Research Article

Evaluation of cytotoxic, antibacterial and antioxidant activities of leaf extract of *Coleus ambionicus*

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Abstract

Objective: *Coleus ambionicus* has therapeutic properties attributed to its natural bio active compounds which are highly valued in the pharmacological industry. The present study evaluated the antibacterial, antioxidant & anticancer activity of *coleus ambionicus* leaf extract. **Material and methods:** The organic solvents such as ethanol, methanol, ethyl acetate and chloroform were used for the extraction of bioactive compounds. The antibacterial activity was screened by disc diffusion method and antioxidant activity was determined by reducing power method. **Results and conclusion**: The ethanol extract showed high antibacterial activity against *Klebsiella pneumonia* and the same extract was showed highest antioxidant activity. So, the ethanol extract of *Coleus amboinicus* was subjected to anticancer activity against A549, the lung cancer cell line and it showed dose dependent inhibition of lung cancer cell line. For the identification of bioactive compounds, the ethanol extract was subjected to column chromatography and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. GC-MS analysis showed the presence of three bio active compounds viz Phenol 2-methyl-5-(1-methylethyl), 2,4-Di-tert-butylphenol and Bis(2-ethylhexyl) phthalate and these bioactive compounds are responsible for the pharmacological properties of *Coleus amboinicus.*

Keywords: *Coleus amboinicus*, pharmacological activity, bioactive compounds, GC-MS

Introduction

Coleus amboinicus (Mexican mint) (Karpuravalli) is a large succulent aromatic perennial herbal well-known plant which belongs to the Lamiaceae family and it is found in almost all over India. This plant is effective in wound healing with very less side effects, so this herb is pretty impressive in this sense. Pharmacological uses of *P. amboinicus* gives impact effective in the treatment of famous diseases such as cephalgia, otalgia, anorexia, dyspepsia bloating, colic, diarrhea, cholera, gums, seizures, asthma, cough, chronic bronchitis, kidney calculi, vesicle calculi, hiccough, strangury, hepatopathy, fever, and malaria (Saraswati *et al*., 2016).

In this modern world, there are many commercial antibiotic drugs which act against microbial activity but indiscriminative for many infectious diseases and also cause several side effects on the host like immune suppression and allergenic reaction, so scientist searched for new and effective drugs from medicinal plants as alternatives (Uddin *et al*., 2021). Certainly, natural products are considered as an important key factor in medicinal sector and extensively used in pharmaceutical industries. This has been led towards the 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 (Swamy *et al*., 2015). 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. This herb is most commonly used by indigenous people in tropical rain forests. This is mainly due to its natural production of an essential oil with high amounts of bioactive compounds such as Carvacrol (Castillo *et al.*, 1999), Thymol (Singh *et al.*, 2002) β-Caryophyllene, α-Humulene, γ-Terpinene, p-Cymene, α-Terpineol and β-Selinene, identified in the oil component of its leaves

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(Senthilkumar *et al.,* 2010). These biochemical components exhibit various biological properties (Bhatt *et al.,* 2012) and are widely used as a medicine to treat conditions like cold, asthma, constipation, headache, cough, fever and skin diseases. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth. Antioxidants which occurs from the plants called natural antioxidant, which increases the antioxidants capacity and help to reduce the risk of certain disease such as cancer, heart disease and stroke (Prior *et al.,* 2016). The secondary metabolic produced from plant are phenolic compounds and flavonoid, which are reported to be free radical scavenger (Zeeshan *et al*., 2022).

Cancer is a health problem of global concern (Graidist *et al*., 2015). Chemotherapy, the most common mode of intervention in cancer, is associated with adverse effects, from nausea to bone marrow failure. In addition, development of drug resistance is also a concern (Raguz and Yague, 2008). Therefore, the search for effective therapeutics for cancer is an ongoing process (Fadeyi *et al.,* 2013). medicines such as Ayurveda are playing an important role in the treatment of cancer. Hence isolation of bioactive products from medicinal plants and herbs is gaining importance too. Eleven percent of the basic drugs are considered as essential by World Health Organization (WHO), which are plant derived and modified products.

The main objective of the present study was to analyze the phytochemicals present in coleus amboinicus plant extract and to investigate its pharmacological activities such as antibacterial, antioxidant and anticancer activity against lung cancer cell line. To identify the bioactive compounds responsible for the anticancer activity is analyzed by GC-MS.

