# Physicochemical Characterization, Phytochemical Analysis And In Vitro Biological Investigations Of Procris repens And Sargassum duplicatum

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**Abstract**

**This review paper describes the Physicochemical characterization, Phytochemical screening and in vitro biological activities of selected plants that is *Procris repens* and *Sargassum duplicatum*. *Procris repens* (Lour.) B.J.Conn & Hadiah belonging to the family *Urticaceae* commonly called as 'Trailing Watermelon Begonia' and 'Wavy Watermelon Begonia'. *Sargassum duplicatum* J.Ag. is one of the main seaweeds in the marine ecosystem that has been used as a source of medicine among brown seaweeds belonging to the family *Sargassaceae*. This review paper discusses in detail study of Physicochemical characterization, Phytochemical analysis and invitro anti microbial activity of selected plants. Physicochemical characterization discusses the Total ash value, Extractive value, Loss on drying, Crude fiber content and Foreign matter of selected plants.[1] Here the phytochemical analysis describe the Quantitative estimation of phytoconstituents and Thin layer chromatography[2]. This review paper discusses in detail study of in vitro antimicrobial which includes anti bacterial and antifungal activities of selected plants[3]. Here the antibacterial activity was conducted by agar cup plate method. Pharmacological studies shows significant anti bacterial and anti fungal activities in *Procris repens* and *Sargassum duplicatum*.[4]**

**Keywords: *Procris repens*, *Sargassum duplicatum*, Physicochemical, Phytochemical, Anti bacterial , Anti fungal.**

## Introduction

*Procris repens* belonging to family *Urticaceae* is commonly known as Trailing watermelon begonia[5]. Almost all the parts ie, stem, leaf, flower are used for different type of ailments.[6]


**Fig 1:** *Procris repens*

**Uses**

It is used by Malays for poulticing boils, swollen areas, and the abdomen when it is painful. A decoction from the plant is used for rheumatism. *Procris repens* (Lour.) B. J. Conn and Hadiah (*Urticaceae*) is traditionally used in folk medicine for the treatment of skin injuries and respiratory conditions. Whole plant of *Procris repens* was used to treat icterus, acute and chronic hepatitis, and allergic dermatitis in Chinese medicine.

***Sargassum duplicatum***

*Sargassum duplicatum*[7] belonging to the family *Sargassaceae*. Mainly found in Rameswaram, Tamil Nadu.

**Fig 2:** *Sargassum duplicatum*

**Uses**

- Antidiabetic
- Anticancer
- Anti- macrofouling agent
- cytotoxic
- Wound healing activities·

## Antimicrobial activity

Plant extract with medicinal value have been used to treat many diseases that can be either be bacterial, fungal or parasitic among many others[8]. The list of plants which exhibit antimicrobial activity ever increasing[9]. Eg: Neem (*Azadiracta indica*), Garlic (*Allium sativum*), Bardana (*Arctium lappa*), Large Indian Cress (*Tropaeo lummajus*), Cumin (*Cuminum ciminum*), Jacaranda (*Jacaranda mimosaefolia*), Eucalyptus (*Eucalyptus globules*), Grapefruit (*Citrus paradise*)[10].

**Materials and Methods**

**Physicochemical characterization**

1. **Ash values**

The ash of any organic material composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica)[11].

**a. Total ash**

Total ash is the measure of total amount of material produced after complete incineration of the ground drug at as low temperature as possible (about 450°C) to remove all carbons[12]. Total ash usually consists of carbonates, phosphates, silicates and silica which includes both,

Physiological ash – derived from the plant tissue itself[12]

Non-physiological ash – residue of the adhering material to the plant surface, e.g., sand and soil.

**Procedure**

Incinerated about 3 g accurately weighed powdered drug in tared platinum crucible at a temperature not exceeding 450° C until free from carbon. Cooled and weighed, repeated to get constant value[13]. Percentage total ash was calculated with reference to the air-dried drug.

**b. Acid-insoluble ash**

It is the ash obtained after extracting the total ash with Hydrochloric acid.

