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Phytochemical Investigation of Roots of Phyllantus Virgatus

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ABSTRACT:

Phyllantusvirgatus originates from the euphorbiaceae family and is an indigenous to India, South china Asia and Polynesia where it has been cultivated as a source of medicinal herb. Traditionally it is used to treat skin diseases and inflammation, jaundice stomach ache and antifeedant activity etc. According to previous studies, this genus possesses many biological activities likeanti-bacterial, hepatoprotective, hepatitis,anti-viral, anti-inflammatory, antioxidant properties and also presence of many active constituents.Phyllantusvirgatus led to the isolation of many structurally active constituents like terpenoids and alkaloids with pharmacological properties. In our present study, phytochemical studies on stem of methanol extract of phyllantusvirgatus which led to isolation of active constituents. The resulted active constituents were determined by using different analytical instruments

Key Words: ***phyllantusvirgatus, 1D&2D NMR, IR, HRMS, column chromatography***

1.1INTRODUCTION TO NATURAL PRODUCTS

Natural product is a chemical compound or substance produced by a living organism-that is found in nature. In the broadest sense, natural products include any substance produced by life. Natural products have been used as medicinal products for centuries globally although the sources and applications vary among different regions. As such ,natural products are the active components of many traditional medicines. Natural products sometimes have pharmacological or biological activity for use in pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. That can be of therapeutic benefit in treating diseases. Furthermore, synthetics analogues of natural products with improved potency and safety can be prepared and therefore natural products are often used as starting points for drug discovery. Plants have always been a rich source of pharmacologically active nature products (e.g. Alkaloids, morphine, cocaine, quinine, digitalis, nicotine, tubocurarine). Many of these compounds are useful drugs in themselves (e.g. Alkaloids, morphine, quinine), and others have been the basis for synthetic drugs. In the early 1900s, before the "Synthetic Era'', 80% of all medicines were obtained from roots, barks and leaves. In more recent times, natural products have continued to be significant sources of drugs and leads. Their dominant role is evident in the approximately 60% of anticancer compounds and 75% of drugs for infectious diseases that are either natural products or natural product derivatives.

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Objective of the study:

To perform phytochemical investigation of root of phyllanthusvirgatus.

Plan of the study:

- To prepare extract from Phyllanthus virgatus stem.
- To develop TLC method for the extract prepared.
- To isolate the phytochemical constituents from the extract of Phyllanthus virgatus.
- To analyze and characterize the isolated phytochemical constituents by various Spectroscopic techniques like^{1} H NMR, ^{13}C NMR, FTR and ESI-MS.
- To screen the estimation of isolated bioactive compounds from Phyllanthus virgatus.

PLANT REVIEW:

General description:

Phyllanthus is the largest genus in the flowering plant of family phyllanthaceae. Estimates number of species in this genus vary widely, from 750 to 1200. Several phyllanthus species are used as traditional medicines. Phyllanthus has a remarakable diversity of growth forms including annual and perennial herbs, shrubs,climbers, floating aquatics, and pachycaulous succulents. Some have flattened leaflike stems called cladodes.

It has a wide variety of floral morphologies and chromosome numbers and has one of the widest range of pollen types of any seed plant genus.Phyllanthus species commonly found around tropical regions of Africa, Asia, America, Australia and Europe.

Plants have serve mankind since centuries as they contain secondary metabolites that exert specific therapeutic effects.Phyllanthusvirgatus – Seed Under Leaf. Seed Under Leaf is a slender, branched, hairless herb, woody in the lower part.This plant extracts have been used since ancient times, for treating several diseases.

MATERIALS AND METHODS

Materials, chemicals and instruments:

Columns of different types, beakers, test tubes, conical flasks, rota evaporator, weighing balance, filter paper, Merck Millipore PLC Silica gel 60 F_{254} plate, 5% $H2SO_4$ in methanol charing solution, TLC chamber, UV chamber, Waters HPLC instrument, twin trough plate development chamber. All the chemicals and reagents used were obtained from Ranboxy Fine Chemicals Ltd, Punjab.

Sample preparation:

Plant material collection:

The whole plants of were collected from the botanical garden located in. these plants were taxonomically identified by Dr A Prasad Rao, senior taxonomist.

EXTRACTION:

After collection, all plant material was dried at room temperature in open air in the laboratory away from direct sunlight, and grind to a powder using a commercial blender. The powdered plant material was stored in tightly closed glass bottles in the dark at room temperature.the drying of plant material makes handling , working and storing plant material much easier. It also improves extraction efficiency as some members of some organells containing phytochemicals are destroyed during drying.