Materials and Methods

Collection of plant sample

Fresh *Coleus amboinicus* (Karpuravalli) leaves were collected from Parthivapuram, Kanyakumari district, India. The leaves were cleaned with running tap water and shade dried for one month to get dry sample and directly subjected to solvent extraction.

Preparation of plant leaf extract

The plant extract was prepared based on the method followed by Khalel Nabih *et al*. (2014). The prepared dry leaf sample was finely grounded into powder and 8 grams of dried leaf powder and 30 grams of fresh leaf paste was extracted using 100ml of solvents such as ethanol, methanol, ethyl acetate and chloroform and stored in dark place at room temperature for one week. Then it was filtered and stored at room temperature for further use (Nikhal *et al.,* 2011).

Screening of antibacterial activity of leaf extract

Antibacterial activity of *Coleus amboinicus* leaf extract was screened based on the disc diffusion method (Kirby-Bauer Method). The pathogenic bacterial test organisms was obtained from Microbial Type culture Collection (MTCC), Institute of Microbial technology, Chandigarh. The bacterial strains used are *Klebsiella pneumoniae* (MTCC 4030), *Escherichia coli* (MTCC 40), *Enterobacter aerogenes* (MTCC 3906), *Streptococcus pyogenes* (MTCC 928) and *Pseudomonas aeruginosa* (MTCC 741). These bacterial cultures were sub cultured in nutrient agar medium. The Whatman filter paper disc of uniform size (6mm) was impregnated with the plant extracts was seeded on nutrient agar plates cultured with test organisms. Then the plates were incubated at 37°C for 24 hours (Bauer *et al*., 1966).

Screening of Antioxidant activity

The evaluation of antioxidant activity for different plant extracts was done by reducing power method. The plant extract (diluted in 1ml of distilled water) of different concentration (25ul, 50ul, 100ul and 200ul) was mixed with 1.5ml of 0.2M sodium phosphate buffer (pH 6.6) and 1.5ml of potassium ferric cyanide (1%) and the reaction mixture was incubated at 50°C for 20 minutes. After incubation, 1.5 ml of tri chloro acetic acid (TCA) was added to the reaction mixture and then centrifuged at 5000 rpm for 10 minutes at 27°C. Then 1ml of supernatant obtained was treated with 100ul of 0.1% ferric chloride and 400ul of distilled water and the absorbance was measured at 700nm (Abubakar *et al.,* 2020).

Isolation and purification of bioactive compounds

The fresh and dry leaf ethanol extract of *Coleus amboinicus* which showed higher activity in antibacterial and antioxidant activity was subjected to Column chromatography. The silica gel column was prepared with Chloroform and methanol in the ratio of 99:1. The extract of 10ml was chromatographed over silica gel column (100-200 mesh) using ethanol as solvent with increased polarity. The eluents were collected and subjected to antibacterial activity against *Klebsiella pneumonia*. The eluent which showed higher activity was subjected to further analysis (Gini *et al*., 2018). The collected eluents were subjected to antibacterial activity against *Klebsilla pneumonia* & the highly active eluent was utilized for further analysis.

Screening of anticancer activity against lung cancer

The Human lung cancer cell line **(**A549) was procured from (NCCS), Pune, India and maintained in Dulbecco's modified

Eagles medium, DMEM. The cultured cell lines was incubated at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation by Inverted phase contrast microscope and followed by MTT assay. Then the two days old confluent monolayer of cells were trypsinized and suspended in 10% growth medium and then 100µl of cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator (Raj *et al*., 2023).

For the preparation of sample extract 1ml of highly active eluent was dissolved in 1mL DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. Then the entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation was recorded as images. After 24 hours the growth medium was removed and freshly prepared sample in DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM) and each concentration of 100µl are added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator. Untreated cell was maintained as control.

Anticancer Assay by MTT Method:

After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution (15mg MTT in 3ml PBS) was added to all test and control wells. The plates were gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed to solubilize the formazan crystals. The absorbance values were measured by microplate reader at a wavelength of 540 nm (Thomas *et al*., 2017).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

Identification of bioactive compound by GC-MS analysis

The GC–MS analysis of ethanol extract (highly active eluents) of *Coleus amboinicus* was carried out using GC–MS QP2010 Ultra (Shimadzu) with Rxi-5Sil MS fused-silica capillary column of 30 m length, 0.25 mm diameter, and 0.25 mm film thickness. An electron ionization system with ionization energy of 70 eV was used for analysis. Helium gas (99.99%) was used as the carrier gas at the constant flow-rate of 8.9 ml/min. The temperatures of the injector and mass transfer line were set at 250°C and 240°C, respectively. The oven temperature was programmed from 80°C to 200°C at 3°C/min, and finally increased to 260°C at 10°C/min. Aliquots of 1 ml of the diluted samples were injected in split mode with a split ratio of 1:10 and with a mass scan range of 45–900 AMU. The total running time of the GC–MS analysis was 51 min.

