Comparative Assessment of the Phytochemical and Antimicrobial Efficacy of Cow Urine Extracts of Selected Plants and Microalgae against Microbes

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ABSTRACT

Objective: Comparative screening of the phytochemical and antimicrobial efficacy of cow urine extracts from selected algae and medicinal plants against fungi and bacteria.

Methods:

The work is emphasised on the comparative study of the phytochemical and antimicrobial potential of the selected medicinal plants and algae against microbes .The Cow urine extracts of selected plants Ficus religiosa, Cynodon dactylon and Tinospora cordifolia were compared with the microalgae selected in the study Kappaphycus alvarezii (Red Algae) and Sargassum species (Brown Algae) against fungi ,Saccharomyces cerevisiae, Aspergillus candidus, Aspergillus niger and Pencillium Species and bacteria, Bacillus subtilis ,Shigella flexneri, Escherichia Coli ,Enterobacter cloacae which were obtained from Microbial Type Culture collection,Indian Institute Of Microbial Technology Chandigarh, India .

Result: The phytochemical analysis of extracts carried out revealed the presence of alkaloids, flavanoids, glycosides, tannins, saponins, reducing sugar and steroids in selected plants and algae. The Ficus religiosa extract showed maximum number of compounds such as saponins to be 19.58-18.43 mg/g dry weight, flavanoids to be 10.11-10.66 mg/g dry weight and alkaloids to be 0.53-0.59 mg/g dry weight compared to the Sargassum species with saponins to be 12.69-11.83 mg/g dry weight, flavanoids to be 5.21-5.13 mg/g dry weight and Alkaloid to be 0.00 mg/g dry weight. The comparative assessment of the cow urine extracts of selected plants and microalgae revealed that Tinospora cordifolia showed highest zone of inhibition(13mm)compared to Kappaphycus alvarezii and Sargassum species which showed zone of inhibition 6.5mm against Saccharomyces cerevisiae.Saccharomyces cerevisiae was the most inhibited fungal isolate with 13mm inhibition by Tinospora cordifolia extracts inhibiting its growth.The cow urine extract of Sargassum species showed maximum zone of inhibition 15mm compared to Tinospora cordifolia which showed 13.5mm against Bacillus subtilis.

Conclusion:In the present study we compared that the antibacterial activity and antifungal activity of cow urine extract of Tinospora cordifolia ,Ficus religiosa, Cynodon dactylon against Kappaphycus alvarezii and Sargassum species against selected microbes and this inhibitory activity can be used in the control ofmicrobes of various origins .The Kappaphycus alvarezii and Sargassum species revealed significant antimicrobial activity against all pathogens. The compilation indicates that cow urine extracts of Sargassum species and Kappaphycus alvarezii exhibit better antimicrobial action against different clinical microbial strain.. The algae cow urine extracts are sources for development of new novel pharmaceutical agents. The future research lies in utilisation of these extracts to develop algae and plant extracts by making proper formulations of cow urine extracts and to counter various infections caused by microbes, thus boosting the human immune system and development of novel drugs.

Keywords:
Antimicrobial activity, cow urine extracts Cynodon dactylon, .Ficus religiosa, phytochemical activity, Sargassum species, and Tinospora cordiflora.

INTRODUCTION

Sea weeds and medicinal plants have unique place in the ancient and traditional medicine being a good source of antimicrobial potential with a promising sourcefor natural products. Algae are eukaryotic organisms inhabited in salty sea water, can be broadly divided into microalgae and macro algae. These seaweeds are classified on the chemical composition and nutrition basis as Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) [1].Gelidiumacerosa is the genus of red algae which contains phycoerthyrin and phycocyanin as pigments responsible for red colourwith a high economic value, in food industries, and pharmaceutical industries. [2]. The Gelidiumacerosa contain large amount of valuable phytochemicals like sapononins, flavanoids and alkaloids etc., which are known for its medicinal uses and is recognized to synthesize several bioactive compounds which show antimicrobial property [3].Brown algae are a group of algae with possession of pigment called fucoxanthin.The phlorotannin contents as marine phenolic compounds have reported in good amount in brown algae.[4].Cynodon dactylon is also known as the Bermuda grass or the Doob grass is a creeping grass, light green in color, very tough and has a rough texture. C.dactylon is having wide range of antimicrobial activity which includes explanation of its antiviral and antibacterial nature. In traditional medicine it was used for indigestion and the treatment of wounds and in folk remedy for calculus, cancer, carbuncles, convulsions, cough, cramps, cystitis, diarrhea, dropsy, dysentery, epilepsy, headache, hemorrhage, hypertension, hysteria, insanity, laxative, measles, rubella, snakebite, sore stones, tumors, urogenital disorders, warts, and wounds.[5].Tinospora cordiflora(family Menispermaceae) commonly called Guduchi is a glabrous climbing shrub ,leaves are heart shaped and rich in protein (11.2%) and are fairly rich in calcium and phosphorus.. The aqueous extract of guduchi stem has shown the presence of arabino galactan that showed immunological activity. The bitter principle present shows

