

Review of Extraction, Isolation and Characterization of Bioactive Compounds from Medicinal Plant

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Abstract: *The medicinal plant are pure compound or as standardized extract , provide idea to isolate new drug because medicinal plant consist of unlimited sources of chemical compound .It also consist of essential oil being used for perfuminary purpose .the focus of this paper is method used for extraction , isolation , characterization of bioactive compound from medicinal plant. Method used for extraction, isolation and characterization of bioactive compound extraction by soxhlet apparatus, microwave assisted extraction, ultrasonic assisted extraction are used. the purification of bioactive compound by TLC . the structural determination of bioactive compound by UV –visible, Infra Red IR, Nuclearmagnetic Resonance NMR and mass spectroscopy is used. The biochemical characterization done by using the toxicity assay, in vivo evaluation, clinical study.*

Keywords: Isolation, Characterization, Bioactive Compound

I. INTRODUCTION

Medicinal plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for discover new drug [1].According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs[2]. The Bioactive compounds as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity is reported[3]. Bioactive compounds can be used as food additives due to their antioxidant properties. Antioxidants are substances that reduce oxidative stress in foods[5]. Synthetic antioxidants are widely used because of their stability and their widespread availability; however, they are related to mutagenic and carcinogenic effects and this has led to the search for antioxidants extracted from plant matrices[6].The aim of this paper was how to extract bioactive compound and which method is used to isolate , characterization of bioactive compound [7].

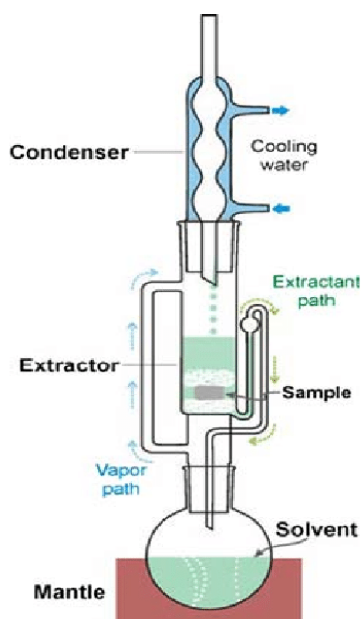
1.1 Extraction of Bioactive Compound

A. Extraction by Soxhlet

Bioactive compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered[8]. soxhlet extraction and others are commonly used for the plant samples extraction[9]. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems[10].

