Advances in Bioresearch
Adv. Biores., Special Issue (2) 2025: 122-130

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585. SPL2.25.122130

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Effects of plant extracts of *Coleus amboinicus* against Antioxidant and antimicrobial activity

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ABSTRACT
To aim of this study was to investigate the effects of Coleus amboinicus by analyse the anatomy, morphology, antioxidant activity and antimicrobial activity. Coleus amboinicus plant extract was collected, air dried, powdered and the plant extract was made with ethanol, hexane and ethyl acetate and tested for phytochemical analysis, antimicrobial and antioxidant activity. The phytochemical analysis was confirmed by significant presence of alkaloids, saponins, Phenols, tannins, terpenoids and flavonoids. The antimicrobial activity was tested against the three plant extracts like hexane, ethyl acetate and ethanol was tested against E-coli, Pseudomonas and Staphlococcus. The zone of inhibition was observed clearly in hexane and ethyl acetate plant extract when compare to ethanol. Similarly, the plant extract was tested against antioxidant activity, the hexane and ethyl acetate showed good scavenging activity when compare to ethanol. In conclusion, hexane and ethyl acetate plant extract of Coleus amboinicus could be act as a good antioxidant scavenger and has good antimicrobial activity by the presence of phytoconstituents.
Keywords: *Coleus amboinicus, phytochemical analysis, antimicrobial and antioxidant activity*

Received 0101.2025 Revised 11.02.2025 Accepted 20.03.2025

How to cite this article:
S. Sivaranjani, V.Ramabhai, Vijaya S, A.Sarumathi, D.K.Usha and P. Sathiyavani. Effects of plant extracts of *Coleus amboinicus* against Antioxidant and antimicrobial activity. Adv. Biores. Special Issue [2] 2025. 122-130

INTRODUCTION

The art and science of utilizing the medicinal properties of the herbs in Ayurveda is well documented. Increased incidences of documented side effects of the allopathic drugs with ever increasing cost of the same has paved way for a renewed interest in the concept of Ayurveda. The medical properties of the plants are based on the antioxidant, antipyretic, antimicrobial and/or analgesic properties of certain phytochemicals in them [1]. Herbal medicine has become a very safe, non-toxic, and easily available source of cancer-treating compounds. Herbs are believed to neutralize the effects of diseases in a body because of various characteristics they possess [2]. It is a polyhydroxy phenolic compound and a natural antioxidant that can be obtained from a variety of natural products i.e., grapes, strawberries, bananas, green tea, and vegetables [3].

Plant phytochemicals possess antimicrobial activity against a wide range of bacteria, yeast and mold, but vary in quantity and quality depending on the bioactive constituents. Its wide range of chemical diversity containing phytochemicals such as terpenes, alcohols, acetones, phenols, acids, aldehydes and esters is often used as components in the pharmaceutical industry. Bacteria are prokaryotic microorganisms usually found on the surface of the skin, mucosal layer and intestinal tract of humans and animals [4]. The genus *Staphylococcus* is one example mostly he of common bacteria found to reside on the skin and in mucous membrane and is armless. Yet, there are dangerous bacteria classified as human pathogens, causing contagious diseases with a fatal prognosis. These bacteria are usually inhibited by taking antibiotics. However, recently drug resistance developed by microbes is increasingly observed and is a global phenomenon. Therefore, continuous exploration of medicinal plants for effective drugs is an

ongoing process. From early years, *P. amboinicus* has been used as folk medicine to fight pathogenic bacterial activity. In Cuba, a decoction of the leaves was given to patients suffering from chronic cough or tuberculosis and later scientific studies revealed *P. amboinicus* having anti-Mycobacterium tuberculosis activity [5].

