

Full Length Research Paper

Isolation of piperdine from *Piper nigrum* and its antiproliferative activity

S. K. Reshmi, E. Sathya and P. Suganya Devi*

Department of Biotechnology, N. G. M. College, Pollachi, Coimbatore, Mahalingam Centre for Research and Development, Tamilnadu (India)-642001, India.

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Many plant derived molecules have shown a promising effect in therapeutics. Among the plants investigated to date, one showing enormous potential is the Piperaceae. Piperine is an alkaloid found naturally in plants belonging to the pyridine group of Piperaceae family, such as *Piper nigrum* and *Piper longum*. It is widely used in various herbal cough syrups and it is also used in anti inflammatory, anti malarial, anti leukemia treatment. So the present study was aimed to extract the phytochemical compounds in different solvent system in *P. longum*, *P. nigrum* and *Piper cubeba*. In preliminary screening and confirmatory test it was identified as alkaloid. The methanol extract of *P. nigrum*, *P. longum* and ethanol extract of *P. nigrum* and ethyl acetate extract of *P. cubeba* showed good result in TLC so these extracts were taken for HPTLC analysis. In HPTLC analysis of *P. nigrum* sample showed six alkaloid bands, two alkaloid bands were similar to Piperine standard 1 and 2, the other alkaloid may be piperidine, piperettine and piperanine. In *P. longum* sample contain three alkaloid bands one band was similar to Piperine standard 1, the other may be piperlongumine and piperlonguminine and no alkaloid band was found in *P. cubeba*. The anti bacterial was tested against gram positive and negative organism using agar well diffusion method. High activity was found in *P. nigrum* ethanol extract against the organism *Salmonella typhi*. The alkaloid piperdine was purified by refluxion method to check the antitumour activity which shows 51.38% of inhibition at 5 µg/ml concentration that conforms the compound piperidine to be used as anticancer drug for further mechanistic works.

Key words: Alkaloids, piperine, HPTLC, antibacterial activity, purification, piperidine, HEp2 cell lines.

INTRODUCTION

Many plant derived molecule have shown a promising effect in therapeutics (Lokhande et al., 2007). Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as Piperaceae (Dodson et al., 2000). *Piper longum*, *Piper nigrum* and *Piper cubeba* is a flowering vine in the family Piperaceae. *P. longum* (long pepper) is a small shrub with large woody root and numerous creeping, jointed stem, thickened at the node. The fruit contain 1% volatile oil, resin, a waxy alkaloid. It is used for several medicinal

properties. It has much pharmacological action such as antifungal, anti-inflammatory, antioxidant and anti cancer effect (Atal et al., 1985) and it is known to have insecticidal activity against mosquitoes and flies (Miyakado et al., 1989). The plant grows all over India, in evergreen forests and is cultivated in Assam, Tamil Nadu and Andhra Pradesh. *P. nigrum* (black pepper) it is a monocious or decorous climbing vine native to southern India and Srilanka and is extensively cultivated there and elsewhere in tropical regions. The shout climbing stem are very flexible with leathery blackish green leaves, they are widely cultivated in tropics. They have several uses such as they help in pain relief, rheumatism, chills, flu, colds, muscular aches and fever. Externally it is used for its rubefacient and as a local application for relaxed sore,

*Corresponding author. E-mail: suganyabiotech@yahoo.co.in.
Tel: 09942625387.

throat and some skin disorder. It has antimicrobial (Dorman and Deans, 2000), antimutagenic (El-Hamss et al., 2003), antioxidant and radical scavenging property (Gulcin, 2005) and inhalation of black pepper oil increase the reflexive swallowing movement (Vijayakumar et al., 2004). *P. cubeba* (Java pepper or tailed pepper) the berries of *P. cubeba* are commonly known as cubeb. It is mostly grown in Java and Sumatra. This is a perennial plant, with a climbing stem, round branches, about as thick as a goose-quill, ash-colored and rooting at the joints. The leaves are from four to six and a half inches long by one and a half to two inches broad, ovate-oblong, acuminate and very smooth. Flowers arranged in spikes at the end of the branches; fruit, a berry rather longer than that of black pepper. It is used to treat gonorrhoea, dysentery, syphilis, abdominal pain and asthma (Eisai 1995) and has also inhibitory effect on hepatitis C virus protease. Choi and Hwang (2003) demonstrated anti-inflammatory and analgesic activity of methanol extract from the fruit of *P. cubeba* it accumulates lignans and essential oil in a relatively high amount. The alkaloids, of which some 5,500 are known, comprise the largest single class of secondary plant substance. Alkaloids are often toxic to man and many have dramatic physiological activities; hence their wide use in medicine. They are usually colorless, often optically active substances, most are crystalline but a few (e.g. nicotine) are liquids at room temperature.

Piperine is an alkaloid found naturally in plants belonging to the pyridine group of Piperaceae family, such as *P. nigrum* and *P. longum*. Piperine is the trans stereoisomer of 1-piperoylpiperidine. It is also known as (E, E)-1- piperoylpiperidine and (E, E)-1- [5-(1, 3-benzodioxol-5-yl)-1-oxo-2, 4-pentdienyl] piperidine. Piperine is the alkaloid responsible for the pungency of black pepper and long pepper, along with chavicine (an isomer of piperine). It has also been used in some forms of traditional medicine and as an insecticide.

Majeed (1999) reported that piperine is widely used in various herbal cough syrups for its potent anti-tussive and bronchodilator properties. It is used in anti-inflammatory, anti-malarial, anti-leukemia treatment. Recent medical studies have shown that it is helpful in increasing the absorption of certain vitamins, selenium, beta-carotene, also increase the body's natural thermogenic activity.

Recently, many bacterial pathogens are becoming resistant to existing antibiotics due to their indiscriminate use in the treatment of infectious diseases (Davis, 1994; Service, 1995; Shears, 2000). Therefore, there is exigency to discover new and efficient antimicrobials from other source such as plant (Cordell, 2000; Karaman et al., 2003). In the present study an attempt was made to screen different extracts prepared from dried fruit of *P. nigrum*, *P. longum* and *P. cubeba* for its antimicrobial action against gram positive and gram negative bacteria. The alkaloid piperidine was purified for further studies in antitumour activity. The results of antitumour activity of piperidine, isolated for the first time from the plant, have

been reported in present communication.

OBJECTIVE OF THE STUDY

1. To assess the bioactive compound (alkaloids) from *P. longum*, *P. nigrum* and *P. cubeba*.
2. To evaluate the antimicrobial activity of bioactive compound (alkaloids).
3. To purify the compound piperidine from *P. nigrum*.
4. Antitumour activity of *Piperidine*.

MATERIALS AND METHODS

Plant material

The dried fruit of *Piper longum*, *Piper nigrum* and *Piper cubeba* were obtained from in and around Coimbatore district, Tamilnadu and stored in deep freezer.