However, liable or volatile compounds can be and some undesirable artefacts may be formed so caution is taken to dry plant material atambient temperatures away from direct sunlight. To avoide the formation of mould plant material is preferably dried in open air and turned over periodically. We have adopted two types of extraction techniques for our study.

Cold extraction:

Cold extraction is the process where the desired solvent like methanol is poured directly into plant material and the process of soaking is kept for 48hours. The solution can then be separated by filtrarion and the extract is concentrated on rotary evaporator etc.

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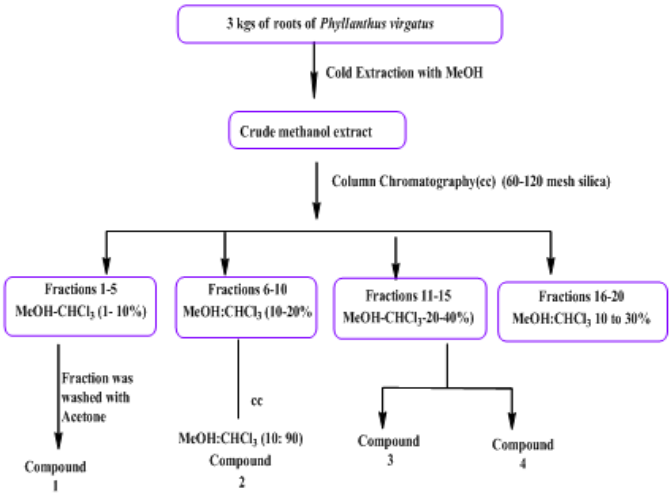
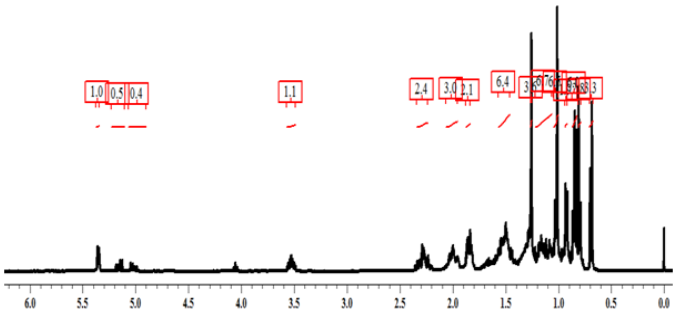
Isolation by column chromatography

