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STRUCTURAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF COMPOUNDS ISOLATED FROM ACETONE EXTRACT OF *PIPER LONGUM* FRUITS

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ABSTRACT

Acetone has been very rarely employed as an extraction medium for isolation of phytochemicals from *Piper longum* though it has been rated as a very good solvent for extraction. In the present study two compounds were isolated from the acetone extract of *Piper longum* fruits, characterized using IR and NMR spectroscopy and the antibacterial activity against *E.coli* and *M.smegmatis* was tested. Based on the spectral studies it was observed that one of the compounds designated P had the chemical groups similar to piperine. The compound designated Y had chemical groups similar to that of piperine with the absence of N- $(CH_2)_2$ group of the pyridine ring. Compound P had no activity against *E.coli* and significantly less activity against *M.smegmatis* compared to standard drug. Compound Y had no activity against M.smegmatis and significantly less activity against *E.coli* compared to standard drug. Though the two isolated compounds did not possess significant antibacterial activity , the study demonstrates the use of acetone as a potential solvent for extraction.

KEY WORDS
Piper longum
Antibacterial activity
IR spectra, NMR spectra

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INTRODUCTION

Plants produce a host of bioactive molecules which have probably evolved as chemical defences against infection, predation or physical agents. (Richard .A. Dixon, 2001) Hence the interest of researchers in Ethno pharmacology continues to remain unabated and current research focuses on evaluating the anti bacterial , hepato protective potential of plant extracts. (Sharad Bissa, 2015) (Parvathi, Ramesh *et al*., 2013). Among the various medicinal plants , *Piper longum* holds special interest because piperine, the alkaloid in *Piper longum* is reported to act as a bioenhancer of rifampicin , bioavailability enhancer of anti tuberculosis drugs and nutrients and as a hepatoprotective agent. (Veena Balakrishnan *et al*., 2001), (Atal & Bedi, 2010), (Koul & Kapil., 1993).

Acetone has been ranked as the best solvent for extraction based on the ability to solubilize antimicrobials, ease of removal of solvent and also based on its ranking as biohazard. (Cowan, 1999) Hence the objective of the present study was to isolate compounds from acetone extract of *Piper longum* followed by characterisation and testing for antibacterial activity

MATERIALS AND METHODS

The fruits of *Piper longum* purchased from local store was authenticated by Dr.S.Brinda of Central Institute for Siddha Research, Chennai. The fruits were shade dried and ground to a powder. The powder was stored in a sealed bottle at 4°C. 20g of the powder was soaked in 100 ml of acetone in sealed bottles for 24 hrs. In a separating funnel the extracts were partitioned with hexane and subsequently with chloroform similar to the procedure by Sung Eun Lee ,2000. Preparative thin layer chromatography of the hexane fraction in silica gel with cyclohexane gave a yellow compound designated Y with R_f = 0.9. The purity of the compound was ascertained by a check TLC. Chromatography of the chloroform fraction with a solvent system of hexane/acetone (65:35) similar to the procudure by Epstein *et al.,* 1993 gave a compound designated P with R_f 0.7. The purity of the compound was ascertained by a check TLC.UV light from a Hanovia lamp was used to view the developed chromatogram. The compounds Y and P were characterized by IR and NMR spectra. NMR spectra of Piperine obtained from Sami Labs, Bangalore was also recorded. E.*coli* (gram negative), and *M. smegmatis* (mycobacteria). cultures were obtained from Bacteriology Department of Tuberculosis Research Centre. *E.coli* was sub cultured in nutrient agar and *M. smegmatis* was subcultured in Lowenstein Jensen medium. For comparison of anti bacterial activity, standard drugs Ofloxacin (E.coli) and Ethambutol (M.smegmatis) were used. The anti bacterial activity of compounds

Y and P was studied by disc diffusion method in nutrient agar (E.coli) and solid sauton's medium. (M.smegmatis). (Robert. F.Boyd,1984).

RESULTS

Figure 1 & 2 gives the IR spectra of compound P and compound Y respectively. Figure 3, 4, & 5 gives the NMR spectra of piperine obtained from Sami labs, compound P and compound Y respectively. Table 1 gives the antibacterial activity of compounds P and Y.

