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Original Article

# Phenolic constituents and biological activities of leaf extracts of traditional medicinal plant *Plectranthus amboinicus* Benth (Lamiaceae)

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## ABSTRACT

*Plectranthus amboinicus* Benth (Lamiaceae) is a medicinal plant native to India, and its leaves are widely used in several traditional medicinal preparations. The purpose of this study was to detect and quantify phenolics present in ethyl acetate and acetone extracts of *P. amboinicus* leaves, and evaluate their antioxidant, antibacterial, antimutagenic and anticancer activities. The HPLC chromatograms of crude leaf extracts indicated the presence of phenolics like caffeic acid, coumaric acid, rutin, quercetin and gallic acid, which were present in the range of 0.01 - 1.41 mg/g in ethyl acetate and 0.03 - 1.93 mg/g in the acetone extract. The acetone extract showed statistically ($p < 0.05$) higher antioxidant activity ($IC_{50}$, 99.59 µg/ml) than ethyl acetate extract ($IC_{50}$, 149.96 µg/ml). Statistically ($p < 0.05$) higher antimutagenicity was shown by acetone extract (46.16%) as compare to ethyl acetate extract (12.16%) at 500 µg/plate concentration. The acetone extract showed higher antibacterial activity than ethyl acetate extract, and both the extracts showed highest activity against *B. cereus* (375 and 625 µg/ml, respectively) and lowest activity against *Y. enterocolitica* (1000 and 1125 µg/ml, respectively). Both the extracts also showed inhibitory effect on cancer cell lines HCT-15 and MCF-7. These results suggest that the leaves of *P. amboinicus* possess various biological activities, and validate the traditional use of the leaves of *P. amboinicus* against cold, infection and ulceration.

**Keywords** antibacterial, anticancer, antimutagenic, antioxidant, phenolics, *Plectranthus amboinicus*

## INTRODUCTION

*Plectranthus amboinicus* Benth (Lamiaceae) is a plant native to India (Khare et al., 2011), and its leaves are traditionally used in several medicinal preparations, especially for the treatment of coughs, sore throats, nasal congestion, and for a range of other problems such as infections, rheumatism and flatulence (Khory et al., 1999; Sharma and Singh, 2002). The plant is cultivated in home-gardens throughout India for use in traditional medicine to treat malarial fever, inflammation, hepatopathy, renal and gallstones, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsions, and epilepsy (Kaliappan and Viswanathan, 2008). Other traditional medicinal uses of leaves in India include against skin ulcerations, scorpion bite, skin allergy, wounds, diarrhoea, hepatoprotective, and to promote liver health (Shenoy et al., 2012). The plant is commonly used in home medication in several other countries, such as Indonesia, to stimulate lactation following childbirth; Cambodia to protect children from colds; and Brazil to treat skin lesions (Franca et al., 1996; Sousa et al., 2011).

There have been numerous research studies on hydroalcoholic extract of Indian borage leaves, and it was found to have anti-inflammatory (Patel et al., 2010), antitumorigenic and genotoxic, nephroprotective (Palani et al., 2010), anticancer (Gurgel et al., 2009) and antifungal properties (Murthy et al., 2009). In our earlier study on Indian borage leaves (Bhatt and Negi, 2012), we found that acetone and ethyl acetate extracts in sequential extraction showed higher activity than the hydroalcoholic extract. Therefore, in the present study, we have used the extracts prepared by using these solvents (acetone and ethyl acetate) individually to study their antioxidant, antimutagenic, cytotoxic and antibacterial potency. We have also identified and quantified a few potent bioactive molecules in these extracts.

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## MATERIALS AND METHODS

### Plant material

The leaves of *P. amboinicus* were collected from the plants grown in the campus of Central Food Technological Research Institute (CFTRI), Mysore, India. The plant material was identified by Mr. A. S. Chauhan, Scientist, Fruit and Vegetable Technology Department, CFTRI, Mysore, and a specimen voucher was deposited in the Fruit and Vegetable Technology departmental herbarium (FVT DH No. LGMGHCC-PA-8/2011).