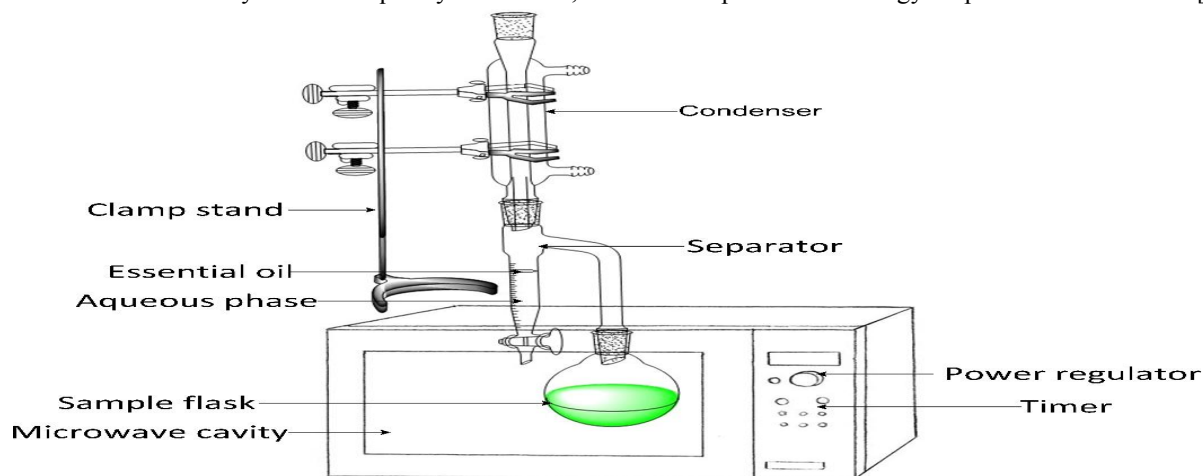
B. Extraction by Maceration Process

5.0 g of dried powder bioactive compound were placed into macerated in 75 mL of distilled water and placed into shaking bath (Clifton Range®) at constant speed of 220 rpm and retention time of 30 minutes[10]. The shaking maceration process was performed at different temperature of 25 °C, 50 °C and 75 °C. Meanwhile, shaking maceration process was also done at different retention time of 30, 60, 90 and 120 minutes at constant temperature of 75 °C with constant speed of 220 rpm. The crude extracts were freeze dried[11].



C. Microwave-Assisted Extraction (MAE)

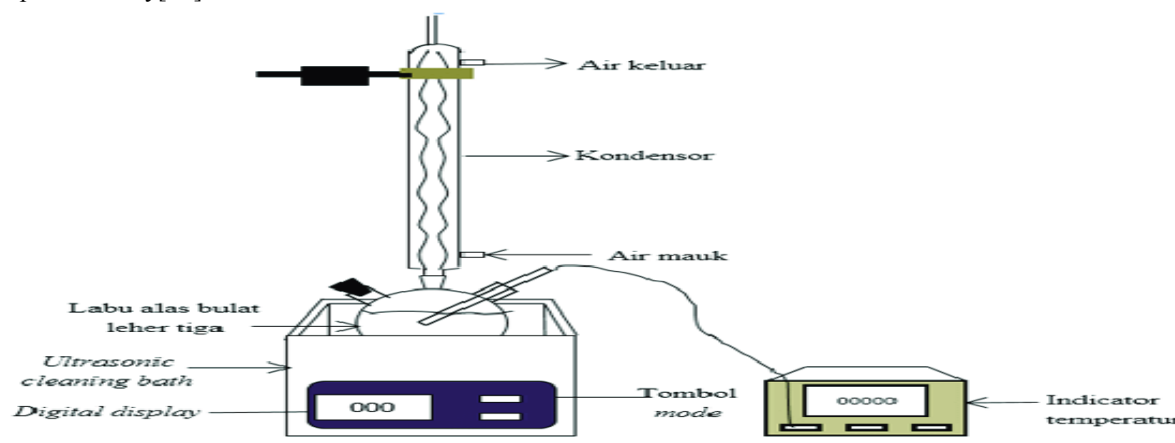
MAE has attracted the attention of researchers as a technique to extract bioactive compounds from a wide variety of plants and natural residues [12]. Microwaves have electromagnetic radiation that occurs at frequencies between 300 MHz to 300 GHz, and wavelengths between 1 cm and 1 m. These electromagnetic waves consist of both an electrical field and a magnetic field. These are described as two perpendicular fields[13]. The first application of microwaves was to heat up objects that can absorb a part of the electromagnetic energy and convert it into heat. Commercial microwave instruments commonly use the frequency 2450 MHz, which corresponds to an energy output of 600–700 Watts[14].



D. Ultrasonic-Assisted Extraction (UAE)

Ultrasound-assisted extraction has been used in diverse applications of food-processing technology to extract bioactive compounds from plant materials[15]. Ultrasound, with levels greater than 20 kHz, is used to disrupt plant cell walls, which helps improve the solvent's ability to penetrate the cells and obtain a higher extraction yield. UAE can use a low operating temperature through processing, maintaining a high extract quality for compounds. UAE is known to be one of the easiest extraction techniques because it uses common laboratory equipment such as an ultrasonic bath[16]. In this technique, a smashed sample is mixed with the suitable solvent and placed into the ultrasonic bath, while temperature

and extraction time are controlled [17]. UAE of various organic and inorganic samples can use a wide range of solvents. Common equipment used in ultrasound-assisted extraction includes an ultrasonic bath and an ultrasonic probe system. Unfortunately, ultrasonic probe has two main negative properties mainly related to experimental repeatability and reproducibility[18].