DISCUSSION

Peaks obtained in IR spectra of Compound P (Table 2) indicate that compound P had all groups similar to piperine as reported in previous studies. (Aditi Gupta *et al.*, 2013) (Deepthi Swapna *et al.*, 2012). The NMR peaks of compound

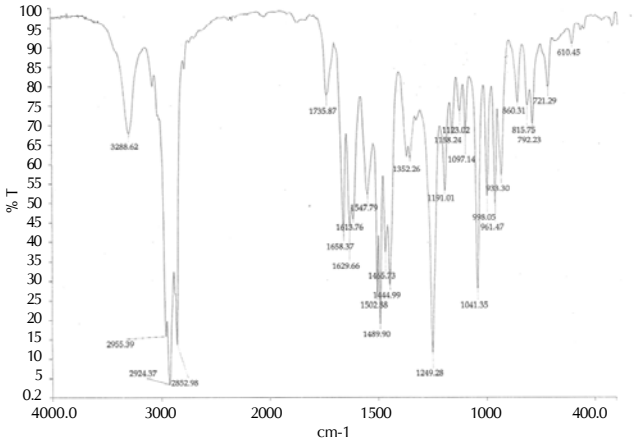


Figure 1: IR Spectra of Compound P

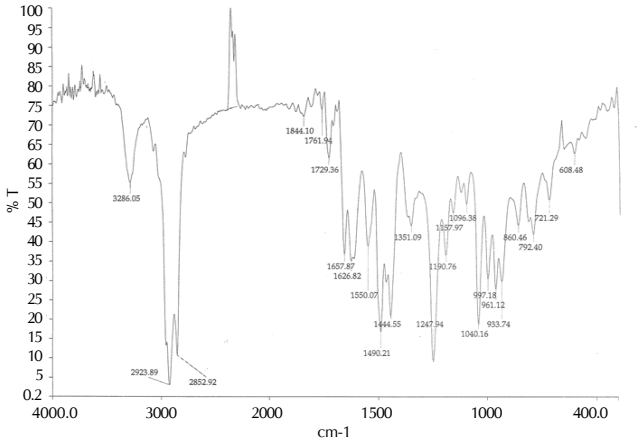


Figure 2: IR Spectra of Compound Y

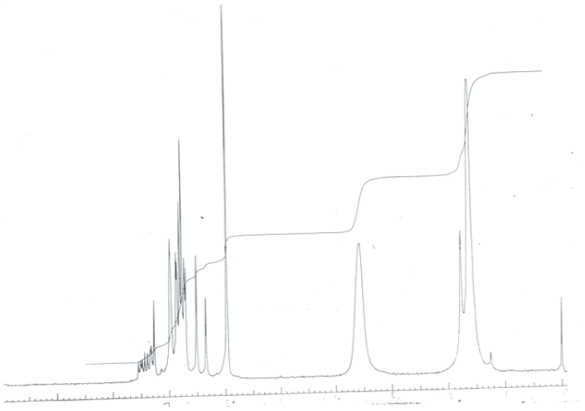


Figure 3: NMR Spectra of piperine

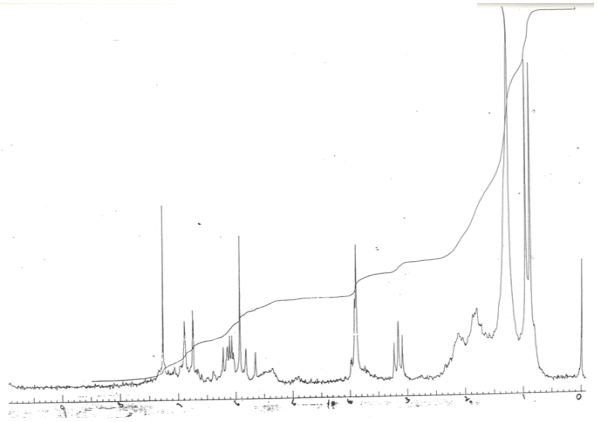


Figure 4: NMR Spectra of Compound P

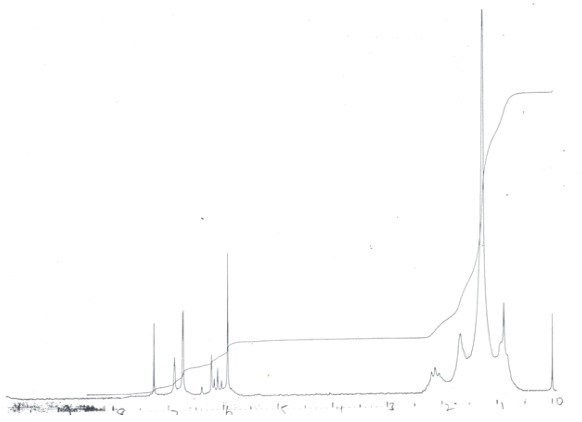


Figure 5: NMR Spectra of Compound Y

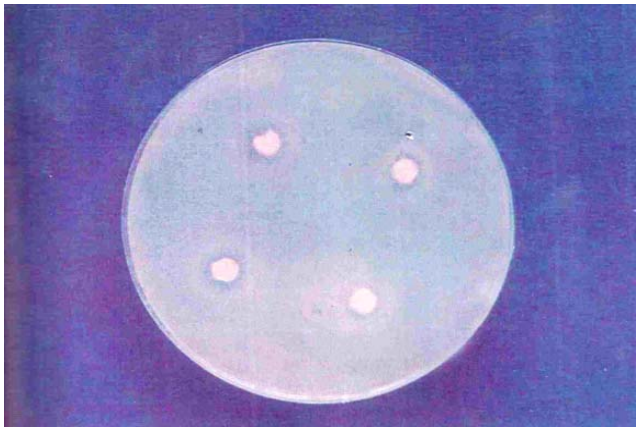


Photo 1: Activity of compound Y against E.coli

Table 1: Antibacterial Activity by Disc diffusion method

Microorganisms	Minimum μg on the disc that produced zone of inhibition		
	Compound Y	Compound P	Standard Drug
E.coli (gram negative)	115	No activity	0.016 (ofloxacin)
M. smegmatis (mycobacteria)	No activity	128	0.06 (ethambutol)

Table 2:Ir Spectra

Groups	Compound P	Compound Y
Asymmetric and symmetric CH_2 stretching ,aliphatic stretching	2925/cm2853/cm	2923/cm2852/cm
Aromatic stretching C=C benzene	1610/cm1597/cm1490/cm	1626/cm1550/cm1490/cm
CH_2 bending	1440/cm	1444/cm
C-O stretching (most common)	932/cm	933/cm
CH bendingTrans double bond	998/cm	/997/cm

Table 3: Nmr Spectra

Groups	Piperine	Compound P	Compound Y
3 X CH_2	1.6 δ	1.41δ	1δ
$N<^{CH_2}_{CH_2}$	3.5δ	3.2δ	Nil
$O>^{O}_{CH_2}$	5.9δ	5.9δ	5.9δ
