



Original Article

CODEN: IJPNL6

Extraction and Qualitative Phytochemical Screening of Medicinal Plants: A Brief Summary

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Received on: 16-02-2018; Revised on: 23-03-2018; Accepted on: 31-03-2018

ABSTRACT

Phytochemicals are the medicinally active compounds found in plants; parts such as roots, barks, leaves, flowers, seeds, and even pulps. This review focuses on the collection and preparation of plants, the extraction of active compounds, and the qualitative analysis of the phytochemicals present in the plant sample.

Novelty is not claimed for the methods described in this paper. Instead, the aim is to review those methods which have been widely used in photochemistry.

Keywords: Phytochemicals, Crude drugs, Extraction, Phytochemical screening

INTRODUCTION

Asia, the largest continent in the world, has diverse plant flora but species richness is concentrated mainly in tropical and subtropical regions, one of which is the Philippines.

Medicinal plants are the major bio resource of drugs for both traditional and conventional systems of medicine [1-4]. However, the medicinal value of plants lies in their phytochemical constituents, which produce a definite physiological action on the human body. These phytochemicals can be classified in a number of different ways: based on their biosynthetic origin, solubility properties, and the presence of certain key functional groups.

This paper mainly deals with the preparation, extraction, and qualitative analysis of phytochemicals present in medicinal plants.

MATERIALS AND METHODS

Collection of plant

Ideally, fresh plant samples should be used for phytochemical

analysis. But sometimes, samples are not available and may have to be provided by someone living in another country. In such cases, freshly picked tissue, stored dry in a plastic bag, must remain in good condition for analysis during the several days of its transportation [4,5].

The botanical identity of the plants studied must be authenticated by an acknowledged authority, especially at the early stage of the investigation, and be specific with not only its genus and species but also with its variety. This practice is more applicable if the plant is obtained from a wild forest, in which the plant can be mistaken with a similar plant, rather than obtained from a registered herbarium. The plants obtained from wild forests, however, grew in their natural home ground and can be deemed unexposed to pesticides and city pollution.

These days, it is now a common practice in phytochemical research to deposit a voucher specimen of a plant examined in a recognized herbarium. This is to secure that future reference is available for the plant studied if this becomes necessary.

The selection of correct plant part (e.g. leaves, roots, seeds) is important prior to testing to know which part of the plant contained most of the phytochemicals that the researcher needs for his study. Also, for quality control purposes, the age of the plant and the time, season, and place (including the coordinates of the location) of its collection must be recorded.

Cleaning

After collection, plant undergoes washing with tap water to remove the dust, dirt, and other foreign matters attached to the surface of the plant. Cleaning is continued by stripping the leaves from stems, or peeling if necessary. Cleaning using the hands leads to better results and less damage on the plant. Wiping the samples with clean and dry cloth enhances the drying process [4,5-11].

Drying

The main purpose of drying is to remove water after cleaning. This should be done immediately to prevent spoilage or microbial growth of the plant sample.

Conditions used for drying the plant material largely depend on the nature of its chemical constituents. Drying can be done in various ways [4,5,12].

Natural drying

This type of drying includes sun-drying and air-drying in room temperature or sheds, in which the latter prevents direct sun exposure that can wither the plant and lose its heat or light-sensitive bioactives. This method of drying, however, may take a few weeks for complete drying and depends mainly on temperature and humidity.

Artificial drying

This drying method is done with the help of artificial driers such as oven. Although this method reduces the drying time to several hours or minutes, this requires large number of workers since loading and unloading of plant samples has to be done manually. The exposure to heat, if oven is used, is a disadvantage as this may damage bioactives that are sensitive to heat or elevated temperature. As a general rule, leaves, herbs, and flowers are dried between 20°C and 40°C, and barks and roots between 30°C and 65°C.

Freeze drying

This is also known as lyophilization or cryodesiccation. This dehydration process is used to preserve the temperature-sensitive constituents in the plant, by combining the processes of freezing and sublimation.

Supercritical drying

This procedure allows the plant sample being dried to cross the boundary from liquid to gas, while the amount of liquid decreases. This eventually leads to drying of the plant sample.

Moisture content determination

After drying and before extraction, it is significant to determine the amount of moisture in the plant. This is because moisture can add up to the weight of the plant and can affect its ratio with the solvent during extraction [1,12].

