CHAPTER III

MATERIAL & METHODS

Plants were collected from district Meerut of Uttar Pradesh as well as from Garhwal hills of Uttrakhand, India. Identification of plants was done through herbarium available in the dept of Botany, Meerut College, Meerut & Forest Research Institute, Dehradun.

5 gm of fresh plant part was washed 2-3 times with distilled water & then surface sterilized with 90% alcohol. Subsequently the plant material was grounded in 50 ml of distilled water & alcohol separately for aqueous and alcoholic extracts, respectively. The alcoholic extracts were kept for 24 hrs. at room temperature to evaporate the alcohol. In the remaining residue, 50 ml of distilled...
water added. The macerates were squeezed through double layered Muslin cloth & filtered through filter paper. After filtration, the aliquot was centrifuged at 5000 rpm for 30 minute. The supernatants were filtered through whatmann no. 1 filter paper & then sterilized by passing through 0.2 micron disposable filters. The extract made 10% & thus obtained were used in studies.

The plant material were collected from wild areas of Meerut district and also of Garhwal Himalaya. The method was followed based on work of Natarazan et al. (2001) with some modifications.

Five gms of each aerial parts of -

_Dhatura metel_ Solanaceae
_Calatropis procera_ Asclepiadaceae
_Ocimum basilicum_ Lamiaceae (=Labiatae)
_Cheilanthes albomarginata_ Polypodiaceae (Fern)

were initially treated with 0.1% HgCl$_2$ solution for sterilization and subsequently washed thoroughly with sterile distilled water & grounded in motor & pistil with 50% methanol. The homogenized liquil was filtered & configuged at 5000 rpm. The supernatant was used as test extract & make up into 20 ml using 50% methanol. Further, the extract was diluted into different concentrations, i.e. 10%, 25%, 50%, 75%, 20 ml of DA (Sabourands Dextro Agar) culture medium with 5 ml of the above concentration of the
extract was poured in sterile petriplates and allowed to solidify. Then the test fungus was inoculated at the centre of the medium and incubated at room temperature $25^0\text{C} \pm 2^0\text{C}$. Replicates and controls were maintained throughout the study. The diameter of the fungal growth was measured on $5^{\text{th}}$ and $7^{\text{th}}$ day.

To study the effects of antifungal alcoholic extract and aqueous extract of selected plants two sets of culture media were prepared separately for control and treatment. In the test sets of neutral pH 7, requisite amount of the experimental material were mixed and then added into the sterilized Sabouraud dextrose agar (SDA) medium of respective pH level. In the control set of each experimental set, the same volume of distilled water (in place of experimental material) was mixed in appropriate amounts whenever found necessary.

Mycelia discs of 5 mm diameter, were cut from the periphery of 7 day old culture of the test organisms were aseptically inoculated upside down on the surface of the SDA medium in plaster. Inoculated petri plates were incubated at $25^0\text{C} \pm 2^0\text{C}$ and observations were recorded at $5^{\text{th}}$ and $7^{\text{th}}$ day. Fungal growth taken as measurement parameter. The absence of Fungi denoted antifungal property of fungicidal nature.

Percentage of mycelial growth inhibition on different pH levels were calculated using following equation:
I = (C-t) x 100

Test fungi isolated and were used for in vitro studies. The culture was purified by hyphal tip technique. The stock culture of the test fungus was maintained on SDA medium at 25 degree C ± 1° Degree C.

Effect of different concentration of aqueous and alcoholic extract against the fungus was studied employing techniques.

2. Poisoned food technique. (Nene and Thapliyal, 1979) Poisoned food technique used to assess the antifungal activity of selected plant extracts. A series of double strength of test plant extracts viz. 10%, 25%, 50% and 75% were prepared using sterile distilled water. 30 ml of test extract was poured into 100 ml conical flask containing 30 ml sterilized melted SDA of double concentration. 30 ml of this mixed medium was then poured in each petriplate aseptically. The patriplates were inoculated with previously maintained 7 days old culture. 5 ml mycalial disc was cut with sterilized cork borer and transferred aseptically in the centre in inverted position. All petriplate including control and experimental were incubated at 25 degree C ± 1 degree C for 7 days. After 7 days of incubation, observation were recorded and percent inhibition of radial growth was calculate using following formula:
\[ I = \frac{C - T}{C} \times 100 \]

where, \( I \) = percent inhibition

\( C \) = radial growth in check in mm/cm

\( T \) = radial growth in treated set in mm/cm

**METHODS OF STUDY IN FLOWERING PLANTS**

The present work is the result of extensive study conducted during 2004 and 2006. During this period plant collection trips were made each month, to collect plants in vegetative, flowering and fruiting stages. If the same plants were found in different localities specimens were also collected but they were numbered only when some variation was found, otherwise the specimens were left unnumbered. Side by side climatic condition and soil characteristics also recorded. Plants also collected from different forest ranges of Himalaya.

During the field work, observations on habit, habitat, ecological features, size of the plant, colour of flower, fruit and seed characters, abundance of a particular species in the area and its association were recorded in the field book and for each species 5-7 specimens were collected. Localities of plants that were in vegetative conditions, were carefully marked to relocate them in subsequent trips for collecting, flowering and fruiting specimen. During collection all instructions given in the booklet of
H. Santapau (1955) instructions for field collectors of the Botanical Survey of India" was taken into consideration.

Small medicinal herbs were carefully uprooted and collected entirely, for larger species representative portions were preserved. Immediately upon collection the specimens were numbered with appropriate field labels and were placed in light plywood field plant press or packed. In polythene bags to avoid excessive desiccation and wilting. On return to the laboratory/residence, all the specimens were carefully checked and field notes restudied. The 4 or 5 specimens of each species were pressed. The Specimens were pressed. The specimens were pressed in herbarium press between blotting papers or news papers, which were changed every day until the plants dried up. During high humid periods, the plants were exposed to sun light on alternate days to prevent the specimen from fungal attack. Large seeds, fleshy fruits and flowers were packed in the polythene bags and numbered. Some aquatic plants and flowers were also preserved in FAA solution.

After drying each specimen was glued on to a relatively stiff herbarium sheet. Bigger specimens were folded and then glued. A label showing the name of the family, name of the plant, local name(s) if any, place and date of collection and number etc. was affixed at the bottom right hand corner of the sheet.
All the specimens were identified with the help of available literature and floras and then compared with authentic specimens. All such studies are also based on work done by Nayar (1977) and Murti and Singh (1994).