Int J Pharm Bio Sci 2015 Jan; 6(1): (B) 427 - 432

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

TLC-BIOAUTOGRAPHY GUIDED SCREENING OF THE METHANOLIC EXTRACT OF RICINUS COMMUNIS

NATHANIEL SANDAM* AND PONNAMMA SU

Department of Biotechnology, Presidency College, Bangalore, India.

ABSTRACT

New medicines are required to treat various kinds of bacterial diseases since bacteria tend to develop characteristics like drug resistance resulting in the ineffectiveness of the presently used drugs. Therefore, there is a need to exploit the antimicrobial activities of plants for the treatment of infections and to treat infections caused by drug resistant bacteria as an alternative.[1]The antimicrobial activity of *Ricinus communis* was investigated by TLC-Bioautography. Preliminary screening of the methanolic extracts of *Ricinus communis* showed that the extract contains alkaloids, saponins, oils and fats, phenols, flavanoids, tannins and glycosides. From among the various solvent systems tested, the 3 solvent system of Chloroform: Petroleum ether: Ethyl acetate in the ratio 8:2:2 was found to be the best as it resolved the methanolic extract into 8 good fractions. Among the 8 fractions, 4 were found to be antimicrobial by TLC-bioautography technique. The active fraction corresponding to Rf value 0.2750 showed distinct inhibitory zones against both *Klebsiella pneumoniae* (MTCC 3384) and *Pseudomonas aeruginosa* (PC 002) [2]. GC-MS analysis of this fraction revealed fatty acids as the major component. Our work has shown that the fraction corresponding to Rf value 0.2750 has potential to be used as an antimicrobial agent against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

KEY WORDS: **Bioautography, TLC, GC-MS, *Ricinus communis*.**

NATHANIEL SANDAM
Department of Biotechnology, Presidency College, Bangalore, India.

***Corresponding author**

INTRODUCTION

The medicinal use of natural products—compounds that are derived from natural sources such as plants, animals or micro-organisms—precedes recorded human history probably by thousands of years[3]. Herbal products and secondary metabolites formed by plants have shown great potential in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases[4, 5]. 65 - 80% of the world population relies on traditional medicine to treat various diseases[6]. To date, many plants have been claimed to pose beneficial health effects such as antioxidant and antimicrobial properties. Plants have also been known to control and cure various other diseases such as diabetes, malaria etc. Therefore there is a need to exploit the antimicrobial activities of plants for the treatment of infections especially those caused by drug resistant bacteria. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are both human pathogens, responsible for causing nosocomial infections[2]. Various drug resistant forms of these bacteria have been isolated. Since the treatment of drug resistant pathogens has proved to be a very difficult task, it is important to develop new drugs that act on these resistant varieties. As an attempt to discover new lead compounds, plant extracts are screened by many researchers to detect secondary metabolites having relevant biological activities, including antimicrobial activities. In this regard, several bioassays were developed for screening purposes[7]. Bioautography is a highly efficacious assay for the detection of antimicrobial compounds because it allows the localization of activity even in a complex matrix, and therefore facilitates the target-directed isolation of the active constituents[8]. A number of bioautographic assays have been developed, which can be divided into three groups[9]. These include: direct bioautography- where the microorganisms grow directly on thin-layer chromatography (TLC) plates; contact bioautography- where the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact, and agar overlay or immersion bioautography- where a seeded agar medium is applied onto the TLC plate. The latter technique can be considered as a hybrid of the direct and contact bioautography[10]. Bioautography is a sensitive method for detection of antimicrobial compounds even in small amounts. Hence for the detection of antimicrobial compounds, bioautography is suitable method in the initial stages as compared to agar dilution methods; also Bioautography is particularly important to avoid the time-consuming isolation of inactive compounds. Gas Chromatography- Mass Spectroscopy (GC-MS) is a method that combines the feature of gas chromatography and mass spectroscopy to identify different substances within a test sample. GC-MS has been widely accepted as 'gold standard' for chemical identification of volatile and semi-volatile organic compounds in mixtures, drug detection, environmental analysis, explosives investigation, and identification of unknown samples. Additionally, it can identify trace elements in materials that were previously thought to go undetected by other technologies. Following TLC-Bioautography, active fractions can be subjected to GC-MS to identify the active compounds.