The presence of high moisture can also affect size reduction (or grinding) which is a pre-requisite of extraction. Moisture content determination is expressed in percent and can be obtained by:

Moisture analyser

In this device, a small amount of plant sample (e.g. 10 grams) is placed inside the compartment and is allowed to be analysed for its % moisture in a few minutes. For leaves, they are cut or shredded, but seeds and fruits smaller than 3 mm should be cracked.

Gravimetric method

In this method, the plant is dried and cooled and then percent moisture is calculated. The disadvantage of gravimetric method is that it coincides with the drying procedure. Before drying, the weight of the fresh sample is recorded because this is needed in calculating the % moisture.

$$\% \text{ Moisture} = \frac{\text{Loss of weight of the plant sample}}{\text{weight of fresh plant sample}} \times 100\%$$

Herein, the loss of weight of the plant sample is computed by subtracting the weight of the dried sample from the weight of the fresh plant sample.

In addition, during gravimetric method, it is not necessary that the researcher uses the entire amount of plant sample to determine the % moisture. Ten (10) grams of the fresh plant is

enough to represent the whole. Like moisture analyzer, leaves are cut or shredded while seeds and fruits smaller than 3 mm are cracked.

Accepted % moisture ranges from 10 to 12%.

Extraction

Extraction is defined as the separation of medicinally active portions of plant tissues from the inactive components through the use solvents. *Marc* is the damp solid material or the plant being used and *menstruum* is the liquid material or solvent [1-11].

During extraction, the solvent diffuses into the marc and solubilizes compounds with similar polarity. The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance that is being isolated.

Pre-requisites of extraction

Recording of the weight of the plant sample

This is performed because the weight of the dried sample is used for the computation of percentage yield later on in the experiment [4,5].

Size reduction or grinding or powdering of the plant sample

This is carried out to maximize the surface area, which in turn enhances the mass transfer of active principle from plant material to the solvent. Pulverization is done by the use of mills, blenders, or mortar and pestle. Then the powders are passed through sieves to confirm size uniformity. The 30-40 mesh size is optimal, while smaller particles may become slimy during extraction and could cause difficulty during filtration.

Parameters for selecting an appropriate extraction method

If the phytoconstituents are heat-sensitive, extraction methods that do not require heat are used such as cold maceration and percolation. For heat-stable constituents, Soxhlet extraction and decoction can be used.

In case of hot extraction, higher than the required temperature should be avoided. Some bioactives like glycosides are likely to degrade upon continuous exposure to elevated temperature [4,5].

The design and material of fabrication of the extractor must also be taken into consideration.

Parameters for selecting an appropriate solvent

If the therapeutic value lies in non-polar constituents or bioactives, then a non-polar solvent is used. This applies the “like dissolves like” principle. For example, if the active constituents are glycosides, polar solvents like aqueous methanol should be used [4,5].

Suitable precautions should be considered when dealing with constituents that degrade while being kept in organic solvents, like the case of flavonoids and phenyl propanoids.

The quality of water or menstruum used should be specified and controlled.

Other parameters

Time of extraction must be noted. Insufficient time means incomplete extraction. If the duration of extraction is prolonged, unwanted constituents may also be extracted. For example, in teas, if they are boiled for a longer period of time, tannins are extracted, as well, which impart astringency to the final product.

The number and frequency of extractions required to complete the entire extraction is as important as the duration of each extraction [4,5].

Methods of extraction

Maceration

In this process, the powdered plant sample is placed in a covered container with the solvent and allowed to stand for a period of at least 3 days, in room temperature. Frequent agitation is required until the soluble matter has dissolved. The mixture is then strained, the marc is pressed, and the combined liquids are clarified by filtration or decantation after standing [1,2,3,4,5,11].

Infusion

This is executed by macerating the crude drug for a short period of time with cold or boiling water. This method is performed for plant samples containing readily soluble phytochemicals.

Digestion

This is a form of maceration in which a gentle heat is used during extraction. It is used when moderate and controlled elevated temperature is acceptable for the phytochemicals not to deteriorate.

Decoction

In this process, the marc is boiled in a specified volume of water for a defined time; usually 15 minutes, then it is cooled and filtered. This procedure is suitable for extracting water-soluble and heat-stable constituents.

