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Research Article

Isolation and characterization of lead molecule from *gramineae* and *piparaceae* drugs

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ABSTRACT

The classes of phytochemicals have been isolated from Piper species, phenylpropanoids, lignans and neolignans, alkaloids and several compounds of mixed-biosynthetic origin. *Piper betle* Linn. (Piperaceae) is a perennial dioecious creeper, cultivated in India for its leaves, used for chewing. The leaf is carminative, aphrodisiac, tonic, laxative and improves appetite. *Cynodon dactylon* (L.) Pers. (Fam: Gramineae) is commonly known as "Doob" in India (Arugampul: Tamil). It is a weed and possesses varied medicinal properties. Leaf, root and rhizome of the plant have been used in folk medicine in different countries, as anti-inflammatory, anticystitis, antihypertensive antiviral, hypolipidemic agent, antihysteria, antipsychotic, and antigonorrheal infection. A weighed quantity (500 g) of the air-dried powdered drugs of C. dactyon and P. Betle was taken, in a soxhelet apparatus. The filtrate was concentrated in a rotary flash evaporator at a temperature not exceeding 60°C (yield 15.0% for PB extract and 13.5% for CD extract, dry weight basis). The isolation was performed by using column chromatography having the dimension of 63.3 cm in length, 1.3 cm in diameter by using silica gel (100-200 mesh) as the stationary phase for the collected ethyl acetate extract of *Piper betle* by wet packing method. The collected fraction of the solvent system having 50:50 ratio of toluene: ethyl acetate has shown the R_f value of 0.6, and for *Cynodon dactylon* having 90:10 ratio of Chloroform and ethyl acetate has shown the R_f value of 0.76, it was compared with standard R_f and the fraction was characterized by using IR, NMR, and mass spectroscopy studies. The Spectral data revealed that the isolated compounds from the selected plant materials were 5-Phenyl-3,4-alkynyl-2-pentanone and 1-methyl ethyl propanoate, these componenets were new compounds and they not yet isolated.

Keywords: *Cynodon dactylon; Piper betle;* 5-Phenyl-3,4-alkynyl-2-pentanone; 1-methyl ethyl propanoate

INTRODUCTION

In the last few years there has been an exponential growth and changes in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. Combined therapies have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves that work together in a dynamic way to produce therapeutic efficacy with minimum side effects (Sheetal Verma et al., 2008). The classes of phytochemicals have been isolated from Piper species, phenylpropanoids, lignans and neolignans, alkaloids and several compounds of mixed-biosynthetic origin (Massuo J. Kato1 et al., 2007). *Piper betle* Linn. (Piperaceae) is a perennial dioecious creeper, cultivated in India for its leaves, used for chewing. The leaf is carminative, aphrodisiac, tonic, laxative and improves appetite. Leaves contained caryophyllene, cadinene, γ-lactone, allyl catechol, p-cymene and eugenol methyl ether in varying amounts (Massuo J. Kato1 et al., 2007). *Cynodon dactylon* (L.) Pers. (Fam: Gramineae) is commonly known as "Doob" in India (Arugampul: Tamil). It is a weed and possesses varied medicinal properties. Leaf, root and rhizome of the plant have been used in folk medicine in different countries, as anti-inflammatory, anticystitis, antihypertensive antiviral, hypolipidemic agent, antihysteria, antipsychotic, and antigonorrheal infection. In India, the plant is used in the treatment of melena, thirst, anorexia, burning sensations in the body, pruritis, miscarriage and erysipelas and its leaf juice with a pinch of common salt has been used orally to treat stomachache. Decoction of whole plant is given orally to cure menstrual problem (Badri prakash Nagori et al., 2011). The aim of this study is to mainly investigate to isolate *Cynodon dactylon* and *Piper betle* lead molecule and characterized through spectral studies.

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MATERIALS AND METHODS

Fresh leaves of C. dactylon And P.Betle were collected from Local area. The plants were taxonomically identified and authenticated by Dr. Madhavachetty, Department of Botany, S.V. University, Tirupathi, Andhra Pradesh. The materials were washed, shade dried, powdered, passed through sieve no. 60 and stored in air tight containers for experimental work.

