# Recent Advances in Screening of Natural Products for Antimicrobial Agents

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**Abstract:** It has been a very long history for human to resist diseases. During this period, a large number of drugs that could kill or inhibit the growth of microbe has been discovered, most of which were natural products. However, there may still be a large amount of antimicrobial medicines in natural compounds which have not been found yet. The ways of screening for antimicrobial always cost a long time and need a lot of manpower before. However, in recent years, a lot of new antimicrobial targets, antimicrobial drugs and screening methods which are simpler, faster and more efficient have been invented. In this paper the newly discovered targets, natural products and representative technologies were reviewed, which were expected to make some contributions to the research and development of medicines.

**Keywords:** Antimicrobial drugs, antimicrobial targets, natural products, screening methods.

## INTRODUCTION

Infectious disease is a global problem. Since the discovery of penicillin, antibiotics have become the most powerful weapon against bacteria. Most of the currently used antibacterials were developed between the 1940s and 1960s [1]. However, the emergence of drug resistance in the clinic is the major difficulty for all classes of antibiotics. World health problems caused by drug-resistant microbe are increasing, so more and more scientists begin to research on novel antimicrobial targets and expect to find new drugs of antibacterial.

Herbal medicine is an important part of alternative medicine, which has been successfully applied in clinical for thousands of years in China, Japan and other East Asian countries, and it is also gradually accepted by medicinal community in some Northern America and European countries. There are many kinds of natural products, so development of new high throughput assays which can simultaneously screen multiple targets to rapidly identify novel antibiotics is crucial. However, natural products often contain nuisance compounds such as tannin and chlorophyll which impede the procedure of isolation and purification [2]. Therefore, the invention of new screening method is urgent if screening of natural products continues to be an important engine for lead generation. The screening of chemical compounds for pharmacological activity has been ongoing in various forms for at least 40 years, new antimicrobial natural products and numerous ways of screening have been developed during this time, such as, agar dilution methods, spectroscopic techniques, fluorescent assay and other high throughput screening methods. Besides, the discovery of novel antimicrobial targets and mechanisms plays an essential role in screening for antibacterial compounds in a short period of time. Hence, the new antimicrobial targets, antibacterial natural products and the representative screening assays will be reviewed in this article.

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## ANTIMICROBIAL TARGETS

Bacteria cell is mainly composed of cell wall, cell membrane, cytoplasm, nuclear plastid and other sections, and if physiological activity of any important cell organs is inhibited, the microorganism will die. Because of the emergence of drug resistance, the finding of novel antimicrobial targets has become more and more urgent nowadays. There are several new antimicrobial targets and improved targets discovered from established classes of antibiotics, which have recently been approved or are in late-stage development, and these targets will be produced next.

***Cell wall:*** Some antimicrobial drugs in early stage can interfere with the biosynthesis of the bacterial cell wall, such as β-lactam antibiotics, vancomycin, fosfomycin and penicillin. Among them, β-lactam antibiotics can also inhibit multiple catalytic sites, a family of D,D-transpeptidases and D,D-carboxypeptidases which are called penicillin-binding proteins (PBPs) [3]. Lipid II is an important cell wall antibiotics target and many antibacterial agents act through this target, such as glycopeptides, ribosomally-made peptides and depsipeptides. Moreover, depsipeptides can also complex with undecaprenyl-pyrophosphate and inhibit the dephosphorylation of it [4]. Recent researches have shown that inhibition of D-alanyl-D-alanine ligase, which catalyzes the dimerization of D-alanine and plays an essential role in bacterial cell wall biosynthesis, will prevent bacterial growth. Currently, a new series of inhibitors for D-alanyl-D-alanine ligase have been developed [5].

***Cell membrane:*** Bacterial cell membranes are mainly composed of lipids and protein, and bacterial will die if the cell membranes are damaged. For example, polymyxin B can bind tightly to phosphatides while nystatin and amphotericin B may bind to sterols of cell membranes. Magainin 2, Idolicidin and Lactoferricin B can interact with lipopolysaccharide (LPS) of cell membranes [6-8], and finally leads to the death of bacterial.