### Chemicals and reagents

All the phenolics like caffeic acid, coumaric acid, rutin, quercetin and gallic acid; and 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and

sodium pyruvate were purchased from Sigma Chemic Co. (Bengaluru, India). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Fluka (Bengaluru, India). All the microbiological media and sterilized plates were procured from HIMEDIA (Mumbai, India). Solvents and other chemicals used were of analytical grade.

**Extraction of polyphenols**

The fresh leaves of *P. amboinicus* were washed in running tap water and dried at low temperature (55℃) in a hot air oven for 48 h. The dried leaves (4 - 5% moisture) were powdered using a mixer grinder and used for extraction with ethyl acetate and acetone (1:4 w/v; leaf and solvent ratio) individually to obtain bioactive fractions. The extraction was done at room temperature by constant shaking for 24 h, it was filtered with Whatman No 1 filter paper and the filtrate was concentrated in a fume hood to obtain the extract. The solvent free extracts were dissolved in methanol for antioxidant, antimutagenic and cytotoxic assays, and in propylene glycol (PG) for use in the antibacterial experiments.

**Determination of total phenols**

The concentration of phenols in the ethyl acetate and acetone leaf extracts was determined by the method of Singleton and Rossi (1965). Aliquots of the samples prepared in methanol were mixed with 1.0 ml of 10 fold diluted Folin-Ciocalteu reagent and 1 ml of saturated sodium carbonate solution. After allowing it to stand for 30 min at 30℃, the absorbance was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu, Japan). Total phenolics were calculated using a standard gallic acid curve and results were expressed as mg gallic acid equivalents (GAE)/g extract.

**High performance liquid chromatography (HPLC)**

HPLC was performed in Shimadzu unit (LC 10A, SPD-MOAVP, Kyoto, Japan) equipped with a quaternary pump and a diode array detector. The mobile phase involved a gradient of water: acetic acid (100:1) as solvent A and methanol: acetonitrile: acetic acid (95:5:1) as solvent B at a constant flow rate of 1.0 ml/min. The gradient comprised of an initial isocratic period of 2 min with 5% solvent B, followed by a linear increase to 25% with B over 10 min with further stepwise linear increase to 40% B in 20 min, 50% B in 30 min and 100% B in 40 min, which was maintained for 5 min and returned to initial conditions in next 10 min. Samples were dissolved in methanol, and 20 µl sample was injected for chromatographic separation. The compounds were identified by comparing their retention times with those of standards at 325 nm with the exception of gallic acid (observed at 280 nm). The quantity of the various compounds present was determined from the standard curve using the standards for individual phenolic compounds obtained from Sigma Aldrich, Bengaluru, India.

**Free radical scavenging assay**

The free radical scavenging activity of the acetone and ethyl acetate leaf extracts was measured *in vitro* by DPPH assay (Blois, 1958). Aliquots of the samples (1000 µg/ml) adjusted to a final volume of 2.5 ml were mixed with 5 ml of 0.1 mM DPPH solution. The tubes were shaken properly and incubated for 20 min in the dark. The change in the absorbance of the samples was measured at 517 nm using a spectrophotometer. The radical scavenging activity of the extracts at different concentrations was determined and compared with that of butylated hydroxyanisole (BHA), which was used as the standard. DPPH solution without extract/standard formed the control. The percentage DPPH radical scavenging activity was calculated as following-

% DPPH radical scavenging activity

$$= 100 \times (A_0 - A_s)/A_0 \quad (1)$$

where $A_0$ is absorbance of control and $A_s$ is the absorbance of the sample. Antioxidant activity was also expressed as half maximal inhibitory concentration ($IC_{50}$), defined as the concentration of extracts (µg/ml) causing a 50% decrease in initial DPPH radical absorbance at 517 nm. $IC_{50}$ values in DPPH assay were calculated from the graph plotting inhibition percentage against extract concentration.

