International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Evaluation of Antioxident and Antifungal Activity of Caesalpinia Bonducella Root Extract

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Abstract

Medicinal plants are a vital source of medications globally, offering low-cost treatments for various ailments. The seeds of *Caesalpinia bonducella* have been traditionally utilized to treat conditions such as leprosy, fever, edema, colic, malaria, and stomach pain. This study aimed to investigate the polar phytoconstituents and assess the antioxidant and antifungal activities of *Caesalpinia bonducella* root extract using solvent ethanol through the Soxhlet extraction method, potentially providing a natural alternative to synthetic antibiotics. Root of *Caesalpinia bonducella* was collected, authenticated, dried, and extract with ethanol, followed by evaluating the antioxidant properties using a DPPH assay method, the extract was mixed with DPPH solution and incubated in darkness. Absorbance was measured at 520 nm, with ascorbic acid as the standard for comparison. Antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* was assessed using microbiological assays. The ethanolic extract showed antioxidant scavenging activity of 87.83 µg/ml compared to ascorbic acid's 93.55 µg/ml. The extract was rich in secondary metabolites, including flavonoids, phenolic compounds, and alkaloids, which contributed to its antioxidant activity by effectively reducing DPPH radicals. The antifungal tests indicated notable efficacy against the tested fungi, the *Caesalpinia bonducella* extract showed good antifungal activity further supporting the potential medicinal application of this plant.

The current study concluded the *Caesalpinia bonducella* extract significant effect of antioxidant and antifungal property

KEYWORDS: *Caesalpinia bonducella*, Antioxidant, Antifungal.

Introduction

The thorny shrub *Caesalpinia bonducella* (L.) Flem. Fever nut, also known as *bonduc* nut (Family: *Caesalpiniaceae*) and sometimes called Nata Karanja. It's a key medication in traditional medicinal practices including Ayurveda, Siddha, Unani, and homoeopathy and is found in the hotter regions of India, Myanmar, and Sri Lanka[1,2]. Because the plant has therapeutic values in all parts, it is a very useful medicinal plant that is used in conventional medicine. The vegetation has been claimed to have antinociceptive, anti-inflammatory, antimalarial, antimicrobial, antipyretic, analgesic, antibacterial, antispasmodic, antioxidant, antiproliferative, antitumor, larvicidal, muscle contractile, hepatoprotective, anticonvulsant, and ant filarial properties.

Free radicles are highly reactive molecule that can cause significant cellular injury by damaging lipids, proteins, and the DNA. This oxidative stress occurs when there is a imbalance between free radicles and

antioxidants in the body leading to cellular dysfunction and contributing to various diseases, including cancer, CVD and neurodegenerative diseases. The mechanism of action of free radicles includes lipid peroxidation, which disrupts cell membranes and mutations in DNA that can lead to cell death.

Antioxidants play a crucial role in neutralizing free radicals, which are unstable molecules that can cause cellular damage, potentially leading to various diseases and aging processes. *Caesalpinia bonducella*, a medicinal plant known for its pharmacological properties, contains bioactive compounds with potential antioxidant activity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used to evaluate the free radical scavenging ability of plant extracts and other compounds due to its simplicity, speed, and reproducibility.

Globally, infectious illnesses are a major cause of morbidity and mortality and a serious threat to public health, which are caused by a diverse array of pathogenic fungi, bacteria and others. Public health is still seriously threatened by infectious diseases brought on by microbes like fungus, even with the tremendous advancements in human medicine.[3] Pathogenic fungal infections are becoming more widely acknowledged as a growing concern to public health.[4] The prevalence of potentially fatal systemic fungal infections has sharply increased throughout the past 20 years. Creating efficient treatment plans for fungal infections like candidiasis has proven to be difficult.[5]

Historically, the arsenal of antifungal drugs has been relatively limited compared to antibacterial agents. Current antifungal therapies mainly include azoles, echinocandins, and polyenes, each with its own set of limitations and challenges, such as toxicity, narrow spectrum, and the emergence of resistant strains. As a result, there is a critical demand for innovative approaches that not only combat existing fungal infections but also anticipate and address the evolving landscape of fungal resistance.[5]

The roots are regarded as febrifuge and anthelmintic in Madagascar; they are much utilized as an astringent in cases of blennorrhoea and leucorrhoea. In fever a root decoction is recommended in Guinea. The root bark has antitumor and placental removal properties. And it has several characteristics, such as anthelmintic and febrifuge, etc. It is applied locally to lesions and used as a rubefacient in Jamaica. In situations of hernia, the powdered bark consumed with honey is used. The roots are eaten in cases of hernia and used in Himachal Pradesh for diabetes and intermittent fever[6]

The primary goal of this work is to scrutinize the root bark of *Caesalpinia bonducella* using microscopic, and phytochemical examination. *Bonducella (L.) Flem*, which could be utilized to correctly identify this medication.

