

THE ISOLATION, CHARACTERISATION AND ANTI-MICROBIAL ACTIVITY OF ALKALOIDS OF *JATROPHA CURCAS* AND *PIPER LONGUM*

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Alkaloids from *Jatropha curcas* and *Piper longum* leaf extracts were isolated, characterised and tested against five human pathogenic microbes. *J. curcas* alkaloid identified by TLC and NMR as tetra methyl pyrigine (0.11 mg/gdw) and that of *P. longum* as piperidine (0.89 mg/gdw). *J. curcas* extract showed maximum antimicrobial activity against *Salmonella typhie* while *P. longum* extract was most efficient against *S. aureus*.

Keywords : Alkaloids; Isolation and characterization; *Jatropha curcas*; *Piper longum*; Piperidine; Tetra methyl Pyrigine.

Introduction

Alkaloids are naturally occurring amines having pharmacological importance. They are nitrogenous base and derivative of amino acid, having bitter taste. Much work has been done on family Euphorbiaceae^{1, 2} and Piperaceae³⁻⁵. The present study has been undertaken to assess the effectiveness of anti-microbial alkaloids of *Jatropha curcas* (Euphorbiaceae) and *Piper longum* (Piperaceae).

Jatropha curcas belongs to family Euphorbiaceae. It is a multipurpose shrub having significant importance because of its several industrial and medicinal uses. It grows in number of climatic zones in tropical and subtropical region. *Jatropha* oil is a potential source of biofuel. It is native of South America. In India it is mainly found in Maharashtra, Rajasthan, Gujarat and Tamil Nadu. The plant is also used to treat skin diseases, burn and to dress sores. The latex of the plant is used as an anti-cancerous medicine.

Piper longum is among the best known species from the Piperaceae family. It is used in many medicines since centuries. It is also used to treat gastro-intestinal diseases, constipation, neurological disease, dyspepsia, fever and malaria.

Jatropha sps. has been examined for its anti-viral activity⁶, anti-parasitic activity⁷, anti-feedent activity⁸ and antifungal activity⁹. Hexane, Chloroform and Methanol extracts of *Jatropha podogriea* roots have been tested for anti-microbial activity¹⁰. Susceptibility of selected bacterial species for *Jatropha curcas* has been examined¹¹ as also in the case of *Piper longum*, the antibacterial

activity has been reported¹² on gram⁺ and gram⁻ bacteria, but in both the cases the active principles have not been stated. However, the present study pertains to the isolation, identification and characterization of active principles of *Jatropha curcas* and *Piper longum* leaves, which were further examined for anti-bacterial activity against *Salmonella typhie*, *Staphylococcus aureus*, *Enterobacter*, *Pseudomonas* and *E.coli* i.e. human pathogens. The study also includes the separate and combined effect of alkaloids of both plant species against human pathogens.

Tetra methyl pyrazine, an amide alkaloid, which is present in *Jatropha podogriea*¹³ and *Ephydra*¹⁴, was reported as anti-bacterial, anti-hypertensive, and anti-asthamatic compound¹⁵. Tetra methyl pyrazine is also reported to be found in *Jatropha curcas*¹⁶. Many genera contains Piperidine such as *Piper nigrum*, *Nicotiana*, *Labolia* and *Pinus* etc.¹⁷ Anti-microbial activity of Piperidine chloride derivative has been found¹⁸. In the present study, thin layer chromatography was used for separation, identification and isolation of the bioactive compounds (alkaloids) of leaves of *Jatropha curcas* and *Piper longum*. The characterization was carried out using Nuclear Magnetic Resonance (NMR) spectrometer analysis.

Material and Methods

Leaves of *Jatropha curcas* and *Piper longum* was dried at 100°C for 15 minutes and then at 40°C till a constant weight was achieved. The samples were powdered, weighed and ground in a mortar and transferred to separate vessels. The samples were extracted for alkaloidal

Table 1. Antimicrobial activities of isolated alkaloids.

Name of Pathogen	Tetramethyl pyrazine (<i>J. curcas</i>) (10mg)	Piperidine (<i>P. longum</i>) 10mg	Tetramethyl pyrazine (10mg) + Piperidine(10mg)
<i>Salmonella typhie</i>	10mm	10mm	7mm
<i>Staphylococcus aureus</i>	7mm	12mm	8 mm
<i>Enterobacter</i>	10mm	7mm	6mm
<i>E.coli</i>	7mm	12mm	8mm
<i>Psuedomonas</i>	7mm	7mm	7mm

