PHYTOCHEMICAL SCREENING OF

PLECTRANTHUS AMBOINICUS

DEPARTMENT OF BIOCHEMISTRY

T.K.M COLLEGE OF ARTS AND SCIENCE

(Affliated to University Of Kerala)

KOLLAM-5

APRIL 2022

PHYTOCHEMICAL SCREENING OF *PLECTRANTHUS AMBOINICUS*

PROJECT REPORT

Dissertation Submitted to the University of Kerala in partial fulfillment of the requirement for the award of the Degree of Bachelor of Science in Biochemistry

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DECLARATION

We hereby declare that the project titled 'Phytochemical Screening of *Plectranthus amboinicus* ' is based on the original work carried out by us under the supervision of Dr. Hari Sankar.H.S Assistant Professor, Department of Biochemistry, T.K.M. College of Arts and Science, Kollam.

We also declare that the project report hasn't been submitted either partly or completely for the award of any other degree or diploma or other similar titles of any other University/ Institutions in India or abroad.

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CERTIFICATE

This is to certify that the dissertation entitled 'Phytochemical Screening of *Plectranthus amboinicus* ‘submitted to the University of Kerala in partial fulfilment of the requirements for the award of the degree of Bachelor of Science in Biochemistry, is a record of original research work carried out by the following candidates under my guidance and supervision.

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It is also certified that no part thereof has been presented for the award of any other degree or diploma or other similar titles of any other university .

Dr. Hari Sankar. H. S

Project Guide

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We would like to show our heartfelt gratitude to our guide Dr. Hari Sankar. H.S, Assistant Professor, Department of Biochemistry, T.K.M. College of Arts and Science, Kollam for his guidance and encouragement that lead us to the successful completion of the work.

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1.INTRODUCTION

At present, plant-based medicines are widely employed in various public health practices throughout the globe as they are safe and cost-effective, and efficiently combat various deadly diseases and help in maintaining good health(Kumara Swamy *et al*.,2011). Herbal medicines are very commonly used in Unani, Ayurveda, Sidda, Folk and other traditional practices of healthcare management (Swamy *et al.*,2015).

According to the estimation by the World Health Organization, about 80% of people on the globe are still dependent on traditional herb-based medications due to their low cost, easy accessibility and likely negligible side effects in comparison to allopathic medicines . Certainly, many of the leading active drug molecules of plants and their derivatives used presently in allopathic medicine are mainly due to the understanding of traditional medical practices for curing diseases . Modern drug discovery research is governed by natural plant-based compounds and their products, followed by synthetic chemical drugs(Swamy *et al*., 2015).

Currently, natural products are considered as a major source of medicaments and hence they are extensively used by pharmaceutical industries. This has led towards increased global demand for medicinal plants in the modern era of natural medicine, leading to exploration and exploitation of new plant sources for their medicinal properties.

The Lamiaceae members of plant species belonging to commercially important genera, such as *Plectranthus, Salvia, Ocimum* and *Mentha,* are attributed with a rich diversity of ethnobotanical benefits. More than 300 species of Plectranthus are reported all over the tropical and warm regions of the old world,

including Asia, Africa and Australia (Sandhya *et al*.,2011). In over 85% of the literature, documentation of Plectranthus is on the therapeutic values of this genus followed by its nutritional and horticultural properties attributed to its aromatic nature and essential oil producing capability (Mohanty *et al*., 2014).

Plectranthus amboinicus (Loureiro) Sprengel is one of the most documented species in the family Lamiaceae. *P. amboinicus*, also commonly known as Indian borage, and is a fleshy, succulent herb famous for its distinct oregano-like flavor and odor. It is one of the most cited species in the Lamiaceae family, especially for its medicinal properties, accounting for 68% of all customary applications of this genus (Kumara *et al*.,2012). This herb is widely used by indigenous people of tropical rain forests, either in folk medicine or for culinary purposes. This is mainly due to its natural production of an essential oil with high amounts of bioactive compounds such as Carvacrol , Thymol , β-Caryophyllene, α-Humulene, γ-Terpinene, p-Cymene, α-Terpineol and β-Selinene in its leaves . These biochemical components exhibit various biological properties and are widely used in folk medicine to treat conditions like cold, asthma, constipation, headache, cough, fever and skin diseases (Retief *et a*l.,2000).

The leaves of the plant are often eaten raw or used as flavoring agents, or incorporated as ingredients in the preparation of traditional food. The chopped leaves are also used as a substitute for sage (*Salvia officinalis*) in meat stuffing.

(Alasbahi *et a*l.,2010) With this background, the present review was undertaken to present complete facts on the multifarious medical benefits of *P. amboicus*. This review is a compiled survey of information on various aspects of *P. amboinicus* including botany, distribution, wild relatives, phytochemistry, medicinal and nutritional properties and other benefits.

Plectranthus amboinicus

Cuban oregano

Flower

2.REVIEW OF LITERATURE

Plectranthus Amboinicus is commonly known as Indian/ country borage(Lukhoba *et al*.,2006). It is recorded in the Indian system of medicine as one of the sources of *Pashanabheda*. It is large succulent aromatic perennial herb, shrubby below. It is found throughout India, Ceylon and Moluccas. The leaves of the plant are bitter, acrid and were being widely used traditionally for various purposes. The plant has been worked out very well and isolated several chemical constituents and had shown various biological properties(Castillo *et al*.,2001). This review is an effort to compile all the information reported on its macroscopic, microscopic features, nutritional content, phytochemistry, pharmacology and therapeutic uses.