adaptogenic, antispasmodic, anti-inflammatory, antipyretic, anti-neoplastic, hypolipidemic. [6].**Ficus religiosa** also known as Peepal, Pipal, Ashwattha tree, or Bodhi tree is commonly planted as boulevard and roadside tree. It is an efficient medicine prescribed in either dried powdered form for the treatment of asthma, or along with hard for the treatment of diabetes; it is consumed in the fresh form for the treatment of dehydration. It is consumed as famine food during scarcity, figs of Ficus religiosa are an excellent source of flavanoids, phenols, fiber, antioxidants and other compounds like vitamins, proteins, minerals, carbohydrates, serotonin, etc.[7]**Cow urine** is a liquid discharge consisting of nontoxic waste material from the cow body. The main constituents of cow urine are Water: 95%, followed by Urea: 2.5%, and the rest 2.5% is a mixture of different minerals, salts, hormones, and enzymes. Antimicrobial and germicidal properties of cow urine are due to the presence of urea, creatinine, aurum hydroxide, carbolic acid, phenols, and salts of calcium, and manganese.[8]Shigella flexneri**,** a most gram negative bacteria recognized as the etiologic agents of bacillary dysentery or shigellosis. [9]Enterobacter a gram-negative bacteria classified as facultative anaerobes, can cause eye and skin infections, meningitis, bacteremia (bacterial blood infection), pneumonia, and urinary tract infections .[10]Bacillus subtilis cells are rod-shaped, Gram-positive bacteria that are found naturally in soil and vegetation [.11]Bacillus subtilis are non-pathogenic They can contaminate food and seldom result in food poisoning. Bacillus subtilisstrains can cause rots in potatoes. .[12]].E.coli is a Gram-negative, facultative anaerobic virulent strain that can cause gastroenteritis ,urinary tract infections, and neonatal meningitis.[.[13.].Aspergillus niger spores if inhaled in large amounts causes Aspergillosis, a serious lung infection. [14].Species of Penicillium are recognized by their dense brush-like spore-bearing structures called penicillin some species produce toxins and may render food inedible or even dangerous.[15].Saccharomyces is a genus of fungi that includes many species of yeasts. Saccharomyces cause food spoilage of sugar-rich foods, such as maple sap, syrup, concentrated juices and condiments [16]

Aspergillus candidus is a fungus which is a common contaminant of grain dust and which causes respiratory disease [17].Therefore, the present investigation aims to compare thefungal and bactericidal efficacy of cow urine extracts of selected plants Ficus religiosa, Cynodon dactylon and Tinospora cordifolia and selected algae Kappaphycus alvarezii and Sargassum species against microbes. This comparative assessment paved the path for the utilisation of these extracts to develop medicinal plant and algae extracts by making proper formulations with cow urine counter various infections caused by microbes, thus boosting the human immune systems and a boon for future development of novel drugs.

MATERIAL AND METHODS

Plant material

The Sea weeds, Kappaphycus alvarezii was collected from the seacoast of Rameshwaram, Tamilnadu, India. The three selected medicinal plants, Cynodon dactylon, Ficus religiosa, Tinospora cordifolia, were collected from wild population around Udaipur district and identity was confirmed at Maharana Pratap University of Agriculture and Technology, Udaipur. Sargassum species was collected from was collected from the sea coast of Kanyakumari and Ramanathapuram district of Tamilnadu, India. The selected plants and algae are used for experimental purpose and their bioactivity and identity was confirmed at Maharana Pratap University of Agriculture and Technology Udaipur. The algae samples were bought in the laboratories in sterile conditions and were dried and kept for a week in the sun and then were grinded to small powder.

Fungal and Bacterial Cultures:

The test organisms B1-Bacillus subtilis-MTCC 441,B2-Shigella flexneri-MTCC 1457,B5-Escherichia Coli-MTCC 739,B6-Enterobacter cloacae, **.**A.niger-MTCC 282,A. candidus- MTCC 1989,S. cerevisiae-MTCC 170 were obtained from Microbial Type Culture collection, Indian Institute Of Microbial Technology Chandigarh, India .

The cultures were allowed to grow on their respective selective media to check and ensure their purity and optimum growth before subjected to further analysis.