Results

Antibacterial activity of *C. amboinicus* leaf extract

In dry leaf extract, the ethanol extract shows highest activity against *Streptococcus pyogenes* (2.13±0.094) and in fresh leaf extract, the ethanol extract shows highest activity against *Klebsiella pneumoniae* (2.26±0.057) and *Streptococcus pyogenes* (2.13±0.05) moderate activity was noticed in other tested pathogens. The antibacterial activity of both leaf extract showed that the ethanol extract is the best one for the antibacterial activity.

Table 1: Antibacterial activity of dry and fresh leaf extract

Pathogenic bacteria	Dry leaf				Fresh leaf			
	Ethanol	methanol	Ethyl acetate	Chloroform	Ethanol	methanol	Ethyl acetate	Chloroform
Klebsiella pneumoniae	1.73±0.205	0.03±0.047	0.3±0.081	0.36±0.047	2.26±0.057	1.367±0.047	1.57±0.05	-
Escherichia coli	-	0.23±0.047	0.67±0.047	0.36±0.047	1.5±0.1	0.967±0.047	2.33±1.1	0.97±0.5
Pseudomonas aeruginosa	1.1±141	0.63±0.047	0.03±0.047	0.36±0.047	-	0.63±0.047	0.97±0.05	-
Streptococcus pyogenes	2.13±0.094	1.1±0.141	-	0.16±0.047	2.13±0.05	1.13±0.047	0.97±0.05	-
Enterobacter aerogenes	1.567±0.047	0.367±0.047	-	0.16±0.047	0.6±0.057	1.167±0.047	0.37±0.05	0.37±0.11

Antioxidant activity of *C. amboinicus* leaf extract

The antioxidant activity of different extracts of *C. amboinicus* was measured by reducing power assay method. This method used to compare the anti-oxidant activity of dry and fresh extracts (Ethanol, Methanol, Ethyl acetate, Chloroform). In dry leaf extract, ethanol extract exhibited highest reducing power ability of 62.3±0.27 at a concentration of 200µg/ml and the lowest activity was noticed as 10.61±0.30 at 25 µg concentration. All other extract showed moderate antioxidant activity and in fresh leaf extracts ethanol extract exhibited highest reducing power ability of 49.9±0.18 at a concentration of 200µg/ml and lowest activity was noticed as 10.61±0.30 at 25 µg concentration. All other extract showed moderate antioxidant activity. The extracts showed good reducing power ability in a dose dependent manner. When compared with fresh leaf extract, the dry leaf ethanol extract showed highest anti-oxidant activity. The extracts showed good reducing power ability in a dose dependent manner showed in table 2. Reducing power of the extracts increases with the increase in concentration The result suggests that the ethanol extract possess antioxidants which can be used in pharmaceutical industry.

Purification of *C. amboinicus* leaf extract by Column chromatography

Column Chromatography was performed for the purification of bioactive compound both in dry and fresh leaf extract. Totally 8 fractions were collected from both dry and fresh leaf extract with an increased polarity of solvents. The collected fractions were subjected to antibacterial screening against *Klebsiella pneumoniae* for the selection of best fraction and the concentration of inhibition (cm) of dry and fresh leaf extract of *C. ambionicus* was shown in table 3. The 4th fraction of dry leaf extract showed the best antibacterial activity (2.1±2.1 cm) against *Klebsiella pneumoniae* so it was chosen for further analysis.

Screening of Anticancer activity of *C. amboinicus* dry leaf extract

Anticancer activity of *C. amboinicus* dry leaf extract (4th fraction) was done on Human Lung Cancer Cell line (A549) using MTT cell viability assay method. The anticancer result showed dose dependent inhibition of cancer cell line. The 80% of cell viability was noticed at 6.25 concentrations, 75% cell viability at 12.5 µg/ml concentrations, 69% cell viability at 25µg/ml concentration, 63% cell viability at 50 µg/ml concentrations and 55% cell viability at 100µlg/ml. The dry leaf extract of *C. amboinicus* showed a high activity against the lung cancer Cell line (A549) as it killed 45% of Lung cancer cell at 100µg/ml concentration (figure 1).