Solvent extraction

The powdered plant material (10 g) was extracted with 50 ml of ethanol, methanol and ethyl acetate in a shaker for 72 h. The extract was concentrated to remove the solvent and filtered through Whatmann no: 1 filter paper (normal shaded). The clear extract was used for preliminary screening for alkaloids (Harbone, 1998).

Preliminary screening for alkaloids

The presence of alkaloids was screened by precipitating with the reagents like Dragendorff, Wagner and Mayer's (Harbone, 1998).

Confirmation of alkaloids by ultra-violet and TLC analysis

The presence of alkaloids was confirmed by ultra-violet and TLC analysis (Harbone, 1998).

Ultra-violet (UV) analysis

The samples were run on chromatography paper and were confirmed by viewing the chromatogram under UV light.

TLC analysis

The presence of alkaloids were analyzed using TLC by spraying the TLC plate with Dragendorff reagent.

HPTLC analysis for alkaloid profile (Dalmia Research center, CBE)

Samples given

P. longum (Methanol extract) - coded as 'A'.
P. nigrum (Methanol extract) - coded as 'B'.
P. cubeba (Ethyl acetate extract) - coded as 'C'.
 Standard piperine solution - coded as 'D'.

Procedure

Test solution preparation: All the 3 samples were centrifuged at 3000 rpm for 5 min. Samples 'A' and 'C' were taken for analysis as given and sample 'B' was diluted 4 times with methanol. These solutions were used as test solution for HPTLC analysis.

Samples and standard piperine loading

Three microlitre of the above test solutions and standard piperine solution (0.5 µg/1 µl) were loaded as 8 mm band length in the 5 x 10 Silica gel 60F₂₅₄ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (alkaloid) and the plate was developed in the respective mobile phase up to 90 mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV 366 nm.

Derivatization

The developed plate was sprayed with respective spray reagent (alkaloid) and dried at 110°C in hot air oven. The plate was photo-documented at white light using photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning

Finally, the plate was fixed in scanner stage and scanning was done at 500 nm. The peak table, peak display and peak densitogram were noted.

Analysis details

Mobile phase

Benzene-ethyl acetate (8:4)

Spray reagent

Dragendorff's reagent followed with 10% ethanolic sulfuric acid after dried and kept the plate in hot air oven at 120°C for 5 min.

Antibacterial activity

Bacterial strains

Pseudomonas fluoresces (gram negative), *Bacillus subtilis* (gram positive), *Escherichia coli* (gram negative), *Salmonella typhi* (gram negative).

Agar well diffusion method (Lokhande et al., 2007)

Antibacterial activity of the crude extract was determined by well diffusion method (Rios et al., 1988; Perez et al., 1990; Mosquera et al., 2004). The microbial culture were grown at 37°C for 18 h and then approximately diluted by sterile saline (0.9% w/v) solution to obtain a cell suspension of 10⁵ CFU mL⁻¹. Diluted inoculum was spread on Muller Hinton Agar plates. Wells of 6 mm diameter was punched into the agar medium and filled with 25 and 50 µl of the crude extract from *P. longum* (methanol), *P. nigrum* (methanol and ethanol) and *P. cubeba* (ethyl acetate). The plates were incubated for 18 – 24 h at 37°C. The antibacterial activity was evaluated by measuring the zone of Inhibition (ZOI). The antibiotic streptomycin was used in the test system as positive control.

Purification

Fifty gramme of powered samples of *P. nigrum* and *P. longum* were taken and mixed with 100 ml of Dichloromethane and kept in magnetic stirrer for 20 min for equal mixing. The extract was filtered through whatmann filter paper: 1 and kept for evaporation in the water bath at 100°C until dark brown oil is left. After cooling it in the ice packet for 5 min to that add 25 ml of cold Diethyl ether and stir for 5 min and evaporate the content again cool and add diethyl ether after 15 min formation yellow crystal piperidine is observed (if not repeat the step). The yellow crystal were filtered through whatmann filter paper 3. To recrystallize, the piperidine crystal was dissolved it in 8 ml of acetone:hexane (7:5) solution. Centrifuge at 10,000 rpm for 5 min. Dissolve the pellete and wash it with cold ether by again centrifuging at 10,000 rpm for 5 min. Finally dissolve the crystals in DMSO or methanol for further studies.

In-vitro studies

The purified compound piperidine isolated from *P. nigrum* was taken for cytotoxicity screening and microculture tetrazolium (MTT) assay

Cytotoxicity screening

HT 29 cell line (Human colon carcinoma) and HepG2 (Human liver carcinoma) were cultured in McCoy's 5A and DMEM (Dulbecco's modified eagles medium) medium respectively, containing 10% fetal calf serum, penicillin (100 U) and streptomycin (100 µg). 10 ml of DMEM or McCoy's 5A containing 10% serum was added to the flask and pipetted to breakdown the clumps of cells. Total cell count was taken using a haemocytometer and calculated the total number of cells. The medium was added according to the cell population needed. Required amount of medium containing the required number of cells (0.5-1.0 x 10⁵ cells/ml) was transferred into bottles according to the cell count and the volume was made up with medium and required amount of serum (10% growth medium and 2% maintenance medium) was added. The flasks were incubated at 37°C for 48 h in 5% CO₂ and the cells were periodically checked for any morphological changes and contamination. After the formation of monolayer, the cells were further utilized.

Determination of mitochondrial synthesis by microculture tetrazolium (MTT) assay (Mosmann, 1983)

This is a colorimetric assay that reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by succinate dehydrogenase. The MTT enters into the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells were then solubilised with



Figure 1. Test with Dragendroff's reagent (Bioactive alkaloid analysis).



Figure 2. Test with Wagner's reagent (Bioactive alkaloid analysis).

an organic product (e.g isopropanol) and solubilised formazan product is measured spectrophotometrically. The reduction of MTT level in the assay can occur only if the cells are viable. So the viability of the cells indicates the level of activity is measured based on the viability of the cells. In the MTT assay the number of viable cells was found to be proportional to the extent of formazan production. The percentage growth inhibition of the cell was calculated using the formula below:

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of Individual Test Group}}{\text{Mean OD of Control Group}} \right) \times 100$$

RESULT AND DISCUSSION

Preliminary screening for alkaloids

Test for alkaloids

Two milliliter of aliquots of the extracts were treated with the following reagents for the presence of alkaloids. When the extract was mixed with Dragendroff's reagent it gives orange coloured precipitate (Figure 1), when it was mixed with Wagner's reagent it gives reddish orange coloured precipitate (Figure 2) and it gives cream coloured precipitate when it was mixed with Mayer's reagent (Figure 3).



Figure 3. Mayer's reagent (Bioactive alkaloid analysis).



Figure 4. TLC analysis (Bioactive alkaloid analysis).