Table 1: Scientific Classification

Kingdom	Plantae
Phylum	Angiosperms
Class	Eudicots
Subclass	Asterids
Order	Lamiales
Family	Lamiaceae
Tribe	Ocimeae
Genus	Coleus
Species	C.amboinicus

2.1 GEOGRAPHICAL DISTRIBUTION

Plectranthus Amboinicus is native to Southern and Eastern Africa, from South Africa (KwaZulu-Natal) and Eswatini to Angola and Mozambique and north to Kenya and Tanzania, where it grows in woodland or coastal bush, on rocky slopes and loamy or sandy flats at low elevations. From Southern Africa it would have been carried by Arabs and other traders to Arabia, India and Southeast Asia along the Indian Ocean maritime trade routes. (Singh *et al.*, 2002).The plant also currently grows in mainland India. The plant was later brought to Europe, and then from Spain to the Americas, hence the name Spanish thyme(Murthy *et al.*, 2009).

2.2 BOTANICAL DESCRIPTION

P. amboinicus is a member of the family, Lamiaceae or mint family. The paleotropical oil-rich genus, plectranthus belongs to the subfamily Nepetoideae. It comprises about 300 species of annual or perennial herbs or subshrubs which are often succulents(Gonçalves *et al*.,2012.). Many species of Plectranthus have economical and medicinal values. Among them, *P. amboinicus* is one the most important aromatic medicinal succulent plants that possess distinctive smelling leaves with short soft erect hairs(Bhatt P *et al.*, 2012).

2.3 MORPHOLOGICAL FEATURES

P. amboinicus is a succulent shrub with a tendency for climbing or creeping. This sprawling large succulent herb is fleshy and highly aromatic. The fleshy stems grow about 30–90 cm, either with long rigid hairs . Leaves are undivided, and very thick; they are pubescent (thickly studded with hairs) , with the lower surface possessing the most numerous glandular hairs, giving a frosted appearance . The taste of this leaf is pleasantly aromatic with an agreeable and refreshing odor. Flowers are on a short stem (shortly pedicelled), pale purplish. Flowers have a bell shaped calyx. The

corolla is pale purplish and five times longer than the calyx. Fruit nutlets are smooth, pale brown. *P. amboinicus* rarely flowers and seeds are difficult to collect.

2.4 NUTRITIONAL COMPOSITION

Herbs have been used extensively in culinary purposes since ancient times. Many delicious cuisines we enjoy contain various dietary herbs to increase the taste and flavor of the food. Herbal plants also have lots of health benefits attributed to their nutritional content. Hence, *P. aromaticus* can be a good source of nutritive compounds which help to enhance the taste and also prolong the shelf life of food products. A study validates the presence of high minerals, precisely calcium and potassium, at 0.158% and 0.138%, respectively. These minerals are necessary to build and maintain strong bones and to retain normal function of heart, kidneys, muscles and nerves. *P. amboinicus* also has a significant content of iron at 0.262%. Iron is an important component of hemoglobin aids red blood cells to carry oxygen throughout the body. Hemoglobin represents about two-thirds of the body's iron and its deficiency causes anemia. Adding to that, this plant also contains total Xanthophylls (0.356 mg/g of dry weight of the plant) which consist of Neoxanthin, Violaxanthin, Leutin, Zeaxanthinics. It also has α-Carotene (0.157 mg/g of dry weight) and β-Carotene (0.0035 mg/g of dry weight) (Khare R.S et al., 2011). All this makes *P. amboinicus* a unique dietary supplement.

2.5 ORIGIN AND WILD RELATIVES

The name *Plectranthus* derives from the Greek words “plectron”, meaning spur, and “Anthos”, meaning flower, in reference to the spur-shaped flowers of some members of the genus . Due to lack of precise morphological features to distinguish species within the genus Plectranthus and its closely associated genera, numerous taxonomic problems with the naming of species have resulted in misplacement of species in some closely linked genera such as Coleus, Solenostemon and Englerastrum . The species *P. amboinicus* was originally classified under the genus Coleus but was moved to the Plectranthus genus, although both names are sometimes seen in the literature today. This species also has the greatest number of synonyms (Wagner *et al.*,2001).

2.6 PHYTOCHEMISTRY

A large and growing body of literature has investigated the chemical composition and pharmacological properties of *P. amboinicus*. The literature survey has emphasized the occurrence of different classes of phytocompounds including 76 volatiles and 30 non-volatile compounds(Hassani *et al* .,2001). The chemical profile and the accumulation pattern of bioactive constituents in different parts of the plant and their essential oil content varies depending on various parameters, such as geographical features, climate and different stages of plant material collection. Moreover, the method of extraction and identification can also lead to variations in phytochemical composition. Correct identification, isolation and quantification of phytocompounds is very much necessary in order to understand their pharmacological and biological significance(Roshan P *et al*.,2010). *P. amboinicus* is reported to contain several classes of phytochemicals including monoterepenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids and ester(Khan *et al*.,2013).

2.7ANTIMICROBIAL ACTIVITY

2.7.1 ANTIBACTERIAL ACTIVITY

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. Hot water extract of *P. amboinicus* leaves inhibited growth of pathogens, Escherichia coli and Salmonella typhimurium. This antibacterial activity of plant extracts is most likely due to the combined effect of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents and the generation of hydro peroxide from polyphenols. Further, it was shown that unsterilized ethanolic leaf extract of *P. amboinicus* exhibits antibacterial activity against diabetic wound pathogens, *E. coli, S. aureus, P. mirabilis, P. aeruginosa* and *K. pneumonia.*

P. amboinicus which is rich in carvacrol, has shown an antagonistic effect when used with mouthwash to avoid bacterial growth in the oral cavity (Santos *et al.,* 2016). The alcoholic extract of the leaves have shown the lowest MIC against the oral pathogens *Streptococcus mutans* and *Lactobacillus acidophilus* suggesting the incorporation of this extract in toothpaste or mouth wash preparations (Seham *et al*., 2012).

2.7.2 ANTIFUNGAL ACTIVITY

There is also vast evidence that *P. amboinicu*s plays a crucial role in hindering the growth of disease causing fungus. *P. amboinicus* oil was found to be effective against various fungi tested, as it inhibited the radial growth of mycelia and exhibited broad fungitoxic properties against *Aspergillus flavus, Aspergillus niger, Aspergillusochraceus CFR 221, Aspergillusoryzae, Candida versatilis, Fusarium sp. GF-1019, Penicillium sp., and Saccharomyces cerevisiae* (Murthy *et al*., 2009).

