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Evaluation of antibacterial property of anthyocyanin extracted from *brassica oleracea* against gram positive and gram negative bacteria by using erythromycin as a standard drug.

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Abstract

Anthocyanin was extracted from Brassica oleracea and identified by UV and TLC.The benzene test initially helps us to identify Anthocyanin in residue. Crystals were purified by recrystallization from ether. Acetone –petroleum ether extraction resulted in higher crude Anthocyanin yield than hexane extraction The compounds were tested against two microorganisms namely E. coli (gram-ve) and bacillus (gram+ve) and the compounds showed significant activity against two species. Test sample one Showed significant activity with zone of inhibition (18.21mm). When compared to standard Erythromycin with zone of inhibition (20.83mm) at 100,200,400mg/ml against E. coli organism. Test sample two Showed significant activity with zone of inhibition (15.56mm) when compared to standard Erythromycin with zone of inhibition at (100,200,400mg/ml) against Bacillus.

Keywords: Brassica Oleracea, Anthocyanin, Erythromycin, Bacillus.

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Introduction

Brassica oleracea is a plant species that includes many common foods as cultivars, including cabbage, broccoli, cauliflower, kale, collard greens, savoy, kohlrabi and gailan. In its uncultivated form, it is called "**wild cabbage**," and is native to coastal southern and western Europe. Its high tolerance for salt and lime, and its intolerance of competition from other plants typically restrict its natural occurrence to limestone sea cliffs, like the chalk cliffs on both sides of the English Channel,1 and the windswept coast on the western side of the Isle of Wight.

Wild *B. oleracea* is a tall biennial plant that forms a stout rosette of large leaves in the first year. The leaves are fleshier and thicker than other *Brassica* species—an adaptation that helps it store water and nutrients in its difficult growing environment. In its second year, it uses the stored nutrients to produce a flower spike 1 to 2 metres (3–7 ft.) tall with numerous yellow flowers.

Materials and Methods

Collection of plant material

The plant material was collected from the local market, Nacharam, Medchal, Telangana.

Extraction:

Weigh 250gms of Brassica oleracea paste in a first add 35 ml of ethanol and heat for 5min. Filter with filter and press it off all the filtrate. Keep it aside to the filter cake

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add 70 ml of benzene and start condensation or keep it in separating funnel and shake it. Thus separate the supernant and add it to the first filtrate. Repeat the step for 3 times and collect the filtrate separating funnel and add 100ml of saturated Nacl solution. Shake gently and allow it to separate it for 2 layers. Add few drops of anhydrous Na2So4 and allow to stand for 15min.Filter and keep the filtrate in dark bottle away from light. Otherwise the color of Anthocynin will disappear **[2-3].**

Chemicals and apparatus

Hexane, ethyl acetate, ethanol, methanol, electric mixer and rotary vacuum evaporator.

Microorganisms tested

Microorganisms studied in this project are provided from NNRG Institute. Which include Staphylococcus aureus, Escherichia coli, Lactobacilli acidophilus, and streptococci.

Preliminary phytochemical analysis

Preliminary phytochemical screening, the Ethanolic extract was tested for carbohydrates, alkaloids, glycosides, sterols, phenolic compounds, tannins, Flavonoids, saponins, proteins and amino acids using standard procedure. The Ethanolic extract was subjected to preliminary phytochemical screening **[4-6].**

Test for Alkaloids:

The Ethanolic extract was treated with diluted HCl acid and filtered. The filtrate was treated with various Alkaloidal agents **[7-8].**

Mayer's Test: Sample was treated with Mayer's reagent; appearance of cream colour indicates the presence of alkaloids.

Dragendroff's Test: Sample was treated with Dragendroff's reagent; appearance of reddish brown precipitate indicates the presence of alkaloids.

Hager's Test: Sample was treated with Hager's reagent; appearance of yellow colour indicates the presence of alkaloids.

Wager's Test: Sample was treated with Wager's reagent; appearance of brown precipitate indicates the presence of alkaloids.

Test for Carbohydrates:

The Ethanolic extract was treated with 3ml of α-naphthaol in alcohol and Conc.H2so4 was carefully added to side of the test tube. Formation of a violet ring at the junction of two liquids indicates presence of carbohydrates **[9].**

Fehling's Test: To the sample Fehling's A and B were added and heated for 2mins appearance of reddish brown colour indicates presence of reducing sugars.

