

PAPER ID: NITET22

EVALUATION OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF SELECTED MICROALGAE AND PLANTS AGAINST MICROBES

AGGARWAL AMONIKA,

Research Scholar, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, India,
amonikaaggarwal@gmail.com

DR.GARIMA SAXENA

Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, India
Corresponding Author drgarimasaxena@gmail.com

ABSTRACT

OBJECTIVE

To study and evaluate the phytochemical and antimicrobial activity of selected algae and medicinal plant extracts against bacteria.

METHODS

The study was emphasised on evaluating the antimicrobial potential of the selected algae and medicinal plants against microbes. The plants selected in the study are *Ficus religiosa*, *Cynodon dactylon* and *Tinospora cordifolia*. The microalgae selected in the study are *Kappaphycus alvarezii* (Red Algae) and *Sargassum* species (Brown Algae). The extracts were made from the following solvents, Cow urine, Benzene, acetone, ethanol from selected plants and algae.

The different extracts were tested for their antimicrobial activity against microbes such as bacteria, *Bacillus subtilis*, *Shigella flexneri*, *Escherichia coli*, *Enterobacter cloacae* which were obtained from Microbial Type Culture collection located at Indian Institute of Microbial Technology located in Chandigarh, India.

RESULT

The ethanolic extract of *Cynodon dactylon* showed highest antimicrobial activity and 29.5mm zone of inhibition against *Bacillus subtilis*. The significant inhibition (23mm) against *Enterobacter cloacae* was shown by the ethanol extract of *Cynodon dactylon* and *Ficus religiosa*. In cow urine extract, highest zone of inhibition was shown by *Sargassum* species (15mm) against *Bacillus subtilis*. The most inhibited bacterial isolate was *Bacillus subtilis*. The TLC plates were used for separation with 17 extracts (85%) inhibiting its growths tested from plants and algae whereas *Escherichia coli* and *Shigella flexneri* exhibited resistance with only 9 extracts (45%) showing inhibition. *Bacillus subtilis* was also inhibited from extracts of Cow urine, Benzene, acetone, ethanol from *Tinospora cordiflora*. The benzene extract of *Tinospora cordiflora* showed highest zone of inhibition (15.5mm) against *Shigella flexneri*. The ethanol extract of *Tinospora cordiflora* also showed significant inhibition (13mm) against *E. coli*.

CONCLUSION

In the present study, it was determined that the cow urine extract of *Tinospora cordifolia*, *Ficus religiosa*, *Cynodon dactylon*, *Kappaphycus alvarezii* and *Sargassum* species revealed inhibitory activity and this inhibitory activity can be used in the control of bacteria of various origins [2]. The *Kappaphycus alvarezii* and *Sargassum* species also showed significant antimicrobial activity against all pathogens. The susceptibility of the bacteria to the crude extracts varied on the basis of zones of growth inhibition according to microorganism and extracting solvent [3]. The algal extracts are a source of highly bioactive prolific secondary metabolites that might lead in the development of new innovative novel pharmaceutical agents [4]. The results emphasise on the enhanced activity of extract of cow urine in *Sargassum* species and *Kappaphycus alvarezii* was due to presence of acrylic acid and dimethyl sulphide in algae [5] and antimicrobial property of cow urine due to the presence of amino acids in urinary peptides and its low pH, which enhance the bacterial killing by increasing bacterial cell hydrophobicity exhibiting antimicrobial action against different clinical microbial strains [6].

KEYWORDS: *Ficus religiosa*, *Cynodon dactylon*, *Tinospora cordifolia*, *Kappaphycus alvarezii*, *Sargassum* species, Antimicrobial activity, phytochemical activity, cow urine extract.

INTRODUCTION

The new innovative and inventive methods for the development of novel drugs vast potential lie in use of medicinal plants and marine species as rich source and of antimicrobial agents. These seaweeds are classified based on chemical composition and their nutrition such as Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) [7]. Among marine algae, red algae contains phycoerythrin and phycocyanin as pigments responsible for red colour [8]. Brown algae are a group of algae with possession of pigment called fucoxanthin [9]. The phlorotannin contents as marine phenolic compounds have reported in good amount in brown algae [10]. The phyto constituents such as

flavanoids, phenols and tannins are present in algae indicating a possibility that the extracts may have antioxidant property[11].The phytochemical in algae are extensively used in textiles, gelling, thickening and as stabilising agents .