**Procedure**

The total ash obtained was boiled with 25 ml of 2 M HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper (Whatman- 41), washed with hot water[14]. Transferred the filter paper containing insoluble matter to the original crucible, ignited, weighed and calculated the percentage acid-insoluble ash with reference to the air-dried drug.

**c. Water soluble ash**

It is the part of total ash content which is soluble in water. It is a good indicator of either water-soluble salts in the drug or incorrect preparation[15]. It is the difference in weight between the total ash and the residue obtained after treatment of total ash with water.

**Procedure**

The total ash obtained was boiled for 5 minutes with 25 ml of water; collected the insoluble matter on an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug.

**d. Sulphated ash**

**Procedure**

Heated a platinum crucible to redness for 10 minutes, allowed to cool in a desiccator and weighed[16]. Put 3g accurately weighed powdered drug, into the crucible, ignited gently at first, until the substance is thoroughly charred. Cooled, moistened the residue with 1 ml Sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800 ± 25°C until all black particles have disappeared. The crucible was cooled, few drops of Sulphuric acid was added and again heated. The ignition was carried out as before, cooled and weighed to get a constant weight. Calculated the percentage of sulphated ash with reference to the air-dried drug.

**2. Extractive values**

It is the number of active constituents in a given amount of medicinal plant material when extracted with a particular solvent. The composition of phytoconstituents in that solvent depends upon the nature of the drug and solvent used[17]. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample; for example, in a drug where the extraction procedure for the constituents commence with water as solvent, any subsequent aqueous extraction on the re-dried residue will give a very low yield of soluble matter. Water soluble, alcohol soluble and ether soluble extractive values are an indicative of poor quality, adulteration with any unwanted materials or incorrect processing of the crude drug during the process of drying, storage etc.

**a. Alcohol soluble extractive**

Macerated 5 g of air-dried, coarsely powdered drug with 100 ml of 90% ethanol in a closed flask for 24 hours[18]. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered rapidly without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105°C, to constant weight. Calculated the percentage of alcohol soluble extractive with reference to air dried drug.

**b. Water soluble extractive**

Macerated 5 g of the air dried coarsely powdered drug with 100 ml of Chloroform water in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105°C, to constant weight. Calculated the percentage of water soluble extractive with reference to air dried drug.

**c. Ether soluble extractive**

Macerated 5 g of the air dried, coarsely powdered drug with 100 ml of Diethyl ether in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered, without any loss of solvent[19]. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105°C, to constant weight. Calculated the percentage ether soluble extractive with reference to air dried drug.

3. **Loss on drying**

It is the loss of weight expressed as percentage w/w. It determines the amount of volatile matter of any kind (including water) that can be driven off under the conditions specified.

**Procedure**

Accurately weighed about 1.5 g of fresh leaves of in a tarred porcelain dish and dried in an oven at 105°C to constant weight, cooled in desiccator and weighed[20]. From the difference in weights percentage loss on drying was calculated.

4. **Crude fibre**

Crude fibre consist of the material other than ash which is insoluble in water and cannot be digested by boiling with dilute acid or alkali. Excess of crude fibre indicate the adulteration with woody tissues.

**Procedure**

Accurately weighed about 2 g of powdered drug in beaker and added 50 ml of 10% v/v Nitric acid. Heated to boil with constant stirring for 30 minutes[21]. The mixture was then strained through fine cotton cloth and the residue was washed with hot water until free from acid. The residue was transferred to the beaker and added 50 ml 2.5% sodium hydroxide solution. Again, heated to boil with constant stirring for 30 minutes. Strained and washed with hot water as earlier. Transferred the residue in tarred crucible, dried, weighed and the percentage crude fibre content was calculated with reference to the air-dried drug.

**Phytochemical analysis**

The whole plant or organism serves as an active laboratory for the production of natural products from primary metabolites. Primary metabolites are the products of vital metabolic pathways such as respiratory chain, TCA cycle etc. Secondary metabolites are varieties of simple to sophisticated bizarre molecules[22]. They are fascinating chemical molecules, very useful and of great importance in nature, as well as highly diversified in structures, properties, uses, chemistry etc.