Benedict's Test: To the sample Benedict's was added and heated appearance of reddish orange precipitate indicates presence of reducing sugar.

Barfoed's Test: The sample was treated with Barfoed's reagent and heated appearance of reddish orange precipitate indicates the presence of reducing sugar [10-11].

Test for Proteins

Biuret's Test: To the Ethanolic extract, copper sulphate solution followed by sodium hydroxide was added; a violet colour precipitate indicates the presence of proteins.

Million's Test: To the Ethanolic extract, Million's reagent was added; appearance of pink colour indicates presence of proteins. 12.

Test for Steroids:

LibermannBruchard's Test:

The Ethanolic extract was treated with Conc.H2SO4 and glacial acetic acid followed by acetic anhydride, a violet ring appears at the junction of the liquids appearance of green colour in the aqueous layer indicates the presence of steroids [13].

Test for Phenols:

The Ethanolic extracts were treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenols.

The Ethanolic extract was treated with 10% sodium chloride solution; appearance of cream colour indicates presence of phenol [14-18].

Test for Tannins:

The Ethanolic extract was treated with 19% lead acetate solution appearance of white precipitate indicates the presence of tannins.

The Ethanolic extract was treated with aqueous bromine water; appearance of white precipitate indicates the presence of tannins **[19].**

Test for Flavonoids:

5ml of the Ethanolic extract solution was hydrolysed with 10% sulphuric acid and cooled. It was then extracted with diethyl ether and divided into 3 portions in three separate test tubes. One ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide and 1ml of diluted ammonia solutions were added to the first, second and third test tubes respectively. Development of yellow colour in each test tube indicates the presence of Flavonoids **[20].**

Shinoda's Test:

The Ethanolic extract was dissolved in alcohol, to which a piece of magnesium followed by drop wise addition of

Conc. HCl and heated. Appearance of magenta colour indicates the presence of Flavonoids **[21,22].**

Test for Saponins:

Foam test:

One ml of the Ethanolic extract was diluted to 20 ml with distilled water; formation of foam in the upper part of the test tubes indicates the presence of saponins.

Test for Terpenes:

The Ethanolic extract was treated with tin and thionyl chloride; appearance of pink colour indicates the presence of terpenes.

Test for Amino acids:

Ninhydrin test: Two drops of ninhydrin solution were added to the extract, a characteristic purple colour indicates the presence of amino acids **[23].**

Test for glycosides

The extract was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added and made into a paste, warmed gently over water bath. The presence of glycosides was identified by dark green colour **[24].**

Preparation of inoculums

Inoculum was prepared by using isolated bacterial colonies. The bacterial colonies were inoculated in nutrient broth. All bacterial cultures were maintained by weekly transferring into nutrient broth and storing in sterile test tubes at low temperature **[24].**

Inoculation of plates

Muller Hinton agar plates were used. Muller Hinton agar was prepared and autoclaving at 121°C for 15 minutes was done. The medium was poured in sterile Petri plates under aseptic conditions. Then allowed the media to solidify at room temperature and stored at 4°C until use. Inoculation of plates was done by the modified method of Acar and Goldstein using flood-inoculation technique. A small single well isolated colony was emulsified in 2ml sterile saline in test tubes and the turbidity of the bacterial suspension was adjusted equivalent to 0.5 Mc Farland and 2 ml of this was transferred onto the Muller Hinton Agar plate and distributed gently over surface of medium with sterile glass spreaders to obtain uniform inoculums polysorbate 80 (0.05 %) was added to the agar base. The plates were dried for 5 minutes **[25].**

Determination of antimicrobial activity

Agar Disc diffusion method was used for screening of antimicrobial activity of Prickly pear extract **[26]**. The sterile filter paper discs of diameter 6 mm were impregnated with the test material (20 µL of Akkalkara extract) and aseptically placed on the inoculated plates. The plates were left at ambient temperature for 30 minutes to allow exceed pre diffusion prior to incubation at 37 C for 24h. The broad spectrum antibiotics i.e. Ampicillin was used as positive control for obtaining comparative results. Plates were observed after 24 - 48 h incubation for appearance of zones of inhibition around the discs. Antibacterial activity was evaluated by measuring diameter of zones of inhibition (in millimetres) of bacterial growth.

