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EXTRACTION AND ISOLATION OF BIOACTIVE COMPOUNDS FROM A THERAPEUTIC MEDICINAL PLANT –SENNAALATA (L.) ROXB

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Abstract:

A source of great economic benefit all over the world is medicinal plants. Some bioactive compounds that create a definite physiological action on the human body are of medicinal value to plants. Alkaloids, flavonoids, tannins, and phenol compounds are the most essential bioactive (chemical) compounds for plants. The object of the present investigation was to examine the bioactive compounds of therapeutically effective Sennaalata (L.) Roxbhydroalcoholic leaf extracts.Different studies were performed in Sennaalata, i.e., thin layer chromatography, column chromatography, MASS spectroscopy, H^1 NMR, C^{13} NMR and FTIR spectroscopy. The results of the thin layer chromatographic analysis showed visible spots with different R_f values in different solvent systems and a strong response in hydroalcoholic extracts. Of the hydroalcoholic extract of Sennaalata, a total of eighteen compounds were eluted.

Introduction:

For the wellbeing of individuals and populations, medicinal plants are of great importance. Some bioactive compounds that produce a definite physiological effect on the human body are of medicinal value to these plants (McGraw Hill, 1952). Plants have formed the basis of

advanced conventional therapeutic systems that have existed for thousands of years and continue to give new remedies to humanity (Gurib-Fakim, 2006). The increasing presence of natural medicines in modern medicine demonstrates the importance of plants in human and animal health.Indeed, in the world today, natural products and their derivatives account for more than 50 percent of all medications in clinical use. At least a dozen potent medicines have been produced from flowering plants over the last 40 years.

Current advances in research have made it possible to extract medically essential compounds from plants used in conventional medicinal practices. The bio-active compounds in plants known as phytochemicals are present. These phytochemicals are obtained from various plant components such as leaves, barks, and seeds, coats of seeds, flowers, roots and pulps and are thus used as direct medicinal agents (Ingle et al., 2017). In certain cases, it has been shown that extracts are biologically active in both in vitro and in vivo test systems (Van Wyk and Wink, 2004). In combination with others, certain plant-derived compounds are successful, while others are active as single entities (Balunas and Kinghorn, 2005). The separation of substances, however, remains a daunting task and a mammoth task. The isolation of bioactive compounds is conventionally followed by the determination, via a variety of bioassays, of the presence of certain compounds within plant extracts.

More than 80 percent of the global population depends on traditional medicine for their primary health care needs, according to the World Health Organization (Sasidharanet al., 2011). For their pharmacological function, plants are being extensively investigated as the source of material for major modern drugs. In the Ayurvedic method of medicine, crude extracts of various sections of medicinal plants were used to treat various forms of infectious disease (Singh et al., 2015).By suppressing their growth (a natural phenomenon known as allelopathy), plants that release these active compounds are able to compete and invade other plant species in their vicinity (Kruse et al., 2000; Dayan and Duke, 2009). Numerous phytoalexins have also been documented to possess strong antioxidant, antibacterial, and herbicidal properties in addition to their physiological functions in plants. In a wide variety of applications, including food, pharmaceutical, cosmetic, and agricultural industries, a number of bioactive compounds have been isolated, refined and used(Osbourn and Lanzotti, 2009; Mierziaket al., 2014; Upadhyay, 2011; Takshak, 2018). Therefore, it has become important to investigate active medicinal plants and their natural bioactive molecules in order to take advantage of the potential additional values of natural sources.

Traditional treatment procedures are an important part of complementary or alternative medicine today (Vasudevaet al., 2012). While their effectiveness and mechanisms of action have not been scientifically tested in most cases, due to their active chemical constituents, these simple medicinal formulations frequently mediate beneficial response (Bhatet al., 2012). The extraction of plant metabolites is therefore important to isolate biologically active compounds and to understand their role in preventing and treating diseases and to understand their toxic effects.

In many developing countries, Sennaalata (L.) Roxb, a soft wood shrub known as winged Senna (Parkin et al., 2001), ringworm shrub or craw-craw vine, forest king, or candle cassia (Tagneet al., 2014), is an important medicinal plant. For the treatment of skin diseases and many eruptive skin infections, leaf extracts are added alone or combined with lime juice, local gin or oil (Palanichamyet al., 1991; Palanichamyet al., 1990; Dalziel, 1937; Ganapathyet al., 2015).The method of application in Ghana is to rub the skin affected until it bleeds and then rub the mixture into the sores of Sennaalata plants. Preliminary studies have been published on the biochemical constituents of Sennaalata, but there are no records of

bioactive components derived from hydro-alcoholic leaf extracts. The present study was therefore aimed at extracting, isolating and identifying bioactive compounds through different chromatographic and spectroscopic techniques from the leaves of Sennaalata.