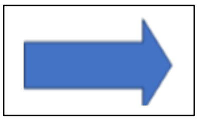
**Extraction**

The process of separating active principles from powdered crude drugs by using suitable solvents is called extraction. The choice of solvent depends upon the characteristics of the secondary metabolites like polarity, pH and thermal stability. Successive solvent extraction is suitable to extract the constituents of different polarity. It involves the extraction of the same plant material with solvents of different polarity ranging from non-polar to polar.

**Soxhlet extraction**

100 g of leaves of *Procris repens* and whole plant of *Sargassum duplicatum* were shade dried for seven days and size reduced by using a home mixer and extraction was carried out by continues hot extraction method using soxhlet apparatus with 1 liter of methanol in the round bottom flask and extraction was continued for 10 hr. The extract obtained was collected and concentrated by evaporator. The concentrated extract was weighed and stored.

**Fig 3:** Soxhlet extraction



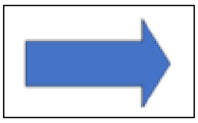


**Fig 4:** Methanolic extract of *Sargassum duplicatum*

**Fig 5:** Methanolic extract of *Procris repens*

**Quantitative estimation of phytoconstituents**

- **Estimation of Alkaloid**

**Procedure**

Stock solution: 100µl of test sample was pipetted out and the volume in each tube was made up to 1.0 ml with distilled water.

The 100µl of test sample from stock solution was dissolved in 1ml dimethyl sulphoxide (DMSO), added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5ml of bromocresol green solution and 5ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100µg) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470nm with an UV/Visible spectrophotometer.

- **Estimation of Glycosides**

**Procedure**

Stock solution: 100µl of test sample was pipetted out and the volume in each tube was made up to 1.0 ml with distilled water.

About 100µl of the extract from stock and 1ml of Baljet's reagent are taken and allowed to stand for one hour. Then dilute the solution with 2ml distilled water and mix. Read the intensity of the colour obtained against blank at 495nm using a spectrophotometer. Standard graph can be prepared using varying concentration of standard digitoxin (2-14µg/ml).

**Thin layer chromatography**

**Procedure**

TLC plates were prepared by using silica gel G and the coated plates were allowed to dry in air and activated by heating in hot air oven at 105°C for 1 hour[23]. The extracts were dissolved in respective solvents and applied in TLC plates by means of capillary tube, a few centimeters above from the edge of plate. The plates were then developed in TLC chamber previously saturated with different solvent systems. By trial and error method appropriate solvent system were developed and different spots developed were. The colored substances were visual on the chromatogram. Colorless components were detected using visualizing agent, UV light and spray reagents and $R_f$ values were calculated.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

## ANTI MICROBIAL ACTIVITY

- **ANTIBACTERIAL ACTIVITY**

➢ Using the agar cup plate method, the antibacterial activity of *Procris repens* and *Sargassum duplicatum* was assessed against *Staphylococcus aureus* , *Bacillus cereus* , *Escherichia coli* and *Klebsiella pneumonia* .
➢ After being prepared and sterilized, Mueller Hinton agar (MHA) was transferred into plates.
➢ 100 µl of the pure cultures of test strains were swabbed uniformly using a sterile swab on the surface of the MHA plate to obtain an even inoculum.
➢ The plates were allowed to dry for 5 minutes. Then, a hole with a diameter of 8 mm is punched aseptically with a sterile cork borer, and 100µL of the test samples at the desired concentration is introduced into the well[24].
➢ The antibacterial activity was observed after incubating the plates for 24 hours at 35±2℃ and the zone of inhibition surrounding the well was noted in mm.

- **ANTIFUNGAL ACTIVITY**

➢ Using the agar cup plate method, the antifungal activity of *Procris repens* and *Sargassum duplicatum* was assessed against *Aspergillus niger* and *Candida albicans*.
➢ After being prepared and sterilized, Sabouraud Dextrose Agar (SDA) was transferred into plates.
➢ 100 µl of the pure cultures of test strains were swabbed uniformly using a sterile swab on the surface of the Sabouraud Dextrose Agar [(SDA) plate to obtain an even inoculum.
➢ The plates were allowed to dry for 5 minutes.
➢ Then, a hole with a diameter of 8 mm is punched aseptically with a sterile cork borer, and 100µL of the test samples at the desired concentration is introduced into the well.
➢ The antibacterial activity was observed after incubating the plates for 24 hours at 35 ± 2℃ and the zone of inhibition surrounding the well was noted in mm[25].