***Protein:*** Most of the proteins are involved in the physiological activity of bacteria, and some drugs work functionally by combining with protein. For example, chloramphenicol, cillimycin and the macrolide antibiotics

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can bind to 50s subunit of the bacterial ribosome, and prevent bacterial growth sequentially. *Plasmodium falciparum* thymidylate kinase is essential for the synthesis of dTTP, a critical precursor of DNA, and recent studies show that this enzyme represents an attractive antimicrobial target [9]. Paige Carleitta found a new antimicrobial target in *Bacillus anthracis*, the novel type III pantothenate kinase (PanKBa; encoded by coaX), which catalyzes the first committed step in CoA biosynthesis and has demonstrated that PanKBa is an essential enzyme by analyzing the growth characteristics of a conditional coaX mutan [10]. Moreover, Velasco G R has been researching on betaine aldehyde dehydrogenase of the human pathogen pseudomonas aeruginosa which may play a dual role of assimilating carbon and nitrogen from choline or choline precursors-abundant at infection sites and producing glycine betaine, potentially function against the high-osmolality stress prevalent in the infected tissues [11].

***Genetic and RNA:*** In recent years, the genomics have provided staggering amounts of sequence information and simultaneously expanded the targets for new antimicrobials. Genome sequence information supplements the targets that can provide additional information to assess the potential breadth of spectrum and potential selectivity that inhibitors of the targets may provide [12]. It is known to all that genetic mutation can lead to death of cells, and one method is using conditionally lethal mutants, such as high temperature, radiation and so on. While temperature-sensitive mutant strategy is more feasible. Harris, S.D. and coworkers had successfully achieved mutant forms [13]. Dihydrofolate reductase FolA was considered to be essential for bacterial growth, however, comparative genomics has revealed that several bacterial genome sequences appear to lack the FolA gene. The report [14] shows that life without two "essential enzymes", ThyA and FolA, is still possible. Therefore, there may be a new way in designing new compounds for inhibiting microbial growth. This instance means that we can explore new targets in known fields, even fairly well studied areas. The similar examples include discoveries in isoprenoid, thiamin, and adenylate metabolism [15]. The completion of numerous microbial genome sequences has provided numerous new targets, and also means to assess the anticipated spectrum and selectivity of the inhibitors of these targets [12].

Genetic studies have shown that mRNA splicing is essential in *Saccharomyces cerevisiae* [16], and messenger RNA splicing in fungi is an attractive target for the development of novel antifungal antibiotics [17].

***Other targets:*** There are also many other targets, for example, Jodi A L found fungal fatty acid synthase (FAS) may be a good potential antifungal drug target [18], and recent evidence has proved it [19, 20]. Iron is necessary for the survival of most organisms, depriving pathogenic microbes of iron is therefore a potential antimicrobial strategy. Frederick R E has been researching on this field and got some inhibitors [21]. Andreassi, J.L. reported a promising target, diphosphomevalonate (DPM), which can bind with high affinity to the mevalonate kinase and switch off the mevalonate pathway in *Streptococcus pneumoniae* [22]. The acquisition of manganese is vital for the virulence of several bacterial species. Latest studies have identified a cation diffusion facilitator (CDF) protein of specific unknown substrate that functions as a manganese export system in streptococcus pneumonia, and indicates that manganese efflux is required for invasive disease and may provide a useful antimicrobial target to devise future therapeutics [23].

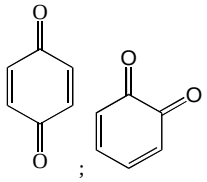
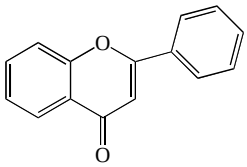
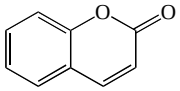
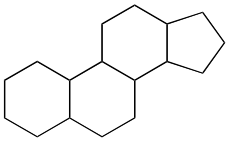
## NATURAL PRODUCTS

Since the emergence of drug resistance, scientists have realized that the effective life span of any antibiotic is limited, and more and more people turn their eyes to natural products. To date, antimicrobial agents have been widely discovered in natural products, plants and also animals. Finding healing powers in plants is an ancient idea, and more than half of the current clinically used chemotherapeutic agents were derived from natural products. The actual natural products are effective, and have low enough toxicity, a broad enough spectrum and sufficiently good pharmacokinetics to be clinically useful without chemical modification.