Preparation of Ethyl acetate Extract of C. dactylon and P. Betle Leaves

A weighed quantity (250 g) of the air-dried powdered drugs of C. dactyon and P. Betle was taken, in a soxhelet apparatus. The extraction was carried out by using solvents of increasing polarity starting from Diethyl ether, benzene, dichloromethane, acetone and ethyl acetate for a period of 72 hours. The filtrate was concentrated in a rotary flash evaporator at a temperature not exceeding 60°C (yield 20.36 % w/w, dry weight basis) and was dissolved in distilled water for experimental studies (C. K. Kokate et al., 22nd Edition).

ISOLATION OF LEAD MOLECTULES FROM C. dactylon and P. betle

The isolation was performed by using column chromatography having the dimension of 63.3 cm in length, 1.3 cm in diameter by using silica gel (100-200 mesh) as the stationary phase for the collected ethyl acetate extract of *Piper betle* by wet packing method. The compounds are eluted by using Chloroform, Toluene and Ethyl acetate and chloroform and ethyl acetate for *Cynodon dactylon* in the ratio of 50:50, 90:10. The purity of all isolated substances was examined by thin layer chromatography (TLC) using Merck silica gel coated aluminum plates 200 µm layer thickness, with several solvent systems of different polarity. Spots were visualized by short-wave UV light, and FeCl3 reagents (H. Wagner et al., II edition), (Agarwal et al.,)

Characterization

The sample was subjected to IR, NMR and Mass spectroscopic studies. IR spectroscopy is performed by using pressed pellet technique and it is useful in determining the important functional groups of the compound as a part of its structural identification. The sample was subjected for the proton and ^{13}CNMR.The simple procedure involves continuous wave method. In this, a solution of sample in a uniform 5mm glass tube is oriented between the poles of a powerful magnet, and is spun to average any magnetic field variations, as well as tube imperfections. Radiofrequency radiation of appropriate energy is broad cast into the sample from an antenna coil. A receiver coil surrounds the sample tube, and emission of absorbed R_f energy is monitored by dedicated electronic devices and a computer. Mass Spectrum is a record of the masses and the relative abundances of the molecular ion and the positively charged fragments formed from it by the electron bombardment. The mass spectrum of a pure compound provides several kinds of data which are useful for its identification (B.K. Sharma et al., 24th edition), (Y.R. Sharma et al., 4th edition).

RESULTS AND DISCUSSION

Isolation has been carried out with the solvent system of toluene: ethyl acetate in various proportions and the fractions were collected and tabulated.

Table 1: Isolation of ethyl acetate fraction of *Cynodon dactylon*

S. No	Name of the Solvent system	polarity	Fraction	Compound
1.	Chloroform	100%	1-5	--
2.	Chloroform: Ethyl acetate	50:50	6-10	--
3.	Ethyl acetate	100%	11-16	--
4.	Chloroform: Ethyl acetate	60:30	17-22	--
5.	Chloroform: Ethyl acetate	70:30	23-29	--
6.	Chloroform: Ethyl acetate	80 :20	30-35	--
7.	Chloroform: Ethyl acetate	90:10	36-40	Spot(Dark Brownish yellow)

The collected fraction of the solvent system having 50:50 ratio of toluene: ethyl acetate has shown the R_f value of 0.6, it was compared with standard R_f and the fraction was characterized by using IR, NMR, and mass spectroscopy studies.