The *Ficus religiosa* is a sacred fig tree whose leave contains tannic acid and stem rich in vitamin K .This herb found as a relieve for many ailments like gout ,constipation,swollen glands ,skin disease ,dehydration etc[12].*Cynodondactylon* is a Bermuda grass and is of good nutritional value ,is beneficial in wounds ,skin problems ,diabetes ,epilepsy ,gynaecological problemsetc[13].*Tinosporacordifolia* ,also known as guduchi or Amrita is an immunity boosting herb and is a promising anti cancer herb and found use in diabetes[14].*Shigella flexneri*, a most common bacteria of a group ,gram-negative, recognized as the etiologic agents of bacillary dysentery or shigellosis. [15]*Enterobacter*, a gram-negative bacteria classified as facultative anaerobes, can cause eye and skin infections, meningitis, bacteraemia (bacterial blood infection), pneumonia, and urinarytractinfections [16]. *Bacillus subtilis*cells are rod-shaped, Gram-positive bacteria that are found naturally in soil and vegetation [17].*Bacillus subtilis*bacteria are non-pathogenic [18]. They can contaminate food and seldom result in food poisoning. *Bacillus subtilis*strains can cause rots in potatoes.*E.coli*is a Gram-negative, facultative anaerobic virulent strain that can cause gastroenteritis, urinary tractinfections, and neonatal meningitis [19].Plants and Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities [20].Seaweeds have become a recognized potential natural product in pharmaceutical industries and an important resource to combat serious diseases in the world. The medicinal value of these plants lies in some bio active substances that produce a definite physiological action on the human body. Plants and algae are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial growth and have a great potential for producing new drugs for human benefit.The most important of these secondary metabolites of plants are flavanoids alkaloids, tannin, and phenolic compounds.. Therefore, it is of great attraction to carry out a systematic way of screening in order to utilise these plants in folk medicines and disclose the active principle forisolation and characterization of their constituents which may result in the discovery of novel active compounds Therefore, the present investigation aims to detect the bactericidal efficacy of *Ficus religiosa*, *Cynodondactylon* and *Tinosporacordifolia*. *Kappaphycusalvarezii* and *Sargassum* species.

MATERIAL AND METHODS

PLANT MATERIAL

The three selected plants, *Cynodondactylon*, *Ficus religiosa*, *Tinosporacordifolia*, were collected from forest region and wild population around Udaipur district and identity was confirmed at Maharana Pratap University of Agriculture and Technology, Udaipur.

The Sea weeds, *Kappaphycusalvarezii* was gathered from the seacoast of Rameshwaram, Tamilnadu, India *Sargassum* species was gathered from sea coast of kanyakumari district ofTamilnadu, India. Their bioactivity and identity was confirmed at Maharana Pratap University of Agriculture and Technology Udaipur. The *Kappaphycusalvarezii* and *Sargassum* species were carried in sterile bags. The selected algae samples were washed with water and dried to remove any waste which was stuck on the surface .The sample was then powdered in a mixer grinder.

BACTERIAL CULTURES

The test bacterial organisms: B1-*Bacillus subtilis*-MTCC 441, B2-*Shigella flexneri*-MTCC 1457, B5-*Escherichia Coli*-MTCC 739, B6-*Enterobacter cloaca* were obtained from Microbial Type Culture collection, Indian Institute of Microbial Technology located in Chandigarh, India.

The purity of the bacterial culture was ensured and checked by their growth in selective media .Then they were further utilised for studying antimicrobial property of the samples.

PREPARATION OF LEAF EXTRACT

The healthy leaf samples of selected plants, *Ficus religiosa*, *Tinosporacordiflora*, *Cynodondactylon* were gathered and washed with tap water. The leaves were dried under sunlight for 5 days by covering them with a dry newspaper. The dried leaves were converted to a powder form using a mixer. The total weight of each sample was noted and they were stored in a dry container. Equal amount of each powdered sample (4g) was dissolved in 4 selected (40ml)- ethanol, acetone, benzene and distilled cow's urine.The dissolved ratio (1:10) for the powder to solvent was taken in a wide mouth test tube. The solutions were mixed and kept aside for 3 days to provide enough time for extraction. This process was repeated thrice for ample extraction of the samples in the same test tube .After 3 days ,the supernatant of the powder and solvent solutions were pipette out and placed in glass bottles. The extracts in the bottles were then exposed for evaporation to get powder form of the extract.

PREPARATION OF AQUEOUS SEA WEED EXTRACT

4g of powdered sample was soaked in 40 ml of the solvents cow urine, benzene, acetone and ethanol for 3 days for the preparation of aqueous extract. The remaining extracts were filtered and concentrated in a rotator evaporator .The vacuum pump was used to remove the residual water .The weighted crude extract were suspended in dimethyl sulfoxide (DEMSO) to a final concentration of 50mg/ml and stored in a refrigerator.

