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Potential antibacterial activity of isolated Caldine alkaloid from *Parthenium hysterophorus* L.

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Abstract

Parthenium hysterophorus L. is a multifaceted plant that has a wide range of chemical constituents. Aim of this study was to isolate alkaloid from leaf extract of *Parthenium hysterophorus* L. Isolation of compounds was done through column chromatography techniques, followed by further characterization using the LC-MS/MS, FTIR. An alkaloid Caldine was found present in the leaf extract of the P. *hysterophorus*. Antibacterial activity of Caldine was evaluated against bacteria *Erwinia carotovora* and *Ralstonia solanocearum*. Growth of both bacteria was inhibited by the Caldine. *R. solanocearum* was more susceptible to the antibacterial action with 18mm zone of inhibition at 150 µL Caldine concentration.

Key words : *Parthenium,* Antibacterial activity, Alkaloid, Caldine.

Phytopathogenic bacteria are found associated with wide range crops throughout the world wide[25]. They can cause blights, stunting, leaf epinasty and hormonal balance[11]. Phytopathogens are cause of losses per year to the food production, an over $1 billion dollars entire world[18]. A key element driving this issue is the extensive rise of antibiotic resistance in bacteria, which presents a serious challenge to the agriculture crop and human being[3]. Multiple antibiotics drugs present in the market used to treat bacterial infection[7]. These medications can cure infections, but along with have a number of negative side effects. Therefore, in this context, natural, safer, and less expensive sources of antibiotics drugs are needed[6]. The plant *Parthenium hysterophorus* L. is easily accessible and thrives across India and is known to contain the potent antimicrobial compounds. Previous investigations have also confirmed its antibacterial potential against bacteria *Rhizobium, Actinomycetes*, *Azotobacter*, and *Azospirillum*[12]. *P. hysterophorus* plant has been reported to contain large number of

biologically active substances, such as alkaloids, phenols, terpenoids, tannins, glycosides, parthenin, coronopilin, 2β-hydroxycoronophilin, and tetraneurin-A. Caldine is alkaloid [https://doi.org/10.1201/EBK1420077698; Dictionary of Alkaloids] also known as a norspermidine (https://pubchem.ncbi.nlm.nih.gov/#query=Caldine), is a polyamine. Most eukaryotes, as well as bacteria and archaea, produce spermidine, the most prevalent polyamine in the biosphere[19]. Caldine, or norspermidine, is a structural counterpart of spermidine. A central amine connects to the two aminopropyl groups in symmetrical structure of caldine[5]. Norspermine and norspermidine could possibly be combined to form alkaloids in plants[17]. *Erwinia* and *Ralstonia* are phytopathogenic bacteria, which cause a range of plant diseases, including bacterial wilt, brown rot of potato, fire blight, and blackleg[22]. Though these bacteria are not considered major human pathogens, they have been associated with bacteremia, neonatal sepsis, endocarditis, meningitis, conjunctivitis, bloodstream infections[21] and urinary tract infections[1]. In present study Caldine was isolated from the leaf extract of *P. hysterophorus* L. and evaluated against two phytopathogenic bacteria namely, *Erwinia carotovora* and *Ralstonia solanocearum*.

Collection of plant samples :

The whole plant of *P. hysterophorus* L. was collected from the university campus. The plant was identified and authenticated by Botanical survey of India, Central regional center Allahabad, India. The plant parts were washed, shade dried at room temperature and grinded by mechanical grinder and stored at -20°C.

Extraction of Alkaloids :

The total alkaloids content of the plant leaf was extracted by following the Stas-otto extraction procedure[23]. Plant powder was moist with water and mixed with lime, filtered using filter paper. The solvent methanol was added in same amount of filtrate and shake vigorously. After that two-layer was obtained, aqueous layer was discarded and organic layer was concentrated at 40°C for 2 hrs., followed by mixing with diluted HCL for digestion of fat and resin, resulted two layers of solution. Now aqueous layer was used and washed with chloroform and collected the precipitate, and dried.

Preliminary biochemical test and TLC for alkaloids :

An amount of dried material was added in methanol. A small quantity of methanolic extract of *P*arthenium was subjected to dragendroff biochemical test to determine the presence of alkaloids. To separate the band through TLC, chloroform and methanol was used as mobile phase in 9:1 ratio and dragendroff reagent was used for visualization as well as identification of alkaloids.

Isolation and Purification of extract :

Isolation and purification of extract was done by the column chromatography. The extract was loaded on a silica gel column packed with chloroform and eluted with chloroform and methanol in 9:1 ratio to yield 20 fractions. Each fraction was collected and employed for biochemical test as well as spraying of dragendroff's reagent on TLC plates for the presence and identification of alkaloids.

Fourier Transform Infrared Spectroscopy: The functional group was obtained from the column chromatography and was measured at 500-4000 cm^{-1} range using Fourier Transform Infrared Spectroscopy (FTIR).

