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COMPARISON OF ETHANOLIC EXTRACT OF *COLEUS AMBOINICUS* AND *COLEUS BLUMEI* LEAVES FOR EVALUATION OF ANTIOXIDANT AND ANTI BACTERIAL ACTIVITY

Rangam Chariitha*, Polaky Samuel, M. Aishwarya, Sara Naaz, Madiha Fathima
Department of Pharmacology, Smt. Sarojini Ramulamma College of Pharmacy, Mahbubnagar-501001,Telangana,India.

Abstract:

To evaluate & compare the antioxidant and antibacterial activity of ethanolic extract of Coleus amboinicus and Coleus blumei leaves.The ethanoic extract of Coleus amboinicus and Coleus blumei leaves was prepared by maceration and were screened for the presence of various phytochemical constituents. The antioxidant activity of ethanolic extract of Coleus amboinicus and Coleus blumei leaves was determined by Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and H2O2 radical scavenging assay. And the antibacterial activity was evaluated by Agar well diffusion method for determining mean diameter of inhibition zone and Broth dilution method for determining minimum inhibitory concentration. The results showed that direct crude extract of Coleus amboinicus having good antioxidant activity than Coleus blumei. And it is also having antibacterial activity against the gram-positive bacteria Streptococcus pneumoniae than the gram- negative bacteria Pseudomonas aeruginosa. But whereas, Coleus blumei shows less antibacterial activity towards the both bacteria. Coleus amboinicus showed better antioxidant activity and antibacterial activity and this justifies its better use in ethnomedicine.

Keywords: *Coleus amboinicus, Coleus blumei, Antioxidant activity, Antibacterial activity, Chloramphenicol.*

Corresponding author:
Miss. Rangam Chariitha,
Assistant Professor,
Department of Pharmacology,
Smt. Sarojini Ramulamma College of Pharmacy,
Seshadri Nagar,Mahbubnagar-501001,Telangana,India.
E-mail: rangamcharitha3@gmail.com
Mobile-+919052508290

QR code

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INTRODUCTION:

Antioxidants

The compounds known as antioxidants prevent other molecules from oxidizing and causing damage to cells. The chemical process of oxidation involves the transfer of electrons from a molecule to an oxidizing agent. Free radicals are known to be produced during oxidation reactions. The outermost shell of these extremely reactive free radicals comprises one or more unpaired electrons.

The best sources of antioxidants are plant-based foods, especially fruits and vegetables. Foods that are particularly high in antioxidants are often referred to as a **"superfood"** or **"functional food".**

Based on their solubility, antioxidants are broadly categorized into two groups: water soluble and lipid soluble. In general, water-soluble antioxidants, such as ascorbic acid, glutathione, and uric acid, have functions in the cell cytosol and the blood plasma. Ascorbic acid is a redox catalyst which reduces and neutralizes the reactive oxygen species (ROS), glutathione has antioxidant properties as reducing agent and can be reversibly oxidized and reduced, while α tocopherol, carotenoid, and ubiquinol are the examples of lipid-soluble antioxidants and protect the cell membranes from lipid peroxidation.[1]

Hydroxyl radical (OH^-)

Hydroxyl radical is the neutral form of hydroxide ion and is a highly reactive free radical. It can strongly react with both organic and inorganic molecules including DNA, proteins, lipids, and carbohydrates and cause severe damage to the cells than any other ROS can do. It is formed in a Fenton reaction, in which H_2O_2 react with metal ions (Fe^{+2} or Cu^+), often bound in complex with different proteins such as ferritin (an intracellular protein that stores iron) and ceruloplasmin (plasma copper carrying protein) or other molecules. Under stress conditions, an excess of $O_2^{\bullet-}$ releases free iron from ferritin and the released free iron participates in Fenton reaction to form $OH^{\bullet}$. It is also formed by the reaction between superoxide radical and H_2O_2 in a reaction called Haber – Weiss reaction

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH^- \text{ (Fenton reaction)}$$

$$O\cdot\!-2+H2O2 \rightarrow O2+OH\cdot+OH^- \text{ (Haber-Weiss reaction)}$$

Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide is formed in vivo in a dismutation reaction catalyzed by the enzyme superoxide dismutase (SOD). H_2O_2 has no direct effect on DNA but can damage DNA by producing hydroxyl radical (OH^-) in the presence of transition metal ions. The major antioxidant enzymes that can eliminate the H_2O_2 include catalase, glutathione peroxidase and peroxiredoxins.[2]

$$O_2 + O_2^{\cdot-} + 2H_20 \xrightarrow{Cu,\ Zn,\ Mn\ -\ SOD} H_2O_2 + O_2$$

Nitric Oxide or Nitrogen Monoxide ($NO^{\bullet}$)