The plant *Coleus amboinicus* is an Indian borage, dicotyledonous plant belonging to the family Lamiacea [6,7]. It is a large succulent aromatic perennial herb, branched, fleshy highly aromatic pubescent herb with distinctive smelling leaves. The plant is distributed throughout India and also cultivated in the gardens. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy [8] renal and vesical calculi, cough, chronic asthma [9], hiccough, bronchitis, helminthiasis, colic, convulsions and epilepsy [10]. It is used to treat cold and cough as well as arthritic inflammations [11]. It has insect repellent properties [12] and another member of the Coleus genus, *C. aromaticus*, has been found to cause reduction in egg laying capacity, retard in adult emergence and weight loss in the pulse beetle *Callosobruchus maculatus* [13]. A moderate allelopathic effect of the powdered leaves of *C. amboinicus* against the water hyacinth is also have in it [14]. Detailed Studies demonstrated the "fungistatic" properties of the essential oil of this plant [15]. The Phytochemical study confirmed the presence of various flavonoids like quercetin, apigenin, luteolin, salvigening enkwanin and volatile oils in the leaves [16]. Ethanolic and aqueous leaf extracts of the plant has been found possess significant diuretic activity [17]. The plant is also known to contain the constituents responsible for antioxidant and anti bacterial activity [18].

MATERIAL AND METHODS

CLASSIFICATION:

Division: Magnoliophyta
Kingdom: Plantae
Clade: Angiosperms
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: Coleus
Species: aromaticus

STUDY DESIGN

Experimental design*: In vitro* study laboratory setting, Biotechnology lab, Bon secours college for women

COLLECTION OF PLANT MATERIAL

Coleus aromaticus was collected from the uranthairayan kudikadu, Tamilndu, India (Lat N10◦ 35' 53124"; Long E 79'16" 118452" 26/01/2021; 09:55 Am) . The collected samples were washed thoroughly with water and dried in shade at room temperature and powdered using blender to coarse powder. Then they are stored in well closed light resistant container until further use.

Fig.1: Collection of Leaves

MORPHOLOGY

Leaf samples were subjected for morphological examination based on colour, odor, taste, size and shape.

ANATOMY

The most common practice used in the study of plant tissues is the preparation of temporary or permanent histological slides that can be examined with the aid of an optical microscope. Fresh leaves can be studied conventionally by examining the nature and arrangement of cells and tissues in thin section cut transversely. Under the Light microscope the tissues are examined.

EXTRACTION METHODS USED ON *COLEUS AMBOINICUS*

The dried leaf samples are placed into a blender to be grounded into coarse powder. Three solvents were arranged in increasing polarity; hexane, ethyl acetate, ethanol are taking 100ml all the reagents are equally transferred into the conical flask and the leaf powder was added to 10 gm each of solvents then they are transferred into orbital shaker for one day using maceration extraction procedure. Then the mixtures were closed with cotton and wrapped in aluminum foil to avoid evaporation and the mixture was transferring the flasks were placed on a platform shaker at 5000 rpm. After one day of soaking in solvent, the mixtures were filtered using Whatman filter paper. The samples were collected and they are evaporated at room temperature condition for four or five days after evaporating the small quantity of sample collected then it is transfer the tube and stored the sample for further process such as phytochemical Analysis, anti-microbial activity and anti-oxidant activity.

PHYTOCHEMICAL ACTIVITY

Phytochemical tests for the screening and identification of bioactive chemical constituents in the *Coleus amboinicus* was carried out with the extracts using the standard procedure as described. For each test, 1 mL of each solvent extract was used for analysis. The following methods are followed with the standard protocol [19,20].

TEST FOR ALKALOIDS

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids

TEST FOR SAPONINS

Extract was placed in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

TEST FOR PHENOLS AND TANNINS

Extract was mixed with 2 mL of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

TEST FOR TERPENOIDS (SALKOWSKI'S TEST)

Extract was mixed with 2 mL of chloroform. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish-brown coloration of the interphase was formed to show positive results for the presence of terpenoids.

TEST FOR FLAVONOIDS (SHINODA TEST).

Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids.

ANTIOXIDANT ACTIVITY:

DPPH FREE RADICAL SCAVENGING ACTIVITY

The free-radical scavenging activity of all the three extracts was measured as decrease in the absorbance of methanol solution of DPPH. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493 and5 ml of this stock solution was added to 1 ml of Hexane, Ethanol & Ethyl acetateextract solution at different concentrations (20,40,60,80,100µg/ml). After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 µg/ml) [21]. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\%\text{Anti-radical activity} = \frac{\text{Control Absorbance - Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

ANTIMICROBIAL ACTIVITY

Antimicrobial Potential of the Extracts the leaf extracts of *Coleus amboinicus* were tested against *Staphylococcus aureus, Escherichia coli and Pseudomonas aregnosa*. The 100 µl of overnight grown bacterium diluted to 103 cfu/ml was inoculated into the flask containing 20 ml nutrient agar and different concentrations of leaf extracts, and the contents were poured into sterilized petriplates. The plates were observed for bacterial growth after overnight incubation at 37˚C and minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound capable of inhibiting the complete growth of bacteria [22].