**Total antioxidant capacity**

The total antioxidant capacity of acetone and ethyl acetate extract of the leaves of Indian borage was evaluated using the phosphomolybdenum method (Prieto et al., 1999). An aliquot of 0.3 ml of extract samples (1000 µg/ml) was combined with a mixture of 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were capped and incubated at 95℃ for 90 min. After the samples had cooled to ambient temperature, the absorbance of the solution was measured at 695 nm against reagent blank containing the respective solvents. Ascorbic acid was used as a positive control and results were expressed as µM Ascorbic acid equivalents (AAE)/g extract.

**Antimutagenicity assay**

*Salmonella typhimurium* mutant strains TA-98 and TA-1538 were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMT), Chandigarh, India, and grown according to the supplier's instruction. The antimutagenicity of acetone and ethyl acetate extracts of leaves at different concentration (500, 1000 and 2000 µg/plate) was assayed by plating the extracts with molten soft agar containing 10 h old *S. typhimurium* culture onto minimal glucose agar plates (Maron and Ames, 1983). Sodium azide was used as a diagnostic mutagen (1.5 µg per plate) in positive control and plates without sodium azide and without test samples were considered as negative controls. $His^+$ revertants were counted after incubation of the plates at 37°C for 48 h. Each sample was assayed using duplicate plates and the data presented here are mean ± SD of three independent assays. The inhibition of mutagenicity was calculated as-

% inhibition

$$= [1 - T/M] \times 100 \quad (3)$$

where T is the number of revertants per plate in the presence of mutagen and test sample, and M is the number of revertants per plate in positive control. The number of spontaneous revertants (negative control) was subtracted from the numerator and denominator. Based on % inhibition, the antimutagenic effect was defined as weak (< 25%), moderate (25 - 40%), and strong (> 40%) (Ikken et al., 1999).

**Anticancer activity**

The human cancer cells, Caco-2 (human adenocarcinoma), HCT-15 (human colon adenocarcinoma) and MCF-7 (human mammary gland; breast adenocarcinoma) were purchased from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in DMEM with 10 or 20% FBS, sodium pyruvate and penstrep and maintained at 37°C with 5% $CO_2$ in a humidified atmosphere (Galaxy 170S, Newbrunswick, USA). The cytotoxic activity of the extracts on various cell lines was measured by MTT assay essentially as described in Bhatt et al. (2013). Cells in exponential growth phase were placed in 96-well plate to make - 6000 cells/well, and extract was added at

concentrations ranging from 3.125 - 100 µg in 100 µl final volumes in different wells, and it was incubated at 37°C with 5% $CO_2$ for 68 h. DMEM was used in place of the extracts as a negative control. Cell proliferation was determined by adding 20 µl of MTT (0.5 mg/ ml), and it was incubated further at 37°C with 5% $CO_2$ for 4 h. After incubation, the formazone crystals were dissolved in equal volume of MTT solubilizing solution (0.1 N HCl in anhydrous isopropanol with 10 % TRITON X-100). The quantity of blue formazone was measured at 595 nm using a microplate reader (iMARK, Bio-RAD, Japan). Data were obtained from three independent assays, using 3 wells for each assay. Cytotoxicity was determined based on the percent cell viability.

**Antibacterial activity**

The bacterial cultures, *Bacillus cereus* F 4810 (Public Health Laboratory, London, UK), *Staphylococcus aureus* FRI 722 (Public Health Laboratory, The Netherlands), *Escherichia coli* MTCC 108 (MTCC, IMT, Chandigarh, India) and *Yersinia enterocolitica* MTCC 859 (MTCC, IMT, Chandigarh, India) were grown in Brain Heart Infusion (BHI) Agar at 37℃. Each bacterial strain was transferred from stored slants at 4 - 5°C to 10 ml BHI broth and cultivated overnight at 37℃ for 24 h. A pre-culture was prepared by transferring 1 mL of this culture to 9 ml BHI broth and cultivated for 24 h at 37°C. The cells were harvested by centrifugation at 5000 rpm for 10 min, washed and suspended in saline solution (0.85% NaCl, w/v).