Table 1. Peak HPTLC analysis.

| Track | Peak | R _f | Height | Assigned substance |
|-------|------|----------------|--------|--------------------|
| A | 1 | 0.24 | 41 | Alkaloid 1 |
| A | 2 | 0.4 | 231.5 | Piperine 2 |
| A | 3 | 0.48 | 250 | Alkaloid 2 |
| B | 1 | 0.15 | 333.9 | Alkaloid 3 |
| B | 2 | 0.19 | 226 | Alkaloid 4 |
| B | 3 | 0.37 | 655.5 | Piperine 1 |
| B | 4 | 0.41 | 558.4 | Piperine 2 |
| B | 5 | 0.51 | 72.5 | Alkaloid 5 |
| B | 6 | 0.59 | 46.2 | Alkaloid 6 |
| C | - | - | - | No alkaloid band |
| D | 1 | 0.35 | 176.4 | Piperine 1 |
| D | 2 | 0.4 | 15.2 | Piperine 2 |

Confirmation of alkaloids by UV and TLC analysis

UV analysis: The florescent bands were also observed when the chromatogram was viewed under UV light.

TLC analysis of alkaloids: The methanol extract of *P. longum* And *P. nigrum* and ethanol extract of *p. nigrum* and ethyl acetate extract of *P. cubeba* was taken for TLC and HPTLC analysis. The presences of various alkaloids were analyzed using TLC. The samples were spotted individually in TLC plate and the solvent system used for this study was acetic acid and methanol in ratio of 8:2. Characteristic orange colour (Figure 4) bands were obtained when sprayed with Dragendroff's reagent and with R_f value *P. longum* (Methanol) -0.61, *P. nigrum* (Ethanol)-0.64, *P. nigrum* (Methanol)-0.65. No orange coloured bands were observed in *P. cubeba*.

HPTLC analysis for alkaloid profile

Detection: Bright orange colored zones were present in sample A and B at various R_f values in the chromatogram