**Result and Discussion**

**Determination of physicochemical constants**

| PARAMETER | *Procris repens* | *Sargassum duplicatum* |
|---|---|---|
| ASH VALUE<br>Total ash | 3.84 ± 0.0013 %w/w | 7.15 ± 0.0012 %w/w |
| Acid insoluble ash | 1.24 ± 0.0020 %w/w | 3.65 ± 0.0013 %w/w |
| Water soluble ash | 2.43 ± 0.0010 %w/w | 2.14 ± 0.0021 %w/w |
| Sulphated ash | 7.82 ± 0.0010 %w/w | 10.92 ± 0.0014 %w/w |
| EXTRACTIVE VALUE<br>Ether soluble extractive | 0.69 ± 0.0015 %w/w | 5.45 ± 0.0020 %w/w |
| Alcohol soluble extractive | 1.8 ± 0.020%w/w | 10.45 ± 0.001 %w/w |
| Water soluble extractive | 0.8 ± 0.0425 %w/w | 2.32 ±0.0012 %w/w |

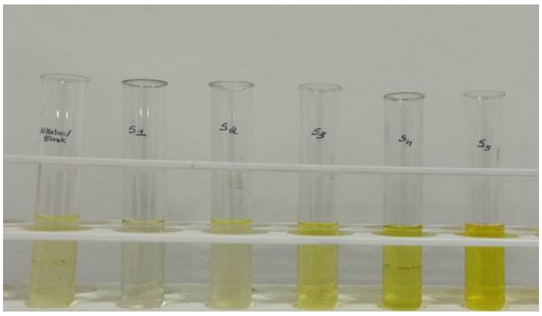
| | | |
|---|---|---|
| LOSS ON DRYING | 1.09 ± 0.0057 %w/w | 7.45 ± 0.0041 %w/w |
| CRUDE FIBER CONTENT | 12 ± 0.011%w/w | 10 ± 0.002 %w/w |
| FOREIGN MATTER | 0.8 %w/w | 0.4 %w/w |

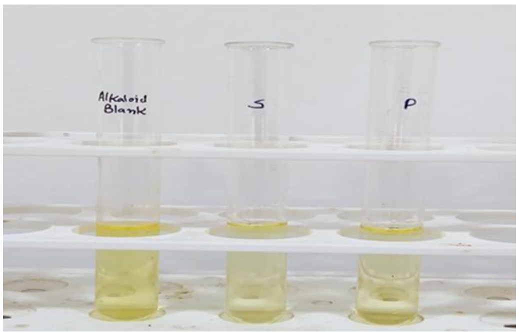
**Phytoconstituents Estimation**

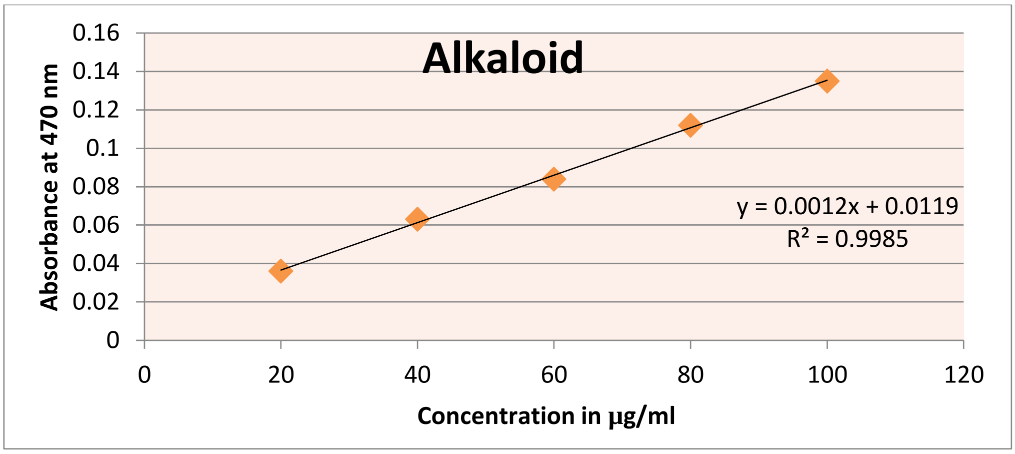
**A. <u>ESTIMATION OF ALKALOID</u> (GRAVIMETRIC METHOD)**