The leaf extracts of *P. amboinicus* were tested against selected food borne pathogens by the agar dilution method (Negi et al., 1999). One hundred µl of bacterial culture diluted to $10^3$ 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 petri plates. The plates were observed for bacterial growth after overnight incubation at 37℃, and the minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extract capable of inhibiting the complete growth of bacteria.

**Statistical analysis**

All the experiments were performed in triplicate, and the values were reported as mean ± SD (n = 3). The effect of acetone and ethyl acetate extract on various bioactivities was compared using *t*-test performed in Microsoft Excel.

## RESULTS AND DISCUSSION

**Phenolics in leave extracts**

The total phenolics in terms of GAE were significantly higher ($p < 0.01$) in the acetone extract (85.15 ± 1.18 GAE/g extract) than ethyl acetate (67.83 ± 1.10 GAE/g extract). In HPLC, the presence of some known bioactive molecules was observed (Fig. 1). The acetone extract contained caffeic acid (0.068 mg), coumaric acid (0.137 mg), rutin (0.283 mg), quercetin (1.933 mg) and gallic acid (0.033 mg) per gram of dried extract, while ethyl acetate extract had caffeic acid (0.054 mg), coumaric acid (0.097 mg), rutin (0.207 mg), quercetin (1.418 mg) and gallic acid (0.012 mg) per gram of dried extract. Although, the amount of the identified phenolics was less in the ethyl acetate crude extract in comparison to acetone, the order of phenolics in terms of quantity was similar for both the extracts (quercetin > rutin > coumaric > caffeic acid > gallic acid). Both the extracts showed appreciable antioxidant, antibacterial and anticancer properties, and the difference in quantities of individual phenolics may be responsible for the differences in the biological activities of these extracts. It is possible that these biomolecules may be responsible for the observed biological activities in the acetone and ethyl acetate extracts of the leaves. Bioactive molecules in the leaves *P. amboinicus,* especially phenolics and flavonoids like carvacrol, thymol, β-caryophyllene, quercetin, chlorogenic acid, rosmarinic acid and eugenol have been thought to be responsible for their pharmacological properties (Khare et al., 2011).

**(A)**
**(B)**

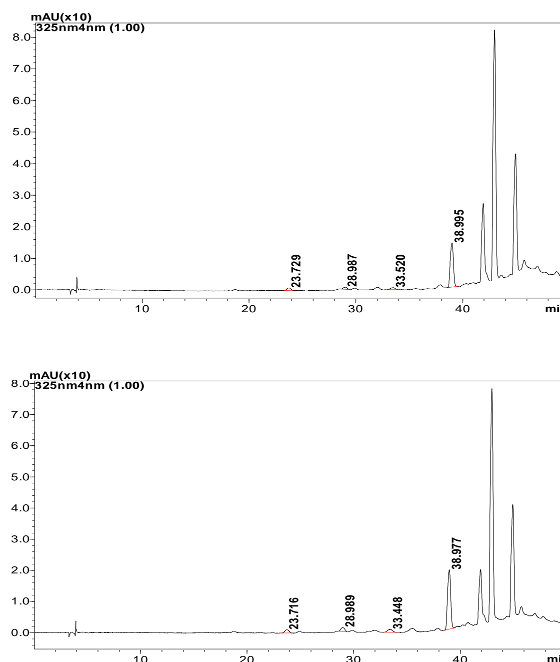


**Fig. 1.** HPLC chromatograms of (A) acetone extracts and (B) ethyl acetate of extracts of the leaves of *P. amboinicus*- Retention time in min at 325 nm [caffeic acid (23.7 min), coumaric acid (28.9 min), rutin (33.4 min), and quercetin (38.9 min)]

**Antioxidant activity of leaves extracts**

Although, the total antioxidant capacity determined using the phosphomolybdenum method showed higher total antioxidant capacity for the acetone extract (1813.48 ± 0.046 µM AAE/g extract) as compared to ethyl acetate extract (1778.34 ± 0.027 µM AAE/g extract), the difference was not statistically significant ($p < 0.05$). However, acetone extract of the leaves showed an appreciable DPPH radical scavenging activity at 200 µg/ ml (~79%), which was significantly higher ($p < 0.001$) than ethyl acetate (58%) at the same concentration (Table 1).