Table 2. Antioxidant activity of different extracts by reducing power method for dry leaf and fresh leaf samples

Conc. µg/ml	Ascorbic acid	Dry leaf				Fresh leaf			
		Ethanol	Methanol	Ethyl acetate	chloroform	Ethanol	methanol	Ethyl acetate	Chloroform
25	19.53±0.29	16.6±0.27	11.33±0.21	19.51±0.43	13.33±0.34	19.5±0.43	12.32±0.49	12.09±0.41	14.24±0.11
50	37.4±0.18	28.8±0.16	14.24±0.11	12.32±0.49	10.61±0.30	24.4±0.18	16.39±0.06	10.61±0.30	12.09±0.41
100	88.8±0.13	61.1±0.14	16.39±0.06	10.60±0.30	12.09±0.41	36.15±0.17	16.6±0.27	16.39±0.06	13.33±0.34
200	97.13±0.17	62.3±0.27	13.59±0.36	15.31±0.32	16.39±0.06	49.9±0.18	15.31±0.32	14.24±0.11	11.33±0.21

Table 3. Purification of *C. amboinicus* leaf extract by Column chromatography of ethanol dry and fresh leaf extract

Eluted fraction	*Klebsiella pneumoniae* (Zone of Inhibition)	
Control	Ethanol Dry leaf extract	Ethanol Fresh leaf extract
1	-	-
2	1±1	0.2±0.02
3	1.1±0.06	0.5±0.5
4	2.1±.0.21	0.96±
5	1.6±0.06	0.6±0
6	1.3±0.21	0.2±0.02
7	0.5±0.5	0.1±0.01
8	-	-

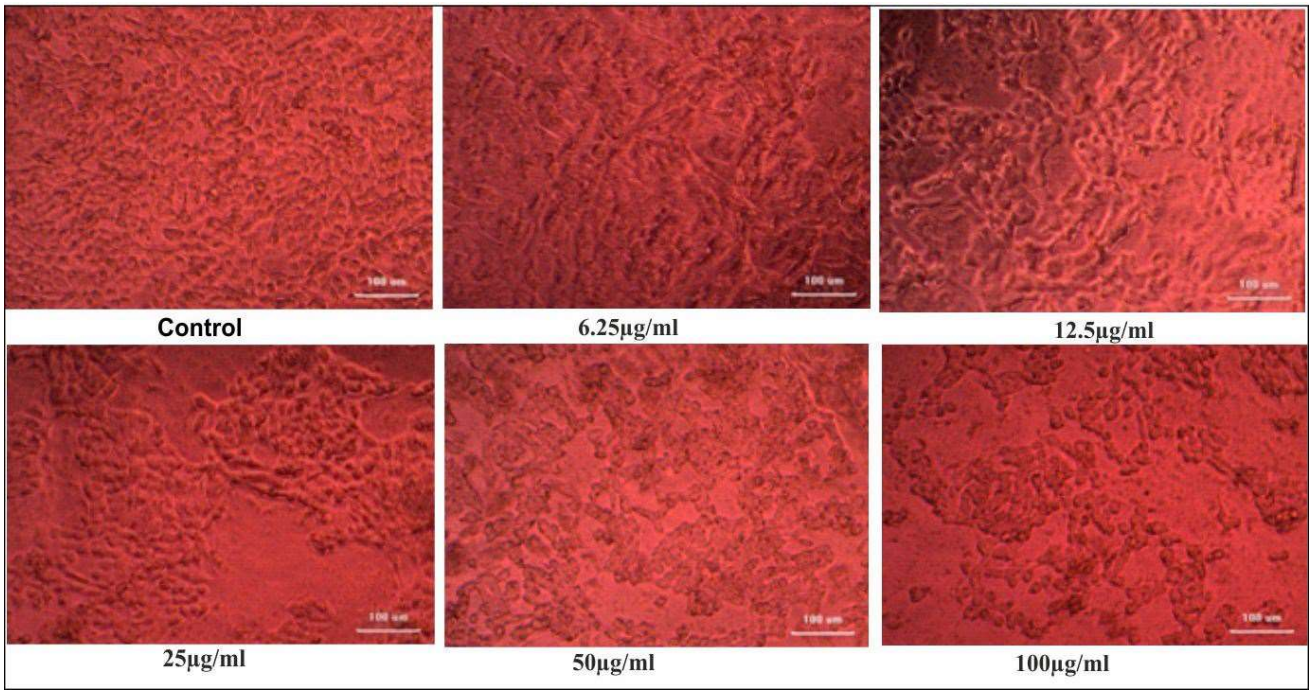

Figure 1. Anticancer activity of dry leaf extract of *C. amboinicus* on Lung cancer cell lines A549.