ANTIMICROBIAL ASSAY

PROCEDURE

1. PREPARATION OF PLATES

- i. NAM for bacteria was prepared according to the accurate composition and immediately after autoclaving, it was cooled in a 45 - 50°C. The freshly prepared and cooled medium was poured into petri plates.
- ii. The agar medium was cooled to room temperature unless the plate is used the same day; and stored in a refrigerator (4°C).

2. SPREADING OF BACTERIA ON THE PLATES

- i. 100 µL of bacteria from freshly prepared culture was taken in the pipette and poured in the middle of the respective petri plate.
- ii. Using a cotton swab that has already put in UV light, the bacteria was spread evenly on the surface of the plate so that bacteria were spread in each corner of the plate and dried for 4-5 minutes.

3. ANTIMICROBIAL DISKS

- i. Using a flame - sterilized forceps the disc was dipped in the sample or antibiotics for 5-10 seconds. Each disc was then gently placed on the agar plate to ensure that the disc was attached into the agar. The plate was kept in laminar air flow for 30 minutes so that the drug was properly absorbed in the gel.

4. INCUBATION OF PLATES

- i. Plates were inverted and incubated at 24h at 30°C for bacteria.

5. MEASUREMENT OF DIAMETER

- i. Zone of inhibition is measured with the help of the scale and noted down.

RESULT

The ethanol, acetone benzene, cow urine extracts of *Ficus religiosa*, *Cynodondactylon*, *Kappaphycusalvarezii* (Red Algae), *Sargassum* species (Brown Algae), *Tinosporacordifolia* were tested against the pathogenic microbes. Out of plants and algae tested for antimicrobial activity, plant species and algae showed antibacterial activity by inhibiting one or more microorganisms. The results of the antimicrobial activity of plant extracts tested against bacteria by disc diffusion method shown in Table 2. Among the plants screened, the ethanolic extract of *Ficus religiosa*, *Cynodondactylon*, *Kappaphycusalvarezii* (Red Algae), *Sargassum* species (Brown Algae), *Tinosporacordifolia* showed significant inhibition of all tested bacteria whereas ethanolic extract of *Kappaphycusalvarezii* and *Sargassum* species (Brown Algae), showed less inhibition activity. Cow urine extract of marine species *Kappaphycusalvarezii* and *Sargassum* species showed significant inhibition compared to less inhibition activity of *Ficus religiosa* and *Cynodondactylon*. Acetone extracts of *Ficus religiosa*, *Cynodondactylon*, and *Tinosporacordifolia* showed very less activity as compared to good inhibition activity of ethanolic

extracts against *Bacillus subtilis*. Benzene extract showed good activity in both algae extracts as well as plant extracts. The antimicrobial activity of *Bacillus subtilis* against *Ficus religiosa*, *Cynodondactylon*, *Tinosporacordifolia*, *Kappaphycusalvarezii*, *Sargassum* species of ethanol, acetone, cow urine and benzene extracts was maximum for *Cynodondactylon* (29.5) mm in ethanol extract.

Tinospora cordiflora showed antibacterial activity against all bacteria used in this study. Cow urine extracts of *Kappaphycus* (Red Algae) *Sargassum* species showed good inhibition activity against *Bacillus subtilis* and *Shigella flexneri*. *Escherichia Coli*, *Enterobacter cloacae* showed minimal activity against cow urine extracts of all the selected algae and plants. Ethanol extract showed a maximum activity against pathogens like *Bacillus subtilis* (29.5 mm), *Enterobacter cloacae* (23mm). The antimicrobial activity of *Ficus religiosa*, *Cynodondactylon*, *Tinosporacordifolia*, *Kappaphycusalvarezii*, *Sargassum* species of ethanol, acetone, cow urine and benzene extracts was highest for *Tinosporacordifolia* (15.5) mm in benzene extract against *Shigella flexneri*.

The antimicrobial activity of *E. coli* against *Ficus religiosa*, *Cynodondactylon*, *Tinosporacordifolia*, *Kappaphycusalvarezii*, *Sargassum* species of ethanol, acetone, cow urine and benzene extracts was highest for *Tinosporacordifolia* (13) mm in ethanol extract. The antimicrobial activity of *Enterobacter cloacae* against *Ficus religiosa*, *Cynodondactylon*, *Tinosporacordifolia*, *Kappaphycusalvarezii*, *Sargassum* species of ethanol, acetone, cow urine and benzene extracts was highest for *Ficus religiosa* and *Cynodon dactylon* (23) mm in ethanol extract. Analysis of plant extracts revealed the presence of flavanoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antimicrobial property [21]. Flavanoids, tannins and saponins are present in all plant and algae extracts [22]. These bioactive compounds are known to exert antimicrobial action and act by different mechanism [23]. Tannins bind and interfere protein synthesis by binding to proline rich proteins [24]. Flavanoids are synthesized by plants in response to microbial infection, are hydroxylated phenolic substance and effective antimicrobial substances against microorganisms [25]. Antimicrobial property of saponin is due to its ability to cause leakage of certain enzymes and proteins from the cell [26].