DPPH radical scavenging activity in terms of $IC_{50}$ values for acetone extract (99.8 µg/ ml) was significantly lower ($p < 0.01$) than ethyl acetate extract (149.9 µg/ ml) indicating better free radical scavenging capacity of acetone extract. Salar and Seasotiya (2011) also reported exceptionally high scavenging activity in acetone extract as compared to ethyl acetate extracts of *B. monosperma*. Higher DPPH radical scavenging activity and total antioxidant capacity observed in the acetone extract of leaves relative to ethyl acetate extract in the present study may probably be due to differences in their phenolics (Aydemir and Becerik, 2011; Guleria et al., 2013; Negi et al., 2008; 2010), as phenolics are reported to contribute to the antioxidant activity of leaf extracts (Fu et al., 2013; Guo et al., 2013; Huang et al., 2013). However, the DPPH radical scavenging activity of both the extracts was lower compared to the synthetic antioxidant, BHA (~95% at 60 µg/ml), which was used as the standard antioxidant.

**Table 1.** DPPH radical scavenging activity of ethyl acetate and acetone extracts of leaves of *P. amboinicus*

| Concentration (µg/ml) | % DPPH radical scavenging activity | | |
|---|---|---|---|
| | BHA | Ethyl acetate extract | Acetone extract |
| 20 | 89.89 ± 0.20 | 23.93 ± 0.28 | 21.85 ± 0.29$^{*}$ |
| 40 | 92.38 ± 0.10 | 29.46 ± 0.13 | 27.81 ± 0.45$^{**}$ |
| 60 | 94.07 ± 0.03 | 32.58 ± 0.08 | 33.50 ± 0.31$^{**}$ |
| 80 | 94.41 ± 0.02 | 34.57 ± 0.94 | 41.89 ± 6.18$^{NS}$ |
| 100 | 93.83 ± 0.10 | 38.83 ± 0.07 | 55.50 ± 0.28$^{***}$ |
| 120 | 96.83 ± 0.02 | 44.77 ± 2.24 | 61.48 ± 0.23$^{**}$ |
| 140 | 96.83 ± 0.01 | 50.22 ± 3.06 | 66.36 ± 0.13$^{**}$ |
| 160 | 96.83 ± 0.01 | 52.68 ± 0.53 | 71.24 ± 2.06$^{**}$ |
| 180 | 96.83 ± 0.01 | 56.37 ± 0.60 | 76.12 ± 0.00$^{**}$ |
| 200 | 96.83 ± 0.02 | 58.05 ± 0.24 | 79.86 ± 0.33$^{***}$ |

All values are mean ± SD (n = 3); Statistical analysis by *t*-test; Difference between ethyl acetate and acetone extract at a particular concentration. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS, Non-significant.

**Antimutagenic activity of leaf extracts**

The antimutagenic effect of acetone and ethyl acetate extracts against sodium azide induced mutation in *S. typhimurium* strains TA-98 and TA-1538 was evaluated by Ames test at various concentrations ranging from 500 - 2000 µg/plate (Fig. 2). Both the extracts showed strong antimutagenic activity at 2000 µg/plate concentration for both the strains. In case of TA-1538, both the extracts showed moderate antimutagenic activity at 500 µg/plate and strong activity at 1000 µg/plate concentration. However, in case of TA-98, acetone extract showed strong antimutagenic activity (46.16%) at 500 µg/plate, whereas ethyl acetate extract had weak activity (12.56%) at similar concentration. Although, acetone extract showed higher antimutagenicity in both the strains than ethyl acetate extract, the difference in antimutagenicity was significant ($p < 0.01$) only for TA-98 strain at 500 µg/plate concentration.