Review of Literature

Caesalpinia bonduc (Linn.) Roxb., commonly known as fever nut, is a medicinal plant widely used in traditional systems of medicine, including Ayurveda and Unani. It is a thorny shrub found in tropical regions, recognized for its therapeutic properties. The seeds of this plant have been used in the treatment of a variety of ailments, such as fevers, diabetes, asthma, and gastrointestinal disorders. The plant's traditional use as an antipyretic and anti-inflammatory agent has gained attention, leading to studies that validate its role in modern medicine[7]

The leaves of the shrub *C. bonducella* are big, branching, and measure between 30 and 60 centimetres in length. The petioles on the dorsal side of the leaf are prickly. Six to eight sets of pinnae with a few stipulary spines can be found near the base of the leaf, along with reduced pinnae with an elongated mucronate point.[8,9]

The hard-coated seeds exhibit a greenish or grey hue and a slight compression on one side due to the close-

knit pressing of adjacent seeds. The seeds are spherical, black, and have vertical crack marks on them. The testa, which consists of three layers and is between one and two millimetres thick, is found to be separated from the kernels of dried seeds It displays a closed hilum and micropyle. Hilum typically has a pale portion remaining to the funicle, surrounded by a dark area. Micropyle is close to a dark district's boundaries. It has a seed coat that is generally dim pale blue in nature and dim greenish to greyish

The stem of *Caesalpinia bonducella* is woody, slender, and covered with sharp, recurved spines. It is green when young and turns brown and tough as it matures. The plant is a scrambling shrub, often using its thorny stems to climb and support itself on nearby vegetation. These spines help protect the plant from herbivores and also assist in anchoring the plant in its natural environment.[10]

Caesalpinia bonducella has a well-developed taproot system that penetrates deep into the soil, allowing the plant to survive in arid conditions. The roots are fibrous and strong, providing stability to the plant, especially in sandy or loose soils. Traditionally, the root has been used in various medicinal preparations for treating fevers and digestive issues due to its reputed tonic and purgative properties.[11]

Phytoconstituents

- **Leaves**: Pinitol, glucose, calcium, brazzillin
- **Bark**: Homoisoflavonoids, 6-Omethylcaesalpinianone, and caesalpinianone
- **Seed Kernel**: Phytosterols- sitosterol, heptocosane noncrystalline, bitter glycoside bonducin ,natural saponins
- **Root**: Cassane furanoditerpene, caesalpinin, bonducellpins A, B, C, D, and diosgenin
- **Seed**: Neutral saponin, terpenoids, caesalpin, β-caesalpin and α- caesalpin

Traditional and Modern Uses:

Indigenous people all around the world have linked *C. bonducella* to a variety of illnesses. The seeds have been utilized as a styptic and to address ailments colic discomfort, helminthiasis, and skin illnesses, as well as inflammation. In Chennai's Madras, an ointment prepared from the plant's ground seeds in Castor oil is a useful tool for reducing the symptoms of hydrocele. and external application of orchitis. It has also been discovered that the oil extracted from the plant's seeds can regulate seizures and paralysis episodes. In Guinea the shrub's ground seeds are utilized as a vesicant. when combined with an equal amount of pepper, powdered seeds given to patients suffering from malaria were discovered to have weak antiperiodic characteristics.[8]

When administered internally, a paste made of powdered seeds and water has been proven to be highly helpful in cases of snake bite, although it cannot be regarded as an antidote to snake poison. Plant seeds, when crushed and eaten with honey and sprinkled with long pepper, has been discovered to have expectorant properties. Within the roasted seeds have been utilized by the West Indies indigenous people to manage diabetes problems. Adult dosage of 15–30 grains of powdered seed kernel with equal amounts of black pepper eaten three times a day has been discovered to be extremely helpful in all instances of basic, ongoing, and sporadic fevers. The plant's twigs and leaves have been used historically to address liver problems, inflammation, and malignancies as well as dental pain The climber's fluids and leaves have been traditionally employed to treat ailments like Smallpox and elephantiasis.[12]

TABLE 03: PHARMACOLOGICAL ACTIVITIES:

SL NO	Activities	References
1.	Antimicrobial activity	Arif T. *et al*., 2009
2.	Antioxidant activity	Mandal S *et al* 2009
3.	Anti-cancer activity	Gupta M. *et al*.,2005
4.	Anti-convulsant activity	Ali N. *et al*.,2008
5.	Anti-inflammatory activity	Shukla S. *et al.,*2009
6.	Anti-fungal activity	Ata A, et al, 2009
7.	Anti diabetic activity	Kannur D.M. *et al.,*2006
9	Anti filarial activity	Fatma N et al 2008
10.	Antipyretic activity	Archana P. *et al.,* 2005
11.	Antibacterial activity	Raman N. *et al*.,2000

Materials and Method:

Plant collection and authentication of *Caesalpinia bonducella* root.