| Standard | Concentration of Atropine (µg/ml) | Absorbance at 470 nm |
|---|---|---|
| S1 | 20 | 0.036 |
| S2 | 40 | 0.063 |
| S3 | 60 | 0.084 |
| S4 | 80 | 0.112 |
| S5 | 100 | 0.135 |

| Sl. No: | Sample code | Absorbance at 470 nm | Concentration of alkaloid (µg/ml) |
|---|---|---|---|
| 1 | P | 0.062 | 40.59 |
| 2 | S | 0.105 | 75.36 |



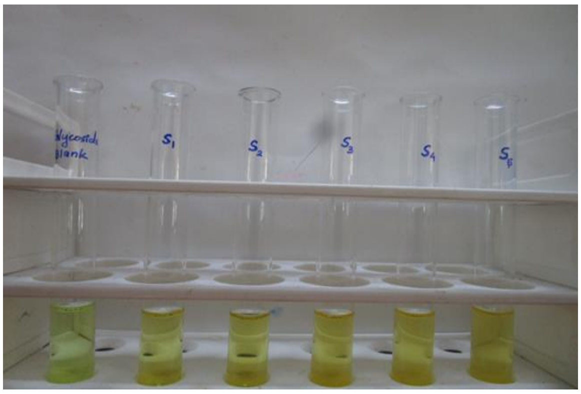



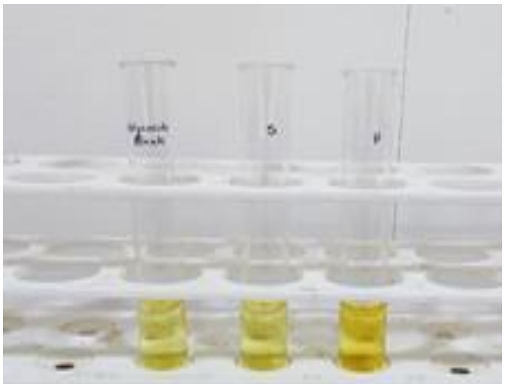


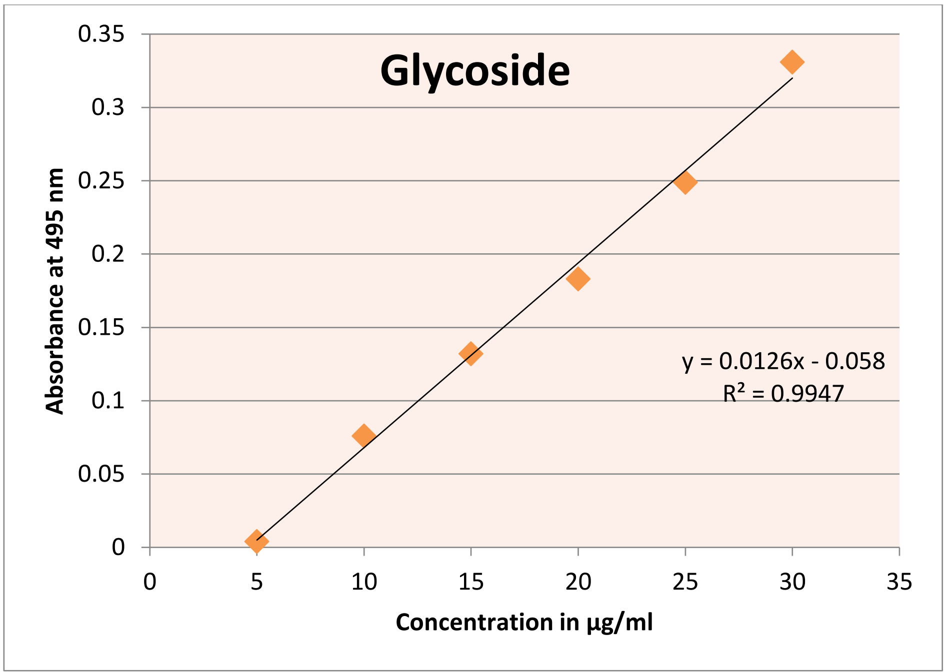

## B. ESTIMATION OF GLYCOSIDES (SPECTROPHOTOMETRIC METHOD)

| Standards | Concentration of Digitoxin (µg/ml) | Absorbance at 495 nm |
|---|---|---|
| S1 | 5 | 0.004 |
| S2 | 10 | 0.076 |
| S3 | 15 | 0.132 |
| S4 | 20 | 0.183 |
| S5 | 25 | 0.249 |
| S6 | 30 | 0.331 |

| Sl. No. | Sample code | Absorbance at 495 nm | Concentration of glycosides (µg/ml) |
|---|---|---|---|
| **1** | P | 0.099 | 103.41 |
| **2** | S | 0.019 | 52.85 |








**Thin layer chromatography**

**Table 1: Thin layer chromatography**

| Sl.No. | Sample | Mobile Phase – (Solvent System Used) | Proportion of Solvent system | Spraying Reagent | Observation (Visible light) | Observation (UV light) |
|---|---|---|---|---|---|---|
| **1.** | ***P. repens*** | **Butanol:Water: Acetic acid** | **8.4:1.4:1.7** | **10% Sulphuric acid** | **Yellow/Black band** | **Yellow Orange and blue fluorescent bands in UV** |
| **2.** | ***S. duplicatum*** | | | | **Yellow Black band** | |

| Sample | Solvent front (cm) | Solute front (cm) | Rf value |