DISCUSSION

The cow urine extracts of *Kappaphycusalvarezii* *Sargassum* species and *Tinospora cordiflora* are among alternatives for bacterial disease management. It has been found cow urine extracts of plants possess significant antibacterial activity against pathogenic bacteria. In the early reported papers, there are a

number of reports which evaluated the antimicrobial properties of marine algae and medicinal plants but very few work was done on cow urine extracts of selected algae species and plants against *Bacteria subtilis*, *E.coli*, *Enterobacter cloacae*, *Shigella flexneri*. In the present investigation, the high susceptibility was recorded for cow urine extracts of *Kappaphycus alvarezii* and *Sargassum* species, *Tinospora cordiflora* against *Bacillus subtilis*. In a study, Tiwari and Das [27] showed inhibitory effects of cow urine extracts against medicinal plants. In another study Venkatesh et al. [28] found inhibitory effect of cow urine extract of red algae against *Xanthomonas oryzae*. Rakesh et al. [29] showed inhibitory effects of cow urine extract of plants against *F. axysporum*, *F. sp. zingiberi* and *P. aphanidermatum* causative agent or rhizome rot of ginger. Venkatesh et al. [30] studied *Antixanthomonas* activity, phytochemical analysis and characterisation of antimicrobial compounds from *Kappaphycus alvarezii*. It has been experimentally shown that cow urine extract possesses inhibitory activity against phytopathogens [31]. The results provide justification for the use of these algae in folk medicine to treat various infectious diseases. When four bacterial organisms were compared it was found that, maximum suppression was observed against *Bacillus subtilis* followed by *Enterobacter cloacae*, *E.coli* and *Shigella flexneri* against all selected extracts. The enhanced activity of extract of cow urine in *Sargassum* species and *Kappaphycus alvarezii* was due to presence of acrylic acid and dimethyl sulphide and antimicrobial property of cow urine itself was due to its low pH, presence of amino acids in urinary peptides which enhance the bacterial killing by increasing bacterial cell hydrophobicity exhibiting antimicrobial action against different clinical microbial strains. This synergistic effect was responsible for inhibition activity. The inhibition activity of ethanol extracts of *Cynodon dactylon* and *Ficus religiosa* was due to the presence of secondary metabolites like saponins, flavonoids etc. From the results it dictates that the greater activity resides in benzene, cow urine extracts of *Tinospora cordifolia* since other extracts of acetone which did not effectively inhibit the growth of the bacteria. This may be due to the chemical constituents responsible for the antibacterial activity are more soluble in ethanol extracts. [32] It can be interpreted that the antibacterial activity against microorganisms is due to any one or more alkaloids of the plants. Further work is necessary to isolate and purification compounds in *Tinospora cordifolia* which will allow the scientific community to recommend their utilization as an accessible alternative to synthetic antibiotics. Further research in this direction will help to develop medicinal plant extracts by making proper formulations of cow urine and to counter various infections caused by microbes, thus boosting the human immune system. It was concluded that cow urine itself has antimicrobial

property and this inhibitory activity in combination with *Kappaphycus alvarezii*, *Sargassum* species and *Tinospora cordiflora* extracts can synergistically be used in the control of bacteria.

Table 1. Shows the antimicrobial activity, Ethanol extract showed a maximum activity against pathogens like *Bacteria subtilis* (29.5 mm), *Enterobacter cloacae* (23mm). Benzene extract against *Enterobacter cloacae* showed promising activity.

Selected Plants and algae	Bacteria	Ethanol	Acetone	Benzene	Cow Urine	Positive Control	Negative Control
<i>Ficus religiosa</i>	<i>Bacillus subtilis</i>	27	6	7.5	10	31.5	6
	<i>Shigella flexneri</i>	8.5	6	6	6	28.5	6
	<i>E.coli</i>	10.5	6	7	6	10	6
	<i>Enterobacter cloacae</i>	23	12.5	15	6	15.5	6
<i>Cynodon dactylon</i>	<i>Bacillus subtilis</i>	29.5*	6	13	8.5	31.5	6
	<i>Shigella flexneri</i>	8	6	6	6	27.5	6
	<i>E.coli</i>	6	6	6	6	13.5	6
	<i>Enterobacter cloacae</i>	23	17.5	18.5	6	18.5	6
<i>Tinospora cordifolia</i>	<i>Bacillus subtilis</i>	15.5	8	12	13.5	18	6
	<i>Shigella flexneri</i>	14	12	15.5	6	34	6
	<i>E.coli</i>	13	12.5	9.5	6	18	6
	<i>Enterobacter cloacae</i>	8.5	6	6	8	26.5	6
<i>Kappaphycus alvarezii</i>	<i>Bacillus subtilis</i>	6	15	8	12	50	.
	<i>Shigella flexneri</i>	9	9	11.5	13	46	.
	<i>E.coli</i>	6	11.5	6	6	13	.
	<i>Enterobacter cloacae</i>	6.5	6.5	6.5	6	16	.
<i>Sargassum species</i>	<i>Bacillus subtilis</i>	9	9	10	15	48	.
	<i>Shigella flexneri</i>	9	9	10.5	11	44	.
	<i>E.coli</i>	8	12	11.5	6	14	.
	<i>Enterobacter cloacae</i>	8.5	6	10	11	10	.