Determination of Minimum inhibitory concentration (MIC)

To determine Minimum Inhibitory Concentration (MIC), Two-fold dilution series (40, 20,10,5,2.5and1.25 mg/ml) in the solvent of Ethanol were prepared and bio assayed in well diffusion agar assay as mentioned above. Theresulting solutions were vortexed at high speed until completely dispersed, immediately poured into sterile Petri plates then allowed to set for 30 minutes **[25]**. The plates were then inoculated with the samples of Staphylococcus aureus, Escherichia coli, Lactobacilli acidophilus, for 24 h at 37C. Following the incubation period, the plates were observed and recorded for the presence or absence of growth. From the results, the MIC was recorded as the lowest concentration of test substance where the absence of growth was observed **[25, 26]**.

Results and Discussion

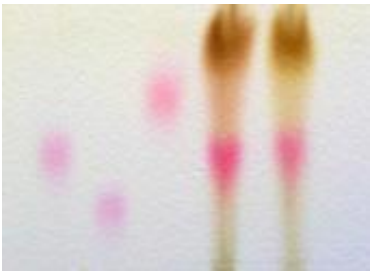
Identification of Anthyocyanin

Thin Layer Chromatography (TLC) Analysis Sample Details: lycopene Adsorbent: Precoated Silica Gel Solvent System: pet ether; acetone (40: 10)

Sample Preparation: lycopene (1 mg) was dissolved in n hexane (1 ml) and this solution was applied on the TLC plate with the aid of capillary tube.

Detection: Saturated Iodine Chamber.

Procedure: The lycopene was subjected on to the Precoated and activated Silica gel TLC plates. (Plates were kept in oven for 1hr at 70°C). The mobile phase is pet- ether: acetone ratios. After the TLC run and spraying the detecting agent reddish spots of lycopene were identified visually. Rf value was calculated. Rf-value =0.40



b. **Ultra Violet- Spectrophotometer Analysis**: The 0.01% w/v solution of Anthyocyanin in methanol was prepared and λmax was determined.

Antibacterial activity: Antibacterial activity was studied against Gram negative bacteria pseudomonas aeruginosa (MTCC- 3541) Procedure: The in vitro antibacterial activity of the methanol benzene and acetone extracts of lycopene was carried out by agar dilution method.

In first plate we took LB[1.5gm] +agar[0.5gm] in 25ml distilled water. After autoclave spreading of Pseudomonas aeruginosa (MTCC-3541)was done and results were noted down next day

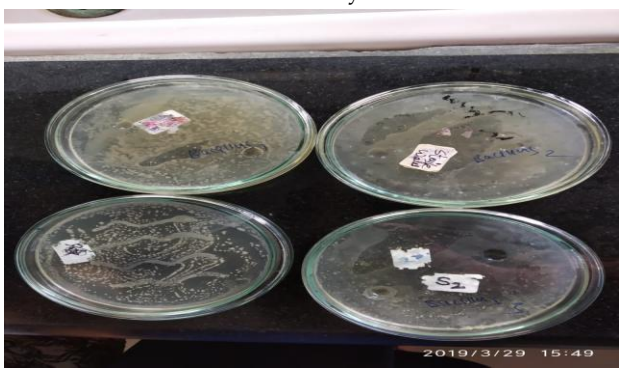


Fig: 3

In second plate we took the LB[1.5gm] agar+[0.5gm] in 25ml distill water and + lycopene(2.5ml). After autoclave, spreading of bacteria pseudomonas aeruginosa (MTCC-3541), results were noted down next day(fig. b).

Results

The compounds were tested against two microorganisms namely E. coli (gram-ve) and bacillus(gram+ve) and the compounds showed significant activity against two species.

Erythromycin was used as standard drug.

Test sample-1: Showed significant activity with zone of inhibition (18.21mm). When compared to standard Erythromycin with zone of inhibition(20.83mm)at 100,200,400mg/ml against E. coli organism.

Test sample-2:Showed significant activity with zone of inhibition (15.56mm)when compared to standard Erythromycin with zone of inhibition at (100,200,400mg/ml)against Bacillus.

Conclusion

Anthocyanin was extracted from Brassica oleracea and identified by UV and TLC. The benzene test initially helps us to identify Anthocyanin in residue. Crystals were purified by recrystallization from ether. Acetone – petroleum ether extraction resulted in higher crude Anthocyanin yield than hexane extraction. The study showed that extracted Anthocyanin showed antimicrobial activity against.

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