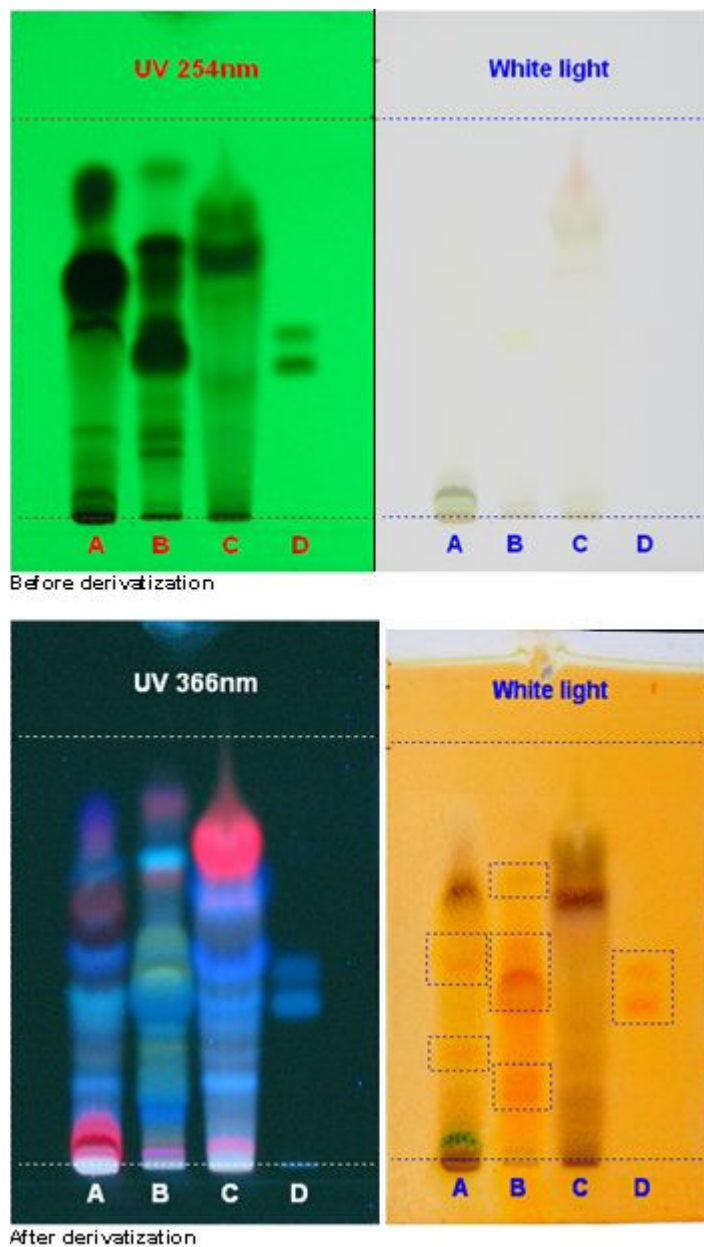
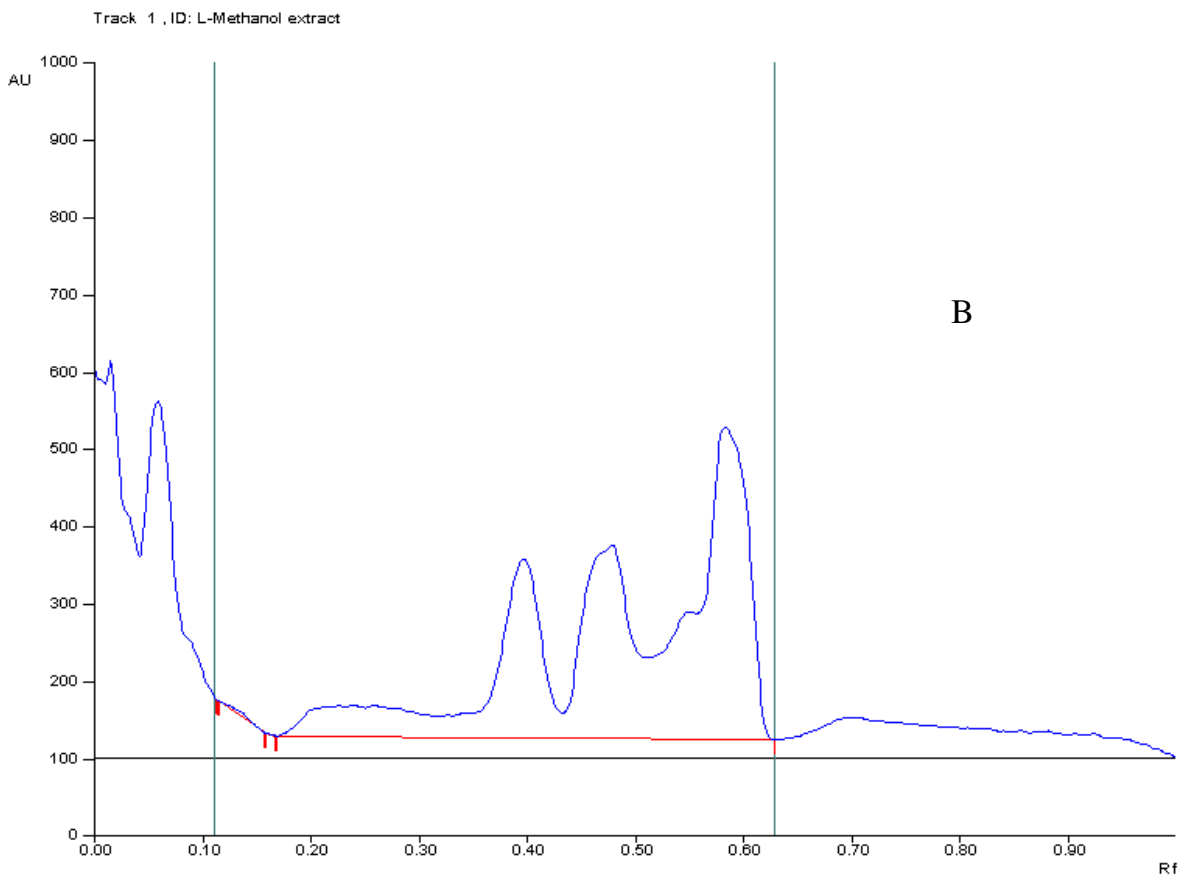
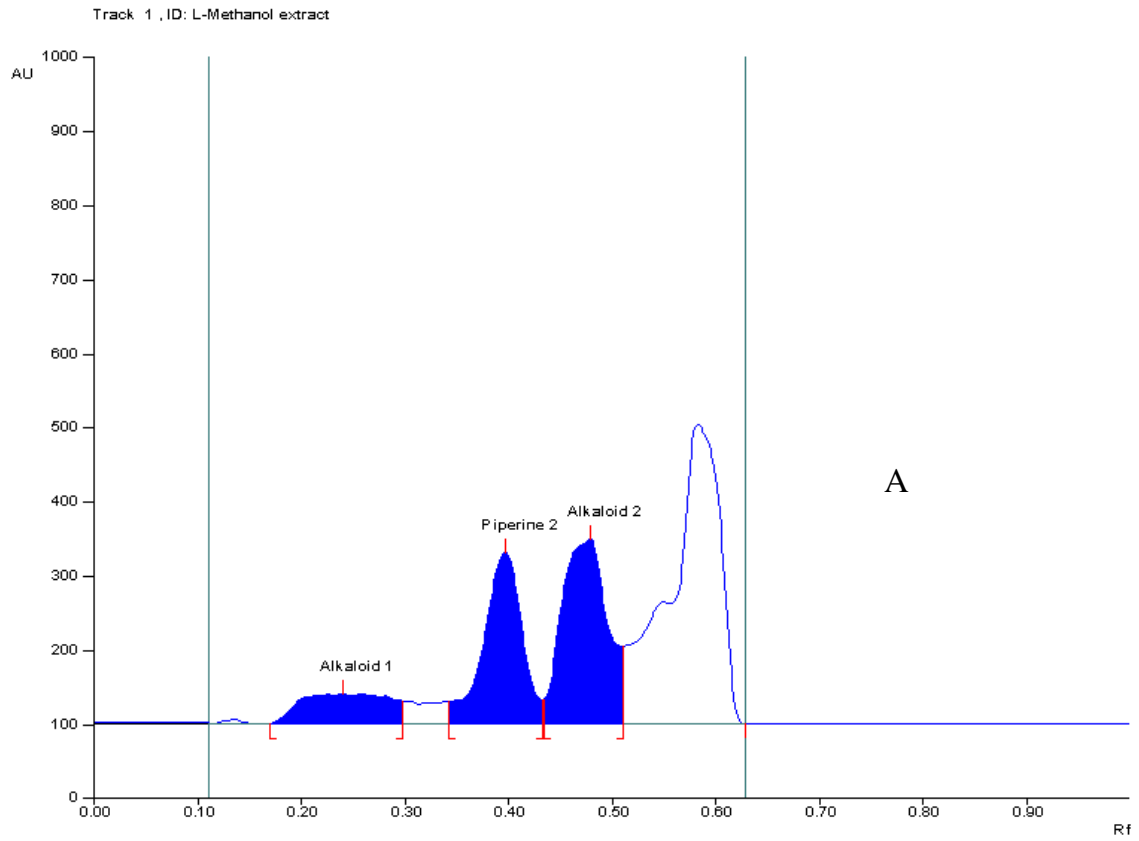
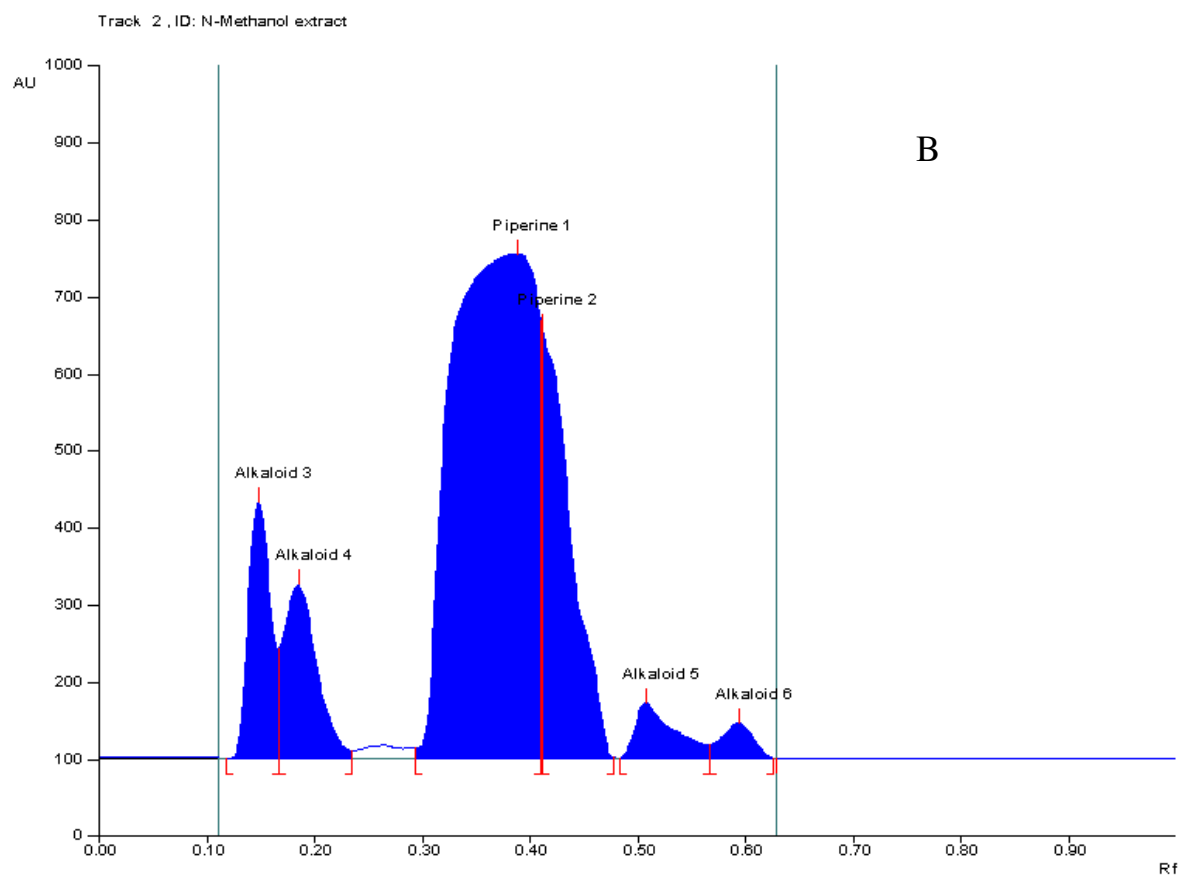
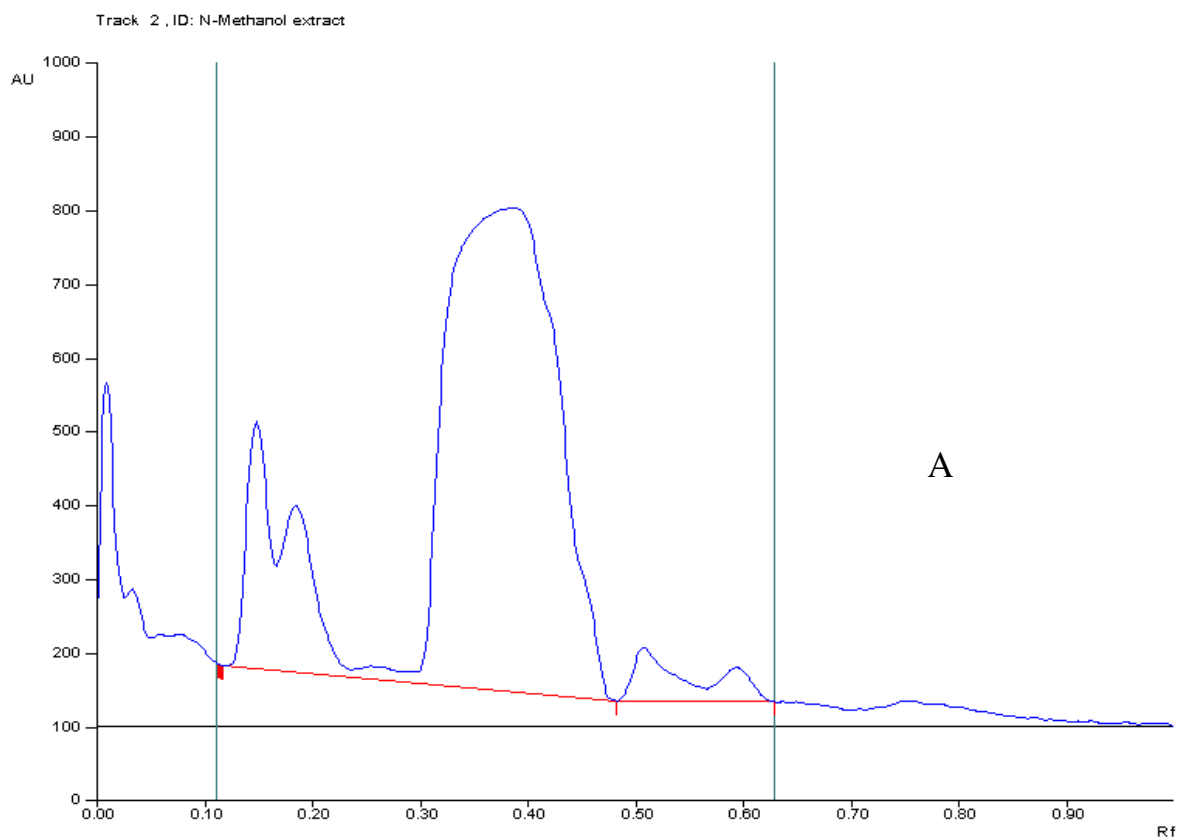