Percolation

This procedure uses a percolator, a narrow, cone-shaped vessel that is open at both ends. The marc is moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 hours, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 hours. After that, the outlet of the percolator is then opened, and the liquid contained therein is allowed to drip slowly.

Hot continuous extraction (soxhlet)

In this method, the finely pulverized marc is placed in a porous bag or “thimble” made of strong filter paper, which is placed in a chamber of the Soxhlet apparatus. The menstruum in the flask beneath is then heated, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the marc, and extracts it by contact. The advantage of this method is that large amounts of marc can be extracted with a much smaller volume of extractant.

Ultrasound extraction (sonication)

This procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation and eventually, extraction. This method, however, is limited due to higher costs and unavailability in some institutions.

Supercritical fluid extraction (SFE)

This is an alternative extraction method with general goals of reduced use of organic solvents and increased sample output. The extracting fluid used is CO₂, which is favorable in physical properties, inexpensive, safe, and abundant. However, since the polarity of CO₂ is low, a polar modifier is often added to carbon dioxide. It is called “supercritical” because if CO₂ behaves above its critical temperature (31.10 deg Celsius) and critical pressure (72.9 atm), CO₂ expands to fill its container like a gas but with a density like that of a liquid. In addition to its advantages, this method strictly avoids damage from heat and some organic solvents, no

solvent residues, and is environmentally friendly.

Plant tissue homogenization

In this method, the crude drug is mixed with a certain quantity of solvent and shaken vigorously for 5 to 10 minutes or left for 24 hours after which the extract is filtered.

Serial exhaustive extraction

This involves successive extraction with solvents of increasing polarity, from a non-polar (e.g. hexane) to a more polar solvent (e.g. methanol) to ensure that a wide polarity range of compounds is extracted using soxhlet or percolation types of extraction. This method, by some researchers, is also called “sequential extraction.”

Choice of solvents

Successful determination of active phytochemicals from plant material is largely affected by the type of solvent used during extraction [2,4].

Water

Water is the universal solvent and the safest among solvents. It is cheap and readily available. It is highly polar, and its inorganic nature is of advantage if organic solvents are avoided. It is used to extract plant products with anti-microbial activity compared to other solvents like alcohols which have their own anti-microbial activity. To add, it is the standard solvent for decoction.

Alcohols

Methanol is more polar than ethanol due to its shorter carbon chain. But because of methanol’s cytotoxic nature to cause blindness to the bioassay used, it is unsuitable for extraction for some studies as it may lead to incorrect results. Butanol is another alcohol that can be used but is less polar compared to ethanol and methanol due to its longer carbon chain.

Chloroform, dichloromethane, diethyl ether, and ethyl acetate

These are low polar solvents used to extract less polar bioactives.

Acetone

Acetone is the simplest ketone. It dissolves many hydrophilic and lipophilic components from plant materials, is miscible with water, is volatile, and has a low toxicity.

Hexane

Hexane is a common non-polar solvent used to remove fats and fixed oils in plants.

Other steps involved during extraction*Filtration*

After extraction, filtration is performed by using Whatmann No. 1 filter paper to separate the menstruum from the marc. The marc can be further pressed using clean cloth to attain all bioactives from the plant. Another way of filtration is the use of Buchner funnel with gentle suction, which is faster than the previous [1,2,4,8-12].

Concentration

The filtrate or the enriched extract known as miscella is placed into an evaporator (e.g. rotary evaporator) where it is concentrated under vacuum to produce a thick concentrated extract.

Drying

The concentrated extract is further placed into a vacuum chamber dryer to produce a solid mass devoid of the solvent. The dried mass is then pulverized which can be used directly for the desired pharmaceutical formulations or further processed for identification or isolation of its phytochemical constituents.

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. % yield is calculated using the formula below:

$$\% \text{ Yield} = \frac{\text{Weight of the actual (final) dried extract}}{\text{(initial) dried plant sample}} \times 100\% \text{ weight of}$$

Organoleptic evaluation

The colour, taste, appearance, and physical state of the dried mass are described during organoleptic evaluation.