It is a small molecule generated in tissues by different nitric oxide synthases (NOS)s which convert L-arginine to L-citrulline. In this reaction one of the terminal guanido nitrogen atoms undergo oxidation and produces $NO\cdot$. Three types of isoforms of NOS such as neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) are involved in the formation of the NO radical.

$$L\text{−}Arginine + O_2 + NADPH \xrightarrow{NOS} L\text{−}Citrulline + NO\cdot + NADP^+$$

Micro organisms

Microorganisms, are tiny organisms that are invisible to the human eye. Bacteria are ubiquitous, which means they are found everywhere. They inhabit a variety of ecosystems and can tolerate extreme conditions like those found in toxic waste, arctic ice, and hydrothermal vents.

They can be prokaryotic, which means they have a nucleus and membrane-bound organelles, or eukaryotic, which means they don't. Chloramphenicol: Many bacterial infections can be treated with the antibiotic chloramphenicol. In 1947, chloramphenicol was isolated from Streptomyces venezuelae and subsequently discovered. ts chemical structure was identified and it was first synthesized in 1949. It is on the World Health Organization's List of Essential Medicines. It is available as a generic medication. Because of its wide range of action, chloramphenicol has proven to be beneficial in treating eye infections, including blepharitis and conjunctivitis, which can be brought on by a variety of bacteria, including Escherichia coli, Streptococcus pneumoniae, and Staphylococcus aureus.[3]

Plant profile of *Coleus Amboinicus*

Taxonomic classification:

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Lamiaceae
Genus	:	Coleus
Species	:	*Coleus amboinicus*

Plant profile of *coleus blumei*

Taxonomic classification:

Kingdom : Plantae
Phylum : Spermatophyta
Subphylum : Angiospermae
Class : Dicotyledonae
Order : Lamiales
Family : Lamiaceae
Genus : Coleus
Species : *Coleus blumei*

Plant Collection, identification & Drying

Fresh plant of *Coleus amboinicus* & *Coleus blumei* was collected from the Govt. Degree College, R.R Dist., Hyderabad, Telangana. The plant materials were identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupathi. A voucher was kept in the Department of Pharmacology for reference. Crude *Coleus amboinicus* and *Coleus blumei* leaves were dried under shade and coarsely powdered. The powered material was taken for the extraction process.

Preparation of Plant Extracts

Maceration is the technique of smoothing by immersing in a solution. The primary goal is to acquire soft materials in liquid media.

Preliminary Phytochemical Screening: Preliminary phytochemical screening was performed on the EECA & EECB to determine several phytochemical constituents found in *Coleus amboinicus* & *Coleus blumei.*

Chemicals, Reagents and Apparatus: The reagents used in this study comprised Ethanol, Hydrogen peroxide, Sodium hydroxide, Ferric chloride, Potassium dihydrogen Phosphate, EDTA, Sodium Nitroprusside, Glacial acetic acid, Sulphanilic acid, Potassium hydroxide, Ascorbic acid, Thiobarbituric acid, Trichloro acetic acid[2]

***In vitro* antioxidant Assay**

Hydroxyl radical scavenging activity

Procedure:

500 L of the EECA & EECB, 100 L of 2-deoxy-D ribose (28 mM was prepared in 20 mM concn of KH_2PO_4 - KOH buffer at pH 7.4), and 1 mL of the reaction mixture is incorporated. The reaction mixture about 1 ml was added to 500 L of Nanosuspension of ethanolic extract of *Coleus amboinicus* & *Coleus blumei*, 200 M $FeC1_3$ (1:1 v/v), 200 L EDTA (1.04 mM), and 1.0 mL of trichloroacetic acid (2.8%) and 1mL of thiobarbituric acid (1%) are added and incubated for 20 minutes at 100°C. Upon cooling, 532 nm absorbance is evaluated in comparison to a blank sample.[4]

Nitric oxide scavenging activity

Procedure:

EECA & EECB in amounts ranging from 0.2 - 0.8 mg/mL is added to 0.5 mL of phosphate buffer saline (pH 7.4) and 2 mL of 10 mM sodium nitroprusside to the mixture, 500 L of EECA, and 500 L of EECB is maintained at 25°C. The Griess reagent is prepared by adding 1 mL of naphthyl ethylenediamine dichloride (0.1% w/v) along with 1.0 mL of Sulfanilic acid reagent at a concentration of 0.33% in 20% glacial acetic acid at room temperature for 5 minutes is combined with 0.5 mL of the incubated solution after 150 minutes. The mixture's absorbance is measured at 546 nm. The amount of nitric oxide radical inhibition is determined using the equation below:

$$\% \text{ Inhibition of NO radical} = [A_0 - A_1] = A_0 \times 100$$

Where A_0 - absorbance before the reaction

A_1 - absorbance following the Griess reagent reaction.[5]

H_2O_2 radical scavenging activity

Procedure: The hydrogen peroxide radical scavenging assay prepared a hydrogen peroxide solution (40 mM) in phosphate buffer (pH 7.4). 4 mL of EECA & EECB at different concentrations (10, 20, 30, 40, 50 mg/mL) was added to a hydrogen peroxide solution (0.6 mL, 40mM). The absorbance of the resultant solution was compared to a blank solution after 10 minutes. The degree of H_2O_2 inhibition is calculated using the equation below[6]

$$\% H_2O_2 \text{ activity} = \frac{Abs_{control} - Abs_{sample} \times 100}{Abs_{control}}$$

***In Vitro* ANTI-bacterial Activity**

Agar well - diffusion method

This widely used method involves inoculating agar plates with a standardized inoculum of the test microorganism. Next, the test substance is added to filter paper discs (approximately 6 mm in diameter) and deposited on the agar surface at the specified concentration. The Petri dishes are incubated in the proper atmosphere. The test microorganism's germination and growth are usually inhibited by an antimicrobial substance that diffuses into the agar. The diameters of the inhibitory growth zones are then determined.

Antibiogram classifies bacteria as susceptible, intermediate, or resistant, yielding qualitative results. However, since the bacterial growth inhibition does

not mean the bacterial death, this method cannot distinguish bactericidal and bacteriostatic effects Agar well - diffusion method is used to determine the mean diameter of inhibition zone. In addition, as it is difficult to measure how much of the antimicrobial agent diffuses into the agar medium, the agar disk-diffusion method is inappropriate for determining the minimum inhibitory concentration (MIC).
nevertheless by comparing the inhibition zones with stored algorithms, an approximate minimum inhibitory concentration (MIC) can be determined for a variety of bacteria and drugs.
This method is frequently used for the antimicrobial screening of plant extracts, essential oils, and other pharmaceuticals due to its benefits, which include primarily its simplicity and low cost.[7]

- **Broth Dilution Method for MIC Determination**

The least inhibitory concentration (MIC) is the lowest concentration at which the isolate is totally inhibited (as shown by the lack of observable bacterial growth).

There are two ways to carry out dilution methods: agar dilution and broth dilution.
Broth dilution testing allows providing both quantitative (MIC) and qualitative (category interpretation) results. MIC is used to determine a bacterial strain's level of resistance and has a significant impact on the choice of which antimicrobial drugs to use.
There are two methods available for dilution of broth.
1. Macro dilution: Standard test tubes with a broth volume of 1 ml are used.
2. Micro dilution: Uses about 0.05 to 0.1 ml total broth volume and can be performed in a microtiter plate or tray.
Apart from the broth volume, the process for both macro and microdilution is the same.
The following process is used to calculate the minimum inhibitory concentration (MIC) of an antibiotic using the broth dilution method.

1. Preparation of antibiotic stock solution

2. Preparation of antibiotic dilution range

3. Preparation of agar dilution plates

4. Preparation of inoculum

5. Inoculation

6. Incubation

7. Reading and interpreting results[8]

1. Preparation of antibiotic stock solution: Commercially available antimicrobial powders can be used to produce antibiotic stock solutions (with given potency).
Either of the following formulas can be used to determine the amount of antimicrobial powder (1) or diluent (2) needed for a standard solution, as well as the diluents in which it can dissolve:

A minimum of 1000 µg/mL (for instance, 1280 µg/ml) or ten times the highest concentration to be tested, whichever is higher, should be used to prepare antimicrobial agent stock solutions.

Because microbial contamination is extremely rare, solutions prepared aseptically but not filter-sterilized are generally acceptable.