Figure 5. Chromatogram (HPTLC analysis). *P. longum* (Methanol extract) - coded as 'A', *P. nigrum* (Methanol extract) - coded as 'B', *Piper cubeba* (Ethyl acetate extract) - coded as 'C', Standard Piperine solution - coded as 'D'.

at white light after derivatization (peak Table 1), which belongs to alkaloid class compounds present in the given sample. From the standard piperine Track 4(a and b), it was confirmed that piperine is present in sample A and B with other alkaloids (Figure 5). From peak Table 1 given and from the graph the peak, R_f value, area and substance are determined. Track 1 (a and b) (*P. longum*) has piperine 2 in its second peak with the R_f value of 0.4. Track 2 (a and b) (*P. nigrum*) has piperine 1 and piperine 2 with the R_f value of 0.34 and 0.41. No alkaloids were found in *P. cubeba* sample under HPTLC analysis. Track 4



Track 1. A. Baseline display (Scanned at 500 nm). B. Peak densitogram display.



Track 2. A. Baseline display (Scanned at 500 nm). B. Peak densitogram display.

(Standard piperine¹ and 2) that is used to detect the presence of piperine in Tracks 1, 2 and 3. Thus from the result Track 2 (*P. nigrum*) show higher Rf value when compared to Track 1. Shanmugasundaram et al. (2008) reported that piperine was found in the cough syrup with an Rf value of 0.37 by HPTLC analysis and quantation was carried out at an UV 330 nm. Thus the concentration of piperine is more in *P. nigrum* than in *P. Longum*.

Chromatogram

The wavelength of alkaloid is between 254 to 366 nm, after derivatization at white light in sample A, 3 alkaloids were identified and one is piperine 2, in sample B, 6 alkaloids were identified and contain both piperine¹ and piperine 2. In sample C no alkaloid band was identified. Badheka et al. (1987) reported that the piperine bands were detected by UV light at 254 nm.

Peak densitogram

In densitogram of Track 1 contains three peaks the second peak was similar to standard piperine 2 Netz et al. (1993) reported that dried fruit of *P. nigrum* (Black pepper) contains piperine, piperidine, piperettine and piperanine. From the above results the other alkaloids may piperidine, piperettine and piperanine. Track 2 has six peaks in that 3rd and 4th was piperine 1 and 2. Desai et al. (1989) reported that *P. longum* showed the presence of Piperlongumine, piperlonguminine, piperine and pipartine.