The collection of *Caesalpinia bonducella* roots around davanagere. Plant authentication was done according to the references by taxonomists and whole plant was procured from davanagere, Karnataka state. The shade dried whole plant material was subjected to Soxhlet extraction by ethanol as solvent (70 - 80 °C). The extract was evaporated to dryness and was preserved in desiccator.

Preparation of the extract:

In this method, crushed root powder of *Caesalpinia bonducella* was placed in thimble chamber of the Soxhlet apparatus. The extraction solvent ethanol was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reached the siphon arm,the liquid contents emptied into the bottom flask again and the process was continued [13-14]

The Fresh *Caesalpinia bonducella* dried root was procured during rainy season from davangere district, Karnataka state. It was Authenticated by Dr. Halesh.C Assistant professor DOS in botany davangere University, Shivagangotri Davangere, Karnataka, India. About 300 g of root part of *Caesalpinia bonducella* dried cource powder was weighed and extraction process is carried out by using 600 ml of ethanol in Soxhlet apparatus for 48 hrs. Appearance of colourless solvent in the siphon tube was taken as the end point of extraction (approximately 25-30 cycles). The extract was then concentrated to 3/4th of its original volume by distillation. The extract was concentrated by evaporation at 70 °C for 8 h and then dried. The concentrated extract was made in Gel form preserved in a desiccator. The obtained extract was subjected to phytochemical investigation, antioxidant & antifungal activity.

QUALITATIVE TESTS FOR PHYTOCONSTITUENTS

1. TEST FOR CARBOHYDRATES

Molisch's test: The extract with Molisch's reagent mixes and added concentrated sulphuric acid along the sides to form layers. A violet ring at the interference show the presence of carbohydrates.

Benedict's test: With Benedict's reagent the carbohydrates on boiling and cooling a green reddish-brown precipitate was formed which shows the presence of reducing sugar.

Fehling's test: The extract was heated with Fehling's A and Fehling's B solution it gives a range red precipitate shows the presence of reducing sugar.

Barfoeds test: To the extract Barford's reagent was added and it was boiled on a water bath, reddish precipitate was observed within 90 minutes show the presence of monosaccharide.

2. TEST FOR PROTEINS

Biuret test: Reagent 40% sodium hydroxide and dilute copper sulphate solution. Protein shows blue, pink, or violet colour whereas amino acid fails to show the colour.

Ninhydrin test: With Ninhydrin amino acid show blue colour. But protein may give the positive test very rarely.

Million's test: To the 3 ml of extract 2 ml of mercuric sulphate in concentrated sulphuric acid was added boiled for a minute. Added 2 drops dilute sodium nitrate and heated.

3. TEST FOR GLYCOSIDES

Keller-Kailani test: To the various extracts 1 ml of glacial acetic acid and few drops of ferric chloride solution was added and then slowly concentrated sulphuric acid
was added through the sides of test tube. A reddish-brown ring at the junction of liquids was observed which shows the presence of de-oxy sugar.

Raymond's test: To the various extract dinitro benzene in hot methanolic potassium hydroxide was added.

Lugol's test: To the various extract's sodium nitroprusside solution and sodium hydroxide was added. Bioactive significance of *caesalpinia bonducella* antifungal and antioxidant activity

Keddie test: Alcoholic solution of the extract with alcoholic solution of 3, 5, dinitro benzoic acid and hot potassium hydroxide gave violet colour.

4. TEST FOR ALKALOIDS

Mayer's test: The various extracts were dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Mayer's reagent (Potassium Mercure Iodide).

Wagner's test: The various extracts were dissolved in chloroform. The chloroform layers were evaporated, to the residue were acidified and added few drops of
Wagner's reagent (Iodine in Potassium Iodide).

Hager's test: The various extracts were dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Hager's reagent (Saturated Picric Acid solution).

Dragandorffs test: The various extracts were dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Dragandorffs reagent (potassium Bismuth Iodide).

5. TEST FOR FLAVONOIDS

Ferric Chloride test: To a small quantity of the alcoholic solution of the extract a few drops of neutral Ferric chloride solution was added. A green colour was produced due to the phenolic nucleus. Bioactive significance of *caesalpinia bonducella* antifungal and antioxidant activity

Lead acetate test: To the alcoholic solution of extract few drops of Lead acetate solution (10%) was added.