In evaluating the interference of *P. amboinicus* essential oil on the anti-Candida activity of some clinically used antifungals (itraconazole, ketoconazole and amphotericin B), it showed a diverse level of interference. Essential oil exhibited prominent interference on the activity of itraconazole, providing a synergic effect on *C. albicans, C. tropicalis, C. krusei and C. stellatoidea.*

In another research, antifungal activity of the volatile oil was studied against various fungi by an agar well diffusion susceptibility test. In that, growth of *Aspergillusochraceus*, *Aspergillusniger* and *Penicillium sp.* was inhibited by 60%, 64% and 60%, respectively, with 10 µL of volatile oil.

2.7.3 ANTIVIRAL ACTIVITY

A large number of active agents are available for the symptomatic treatment of sexually transmitted diseases (STDs) and acquired immune deficiency syndrome (AIDS). Nevertheless, the emergence of drug-resistant strains and dose-limiting toxic effects has complicated the treatment of these diseases and necessitated the search for new antimicrobial substances from various sources(Staples *et al.*, 2001).

Extracts of *P. amboinicus* were tested and reported to have antiviral activity against Herpes Simplex Virus-1 (HSV1) and anti-HIV inhibition activity. Besides that, ethanolic extract of *P. amboinicus* was reported to have selective antiviral activity on Vero cell lines at 0.1 mg/mL minimum inhibitory concentration when tested against HSV1 and Vesicular Stomatitis viruses (VSV).

2.8 THERAPEUTIC ATTRIBUTES

2.8.1 RESPIRATORY DISORDERS

P. amboinicus is frequently cited in the treatment of chronic coughs, asthma, bronchitis and sore throat in India and the Caribbean Islands. In accordance with that, leaves of *P. amboinicus* had positive bronchodilator activity when tested on guinea pigs. In Eastern Cuba, essential oil from aerial parts of *P. amboinicus* is used to treat asthma(Staples *et al*.,1999). Decoction or juice made from leaves together with other herbs is also taken orally to control asthma. This decoction is also used to treat catarrhal infections where it clears the excessive build-up of thick phlegm or mucus in an airway or cavity of the body (Can Baser *et al*.,2008).

2.8.2ACTIVITY AGAINST DIGESTIVE DISEASES

P. amboinicus is a popular treatment for dyspepsia, indigestion and diarrhea, and a carminative in India and Africa. In India, the leaves of *P. amboinicus* are consumed along with buttermilk, yogurt, or any other probiotic sources during pathogen-induced diarrhea. The leaves are known to have a prebiotic effect on the probiotic bacteria Lactobacillus plantarum. They utilize the phytoconstituents of the leaves by producing necessary metabolic enzymes. A detailed examination by describes the mode of hot water extract (HWE) of *P. amboinicus* leaves on growth inhibition of pathogens *Escherichia coli* and *Salmonella typhimurium* while stimulating the growth of *Lactobacillus plantarum.*

2.8.3 ANTIPILEPETIC ACTIVITY

Various literatures have reported the use of *P. amboinicus* in the treatment of nervous disorders, including epilepsy and convulsions. In Cuba, it is used as an anticonvulsive and antiepileptic drug., tested the anticonvulsant activity of the leaf,

stem and root alcoholic extract separately on Swiss albino mouse models by maximal electric shock-induced seizures and pentylenetetrazole-induced seizures. They found significant anticonvulsant activity in both the models with alcoholic leaf extract recording the highest activity(Pino *et al.*,1996).

2.8.4 ANTITUMORIGENIC ACTIVITY

The antitumor activity of hexane extracts of *P. amboinicus* has been reported. The results showed a significant inhibition on the growth of Sarcoma-180 tumor in mice treated with the hexane extracts of *P. amboinicus*. A dose of 350 mg/kg of hexane extracts of *P. amboinicus* significantly reduced the growth of S-180 tumor with 66% inhibition, while doses of 100, 150 and 250 mg/kg reduced the inhibition to 44%, 45% and 47%, respectively(Rout *et al*.,2012).

2.8.5 EFFECTS AGAINST SKIN DISEASES

P. amboinicus has been used in Brazil since the early days for the treatment of skin ulcerations caused by *Leishmania braziliensis*. In India, the juice of the leaves is used to treat skin allergies. It is also used to treat burns in Asian regions. When the leaf paste is baked on a flame and applied to cuts or burns, it acts as an antiseptic and promotes healing.

2.8.6 EFFECTS AGAINST ANIMAL AND INSECT BITE

Leaves of *P. amboinicus* are also used as a poultice for centipede and scorpion bites in Asian regions, including Malaysia. It is reported that aqueous extracts (0.706 mg/mL and 0.406 mg/mL) of *P. amboinicus* to be more than 70% efficient when tested against fibroblast cell lysis. This implies the aqueous extracts to have a tendency to be scorpion venom antidotes. However, the same paper also reported its cytotoxicity to be questionable.

2.8.7 ORAL DISEASES

Caries and periodontal disease are especially of concern to public health, where they affect a large part of the population. *P. amboinicus*, rich in carvacrol, has shown an antagonistic effect when used with mouthwash to avoid bacterial growth in the oral cavity. This could be a potential alternative treatment for diseases related to oral cavities(Asiimwe *et al.*, 2014).

2.8.8 ACIVITY AGAINST CARDIOVASCULAR DISEASE

*P. amboinicu*s is also used in the Caribbean, to treat congestive heart failure. The aqueous extracts of the fresh leaves of *P. amboinicus* exhibited dose-dependent positive inotropic activity in the isolated frog heart without affecting the heart rate. (Mallavarapu *et al.*,1999).This may be attributed to the increase in sodium influx thereby causing greater intracellular availability of calcium. In this report the bioactivity of the tissue-cultured extracts of *P. amboinicus* to the parent plant was also described(Gurib-Fakim , *et al.*, 1995).