From the above results the other alkaloids may Piperlongumine, piperlonguminine and pipartine. No alkaloids were found in *P. cubeba* sample under HPTLC analysis. From the above results the sample B had a higher concentration of piperine when compared in sample. Jeganathan et al. (2008) reported that the solvent system for HPTLC analysis for piperine was found to be toluene and ethyl acetate as mobile phase and similarly the mobile phase in this paper is benzene and ethyl acetate.

Antibacterial activity

The result of antibacterial activity of crude extract against the test bacteria are represented in Table 2. In comparison to the reference standard streptomycin (10 mg/disc) the crude extract exhibit significant antibacterial activity at a concentration of 50 µl. The ethanol extract of *P. nigrum* showed higher activity against the gram negative organism *Salmonella thypii* and lowest against ethanol extract of *P. nigrum* against *E. coli*. The zone of inhibition against gram positive and gram negative ranged from 0.4 to 1.6 µg/ml, respectively. Although, the crude extract showed activity against all tested bacterium it was

better against gram negative bacteria than gram positive bacteria. This analysis suggests that the ethanol extract of *P. nigrum* and ethylacetate extract of *P. cubeba* showed highest activity against *Salmonella thypii*. Chung et al. (1995); Vlietinck et al. (1995) had suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents. Bayer et al. (1966) also reported *in vitro* antibacterial activity of ethyl acetate extract and piperine exhibited good sensitivity against *Shigella dysenteriae*, *Staphylococcus aureus* and *Bacillus subtilis*. Ifra ghorri et al. (2009) also reported that *P. nigrum* was found to be effect against *Salmonella* and *B. subtilis*. Reddy et al. (2001) similarly reported about antibacterial activity of pure isolates from black pepper against *B. subtilis* and piperine against *S. aureus*.

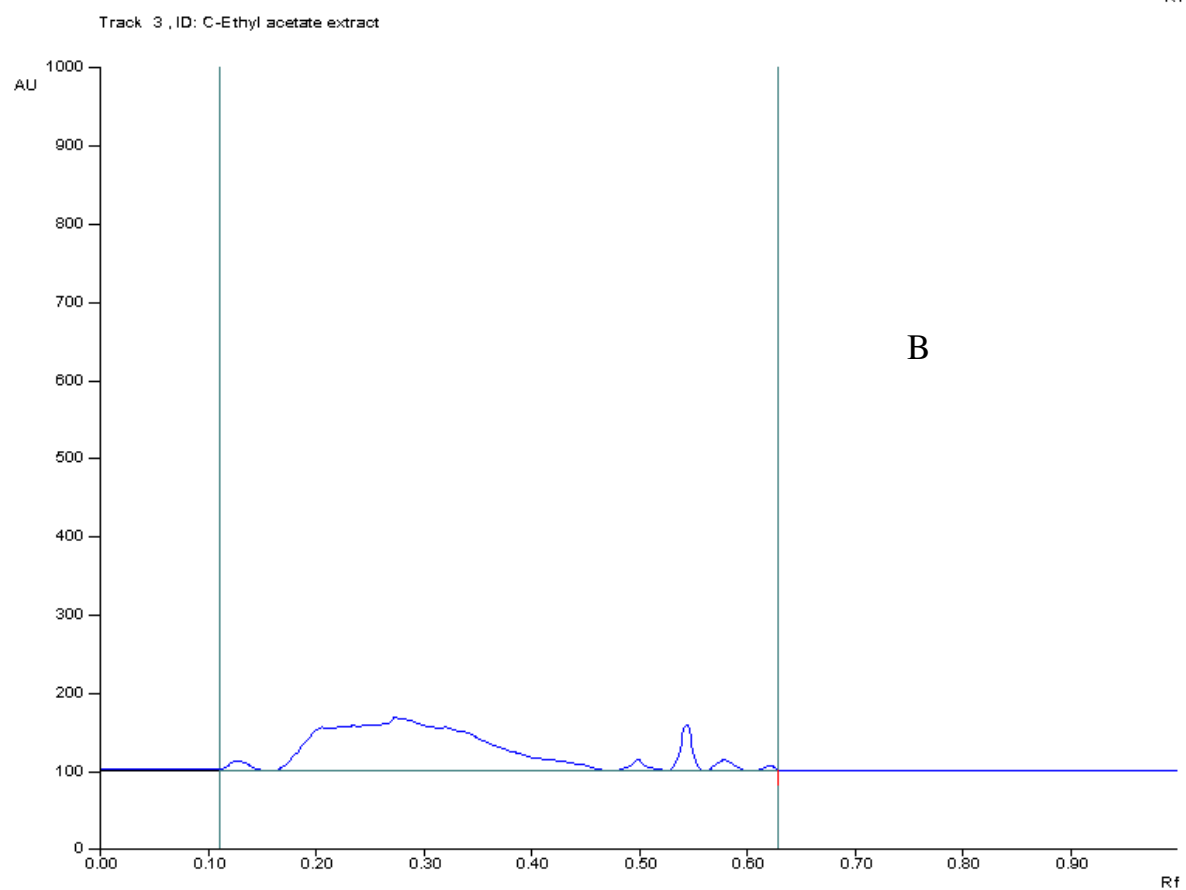
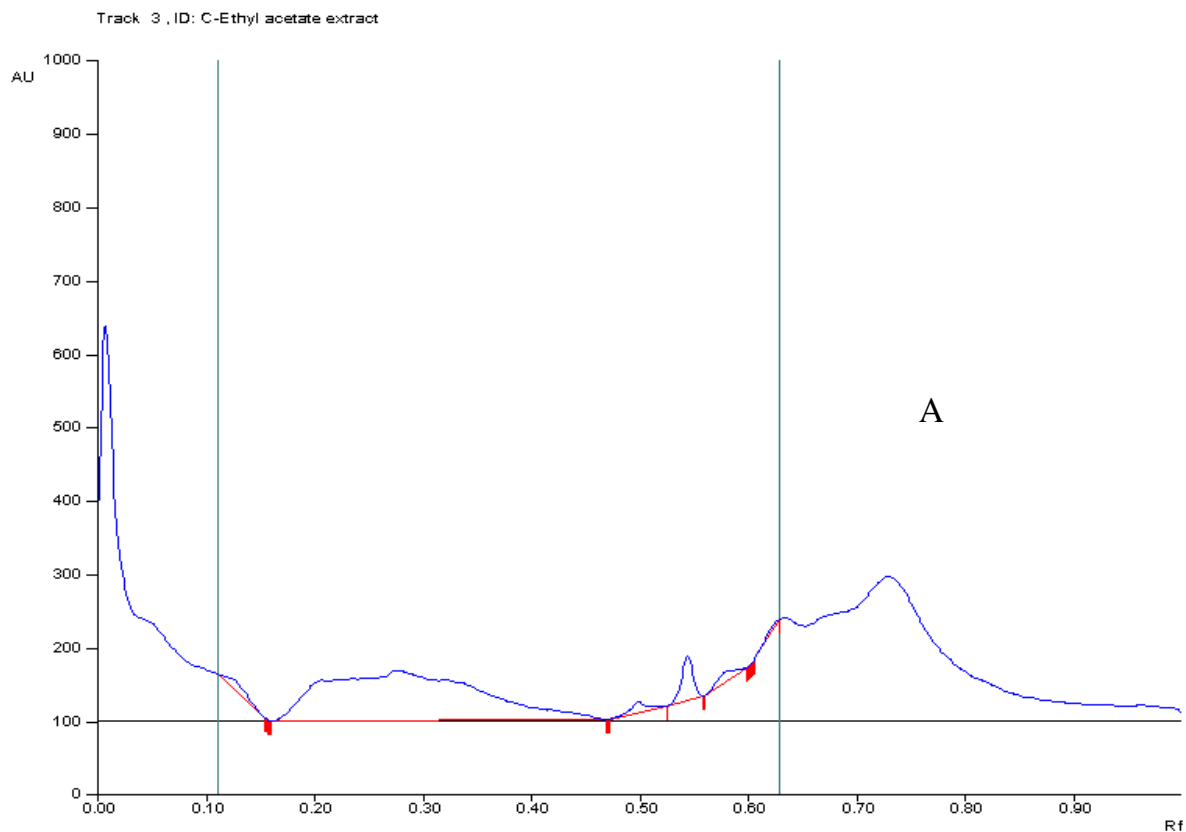
However, further research is needed to optimize the effective use of this agent in clinical practice (Molen, 2001). This assertion is also conformed as their extract indicates a relatively moderate number of photochemical present. From HPTLC analysis it as reported that the major phytochemical present in the crude extract of *P. nigrum*, *P. longum* was found to be piperine thus the inhibitor effect of crude extract of *P. nigrum*, *P. longum* and its active constituent was found to be piperine. Piperine extracted from *Ludwigia hyssopifolia* reported to have antitumour and antimicrobial activity was reported by Banibrata das et al. (2007). The antimicrobial activity of ethylacetate extract of *P. cubeba* was due to the presence of other phytochemical in the extract. It is suggested that more research to be conducted that will further elucidate and characterize the active component of *P. cubeba*.