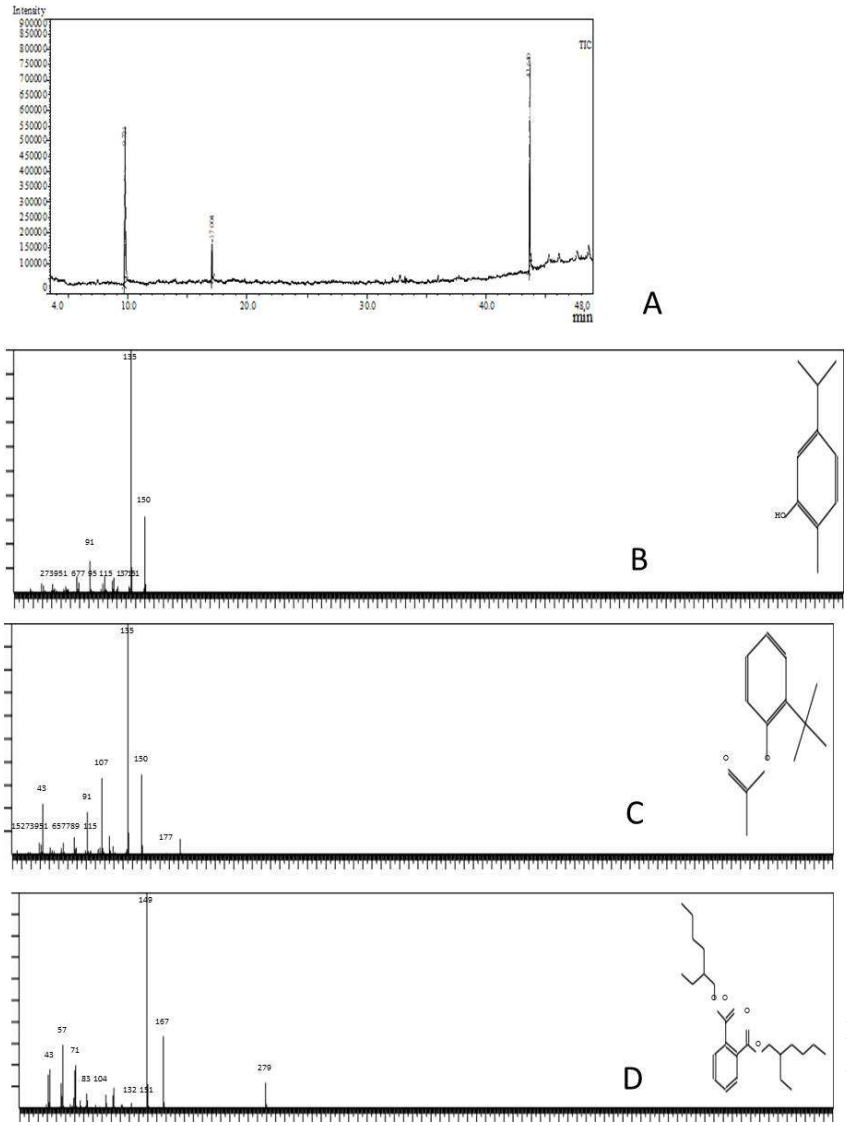

Figure 2. Analysis of bioactive compounds by GC-MS

Table 4. Characterization of bioactive compounds

Peak	R Time	Area	Area%	Name
1	9.721	2017999	44.78	Phenol, 2-methyl-5-(1-methylethyl)-
2	17.004	451715	10.02	2,4-Di-tert-butylphenol
3	43.640	2036918	45.20	Bis(2-ethylhexyl) phthalate
		4506632	100.00	

Analysis of bioactive compounds by GC-MS

GC-MS analysis of ethanol extract (4th fraction) of *C. amboinicus* dry leaf extract showed three peaks which indicated the presence of three bioactive compounds (figure 2A). On comparison of the spectra with Pub Chem database, the identified compounds are identified such as Phenol,2-mehyl-5-(1-methyl ethyl) molecular weight 150 (figure 2B), 2,4-Di-tert-butylphenol ($C_{14}H_{22}O$) molecular weight of 206 (figure 2C), Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$) molecular weight of 390 (figure 2D). Among these bioactive compounds the most prevailing compound was Bis (2-ethylhexyl) phthalate.