2.8.9 ACTIVITY AGAINST GENITOURINARY DISEASE

The leaves of *P. amboinicus* are frequently utilized in the treatment of urinary diseases in the Amazon and India. This species is also reported to relieve kidney troubles and treat vaginal discharges, and is taken as a drink after childbirth. Urolithiasis is a condition when stony concretions form in the bladder or urinary tract. Many remedies have been employed during the treatment of urinary stones. The juice of *P. amboinicus* has been used as a natural remedy to dilute the crystals in the urinary tract in India from ancient times. The antilithiotic activity of the concentrated fresh juice of the leaves of *P. amboinicus* is proved by (Jose *et al.*,2012).

3.OBJECTIVES

The objectives of the present study are

- Identification of the major phytochemical constituents in *P.amboinicus*
- Determination of the total phenolic contents in the aqueous extract of *P.amboinicus*
- Estimatimation of the total antioxidant activity in the aqueous extract of *P.amboinicus*

4.MATERIALS AND METHODS

4.1 Collection of *Plectranthus amboinicus*

P.amboinicus plant were collected from Kollam, Kerala.

4.2 Extraction

The collected plant was dried in shade and after a week dried material were made as fine powder by mechanical grinding. About 50gm of powdered leaf was mixed in 100ml of distilled water and subjected to 48 hours of occasional stirring under room temperature. After two days the extract was filtered with cotton cloth and preserved in refrigerator for future usage.

REAGENTS

1.**Mayer's reagent**: is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml).

2.**Wagner's Reagent**: 2.5 gm iodine is dissolve in 12.5 gm of potassium iodide (KI 2); add 250 ml of water to produce solution.

3.**Dragendroff reagent**: DR is a solution of potassium bismuth iodide composing of basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid, and potassium iodide (KI), and when contact with alkaloids DR produces an orange or orange red precipitate

4.**A Lead Acetate Solution** : It is prepared by dissolving 40 g Pb(CH3COO)2·3H2O in water, adding 0.5 mL CH3COOH, and diluting to 100 mL.

5.**NaOH solution**: To make 1 M NaOH solution, you have to dissolve 40.00 g of sodium hydroxide pellets in 250 mL distilled water and then make up the solution to 1 liter

6.Preparation of **Ferric Chloride Solution**:

By dissolving iron ore in HCl (hydrochloric acid) $Fe_3O_4 + 8HCl \rightarrow FeCl_2 + 2FeCl_3 + 4H_2O$.

By oxidizing iron (II) chloride with chlorine (Cl) $2FeCl_2 + Cl_2 \rightarrow 2FeCl_3$

By oxidizing iron (II) chloride with oxygen.

7.**Potassium dichromate** : It is produced industrially by reacting potassium chloride (KCl) with sodium dichromate ($Na_2Cr_2O_7$).

8.**Sodium nitroprusside**: Diluted preparation is stable for 24 hours at room temperature (must be protected from light). There are TWO STEPS to this process. STEP TWO: Dilute 0.5mL sodium nitroprusside (10mg/mL) with 49.5mL of glucose 5% injection. This makes a 100microgram/mL solution.

4.3 Phytochemical screening tests

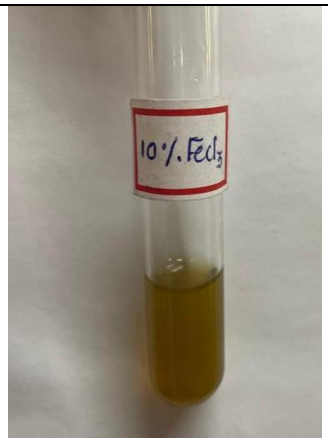
- **TEST FOR ALKALOIDS**

a) Mayer's test: To 1ml of extract added 2ml of Mayer's reagent (Potassium Mercuric lodide). Formation of a cream-colored precipitate indicated the presence of alkaloids.	
b) Wagners test: To 1ml of extract added 2ml of Wagner's reagent (lodine in potassium iodide). Formation of brown/ reddish precipitate indicate the presence of alkaloids.	
c)Dragendroff's Test: To 1ml of extract added 1ml Dragendroff's reagent(solution of potassium bismuth iodide)was added. Formatio of orange red precipitate indicate the presence of alkaloids.	

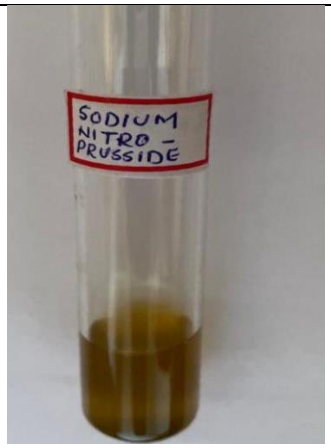
- **<u>TEST FOR FLAVANOIDS</u>**

a) <u>Lead acetate Test</u>: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids.	
b) The extract was treated with sodium hydroxide, formation of yellow color indicated the presence of flavonoids.	
c)Few drops of 1% ammonia added to 1 ml of sample, formation of yellow colour indicated the presence of flavanoids	

- **<u>TEST FOR PHENOLIC COMPOUNDS AND TANNINS</u>**

a)Iml of the test solution was mixed with basic lead acetate solution and the formation of white precipitate indicated the presence of tannins and phenolic compounds	
b) To 1ml of the extract, ferric chloride solution was added. Formation of a dark blue or greenish black color product confirmed the presence of phenolic compounds and tannins.	
c) Strong potassium dichromate solution was added to the test extract, yellow color precipitate confirmed the presence of tannin and phenolic compounds.	