Isolation and purification of piperidine

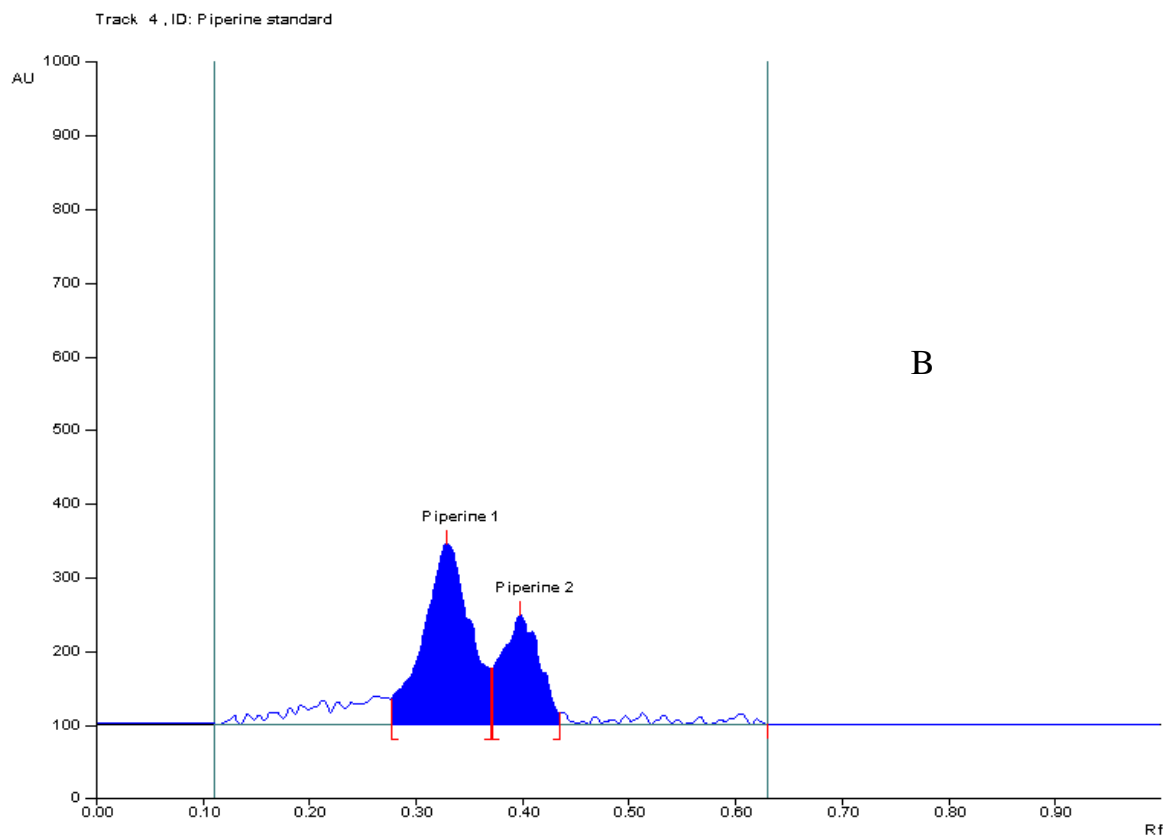
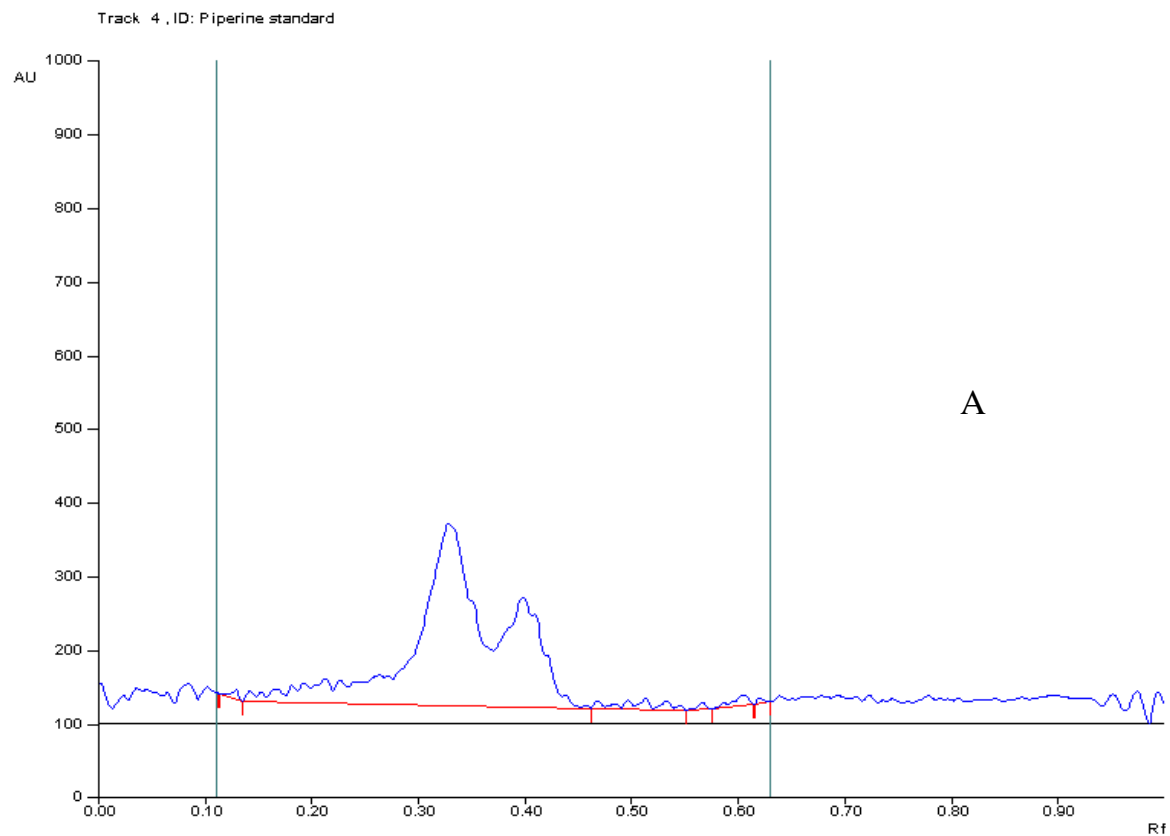
The purified alkaloid piperidine was observed only in *P. nigrum* but not in *P. longum*. Lim et al. (2009) identified the alkaloids like pellitterine, piperidine, piperine and peltorine in *P. nigrum* and *Piper betle* and that was the first report on (E)-1-[3'4'-(Methylenedioxy)cinnamoyl] piperidine 2 from *P. nigrum* as a natural product.