Prepare antimicrobial agent stock solutions at concentrations of at least 1000 µg/mL (example: 1280 µg/ml) or 10 times the highest concentration to be tested, whichever is greater.

Because microbial contamination is extremely rare, solutions prepared aseptically but not filter-sterilized are generally acceptable However, membrane filtration can be used to sterilize liquids if required. Dispense small volumes of the sterile stock solutions into a sterile glass, polypropylene, polystyrene, or polyethylene vials; carefully seal; and store (preferably at -60°C or below, but never at a temperature warmer than -20°C and never in a self-defrosting freezer). Vials can be used the same day after being thawed as needed.[9]

2. Preparation of antibiotic dilution range:
• To perform the test, use sterile 13 x 100 mm test tubes. Make sure the tubes can be frozen if they are going to be preserved for later use.
• Use cotton plugs, plastic or metal closure caps, or loose screw caps to seal the tubes.

• Make the last volumetric dilutions of the antimicrobial ingredient in the broth, either twofold or in another way. For the test, a minimum final volume of 1 mL of each dilution is required. Note: Only 0.1 ml is dispensed into each of a standard tray's 96 ells for micro dilution.

3. Preparation of inoculum:

1. To prepare the inoculum, take an 18 to 24 hour agar plate and select isolated colonies to make a direct broth suspension (use a non-selective medium, such as blood agar).
2. Modify the suspension until the turbidity reaches a standard of 0.5 McFarland turbidity. For Escherichia coli ATCC ®a 25922, this yields a suspension that has between 1 and 2 x 10^8 colony forming units (CFU)/mL.
3. Compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.
4. Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in broth so, after inoculation, each tube contains approximately 5 x 10^5 CFU/mL.

Note: To do this, dilute the 0.5 McFarland suspension 1:150, which will yield a tube that has about 1 x 10^6 CFU/mL. The last inoculum is 5 x 10^5 CFU/mL after the step 3 1:2 dilution.[10]

4.Inoculation:

After the inoculum has been standardized as previously mentioned, add 1 mL of the adjusted inoculum, along with 1 ml of the antimicrobial agent in the dilution series, to each tube (as well as a positive control tube that contains only broth), and mix within 15 minutes. This results in a 1:2 dilution of each antimicrobial concentration and a 1:2 dilution of the inoculums.

5. Incubation:

Place the inoculation tubes in an ambient air incubator and incubate them for 16 to 20 hours at 35 ± 2 °C. Microdilution trays should not be stacked more than four high in order to preserve the same incubation temperature for each culture.

6. Interpretation:

To determine the growth endpoints for each set of tests, compare the quantity of growth in the antimicrobial agent-containing wells or tubes with the development in the growth-control wells or tubes (no antimicrobial agent) used in each set of testing. Acceptable growth (≥ 2 mm button or definite turbidity) in the growth-control well is required for a test to be considered valid.[11]

Results

The results obtained from different evaluation parameters are presented below systematically.

Preparation of plant extract

Maceration procedure was used to obtain an ethanolic extract of *Coleus amboinicus* and *Coleus blumei*. [11]

Table 1: Percentage yield of Ethanolic extract

S. NO	Name of the Plant	% Yield
1.	***Coleus amboinicus***	12.0
2.	***Coleus blumei***	8.6



Figure 1: Extract of *Coleus amboinicus* and *Coleus blumei*

Preliminary phytochemical analysis

The Alcoholic extract showed the presence of steroids,
tannins, alkaloids and flavonoid
EECA showed the presence of alkaloids, flavonoids, tannins, sterols, proteins, glycosides.
EECB showed the presence of alkaloids, carbohydrates, flavonoids, phenols.

Table 2: Phytochemical screening of *Coleus amboinicus* and *Coleus blumei*

Chemical Constituents	Ethanolic extract of *Coleus amboinicus*	Ethanolic extract of *Coleus blumei*
Alkaloids	+	+
Carbohydrates	-	+
Glycosides	+	+
Flavonoids	++	+
Phytosterols	+	-
Saponins	+	-
Phenolic compounds	+	++
Tannins	+	-
Proteins and amino acids	+	-

Note: "+" indicates presence and **"-"** indicates absence of the phytochemical constituents.[12]



Figure 2: Preliminary phytochemical analysis of EECA and EECB

***In vitro* antioxidant assay**

In vitro antioxidant assays were performed for EECA and EECB with nitric oxide, hydroxyl radical and hydrogen peroxide radical scavenging assays.[14]