|---|---|---|---|
| ***P.repens*** | **5.5** | **5.0** | **0.91** |
| ***S.duplicatum*** | **5.0** | **4.0** | **0.80** |

**Fig.6:TLC of *P.repens* before spraying**

**Fig.7:TLC of *P.repens* after spraying**

Fig.8:TLC of *S. duplicatum* before spraying

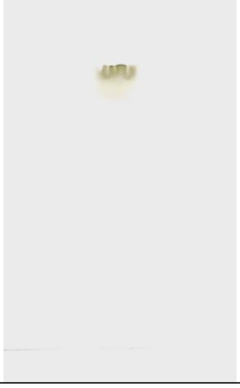


Fig.9:TLC of *S.duplicatum* after spraying

**ANTI MICROBIAL ACTIVITY**

In-vitro anti microbial activity of leaf extract of *Procris repens* plant and whole plant of *Sargassum duplicatum* by Agar cup plate method.

**Table 2: Zone of Inhibition in tested strains**

| Sample | Zone of inhibition ( mm in diameter ) | | | |
|---|---|---|---|---|
| | Staphylococcus aureus | Bacillus cereus | E coli | Klebsiella pneumonia |
| S | 13 ± 0.01 mm | 14 ± 0.12 mm | 15 ± 0.02 mm | 14 ± 0.04 mm |
| P | 9 ± 0.02 mm | 13 ± 0.01mm | 10 ± 0.31mm | 11 ± 0.01 mm |
| +CN | 20 ± 0.10 mm | 21 ± 0.12 mm | 20 ± 0.13 mm | 27 ± 0.04 mm |
| -CN | NA | NA | NA | NA |

**NA : No Activity, +CN : Positive Control (Streptomycin), -CN : Negative Control (Methanol)**

For standard: Values are expressed as Mean ± SEM, n = 3. The values were compared with control using Dunnets multiple comparison and P value obtained was less than 0.001.Therefore, considered as significant.
For methanolic extract: Values are expressed as Mean ± SEM, n = 3. The values were compared with control using Dunnets multiple comparison and P value obtained was less than 0.001. Therefore, considered as significant.