Liquid chromatography-mass spectroscopy:

The selected fraction was eluted in column chromatography subjected to liquid chromatography-mass spectroscopy (LC-MS) equipped with SCIEX QTARP Enabled Triple Quad 5500+. Column: C18 column in reverse phase, Mobile Phase: chloroform: methanol (9:1), Flow Rate: 0.3 mL/min, Injection Volume: 5 µL. Ionization Mode: Positive and negative electrospray ionization (ESI), with 3.0 kV (negative mode) / 3.5 kV (positive mode) capillary voltages. Source Temperature: 150°C, Desolvation Temperature: 400°C, Desolvation Gas Flow: 800 L/h, The MQ4 algorithm was used for data analysis. Processing Technique: 015.Q method Library Search.

Antibacterial activity :

The antibacterial activity of the extract was determined against two phytopathogenic bacteria, *Erwinia carotovora* and *Ralstonia solanocearum* by agar well diffusion method. The test culture was inoculated on the surface of sterilized Luria Bertani Agar medium. An 8 mm well was made using a sterilized cork borer. The stock solution was prepared by dissolving of 10 mg of extract in1 ml DMSO. Three different concentrations, 50, 100 and 150 µl were pipetted out from the stock solution and loaded separately in each well. Throughout the experiment DMSO and Ciprofloxacin was used as negative and positive control respectively. Then the plates were incubated at 37°C for 24 hrs. in a bacterial incubator. The antibacterial potential of extract was determined by measuring the zone of growth inhibition[16].

Isolation, Purification and characterization of Alkaloid :

The 5g plant material was subjected to an extraction process with methanol solvent that yield 0.871 g of extract. Biochemical test of extract was tasted with Dragendroff reagent and the presence of alkaloids was confirmed by the showing of reddish-brown color precipitate. Thin layer chromatography (TLC) of extract confirmed the presence of alkaloid.

Purification of alkaloid from the solution was carried out by silica gel column chromatography on the basis of Dragendroff test five fractions found to show the presence of alkaloid. But prominent presence was reported in fraction methanol leaf extract-5 (ML-5). Identification and characterization of fraction ML-5 were carried out via a LC-MS/MS SCIEX. The mass spectra of fraction ML-5 led to the identification of an alkaloid (Fig. 2 and 3). The retention time, molecular formula, molecular weight, and area percentage were determined. The chemical structure of the alkaloid fraction identified as Caldine (Fig. 1). Therefore ML-5 was selected for further analysis.

Alkaloid	Mol. Formula	Mol. Mass	R. Time	Precursor Mass	Library Score
Caldine	C6H17N3	132.2359	1.37	132.240	76.0

Fig. 1. Chemical structure of isolated caldine alkaloid from *P. hysterophorus* L.

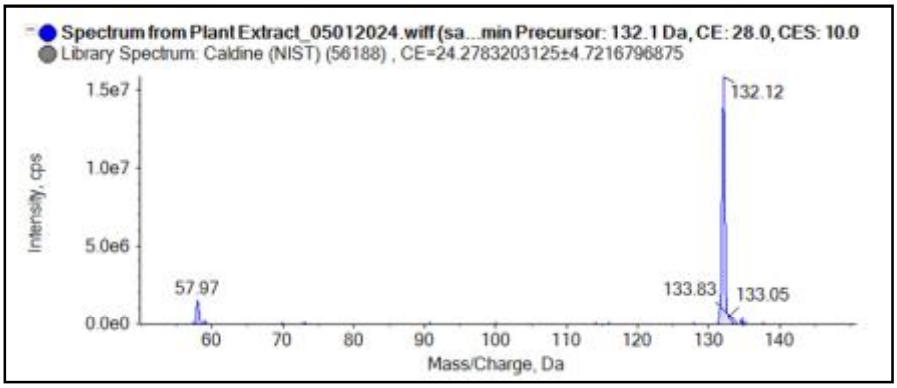

Fig. 2. Acquired / Library MS/MS isolated fraction ML-5 of *P. hysterophorus* L.

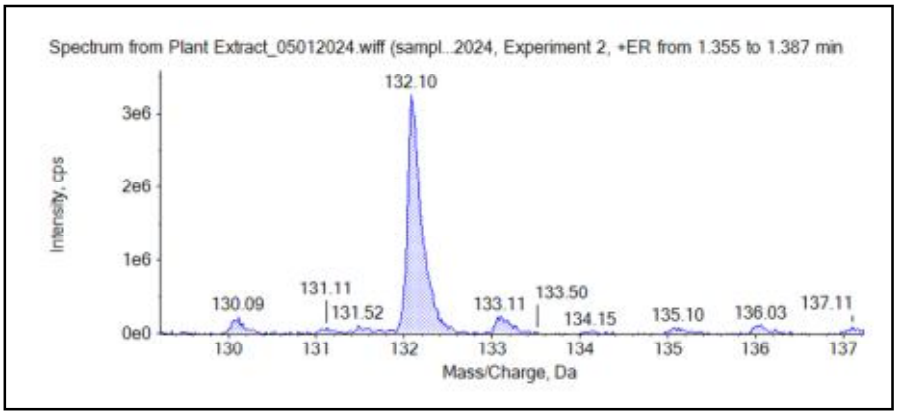

Fig. 3. Acquired / Theoretical MS isolated fraction ML-5 of *P. hysterophorus* L.