Hydroxyl radical scavenging assay:

EECA, EECB and standard drug ascorbic acid showed significant antioxidant action with hydroxyl radical scavenging assay. The assay was carried out in triplicate and percentage inhibition was represented as [MEAN ± SEM].[15]

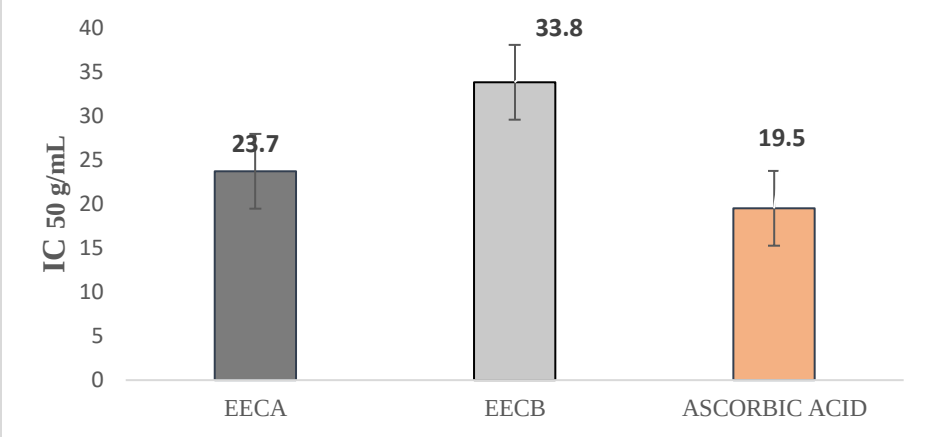

Figure 3: Hydroxyl radical scavenging assay of EECA, EECB & Ascorbic acid

Table 3: Effect of EECA and EECB on Hydroxyl radical scavenging assay

S. No.	Compound	IC_{50}
1.	EECA	23.7
2	EECB	33.8
3.	Ascorbic acid	19.5

Nitric oxide radical scavenging assay

EECA, EECB and standard drug ascorbic acid showed significant antioxidant action with nitric oxide radical scavenging assay. The assay was carried out in triplicate and percentage inhibition was represented as [MEAN ± SEM].[16]

Table 4: Effect of EECA and EECB on Nitric oxide radical scavenging assay

S. No.	Compound	IC_{50}
1.	EECA	29.6
2	EECB	35.45
3.	Ascorbic acid	28.7

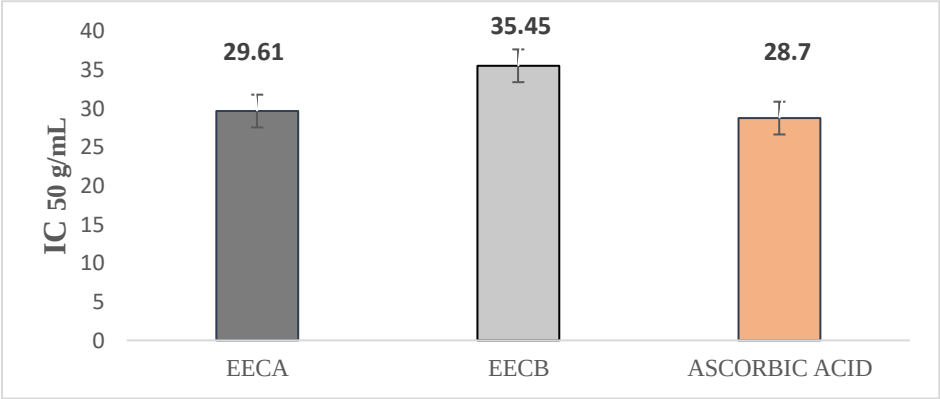

Figure 4: Nitric oxide scavenging assay of EECA, EECB & Ascorbic acid

Hydrogen peroxide radical scavenging assay:

EECA, EECB and standard drug ascorbic acid showed significant antioxidant action with Hydrogen peroxide radical scavenging assay. The assay was carried out in triplicate and percentage inhibition was represented as [MEAN± SEM].[17]