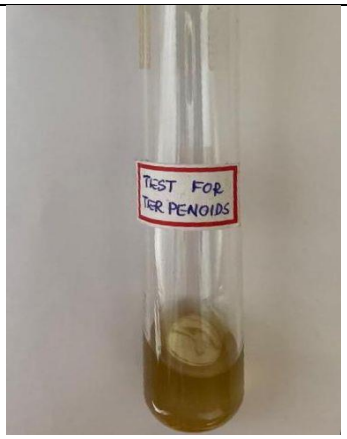
- **<u>TEST FOR GLYCOSIDES</u>**

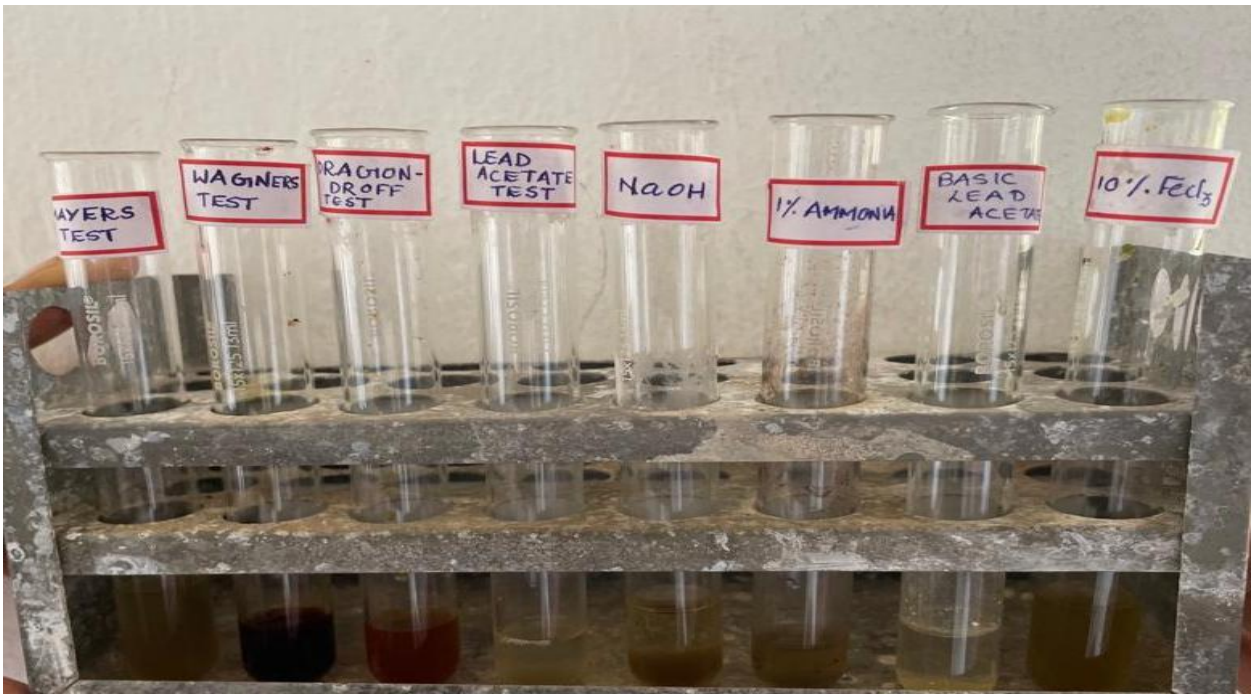
a) <u>Legal's test</u>: 1ml of the extract was treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicated the presence of cardiac glycosides.	
b) 5ml of aqueous extract was mixed with 2ml of glacial acetic acid contains 1 drop of ferric chloride solution carefully added to 1ml of concentrated Sulfuric acid (The concentrated sulphuric acid is seen underneath the mixture). Formation of brown ring indicates the presence of glycosides.	

- **<u>TEST FOR SAPONINS</u>**

a) <u>Froth test</u>: 1ml of the extract was diluted to 20 ml with water and was shaken in a graduated cylinder for 15min. ring indicated the presence of cardiac glycosides.	
b) Foam test: 0.5g of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicated the presence of saponins.	

- **<u>TEST FOR TERPENOIDS</u>**

a) 5ml of extract was added with 2ml of chloroform and 3ml of concentrated H2S04 was added along the sides of all test tube. Appearance of a brownish red color indicates the presence of terpenoids.	


AYERS TEST
WAGNERS TEST
RAGION-DROFF TEST
LEAD ACETATE TEST
NaOH
1% AMMONIA
BASIC LEAD ACETATE
10% FeCl3


SODIUM NITRO - PRUSSIDE
BROWN RING TEST
FROATH TEST-2
FROATH TEST
TEST FOR TER PENOIDS
POTASSIUM DICHROMATE

5.1 DETERMINATION OF TOTAL PHENOLIC CONTENTS IN THE AQUEOUS EXTRCT OF *P.amboinicus*

Aim:To estimate the total phenolic content of the plant in aqueous extract by Folin's Ciocalteu reagent

Principle: In acid medium, the phenolic compounds react with sodium tungstate and sodiummolybdate in the Folin's reagent to form blue colour developed was read at 650 nm.

Materials Required:

1. 100 mg of plant extract
2. Folin'sCiocalteu reagent
3. Na2CO3 (75gm in 1000mL)

Procedure:

The concentration of phenolics in plant extracts was determined using the colourimetric method (SINGLETON et al., 1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml of methanol, 2.5 ml of 10% FolinCiocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The sample was thereafter incubated in a thermostat at 45" C for 45 min. The absorbance was determined using a colourimeter at a wavelength maximum of 650 nm.

The sample was prepared for analysis and absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration lines were constructed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line., then the content of phenolics in extracts was in terms of gallic acid equivalent (mg of GA/g of extract)

The total phenolic content was calculated as gallic acid equivalent (GAE) by the followingequation;

T = CV/M

T is the total phenolic content in mg of the extract as GAE

C is the concentration of gallic established from the calibration curve in mg/ml

V is the volume of the extract solution in ml

M is the weight of the extract in g

A standard graph was plotted with concentration of standard along X axis and O.D along Y axis. From the graph concentration of unknown sample can be calculated.