Storage

The dried mass is then stored in a tight, light-resistant container and labelled prior to phytochemical screening. If phytochemical screening is not performed immediately, the dried mass can be stored in a refrigerator or kept at 4 degree Celsius. To add, it has been reported that storage in a freezer and out of direct sunlight produced no adverse deterioration. However, extracts stored at room temperature for one year is reported to have decreased by 30-40% and 70-80% like in the

case of taxol in *Taxus baccata* in leaves and extracts, respectively. It was also reported that Indian hemp and sarsaparilla deteriorate even when carefully stored.

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods. [1-11,13].

Alkaloids*Dragendroff's test*

The extract is treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Mayer's test

To a few mL of plant sample extract, two drops of Mayer's reagent (potassium mercuric iodide solution) are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

Valser's test

A few drops of Valser's reagent (Potassium iodide solution in Mercuric iodide) are added to a few mL of plant extract. A reddish-brown precipitate indicates a positive result.

Wagner's test

A few drops of Wagner's reagent (iodine solution in potassium iodide) are added to a few mL of plant extract. A reddish- Brown precipitate confirms the test as positive.

Hager's test

The extract is treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids is confirmed by the presence of yellow precipitate.

Flavonoids*Alkaline reagent test*

Extract is combined with a few drops of sodium hydroxide solution. The appearance of intense yellow colour, which turns colourless on addition of dilute acid, signifies the presence of flavonoids.

Shinoda's test

One mL of extract is added with 0.5 mL of hydrochloric acid and magnesium metal. The presence of flavonoids is confirmed by reddish coloration.

Bate-Smith and Metcalf test

0.5 mL of concentrated hydrochloric acid is added to the extract. It is then warmed on a water bath for 15 minutes and is observed for an hour. A strong red or violet colour means a positive result.

Saponins*Foam test*

Fifty (50) mg of extract is diluted with distilled water and made up to 20 mL. The suspension is shaken in a graduated cylinder for 15 minutes. Saponins are detected by the formation of two-cm layer of foam.

Tannins*Ferric chloride test*

The extract (50 mg) is dissolved in 5 mL of distilled water. A few drops of neutral 5% ferric chloride solution are then added. A dark green colour indicates the detection of phenolic compound.

Glycosides

Fifty (50) mg of extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered, and the hydrolysate is subjected to the following:

Legal's test

50 mg of extract is dissolved in pyridine and then sodium nitroprusside solution is added. The solution is made alkaline using 10% NaOH. Pink colour means glycosides are detected.

Phenols*Lead acetate test*

The extract (50 mg) is dissolved in distilled water. Then 3 mL of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Proteins

The extract (100 mg) is dissolved in 10 mL of distilled water and filtered through Whatmann No. 1 filter paper. The filtrate is subjected to test for proteins.

Millon's test

To 2 mL of filtrate, a few drops of Millon's reagent (metallic mercury in nitric acid) is added. A flesh to red precipitate indicates the presence of proteins.

Xanthoproteic test

The extract is treated with concentrated nitric acid and observed. Positive result is indicated by yellow precipitate formation.

Carbohydrates*Molish's test*

To 2 mL of extract, two drops of alcoholic solution of α -naphthol are added. The mixture is shaken well and a few drops of concentrated sulphuric acid are added slowly along the sides of the test tube. The appearance of violet ring at the junction means carbohydrates are detected.

Triterpenoids*Salkowski's test*

Extract is treated with chloroform and then filtered and added with a few drops of conc. sulphuric acid. It is then shaken and allowed to stand. The appearance of golden yellow colour or red brown colour indicates the presence of triterpenes.

Phytosterols*Liebermann-Burchard's test*

Extract is added with chloroform. After filtration, it is treated with a few drops of acetic anhydride, boiled, and cooled. Then conc. sulphuric acid is added. Formation of brown ring at the junction indicates the presence of phytosterols.

Fixed oils and fats*Spot test*

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

CONCLUSION

The various steps given above are necessary in determining phytochemicals of medicinal value. The researcher must take into consideration that standardized procedures and correct selection of solvents and materials lead to accurate and reliable results, within the narrowest possible range.

Even so, with the advent of new technology and emerging safety and environmental concerns, it is important that new methods must be developed to lessen the use of chemicals and methods that may risk the environment.

STATEMENT OF CONFLICT OF INTEREST

The author declares no conflict of interest.

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