E. Purification of the Bioactive Molecule Many Bioactive Molecules

Have been isolated and purified by using paper thin-layer and column chromatographic methods. Column chromatography and thin-layer chromatography (TLC) are still mostly used due to their convenience, economy, and availability in various stationary phases[19]. Silica, alumina, cellulose, and polyamide exhibit the most value for separating the phytochemicals. Plant materials include high amounts of complex phytochemicals, which make a good separation difficult. Therefore, increasing polarity using multiple mobile phases is useful for highly valued separations[20]. Thin-layer chromatography has always been used to analyze the fractions of compounds by column chromatography[21]. Silica gel column chromatography and thin-layer chromatography (TLC) have been used for separation of bioactive molecules with some analytical tools[22].

F. Structural Clarification of the Bioactive Molecules

Determination of the structure of certain molecules uses data from a wide range of spectroscopic techniques such as UV-visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and mass spectroscopy[23]. The basic principle of spectroscopy is passing electromagnetic radiation through an organic molecule that absorbs some of the radiation, but not all. By measuring the amount of absorption of electromagnetic radiation, a spectrum can be produced. The spectra are specific to certain bonds in a molecule[24]. Depending on these spectra, the structure of the organic molecule can be identified. Scientists mainly use spectra produced from either three or four regions—Ultraviolet (UV), Visible, Infrared (IR), radio frequency, and electron beam [25]—for structural clarification.

G. UV-Visible Spectroscopy

UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures[26]. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range[27]. Natural compounds can be determined by using UV-visible spectroscopy [28]. Phenolic compounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with iron. Plants 2017, 6, 42 5 of 23 that have been detected by the ultraviolet/visible (UV-Vis) spectroscopy [29]. Moreover, spectroscopic UV-Vis techniques were found to be less selective and give information on the composition of the total polyphenol content. The UV-Vis spectroscopy was used to determine the total phenolic extract (280 nm), flavones (320 nm), phenolic acids (360 nm), and the total anthocyanids (520 nm). This technique is not time-consuming, and presents reduced cost compared to other techniques[30].

H. Infrared Spectroscopy

Some of the frequencies will be absorbed when infrared light passes through a sample of an organic compound; however, some frequencies will be transmitted through the sample without any absorption occurring[31]. Infrared absorption is related to the vibrational changes that happen inside a molecule when it is exposed to infrared radiation. Therefore, infrared spectroscopy can essentially be described as a vibrational spectroscopy[32]. Different bonds (C–C, C=C, C≡C, C–O, C=O, O–H, and N–H) have diverse vibrational frequencies. If these kinds of bonds are present in an organic molecule, they can be identified by detecting the characteristic frequency absorption band in the infrared spectrum[32]. Fourier Transform Infrared Spectroscopy (FTIR) is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds. FTIR offers a rapid and nondestructive investigation to fingerprint herbal extracts or powders[33].

I. Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR is primarily related to the magnetic properties of certain atomic nuclei; notably the nucleus of the hydrogen atom, the proton, the carbon, and an isotope of carbon[34]. NMR spectroscopy has enabled many researchers to study molecules by recording the differences between the various magnetic nuclei, and thereby giving a clear picture of what the positions of these nuclei are in the molecule[35]. Moreover, it will demonstrate which atoms are present in neighboring groups[36]. Ultimately, it can conclude how many atoms are present in each of these environments. Several attempts have been made in the past by using preparative or semi preparative thin-layer chromatography, liquid chromatography, and column chromatography to isolate individual phenols, the structures of which are determined subsequently by NMR off-line[37].