Table2a. The antimicrobial activity of Bacillus subtilis against Ficus religiosa, Cynodondactylon, Tinosporacordifolia, Kappaphycusalvarezii, Sargassum species. Of ethanol, acetone, cow urine and benzene extracts. In these four extracts Cynodon dactylon showed highest antimicrobial activity.

Bacillus subtilis	Ethanol	Acetone	Benzene	Cow Urine	+ Control	- Control
Ficus religiosa	27	6	7.5	10	31.5	6
Cynodon dactylon	29.5*	6	13	8.5	31.5	6
Tinospora cordifolia	15.5	8	12	13.5	18	6
Kappaphycus alvarezii	6	15	8	12	50	-
Sargassum species	9	9	10	15	48	-

Table 2b shows the antimicrobial activity of Shigella flexneri against Ficus religiosa, Cynodondactylon, Tinosporacordifolia, Kappaphycusalvarezii, Sargassum species of ethanol, acetone, cow urine and benzene extracts. In these four extracts Tinosporacordifolia showed highest antimicrobial activity against Shigella flexneri (15.5)mm in benzene extract

Shigella flexneri	Ethanol	Acetone	Benzene	Cow Urine	+ Control	- Control
Ficus religiosa	8.5	6	6	6	28.5	6
Cynodon dactylon	8	6	6	6	27.5	6
Tinospora cordifolia	14	12	15.5*	6	34	6
Kappaphycus alvarezii	9	9	11.5	13	46	-
Sargassum species	9	9	10.5	11	44	-

Table2c. The antimicrobial activity of E.coli against Ficus religiosa, Cynodondactylon, Tinosporacordifolia, Kappaphycusalvarezii, Sargassum species of ethanol, acetone, cow urine and benzene extracts. In these four extracts Tinosporacordiflora showed highest antimicrobial activity against (13) mm in ethanol extract.

E.coli	Ethanol	Acetone	Benzene	Cow Urine	+ Control	-Control
Ficus religiosa	10.5	6	7	6	10	6
Cynodon dactylon	6	6	6	6	13.5	6
Tinospora cordifolia	13*	12.5	9.5	6	18	6
Kappaphycus alvarezii	6	11.5	6	6	13	-
Sargassum species	8	12	11.5	6	14	-

Table2d. Shows the antimicrobial activity of Enterobacter cloacae against Ficus religiosa, Cynodondactylon, Tinosporacordifolia, Kappaphycus alvarezii Sargassum species of ethanol, acetone, cow urine and benzene extracts. In these four extracts Tinosporacordifolia showed highest antimicrobial activity against Bacillus subtilis (13) mm in ethanol extract. acetone, cow urine and benzene extracts. In these four extracts Ficus religiosa and Cynodondactylon showed highest antimicrobial activity against Enterobacter cloacae (23) mm in ethanol extract

Enterobacter cloacae	Ethanol	Acetone	Benzene	Cow Urine	+ Control	- Control
Ficus religiosa	23*	12.5	15	6	15.5	6
Cynodon dactylon	23*	17.5	18.5	6	18.5	6
Tinospora cordifolia	8.5	6	6	8	26.5	6
Kappaphycus alvarezii	6.5	6.5	6.5	6	16	-
Sargassum species	8.5	6	10	11	10	-