Ricinus communis

The castor oil plant, *Ricinus communis*, is a species of flowering plant which belongs to the family Euphorbiaceae [11]. Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant). An alcoholic extract of the leaf was shown, in lab rats, to protect the liver against damage from certain poisons. Antihistamine and anti-inflammatory properties were found in ethanolic extract of *Ricinus communis* roots[12]. This plant is widely available in India to obtain Castor oil which is used as a coolant and also to cure indigestion, this plant was chosen to be screened for antimicrobial

properties because of its ready availability and promise as a medicinal plant.

MATERIALS AND METHODS

Materials

The organisms used for the study are *Klebsiella pneumoniae (*MTCC 3384*)* obtained from Microbial Type Culture Collection and Gene Bank (MTCC), a national facility established in 1986 is funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India*.,* and a lab grown strain of *Pseudomonas aeruginosa (*PC 002). These organisms were grown on Trypticase Soy Agar, Mueller Hinton Agar (Hi Media), Nutrient Agar and Nutrient Broth (Hi Media).The solvent used for the extraction of the extract was Methanol and the solvents used for TLC were Ethyl acetate, Petroleum ether, Chloroform. Some of the other materials used for the study are Ethyl acetate, Petroleum ether, Chloroform, TLC plates - TLC Silica gel 60 F_{254} (Merck), Ampicillin 10µg (HiMedia)

Methods

Plant collection

Castor leaves were separated from the stems, washed in clean water, and dried at room temperature. The dried plants were milled to a fine powder using a blender and stored in the dark at room temperature in air tight packets until required.

Preparation of extract

9g of the powdered sample was extracted using 90ml of 80% methanol in a conical flask by placing in a water bath maintained at 60°C for 4 hours. The extract was then filtered using Whatman No: 1 filter paper and was stored in the refrigerator at 4°C until use.

Test bacteria

The bacteria *Klebsiella pneumoniae* (MTCC 3384) and *Pseudomonas aeruginosa* (PC 002) were used for this study. They were maintained on Trypticase Soy Agar and Nutrient Agar respectively.

(A) *Preliminary screening for secondary metabolites from Ricinus communis*

Preliminary Screening for secondary metabolites from *Ricinus communis* was carried out using the methods given by Raaman[13].

(B) *Thin Layer Chromatography*

0.1 ml of the extract was spotted onto the TLC plate using a capillary tube and was then kept in a developing chamber containing the eluent system Chloroform: Petroleum ether: Ethyl acetate (CPE system) in the ratio 8:2:2, the mobile phase was run upto a distance of 10cm. Following chromatography, the sheets were dried gently using a dryer and the number of bands formed was observed under UV light and visible light.

(C) *TLC- Bioautography*

TLC- Bioautography by the agar overlay method[14] was employed to determine the antimicrobial activity. 24h nutrient broth cultures were utilized for this antimicrobial assay. The chromogen used to distinguish between living and dead bacteria was 2,3,5-Trimethyl tetrazolium chloride.

The extract was run on two TLC plates separately as described above, following which a thin layer of Muller Hinton Agar (MHA) containing *Klebsiella pneumoniae* (MTCC 3384) and chromogen was carefully poured onto one plate and a thin layer of MHA containing *Pseudomonas aeruginosa* (PC 002) and chromogen was poured onto the other plate. The plates were then placed in a moist chamber and incubated at 37°C for 24 hours to observe for inhibition zones.

(D) *Gas Chromatography- Mass Spectroscopy*

Active fractions corresponding to the Rf value 0.2750 was taken for GC-MS studies since it was inhibitory to the growth of both *Klebsiella pneumoniae* (MTCC 3384) and *Pseudomonas aeruginosa* (PC 002). This fraction was scraped carefully into an Eppendorf tube and 1ml of methanol was added to extract the compounds and the supernatant was subjected to GC-MS analysis.

RESULTS AND DISCUSSION

RESULTS

(A) ***Preliminary screening for secondary metabolites from the methanolic extracts of Ricinus communis.***

Upon preliminary screening of methanolic extracts, the following secondary metabolites were found to be present: Alkaloids, Phenolic compounds, Flavanoids, Tannins, Saponins, Glycosides and Fixed oils.

