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4. Experimental

4.1. General Extraction Protocol used

The shade dried plant materials were ground in an electric mill and extracted at ambient temperature in a sealed container using suitable solvent pool. The extracts were filtered through Whatman No. 1 filter paper atop Buchner funnel applying suction. Residue was subjected to re-extraction, each time followed by filtration. The pooled filtered extracts were subjected to rotary vacuum evaporation at 40°C. The resulting extracts were freeze dried to remove traces of solvent and preserved at -20°C for further exploration.

4.2. Isolation of Compounds from the crude Extract

Pure compounds were isolated from crude extracts after antimicrobial and antioxidant screening. The selected active extracts were subjected to solvent fractionation followed by chromatographic separation (TLC, Column, Flash) to bring out the pure compound.

4.3. Phytochemical Analysis

Phytochemical analysis of the crude extracts were executed to ascertain the presence of phenolic compounds, flavonoids and saponins. Further Total Phenolic Contents (TPC) were estimated.

4.4. Callus Initiation

Callus development was done in modified Murashige & Skoog (1962) medium. Medium was supplemented with NAA (2.0mg/L), BAP (1.5mg/L) and sucrose (30g/L). Two month old cultures were harvested and taken for antioxidant study after drying, milling and extraction with methanol.

4.5. Antimicrobial analysis

Nutrient agar (NA) and nutrient broth (NB) were used for culturing the bacteria. For dermatophytes and yeasts, sabouraud dextrose agar (SDA) and sabouraud dextrose broth (SDB) were used. The microorganisms used in the present study consisted of 9 strains of dermatophytes, 7 strains of yeasts, 10 strains of gram positive bacteria and 5 strain of gram negative bacteria.
The activity of the extracts was analyzed by standard Agar well diffusion and Agar disc diffusion methods. Minimum inhibitory concentration (MIC) was determined by Broth microdilution method.

4.6. Antioxidant studies

Antioxidant studies of the extracts were done \( \text{(in vitro/in vivo)} \) by literature methods (DPPH, FRA, TBARS assays) and by Photochem Antioxidant analyser.

4.7. Biochemical Analysis

The extracts were subjected to Biochemical analysis viz, LDL, HDL, Urea, Cholesterol, SGOT, Creatine, SGPT

4.8. Hæmatological Studies

Hæmatological studies were performed on the following parameters - WBC, Lym, Mon, Neu, Eo, Ba, RBC, MCV, Hct, MCH, MCHC, RDW, Hb, THR, MPV, Pct, PW

4.9. Histopathological studies

Tissues of liver, heart and kidney of the treated animals were fixed on glass slide using standard protocol and stained with Hematoxylin & Eosin, covered with glass slip and sealed the edge of the cover slip with DPX. Observations were made under flourescence microscope (Cos Lab)

4.10. Instruments used

**UV-visible spectroscopy:**

UV visible absorption spectra of the compounds in CHCl\(_3\) at different concentrations were recorded on a Specord spectrophotometer from Analytic Jena AG, Jena, Germany (\( \lambda_{\text{max}} \) in nm).

**Automated flash column chromatography:**

Isolation of active components from the crude extract were executed through CombiFlash Automated flash column chromatography (!elodyne isco, USA) and the data were handled on Pic Track suite.

**Antioxidant analyser:**

The antioxidant properties of the extracts were evaluated using Photochem (Analytic Jena AG, Jena, Germany)
Automated Biochemical analyser:

The biochemical analyses were performed on Automated Biochemical analyser (Tulip Group of companies, Kolkata)

Haematological analyser:

Haematological analyser (Melet Schloessing Laboratories, France)

Infrared Spectrometer:

IR spectra were recorded on a Perkin-Elmer L 120-000A spectrometer ($\nu_{\text{max}}$ in cm$^{-1}$) on KBr disks.

Nuclear Magnetic Resonance:

The nuclear magnetic resonance spectra were recorded on JEOL multinuclear spectrometer in CDCl$_3$ (chemical shift in $\delta$) solution with TMS as internal standard.

Apart from these some routine instruments like Rotary evaporator (Heidolf Laborota 2003, Germany), Sonicator (Equitron), Freeze dryer (Benchtop Free Zone plus Cascade 4.5L Freeze Dry System, Labconco, USA) Micropipette (Eppendorf) were used during the course of the work.

4.11. Chemicals used

All chemicals were purchased from Merck, India & Germany, except DPPH (Sigma) and Media for microorganism (HiMedia). Silica gel (60-120, 100-200, 230-400 mesh) from Merck was used for chromatographic separation. Silica gel G Merck (India) was used for TLC. Hexane, ethyl acetate, n-butanol, petroleum ether ($60^\circ\text{C}-80^\circ\text{C}$) were used as eluents.

4.12: Other accessories used

Glasswares were purchased from JSGW and Borosil. Filter papers were purchased from Whatman. Plasticwares from Tarson, India.