Shinoda test: To the alcoholic solution of the extract a few fragments of magnesium ribbon were added. To this concentrated hydrochloric acid was added drop wise. Magenta colour was produced after few minutes which are the characteristic reaction of flavonoid.

6. TEST FOR STEROIDS

Lieberman Burchardt test: To the chloroform solution in a test-tube a few drops of acetic anhydride were added and mixed well. 1 ml of concentrated sulphuric acid was added from the side of the test tube

and allowed to stand. A reddish ring was formed at the junction of two layers.

Salkowski test: To the chloroform solution in a test-tube concentrated sulphuric acid was added from the side of the test-tube. A reddish-brown colour will be seen in positive.

7. TEST FOR TANNINS

Neutral Ferric chloride test: 2 ml of extract was taken in a test tube and ferric chloride solution was added drop by drop.

Gelatine solution test: To the extract few drops of 1% of solution of Gelatin containing 10% sodium chloride was added. Bioactive significance of *caesalpinia bonducella* antifungal and antioxidant activity

8. TEST FOR SAPONINS

Foam test: Small amount of extract was shaken in a test tube with a little quantity of water, the foam produced persisted for 10 minutes.

Haemolysis test: To the 2 ml of 1.8% sodium chloride solution to the two test tubes. To one of these added 2 ml of distilled water and to the other 2 ml of 1% of the extract. The concentration of the sodium chloride in each test tube now is iso-tonic with blood, serum obtained blood by pricking the thumb at the base of the nail and drawn into a pipette. Added 5 drops of blood to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the tube containing the extract, but no haemolysis in the control indicates the positive test for the presence of saponins.

9. TEST FOR TRITERPENES

Salkowski test: A few drops of concentrated sulphuric acid were added to the chloroform solution, shaken and allow to stand. Lower layer turned yellow.

Lieberman Burchardt test: To the chloroform a few drops of acetic anhydride and 1 ml of sulphuric acid was added. A deep red colour was produced.

ANTIOXIDANT ACTIVITY

Evaluation of Antioxidant Activity by Invitro Method: [15,16]

PROCEDURE FOR DPPH ACTIVITY

Reagent 2, 2-diphenyl 1-picryl hydra Zyl solution (DPPH,0.022%) 4 mg of DPPH was dissolved in 100 ml of methanol. (Stock Solution), 1 ml of stock solution was diluted in 100 ml of methanol. Which gives0.004 mg/ml [40 µg/ml] DPPH solution. (Standard Solution).

Preparation of extract solutions: Extracts/sample (100 mg) dissolved in 100 ml of freshly distilled Met-OH separately to obtain solution of 1 mg/ml concentration. From the above stock solution 1ml is pipetted out and dissolved in 10ml of ethanol separately to obtain 100 µg/ml concentration, solutions were serially diluted separately to obtain to lower concentrations like 50,100, 200, 400, 500 µg/ml

Preparation, of standard solutions: Ascorbic acid (100 mg) dissolved in 100 ml of freshly distilled Me-OH which gives 1mg/ml (Stock solution) to obtain solution of 10 mg/ml concentration.1ml of stock solution is diluted in 10ml of ethanol to obtain solution of 100 µg/ml concentration. Solutions were serially diluted separately to obtain to lower concentrations like 50,100, 200, 400, 500µg/ml.

Procedure: Samples were prepared in methanol at different concentrations as stated above. Sample extract/Standard of 1ml of each concentration was added to 20 ml of 0.022% ethanol solution of DPPH. Incubation period of 30 min was allowed at room temperature in dark place to complete any reaction that is to be occurred. Then absorbance was measured by UV spectrophotometer at λmax 520 nm against blank. Ascorbic acid used as standard free radical scavenger and activity of extract was compared with it. Activity of the sample was calculated from the formula.

% Scavenging = {(A1-A2)/A1} X100.
Where A1 is the absorbance of the Blank, and A2 is the absorbance of the Standard/Sample

ANTIFUNGAL ACTIVITY

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal or antiviral. They all have different modes of action by which they act to suppress the infection.

Agar well-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing.

Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and fungus testing.

