Brief note on regular techniques in phytochemistry.

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Determination of biological metabolites is serious of analytical process to researchers in the field of biological sciences. In this context, the US Food and Drug Administration (FDA) published a draft of Guidance for Industry to the synthesis and produce bioactive substances and drugs [1]. According to this guidance, before a drug can be legally marketed, its spectroscopic or chromatographic fingerprints and chemical assay of characteristic markers are required. Because of the complex nature of a typical drug and the lack of knowledge of its active constituent(s), the FDA may rely on a combination of tests and controls to ensure the identity, purity, quality, strength, potency, and consistency of natural bioactive compounds and drug-like candidates [1,2]. It is well known, approximately the plants are a bio-synthesized wide range of around 2,00,000 metabolites [3]. The chemical composition and biomedical potential of various coastal and traditional plants were reported [4-6]. However, the quantities of the specialized compounds including alkaloids, flavonoids, and essential oils were present in lesser concentrations in the natural products. A number of chromatographic and spectroscopic techniques were implemented to isolate the compounds from the floral system [7]. According to Thunig et al. [8] imaging the secondary metabolites in the leaves and petals of *Hypericum perforatum* by indirect desorption electrospray ionization imaging MS. Secondary metabolites as nutritional components in plant-based foods, nutraceutical applications, and beverages products [9,10]. Here we briefly summarized, most frequently used chromatographic and spectroscopic techniques such as Ultra-violet spectroscopy (UV-Spec), Gas chromatography and mass spectroscopy (GC-MS), and Fourier transform infrared (FT-IR) spectroscopy and High-performance liquid chromatography (HPLC). One of the prominent tools in the field of analytical chemistry is UV-spec. It is also known as Electronic spectroscopy (ES) that involves the promotion of the electrons from the ground state to the higher energy or excited state. Surface Plasmon (SP) excitation causes strong light scattering at specific wavelengths, which results in the formation of strong SP resonance bands depend on reaction medium dielectric constant. The primary conformation of silver nanoparticles synthesized from the reaction medium is analyzed by the UV-Spec. The reduction rate of silver ions observed in different sources viz., callus tissues, leaf extract of plants, the biomass of microalgae and microorganisms (bacteria and fungi) during biogenesis of silver nanoparticles [11,12]. At present, the combination of the spectroscopic and chromatographic techniques was preferred for quantification and identification of metabolites especially, GC-MS and LC-MS methods. GC-MS is used to analysis wide range of substances includes plant metabolites, drug candidates, environmental pollutants, explosives; planet material samples [13]. Recently, usage of GC-MS was increased in many security programs including airports to recognize any foreign or explosive substance were contained in the luggage and human beings respectively. Earlier, we isolated and identified volatile and alkaloid derivatives such as 1-[a-(1-Adamantyl) benzyl idene] thio, propanamide, benzene methanol, 2-(2-amin propoxy, 2-5-Dimethoxy-4-(methylthionyl), 1-Amino-2-(hydroxymethyl) anthraquinone, DL-Cystine, 2-Propen-1-one, 3-(4-nitrophenyl)-, Phenylephrine, alkanes (Hexadecane, Tetradecane and Pentadecane) and Heptaflurobutyric acid/alcohols from coastal medicinal plants and mangroves using GC-MS [14,15]. Fourier transform infrared spectroscopy (FT-IR) is one of the fast, non-destructive techniques (`<1 mg) use for the qualitative and quantitative isolation of compounds [16]. The individual molecules have the ability to absorb the radiations at definite wavelengths. The molecular chemical bond arrangement and the functional group of compounds present in the dried microalgae, plant and nanoparticles samples were analyzed by FT-IR. For qualitative analysis the spectra were recorded over the wave number range of 4000-400 cm^{-1} with 120 scans per sample were recorded. For example, the respective wavelength (1644, 1537, 1398,1237, and 1028 cm^{-1}) and its functional group such as (stretch in enol form; benzene ring in aromatic compounds and stretching C=N, C=C; CH3 deformation; PO_2 asymmetric (phosphate 1); CH=CH in cyclic alcohols and glycogen absorption at CâO and CâC stretching which refer to the characteristic peaks of proteins, carbohydrates, gallic acid, and vanillin in microalgae biomass [12]. High-performance liquid chromatography (HPLC) is one of the outstanding used for separation, identification, and quantification of an individual component in a mixture. The chemical nature, free or conjugated form of compounds with sugar moieties or acids, stability and uneven distribution of bioactive substances in the source substance might causes challenges during the extraction of bioactive compounds from raw materials. Chester [17] reported the recent developments in HPLC stationary phases that include enhanced adsorbents, bonded phases, pellicular particles, control of pore size and silica hybridization. Previously the HPLC also called as high-pressure liquid chromatography. In photochemistry, the HPLC play a vital role in the analysis of secondary metabolites. We identified and quantified bioactive metabolites such as ajmalicine, vindoline, catharanthine and serpentine from different mangrove plants [18]. In conclusion, the purification strategy always includes a step by step procedure that including extraction, pre-purification, purification, isolation, and identification. Chemical structure, solvent polarity, glucoside matrix, degree of polymerization of

phenols, whether the bioactive compounds made interactions with cellular components or not, temperature, solubility, size, derivatization approaches and knowledge about selection of suitable techniques will provide a unique opportunity for the drug development.

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