Table 5: Effect of EECA and EECB on Hydrogen peroxide radical scavenging assay

S. No.	Compound	IC_{50}
1.	EECA	28.3
2	EECB	35.2
3.	Ascorbic acid	25.4

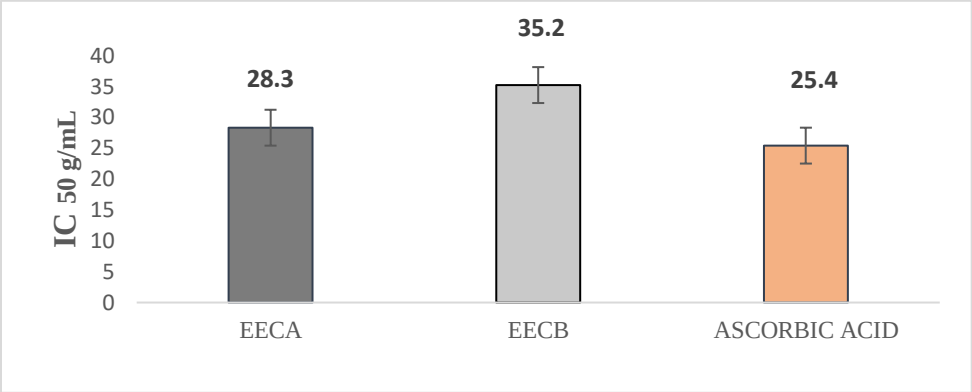

Figure 5: Hydrogen peroxide scavenging assay of EECA, EECB & Ascorbic acid

5.4 *In vitro* antibacterial activity

Table 6: Specific medias used for growth of bacteria

S.NO	NAME OF THE BACTERIA	SPECIFIC MEDIA
1.	**Streptococcus pneumoniae**	Nutrient agar contains Liebig bouillon, peptone, cane sugar and agar
2.	**Pseudomonas aeruginosa (ATCC-10145)**	Trypticase soya Agar with cephalothin-sodium fusidate cetrimide (CFC) Supplements.

Evaluating Antibacterial activity of EECA & EECB

The result of this experiment was presented in Table: 5.7 below, the *in vitro* antibacterial activity of ethanolic extract of *Coleus amboinicus* and *Coleus blumei* leaves was evaluated by agar disc diffusion method and using selected Gram – positive and Gram – negative bacteria. [18]

The diameter of the inhibition zone (ZI) and minimum inhibitory concentration (MIC) are shown in Table 5.7 and 5.8. The data indicate that the extracts exhibited the activity against the investigated food pathogens. Gram-positive bacteria *Streptococcus pneumoniae* demonstrated higher susceptibility than Gram-negative *Pseudomonas aeruginosa*. An important fact is that the both extracts of *Coleus amboinicus* and *Coleus blumei* showed antibacterial activity against *Streptococcus pneumoniae* (MIC = 8 mg/ml & 10 mg/ml) respectively, one of the gram-positive bacteria that causes food poisoning the most frequently.

On the other hand, a weak antibacterial activity was found against *Pseudomonas aeruginosa* (MIC = 15 mg/ml & 17 mg/ml) respectively.

The majority of research on the mechanism of phenolic compounds was on how these chemicals affected the structure and function of cellular membranes, producing swelling and increasing permeability in some cases.

Reduced ATP levels, the loss of the proton motive force, and the loss of the cellular pH gradient all seem to contribute to the increases in cytoplasmic membrane permeability, which ultimately results in cell death.

The primary site of action for flavonoids on gram-positive bacteria is the cell membrane, where they most likely cause phospholipid bilayer disruption, inhibit the respiratory chain or ATP generation, or a combination of these effects. [19]

Table 7: Mean Diameter of Inhibition zone

Pathogenic microorganisms	EECA	EECB	Chloramphenicol 12.5 mg/kg
Streptococcus pneumoniae	14 mm	12 mm	18 mm
Pseudomonas aeruginosa	10 mm	8 mm	30 mm