(B) ***TLC- Bioautography***

TLC- Bioautography with Klebsiella pneumoniae **(MTCC 3384).**

After incubation, zones of growth inhibition were observed. In this plate, a total of 4 active bands were formed (Fig: 1) for the fractions with Rf values of 0.2000, 0.2750, 0.8875 and 0.9125.

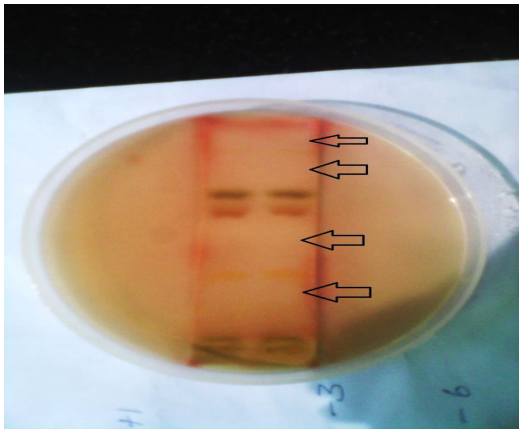

Figure 1

TLC- Bioautography chromatogram of the methanolic extract of Ricinus communis on MHA containing the dye TTC and Klebsiella pneumoniae **(MTCC 3384)** ***showing 4 active bands.***

TLC- Bioautography with Pseudomonas aeruginosa (PC 002)

After incubation, zone of growth inhibition was observed. In this plate 1 active band was formed for the fraction with an Rf value of 0.2750 as shown in Fig: 2.

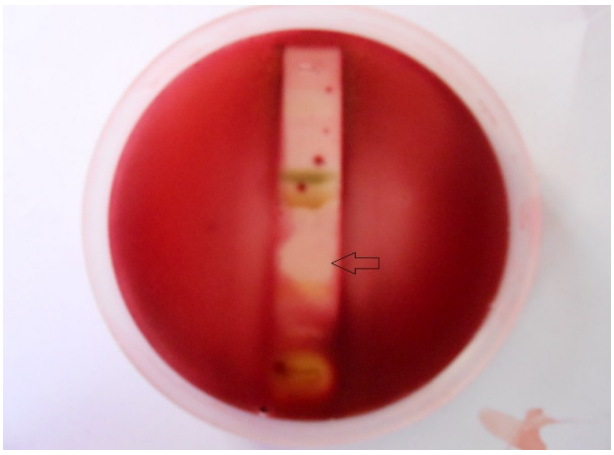

Figure 2

TLC - Bioautography chromatogram of the methanolic extract of Ricinus communis on MHA containing the dye TTC and Pseudomonas aeruginosa (PC 002) showing 1 active band.

D) Gas Chromatography- Mass Spectroscopy

The fraction corresponding to Rf value 0.2750 was taken for GC-MS analysis since it was inhibitory to both *Klebsiella pneumoniae* (MTCC 3384) and *Pseudomonas aeruginosa* (PC 002).Upon analysis, 8 major peaks were obtained. The major compounds are Palmitic acid followed by Hexadecatrienal, Octadecanoic acid, Hexadecanoic acid methylester, Tetradecanoic acid.

.

Serial No.	Retention time	Name
1	14.914	Tetradecanoic acid
2	16.652	Hexadecanoic acid, methyl ester
3	17.003	Hexadecanoic acid (CAS) Palmitic acid
4	18.324	Octadeca-9,12-dienoic acid methyl ester
5	18.583	Octadecanoic acid, methyl ester (CAS) Methyl stearate
6	18.758	Cis,cis,cis-7,10,13-Hexadecatrienal
7	18.906	Octadecanoic acid
8	24.251	2,6,10,14,18,22-Tetracosahexaene

Table1

The 8 major compounds identified after GC-MS.