Materials and method:[17,18]

MATERIALS:

Test Organisms: *Fungi:*

Aspergillus niger MTCC F535, MTCC, Chandigarh
Aspergillus flavus MTCC 2796, MTCC, Chandigarh
Candida albicans MTCC F183, MTCC, Chandigarh
Potato dextrose agar (Cat No: SM096D, Himedia)
SDA agar (Cat No: M063, Himedia)
Fluconazole (Cat No:CMS8387 Himedia)
Double distilled water (Nice chemicals)
Sterile Cotton swabs
Sterile Petriplates (Tarsons)
Laminar Air Flow (Alpha Linear)
Personal Protective Equipment (PPE) i.e., Gloves, Mouth Mosque, Head Cap and Lab Coat etc.
Pipettes (10ul, 200ul and 1ml Pipettes)

STEPS FOLLOWED FOR THE STUDY:

The Fungal strains were maintained on Potato Dextrose Agar (PDA). The microorganisms used for antimicrobial analysis were purchased from Microbial Type Culture Collection and Gene Bank (***MTCC***), Chandigarh, India.

Fungi growth conditions:

Pure cultures from the plate were inoculated into SDA agar plate and sub cultured at 37ºC for 24h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a fungal growth.

Anti-fungal Test by well diffusion method: Antibiotic susceptibility tests were determined by agar well diffusion (Kohner) method. The medium was prepared by dissolving 65 g of SDA agar in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured into Petri plates (25 ml/plate). The plates were swabbed with pathogenic fungal cultures viz *Aspergillus niger, Aspergillus flavus and Candida albicans*. Wells (6 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. About 100 µl of sample with 1mg/ml concentration was added using sterile pipette into the

respective wells. Fluconazole with 150ug/ml was used as a positive control and Double distilled water alone considered as negative control respectively the plates were incubated at 28°C for 48hr for fungal pathogens. The diameter of the inhibition zone (mm) was measured with transparent ruler in millimeters.[19]

Results:

Phytochemical test ethanolic extract of *Caesalpinia bounducella* root was carried out and found the various phytoconstituents are Carbohydrate, Protein, Alkaloids, Flavonoids, Phenol and Tannins are present. The DPPH assay performed showed significant antioxidant activity of *Caesalpinia bounducella* extract. The scavenging activity was dose-dependent and comparable to that of the standard Ascorbic acid.. The reduction capacity of this radical was determined by decrease in its absorbance at λmax 520 nm induced by ethanolic extract of *Caesalpinia bounducella* exhibits potential antioxidant activity. It produces hydrazine by converting the unpaired electrons to paired electrons due to the hydrogen donating ability of the ethanolic extract of *Caesalpinia bounducella*. The concentration of 500 µg/ml ethanolic extract of *Caesalpinia bounducella* showed significant % Scavenging activity (87.83 %). The extract IC_{50} value of ethanolic extract of *Caesalpinia bounducella* was found to be 50.13 µg/ ml by comparing with the standard (Ascorbic acid) % Scavenging activity 93.55 % at 500 µg/ ml and IC_{50} value of Standard (Ascorbic acid) was found to be 25.75 µg/ ml.

GRAPH:

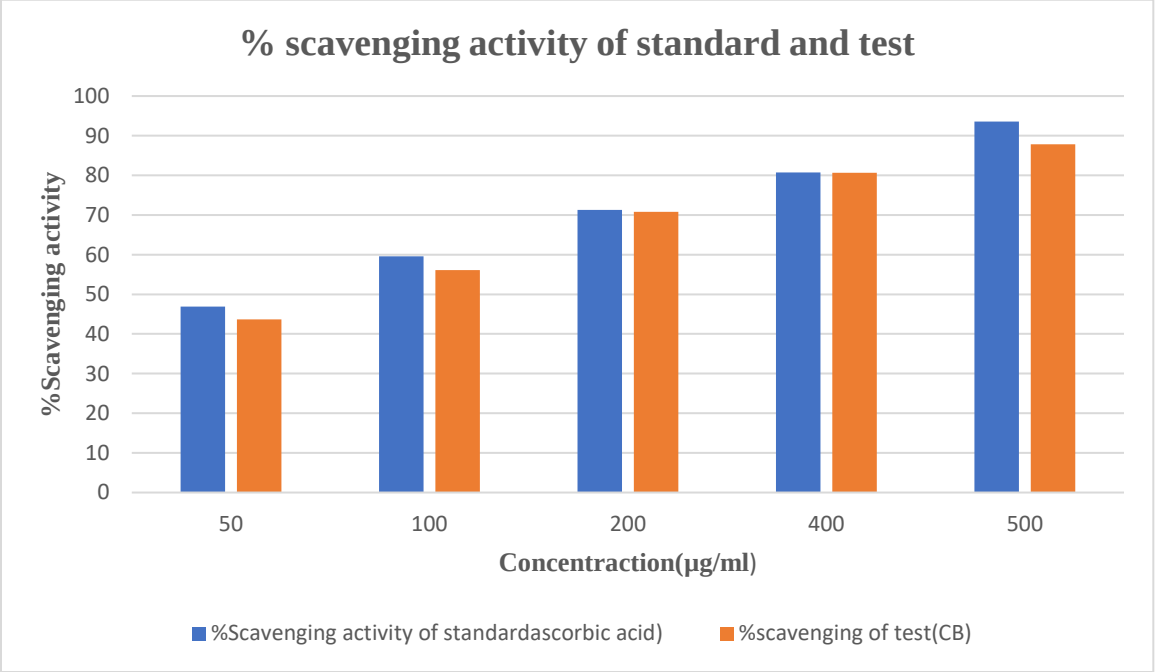

Figure: Bar graph showing the effect of % DPPH radical scavenging of the test sample (CB) and the standard compound (ascorbic acid) against the drug concentrations

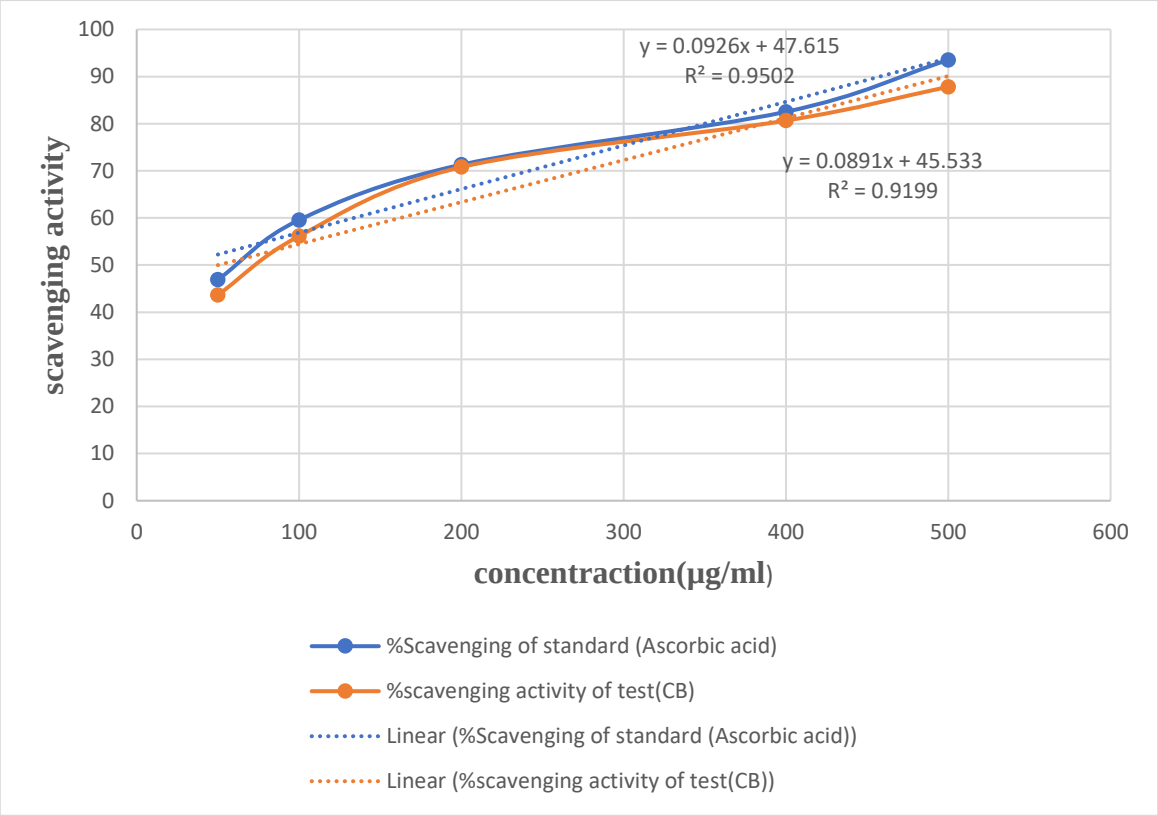

Figure: %scavenging of standard and test compound Linear graph

Sample name	R^2value	Y=mx+c	IC_{50}
Standard (Ascorbic acid)	$R^2 = 0.9502$	y = 0.0926x + 47.615	25.75 µg/ml
Test(CB)	$R^2 = 0.9199$	y = 0.0891x + 45.533	50.13 µg/ml

Table: IC_{50} values Regression equation and r^2 values for antioxidant activity of ascorbic acid and ethanolic extract of *Caesalpinia bonducella.*

The IC_{50} value of Ascorbic acid was found to be 25.75 µg/ml and the CB was found to be 50.13 µg/ml. The DPPH Scavenging activity of the test *Caesalpinia bonducella. Shown* good antioxidant activity

Table: Antifungal activity by well diffusion method:

CB-Well diffusion method-*A.niger, A.flavus&C.albicans*			
Zone of inhibition (mm)			
Microbe	***Aspergillus niger***	***Aspergillus flavus***	***Candida albicans***
Control	0	0	0
Fluconazole-150ug	23	21	20
CB-1mg/ml	15	16	12

Diameter of zone of inhibition (mm) of CB extract against the *A.niger, A.flavus, C.albicans* after the incubation period of 48hrs.

