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Antimicrobial Activity of Senna Plant Extract

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ABSTRACT: Senna (botanical name Cassia augustifolia) is a plant best known for its medicinal properties. It comprises of dianthrone glycosides (compounds consisting of sugar molecules bound to other molecules), as well as mucilage (a thick, gluey substance), tannins and flavonoids. In addition to its use as a safe and effective laxative, senna also has many other health benefits. One of the glycosides present in senna, emodin has many therapeutic benefits including as an anti-inflammatory, antispasmodic, and the ability to inhibit or destroy viruses. The extracts of Cassia augustifolia showed anti-microbial activity. Different extracts (ethanol, methanol, petroleum ether and aqueous solutions) of Cassia augustifolia plant are extracting out. Antimicrobial efficacy of various extracts was assessed by disc diffusion method against Gram positive bacteria Staphylococcus aureus, Gram Negative-Escherichia coli and Pseudomonas aeruginosa and Fungi-Aspergillus niger, Aspergillus flavus, Fusarium oxisporum and Rhizopusstolonifer. Phytochemical screening of the extract showed the presence of alkaloids, flavonoids, carbohydrates, proteins, tannins and triterpenoids in cassia augustifolia. Senna act as anti-fungal agent and act against D.N.A of E. Coli bacterium. Sennosides affect the intestinal tract and induce diarrhea. It has shown that senna produces DNA lesions in Escherichia coli cultures and can act as an antifungal agent.

Keywords: Senna, Escherichia coli, cultures, Salmonella sp, antimicrobial, SCDA.

I. INTRODUCTION:

herbal or traditional medicines is been in the practice for Many centuries. In most of the earlier centuries, plants are the main medicinal source to treat various type of disease. Approximately 20% of the plant found in the world have been Submitted for pharmacological or biological testing or for particular medicinal use. Antibiotics are introduced into the markets based on plants which are obtained from Natural or synthetic sources. Senna flowering plant commonly called as tinivelly Senna or alaxandrian Senna belongs to the family leguminaseae In India senna are cultivated in around 25000 hectare of land producing about 22500 tonnes of leaves and 7500 tonnes of fruits per annum. Senna cultivated in well ploughed, level, rich clayed semi-irrigated land. Pulverization of the soil carried out by the use of plough. First sowing is done in February- March while second in October to November. Before planting prepare the land by ploughing, harrowing and bring the soil to a fine tilt hand apply Benzene hexachloride (10%) or Aldrin (5%) at 25kg/hectare with last operation during land preparation which protects the young seedling from the attack of white ants and worms.

II. MATERIAL AND METHODS:

Collection of plants: The hydro alcoholic extract of Cassia augustifolia were purchased from Amsar Private Ltd. located in No.47 Laxmibai Nagar, Industrial Estate, Fort, Indore – 452006.

ANTIMICROBIAL ACTIVITY OF EXTRACT:

Sample Preparation:

All samples should be collected in 10 ml or 10 grams' portions.

Water soluble products: Dissolve or dilute 10g or 10 ml of product to be examined in 100ml or 90 ml of Soyabean Casein Digest Medium (SCDM).

Total Combined Yeast and Mould Count (TYMC):

- Pipette out I ml of the above treated/pre-enriched sample into two separate sterile Petri plates

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about Q to 10 cm in diameter, for total combined yeast and mould count.

- Pour about 15-20 ml. of Sabouraud Dextrose Agar in to each of above inoculated Petri plates at not more than 45°C.
- Cover the Petri plates properly, mix the sample with the agar by tilting or rotating the dishes and allow the contents to solidify at room temperature. Incubate the Petri plates at 20-25°C for 5 to 7 days.

INTERPRETATION OF RESULTS:

- **For Total Aerobic Microbial count:**

CFU / g or ml = Average CFU obtained on SCDA plates x 10 (Dilution factor)

- **For Total combined yeast and mould count:**

CFU / g or ml = Average CFU obtained on SDA plates x 10 (Dilution factor)

If no microbial colonies observed from the incubated petri plates express the results as < 10 CFU / g or per ml of sample.