STATISTICAL ANALYSIS

The data obtained in the present study was subjected to standard statistical analysis by using Microsoft excel data to find out the STD deviations.

RESULTS

The morphological observation of *Coleus amboinicus* shows the size and colour of the leaf.

Fig 2 Morphological Observation of Leaf

ANATOMICAL STUDY

To understand the internal structure of the cladodes of *Coleus amboinicus*, a cross section was taken, stained with saffranin [23] and viewed under Nikon fluorescent microscope.

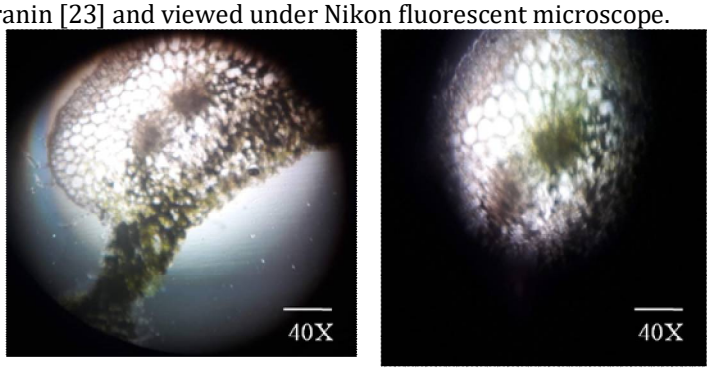


Fig 3: Microscopic observation of *Coleus amboinicus* with 40x magnification

PHYTOCHEMICAL ANALYSIS

Phytochemical Analysis

Phytochemicals	Hexane	Ethyl Acetate	Ethanol
Test for alkaloids	+VE	+VE	+VE
Test for Saponins	+VE	+VE	-VE
Test for Phenols and Tannins	+VE	+VE	+VE
Test for Terpenoids (Salkowski's Test)	-VE	-VE	-VE
Test for Flavonoids (Shinoda Test)	+VE	+VE	+VE

KEYS: +VE= Positive, -VE= Negative

Table 1: Phytochemical screening of *Coleus amboinicus*

Phytochemical screening of the crude Hexane, Ethanol and Ethyl Acetate extract of the revealed the presence of flavonoids, alkaloids, tannins, steroids, saponins and phenolic compounds. Flavonoids and flavones are widely distributed secondary metabolites.

ANTIOXIDANT ACTIVITY:

DPPH FREE RADICAL SCAVENGING ACTIVITY

The free-radical scavenging activity of all the three extracts was measured as decrease in the absorbance of methanol solution of DPPH. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493 and5 ml of this stock solution was added to 1 ml of Hexane, Ethanol & Ethyl acetate extract solution at different concentrations (20,40,60,80,100µg/ml). After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 µg/ml). The Hexane and Ethyl Acetate showed good scavenging activity when compare to Ethanol as shown in the Graph 1

