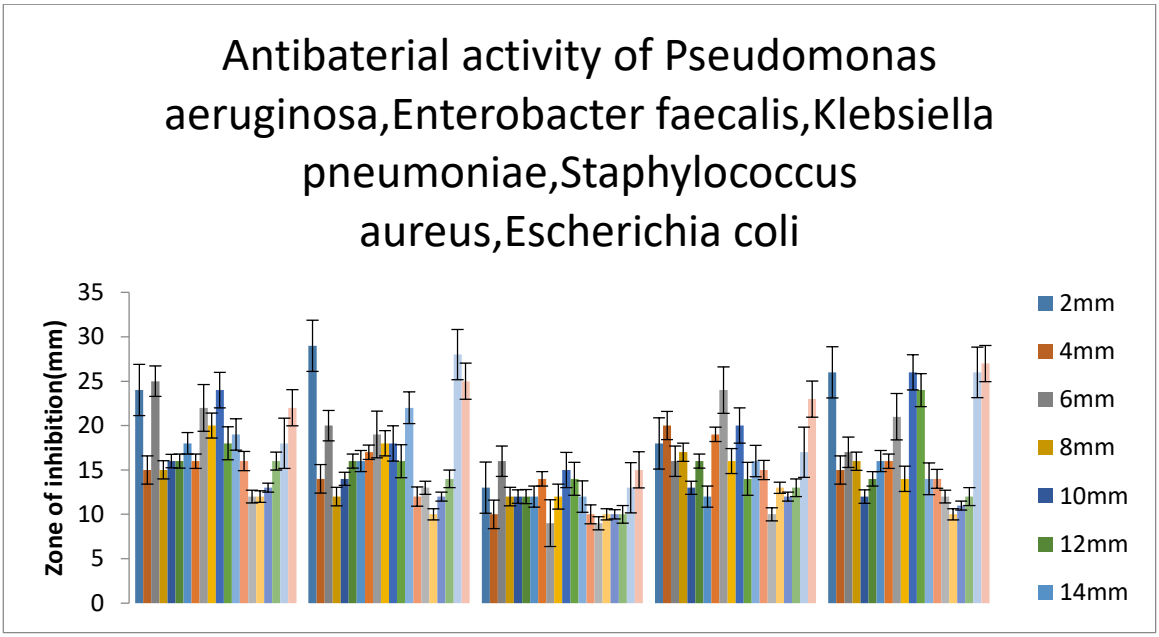
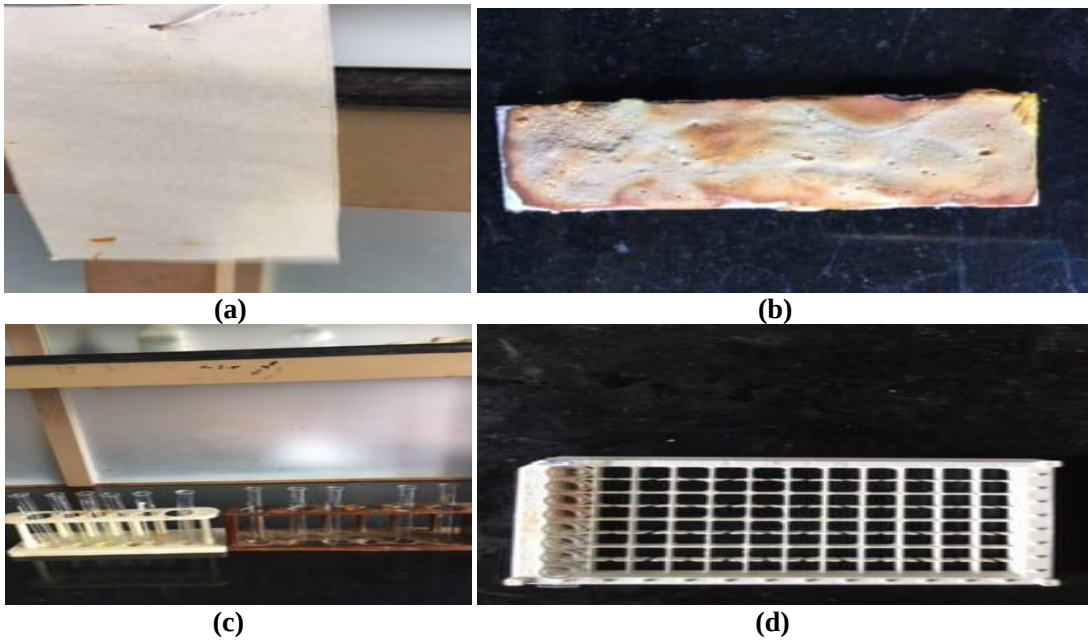