OBSERVATIONS

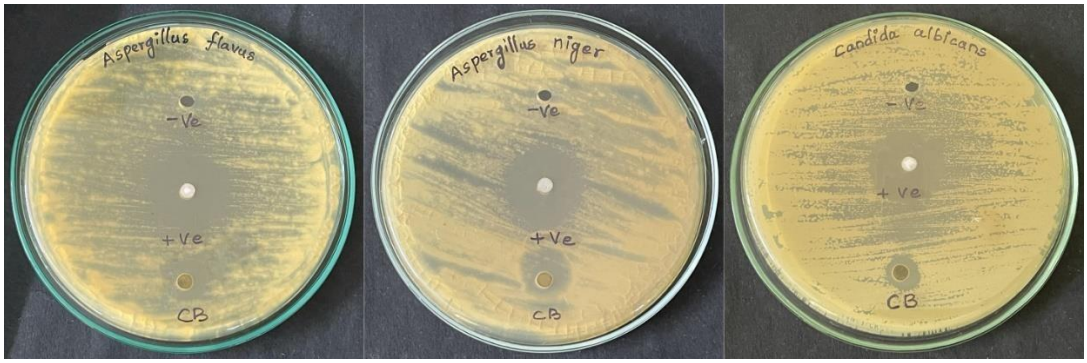

Figure: Anti-fungal activity of CB extract with 1 dilution (1mg/ml) against the *Aspergillus flavus, Aspergillus niger* and *Candida albicans* in comparison to Positive control (Fluconazole with 150ug) and Negative control (Distilled water) and found that the CB extract may have satisfactory anti-fungal activity. Antifungal activity of CB extract was evaluated against some fungi by disc diffusion method. The CB extract Effect on different fungi such as *Candida albicans, Aspergillus niger , Aspergillus flavus* at different concentration shows different antifungal effect which is indicated by zone of inhibition in mm can be compared with that of standard drug fluconazole at concentration of 150µg/ml

GRAPH:

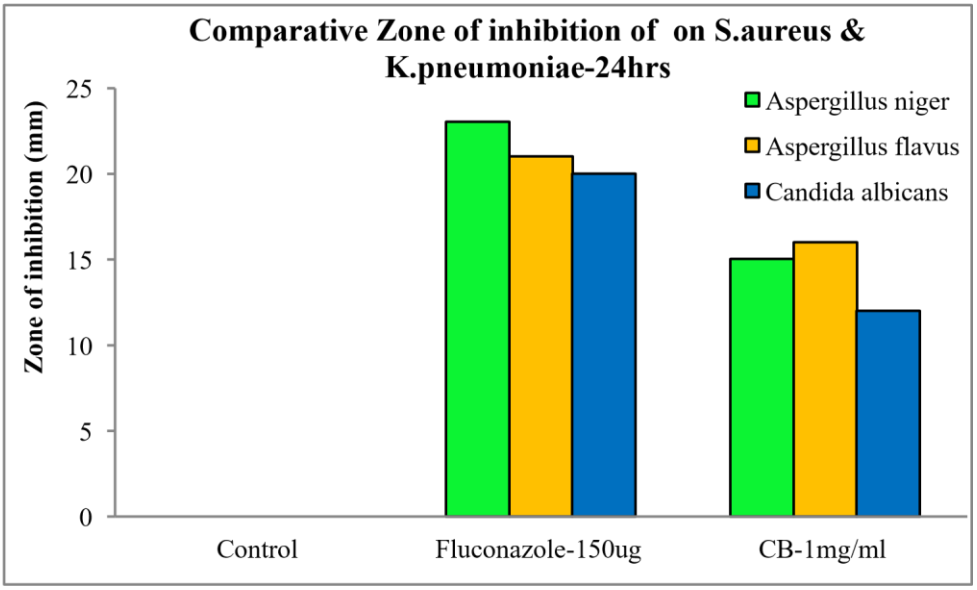

Figure: Overlaid bar graph depicted the anti-fungal activity of CB extract against the *Aspergillus niger, Aspergillus flavus* and *Candida albicans* in comparison to Positive control (Fluconazole-150ug) and Negative control (Distilled water).