Antimicrobial phytochemicals are usually divided into the following skeletons: quinones, alkaloids, flavones, coumarins, tannins, terpenoids and essential oils, sterides, and other compounds, most of which are secondary metabolites. Secondary metabolites have no explicit role in the internal environment of the organism that produces it, but can inhibit organisms outside the producer [24]. Quinones and phenolics have similar mechanisms, oxidation and reduction reactions. Quinones will complex irreversibly with nucleophilic amino acid in protein and lead to inactivation of the protein [25], and phenolics will react with the sulfhydryl groups or interact with the proteins nonspecifically. The more highly phenols are oxidized, the more inhibitory [26, 27], such as lawsonia and rapanone belong to quinines [28, 29]. Berberine is one of the most important alkaloid antibiotics and it can forcefully inhibit a variety of gram-positive and gram-negative bacteria [30]. Trolline and "new kinganone" were found to have the ability to inhibit *Micrococcus aureus, Streptococcu* and *Pneumoniae* [31, 32]. It was reported that teas possessed antimicrobial activity because of catechin which belongs to flavonoids, and catechin probably complexes with extracellular and soluble proteins or bacterial cell walls [33]. Antibiotic properties are also found in coumarins, like carum carvi and galium odoratu [34, 35], while datas on the antibacterial agents of coumarins are still scarce and further research is needed. Terpenoids and essential oils are widely found in nature and they are important sources of seeking and inventing novel antibacterial drugs, moreover, a lot of compounds have been discovered to possess antimicrobial activity [36-41].

In addition to what have been illustrated above, other compounds like steride and tannins can inhibit the growth of microorganism as well. For example, "new pregnane", discroreside E and prtotogracillin can produce a good curative effect to *Pyricularia oryzae* [42, 43]. Tannins are found in most part of a plant, and they may combine with microbial adhesins, enzymes, cell envelope transport proteins, polysaccharides, etc, and inactivate them [44]. However, the mechanisms of action of other compounds are not fully understood and need more investigations. Here we

**Table 1. Natural Products and their Activity**

| Class | Structure | Example | Active | Reference |
|---|---|---|---|---|
| Quinones |  | *Lawsonia* | *M. tuberculosi* | [28] |
| | | *Rapanone* | *Bacillus anthracis, Malarial Protozoa* | [29] |
| Alkaloids | Heterocyclic nitrogen compounds | *Berberine* | Bacteria, *Malarial Protozoa* | [30] |
| | | *Trolline* | *Micrococcus aureus, Streptococcus* | [31] |
| | | *New Kinganone* (not named yet) | *Pneumoniae* | [32] |
| Flavones |  | *Cinchona sp* (Quinine) | Bacteria, Fungi | [46] |
| | | *Camellia sinensis* | *Malarial Protozoa* | [47] |
| | | *Thuidinin* | Bacteria, Fungi, Protozoa | [48] |
| | | *New Flavanol* (not named yet) | Bacteria | [49] |
| | | *New Flavonoid Glycosiodes* (not named yet) | *Micrococcus aureus, Bacillus subtilis, Bacillus coli* | [50] |
| Coumarins |  | *Carum carvi* | Bacteria, Fungi, Viruses | [34] |
| | | *Galium odoratu* | Bacteria, Protozoa | [35] |
| Terpenoids and Essential Oils | $(C_5H_8)_n$ (n=1,2,3…) | *Aegle marmelos* | Fungi | [36] |
| | | *Ocimum basilicum* | *Salmonella, Bacteria* | [37] |
| | | *Dissectol A* | *Tubercle bacillus* | [38] |
| | | *Petroviin A* | Bacteria | [39] |
| | | *Petatrichol A and B* | *Bacillus subtilis* | [40] |
| | | *Iridoids I and II* | Bacreria | [41] |
| Sterides |  | *New Pregnane* (not named yet) *Discroreside E Prtotogracillin* | *Pyricularia oryzae* | [42, 43] |
| Other componds | | *New Pinelloside* (not named yet) | Bacteria | [51] |
| | | *Keisslone* | *Albicans saccharomyces, Aspergillus niger* | [52] |
| | | *Diaporthelactone* | Bacteria | [53] |
| | | *Rhizoctonic acid, monomethylsulochrin* | *Helicobacter pylori* | [54] |

enumerate some representative compounds and some new discovered agents in Table **1** [33, 45].

## SCREENING STRATEGIES

There are considerable ways of screening natural compounds, such as agar dilution and disc diffusion methods that are often used in routine screening before. Some new assays like spectroscopic techniques and high throughput screening methods, which have greatly improved the screening speed, are widely used in nowadays. However, each method has its own advantage and disadvantage, so it is very important to choose a suitable method in different cases.

## AGAR DILUTION AND DISC DIFFUSION METHODS

Among the methods of screening natural products, agar dilution and disc diffusion methods are two of the most commonly used, because their procedure are very simple. In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates containing

dilutions of the antimicrobial agent, and get the lowest concentration of the antimicrobial agent that can visibly inhibit the growth of a microorganism. The procedure of agar dilution assay is as follows: First, prepare the extracts of natural product solutions and get a dilution series of extracts. Then, preparation of inoculums, give standard density of colony-forming units per spot on the agar and culture overnight. The inoculums are diluted with a broth culture and get bacterial suspensions. Transfer the diluted bacterial suspensions to the surface of the series of agar plates, incubate plates at 35-37$^{o}$C (the temperature depends on species of the microbe and stability of the extracts) in air for 18 h. The last step is to get the minimum inhibitory concentration (MIC) at which concentration we can find a single colony or a thin haze within the area of the inoculated spot by the naked eyes [55].