DISCUSSION

TLC of *Ricinus communis* has been performed using various other solvent systems such as benzene/ethanol/ammonium solution (18:2:0.2), chloroform/ethyl acetate/formic acid (10:8:2) Chloroform:Petroleumether:Methanol[15]. Our experiment using the solvent system Chloroform:Petroleumether:Ethyl acetate resulted in good separation of the fractions.To the best of our knowledge, this is the first time that TLC-Bioautography has been carried out using the methanolic extract of *Ricinus communis* using *Klebsiella pneumoniae* (MTCC 3384)and *Pseudomonas aeruginosa* (PC 002.4 active bands were observed against *Klebsiella pneumoniae* (MTCC 3384)and 1 active band against *Pseudomonas aeruginosa* (PC 002), hence it was found that the methanolic extract of *Ricinus communis contains compounds* more inhibitory to *Klebsiella pneumoniae* (MTCC 3384)than against *Pseudomonas aeruginosa* (PC 002).The fraction corresponding to Rf value 0.2750 was used for GC-MS analysis. Upon GC-MS, 8 major peaks were observed. Each of these compounds separately may have been antimicrobial or a combination of these compounds may be antimicrobial. However, Palmitic acid has been proved to be an antimicrobial [16], also, in a work carried out by Ponnamma and Manjunath, similar compound has been identified and has been implicated as an antimicrobial agent[14, 17]. Therefore, it could be stated that palmitic acid might have contributed to the antimicrobial nature of the present extract.

CONCLUSION

TLC-Bioautography of the methanolic extract of *Ricinus communis* followed by GC-MS analysis of the active fraction has yielded 8 compounds that have potential to be antimicrobials; this work lends scope for the isolation and characterization of the individual compounds and exactly determine their antimicrobial property against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, both clinical pathogens that are increasingly becoming drug resistant.

REFERENCES

1. Newman, D. J.; Cragg, G. M. Natural products as sources of new drugs over the last 25years. J. Nat. Prod., 70, 461−477, (2007).
2. Anton Y Peleg and David C Hooper. Hospital acquired infections due to gram negative bacteria. New England Journal of Medicine 362(19): 1804-1813 (2010).
3. Hong-Fang Ji, Xue-Juan Li, and Hong-Yu Zhang. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO report; 10(3) : 194-200. (2009)
4. Ara Tachjian, MD, Viqar Maria, MBBS, and Arshad Jahangir, MD. Use of Herbal Products and Potential Interactions in Patients with Cardiovascular Diseases. J Am Coll Cardiol; 55(6): 515-525. (2010)
5. Aravindaram, K. and Yang, N. S , Anti-inflammatory plant natural products for cancer therapy. Planta. Med. 76(11): 1103-17 (2010)
6. World Health Organisation (WHO), 2002. WHO Traditional Medicine Stratergy. 2002-2005. Geneva.
7. Hostettmann, K. Assays for bioactivity. Methods in Plant Biochemistry, vol. 6, Academic Press,San Diego, pp 360.(1991).
8. Rahalison, L., Hamburger, M Hostettmann, K., Monod, M., Frenk, E. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. Phyto. Anal 2. 2 2: 199-203(1991).
9. Rios, J.L., Recio, M.C., Villar, A. Screening methods for natural products with antimicrobial activity: A review of the literature J. Ethno pharmacol. 23: 127-149.(1988).
10. Islam, N., Parveen, S. A., Nakazawa, N., Marston, A., Hostettmann, K. Bioautography with the fungus *Valsacerato sperma*in the search for antimycotic agents. Pharmaceutical Biology Vol. 41, No. 8: 637-640 (2003).
11. Wedin GP, Jeffrey SN, Everson GW, Krenzelok EP. Castor Bean Poisoning. American Journal of Emergency Medicine, 4(3): 259-261. (1986)
12. Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammatory and free radical scavenging activity of Ricinus communis root extract. J. Ethnopharmacol. 103(3):478-80. (2006).
13. Raaman, N. Phytochemical techniques. New India Publishing Agency, New Delhi.India, pp.19-24.(2006).
14. Ponnamma, S U. Phytochemical studies on *Justicia wynaadensis* by chromatographic techniques. In: Evaluation of the potential antimicrobial properties of *Justicia wynaadensis* and it's in vitro cultivation. Ph.D thesis. Bangalore University, pp.119-145.(2014).
15. Mittal, A., Ali, M. Standardization parameters and HPTLC. International Journal of Drug Development & Research, 5(1):229-234, (2013).
16. Yff, B.T.., Lindsay, K.L., Taylor, M.B., Erasmus, D.G., Jager, A.K.. The Pharmacological screening of Pentanisiaprunelloides and the isolation of the antibacterial compound palmitic acid. J. Ethnopharmacol. 79:101-107. (2002).
17. Ponnamma, S.U. and Manjunath, K. GC-MS analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (Nees) T. Anders. Int J Pharm Bio Sci. 3(3): 570 – 576 (2012).