**Fig.10:*Staphylococcus aureus***


**Fig.11: *Escherichia coli***

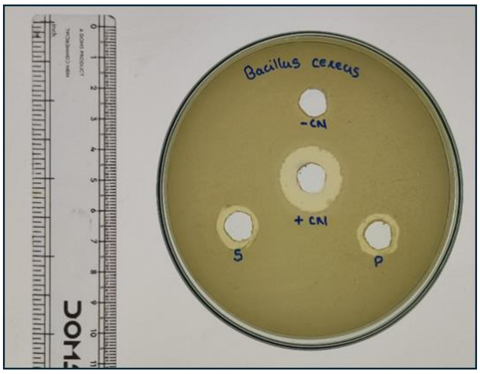



Fig.12:*Bacillus cereus*


Fig.13:*Klebsiella pneumonia*

**Table 3: Zone of Inhibition in tested strains**

| **Samples** | **Zone of inhibition ( mm in diameter )** | |
|---|---|---|
| | **Aspergillus niger** | **Candida albicans** |
| **S** | **12 ± 0.11mm** | **10 ± 0.14 mm** |
| **P** | **13 ± 0.03mm** | **10 ± 0.01 mm** |
| **+CN** | **15 ± 0.13 mm** | **21 ± 0.11mm** |
| **-CN** | **NA** | **NA** |

For standard: Values are expressed as Mean ± SEM, n = 3. The values were compared with control using Dunnets multiple comparison and P value obtained was less than 0.001.Therefore, considered as significant. For methanolic extract: Values are expressed as Mean ± SEM, n = 3. The values were compared with control using Dunnets multiple comparison and P value obtained was less than 0.001. Therefore, considered as significant.

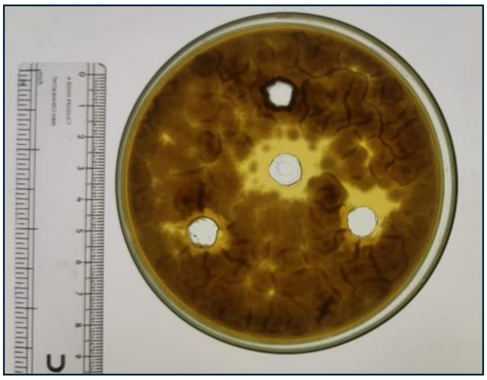


Fig.14:*Aspergillus niger*

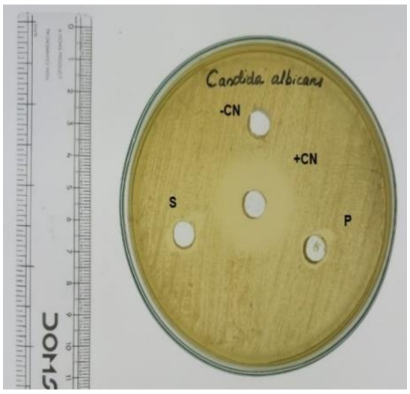



Fig.15:*Candida albicans*

**Conclusion**

In the present study, leaves of *Procris repens* and whole plant of *Sargassum duplicatum* were undertaken to prepare methanolic extract for scientific investigation to validate quality and quantity of phytochemicals. The standardization of the drug was conducted by determination of various physicochemical parameters like ash values, extractive value, crude fibre, loss on drying and these parameters help to develop standards and to determine purity and quality of the drug. Quantitative determination shows the presence of 40.59 µg/ml alkaloids and 103.41 µg/ml glycosides in *Procris repens* while *Sargassum duplicatum* contains 75.36 µg/ml alkaloids and 52.85 µg/ml glycosides. TLC of extract was conducted by trial and error method and suitable solvent system was developed. Rf values of the separated spots were calculated. Anti microbial study by Agar cup plate method revealed that both the plant extract has significant anti bacterial activity against Staphylococcs aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumonia and antifungal activity against Aspergillus niger, Candida albicans. *Procris repens* and *Sargassum duplicatum* is used ethnobotanically, but not much used in natural practices, so it is necessary to standardize this highly usable drug and to incorporate into Indian system of medicine.

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