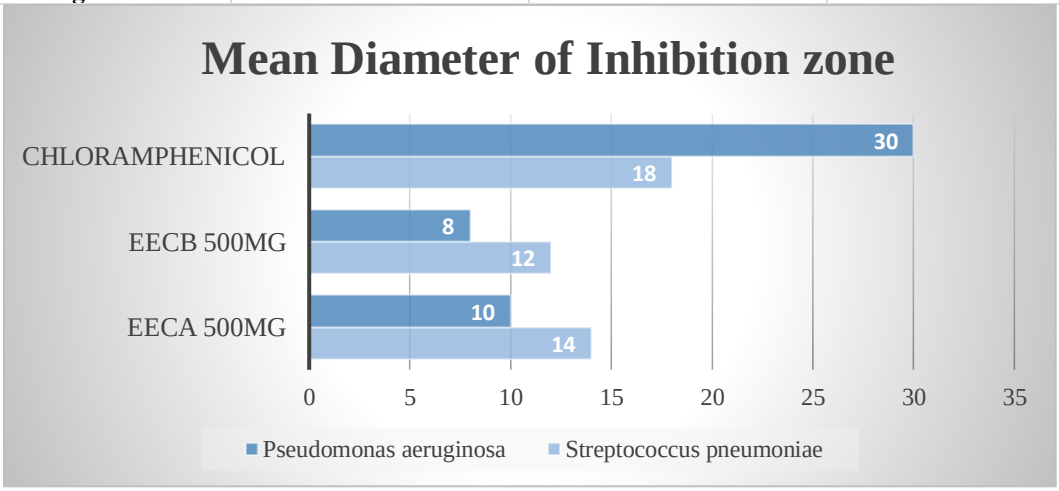

Figure 6: Mean Diameter of Inhibition zone

Table 8: Minimum inhibitory concentration

Pathogenic microorganisms	EECA (mg/ml)	EECB (mg/ml)	Chloramphenicol 12.5 mg/kg
Streptococcus pneumoniae	8	10	5
Pseudomonas aeruginosa	15	17	8

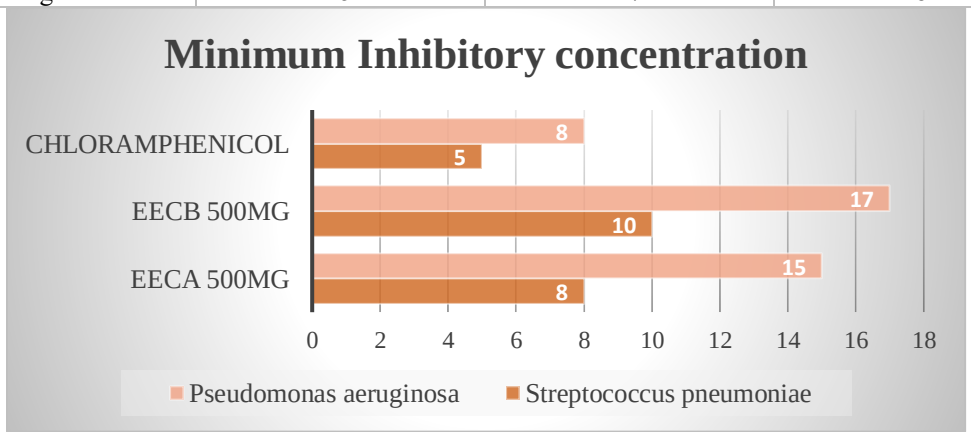

Figure 7: Minimum Inhibitory concentration

DISCUSSION:

Since ancient times, traditional medicine has made use of plants. India is aptly referred to as the "Botanical Garden of the world" and is arguably the world's leading producer of medicinal herbs. Medicinal plants and their products are the oldest and tried as healthcare products. Their importance is growing not only in developed countries but also in many developing countries.

The allopathic drugs are good in onset and have good therapeutic activity but side effects associated are poignant. Thus, the herbal drugs obtained from natural sources with least or no side effects and having similar and better therapeutic activity. Herbal remedies are successful because of the broad therapeutic activities and safety profile of these drugs. In view of above claims and facts, works was undertaken to prove the usage of the plant in the evaluating of antioxidant and antimicrobial activity scientifically.

For future reference, the leaf extracts' physical condition and percentage yield were noted. The percentage yield of the ethanolic extract of *Coleus amboinicus* was considerably good when compared to that of *Coleus blumei.*
Several attempts taken by researchers to determine the chemical constituents present in particular plants. Some of them have already reported regarding the current plants which are selected for present study i.e., *Coleus amboinicus* and *Coleus blumei* which are the rich source of organic compounds with diverse chemical composition.
In order to gather scientific evidence regarding the above claim, that the plant is having specific chemical constituents, we have followed the established procedure of specific test as reported by several practical manuals.

The presence of alkaloids, flavonoids, tannins etc., in plant extracts prepared in solvent such as ethanol has been reported by various workers. The leaves contain glycosides, flavonoids, saponins, phenolic compounds, tannins.