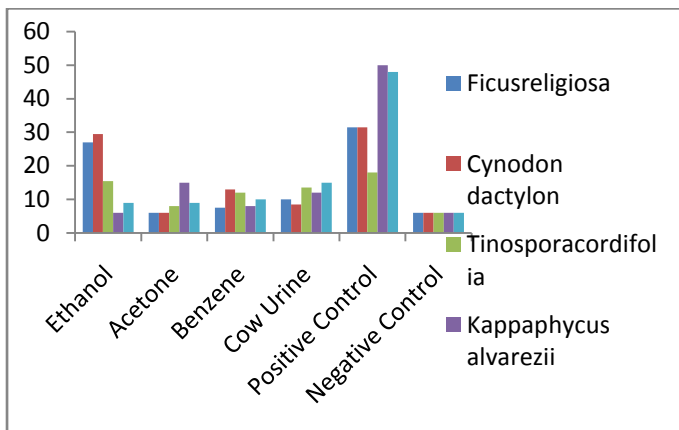


Figure 1: Cynodondactylon showed highest antimicrobial activity against *Bacillus subtilis* (29.5) mm in ethanol extract Zone of inhibition including 6 mm diameter of paper disc.

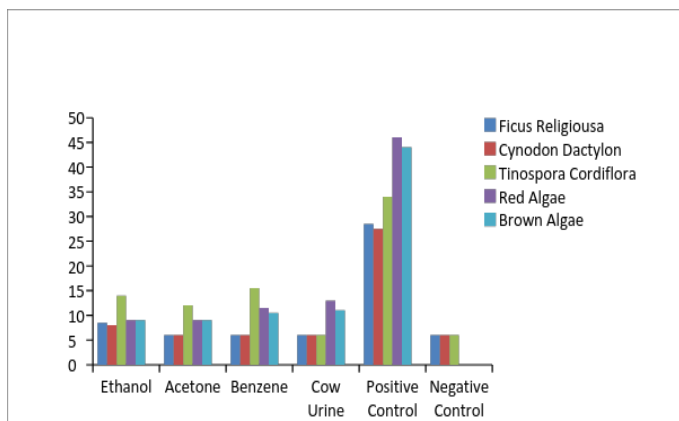


Figure 2: Tinosporacordiflora showed highest antimicrobial activity against bacteria *Shigella flexneri* (15.5) mm in benzene extract Zone of inhibition including 6 mm diameter of paper disc.

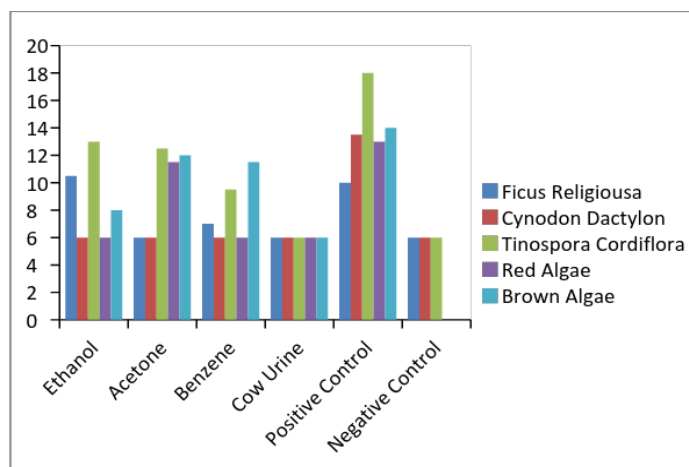


Figure 3: Tinosporacordiflora showed highest antimicrobial activity against (13) mm in ethanol

extract. Zone of inhibition including 6 mm diameter of paper disc.

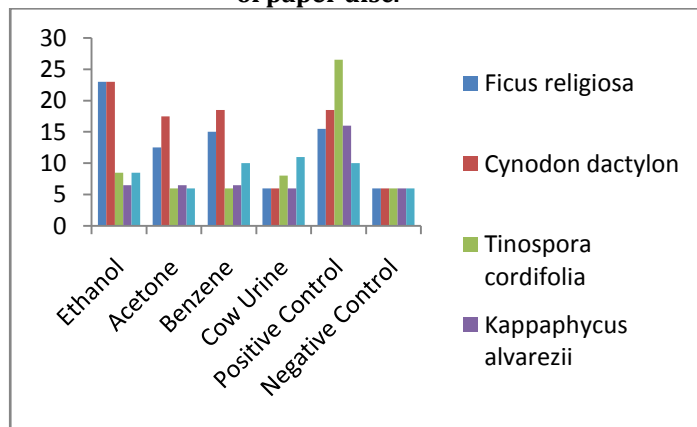


Figure 4: Ficus religiosa and Cynodon dactylon showed highest antimicrobial activity against *Enterobacter cloacae*(23)mm in ethanol extract Zone of inhibition including 6 mm diameter of paper disc.