CH = C<	6.4 δ	6.2δ	6.2δ
Olefenic aromatic	6.7-6.8 δ	6.7-6.9δ	6.7-6.9δ
$OCH - CH - \overset{O}{\overset{\|\|}{C}}$	7.3-7.5 δ	7.2-7.4δ	7.2δ

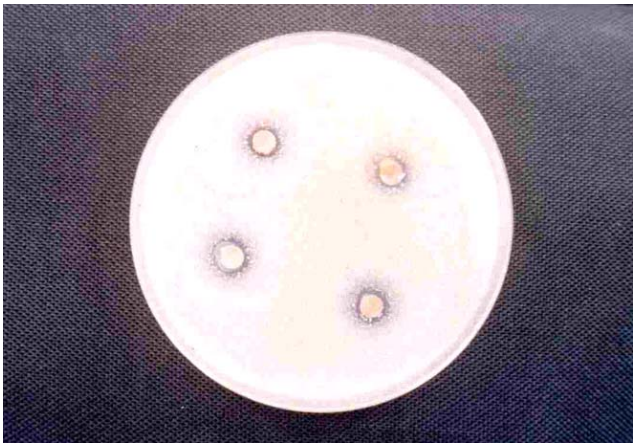
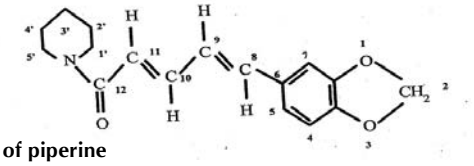


Photo 2: Activity of compound P against M. Smegmatis

P and piperine are also similar (Table 3) indicating structural similarity and also conforms with previous studies. (Berger. and Sicker., 2009) IR peaks of compound Y are similar to compound P(Table 2) but the NMR peaks indicate the absence of N- $(CH_2)_2$ group of the pyridine ring . It is therefore inferred that the compound Y has similar groups like piperine except for the pyridine heterocyclic ring



Structure of piperine

Compound Y had no activity against M.smegmatis and significantly less activity against *E.coli* compared to standard drug.(table 1, photo1) Compound P had no activity against E.coli and possessed significantly less activity against *M.smegmatis* compared to standard drug (Table 1, photo 2). Piperine has been reported to have activity against mycobacterial species. (Deepthi Swapna, 2012).

In the present study an attempt was made to isolate compounds using acetone as solvent which has been very rarely employed in isolation procedures. The compounds isolated were characterized but they did not possess significant activity compared to standard drugs. However the, the study demonstrates the use of acetone as a potential solvent for extraction.

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