In disc diffusion test (also called Kirby Bauer test), a filter paper disc impregnated with a sort of extracts is used and placed on an agar containing a medium that has been inoculated with disease agent. The chemical will diffuse into the medium and kill some of the disease agent around if the agent is susceptible to the chemical, and the area of no growth around the disc is known as "zone of inhibition" (Fig. **1**).

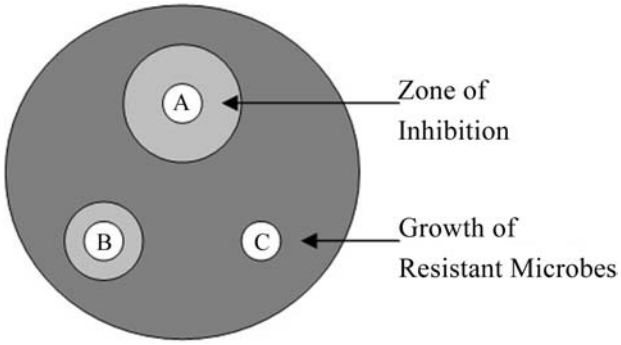



**Fig. (1).** A, B and C are filter paper discs, the circles around A and B are zone of inhibition, the rest portion is the medium filled with disease agent.

## CHAMBER METHOD

Narciso J A explored a chamber method [56], which used a type of autoclavable incubation chamber with a filter paper base, hydrating sponge pieces and a sterile glass sample platform. The filter paper is placed in the bottom of a small crystallization chamber, and put several compact hydrating sponges on. On the top of each sponge place a coverslip, then samples such as fruit or agar plugs are placed on each coverslip, inoculated and treated (Fig. **2**). When finished, the samples are removed and the device can be reused again. The chamber offers a sterile, controlled environment and can be manipulated to serve a number of studies, and contents can be viewed macroscopically or microscopically to see the progress of infection on the samples and the effect of the experimental compounds. This method plays a very important role in Florida's agriculture in order to find antimicrobial compounds which can inhibit or kill the economic pests.

From what have been showing above, it was obvious that the above methods are time consuming and don't suitable for screening of a huge amount of compounds at the same time.

## A HIGH THROUGHPUT YEAST HALO ASSAY

To speed the screening procedure, during these years, other new methods have been developed based on classic disk diffusion assay idea, for example, the high throughput yeast halo assay. This new screen is quantitative and allows inhibitory potencies to be determined, since the diffusion of the sample provides a concentration gradient and a corresponding toxicity halo. The strategy is as follows: A library of compounds are transferred from standard 384-well plates into agar using a pinning robot, immediately after the compound addition, an initial absorbance reading ($Abs_{blank}$) is taken of the plate using a 384-well compatible plate reader at 544 nm. The plate is incubated at 30°C for 24 h and then read again, and halos are verified by visual inspection (Fig. **3**). Inhibition of each compound was quantified using the following equation [57]:

$$F_i = [(Abs_{diagonal} - Abs_{diagonal\text{-}blank}) - (Abs_i - Abs_{i\text{-}blank})]/ (Abs_{diagonal} - Abs_{diagonal\text{-}blank})$$

While the disadvantage of this method is that the computational results are base on visual observation and its MIC data are potentially subjective due to the dependence on the person who is measuring the zone of inhibition. A pattern recognition algorithm was developed to identify halo-like shapes in plate reader optical density measurements, and the

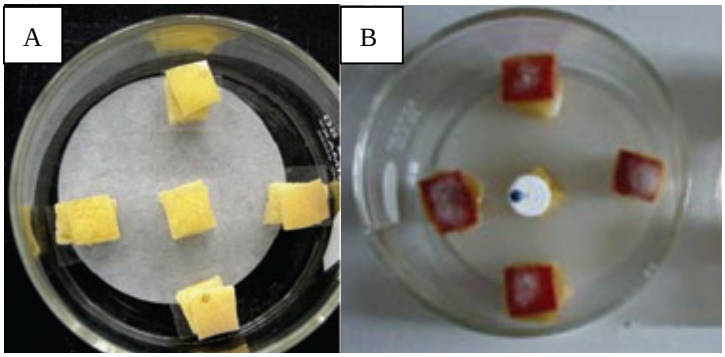



**Fig. (2).** Topical **A**: Grapefruit peel has been inoculated, brushed with antimicrobial coatings. Topical **B**: Tomato pieces were all inoculated with *Fusarium* sp. [56].

author used a plate reader (Fig. **4**) to measure the sizes of each halo. This assay is simple, and the halos provide unmistakable visual confirmation of bioactivity, so the halo assay can be used as a simple and effective way to compare activities among groups of compounds [58].