Table 2: Isolation of ethyl acetate fraction of *Piper betle*

S. No	Name of the Solvent system	polarity	Fraction	Rf Value
1.	Toluene	100%	1-5	No spot
2.	Toluene: ethyl acetate	90:10	6-10	No spot
3.	ethyl acetate	100%	11-16	No spot
4.	Toluene: ethyl acetate	80:20	17-22	No spot
5.	Toluene: ethyl acetate	70:30	23-29	No spot
6.	Toluene: ethyl acetate	60 :40	30-35	No spot
7.	Toluene: ethyl acetate	50:50	36-40	Spot (White color)

The IR spectrum Fig. 1(a), and 1(b) shows an aromatic C-H bending of monosubstituted benzene at 749, C-H stretching in $-CH_2-$ at 1500, C=C stretch in aromatic ring at 1480, C=C stretch in aromatic ring at 1600, C=C stretch in aromatic ring at 1620, C=O stretch in α, β unsaturated ketones at 1670, alkenyl stretch at 2230,

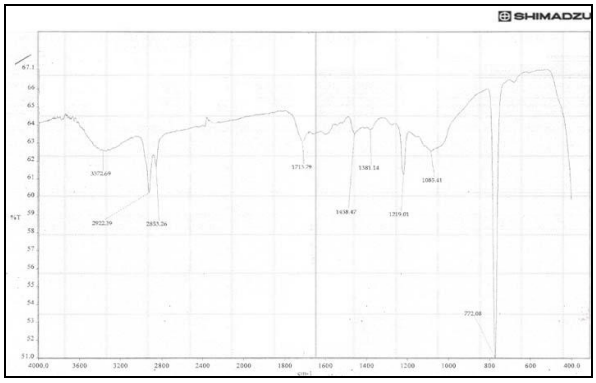


Figure 1(a): IR spectrum of CD isolate

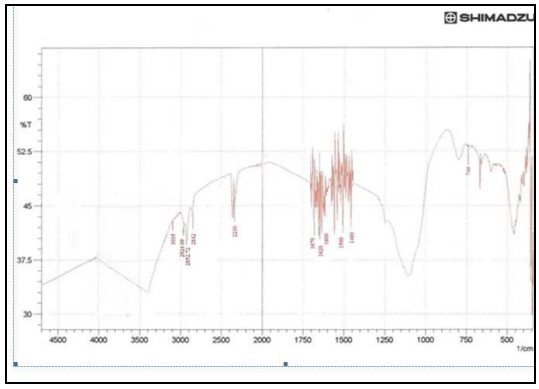


Figure 1(b): IR Spectrum of PB isolate

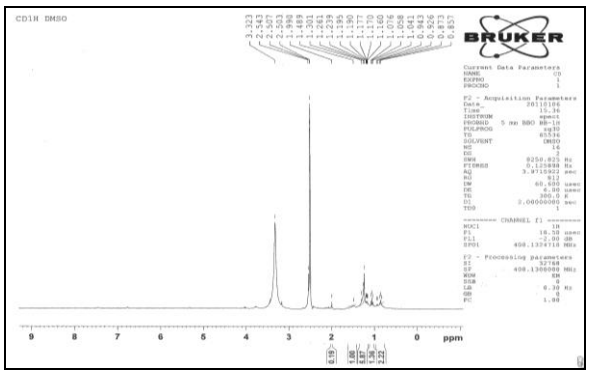


Figure 2(a): ^{1}H NMR of CD isolate

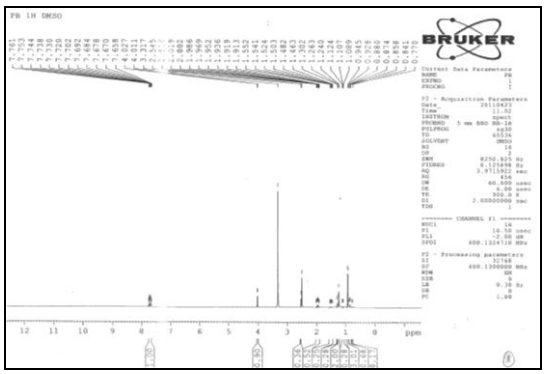


Figure 3(b): ^{1}H NMR of PB isolate

C-H stretch in CH_3 at 2842, C-H stretch in alkanes at 2852.72, C-H stretch in alkanes at 2924.09, Ar-H stretch in aromatic hydrocarbons at 3035 peaks from the interpretation of IR spectrum and the possible functional groups were determined. And for *Cynodon dactylon* the IR spectrum shows may be aromatic hydroxyl group, carboxylic hydroxyl group at 3372, Alkane CH_2 stretch at 2922, may be C=0 stretching at 1713, may be CH_2 and CH_3 stretching at 1458,CH_3 bending at 1381, C=0 stretching at 1219.IR spectrum possible to functional groups were determined.