In vitro antioxidant activity

The primary feature of an antioxidant is its capacity to capture free radicals. Phenolic acids, polyphenols, and flavonoids are examples of antioxidant chemicals that scavenge free radicals such peroxide, hydroperoxide, or lipid peroxyl, hence inhibiting the oxidative pathways that cause degenerative illnesses. Antioxidants shield cells from harm resulting from unregulated ROS production and the lipid peroxidation, protein degradation, and DNA breakage that follows.

Hydroxyl radical scavenging assay

Almost every molecule present in living cells is susceptible to damage by the hydroxyl radical, a highly reactive free radical that is produced in biological systems. These radicals have the ability to attach to DNA nucleosides and break DNA strands. Further, as these species obtain hydrogen atoms from unsaturated fatty acids, they are thought to be quick initiates of the lipid peroxidation process. Lipid peroxidation prevention appears to be closely linked to the hydroxyl radical reducing capabilities of EECA and EECB, so EECA showed good ROS radicle scavenging activity. The antioxidant capability of EECA might be due to the presence of tannins and flavonoids and phenolic compounds.

Nitric oxide radical scavenging assay

Two free radicals that result from the interaction of nitric oxide (NO) and reactive oxygen species (ROS) are NO and RNS. Numerous cellular components have their structure and functional behaviours changed by these substances. The ability of the EECA to directly compete with oxygen and nitrogen oxides in the reaction mixture to prevent the generation of nitrite oxide served as an indicator for its nitric oxide scavenging capability.

Hydrogen peroxide radical scavenging assay

H_2O_2 is a weak oxidizing agent that interacts easily with the thiol group [-SH] to render the enzymes inactive. Many kinds of bodily cells can easily absorb H_2O_2. It interacts with $[Fe^{2+}]$ / $[Cu^{2+}]$ (ferrous / cuprous) after it enters the cell to release H_2O_2 free radicals. H_2O_2 free radical production is the cause of its harmful effects, preventing difficulties brought on by H_2O_2 free radicals, body cells shouldn't permit excessive accumulation of H_2O_2.

In the hydrogen peroxide radical scavenging assay, the IC_{50} value for the EECA, EECB and standard drug ascorbic acid was found to be 28.3, 35.2 & 25.4 µg/mL respectively.

In vitro antibacterial activity

Anti – bacterial activity of these medicinal herbs, if translated into clinical practice would lead to the development of indigenous, chemical free, cost effective, and holistic oral hygiene aids, which can be incorporated into various oral hygiene formulations like dentrifices, mouth rinses, gum paints etc., With continued growth of biotechnology and increasing tools for validation of the bioactive compounds, the potential is high that one day our food will serve as

medicine. The present study extract of *Coleus amboinicus* and *Coleus blumei* were prepared and antimicrobial activity with minimum inhibiting concentration for better application on food industry.

Because of the various therapeutic properties and easy availability of the plants were selected for the present study to evaluate its antimicrobial efficacy on pathogenic microorganisms.

The present study employed Agar well diffusion method to evaluate the antibacterial efficacy of two medicinal plant extracts since it is more reliable and acceptable. The results obtained showed that diameter of zone of inhibition of ethanolic extract of *Coleus amboinicus* is more compared to that of *Coleus blumei.* The literatures showing antimicrobial effect of *Coleus amboinicus* and *Coleus blumei* are scanty.

CONCLUSION:

In Conclusion, this present study investigated the antioxidant and antimicrobial activity of leaves extract of *Coleus amboinicus* and *Coleus blumei*.

Leaves of *Coleus amboinicus* and *Coleus blumei* were collected, authenticated, dried, powdered and extracted with ethanol by maceration process. The yield of *Coleus amboinicus* plant extract was found to be 12.0% and *Coleus blumei* was found to be 8.6%. Phytochemical constituents such as alkaloids, glycosides, flavonoids, phenolic compounds are present in both of the extracts

EECA has shown significant invitro antioxidant action with Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and Hydrogen peroxide radical scavenging assay.

The ethanolic extract of *Coleus amboinicus* showed greater antimicrobial efficacy than ethanolic extract of *Coleus blumei.*

Anti – bacterial activity of these medicinal herbs, if translated into clinical practice would lead to the development of indigenous, chemical free, cost effective, and holistic oral hygiene aids, which can be incorporated into various oral hygiene formulations like dentrifices, mouth rinses, gum paints etc., With continued growth of biotechnology and increasing tools for validation of the bioactive compounds, the potential is high that one day our food will serve as medicine.

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