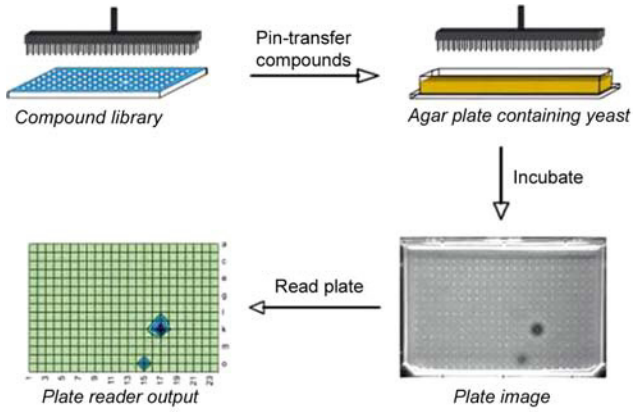



**Fig. (3).** High throughput yeast halo assay strategy [57].

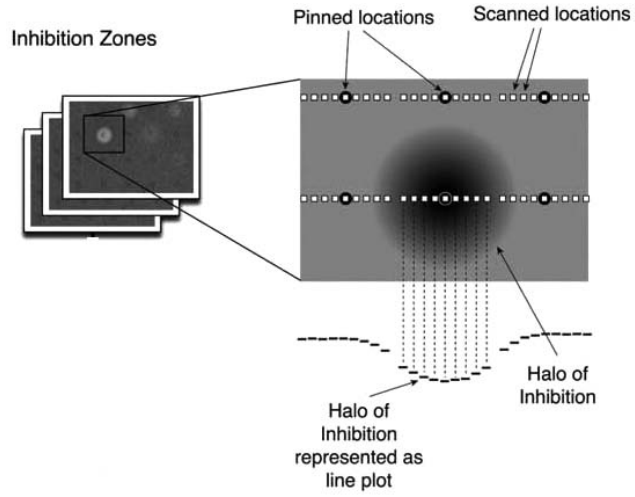



**Fig. (4).** The small black circles are the sites where the compounds are pinned into, the white ones can be viewed to affirm the growth inhibition of each compound, the large shaded portions are the functional area of one compound [58].

## MICROPLATE ASSAY

Many studies have revealed the poor accuracy and laborious nature of the agar and disk diffusion assays that are traditionally used to detect antimicrobial activity of plant extracts. The 96-well microplate assay base on correlating the bacterial growth with the inhibitory effect of the plant extract may solve this problem, and this method have increased accuracy and throughput, and reproducibility is high. The most important point of the microplate assay is how to add materials to each well of a plate (Fig. **5**). Then, the plate is placed in an incubator at 37$^{o}$C and incubated for 22 h, then read the OD (optical density) in the spectrophotometer at 540 nm [59]. This very sensitive assay exposes the lowest concentration of plant extracts to the highest concentration of inhibiting or killing bacterial cells, thus maximizing the potential to detect antimicrobial activity. Furthermore , this method is high throughput, simple and robust and enable rapid screening of plant extracts with a good correlation between the percent inhibition (MIC0-100) and EO (essential oil) concentration [59, 60].

## ANIMAL INFECTION MODEL

In the process of drug development, researchers may used to meet this trouble that they cannot use vitro assays to detect the efficacy of some compounds with high background of toxic or poor pharmacokinetic properties which make them impossible to penetrate across the multidrug resistance barrier of Gram-negative bacteria [61]. In this case, animal infection models could be used, and this idea screen the novel antimicrobials better than *in vitro* models, because animal's body is much more approximate to an infected human host. The whole-animal *C. elegans* screening method identifies not only traditional antibiotics, but also compounds that target bacterial virulence or stimulate host defense [62]. This approach is as follows: first, use a large particle sorter to dispense an exact number of infected animals into each well of a 384-well compound plate; next, stain the worms with SYTOX Orange. Then, use an automated microscope to acquire an image of the SYTOX fluorescence and a bright field image of each well. At last, quantify worm survival in an automated manner by adapting the open-source cell image analysis program CellProfiler and get the inhibitory ability of each compound (Fig. **6**) [63].