NMR Spectroscopy

^{13}C NMR

The δ values Fig. 3(a) and 3(b) , 71.11, 131.5 and 131.71, 166.88 for C_1, C_2, C_3 and the possible groups may be alkenyl, aromatic double bonds and C=O as per the interpretation. For *Cynodon dactylon* The C_{13} NMR spectra of isolated compound revels that shift 173.1,node C shows presence 1-carboxyl(166.0),1-C(C)C(13.5),1-C from 0-carboxyl(-5.0),general corrections (-1.4).The shift 40.9,node CH shows presence aliphatic (-2.3),1 alpha-C(=0)-0(21.8),2 alpha-C(18.2)-1 beta-C(9.4) 1gamma-C(-2.5),general corrections(-3.7). The shift 16.6,node CH_3 shows presence aliphatic (-2.3),1 alpha-C(9.1)1 beta -C(=0)-0(2.0),1 beta-C (9.4), 1gamma-C(-2.5), 1 delta-C(0.3),general corrections(0.6). The shift 52.2,node CH_3 shows presence aliphatic (-2.3),1 alpha-0-C=0(54.9),1 1 gamma-C(2.5),2 delta-C(0.6),general corrections(1.5). The shift 26.6,node CH_2 shows presence aliphatic (-2.3),2 alpha-C(18.2),1 beta -C(=0)-0(2.0),1 beta-C (9.4), 1 delta-C(0.3),general corrections(1.0). The shift 11.0, node CH_3 shows presence aliphatic (-2.3), 1 alpha-C(9.1)1 beta –C(9.4), 1gamma-C(=0)-0(-2.8), 1 gamma-C(-2.5),general corrections (0.1).

Proton NMR

The proton NMR Fig. 2(a) and 2(b) shows δ values of 0.928 and 0.945 for H_1, 2.506 and 2.545 for H_2, 3.317 for H_3,7.658, 7.670, 7.678, 7.684, 7.692, 7.702, 7.720, 7.730, 7.738, 7.744, 7.753, 7.761 for H_4 and the possible groups may be aliphatic alicyclic, alkenyl, aliphatic mono substituted or di substituted and benzene as per the interpretation. For *Cynododn Dactylon* The proton NMR spectra of isolated compound revels that shift 2.49, node CH shows presence methane (1.50),1 alpha-C(0.17), 1alpha-C(=0)-0R,1 beta-C(-0.01). The shift 1 24, node CH_3 shows presence methyl (0.86),1 beta-C(=0)0C(0.28), 1 beta-C-R(0.10).The shift 3.67,node CH_3 shows presence of methyl(0.86),1 alpha-0C(=0)C(2.81).The shift 1.68,node CH_2 shows presence methylene (1.37), 1 alpha-C (0.00), 1 beta-C(=0) 0-C (0.35), 1beta-C (-0.04). The shift 0.96, node CH_3 shows presence methyl (0.86), 1 beta-C-R (0.10).

Mass Spectroscopy

The molecular weight Fig. 4(a) and 4(b) of the compound isolated was found to be 114 from the calculated m/e values. And for *Cynodon dactylon* The molecular weight of isolated compound found to be 114 from the calculated m/e values.