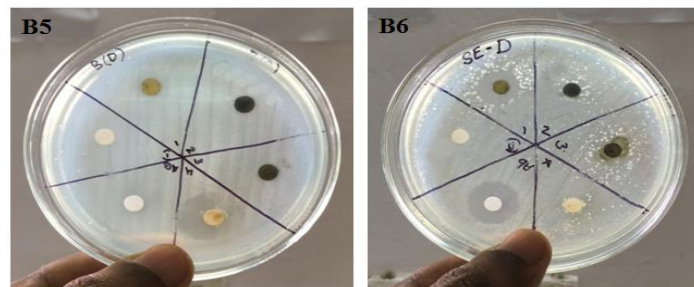


Figure 5: Disc diffusion results of Cynodondactylon against four Bacteria.1: Ethanol, 2: Acetone, 3: Benzene, 4: Cow Urine, Ab: Amoxicillin, (-): Negative control. B1: Bacillus subtilis, B2: Shigella flexneri, B5: E.coli, B6: Enterobacter cloacae

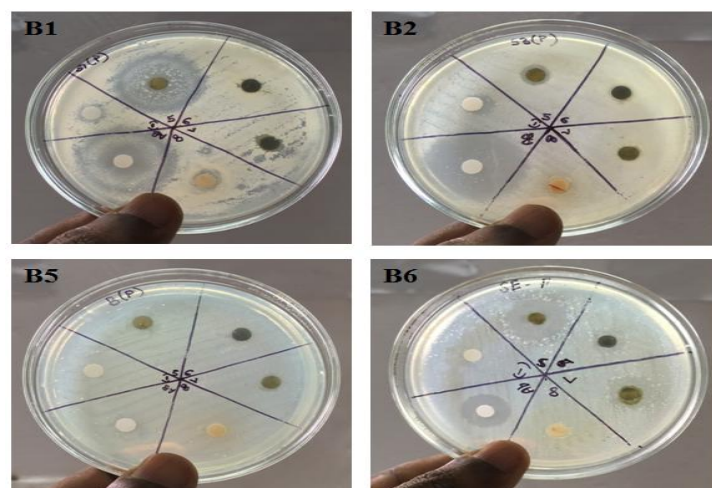


Figure 6: Disc diffusion results of Ficus religiosa against four Bacteria. 5: Ethanol, 6: Acetone, 7: Benzene, 8: Cow Urine, Ab: Amoxicillin, (-): Negative

control. B1: Bacillus subtilis, B2: Shigella flexneri, B5: E.coli, B6: Enterobacter cloacae

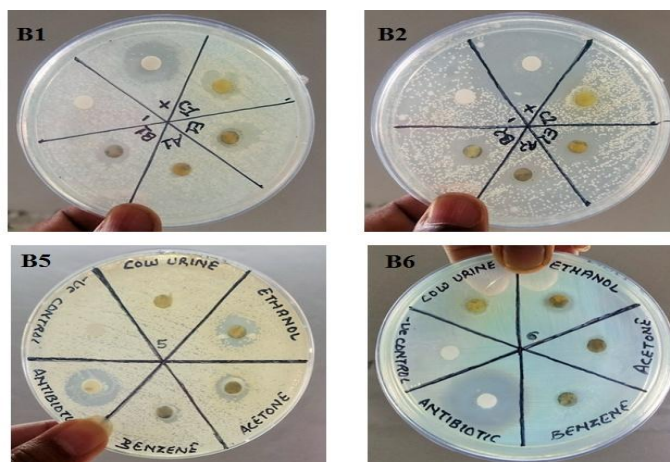


Figure 7: Disc diffusion results of Tinosporacordifolia against four isolated Bacteria. C1: Cow Urine, E1: Ethanol, A1: Acetone, B1: Benzene, +: Amoxicillin, -: Negative control. B1: Bacillus subtilis, B2: Shigella flexneri, B5: E.coli, B6: Enterobacter cloacae