Discussion

Since from the prehistoric times, plants have been used for medical purposes 5 and used as a important ailments human therapeutic aid. Nowadays, due to the high demand for the treatment of various disease increases, herbal plants has been widely explored globally (Khandelwal *et al*., 2011). Antibacterial properties of *Coleus amboinicus* have been reported from different parts of the world. Bhatt *et al.* (2017) studied that the acetone extract of *C. amboinicus* leaves shows the effective antibacterial with least MIC values against the tested food born bacteria namely *Stapylococcus aures, Bacillus cereus, Escherichia coli and Yersinia entereolitica* as the leaf acetone extract had the second highest polyphenolic content. Mallappa Kumara Swamy *et.al*. evaluated in their study that methanol extract shows highest antibacterial activity all the tested pathogen includes *Bacillus subtilis*, Methicillin-*Staphylococcus aureus, Pseudomonas aeruginosa, escherichia coli* and *Candida albicans*, but other two extract which include hexane and acetone failed to inhibit *E. coli, S.aureus and C. albicans* were more susceptible to all the extracts.

In this study, the dry and fresh leaf extract with different solvents including ethanol, methanol, ethyl acetate and chloroform were tested against different bacteria. The ethanolic extract of *C. aromaticus* shows high impact of antibacterial activity against *E. coli*, *Bacillus sp, Pseudomonas sp, Staphylococcus and Klebsiella sp* (Malini, 2013). Various kinds of solvent extract of *C. aromaticus* as well as its essential oils have demonstrated high antimicrobial activity on both Gram positive and Gram negative bacteria. It is also found to be quite effective against drug resistant microorganisms as well as the phytopathogenic microorganisms (Rashmi *et al.,* 2011). The extracted oil of *C. aromaticus* showed great antibacterial activity against *S. aureus, E. coli*, and *K. pneumonia* with mild activity shown against *P. aeruginosa* (Afaf Mohammed *et al.,* 2011). Ethanolic extract has been proven to have impressive antibacterial activity against S. aureus and P. aeruginosa which increase the opportunities for *C. aromaticus* to act as an important source of herbal antibacterial agents (Amar *et al.,* 2012). The ethanol extract of dry leaf shows highest activity compared to other solvents against *Klebsiella pneumonia, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Enterobacter aerogenes*, however it failed to inhibit the activity of *Escherichia coli*. Similarly, in fresh leaf extracts, the ethanol extract of *C. amboinicus* shows highest activity against all the five different tested pathogens compared to other extracts. Bhatt *et al*. (2017) studies that the ethyl acetate extract of *C. amboinicus* leaves shows the highest appreciable DPPH radical scavenging and total antioxidant capacity as it had highest polyphenolic content compared to other extract obtained from solvent including hexane, acetone, methanol, hydro alcohol and freeze dried form. Patel *et al*. (2010) evaluated that the total phenolic compared in *C. amboinicus* contributes in anti-oxidant property. The reducing power of ethanolic and aqueous extracts and antioxidant potential increases with increasing concentration of extract. The most important biomolecules in the body get damaged due to the bio chemical reaction which produces reactive oxygen species which causes several disease conditions. The antioxidant prevents the harmful action of free radicals by blocking them and scavenging and it corrects their damaging effect on the cellular constituents. Rai *et.al*., (2016) investigates the cytotoxicity of two different plants fresh leaf extract with solvents including ethanol and chloroform on two breast cancer cell lines of different grads, MCF-7 and MDA-231 by MTT assay and reported that chloroform extracts of *P.*

heyneanus and ethanol extract of C. *amboinicus* were effective against MCF-7 cells. Likewise, Rosidah *et al*., (2014) studied the cytotoxic activity of *C. amboinicus* extract using different solvents include n-hexane, ethyl acetate and ethanol against HeLa and Vera cell lines using MTT assay. Here n-hexane and ethanol extract were found selective to HeLa cells.