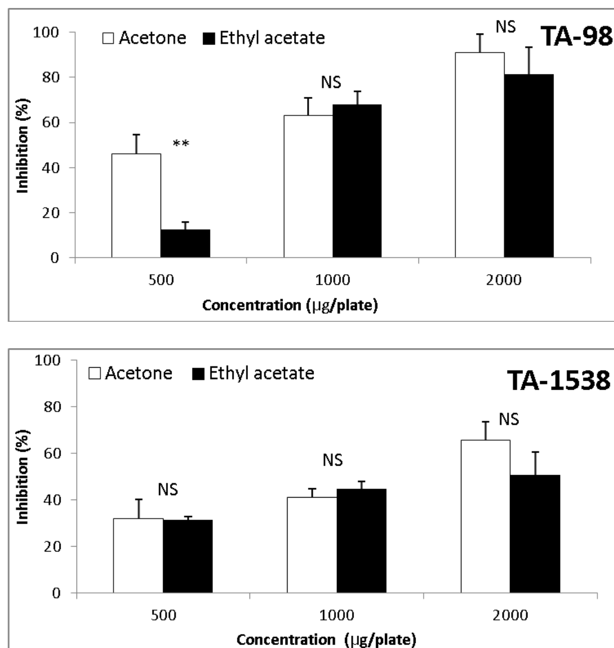


**Fig. 2.** Antimutagenic activity of ethyl acetate and acetone extract of *P. amboinicus* leaves against *S. typhimurium* TA-98 and TA 15 38. All values are mean ± SD (n = 3); Statistical analysis by *t*-test; Difference between ethyl acetate and acetone extract at a particular concentration. $^{**}p < 0.01$; NS, Non-significant.

Mutation induced by mutagens can be reduced by active oxygen scavengers, and compounds that possess antioxidant activity are reported to inhibit mutation and cancer (Hochstein and Atallah, 1988). We also observed both the antioxidant and antimutagenic effects in leaf extracts, similar to *Garcinia* fruit extracts reported earlier (Jayaprakasha et al., 2006; Negi et al., 2008). It has been observed that many plant polyphenols such as catechins, ellagic, chlorogenic, caffeic and ferulic acids act as potent antimutagenic agents (Edenharder and Tang, 1997), and the presence of these compounds in leaf extract may be responsible for their antimutagenic activity in the present study. The variable antimutagenic activity may be attributed to the differences in quality and quantity of phenolics in these extracts as was reported earlier for cinnamon fruit extracts (Jayaprakasha et al., 2007).

**Anticancer activity of leaf extracts**

The acetone and ethyl acetate extracts were evaluated for their cytotoxic effect on three cancer cell lines namely Caco-2, HCT-15 and MCF-7. The extracts showed variable effects on the cell lines but were found to have cytotoxic effect on all three of them (Table 2). The ethyl acetate extract showed a significantly higher ($p < 0.05$) inhibitory effect than acetone extract on cancer cell lines HCT-15 and MCF-7 at 500 and 1000 µg/ml concentrations, however, the cytotoxic effect of the ethyl acetate extract was statistically similar ($p < 0.05$) to acetone extract on Caco-2 at both the concentrations. Plant extracts are known to show cytotoxic effect (Sikdar et al., 2013), and the cytotoxic effect of Indian borage extracts was probably due to the presence of phenolics as reported in *Terminalia chebula* decoction (Pellati et al., 2013). A dose dependent increase in the cell cytotoxicity was seen with both the extracts in the tested concentration range of 31.25 - 1000 µg/ml, similar to carrot oil extracts observed in the range of 10 - 100 µg/ml (Shebaby et al., 2013).