5.2 DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY IN THE AQUEOUS EXTRCT OF *P.amboinicus*

Aim: To estimate the amount of antioxidant activity in a given plant extract

Principle: The antioxidant activity of the given extract is calculated by phosphomolybdenum method. The assay is based on the reduction of Mo(IV) to Mo(V) by the extract and subsequent formation of a green colour phosphate / Mo(V) complex at acidic pH.

Materials required:

1. Plant extract

2. phomolybdinum reagent: 0.6M H2SO4 + 28mM sodium phosphate + 4 ammonium molybdate

Procedure

The antioxidant activity of the extract was calculated by the phosphomolybdenum method. The assay is based on the reduction of Mo (IV) to Mo(V) by the extract and subsequent formation of a green colour phosphate Mo(V) complex at acidic pH. The phosphomolybdenum reagent was prepared by mixing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Blank was prepared by 0.1 g of plant sample ground in 10ml distilled water. Centrifuge at 2000rpm for 10 minutes. Then I ml stock diluted to 5ml distilled water. From this, 0.5 ml was taken as a test. The sample was incubated at 95°C for 90 minutes. The absorbance of the solution was measured at 690 nm against blank after cooling.

A standard graph was plotted by taking the concentration of ascorbic acid on the X-axis.and optical density on the Y-axis. From the graph total antioxidant activity was determined.

5.RESULTS

The powdered leaves and stem of *P.amboinicus* was extracted with water. The resultant cold extracted aqueous solution was subjected to screening of various phytochemical constituents. *Table 2* shown below indicates the presence and absence of various phyto constituents in the aqueous extracts of *P. amboinicus* leaf extract. It revealed the presence of alkaloids, flavanoids, phenols, glycosides, saponins, terpenoids and steroids(Hassani *et al.*, 2001).

Table 2: Phyto constituents of the aqueous extract of *P.amboinicus*

SL.NO	NAME OF TEST	RESULT
1.	Test for alkaloids	+
2.	Test for flavanoids	+
3.	Test for phenolic compounds and tannins	+
4.	Test for glycosides	-
5.	Test for saponins	-
6.	Test for terpenoids	-

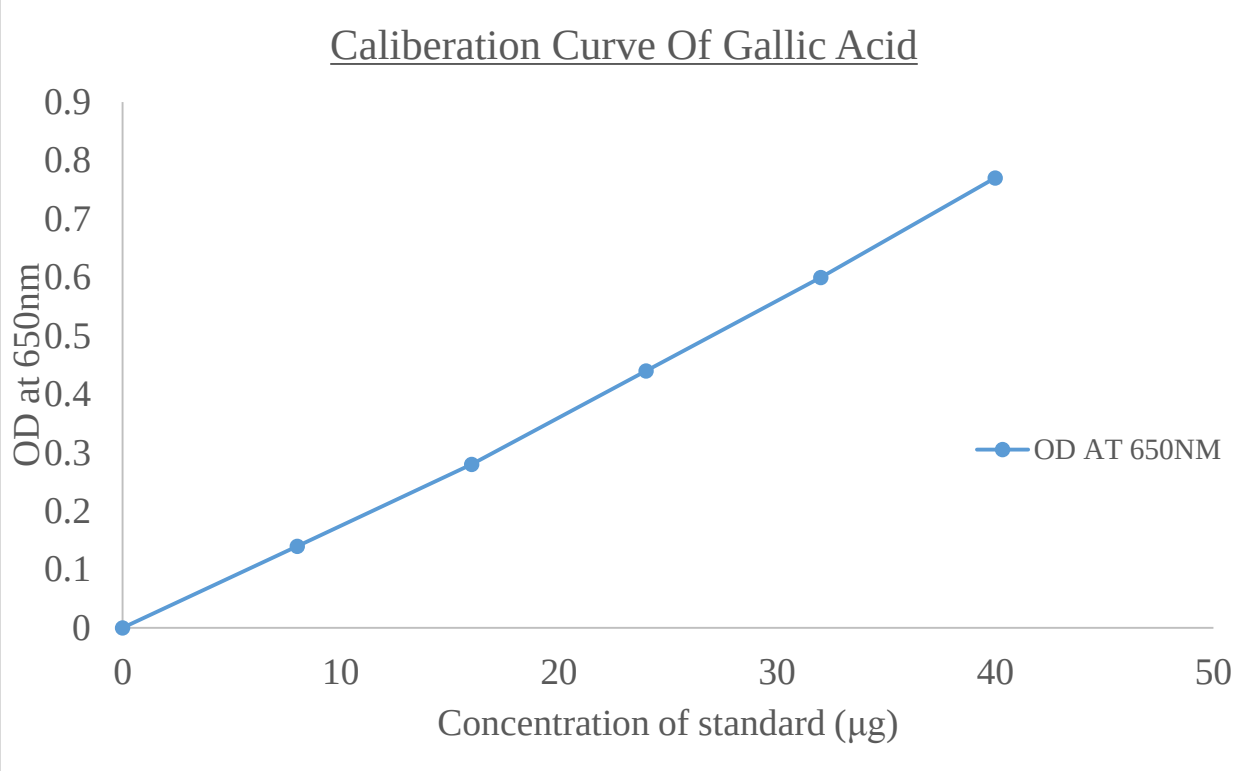
ESTIMATION OF PHENOLIC CONTENT IN *P.amboinicus*

Table 3: Absorbance of gallic acid at 650nm

Sl.No	The concentration of standard(µg)	OD at 650nm
B	0	0
S1	8	0.14
S2	16	0.28
S3	24	0.44
S4	32	0.60
S5	40	0.77
T	-	0.06
TD	-	0.03

Table 3 shows absorbance of gallic acid at 650 nm and Fig no: 1 shows the caliberation curve of gallic acid.