Preparation of leaf extract

The leaf samples of selected plants of Ficus religiosa, Tinosporacordiflora, Cynodon dactylon were collected, washed and dried under sunlight for 5 days. Once the leaves were crunchy dry, a fine powder was made 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 in distilled cow's urine. (40ml)The samples were dissolved in a ratio of (1:10) powder to solvent, in a wide mouth test tube. The test tube mouths were then covered to prevent evaporation of the solvents. The solutions were mixed and left aside for 3 days to provide enough time for extraction. This process was repeated thrice in the same test tube for ample extraction of the samples After 3 days the supernatant of the powder and solvent solutions were pipetted out and placed in glass bottles. The extracts in the bottles were then placed without lids in the open to get powder of the extract.

Preparation of aqueous sea weed extract: The aqueous extract was prepared bydissolving 4g of powdered sample was soaked in 40 ml of the solvents cow urine for 3 days. 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

1. Preparation of plates

i. PDA for fungus and 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; andstored in a refrigerator (4°C).

Spreading of bacteria and fungus on the plates

100 µL of bacteria and fungus from freshly prepared culture was taken in the pipette and poured in the middle of the respective petri plate.

Using a cotton swab that has already put in UV light, the bacteria and fungus was spread evenly on the surface of the plate so that bacteria and fungus 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 and 3-4 days for fungus.

5. Measurement of diameter

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

PHYTOCHEMICAL ANALYSIS

The solvent extracts of Ficus religiosa, Cynodon dactylon and Tinospora cordifolia, Kappaphycus alvarezii (Red Algae) and Sargassum species were subjected to preliminary phytochemical screening to identify the chemical constituents

Different phytochemical was performed.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol

Glycosides. 2ml of chloroform, 2 ml of acetic acid were added to plant extract and allowed to cool, followed by addition of 2ml of concentrated H2SO4 changes the violet to blue then green, indicates the presence of steroidal nucleus that is glycone portion of glycoside, steroidal ring that is glycone portion of glycoside In another way, the available cardiac glycosides are tested by addition of 1-2 drops of glacial acetic acid and 2% of $FeCl_3$ solution in crude plant extract followed by 2ml of H_2SO_4, gives brown ring at the interphase indicates the presence of cardiac glycosides.

Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of phytosterols/triterpenes

Salkowski Test: Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of concentrated sulphuric acid were added along the side of the test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids

Detection of phenols: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols

Qualitative analysis of Phenols and Tannins: Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Qualitative analysis of flavanoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids. (Tiwari et al, 2011).

Qualitative analysis of Terpenoids

Salkowski Test: Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of concentrated sulphuric acid were added along the side of the test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids.

Qualitative analysis of Proteins

a) Biuret test: An aliquot of 2 mL of filtrate is treated with one of 2% CuSo4 solution. To this, 1 mL of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicates the presence of proteins. (Roopalatha et al, 2013).

1. **Qualitative analysis of Steroids:** Plant extracts mixed in 2ml of chloroform and Conc. H2SO4 were added gently, which leads to the development of red color in the lower chloroform layer indicating the presence of steroid, and was further confirmed with addition of acetic acid which develops the greenish color formation.
2. **Qualitative analysis of Napthoquinone:** Dam - Karrer test method: The presence of Napthoquinone was identified by addition of few drops of 10% KOH solution in plant extract, which gives blue color.
3. **Qualitative analysis of Anthocyanins: The** presence of Anthocyanins, were tested by addition of 2ml of HCl and

ammonia with 2ml of aqueous plant extract, gives the development of pink red turn to violet color.

4. **Qualitative analysis of Leuco anthocyanins**: The leuco anthocyanins were tested by adding equal volume of plant extract and isoamyl alcohol, which leads to the development of red color.
5. **Qualitative analysis of fats:**
   a) Solubility test: Oils and fats are soluble in organic solvents like, chloroform, alcohol etc. but are insoluble in water.[18]
6. **Test for anthraquinones**: Five ml of the extract solution was hydrolysed with diluted Conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.[19]

Quantitative estimation of Saponins

The samples were ground and 10 g of each were put into a conical flask and 100 ml of 20%aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated with 60 ml of n-butanol. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight, the Saponin content was calculated as percentage.

$$\text{Saponin content (\%)} = \frac{\text{Weight of extracted material}}{\text{Weight of sample}} \times 100$$

7. **Quantitative estimation of Flavanoids**

10g of the plant sample was extracted repeatedly with 10ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

$$\text{Flavanoids content (\%)} = \frac{\text{Weight of extracted material}}{\text{Weight of sample}} \times 100$$

8. **Quantitative estimation of Alkaloids**

10 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated in water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid, which was dried and weighed.[20]