Materials and Methods:

Collection and Extraction of Plant material

The Sennaalata leaves were collected and the specimen was deposited at the research center for Alpha Omega Hi-Tech Bio. Fresh plant materials have been washed with flowing tap water and dried with shade. The leaves and the bark were crushed by grinder to coarsely powdered. Those coarse powders (25g) were then subjected to successive 250ml hydroalcohol extraction using the Soxhlet unit. The extracts obtained were preserved and then taken up for additional investigations. For these extracts, DMSO (Dimethyl sufloxide) serves as a dissolved solvent.

Identification and characterization

Since plant extracts normally occur as a mixture of different types of bioactive compounds or phytochemicals with different polarities, their separation remains a major challenge for the bioactive compound identification and characterization process. In isolating these bioactive compounds, it is common practice to use a variety of different separation methods, such as TLC, column chromatography, to obtain pure compounds. The pure compounds are then used for structure and biological activity determination.Fourier-transform infrared spectroscopy (FTIR) can also be used to achieve and promote the detection of bioactive compounds in addition to this phytochemical screening assay.

Chromatographic techniques:

Thin-layer chromatography (TLC) and Bio-autographic methods

TLC is a simple, easy, and inexpensive procedure that gives a quick response to the researcher as to how many components are in a blend. When a compound's R_f is contrasted with the R_f of a known compound, TLC is often used to help the identification of a compound in a mixture. Additional tests include the spraying of phytochemical screening reagents, which, depending on the phytochemicals present in a plant extract, cause colour changes; or viewing the plate under UV light. This has also been used to check the purity and identification of isolated substances (Shahverdiet al., 2007).

Column chromatography (CC)

The extract residue (10 g) was applied onto a silica gel column (200 g, 100cm x 3.5 cm), to isolate the active compound from the crude extracts using hexane as a solvent and the polarity was increased by hexane then chloroform and fractions (100 mL) each were collected. The obtained fractions were concentrated and monitored by TLC using hexane, acetone and methanol (97:2:1%) as mobile phase.

Fractions (1-20) Fraction A_1 eluted with chloroform: Methanol (9:1), showed two spots in solvent system chloroform and methanol (9:1). Fraction A_1 was re-chromatographer on silica gel column (25 g, 2cm x 100cm), elution was carried out beginning with chloroform and methanol independently. To separate the compounds, it was tried to increase the polarity of the eluent. For this purpose, polarity was increased gradually with successive addition of methanol. At 1% addition of methanol, the compound 1 and 2 was started to coming out to yield 20 fractions (20 mL each). The solvent evaporated and the fractions were TLC monitored. The R_f value was found to be 5.9 and 4.2. They were combined and re-

crystallized from acetone as pale yellow and light yellow (Compound 1- 2). Again, the solvent polarity was increased gradually to elute another spot. At 1% addition of methanol, the compound 3 to compound 9 was collected to yield 50 fractions (20 mL each). They were combined and re-crystallized from acetone to light green, pale yellow, light brown, green and yellow (Compound 3-9). The R_f value was found to be 5.6, 5.2, 6.1, 5.4, 7.1, 7.9 and 5.7.

Fractions (21-35) with addition of 3% methanol (7:3) was found to contain eight spots. Fraction B_1 was re-chromatographed on silica gel column (25 g, 2cm x 50cm), elution was carried out beginning with methanol and increasing polarity with successive addition. The R_f value was found to be 6.3, 6.6, 7.7, 5.3, 6.8, 6.5, 6.9 and 7.2. They were combined and re-crystallized from acetone as light yellow, yellowish color, light brown, yellow and pale yellow (Compound 10-17). Then the polarity of the compound was increased to 4% of methanol the $R_f$value is 5.5 (Compound 18). The color of the compound was found to be light yellow.