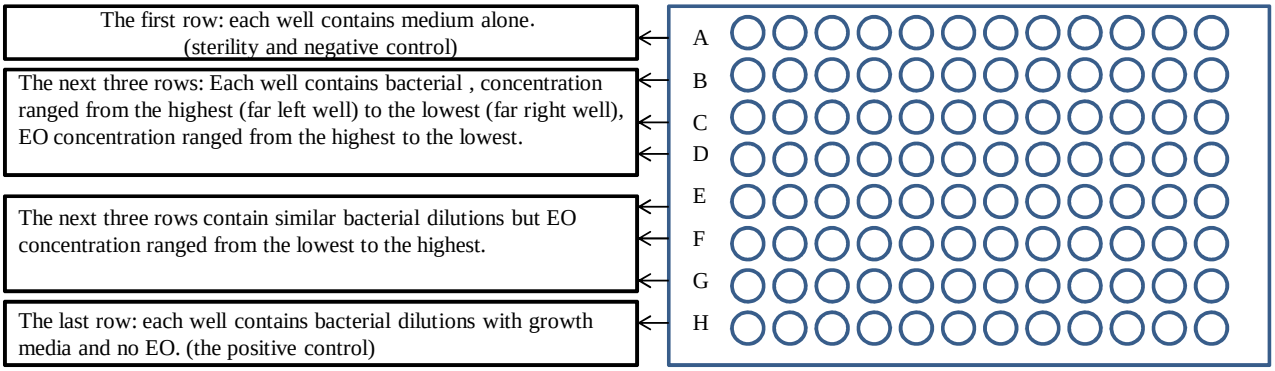



**Fig. (5).** Adding method of each well.

## FLUORESCENT ASSAY

On the way of searching new antibacterial agents, many assays using fluorescence were developed, for example, a simple method was established using a commercially available Live/Dead® Bacterial Viability Kit. Two fluorescent nucleic acid stains are used in this assay: SYTO9 (all cells are stained green) and propidium iodide (cells with damaged membrane are stained red). Using a multi-label plate reader set to measure emissions at two different wavelengths by fluorescence microscopy or their fluorescence emissions, and the activity of drugs are indicated by the percentage of live versus dead bacteria (ratio of green versus red) [64]. The second method can rapidly and simply screen large number of chemicals or natural product libraries, and does not require cell lysis, washing or extraction steps [65]. The screening assay is carried out as follows (Fig. **7**). This method has been used to identify potential new drug candidates for treatment of leishmaniasis [66, 67], therefore, it can be used to develop inexpensive HTS assays for the routine screening of new anti-leishmanial drug candidates [65].