RESULT

The comparative study of cow urine extracts of Ficus religiosa, Cynodon dactylon, Kappaphycus alvarezii (Red Algae), Sargassum species (Brown Algae), Tinospora cordifolia were tested against the Bacillus subtilis ,Shigella flexneri, Escherichia Coli , Enterobacter cloacae, Saccharomyces cerevisiae, Aspergillus candidus, Aspergillus niger and Pencillium Species. The phytochemical constituents of the selected plants investigated are summarized in Table-1. 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 .The Ficus religiosa extract showed maximum number of compounds such as saponins to be 19.58-18.43 mg/g dry weight, flavanoids to be 10.11-10.66 mg/g dry weight and alkaloids to be 0.53-0.59 mg/g dry weight compared to the Sargassum species with saponins to be 12.69-11.83 mg/g dry weight, flavanoids to be 5.21-5.13 mg/g dry weight and Alkaloid to be 0.00 mg/g dry weight. These bioactive compounds are known to exert antimicrobial action and act by different mechanism.. Tannins bind interfere protein synthesis by binding to proline rich proteins. [21] Flavanoids are synthesized by plants and are effective antimicrobial substances against microorganisms. Their binding activity is probably due to their ability to complex with soluble and extracellular proteins and to complex with bacterial cell walls.[22] Antimicrobial property of saponin is due to its ability to cause leakage certain enzymes and proteins from the cell.[23]

The results of the antimicrobial activity of plant extracts tested against bacteria by disc diffusion method shown in Table - 2.The comparative assessment of the cow urine extracts of selected plants and microalgae revealed that Tinospora cordiflora showed highest inhibition 13mm compared to Kappaphycus alvarezii and Sargassum species which showed 6.5mm inhibition against Saccharomyces cerevisiae. The inhibition against Pencillum species in cow urine extract was found to be higher in Kappaphycus alvarezii (9.5mm) and Sargassum species (10mm) comparative to no inhibition in selected plant species. The inhibition against Aspergillus candidus in cow urine extract was found to be higher in Kappaphycus alvarezii (10mm) and Sargassum species (10mm) comparative to 9.5mm inhibition in Cynodon dactylon. The inhibition against Aspergillus niger in cow urine extract was found to be higher in Kappaphycus alvarezii (10mm) comparative to no inhibition found in selected plant species. Saccharomyces cerevisiae was the most inhibited fungal isolate with 13mm inhibition by Tinospora cordifolia extracts inhibiting its growth.

The cow urine extract of Sargassum species showed maximum zone of inhibition 15mm against compared to Tinospora cordifolia which showed 13.5mm against Bacillus subtilis. The Cynodon dactylon cow urine extract showed the lowest inhibition 8.5mm among selected plants and algae against Bacillus subtilis. The extract of Kappaphycus

Alvarezii showed highest inhibition 13mm against Shigella flexneri followed by Sargassum species 11mm.The Ficus religiosa, Cynodon dactylon, Tinospora cordifolia showed no inhibition against Shigella flexneri. The cow urine extracts of all selected plants and microalgae showed no inhibition against E.coli. The extract of Sargassum species showed inhibition 11mm against Enterobacter cloacae whereas inhibition shown by Tinospora cordifolia was 8mm against Enterobacter cloacae. The extract of Ficus religiosa, Cynodon dactylon showed no inhibition against Enterobacter cloacae. Flavanoids, Tannins and saponins are present in all selected plant and algae extracts.

DISCUSSION

The cow urine extracts of Tinospora cordiflora and Kappaphycus alvarezii, Sargassum species possesses significant phytochemical, antibacterial and antifungal activity against pathogenic microbes .The cow urine extract are future alternatives for bacterial and fungal disease management. In the early reported papers, there are a number of reports which evaluated the antimicrobial properties of marine algaeand medicinal plants but very few work was done on comparative study of antifungal, antibacterial, phytochemical activities of selected cow urine extracts of Kappaphycus alvarezii, Sargassum species against Bacteria subtilis, E.coli, Enterobacter cloacae, Shigella flexneri, Saccharomyces cerevisiae, Aspergillus candidus, Aspergillus niger and Pencillium species. In the present investigation, the maximum zone of inhibition was recorded for cow urine extracts of Sargassum species 15mm against Bacillus subtilis and Tinospora cordiflora cow urine extract showed highest inhibition 13mm against Sachromyces cervesaie. This results indicates the enhanced antifungal activity of extract of cow urine in Kappaphycus alvarezii is due to phenolic content which restrains micelle growth because of presence of phenolic content of algae.[24] and enhanced bacterial activity of extract of cow urine in Sargassum species and Kappaphycus alvarezii is due to presence dimethyl sulphide and acrylic acid in algae [25]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.[26]