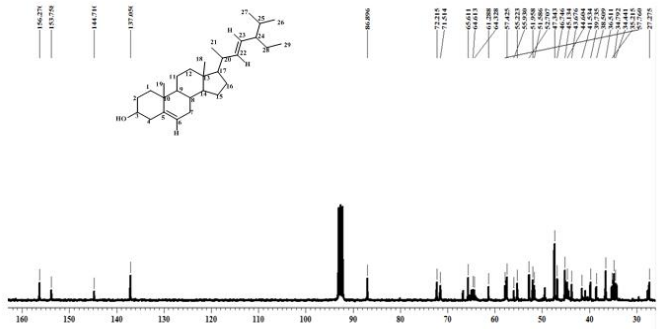


Results andDiscussion

COMPOUND1



H NMR of stigmasterol

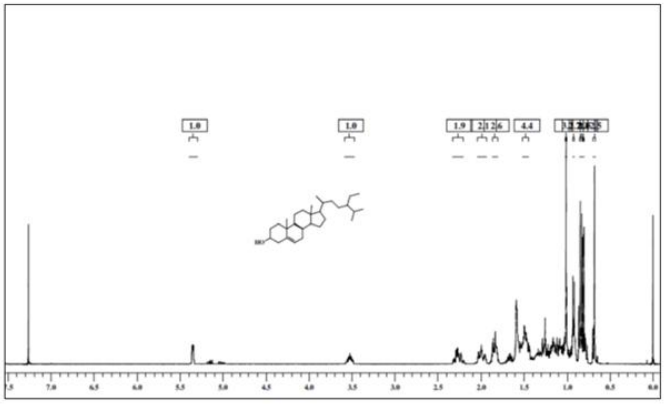


^{13}C NMR of stigmasterol

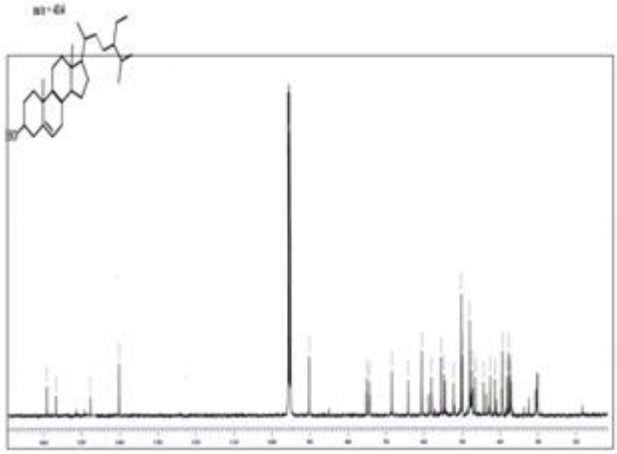
1H NMR (400 MHz, CDCl3) of Stigmasterol: δ 5.35 (1H, m, H-6), 5.15 (1H, dd, *J* = 15.16, H-22), 5.02 (1H, dd, *J* = 15.2, H-23), 3.52 (1H, m, H-3), 2.31 (1H, m, H-4b), 2.23 (1H, m, H-4a), 2.03(1H, m, H-20), 2.00 (1H, m, H-12b), 1.96 (1H, m, H-7b), 1.86 (1H, m, H-1b), 1.83 (1H, m, H-2b),1.68 (1H, m, H-16b), 1.50 (2H, m, H-15b, H-28b), 1.54 (1H, m, H-25), 1.53 (1H, m, H-24), 1.52 (1H,m, H-2a), 1.50 (3H, m, H-7a, H-11a, H-11b), 1.47 (1H, m, H-8), 1.29 (1H, m, H-16a), 1.22 (1H, m, H-28a), 1.18 (1H, m, H-12a), 1.15 (1H, m, H-17), 1.03 (1H, m, H-1a), 1.01 (2H, m, H-14, H-15a, 3H, s,H-21), 1.01 (3H, s, H-19), 0.93 (1H, m, H-9), 0.92 (3H, d, *J* = 6.4 Hz, H-26), 0.83 (3H, s, H-29),0.81 (3H, s, H-27), 0.68(3H, s, H-18).

13C NMR (100 MHz, CDCl3): δ 140.69 (C-5), 138.1 (C-22),129.14 (C-23), 121.4 (C-6), 71.3 (C-3), 56.2 (C-14), 55.9(C-17), 51.1 (C-24), 50.0 (C-9), 42.18 (C-4),41.8 (C-13), 40.3 (C-20), 39.6 (C-12), 37.1 (C-1), 36.3 (C-10), 33.8 (C-8, C-25), 31.7 (C-2, C-7), 29.5 (C-16), 28.15 (C-28), 24.9 (C-15), 22.9 (C-11) 20.9 (C-21, C-26), 19.6 (C-19), 19.2 (C-27), 18.8 (C-29), 11.8 (C-18). IR (KBr) ν_{max}:3428,2936,2860,1641,1461,1375,1256,1057,962,801,592

COMPOUND2



^{1}H NMR Spectrum ($CDCl_3$, 400 MHz) of β-Sitosterol



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^{13}C NMR Spectrum ($CDCl_3$, 100 MHz) of β-Sitosterol

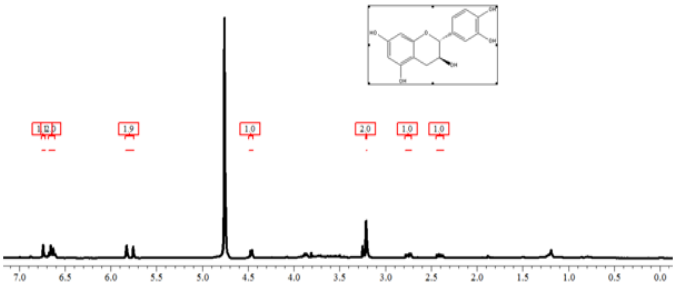
β-Sitosterol:

White amorphous powder (80 mg), The ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.36 (1H, d, *J* = 6.4Hz, H-6), 3.53 (1H, m, H-3), 1.01, 0.68 (3H, s, H-19 and H-18), 0.83 (3H, d, *J* = 6.4 Hz, H-21), 0.81 (3H, d, *J* = 6.4 Hz, H-29), 0.85 (3H, t, *J* = 7.1 Hz, H-26).