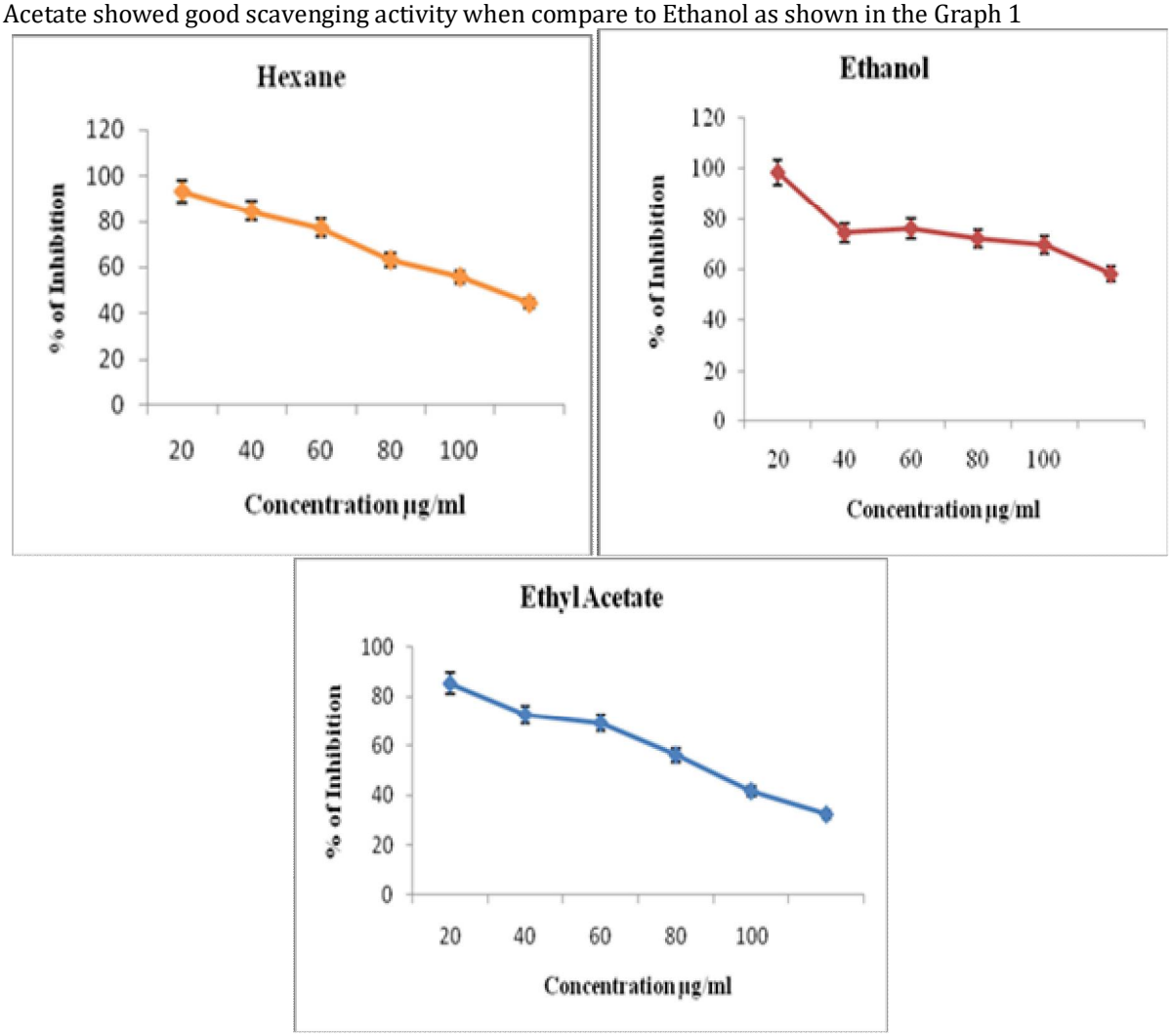
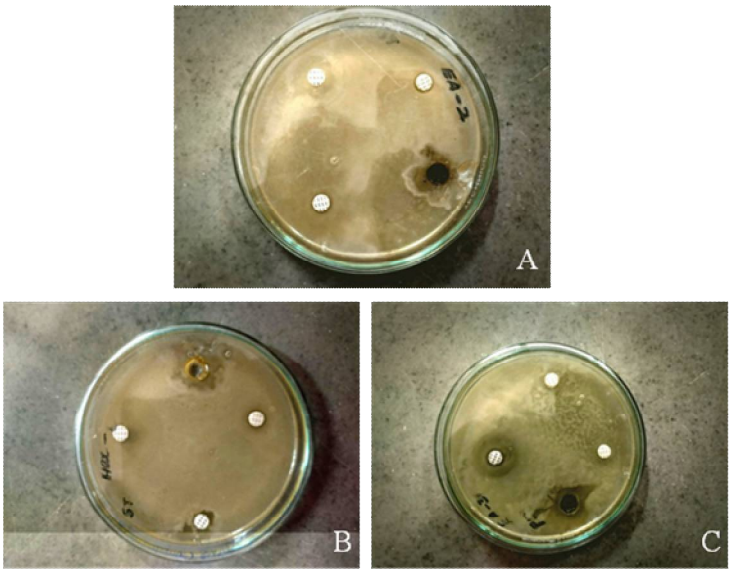