The compilation indicates that cow urine extracts of selected algae exhibit better antimicrobial action against different clinical microbial strain comparative to cow urine extracts of Ficus religiosa and Cynodon dactylon .Kappaphycus alvarezii showed the highest antifungal activity compared to plant wherein Tinospora cordifolia showed the significant antifungal activity. The Sargassum species extract showed significant antibacterial activity against all selected bacteria compared to extract of Tinospora cordifolia which also showed good inhibition activity against all selected bacteria. The algae cow urine extracts are sources for development of new novel pharmaceutical agents. It was concluded that cow urine itself has antimicrobial property and inhibitory activity of plant extracts can synergisticallycan be used as a precursors for the synthesis of useful herbal drugs.Phytochemicals extracted from medicinal plants are known to have antibacterial properties and can lead to the development of new advanced medicines. The production of microbial inhibitory substances from marine species have been carried out to identify novel antimicrobial compounds from marine sources, thus resolution to the growing crisis of antibiotic resistance and their side effects are the breakthrough for search of new antimicrobial compounds from natural resources.New antimicrobial compounds when projected to chemical analysis and biological assay have begun to play an important role in ethnobotanical studies. The cow urine and medicinal plant extracts exhibit better antimicrobial action against different clinical microbial strains; hence can be utilised to control microbial infections.These products essentially help to identify newer structurally novel natural products with new modes of action and exhibiting antimicrobial activity. It is anticipated that phytochemicals with adequate antibacterial efficacy can be used for the treatment of bacterial and fungal infections.

Table1. shows the qualitative phytochemical screening ,among the cow urine extracts of Ficus religiosa, Cynodon dactylon, Tinospora cordifolia, Kappaphycus alvarezii ,Sargassum species, the Ficus religiosa extract showed a maximum number of compounds such as flavanoids, saponins, alkaloids etc.

S. No	Phytochemical tests	Sample				
		Ficus religiosa	**Cynodon dactylon**	**Tinospora cordiflora**	**Kappaphycus alvarezii**	**Sargassum species**
1	Alkaloids	+/-	+/-	-	-	-
2	Carbohydrates	-	+	+	-	-
3	Saponins	+++	+	++	+	+
4	Glycosides	-	+		-	-
5	Flavonoids	+++	+	+	+	+
6	Tannins	+	+++	++	+	+
7	Triterpenes	+	-			
8	Phenolic compounds	+	-	++	+	+
9	Protein	+	-	+	-	-
10	Steroids	-	-	+	-	+
11	Napthoquinone	-	-		-	-
12	Leucoanthocyanins	-	-		-	-
13	Fat and fixed oils	+	+		-	-
14	Anthocyanins	-	-		-	-
15	Anthraquinone	-	-		-	-

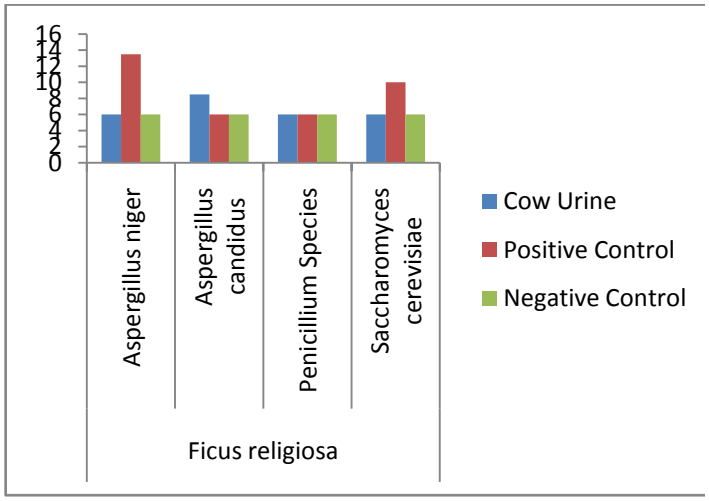
Table1. The qualitative quantitative phytochemical analysis of plant and algae samples (Value in mg/g dry weight) , the Ficus religiosa extract showed maximum number of compounds such as saponins, to be 19.58-18.43 mg/g dry weight. Flavanoids to be 10.11-10.66 mg/g dry weight and alkaloids to be 0.53-0.59 mg/g dry weight.

S.No	Phytochemical	Ficus religiosa	Cynodon dactylon	Tinospora cordifolia	Kappaphycus alvarezii	Sargassum species
1	Saponin	19.58-18.43	3.51-3.48	8.74-8.29	11.65-12.06	12.69-11.83
2	Flavonoid	10.11-10.66	1.28-1.36	1.96-1.99	2.88-2.69	5.21-5.13
3	Alkaloid	0.53-0.59	0.05-0.05	0.00	0.00	0.00

Table2. The antibacterial activity and the antifungal activity of of cow urine extracts of Ficus religiosa, Cynodon dactylon, Tinospora cordifolia, Kappaphycus alvarezii, Sargassum species against Bacillus subtilis, Shigella flexneri, Enterobacter cloacae, E.coli.