Discussion:

The ethanolic root extract of *Caesalpinia bonducella* demonstrated satisfactory antifungal activity against *Aspergillus niger, Aspergillus flavus,* and *Candida albicans*. The observed inhibition zone suggest that the extract possesses bioactive compound with antifungal properties. Although the zone of inhibition produced by the CB extract was smaller than those of the standard antifungal agent Fluconazole, the

extract still exhibited significant activity, particularly against *Aspergillus flavus* and *Aspergillus niger*. Previous studies have reported the presence of various bioactive compounds, including flavonoids, saponins, and alkaloids, in *Caesalpinia bonducella*, which are known to exhibit antimicrobial and antifungal properties. The antifungal effect observed in this study could be attributed to these compounds. The root extract's inhibitory effect on *Candida albicans* is particularly noteworthy, as this pathogen is a common cause of opportunistic infections, especially in immunocompromised patients. The antioxidant activity was performed using DPPH radical scavenging activity. The reduction capacity of this radical was determined by decrease in its absorbance at λmax 520 nm induced by ethanolic extract of *Caesalpinia bonducella* exhibits potential antioxidant activity. It produces hydrazine by converting the unpaired electrons to paired electrons due to the hydrogen donating ability of the *Caesalpinia bonducella* extract. The concentration of 500µg/ml ethanolic extract of *Caesalpinia bonducella* showed significant % of scavenging activity (87.83%). The extract IC^{50} value of *Caesalpinia bonducella* was found to be 50.13 µg/ml by comparing with the standard (Ascorbic acid) % Scavenging activity (93.55) at 500µg/ml and extract IC^{50} value of Standard (Ascorbic acid) was found to be 25.75 µg/m then *Caesalpinia bonducella* confirmed its strong antioxidant potential. The smaller zone of inhibition observed for *Candida albicans* compared to *Aspergillus* species may be due to differences in fungal cell wall structure and resistance mechanisms. Further investigation is required to identify the specific compounds responsible for the antifungal activity and to explore their mechanisms of action. Bioactive significance of *Caesalpinia bonducella*: antifungal and antioxidant activity. This study highlights the potential of *Caesalpinia bonducella* as a natural antifungal agent, particularly for the treatment of infections caused by *Aspergillus* and *Candida* species. Future research should focus on isolating and characterizing the active compounds in the root extract, as well as conducting in vivo studies to evaluate its efficacy and safety in clinical settings.

The ethanolic root extract of *Caesalpinia bonducella* shows promising antifungal activity, likely due to its bioactive compounds that disrupt fungal cell membranes, leading to cell death.[20] Studies suggest that combining such plant extract with conventional antifungal agents like fluconazole can enhance efficacy and reduce the risk of drug resistance.[21] Additionally, similar effects have been observed in other medicinal plants such as neem and turmeric, highlighting *Caesalpinia bonducella* as part of a valuable group of natural antifungals.[22] Its antioxidant properties also make it a strong candidate for wound healing applications, especially in immunocompromised patients, as it can prevent infection and support tissue repair.[23] Future research should focus on isolating the active compounds and developing stable, safe topical formulations.[24]

Conclusion:

This study is significant in that it is the final study reporting the determination of total flavonoid content & phenolic content of *Caesalpinia bonducella* plant. Antifungal activity of *Caesalpinia bonducella* root will shed light on the scientists who will work on this species. Phenolic & flavonoids are very essential & significant components of plants and the ability of phenolic compounds to scavenge radicals is due to their hydroxyl groups. Phenolic & flavonoid compounds can directly contribute to the antioxidative effect & antifungal activities. The present studies were aimed to access antioxidant and antifungal activity of Ethanolic root extract of *Caesalpinia bonducella*. The extract contain important secondary metabolites are responsible for antioxidant activity. The antioxidant screening shows that it showed reducing power to DPPH radicals. But the efficiency showed vitamin C. The Ethanolic root extract of *Caesalpinia bonducella*

the concentration of 500µg/ml showed inhibition (87.83%). of DPPH radicals. Thereby exhibited excellent antioxidant activity. The Ethanolic root extract of *Caesalpinia bonducella* have great potential as antifungal compounds against various fungi. Thus, they can be used in the treatment of infectious diseases caused by resistant fungi. The current study revealed the presence of antioxidant and antifungal activity in Ethanolic root extract of *Caesalpinia bonducella*. It is concluded that Ethanolic root extract of *Caesalpinia bonducella* can be potential additional in pharmaceutical product for improvement of human health. Based on the evidence of investigation it could be concluded that Ethanolic extract of *Caesalpinia bonducella* root, have multiple medicinal property, particularly as antioxidant and antifungal activity.

Reference

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