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Extraction Methods and Phytochemical Analysis in Relation to Medicinal and Aromatic Crops

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SUMMARY

Phytochemical analysis involve methods for extraction, separation, purification and identification of many different constituents present in plants. Medicinal plants are a source of many drugs such as antispasmodics, emetics, antimicrobials, antipyretics, antidiarrheals, antioxidants, and antitumor agents. A large number of the plants are claimed to possess valuable properties in traditional medicine and are also used extensively by tribal people worldwide. Research has emphasized the evaluation and characterization of various plants and plant constituents against a number of diseases. Detection, estimation and extraction of the bioactive plant constituents have always been a challenging task.

INTRODUCTION

For phytochemical analysis, it is best to use fresh plant tissues and to immediately immerse the piece of tissue in boiling alcohol after collection. When the plant being investigated is not available, freshly harvested tissue is often kept dry in a plastic bag. An alternative is to dry the plants before extracting them. In order to prevent excessive chemical changes, drying is done in a well-ventilated area at a regulated temperature. It can be kept for a very long time when it has entirely dried out. In phytochemical analysis, the botanical identity of the plants under study must be verified by a recognized authority. The type of component being isolated as well as the texture and water content of the plant material being extracted naturally influence the specific extraction method. To extract organic contents from dried plant tissue (heartwood, dried seeds, root, and leaf), a soxhlet device is used to constantly extract powdered material using a variety of solvents. This is the traditional chemical method. Plant tissue needs to be killed by immersing it in boiling ethanol to stop enzymatic oxidation or hydrolysis. Extract obtained is clarified by filtration through celite on a water pump and then concentrated in vacuum. Rotary evaporators are highly useful in concentrating bulky solutions at temperatures between 30 and 40°C. When water-soluble components are isolated, lipids are removed before concentration by washing extract repeatedly with petroleum.

Separation:

The separation and purification of plant constituents are mainly carried out using one or other, or a combination, of four chromatographic techniques Choice of technique depends largely on solubility properties and volatility of compounds to be separated. The methods include gas liquid chromatography (GLC), thin-layer chromatography (TLC), paper chromatography (PC) and high performance chromatography (HPLC). PC is especially useful for water-soluble plant components such as organic acids, amino acids, carbohydrates, nucleic acid bases, and phenolic chemicals. GLC is mostly used with volatile components, such as fatty acids, mono and sesquiterpenes, hydrocarbons, and sulphur compounds. TLC is the preferred method for separating all lipid-soluble components, including lipids, steroids, carotenoids, simple quinones, and chlorophylls. As an alternative, HPLC, a technique that combines column efficiency with analysis speed, can be used to separate less volatile chemicals. The methods listed above are often used in combination and depending on the situation, a combination of PC and TLC, TLC and HPLC, or TLC and GLC may be the most effective method for separating a specific plant compound.

Paper chromatography

Partition or adsorption chromatography are the two most common methods used in paper chromatography. Compounds are divided during the partitioning process between water and n-butanol, an alcoholic solvent that is generally water immiscible. The PC operates on a fairly basic technique. Close to one end of the filter paper, a drop of a solution comprising a combination of materials is deposited. After that, the area is left to dry. The spotty

portion of the paper is then submerged in the appropriate solvent. As it moves up the paper, the solvent passes the location and takes the substance with it. The rates at which different chemicals migrate vary depending on a number of factors, including size, nature, solvent, and diffusion coefficient. Paper chromatography is the name given to the completed paper.

$$Rf = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

Thin layer chromatography:

TLC is defined as a chromatographic analytical technique applied to thin adsorbent layers. The method is quick and useful for separating material particles. In the context of science, thin layers can be created on glass plates that are 5 x 20, 10 x 20 or 20 × 20 cm in size. To make five 5 x 20 cm plates, place 10 g of silica gel in a stoppered conical flask, add 22–24 ml of distilled water, shake well for 30 seconds, and then pour the mixture directly onto the plates. Using capillaries, samples are spotted on TLC plates in a horizontal line about 2 cm from the bottom end. After that, the plates are moved into a chamber with a solvent system. Care is used to ensure that the solvent level is lightly below level of spots. TLC chambers are made of rectangular glass jars with airtight lids. Plates are removed and let to dry in the air when the solvent reaches the upper end. To locate chemicals in a chromatogram, specific detection equipment are required because the majority of compounds separated in thin layers are colorless. When non-fluorescent chemicals are separated in adsorbent layers with fluorescent indicators—compounds that show up as black spots on a backdrop of fluorescence—they can be distinguished from fluorescent compounds by their fluorescence. The recovered constituents are obtained by scraping off the adsorbent at the designated locations on the formed plate, eluting the powder using a solvent like ether, and then centrifuging the mixture to eliminate the adsorbent.

Gas Chromatography

When an inert gas passes through a tube that has been filled precisely, the chemicals in a mixture move at various rates in gas chromatography. In GC, gases such as hydrogen and nitrogen are utilized as a moving phase. It is likely to have both partition and adsorption phenomena. Gas solid chromatography (GSC) is the term for the technique when adsorption is occurring, and gas liquid chromatography (GLC) is the term used when partitioning is occurring. The type of column stationary phase and operating temperature are the two main GLC factors. These are adjusted based on the volatility and polarity of the substances that need to be separated.To gradually heat the column from 50 to 350°C, a heater is provided. In GC, a moving carrier gas stream is introduced with the sample, which is then carried through the column by it. Either the low pressure liquid (GLC) or the active solid (GSC) supported on an inert support are contained in the column. Carrier gas forms the mobile phase, while an active solid or non-volatile liquid forms the stationary phase. Sample components divide themselves between the two stages. A substance's solubility or adsorption in a fixed phase might vary. As a result, various components travel through the column at varying rates until emerging at the end in unique zones (peaks) that are divided by carrier gas. Vapors of constituents are identified upon emergence using appropriate instrumental techniques accompained by an automatic recording.

Essential Oil Extraction by Clevengers apparatus.

It is done mainly for oil estimation obtained from any plant part (leaf, stem, root, inflorescence). Essential (volatile oil) oil content of sample was determined by using Clevenger's apparatus (Langenau, 1948) and essential oil yield was calculated by multiplying sample yield by oil content of sample.

Essential oil content (%) = (Weight of extracted oil (ml)/Weight of sample(gm)) ×100

Oleoresin Extraction by Soxhlet extraction.

About 5 kg of fresh sample was dried in an air circulation oven for 20 hours at about 70°C, and they were lyophilized for 72 hours at about -40°C. Following drying, the samples were ground in a knife mill as part of the extraction procedure; homogenization is crucial for them since it lowers resistance to mass transfer. Hexane was chosen as the solvent for an extraction method that might be used for Soxhlet extraction. One 5.0 g batch of freeze-dried sample was added to the extraction apparatus at a time, which was filled with filter paper. Hexane (0.15 L) was introduced to the system after it had been heated to boiling (69°C). Following six hours of reflux maintenance, the solvent was vacuum-evaporated at 25°C, and recovered extract was weighed and stored for further analysis at -18^{o}c (De Aguiar et al., 2013)

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