Sargassum species, showed highest antibacterial activity against (15 mm) Bacillus subtilis .Tinospora cordiflora, showed highest antifungal activity against Saccharomyces cerevisiae (13 mm)

	BACTERIA	**Cow Urine**	**Positive Control**	**Negative Control**		**FUNGI**	**Cow Urine**	**Positive Control**	**Negative Control**
Ficus religiosa	Bacillus subtilis	10	31.5	6	Ficus religiosa	Aspergillus niger	6	13.5	6
	Shigella flexneri	6	28.5	6		Aspergillus candidus	8.5	-	-
	E.coli	6	10	6		Penicillium species	6	-	-
	Enterobacter cloacae	6	15.5	6		Saccharomyces cerevisiae	6	10	6
Cynodon dactylon	Bacillus subtilis	8.5	31.5	6	Cynodon dactylon	Aspergillus niger	6	13.5	-
	Shigella flexneri	6	27.5	6		Aspergillus candidus	9.5	-	-
	E.coli	6	13.5	6		Penicillium species	6	-	-
	Enterobacter cloacae	6	18.5	6		Saccharomyces cerevisiae	6	10	6
Tinospora cordifolia	Bacillus subtilis	13.5	18	6	Kappaphycus alvarezii	Aspergillus niger	10	-	
	Shigella flexneri	6	34	6		Aspergillus candidus	10	-	-
	E.coli	6	18	6		Penicillium species	9.5	-	-
	Enterobacter cloacae	8	26.5	6		Saccharomyces cerevisiae	6.5	-	-
Kappaphycus alvarezii	Bacillus subtilis	12	50	-	Sargassum species	Aspergillus niger	9	20	-
	Shigella flexneri	13	46	-		Aspergillus candidus	6	11	-
	E.coli	6	13	-		Penicillium species	10	10	-
	Enterobacter cloacae	6	16	-		Saccharomyces cerevisiae	6.5	12	-
Sargassum species	Bacillus subtilis	15	48	-	Tinospora cordifolia	Aspergillus niger	6	6	13
	Shigella flexneri	11	44	-		Aspergillus candidus	6	6	11
	E.coli	6	14	-		Penicillium species	6	6	9.5
	Enterobacter cloacae	11	10	-		Saccharomyces cerevisiae	13	25.5	27



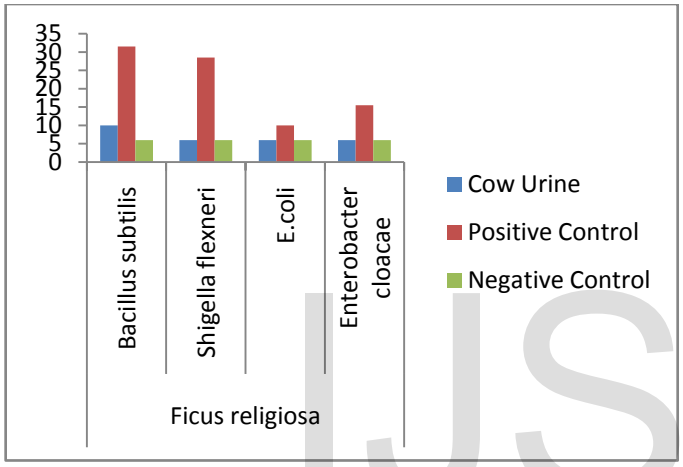
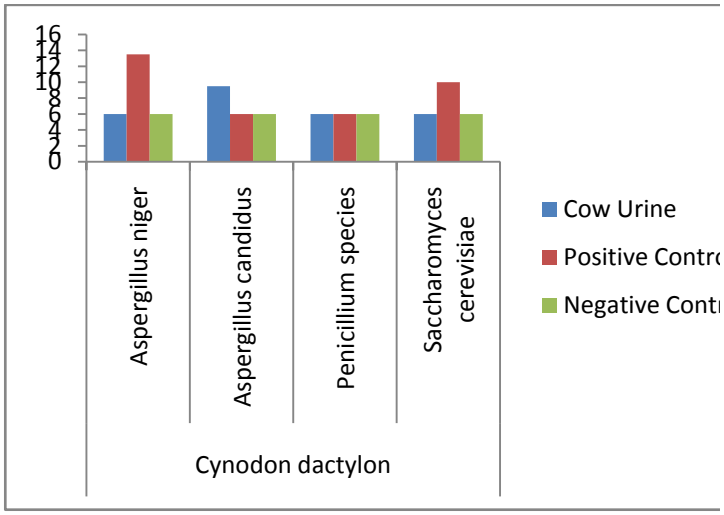


Figure 3: Comparative graphs of antifungal and antibacterial activity of cow urine extract of Ficus religiosa against selected

bacteria and fungi

Zone of inhibition including 6 mm diameter of paper disc.