TEST FOR SPECIFIED MICRO-ORGANISMS:

1. Escherichia coli

- **Sample preparation:**

Prepare a 1 in 10 dilution of the sample using Soybean Casein Digest Medium or Buffered Sodium Chloride Peptone Solution (pH 7) as a diluent. Transfer 10 ml or the quantity corresponding to 1 gm or 1 ml of the sample to Soyabean Casein Digest Medium. Mix and incubate at 30°C-35°C for 18-24 hours.

- **Test:**

After completion of incubation transfer 1 mL of above medium to bottles containing 100ml, MacConkey broth and incubate at 42°C – 44°C for 24 to 48 hours. After incubation. Subculture onto plates of MacConkey agar from the above MacConkey broth bottle. Incubate MacConkey agar plate at 30- 35°C for 18-72 hours. Upon incubation. If characteristic colonies observed on MacConkey agar, this indicates the possible presence of E. coli Identify the presence of Gram-negative rods by Gram's staining. It shall be confirmed by biochemical identification tests.

- **Confirmatory test (Biochemical identification test):**

Transfer well isolated suspected colonies from MacConkey agar plat. To 5 mL of Peptone or Tryptone water contained in a test tube. Incubate at 37°C e 2°C for 24 – 48 hours. After incubation, add 0.5 mL of Kovac's reagent into each tube, shake well and allow standing for 10 minutes. If any red color is observed in the reagent layer, lndole is present. This confirms the possible presence of E. coli.

2. Pseudomonas aeruginosa.

- **Sample preparation:**

Prepare a 1 in 10 dilution of the sample using Soyabean Casein Digest Medium as a diluent.

Transfer 10 ml or the quantity corresponding to 1 gm or 1 ml of the sample to Soyabean Casein Digest Medium. Mix and incubate at 30°C-35°C for 18-24 hour.

- **Test:**

After completion of incubation, subculture onto the surface of Cetrimide Agar Plate with sterile loop. Incubate the plate at 30°C-35°C for 18-72 hour. After completion of incubation period, if characteristics colonies observed on Cetrimide agar, identify the presence of Gram- negative rods by Gram's staining. Further, confirmation is sought by confimatory test.

- **Confirmatory test:**

Streaking the suspect colonies from Xylose Lysine Deoxycholate agar plates on Triple Sugar Iron Agar butt-slant tube. Carry out first streaking on the surface of slant, and then carry out stabbing with wire well beneath the surface (in the butt portion). Incubate the tubes at 30-35°C for 24 hours. After completion of incubation period, a tube showing alkaline (red) stain and acidic (yellow) butt (with or without concomitant blackening of the butt due to production of hydrogen sulphide) further indicates the presences of Salmonella species.

3. Staphylococcus aureus:

- **Sample Preparation:**

Prepare a 1 in 10 dilution of the sample using Soyabean Casein Digest medium or buffered Sodium Chloride Peptone Solution pH 7 as a diluent. Transfer 10 ml or the quantity corresponding to 1 gm or 1 ml of the sample to soyabean casein digest medium. Mix and incubate at 300c – 350c for 18- 24 hours.

- **Test:**

After completion of incubation, subculture onto the surface of Mannitol Salt agar plate with the sterile loop. Agar plate with the sterile loop, Incubate the plate at 300c – 350c for 18- 72 hours. After completion of incubation period, if characteristic colonies observed on Mannitol Salt agar, identify the presence of gram- positive cocci by Gram's staining. Further confirmation is sought by confirmation test.