Thin layer chromatography (TLC)

The TLC plates supplied by Merck, Germany (TLC Silica gel 60 F254) was used to observe the separation of individual compounds as a single spot were trimmed and the position of the origin marked by a straight line.

$$R_f = \text{Distance travelled by substance} / \text{Distance travelled by solvent front}$$

Structure Determination

Data from a broad range of spectroscopic techniques such as Infrared (IR), Nuclear Magnetic Resonance (NMR) and Mass spectroscopy are used to determine the structure of natural products. Spectroscopy's basic concept is to transfer electromagnetic radiation through an organic compound that absorbs some, but not all, of the radiation. A spectrum can be generated by measuring the amount of absorption of electromagnetic radiation. The spectrum in a compound is unique to such bonds. The composition of the natural compound can be defined based on these spectrums. For structural clarification, scientists mainly use spectra generated from either three or four regions: infrared (IR), radio frequency (FTIR), and electron beam (Popovaet al., 2008).

Fourier Transform Infrared Spectroscopy (FTIR)

A valuable method for the detection of functional groups present in the plant extract is Fourier-transform infrared spectroscopy. It helps define the molecule and determine its structure (Eberhardtet al., 2007). To classify the chemical constituents and elucidate the structural compounds, it is a high-resolution analytical instrument. To fingerprint herbal extracts or powders, FTIR provides a quick and non-destructive investigation.

Nuclear Magnetic Resonance Spectroscopy (NMR)

The physical, chemical and biological properties of matter are given by Nuclear Magnetic Resonance Spectroscopy. One dimensional technique is routinely used, but two-dimensional NMR techniques could be used to achieve the complex structure of the molecules. For the determination of the molecular structure of solids, solid state NMR spectroscopy is used. Radiolabeled 13C NMR is used in the compound to distinguish the types of carbon present. 1 H-NMR is used to determine the hydrogen types present in the compound and to determine how the hydrogen atoms are related(Ingle et al., 2017).

Mass Spectroscopy

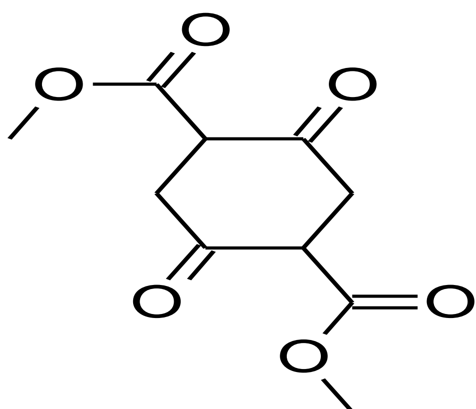
Mass spectrometry is an effective analytical technique used to identify unknown compounds, measure known compounds, and elucidate molecular structure and chemical properties. The molecular weight of the sample can be calculated through the MS spectrum. This approach is mainly used for the structural elucidation of organic compounds, for the sequencing of peptides or oligonucleotides and for monitoring the presence of compounds previously characterized by identifying both the molecular weight and the diagnostic fragment of the molecule simultaneously in complex mixtures with a high specificity (Ingle et al., 2017).

Results and Discussion:

Chromatographic fractionation of the crude sample of Sennaalatacarried out in hydroalcoholic extract. Totally eighteen compounds were isolated from the hydroalcoholic extract, from that compound 12 and compound 15 shows better activity. These two compounds shows better antioxidant activity when compared to the other compounds (22.34 µg/ml and 22.06 µg/ml). The compound which was eluted from the hydroalcoholic extract emerged as the most effective solvent with the lowest IC_{50} values in scavenging ability. Hence, this extract was isolated and purified the bioactive compounds responsible for antioxidant activity.

The spectral analysis was carried out for compound 12 (Dimethyl Succinyl Succinate) and compound 15 (Heptadecanoic acid). In Dimethyl Succinyl Succinate the Molecular Formula is $C_{10}H_{12}O_6$, Molecular Weight is 228.2, Molar Refractivity: 49.83 ± 0.3 cm^3, Molar Volume: 174.6 ± 3.0 cm3, Parachor: 458.2 ± 6.0 cm3, Index of Refraction: 1.482 ± 0.02, Surface Tension: 47.3 ± 3.0 dyne/cm, Density: 1.306 ± 0.06 g/cm^3 and Polarizability: 19.75 ± 0.5 10-24cm^3. Whereas Heptadecanoic acid has its Molecular Formula $C_{17}H_{34}O_2$ and Molecular Weight is 270.45, the Physical properties are Molar Refractivity: 82.37 ± 0.3 cm^3, Molar Volume: 303.7 ± 3.0 cm^3, Parachor: 730.2 ± 4.0 cm^3, Index of Refraction: 1.454 ± 0.02, Surface Tension: 33.4 ± 3.0 dyne/cm, Density: 0.890 ± 0.06 g/cm^3 and Polarizability: 32.65 ± 0.5 10-24cm^3.