Fig 4: DPPH radical scavenging activity of crude extract from *Coleus amboinicus*

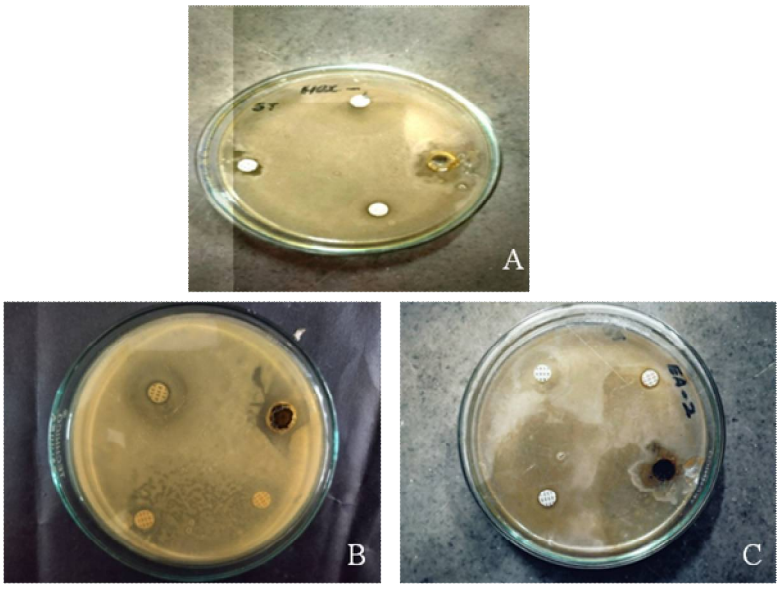
ANTIMICROBIAL ACTIVITY

The inhibitory effect of extracts from *E-coli, Pseudomonas and Staphlococcus* which were tested against diverse enteric pathogens at a concentration of 10 mg ml^{-1}, resulted in different extents of inhibition in Fig. 5-7. The strains *E.coli, Pseudomonas* and *Staphlococcus* were inhibited by the extract of Hexane, Ethyl Acetate and Ethanol. However, extract of Ethanol did not exhibit any potential antimicrobial activity against the 3 tested pathogens whereas Hexane and Ethyl acetate exhibited inhibitory activity.



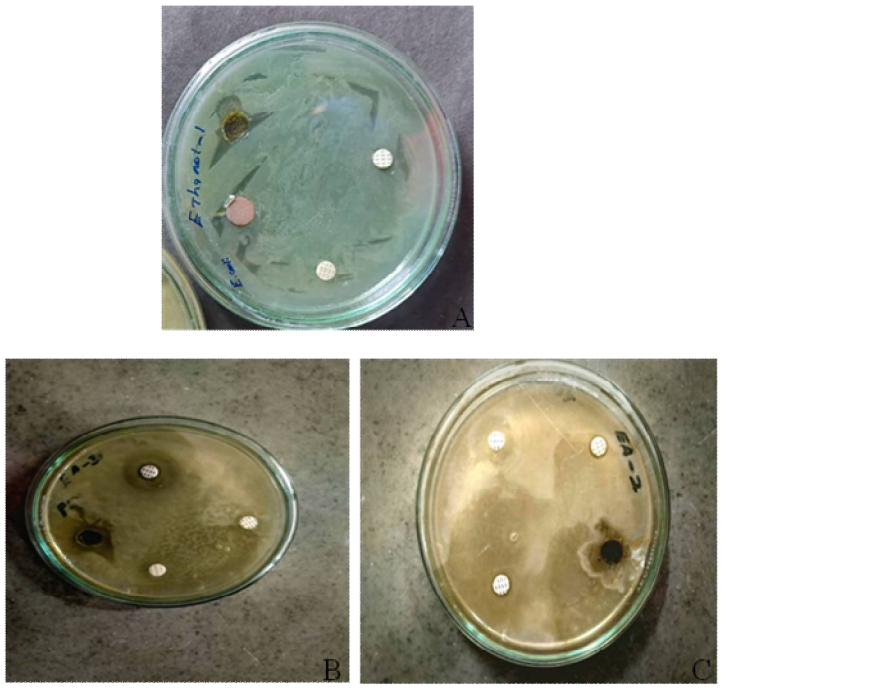
Antimicrobial activity of Ethanol extract from *Coleus amboinicus*
A – Treated with Ethanol extract of *Coleus amboinicus* with *E-Coli*
B - Treated with Ethanol extract of *Coleus amboinicus* with *Staphylococcus*
C- Treated with Ethanol extract of *Coleus amboinicus Pseudomonas*