- **Confirmatory test:**

Transfer representative suspected colonies from surfaces of Mannitol Salt agar to individual tubes, each containing 0.5ml of plasma with or without suitable additives, incubate in a water bath at 370c, examining the tube after 3 hours and subsequently at suitable intervals upto a 24 hours. Run a negative control (only 0.5ml of plasma) for test. Positive control should not show coagulation even after 24 hours of incubation.

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4. Salmonella sp.

- **Sample preparation:**

Prepare a 1 in 10 dilution of the sample using Soyabean Casein Digest Medium as a diluent. Mix and incubate at 30°C-35°C for 18-24 hour,

- **Test:**

Transfer 0.1 mL of above medium into test tubes containing 10 mL Rappaport Vassiliadis Salmonella Enrichment Broth. Incubate the tube at 30-35°C, for 18-24 hours. After completion of incubation period, subcultures on Xylose Lysine Deoxycholate agar plates and incubate the plates in an inverted position at 30-35°C for 18-48 hours. After completion of incubation period, if characteristic colonies observed on XLD agar, identify the presence of Gram-negative rods by Gram's staining. Further confirmation is sought by confirmatory test.

- **Confirmatory test:**

Streaking the suspect colonies from Xylose Lysine Deoxycholate agar plates on Triple Sugar Iron Agar butt-slant tube. Carry out first streaking on the surface of slant, and then carry out stabbing with wire well beneath the surface (in the butt portion). Incubate the tubes at 30-35°C for 24 hours. After completion of incubation period, a tube showing alkaline (red) slain and acidic (yellow) butt (with or without concomitant blackening of the butt due to production of hydrogen sulphide) further indicates the presences of Salmonella species.

III. RESULTS:

This study was carried out on Senna extract to evaluate the anti microbial and anti fungal activity. The successive extract was test against bacteria (Escherichia coli , Pseudomonas aeruginosa , Staphylococcus aureus, Salmonella sp.). Among the test the results were obtained as follows:

1. Escherichia coli

❖ **Interpretation of the results:**

Upon incubation of MacConkey agar, if brick red colored colonies surrounded by a zone of precipitated bile are observed, this indicates the possible presence of E. coli.

❖ **Conclusion:**

The product complies with the test if no colonies are present or if the identification tests arc negative.

2. **Pseudomonas aeruginosa.**

❖ **Interpretation of results:**

Upon incubation of Xylose Lysine Deoxycholate Ago plates, if red color colonies with or without bunk center develops on the media, it indicates the presence of species.

❖ **Conclusion:**

The product complies with the test if no colonies are present or if the identification tests are Negative.

3. Staphylococcus aureus:

❖ **Interpretation of result:**

Upon incubation of Mannitol Salt Agar, if yellowish or white coloured colonies surrounded by yellow zone develops on the media, it indicates the presence of Staphylococcus aureus.

❖ **Conclusion:**

The product complies with the test if no colonies are present or if the identification tests are Negative.

4. Salmonella sp.

❖ **Interpretation of results:**

Upon incubation of Xylose Lysine Deoxycholate Agar plates, if red color colonies with or without bunk center develops on the media, it indicates the presence of species.

Total aerobic microbial count:

average CFU obtained on SCDA plates x 10 (dilution factor) / 2
= 8 x 10
= 40cfu/g

Total combined yeast and mould count:

average CFU obtained on SCDA plates x 10 (D F) / 2
= 6 x 10
= 30cfu/g

IV. CONCLUSION:

This study has found the importance of natural products to control bacteria , that effects human life. The microbial activity is identified by using different species and the results were noted. Conclusion, this study revealed that Senna leaves extracts possess medicinal properties and antibacterial activity that inhibit bacterial growth. The results of the present study show that S. siamea leaves extracts are effective against all tested bacteria tested. The antibacterial activities of the extracts are expected perhaps due to the present of bioactive compounds like alkaloid, terpenoid, saponin, tannin, flavonoids and anthraquinones which were dissolved in the solvents. The results of present study have provided the justification for therapeutic potential of S. siamea leaves and also used as medicinal plant.

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