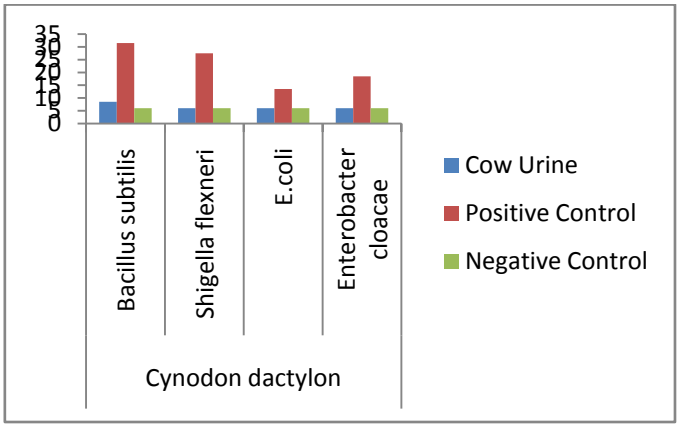
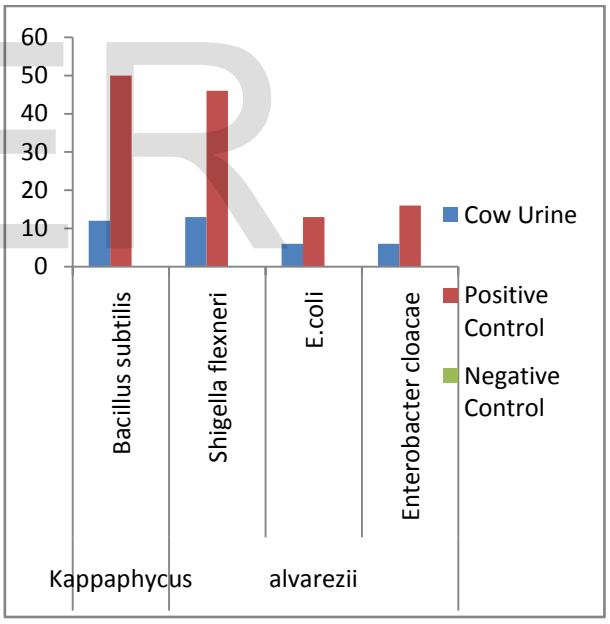


Figure 3: Comparative graphs of antifungal and antibacterial activity of cow urine extract of Cynodon dactylon against selected

bacteria and fungi

Zone of inhibition including 6 mm diameter of paper disc.



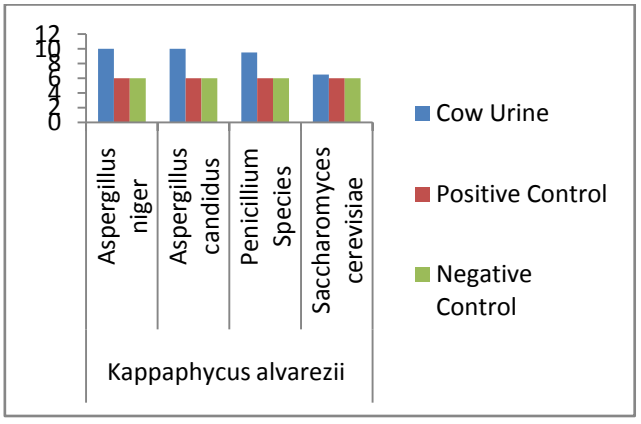
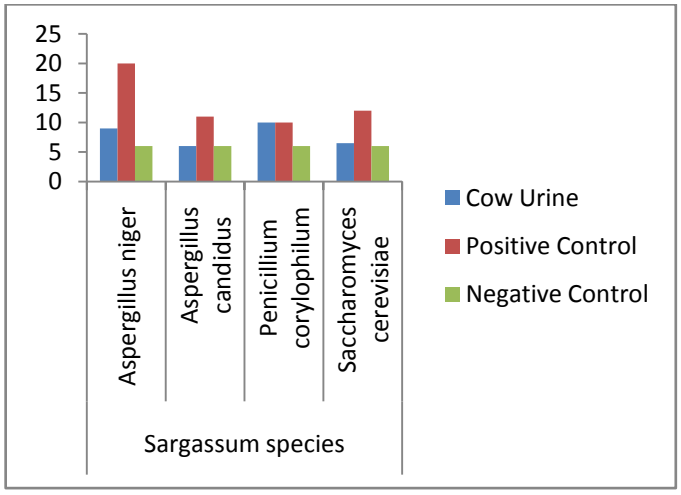
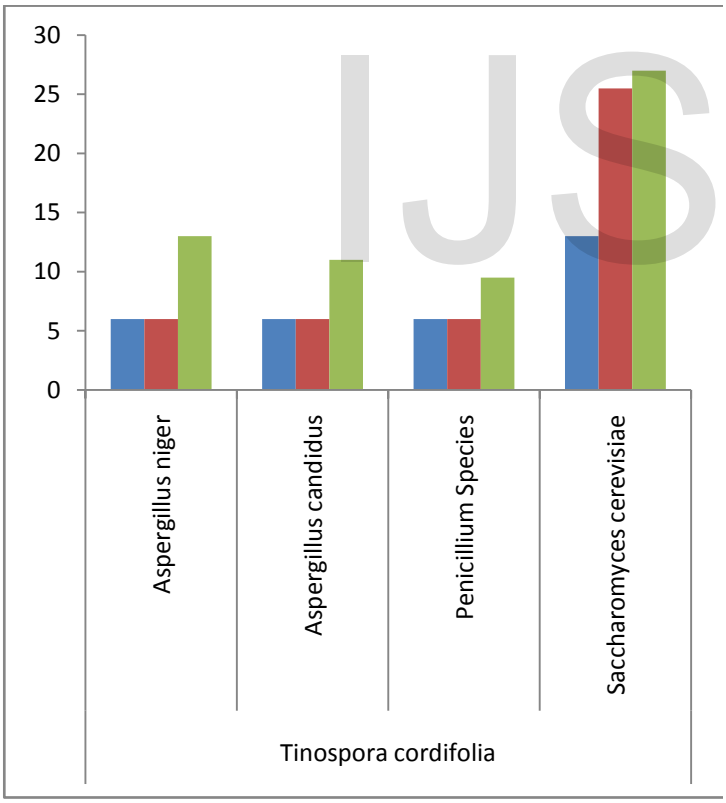