Dimethyl Succinyl Succinate **Heptadecanoic acid**

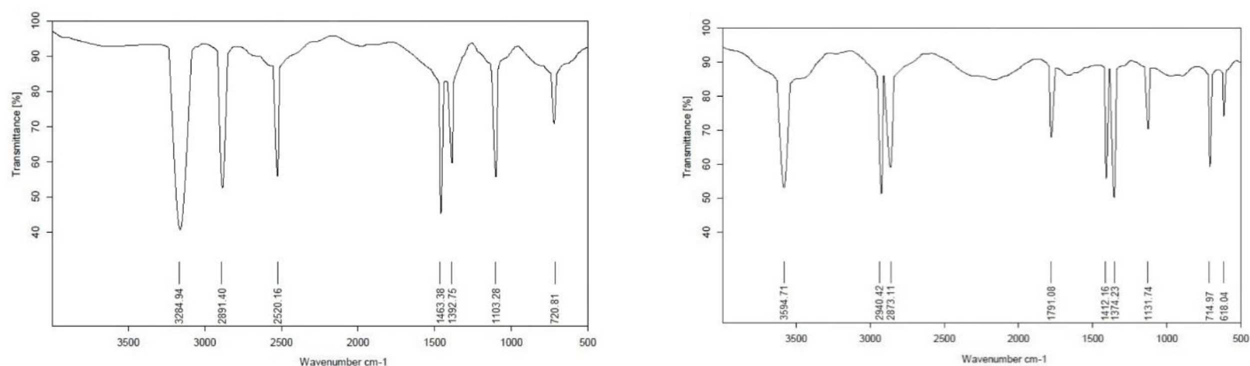


FTIR Analysis

The compound Dimethyl Succinyl Succinateexhibited a characteristic band at 3284.94cm^{-1} indicating the presence of Intermolecular carbonyl group CO (strong), 2891.40, 2520.16 cm^{-1} indicating the presence of Esters, 1463.38 cm^{-1}Methoxy (Strong), 1392.75 cm^{-1}Alkanes, CH_2

(Very strong), 1103.28 cm^{-1}CH stretching and 720.81cm^{-1}Ortho Disubstituted(Medium to strong). In compound 15 (Heptadecanoic acid) the presence of 3594.71cm^{-1}Intermolecular hydrogen bonded OH (Strong), 2940.42cm^{-1}Acids (Medium), 2873.11. 1791.08, 1412.16, 1374.23, 1131.74, 714.97, 618.04cm^{-1}Saturated hydrocarbons.

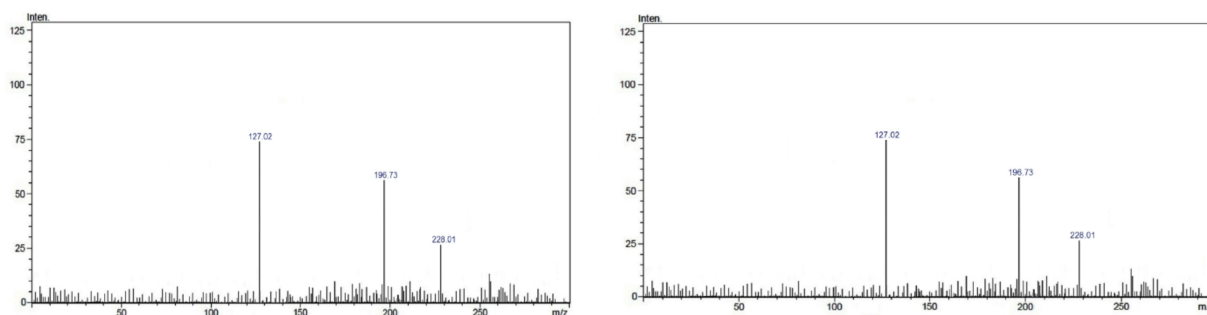
Figure: FTIR analysis of Dimethyl Succinyl Succinate and Heptadecanoic acid



Mass Analysis

EI-Ms m/z: The molecular ion peak is (M+) 228.01, the other fragments are 127.02 and 196.73 in Dimethyl Succinyl Succinate where as in Heptadecanoic acidEI-Ms m/z: The molecular ion peak is (M+) 270.45. The other fragments are 127.02, 196.73 & 228.01.