Fig.No: 1CALIBERATION CURVE OF GALLIC ACID



ESTIMATION OF TOTAL ANTIOXIDANT ACTIVITY IN *P.amboinicus*

Table 4: Absorbance of Ascorbic acid at 690nm

Sl.No	The concentration of standard(µg)	OD at 690nm
B	0	0
S1	40	0.22
S2	80	0.32
S3	120	0.38
S4	160	0.48
S5	200	0.55
T	-	0.06

Table 4 shows absorbance of ascorbic acid at 690 nm and Fig no: 2 shows the caliberation curve of ascorbic acid.

Fig.No. 2: CALIBERATION CURVE OF ASCORBIC ACID

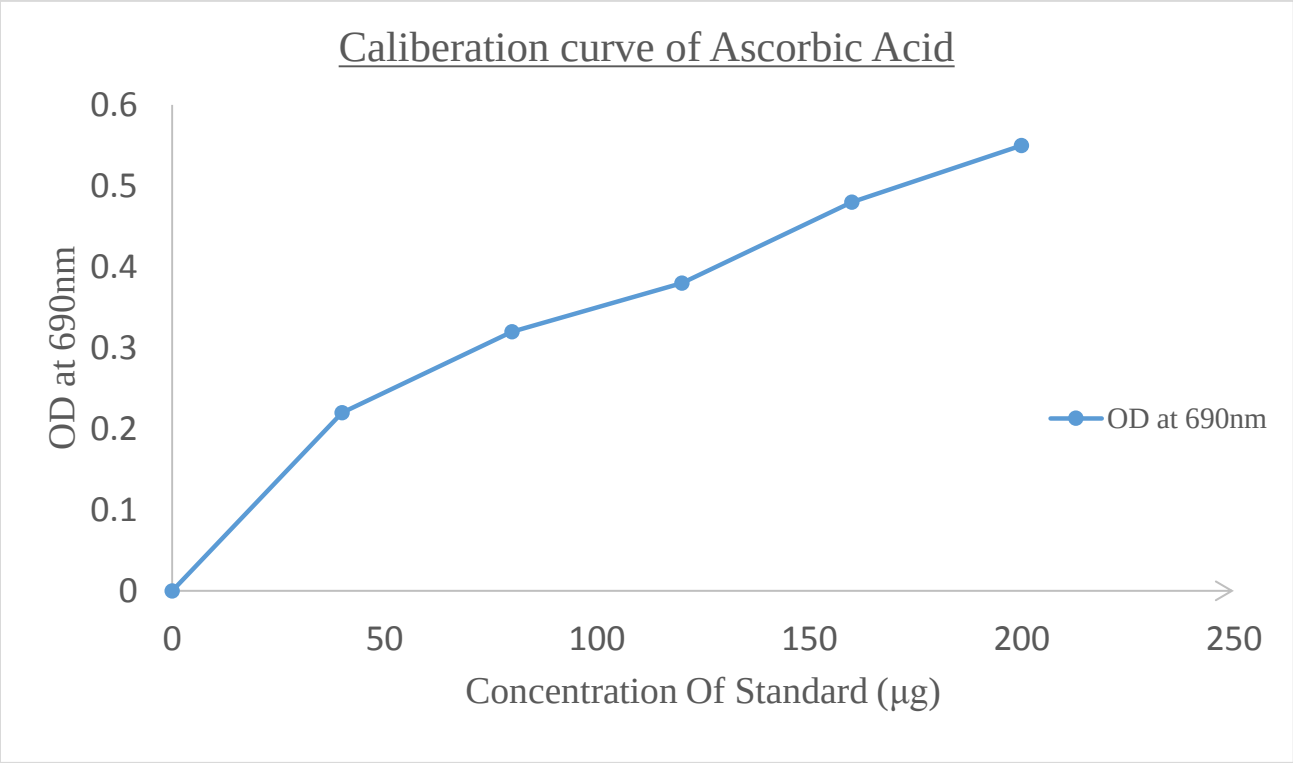


Table 5.1. Table showing total phenolic content

***P. amboinicus* leaf extract**	**Absorbance**	**Total phenolic contents (mg gallic acid/g dry extract)**
T1	0.06	5.06 ± 1.35mg/gram equivalent of gallic acid