**Antibacterial activity of leaf extracts**

The ethyl acetate and the acetone extracts were evaluated for their antibacterial activity against selected food borne pathogens. The acetone extract was found to have the highest antibacterial activity against *B. cereus* (MIC, 375 µg/ml) and lowest against *Y. enterocolitica* (MIC, 1000 µg/ml). A similar trend was observed for the ethyl acetate extract which also showed the highest antibacterial activity against *B. cereus* and *E. coli* (MIC, 625 µg/ml for both) and least activity against *Y. enterocolitica* (MIC, 1125 µg/ml). Although, it is generally reported that plant extracts have higher activity against Gram positive bacteria than Gram negative bacteria because of the difference in their cell wall, the extracts were equally active against both Gram positive as well as Gram negative organisms with antibacterial activity in the order of *B. cereus* < *E.coli* < *S. aureus* < *Y. enterocolitica*. However, Chukwujekwu et al. (2011) reported higher sensitivity of Gram negative bacteria against flavonoids isolated from stem bark of *Erythrina caffra* and the trend was *K. pneumonia* < *E. coli* < *B. subtilis* < *S. aureus*. Different extracts have variable antibacterial activity depending on the type of bioactives present in them, which may vary with the extraction procedure, assay method and bacterium in question (Negi, 2012).

In conclusion, the acetone and ethyl acetate extracts of Indian borage leaves were found to be rich in bioactive compounds. They exhibited appreciable antioxidant, antimutagenic and antibacterial activities. These extracts also showed an anti-proliferative effect on human cancer cell lines. These biological activities can be attributed to the phenolics present in the acetone and ethyl acetate extracts. These results contribute towards validation of traditional use of the leaves of *P. amboinicus* against cold, infection and ulceration.

Table 2. Anticancer activity of ethyl acetate and acetone extract of the leaves of *P. amboinicus*

| Concentration (µg/ml) | % Inhibition of cell proliferation | | | | | |
|---|---|---|---|---|---|---|
| | Caco-2 | | HCT-15 | | MCF-7 | |
| | Ethyl acetate extract | Acetone extract | Ethyl acetate extract | Acetone extract | Ethyl acetate extract | Acetone extract |
| 31.25 | 18.7 ± 1.8 | 14.3 ± 7.6$^{NS}$ | 9.3 ± 1.9 | 9.6 ± 7.2$^{NS}$ | 1.3 ± 0.3 | 7.8 ± 0.5$^{**}$ |
| 62.5 | 26.6 ± 8.4 | 29.3 ± 3.9$^{NS}$ | ND | ND | 6.6 ± 1.7 | 8.4 ± 0.9$^{NS}$ |
| 125 | 31.6 ± 6.8 | 37.4 ± 5.1$^{*}$ | 17.9 ± 2.0 | 11.4 ± 2.7$^{NS}$ | 21.8 ± 1.4 | 10.0 ± 1.7$^{**}$ |
| 250 | 60.0 ± 5.3 | 48.3 ± 6.3$^{NS}$ | 15.2 ± 2.9 | 14.8 ± 11.6$^{NS}$ | 22.8 ± 3.8 | 17.7 ± 2.7$^{NS}$ |
| 500 | 64.4 ± 4.3 | 57.9 ± 7.2$^{NS}$ | 34.1 ± 5.2 | 21.6 ± 3.9$^{**}$ | 32.0 ± 0.8 | 21.8 ± 4.8$^{*}$ |
| 1000 | 62.5 ± 1.5 | 64.1 ± 3.0$^{NS}$ | 76.0 ± 5.1 | 59.1 ± 1.3$^{*}$ | 38.35 ± 3.0 | 25.9 ± 3.4$^{*}$ |

All values are mean ± SD (n = 3); Statistical analysis by *t*-test; Difference between ethyl acetate and acetone extract at a particular concentration for each cell line. $^{*}p < 0.05$; $^{**}p < 0.01$; NS, Non-significant; ND, not determined

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## CONFLICT OF INTEREST

None to declare.

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