In this study, the anticancer activity of dry leaf ethanol extract of *C. amboinicus* was done on Human Lung cancer cell line (A549) using MTT cell viability assay method. The dry leaf ethanol extract from 8th fraction of Column chromatographed sample which is used to kill the cells and it showed the highest against the cell line (A549) as it killed 45% of carcinoma cells with just 100µl/ml concentration of extracts. Gas chromatography Mass Spectrometry can be used to analyze the bioactive components presents in plant extract. Savina *et al.* (2014) evaluated the GCMS analysis revealed the presence of 11 chemical compounds. *C. amboinicus* dry leaf ethanol extract on GC-MS analysis shows three peaks which indicate the presence of three bio compounds namely Phenol,2-methyl-5-(1-mthyl(ethyl), 2,4-Di-butyl phenol and Bis (2-ethyl hexyl) phthalate. Thus it can be concluded that the biological activities of *Coleus amboinicus* of dry and fresh leaf extract can be utilized for the development of medicinal product for human and animal uses.

Conclusion

In conclusion, the present study demonstrates the bioactive compound identification and screening of pharmacological activities such as antimicrobial activity, antioxidant activity and anticancer activity of *C. amboinicus*. The ethanol extract of dry leaf possesses high antibacterial against *Klebsiella pneumoniae* and shows moderate activity against other pathogens. All other extracts also showed moderate activity against most of the pathogens. In antioxidant assay also ethanol extract exhibited highest reducing power ability in a dose dependent manner. The result suggests that the ethanol extract possess antioxidants which can be used in pharmaceutical industry. For the further studies from the ethanol extract the bioactive compounds were purified and the eluents were screened to antibacterial activity of *Klebsiella pneumoniae*. The highest active fraction was subjected to screening of anticancer activity using Human Lung Cancer Cell line (A549). The anticancer result showed dose dependent inhibition of cancer cell line. The extract showed potent anti-n cancer activity against lung cancer cells. The result obtained from GC MS analysis indicated the existences of many valuable bioactive compounds that would be contributed to pharmacological properties of *Coleus amboinicus*. Coleus amboinicus (Lour) shows potential as chemotherapeutic agents for cancer and might become an ingredient for health-beneficial foods to prevent cancer.

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References

Abubakar AR, Haque M. 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12:1–10.

Asiimwe S, Borg-Karlson A, Azeem M, Mugisha MK, Namutebi A, Gakunga NJ. 2014. Chemical composition and Toxicological evaluation of the aqueous leaf extracts of Plectranthus amboinicus Lour : Spreng. International Journal of Pharmaceutical Science Invention 3(2):19-27.

Bauer AW, Kirby WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45:493–496.

Bhatt P, Negi PS. 2012. Antioxidant and antibacterial activities in the leaf extracts of Indian borage (*Plectranthus amboinicus*). Food Science & Nutrition, 3:146–152.

Bhatt P. 2012. Antioxidant and Antibacterial Activities in the Leaf Extracts of Indian Borage (Plectranthus amboinicus). Food and Nutrition Sciences 03(02):2012.

Bhatti Z, Ismail MH, Khan KW. 2022. Plant secondary metabolites: Therapeutic potential and pharmacological properties, in Secondary Metabolites - Trends and Reviews (IntechOpen, 2022).

Castillo RAM, Gonzalez VP. 1999. *Plecthranthus amboinicus* (Lour.) Spreng. Revista Cubana de Plantas Medicinales 4:110–115

DaSilva EJ, Hoareau L. 1999. Medicinal plants: a re-emerging health aid. Electronic Journal of Biotechnology 2:1999.

Firdaus AA. Prihanto, Nurdiani R. 2013. Antioxidant and cytotoxic activity of acanthus ilicifolius flower. Asian Pacific Journal of Tropical Biomedicine, 3(1):17–21.

Gini TG, Jeya Jothi G. 2018. Column chromatography and HPLC analysis of phenolic compounds in the fractions of Salvinia molesta mitchell. Egyptian Journal of Basic and Applied Science 5:197–203.

Graidist. 2015. Cytotoxic Activity of *Piper cubeba* Extract in Breast Cancer Cell Lines: Natural Products for Huma Health. Nutrients *7*(4).

Khandelwal VKM, Koneri R, Balaraman R. 2011. Biological Activities of Some Indian medicinal plants. Journal of Advanced Pharmacy and Rese 1:12-44.

Khare RS, Banerjee S, Kundu K. 2011. Coleus aromaticus Benth-A nutritive medicinal plant of potential therapeutic value. International Journal of Pharma and Bio Sciences 2(3):488-500.