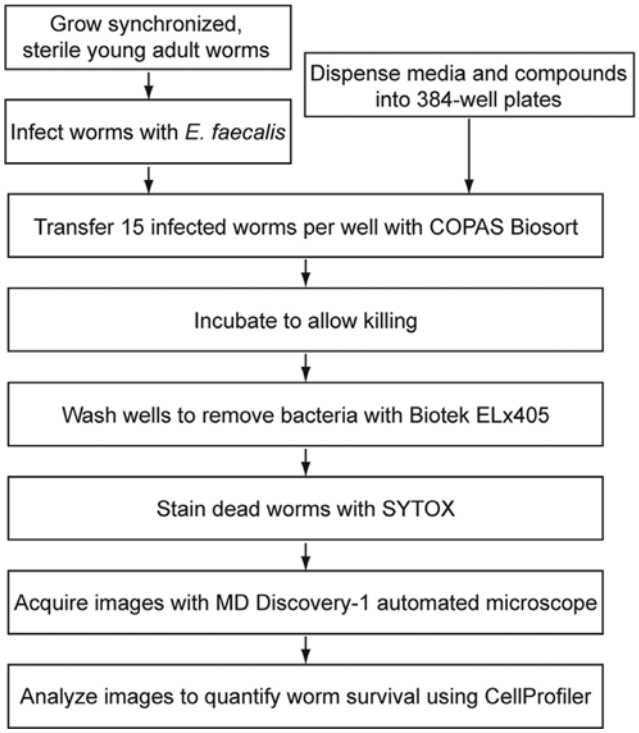



**Fig. (6).** Screening scheme of animal infection model [63].

## TYPE II FATTY ACID SYNTHESIS (FAS II) PATHWAY ASSAY

It is known to all that fatty acids are very essential for all the living beings. The human fatty acid synthase (FAS I) is a multifunctional single polypeptide composed of distinct enzyme domains, and in contrast, bacterial FAS II is carried out by a series of individual enzymes. Therefore the type II fatty acid synthesis pathway is essential to bacterial survival and has the potential to present novel targets for antibiotic discovery [68, 69], and for these reasons, high throughput fatty acid synthesis pathway method was developed. First step of this assay, preparation of type II fatty acid synthesis enzymes, get solutions containing all necessary fatty acid synthesis enzymes. The partially purified protein containing fatty acid synthesis enzymes were preincubated with a serial dilution of natural products in a phospholipid 96-well flash plate at room temperature for 20 min in a buffer solution, which could imitate the environment of intracellular. Then the plate was incubated at 37°C for 30-90 min and terminated by adding perchloric acid. The plates were sealed, incubated at room temperature overnight with mild shaking. The long hydrophobic acyl chains of acyl-ACP could bind to the phospholipids on the surface of the well which were coated with scintillant because of hydrophobic interactions. This binding brought the incorporated $^{14}C$ into the proximate scintillant, and resulted in the emission of a photon which was captured by a scintillation counter [69]. Using this reliable high throughput fatty acid synthesis pathway assay, three new acetylenic allenes named phomallenic acids were isolated from a leaf litter fungus [70], and this assay would play a great role in future drug discovery.

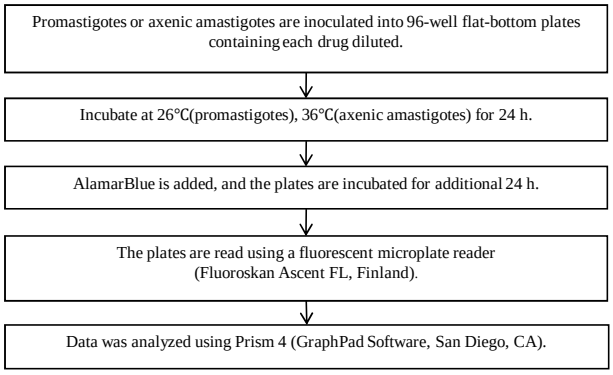



**Fig. (7).** Screening strategy of the fluorescent assay.

## MICROFLUIDIC SENSOR FOR HIGH THROUGHPUT SCREENING

It has been proved for a long time that cell excitement, metabolism, apoptosis, necrosis, division and growth, neurotransmitters, environmental toxic agents, would affect cell volume. Therefore, cell volume has been used as the basis for cell-based screening after the technology of cell volume measuring was developed, such as the microscopy. Among these methods Coulter counter technology has been widely applied, due to its ease of use and relatively rapid sampling rate, while this method requires free-floating cells and is not fast enough to measure the kinetics of cell volume regulation. Daniel, A.A. invented a microfabricated chip (Fig. **8**) which could rapidly screen natural products (only in small quantities), and it was noninvasive and provided real-time measurement of changes in cell volume for both adherent and suspended cells [71]. The usage of this chip is as follows: Bacteria grow overnight in standard culture media in appropriate antibiotics and are diluted with media for the growth assay. Then the cells are suspended in antibiotic-containing medium and perfused into the chip and a cure is obtained. Compare this cure with the cure without any drug. The procedure showed above is suitable for

suspended cells, for adherent cells, they are cultured on glass coverslips and inverted on the top of the chip so that the cells can face the chamber. This method can detect the antibiotic sensitivity of bacteria in less than 15-20 min because it is sensitive to cell growth and does not require the time for cell division.

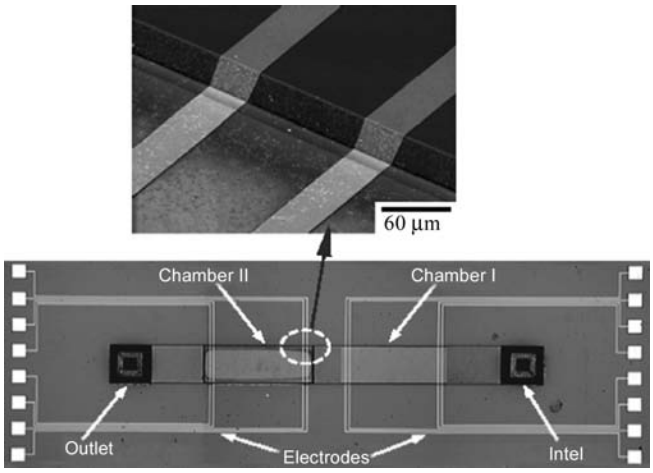



**Fig. (8).** Structure of the volume sensor. Chamber I is used for measuring the cell volume, chamber II is served as a calibrating chamber for monitoring solution resistivity [71].