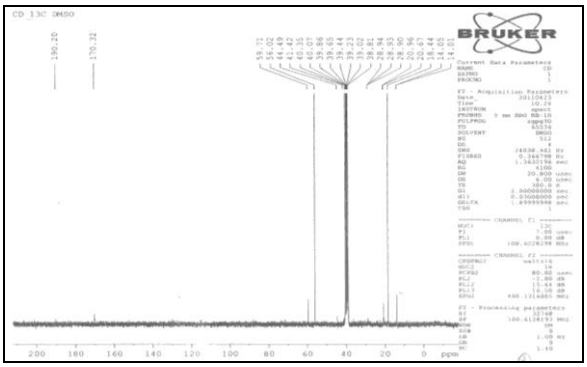


Figure 4(a): ^{13}C NMR of CD isolate

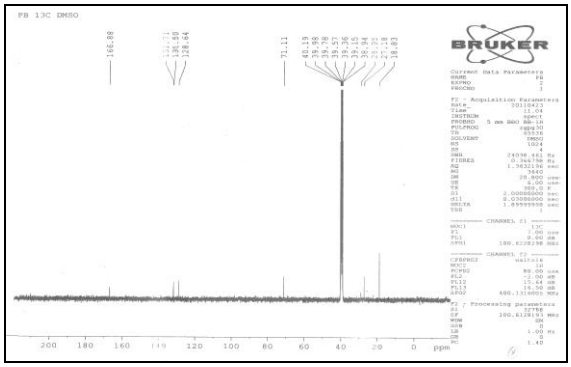


Figure 5(b): ^{13}C NMR of PB isolate

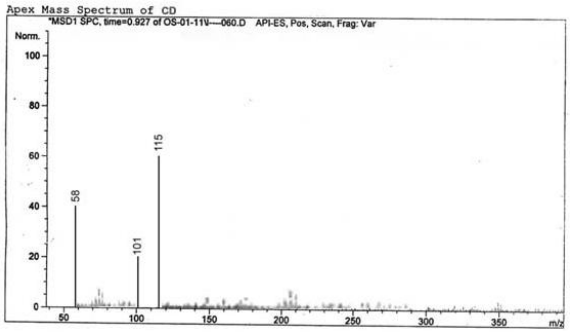


Figure 6(a): Mass spectrum of CD isolate

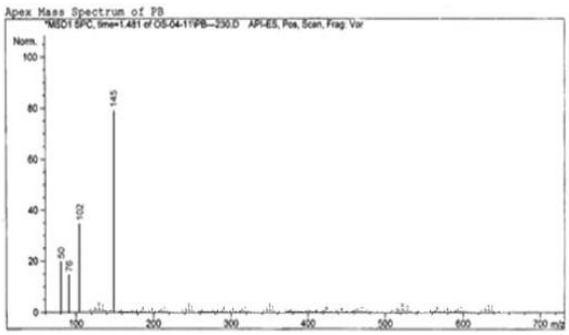


Figure 7(b): Mass spectrum of PB isolate

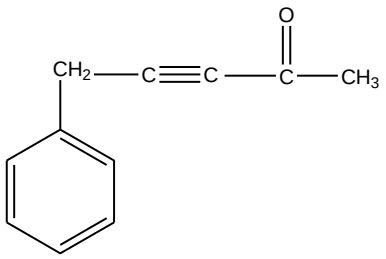
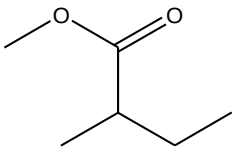


Figure 8: Predicted structure for the isolated compound for *Piper betle*

The isolated compound from *Piper betle* was Colorles crystals and characterized through IR, NMR, and mass spectral data of interpretation and the compound may be 5-Phenyl-3,4-alkynyl-2-pentanone.



1-methyl ethyl propanoate

Figure 9: Predicted structure for the isolated compound for *Cynodon dactylon*

The isolated compound from *Cynodon dactylon* was brow color amorphous powder and characterized through IR, NMR, and mass spectral data of interpretation and the compound may be 1-methyl ethyl propanoate.

CONCLUSION

The shade dried leaves of *Piper betle* belonging to the family piperaceae and *Cynodon dactylon* belonging to the family Gramineae were extracted with the solvents petroleum ether, benzene, chloroform, ethyl acetate, methanol, and water. The residue of ethyl acetate extract on column chromatography gave the isolated compound and the characterization studies showed characteristic functional groups and the H_1NMR spectrum of the isolated compound has shown the signals for assigned protons respectively. From this confirmation (UV, IR, H_1 and C_{13} NMR data), it shows that the isolated compound may be 5-Phenyl-3,4-alkynyl-2-pentanone. For *Cynodon dactylon* shows that isolated compound is 1-methyl ethyl propanoate.

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