Kumar P, Singh S, Kumar N. 2020. Plectranthus amboinicus: A review on its pharmacological and, pharmacognostical studies. American Journal of Physiology, Biochemistry and Pharmacology, 10:55.

Lai PK, Roy J. 2004. Antimicrobial and chemopreventive properties of herbs and spices. Current Medicinal Chemistry 11:1451–1460.

Mahajan, Nikhal S. 2010. Evaluation of antibacterial and antioxidant activity of *Mangifera indica (leaves)*. Journal of Pharmaceutical Sciences and Research 2(1): 2010.

Malini M, Abirami G, Hemalatha V, Annadurai G. 2013. Antimicrobial activity of ethanolic and aqueous ex-tracts of medicinal plants against waste water pathogens. International Journal of Research in Pure and Applied Microbiology 3(2):40–42.

Nabih ES, Abdel Motaleb FI, Salama FA. 2014. The diagnostic efficacy of nectin 4 expression in ovarian cancer patients. Biomarkers 19:498–504.

Patel RD, Mahobia NK, Singh MP, Singh A. 2010. Antioxidant potential of leaves of Plectranthus amboinicus (Lour) Spreng. Der Pharmacia Lettre 2(4):240-245.

Prior RL, Sintara M, Chang T. 2016. Multi-radical (ORACMR5) antioxidant capacity of selected berries and effects of food processing. Journal of Berry Research 6:159–173.

Raguz S, Yague E. 2008. Resistance to chemotherapy: New treatments and novel insights into an old problem. British Journal of Cancer 99:387–391.

Rai V, Pai VR, Kedilaya P. 2016. A preliminary evaluation of anticancer and antioxidant potential of two traditional medicinal plants from Lamiaceae - Pogostemon heyneanus and Plectranthus amboinicus. Journal of Applied Pharmaceutical Science 6(08):073-078.

Raj A. 2023. Exploring the cytotoxicity on human lung cancer cells and DNA binding stratagem of camptothecin functionalised silver nanoparticles through multi-spectroscopic, and calorimetric approach. Scientific Reports, 13: 9045.

Rosidah R, Anjelisa P, Hasibuan Z. 2014. Cytotoxic effect of n-hexane, Ethylacetate and ethanol extracts of plectranthus amboinicus, on HeLa and vero cells lines. International Journal of PharmTech Research 6(6):1806-1809.

Saraswati J, Kaur K, Nagpal AK. 2016. Analytical Techniques for Phytochemicals Screening and Bioactivities of Some Coleus Species: A Review. Journal of Pharmaceutical Sciences and Research, 8(4): 227-237.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines 8:1–10.

Senthilkumar A, Venkatesalu V. 2010. Chemical composition and larvicidal activity of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against *Anopheles stephensi*: A malarial vector mosquito. Parasitology Research 107:1275–1278.

Sharma A, Khanna S, Kaur G, Singh I. 2021. Medicinal plants and their components for wound healing applications. Future Journal of Pharmaceutical Sciences, 7:2021.

Singh G, Singh OP, Prasad YR, Lamposona MP, Catalan C. 2002. Studies on essential oils. Part 33. Chemical and insecticidal investigations on leaf oil of *Coleus amboinicus* (Lour) Flavour and Fragrance Journal 17: 440–442.

Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. 2017. GC-MS Based Metabolite Profiling, Antioxidant and Antimicrobial Properties of Different Solvent Extracts of Malaysian *Plectranthus amboinicus* Leaves. Evidence-Based Complementary and Alternative Medicine. 2017: Article ID 1517683.

Swamy MK, Sinniah UR, Akhtar MS. 2015. *In vitro* pharmacological activities and GC-MS analysis of different solvent extracts of *Lantana camara* leaves collected from tropical region of Malaysia. Evidence-Based Complementary and Alternative Medicine 2015:1–9.

Uddin TM. 2021. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of Infection and Public Health 14:1750–1766.

Walter TM, Swathi K, Nandini VS. 2017. Phytochemical analysis and Invitro Anticancer study of a Siddha formulation KKPN against Cervical Cancer. Siddha Papers 12:3.

Weli AM, Selim D, Akhtar MS, Al-Sabahi JN, Al-Riyami Q, Al-Sulimi ZM et al. Phytochemical investigations and antimicrobial screening of coleus aromaticus grown in Oman. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2011;10(11):3083-3090.