Figure 4: Shows antibacterial activity against 5 urinary tract pathogens



(a) **(b)**

(c) **(d)**

Fig. 5: (a) Paper Chromatography Report Showing Presence of Phenol Compound (b) Thin Layer Chromatography Report Showing Thin Layer Chromatography Report Showing Presence of Phenol Compound (c) Report shows Phytochemical Analysis (d) Lectin Agglutination of *Sargassum wightii*

Flavanoids are structurally characterized as phenolic compounds with a heterocyclic oxygen bound to two aromatic rings which can then differ according to the degree of hydrogenation. This study demonstrates that the antioxidant activity of phlorotannin was 10 times higher than that of other biological compounds. Seaweed phenolic compounds may be involved in the production of anti-aging creams and skin products.

Algae shows Minimum Inhibitory Concentration that the lowest concentration of a drug which prevents visible growth of a bacterium (in vitro test). Algal extract 0.00001 ml concentration of antibiotic shows zone formation.

Results and Discussion

All the extracts showed considerable activities depending on the bacterial isolate sensitivity. Our study reveals that among pathogens, *Klebsiella pneumoniae*, *Pseusomonas aeruginosa* organisms are resistant to antibiotics. Brown algae is highly active against pathogens. Especially, *Laurencia karachiana*, *Sargassum weightii*, *Dunaliella salina*, *Cladophora glomerata*, *Cladophora columbiana* these seaweeds. Acetone and methanol extracts show higher activity against *Enterococcus faecalis*. Seaweeds Phenolic compounds are attracting the attention of the scientific community as well as several Industries due to their high variety and potential uses.

Table 1
Phytochemical analysis of *Sargassam wightii*

S.N.	Test	Inference
01.	Alkaloids	++
02.	Steroids	+
03.	Tannins	+
04.	Phlobatannins	-
05.	Saponins	+
06.	Flavonoids	++
07	Terpenoids	+
08	Cardiac Glycosides	++
09	Phenolic compound	+++
10.	Aromatic acids	+
11.	Xanthoprotein	+

Table 2
MIC (Minimum Inhibitory Concentration)

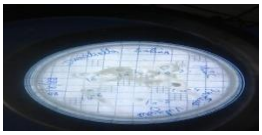
Macro Algae	MIC (10^{-1})	MIC (10^{-2})	MIC (10^{-3})	MIC (10^{-4})	MIC (10−5)	MIC (10−6)
Gelidium spinosum	1.3mm	0.9mm	0.9mm	0.7mm	0.7mm	0.7mm
Gracilaria edulis	1.0mm	0.8mm	0.7mm	0.7mm	0.7mm	0.7mm
Ulva lactuca	0.9mm	0.8mm	0.7mm	0.7mm	0.7mm	0.7mm
Ulva reticulata	1.0mm	0.9mm	0.8mm	0.6mm	0.5mm	0.4mm
Sargassum muticum	1.3mm	0.9mm	0.7mm	0.5mm	0.4mm	0.2mm 
Codium fragile	1.1mm	1.0mm	0.9mm	0.7mm	0.7mm	0.7mm
Caulerpa corynephora	1.2mm	1.1mm	1.0mm	0.9mm	0.8mm	0.7mm
Sargassum wightii	1.8mm	1.5mm	1.3mm	1.1mm	0.9mm	0.4mm
Laurencia karachiana	1.0mm	1.0mm	0.9mm	0.7mm	0.6mm	0.5mm
Padina pavonica	1.6mm	1.3mm	1.1mm	1.0mm	0.9mm	0.8mm
Parphyra umblicalis	1.0mm	0.9mm	0.8mm	0.7mm	0.7mm	0.7mm
Padina tetrastromata	0.9mm	0.7mm	0.6mm	nil	Nil	nil
Cladophora columbiana	1.4mm	1.2mm	1.1mm	1.0mm	0.9mm	0.8mm
Cladophora glomerata	1.2mm	1.0mm	0.9mm	0.7mm	0.7mm	0.7mm
Gracilaria corticata	1.2mm	1.0mm	0.9mm	0.8mm	0.7mm	nil
Caulerpa recemosa	1.2mm	1.1mm	1.0mm	0.9mm	0.8mm	0.7mm
Dunaliella salina (microalgae)	1.5mm	1.3mm	1.2mm	1.0mm	0.8mm	Nil 
Padina boryana	1.1mm	1.0mm	0.9mm	0.8mm	0.7mm	Nil 
Chondrus crispus	1.4mm	1.3mm	1.0mm	0.9mm	0.7mm	0.7mm 