Fig 5: Antimicrobial activity of ethanol extract from *Coleus amboinicus*



Antimicrobial activity of Ethyl Acetate extract from *Coleus amboinicus*
A – Treated with Ethyl Acetate extract of *Coleus amboinicus* with *E-Coli*
B - Treated with Ethyl Acetate extract of *Coleus amboinicus* with *Staphylococcus*
C- Treated with Ethyl Acetate extract of *Coleus amboinicus Pseudomonas*

Fig 6: Antimicrobial activity of ethyl acetate extract from *Coleus amboinicus*



Antimicrobial activity of Hexane extract from *Coleus amboinicus*
A – Treated with Hexane extract of *Coleus amboinicus* with *E-Coli*
B - Treated with Hexane extract of *Coleus amboinicus* with *Staphylococcus*
C- Treated with Hexane extract of *Coleus amboinicus Pseudomonas*

Fig 7: Antimicrobial activity of Hexane extract from *Coleus amboinicus*

DISCUSSION

Phytochemical constituents in the various part of the plant vary significantly. Several medicinal plants are used in traditional medicines for curing many diseases. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. In this study it shows that the phytochemical screening of the crude Hexane, Ethyl Acetate and Ethanol extract of the revealed the presence of flavonoids, alkaloid, tannins, steroids, saponins and phenolic compounds. Flavonoids and flavones are widely distributed secondary metabolites The results of the phytochemical test carried out on the extracts. Preliminary phytochemical screening revealed the presence of Saponins, flavonoids, terpenes, cardenolides and phitobatamin in aqueous extract but absent in ethanol crude extract, Tannin, phenol, glycoside and steroids were also present in ethanol crude extract but absent in ethyl acetate crude extract, phenol was less abundant in ethyl acetate crude extract. Cardenolides was much abundant in ethyl acetate crude extract, found minute in aqueous extract but absent in ethanol crude extract [23].

Our study shows that the free-radical scavenging activity of all the three extracts was measured as decrease in the absorbance of methanol solution of DPPH. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493 and5 ml of this stock solution was added to 1 ml of Hexane, Ethanol & Ethyl acetate extract solution at different concentrations (20,40,60,80,100µg/ml). After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 µg/ml). The Hexane and Ethyl Acetate showed good scavenging activity when compare to Ethanol. The leaves of *P. amboinicus* were extracted by three different methods viz. sequential, hydroalcoholic (HAE) dependant. The DPPH radical scavenging activity of the plant extracts was in the order of ethyl acetate > acetone > HAE > FD > methanol > hexane. The DPPH scavenging activity of the HAE in the present study was 27.62% at 100 ppm, which was at par with the DPPH scavenging activity of the 50% ethanolic extract of Indian borage leaves (30.4%) at similar concentration [24].

The inhibitory effect of extracts from *E-coli, Pseudomonas and Staphlococcus* which were tested against diverse enteric pathogens at a concentration of 10 mg ml^{-1}, resulted in different extents of inhibition in Fig.4,5 and 6. The strains *E. coli, Pseudomonas* and *Staphlococcus* were inhibited by the extract of Hexane,

Ethyl Acetate and Ethanol. However, extract of Ethanol did not exhibit any potential antimicrobial activity against the 3 tested pathogens whereas Hexane and Ethyl acetate exhibited inhibitory activity. The results also indicate that the plant extracts have no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the organisms. The observed inhibition of Gram positive bacteria, *Bacillus cereus and Staphylococcus aureus,* suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol or ethanol as the solvent. Comparisons with related data from the literature indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present finding, P. guajava leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by *B. cereus* and *S. aureus* [25].

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