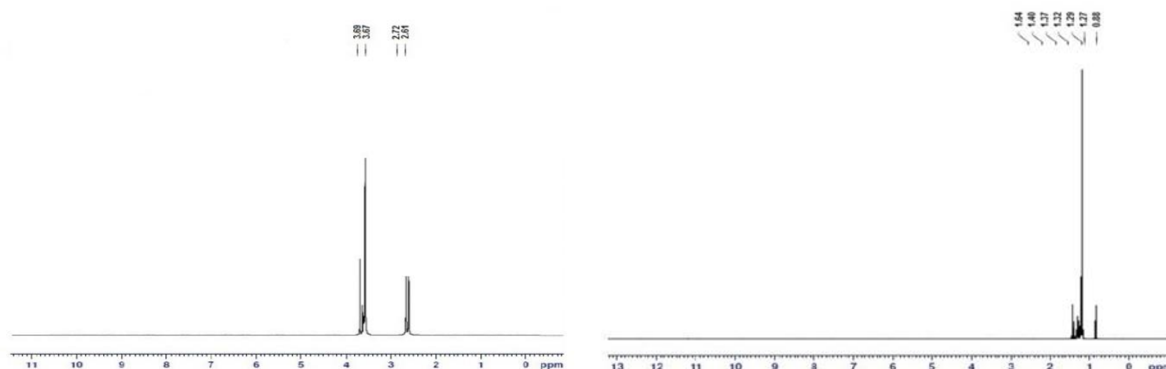
Figure: Massanalysis of Dimethyl Succinyl Succinate and Heptadecanoic acid



H^1 NMR Analysis

The compound Dimethyl Succinyl Succinateexhibited2.61 as Alkanes CH_2 proton, 2.72 as CH proton3.67 and 3.69 as Methoxy (OCH_3) proton. In Heptadecanoic acid0.88 as Primary (RCH_3), 1.27, 1.29, 1.32, 1.37, 1.40 as Alkanes (CH_2) Multiplet and 2.64 as Acid proton.

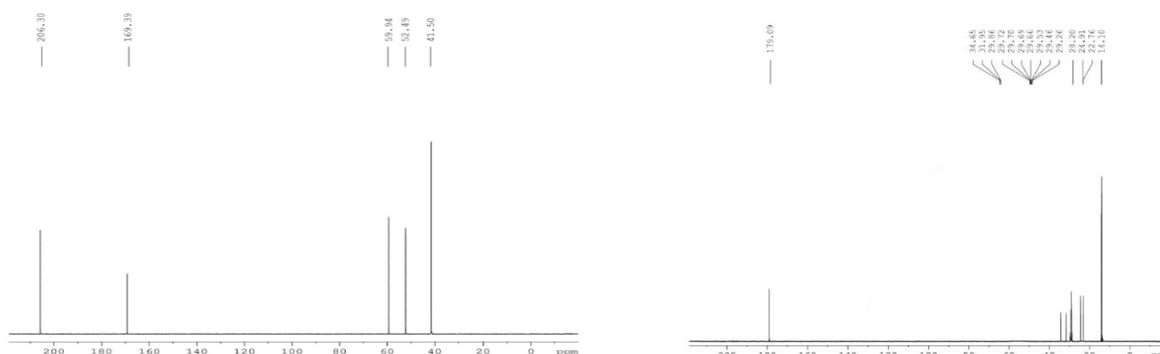
Figure: H^1 NMR Analysisof Dimethyl Succinyl Succinate and Heptadecanoic acid



C^{13} NMR Analysis

The compound Dimethyl Succinyl Succinateexhibited41.50 as CH_3C0-, 52.49, 59.94 as RCH_2, 169.39as C= O (in acids & esters) and 206.30 as C= O (in Ketones). In Heptadecanoic acid14.10 as RCH_3, 22.76, 24.91, 28.20, 29.26, 29.46, 29.53, 29.66,29.69, 29.70, 29.72, 29.86, 31.95, 34.65 as $RCH_2$and 179.09 as COOH (in acids).

Figure: C^{13} NMR Analysis of Dimethyl Succinyl Succinate and Heptadecanoic acid



Conclusion:

As natural products from plant extracts generally contain different mixtures of components with different polarities, their separation poses a major challenge for the identification and characterization process. In the isolation and characterization of various natural products, extraction plays an important part. In order to extract natural products, practically most of them must be purified by the combination of many chromatographic as well as non-chromatographic techniques and numerous other purification methods.

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