## SPECTROSCOPIC TECHNIQUES

Many new screening methods are invented since the development of spectroscopic techniques, among which mass spectrometry (MS) based assays have attracted great attention for high throughput screening in recent years. Screening natural product extract libraries using spectrophotometric assays have poor sensitivity and selectivity because of the strongly absorbing nature of some extracts and the dilute concentrations of active components. However MS does not require analytes to be labeled, either by direct attachment of fluorescent and radioactive labels or by binding of antibodies [72, 73]. At same time, MS-based assays don't need the purification of natural extracts before MS analysis [74] and have the ability to detect and analyze with high sensitivity. Mass spectrometry has recently been used to identify inhibitors of protein aggregation, and if combined with NMR it can also identify protein–protein interactions [75]. Mass spectrometry is usually used in the form of hyphenated techniques, such as GC-MS, ESI-ICRFTMS, FAC-MS and so on, in order to continuously track and determine biological activities and effectively screen molecular libraries. Some new developed assays are as follows: Electrospray ionization fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR) achieved great successes in screening natural product microbial libraries against RNA targets [2]. Quantitative matrix-assisted laser desorption/ionization (MALDI)-MS has been widely developed in most fields of life science for not only high-mass biomolecules, but also low molecular weight compounds because of its high speed, low sample consumption, and high sensitivity [74]. Frontal affinity chromatography mass spectrometry (FAC-MS) has now been most effective in the deconvolution and post-screen identification of active components in crude natural product extracts [76]. Peggy, A.S. and coworkers screened natural product extracts with high-performance liquid chromatography-electrospray mass spectroscopy which were much more sensitive and selective [77].

Another important spectroscopic technique for screening natural products is NMR method. NMR requires the using of expensive instrumentation and the throughput is lower than screening methods based on photometric or chemical detection, however, NMR methods are essential in the screening of libraries with low affinity ligands [78]. Structure-activity relationships (SAR) by NMR is the early achievement in this field [79].

It should be noted that natural products often mix with unknown compositions which are the obstacle to the use of SAR by NMR, so it is more suitable to use saturation transfer difference (STD) spectroscopy for natural products screening [80]. Because if ligand molecules bind to proteins, rapid spin diffusion will result in the signals of the ligand being saturated along with the protein, but the unknown molecules will not bind the protein and don't generate any signals [2].

## AUTOMATIC HIGH THROUGHPUT SCREENING METHOD

As mentioned above, traditional methods, like agar dilution method, will cost a very long time if screening a large number of natural products. In order to relieve scientific staffs from tedious work, high throughput screening (HTS) and robotics are combined together to achieve the desired screening rates. Using automatic equipment, data processing and control software, liquid handling devices, and sensitive detectors, HTS enable a researcher to screen millions of compounds in a very short time, and the existing HTS robots can test up to 100,000 compounds per day [81]. The most important testing vessel of the HTS is the microplates, such as 96, 284, 1536 and 3456 wells. An HTS system typically consist of one or more automatic sections, transfer microplates from station to station for sample and reagent addition, mixing, incubation, and finally detection. This system can usually prepare, incubate, and analyze many microplates simultaneously. Then the machine outputs the result of each experiment as a grid of numeric values, with each number mapping to the value obtained from a single well, therefore, this system can measure dozens of plates in the space of a few minutes. The importance of high throughput robotics not only relate to being able to process very large libraries, but also enable fast turnaround of screening data in a very short time, and these advantage increases the speed of the overall drug discovery process which is a crucial issue for drug discovery [82]. HTS is a relatively recent innovation and feasible through modern advances in robotics and high-speed computer technology, however, it still takes a highly specialized and expensive screening lab to run an HTS operation.

## CONCLUSION

There are tens of thousands of different compounds in nature, screening natural products to find new drugs is an effective way in rational drug discovery. While natural products always mix with other nuisance components which are hard to be removed and screening them costs a

considerably long period of time using general methods in former days. However, we have perhaps never been better equipped to discover new antimicrobial agents in nowadays, with the development of new technology, such as automatic high throughput screening, the screening time has been greatly reduced. Spectroscopic techniques like MS and NMR enable us to screen the natural products libraries more conveniently, sensitively and selectively. We can also use microdilution method, microplate assay, high throughput yeast halo assay which are not very expensive and can be used in routine in-house high throughput screening. Assays like the chamber method can be used if the screening number is small, because its procedures are so simple and the progress of infection can be viewed macroscopically. Nevertheless, some disadvantages still exist in current methods, so it is necessary to continue to research in this area to improve natural products libraries and reduce the time and resources required by screening assays.

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