^{13}C NMR:The^{13}C NMR ($CDCl_3$,100MHz) 37.5 (C-1), 31.9 (C-2), 72.0 (C-3), 42.5 (C-4),140.9 (C-5),121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.3 (C-9), 36.7 (C-10), 21.3 (C-11), 39.9 (C-12), 42.6 (C-13), 56.9 (C-14), 26.3 (C-15), 28.5 (C-16), 56.3 (C-17), 36.3 (C-18), 19.2C-19), 34.2 (C-20), 26.3 (C-21), 36.2 (C-22), 46.1 (C-23), 23.3 (C-24), 12.2 (C-25), 29.4 (C-26), 20.1 (C-27), 19.6 (C-28), 12.0 (C-29).

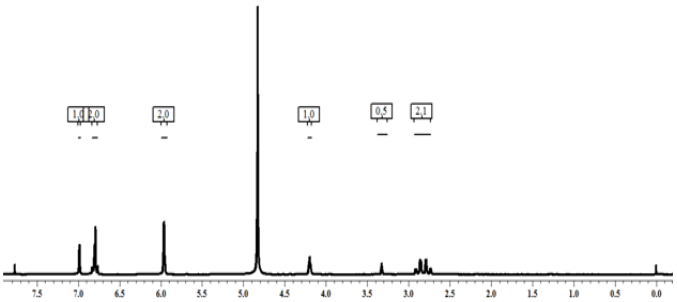
COMPOUND3

^{1}H NMR Of catechin



^{1}H NMR of Catechin: δ 6.7(H, s), 6.6(2H, dd, J=11.004), 5.7(2H, d, J=28.9), 4.4(H, d, J=7.45), 3.2(2H, d, J=1.96) 2.72(H, dd, J=5.3), 2,4(H, dd, J=8.1)

^{1}H NMR of Epicatechin



^{1}H NMR 0f Epicatechin: δ 6.98(1H, d, J=5.25), 6.29(2H, m), 5.9(2H, d, J=1.29), 4.2(2H, m), 3.32(H, m) 2.82(2H, dd, J=4.2).

CONCLUSSION:

The present study describes the isolation of chemical constituents from the roots of *Phyllanthus virgatus* and confirmed by the spectral analysis.

The roots were collected , authenticated, shade dried and powdered using mechanical grinder. The powdered material was extracted by cold extraction process by using high polar solvent methanol. The percentage yield of methanolic extract was found to be 15% w/w in respect to the dried plant material.

The extract was subjected to preliminary phytochemical screening using standard preliminary phytochemical methods and TLC methods in which the extract was found to be rich in many phytoconstituents like alkaloids, glycosides, carbohydrates, volatile oils, tannins, triterpenoids a major part of flavonoids. Based on column chromatography, many fractions were collected and further purified by repeated column chromatography. This was done by simultaneous monitoring of the fractions and mixture of compounds that were eluted using various mobile phase systems.

Fractions 1-5 yielded compound 1, fractions 6-10 yielded compound 2, fractions 11-15 yielded two compounds namely compound 3 and compound 4 respectively. All the compounds were isolated in the MeOH – $CHCl_3$ system and their percentage yield was found to be 1-10%, 10-20% and 20-40% for compound1, compound 2, compound 3 respectively.

Structural elucidation was established by using various modern spectroscopic techniques such as nuclear magnetic resonance (NMR) including (^{1}H, ^{13} C) and MS (GC-MS), infrared (IR), FTIR. The work on *Phyllanthus virgatus* roots yielded a toatal of 4 compounds namely compound 1 stigmasterol which commonly occurs in plantscompound 2β – Sitosterol, compound 3 Catechin, compound 4 Epicatechin. The structures of compounds were also elucidated by comparing their physical properties and spectral data reported in literature. Further studies on *Phyllanthus*

virgatus are necessary for isolation and synthesis of the most potent compounds useful for treating many prevalent diseases.

Despite a period in which pharmaceutical companies cut back on their use of natural products in drug discovery, there are many promising drug candidates in the current development pipeline that are of natural origin. Technical drawbacks associated with natural product research have been lessened, and there are better opportunities to explore the biological activity of previously inaccessible sources of natural products. With increasing acceptance that the chemical diversity of natural products is well suited to provide the core scaffords for future drugs, there should be further developments in the use of novel natural products and chemical libraries based on natural products in drug discovery campaigns.

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