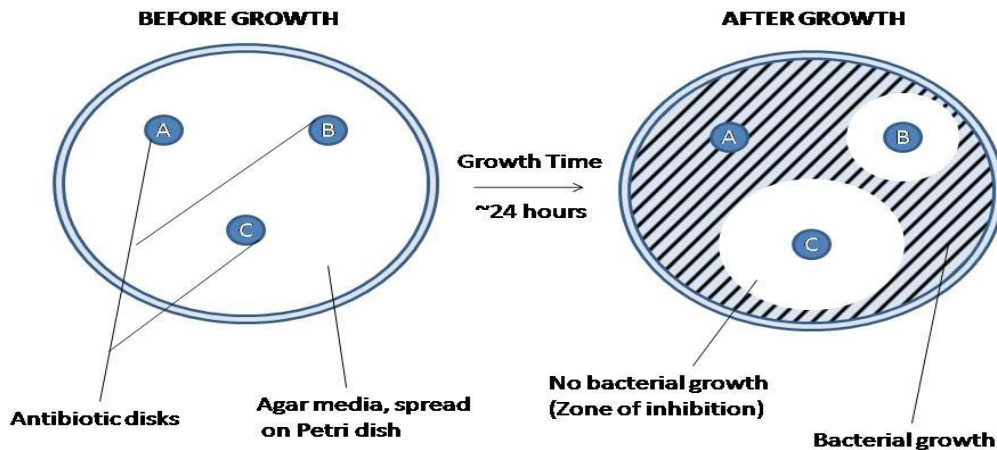
J. Mass Spectrometry for Chemical Compounds

Identification Organic molecules are bombarded with either electrons or lasers in mass spectrometry and thereby converted to charged ions, which are highly energetic. A mass spectrum is a plot of the relative abundance of a fragmented ion against the ratio of mass/charge of these ions[38]. Using mass spectrometry, relative molecular mass (molecular weight) can be determined with high accuracy and an exact molecular formula can be determined with a knowledge of places where the molecule has been fragmented. In previous work, bioactive molecules from pith were isolated and purified by bioactivity-guided solvent extraction, column chromatography, and HPLC [39]. The techniques of UV-visible, IR, NMR, and mass spectrometry were employed to characterize the structure of the bioactive molecule. Furthermore, molecules may be hydrolyzed and their derivatives characterized[40]. Mass spectrometry provides abundant information for the structural elucidation of the compounds when tandem mass spectrometry (MS) is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable [41]. Recently, LC/MS has been extensively used for the analysis of phenolic compounds. Electrospray ionization (ESI) is a preferred source due to its high ionization efficiency for phenolic compounds[43].

K. Determination of Zone of Inhibition Method

In bioactive compound antibacterial and antifungal activities were examined for hydroalcohol extracts[44]. Antibacterial and antifungal activities of plant part extracts against four pathogenic bacteria (two Gram-positive and negative) and three pathogenic fungi were investigated by the agar disk diffusion method[45]. Antimicrobial activity testing was carried out by using agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure Gram-positive, Gram-negative, and fungal strains were taken as a standard antibiotic for comparison of the results[46]. All the extracts were screened for their antibacterial and antifungal activities against the *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes* and the fungi *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*[47]. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Bioactive compound* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes[48]. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 hours[49]. Control

experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin for antibacterial activity and nystatin and griseofulvin for antifungal activity as standard drugs[50]. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C[51]. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms[52].



II. CONCLUSION

Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound.

Medicinal plant extracts showed strong antioxidant capacity., in addition to avoiding the need for longer extraction times compared to the conventional extraction method. Moreover, natural bioactive compounds have been found to interfere with and prevent all kinds of cancer. In fact, many studies have shown that flavonoids play significant multiple roles including mutagenic, cell damage, and carcinogenic, due to their acceleration of different aging factors. In addition to antioxidant activity, the inhibition of cancer development by phenolic compounds relies on a number of basic cellular mechanisms. More comprehensive studies related to these compounds will enhance pharmaceutical exploration in the field of carcinogenic disease prevention.

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