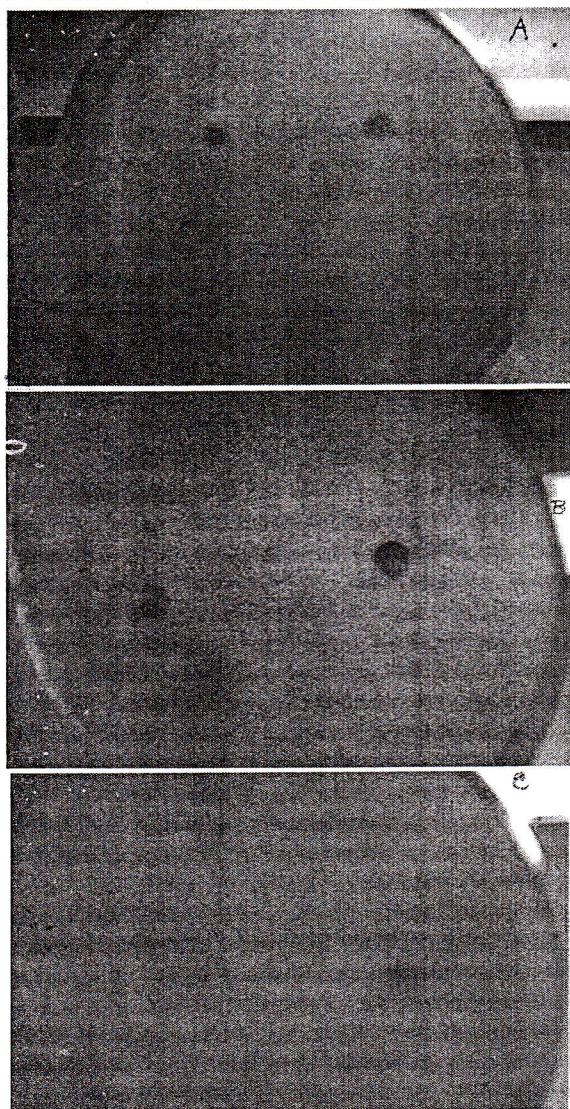


Fig.1. Photograph showing antimicrobial activity (A) *J. curcas* leaf extract against *S. typhie* and (B) *P. longum* leaf extract against *S. aureus*.

content¹⁹. The extracts were dried *in vacuo* and their crude alkaloidal content was calculated.

Thin Layer Chromatography (TLC): TLC was carried out using pre-coated silica-gel plates (Merk Germany). The dried alkaloidal crude sample of *J. curcas* was dissolved in distilled water and applied 1 cm above the edge of the plates along with reference compound of Tetramethyl pyrazine. The plates were dried and developed in various solvent system separately (n-Butanol: acetone: water, 145:5:50; Iso-butanol: water, 85:15) but the best separation was seen in ethyl acetate: dichloro methane (1:1). Developed purple spot was seen under UV light and Rf value was calculated (0.5)²⁰. Similarly, the dried sample of *Piper longum* was also subjected to TLC along with reference compound of Piperidine. It was observed that the separation was best in ethyl acetate:methanol (8:2) solvent system²¹.

Preparative Thin Layer Chromatography (PTLC): The pre-coated silica-gel plates were used for PTLC. Ten plates were developed as done under TLC. The inflorescent spot seen under UV light were collected along with silica-gel and was re-extracted with distilled water. The compounds of both plant species were dried, weighed and subjected to NMR analysis using Meck 300 megahertz Mercury Nuclear Magnetic Spectrometer.

The resulting peaks of NMR analysis were similar to that reported in literature²². The substances that were isolated from the leaves of *J. curcas* and *P. longum* were confirmed as Tetra methyl pyrazine and Piperidine, respectively. The quantification of isolated alkaloids was carried out on weight basis.

Testing of alkaloids for antimicrobial activities: Testing of alkaloids for antimicrobial activities was carried out using Filter Paper Disc Method. Sterilized Petri plates were prepared with 10 ml of growth agar medium and inoculum of five human pathogenic bacteria i.e. *Salmonella typhie*, *Staphylococcus aureus*, *Enterobacter*, *E.coli* and *Pseudomonas*. Paper disc measuring 6 mm diameter absorbing 10 mg of isolated substances of each standard compounds was used simultaneously. Inoculated plates

were kept at 5° C for 45 minutes and then incubated at 35° C for 18 hours. The developed inhibition zone were measured and compared with the standard reference antibiotic.

Results and Discussion

In the present study, active principles were isolated, identified as Tetramethyl pyrazine (Rf 0.5; Ethylacetate:dichloromethane; 1:1) in *Jatropha curcas* and Piperidine (Rf 0.25, ethylacetate:methanol; 8:2) in *Piper longum*. The confirmations of the isolated compounds were done by characteristic peaks of NMR analysis. It was observed that the leaves of *Jatropha curcas* were having 0.11mg/gdw Tetra-methyl pyrazine and *Piper longum* contained 0.89mg/gdw Piperidine. These results support the earlier findings.

Both the isolated alkaloids showed antibacterial activity against all five human pathogenic bacteria (Table 1). Individually, the maximum activity of Piperidine alkaloid was observed against *S.aureus* and *E.coli* (12 mm inhibition zone, Fig. 1-A) as compared to that of Tetramethyl pyrazine against *S.typhie* and *Enterobacter* (10 mm inhibition zone, Fig. 1-B). However, the combination of both the alkaloids did not show marked activity. Thus, it can be concluded that *Jatropha curcas* has a property to cure typhoid due to inhibitory effect of Tetramethyl pyrazine alkaloid against *S.typhie*, the bacteria which causes typhoid. It also has the property to cure many of the skin diseases and respiratory infection which is on account of inhibitory effect against *Enterobacter*. Similarly, Piperidine of *Piper longum* could be helpful to cure diarrhoea and severe cramps, as it has inhibitory effect against *E.coli*, the main cause of these diseases. Piperidine also has a property to inhibit the growth of *S.aureus*, the cause of many skin diseases. Tetramethyl pyrazine and Piperidine both individually have property to cure skin disease.

Acknowledgement

The author thanks Indian Institute of Technology (IIT), Powai, Bombay for granting permission for NMR analysis of isolated compounds, Prof. Deodhar (Chemistry Department, IIT, Powai) for interpreting the NMR results of isolated compounds, Dr. R. N. Mishra, Head of the Pathology Department, INHS Ashwini for the required assistance and guidance.

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