FTIR spectrum of isolated Caldine alkaloid:

The FTIR spectrum analysis of fraction revealed the presence of various characteristics functional groups under infrared region 500-4000 cm^{-1} range. The previously literature study support the presence of alkaloids monocrotaline. Presence of O-H, C-H, C=O, S=O, and C-N stretch bonding structure are responsible for the formation of alkyl group, methyl group, alcohols, ether, amine, and carbonyl group[20].

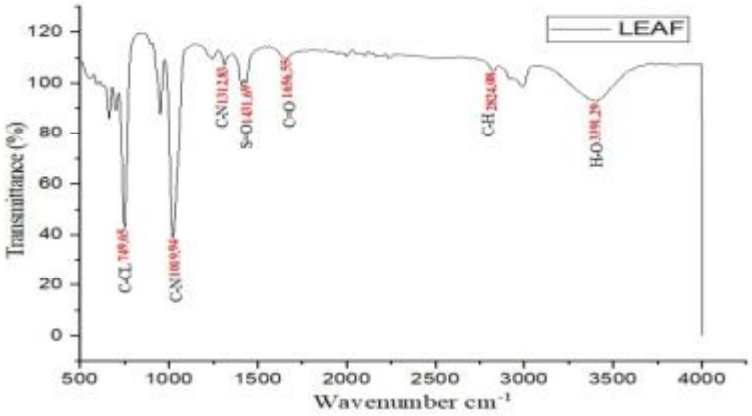

Fig. 4. FTIR spectrum of isolated caldine alkaloid

Antibacterial activity analysis of Caldine :

The extract showed significant antibacterial activity against *Eriwinia carotovora* and *Ralstonia solanocearum* (Fig. 5). The inhibitory zones had widths ranging from 10 mm to 18 mm, the widest zone of inhibition (18mm) against *Ralstonia solanocearum* exhibiting the more potent inhibition. and the least amount (10mm) of inhibition against *Eriwinia* at the concentration of 150µl.

Table-1. Antibacterial activity analysis of caldine

Caldine con. (µl)	*E. caro-tovora*	*R. solano-cearum*
	Zone of inhibition (mm)	
50	00	00
100	11	17
150	12	18
DMSO	00	00
Ciprofloxine	21	22

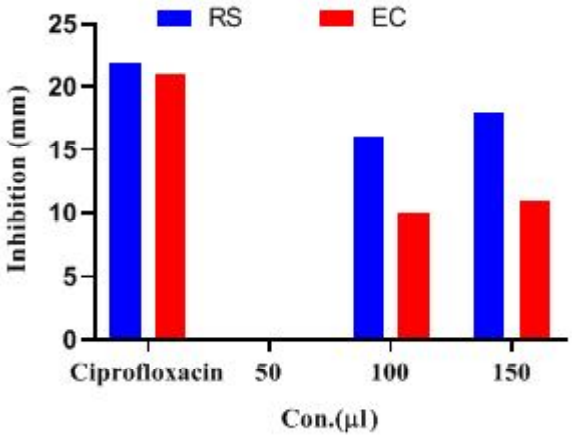

Fig. 5. Graph of antibacterial activity (zone of inhibition) of Caldine alkaloid against *Erwinia carotovora* (EC) and *Ralstonia solanocearum* (RS)

Dragendroff test TLC indicate the presence of alkaloid in the extract and further analysis through LC-MS/MS as well as FTIR spectroscopy confirm the presence of Caldine alkaloid in the leaf extract of *Parthenium*. Earlier identification of Caldine has been restricted to bacteria that found in Bryophyta, mosses[8] and some eukaryotic algae[9]. The originally Caldine (norspermidine) was discovered from thermophilic bacteria[4]. Availability of polyamines including spermidine, spermine and putrescine as well as triamines has been found higher plants such as grass pea[24] and *Heliotropium*[2]. Some researchers have reported and norspermidine from some plant species including alfalfa and cotton[14], *Gossypium barbadense* L.[15]. This is the first report to isolate the polyamines Caldine alkaloids form *P. hysterophorus* L.

Antibacterial effect of Caldine is depicted in Table-1 and Fig. 5. Alkaloid Caldine was found to inhibit the growth of both bacteria *Erwinia carotovora* and *Ralstonia solanocearum.* An alkaloid norspermidine has been reported for antibacterial activity against bacteria, *Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus epidermidis, Streptococcus mutans, Salmonella* and *Klebsiella pneumoniae* and inhibitory effect was approved dose dependent[10]. Konai *et al*.[25] also measured excellent antibacterial activity of norspermidine against *Staphylococcus aureus, Enterococcus faecium,* and *Klebsiella pneumoniae.*

Present study shows the presence of an alkaloid Caldine in the leaf extract of *Parthenium* plant. Previous literature is concerned there, is no report about the isolation of Caldine alkaloid from plant. This is the first study in which Caldine alkaloid has been isolated from the *Parthenium* plant. In addition, Caldine has been selected for antibacterial activity and found to have positive effect. Further investigations are needed for mode of action by Caldine alkaloid against bacteria.

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