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REVIEW PAPER

Bioautography and its scope in the field of natural product chemistry

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Abstract Medicinal plants, vegetables and fruits are the sources of huge number of bioactive lead/scaffolds with therapeutic and nutraceutical importance. Bioautography is a means of target-directed isolation of active molecules on chromatogram. Organic solvents employed in chromatographic separation process can be completely removed before biological detection because these solvents cause inactivation of enzymes and/or death of living organisms. They offer a rapid and easy identification of bioactive lead/scaffolds in complex matrices of plant extracts. Bioautography is a technique to isolate hit(s)/lead(s) by employing a suitable chromatographic process followed by a biological detection system. This review critically describes the methodologies to identify antimicrobial, antioxidant, enzyme inhibitor lead/scaffolds by employing bioautography. A significant number of examples have been incorporated to authenticate the methodologies.

1. Introduction

Planar chromatographic analysis hyphenated with the biological detection method is termed as bioautography [1]. It is an effective and inexpensive technique for the phytochemical analysis of plant extracts to identify bioactive lead/scaffolds. It can thus be performed both in highly developed laboratories as well as in small research laboratories which have minimum access to sophisticated equipments [2]. Despite having sophisticated on-line high-performance liquid chromatography coupled bioassays, bioautography offers a simple, rapid and inexpensive method for the chemical and biological screening of complex plant extracts, with subsequent bioassay-guided isolation [3]. In 1946, Goodall and Levi [4] introduced paper chromatography (PC)-based bioautography for the first time to estimate the purity of penicillin. In 1961, Fisher and Lautner [5] and Nicolaus et al. [6] introduced thin layer chromatography (TLC)-based bioautography. The first review on bioautography was written by Betina in 1973 [7]. Generally, planar chromatographic, viz. TLC and PC are used for bioautography, but the detection method can be successfully improved by the application of advanced chromatographic tools, namely, high performance thin layer chromatography (HPTLC),

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over-pressured layer chromatography (OPLC), and planar electro chromatography (PLC). The major applications of bioautography are the fast screening of a large number of samples for bioactivity, namely, antibacterial, antifungal, antioxidant, enzyme inhibition, etc. and in the target-directed isolation of active compounds [8–11]. In this review, the techniques and application of bioautography are discussed in details with suitable examples.

2. Detection of anti-microbial agents by bioautography

PC and TLC have become tools in the screening of antimicrobial agents through bioautography. Three bioautographic methods, namely, (i) agar diffusion or contact bioautography, (ii) direct TLC bioautographic detection and (iii) immersion or agar overlay bioautography are used to detect antimicrobial agents in a mixture of compounds [12,13].

2.1. *Agar diffusion or contact bioautography*

In contact bioautography, antimicrobial agents diffuse from a developed TLC plate or paper to an inoculated agar plate [14–16]. The chromatogram is placed face down onto the inoculated agar layer for a specific period to enable diffusion. Then the chromatogram is removed and the agar layer is incubated. The zones of inhibition on the agar surface, corresponding to the spots in chromatographic plates, are indicative of the antimicrobial substances. An overall view of contact bioautography has been depicted in Fig. 1. Incubation time for the growth ranges between 16 and 24 h but it can be reduced to 5–6 h by spraying with 2,6-dichlorophenol-indophenol or 2,3,5-tetrazoliumchloride [17]. The disadvantages of contact bioautography are difficulties in obtaining complete contact between the agar and the plate and adherence of the adsorbent to the agar surface. Another problem may arise due to the differential diffusion of components, especially water-insoluble, from the chromatogram to the agar plate. To overcome these difficulties, Wagman and Bailey [12] introduced Chrom-AR and silicic acid/glass fiber sheets for bioautography of antimicrobial compounds. The principle of the method was the same and antimicrobials had to be transferred from the chromatographic plates to agar causing their loss and dilution. Another special case is bioautographic detection of 6-aminopenicilanic acid which is a very weak antibiotic and must be converted through phenyl acetylation to benzyl penicillin by spraying chromatographic plates or paper with acetyl chloride in mild alkaline condition before bioautography [18]. This is a technique familiar to the microbiologists in search for antibiotics from microorganisms, and different procedures have been used to improve its performance [19]. Sphaerococcenol A, a bromoditerpene antibiotic, was isolated by contact bioautography [20]. Three carboxylic polyether antibiotics, namely, monensin, lasalocid and salinomycin, were isolated using contact bioautography by VanderKop et al. [21]. Eight different antifungal agents were isolated from the bark of *Bridelia retusa* using contact bioautography [22]. The development of HPTLC has improved resolution and sensitivity over TLC [23]. Application of HPTLC also reduced the consumption of time and solvents. Through HPTLC-contact bioautography, Ramirez et al. [24] showed multiple antibiotic residues in cow's milk. TLC contact bioautographic assay was introduced by Shahverdi et al. [25] for the detection of antibiotic resistance reversal agents.

2.2. *Direct TLC bioautographic detection*

In direct TLC bioautography, the developed TLC plate is sprayed with or dipped into a fungal or bacterial suspension (Fig. 2). A suspension of test bacteria or fungi is used for the spraying or dipping purpose. An inoculam of absorbance of 0.84 at 560 nm was suggested for bacteria like *Staphylococcus aureus* [26], while using a suspension of 10^6 CFU/mL could be employed for both bacteria and fungi [27]. The bioautogram is then incubated at 25 °C for 48 h under humid condition. For visualization of microbial growth, tetrazolium salts are used. These salts are converted by the dehydrogenases of living microorganisms to intensely colored formazen [28]. These salts are sprayed onto the

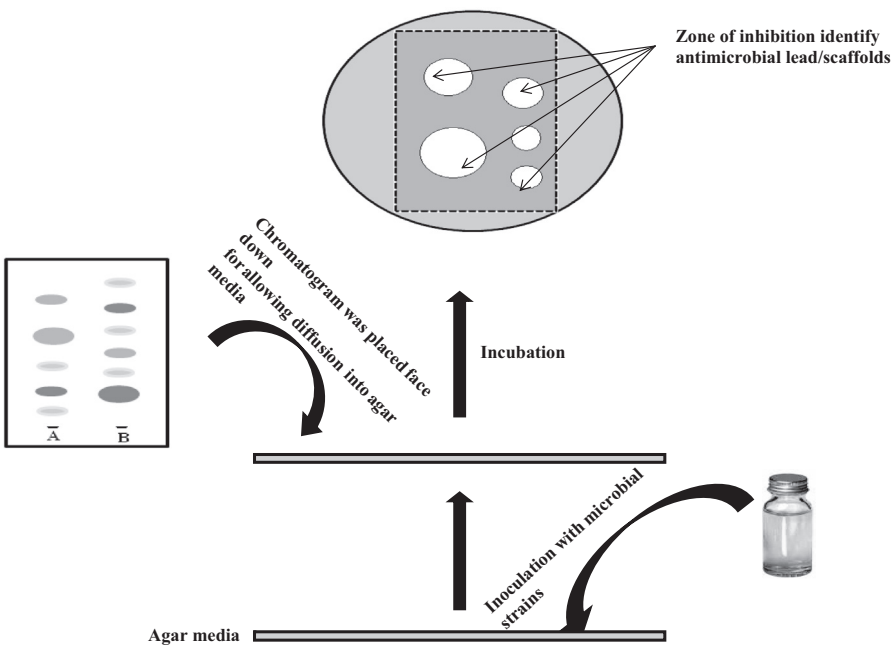

Fig. 1 Schematic overview of contact bioautography.