Antitumour activity

According to a report by World Health Organization (WHO), the use of herbal medicine is in increasing trend in both developing and industrialized countries. Considering the fact that over one-third of the population in developing countries lack access to essential medicines and the provision of safe and effective traditional therapies could become a critical tool to increase access to health care, WHO launched its first ever comprehensive traditional medicine strategy in 2002 (Banibarta et al., 2007). From the extract of *P. nigrum* we have isolated an alkaloidal constituent piperidine. *In vitro* antitumour activity of isolated compound, piperidine was performed by MTT



Track 3. A. Baseline display (Scanned at 500 nm). B. Peak densitogram display.



Track 4. A. Peak densitogram display. B. Baseline display (Scanned at 500 nm).

Table 2. Antibacterial activity against tested organism.

| Samples | Concentration (μ l) | <i>E. coli</i> (mm) | <i>Pseudomonas fluorescens</i> (mm) | <i>B. subtilis</i> (mm) | <i>S. typhi</i> (mm) |
|----------------------------------|--------------------------|---------------------|-------------------------------------|-------------------------|----------------------|
| <i>P. longum</i> (methanol) | 50 | 1.2 | 1.1 | 0.8 | 0.6 |
| | 25 | 0.6 | 0.6 | 0.5 | 0.5 |
| <i>P. nigrum</i> (methanol) | 50 | 0.8 | 0.7 | 1.2 | 1.2 |
| | 25 | 0.7 | 0.4 | 1 | 0.8 |
| <i>P. nigrum</i> (ethanol) | 50 | 0.5 | 1.5 | – | 1.6 |
| | 25 | 0.4 | 0.6 | – | 1.4 |
| <i>P. cubeba</i> (ethyl acetate) | 50 | – | 1 | 0.9 | 16 |
| | 25 | – | 0.6 | 0.8 | 1.2 |

Table 3. Antitumour activity of *P. nigrum*.

| Concentration of sample (μ g/ml) | Percentage cell viability (MTT assay) |
|---------------------------------------|---------------------------------------|
| 5 | 51.38 |
| 2.5 | 62.5 |
| 1.25 | 80.55 |
| 0.625 | 86.11 |
| 0.3125 | 94.44 |
| 0.156 | 95.83 |
| Cell control | - |
| Cytotoxic changes observed | Cell rounding, cell death |

**Figure 6.** Control Hep2 cells showing oval or rod shaped cells with cell to cell anchorage.

HEp2 cells appeared as elongated shape, attached smoothly on the culture cell face and some of the cells grouped together to form colonies (Figure 6).

Following treatment with extract for 24 h, the cells changed to round shape and lost cell contact (Figure 7). In particular the cells lost their surface morphology and died at a concentration of 50%. The study confirms the *in vitro* antiproliferative property of piperidine against HEp2 cancer cell line. Lim et al. (2009) reported that the extract of *P. nigrum* and *P. betle* have cytotoxicity activity against HL60 and HELA cell line. To the best of our knowledge, there is no previous work on the antitumour activity of the compound piperidine.

Recent reports have cited that so many plants and its components could act as antitumour suppressor, apoptotic inducer in cancer cell. The present study is well correlated with previous reports on cancer suppressing activity and anticarcinogenic activity.

Conclusion

Many plant have alkaloid as a major secondary metabolite, the present study points to the probable

assay. The compound piperidine exhibited 51.38% inhibition of HEp2 cells (Human epithiloma cells of laryax) at concentration of 5 μ g/ml of the six different concentration of piperidine the highest concentration displayed a highest inhibition displayed a dose dependent antiproliferative activity on HEp2 cells (Table 3). Untreated

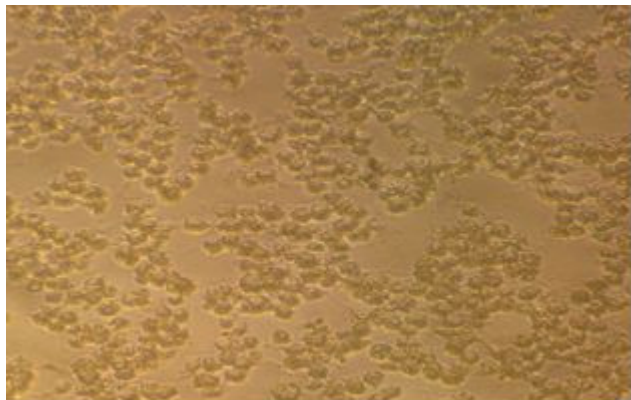


Figure 7. Piperidine treated Hep2 cells showing spherical shaped cells leading to loss of cell anchorage with concentration of 5 µg / ml.

antimicrobial and antitumour potential and also conforms the presence of alkaloid piperine and piperidine by HPTLC analysis. The results of these investigations should be helpful in the better explaining the complex pharmacological activity. Following these studies the study confirms the potential of piperidine can be used as anticancer drug, further more mechanistic work is essential to prove these compounds as one of the specific cancer drug.

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