Figure 3: Comparative graphs of antifungal and antibacterial activity of cow urine extract of Kappaphycus alvarezii against selected

Bacteria and fungi





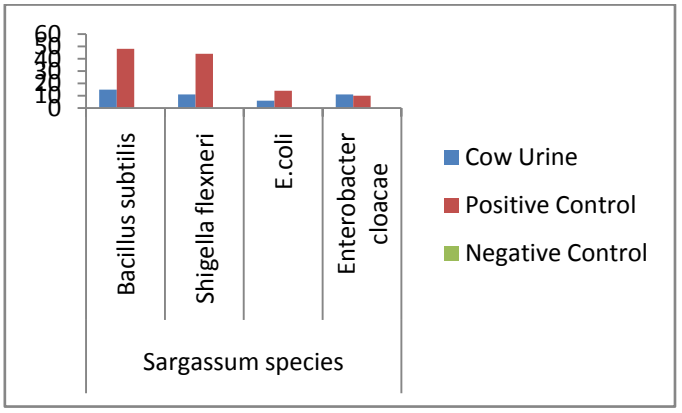


Figure 3: Comparative graphs of antifungal and antibacterial activity of cow urine extract of Sargassum species against selected

Bacteria and fungi

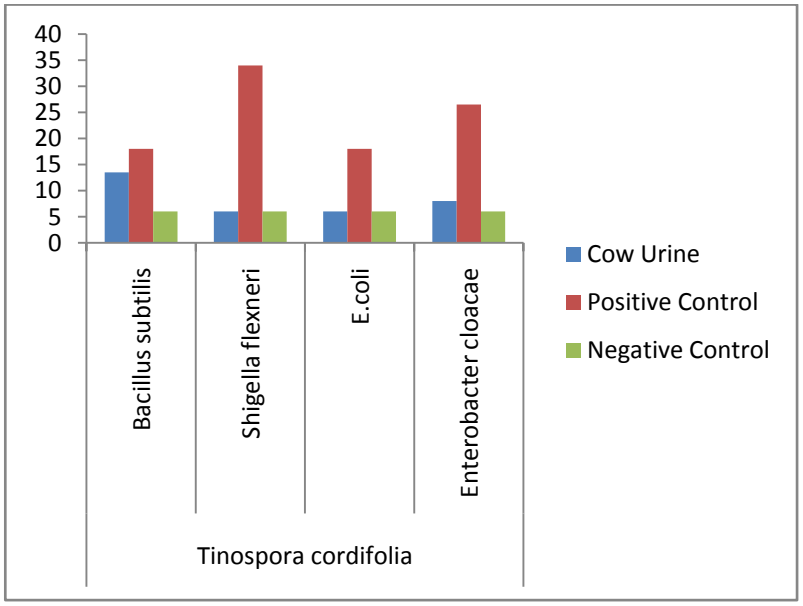


Figure 3: Comparative graphs of antifungal and antibacterial activity of cow urine extract of Tinospora cordifolia against selected bacteria and fungi

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