REFERENCES

- Soni, A, & Sosa, S. (2013). Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts. Journal of Pharmacognosy and Phytochemistry, 2 (4), 22-29.
- Jandaik, S., Thakur, P, &Kumar, V. (2015). Efficacy of Cow Urine as Plant Growth Enhancer and Antifungal Agent. Advances in Agriculture. Volume 2015, Article ID 620368, 7 pages.
- Kavishankar, G.B, LakshmiDevi,N&Mahadeva, Murthy, S. (2011). Phytochemical analysis and antimicrobial properties of selected medicinal plants against bacteria associated with diabetic patients. International Journal of Pharma and Bio Sciences. ISSN 0975-6299 2(4).
- El Gamal, AA. (2010). Biological importance of marine algae. Saudi Pharm J. 18(1), 1-25. Published online 2009 Dec 23.
- Venketash R. S. Shanthi et al. (2011) Preliminary study on Antixanthomonasactivity, phytochemicalanalysis and characterization of antimicrobial compounds from Kappaphycusalvarezii. Asian J Pharm Clin Res, 46-51.
- RandhawaK.Gurpreet.(2015).Chemotherapeutic potential of cow urine :A review J Published online 2015 March 7
- El-Sikaily, A,Ghada, F. El-Said. (2013). Chemical composition of some seaweed from Mediterranean Sea coast, Egypt. Environ Monit Assess. 185(7), 6089-6099. Published online 2012 Dec 5
- Phycoerthyrin From Wikipedia, the free encyclopaedia
- Brown algae From Wikipedia, the free encyclopaedia
- Yadav, M, Chatterji, S, Gupta, S.K, &Watal, G. (2014). Preliminary phytochemical screening of six medicinal plants used intraditional medicine. International Journal of Pharmacy and Pharmaceutical Sciences. 6(5), ISSN- 0975-1491
- Shankhadarwar, S.D. (2015). Phytochemical screening of marine algae Ulvalacuta (Linn.) and Enteromorpha intestinalis (Linn.). Journal of Chemical and Pharmaceutical Research, 7(12), 419-423.
- Ficus religiosa, From Wikipedia, the free encyclopaedia
- Cynodondactylon ,From Wikipedia, the free encyclopaedia
- Tinospora cordifolia, From Wikipedia, the free encyclopaedia
- Jaya Sureshbabu. Shigella Infection. Medscape.
- Kara Rogers Enterobacter bacteria genus. Encyclopaedia Britannica
- Bacillus subtilis. From Microbe Wiki, the student-edited microbiology resource
- Bacillus subtilis. From Wikipedia, the free encyclopaedia
- Escherichia coli From Wikipedia, the free encyclopaedia
- Pérez .M. José, Falqué .Elena. Dominguez Herminia (2016) Antimicrobial Action of Compounds from Marine Seaweed .Mar Drugs. v.14 (3); 2016 .Published online 2016 March.
- ErumIqbal, Kamariah Abu Salimb, Linda B.L. Lima, (2015)Phytochemical screening, totalphenolics and antioxidant activities of bark and leaf extracts of Goniotalamusvelutinus (Airy Shaw) from Brunei DarussalamJournal of King Saud University – Science, Volume 27, Issue 3, July, 224-232.
- Deyab, M., Elkatony, T. & Ward, F. (2016). Qualitative and Quantitative Analysis of Phytochemical Studies on Brown Seaweed DictyotaDichotoma. IJEDR. 4(2), ISSN: 2321-9939.
- Gunjal, S,Ankola, A.V.,Bolmal, U.K., Hullatti, K.K. Formulation and evaluation of antimicrobial activity of Morus alba sol-gel against periodontal pathogens. Journal of Indian Association of Public Dentistry
- Lu, Y, Bennick A. (1998). Interaction of tannin with human salivary proline-rich proteins. Arch Oral Biol. 43(9), 717-28.
- Kumar,S., &Pandey, A.K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal.Volume 2013, Article ID 162750, 16 pages.
- Murugan, T., Albino,Wins, J. & Murugan, M.(2013). Antimicrobial Activity and Phytochemical Constituents of Leaf Extracts of Cassia auriculata. Indian Journal of Pharmaceutical Sciences.
- Tiwari RKS, Das K (2011) Inhibitory effect of cow urine based plant extracts against Rhizoctoniasolani

- causing sheath blight of rice. Ind Phytopathol 64: 265—26827
- 28) T.R Kekuda Prashith(2014) Antifungal effect of cow urine extracts of selected plants against Colletotrichum capsici isolated from anthracnose of chilli Intl J Agri Crop Sci. Vol., 7 (3), 142-146.
- 29) Rakesh KN, Dileep N, Junaid S, Kekuda PTR, Vinayaka KS, Nawaz ASN (2013) Inhibitory effect of cow urine extracts of selected plants against pathogens causing rhizome rot of ginger. SciTechnol Arts Res J 2 : 92—96.
- 30) Venketash R.S.Shanthi S, Rajapandian K, Elamathi S, Thenmozhi S, Radha N (2011) Preliminary study on Antixanthomonas activity ,phytochemical analysis and characterization of antimicrobial compounds from Kappaphycusalvarezii. Asian J Pharm Clin Res4, 46-51.
- 31) Manasa M. Antifungal activity of cow urine extracts of selected plants against helminthosporiumsp.andAlternariasp.Environment &Ecology 32(3):920-922.Published online 28 February 2014.
- 32) Jeyachandran.R, Xavier T. Francis and Anand S.P. (2003) Antibacterial activity of stem extracts of Tinospora cordifolia (wild) hook. F &ThomsonAncient Science of Life VolXXIII (1) July, August, September 2003, 40-43.