Table 3
Shows Minimum Bacterial Concentration

Algae SPS	MBC(10^{-1})	MBC(10^{-2})	MBC(10^{-3})	MBC(10^{-4})	MBC(10^{-5})
GREEN ALGAE- *Ulva reticulata*	Absent	Absent	Absent	Absent	Absent 
RED ALGAE- *Porphyra umbilicalis*	Absent	Absent	Absent	Absent	Absent 
BROWN ALGAE- *Sargassum wightii*	Absent	Absent	Absent	Absent	Absent 

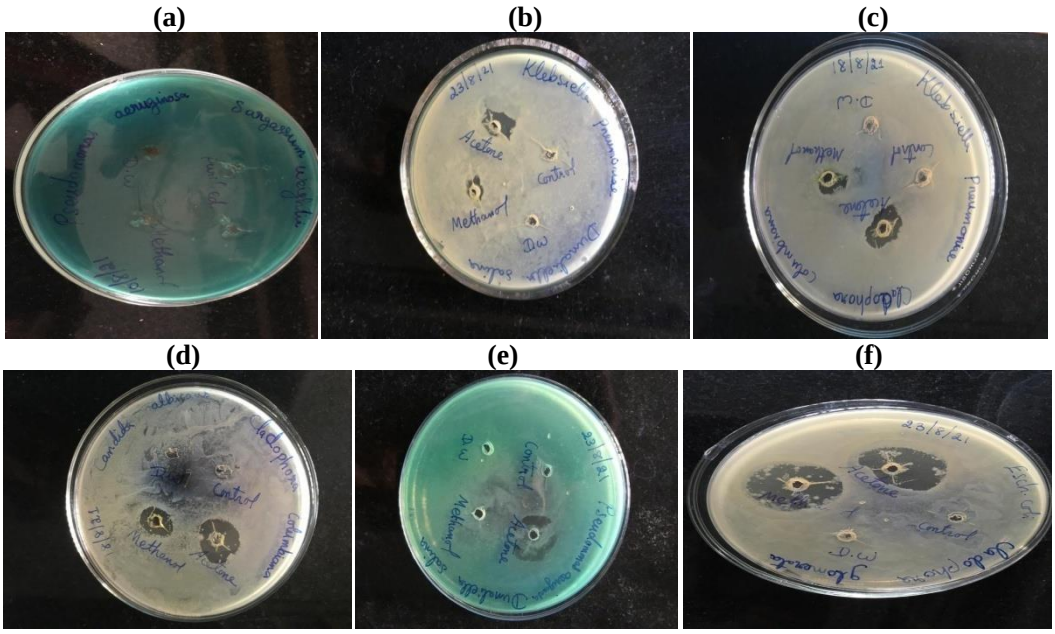


Fig. 6: Antibacterial activity against 5 urinary tract pathogens
(a) *Sargassum wightii* extract against *Pseudomonas aeruginosa,* (b) *Dunaliella salina* extract against *Klebsiella pneumonia,* (c) *Cladophora columbiana* against *Klebsiella pneumoniae,* (d) *Cladophora columbiana* against *Candida albicans,* (e) *Dunaliella salina* extract against *Pseudomonas aeruginosa* and (f) *Cladophora glamerata* extract against *Escherichia coli*

Seaweed extracted polysaccharides are already involved with the pharmaceutical Industry with the aim of replacing synthetic compounds with components of natural origin. Phlonoamnins are well known phenolic compounds synthesized by brown sea weeds. These compounds are constituted by oligomeric units of phloroglucine.

Conclusion

This study recommends seaweed extracts as antibacterial substance for treating multidrug resistant microbes causing acquired infections.

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