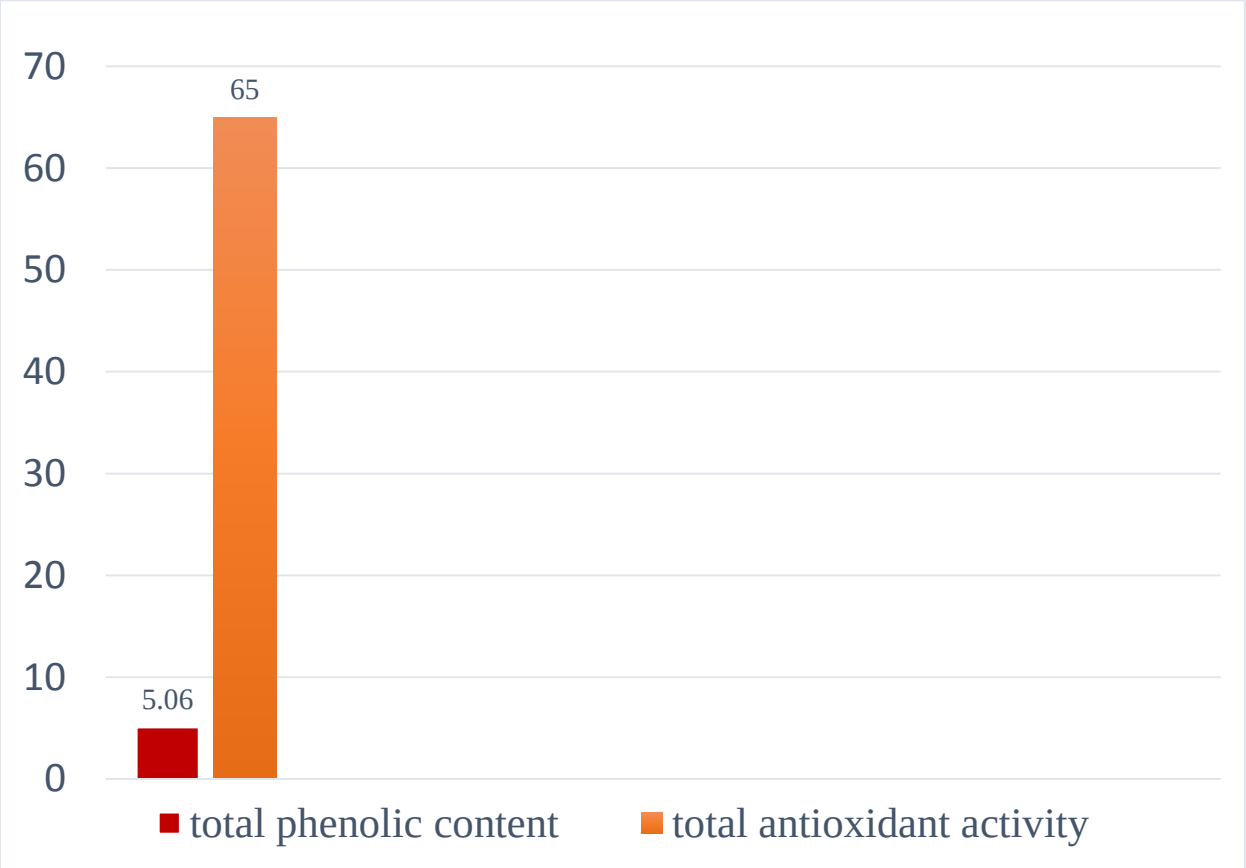
Values are mean± SD(n=3)

Table 5.2. Table showing total antioxidant activity

***P. amboinicus* leaf extract**	**Absorbance**	**Total antioxidant activity (mg ascorbic acid /g dry extract)**
T1	0.06	65 ± 8.1649mg/gram

Values are mean± SD(n=3)

Fig. No : 3 Comparison between concentrations of phytochemicals in *P. amboinicus*



6. DISCUSSION

Phytochemicals have been found to possess a wide range of activities. The phytochemical composition of traditionally used plants has become an important research area as it determines their potential as a source of new drugs. In the present study, the powdered leaf extracts of *P. amboinicus* have been screened for phytochemical constitute in distilled water. Preliminary phytochemical analysis of leaf extract revealed the presence of compounds such as alkaloids, phenolic compounds, tannin and flavonoids.

The leaves of P. amboinicus are traditionally used for the treatment of coughs, sore throats and nasal congestion and other problems such as infections, rheumatism and flatulence, malarial fever, hepatopathy, renal calculi, asthma, hiccoughs, bronchitis, helminthiasis, colic, convulsions and epilepsy (Kaliappan *et al.*, 2008). The leaves were also reported to be useful for urinary diseases, chronic coughs, epilepsy, fever, conjunctivitis, dyspepsia, asthma and colic disorders (Anonymous *et al*., 1950). The plant is used for its essential oils. It is considered nourishing for lactating mothers which enhances breast milk production and also acts as a uterine cleansing agent (Rizal Damanik *et al*., 2009). It is traditionally given in the form of soup for lactating mothers. Studies have cited numerous pharmacological properties including antimicrobial, antiinflammatory, antitumor, wound healing, anti-epileptic, larvicidal, antioxidant and analgesic activities. Also, it has been found to be effective against respiratory, cardiovascular, oral, skin, digestive and urinary diseases.

The total phenolic content was determined using the colourimetric method. A standard graph was plotted by taking the concentration of gallic acid on the X-axis and optical density on the Y axis. From the graph, the total phenolic content was

calculated. The total phenolic content of the given plant extract is 5.06 ±1.35mg/gram equivalent of gallic acid.

The antioxidant activity of the extract was calculated by the phosphomolybdenum method. A standard graph was plotted by taking the concentration of ascorbic acid on the X-axis and optical density on the Y-axis. From the graph, the total antioxidant activity was determined. The total antioxidant activity of the given plant extract is 65 ± 8.1649mg/gram of the extract.

Table 5.1 and Table 5.2 shows total phenolic content and antioxidant activity respectively. Fig.No 3 shows the comparison between the concentration of different phytochemicals. The total phenolic content of the aqeous leaf extract of *C. amboinicus* **=5.06 ± 1.35mg/gram equivalent of gallic acid.** The total antioxidant activity of the aqueous extract of *C. amboinicus* leaf = **65 ± 8.1649 mg/gram.**

6. SUMMARY AND CONCLUSION

P. amboinicus is an important aromatic medicinal herb packed with many bioactive constituents and nutrients, which are important for maintaining good health. The plant has shown a wide range of biological properties and proved to be effective in curing respiratory, cardiovascular, oral, skin, digestive and urinary diseases. The biological properties are attributed to the occurrence of a wide range of bioactive compounds in the plant extracts as well as an essential oil. Thus, it can be stated that *P. amboinicus* has huge future prospects in meeting the global demand for natural, cost-effective and safer bioactive molecules in pharmaceutical and nutraceutical industries. However, additional research efforts are required to isolate, identify and interpret or authenticate the effectiveness of bioactive compounds from *P. amboinicus*. Though several classes of phytocompounds are isolated and authenticated from this herb, their bioactivity and toxicity studies under *in vivo* conditions using animal models are limited to only a few compounds. Till now, no scientific evidence is available on the human safety aspects of *P. amboinicus* even though it is used widely in folk medicine. Further, some detailed investigations should be aimed at understanding the effectiveness of these isolated compounds in treating other human illnesses.

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