CHAPTER - 4

RESULTS AND DISCUSSION
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STANDARDIZATION

Small caltrops, *Tribulus terrestris* Linn. (Fig. 1), belonging to the family Zygophyllaceae is commonly known as 'Chotagokhru', has been described to be of great medicinal value. It is a reputed drug in Ayurvedic system.

The phytochemical study of the fruits of *Tribulus terrestris* was carried out to lay certain standards for the air dried drug. The high value of total ash 12.79% indicated the presence of considerable amount of inorganic constituents in the fruits. The ethanol-soluble extractive and water-soluble extractive values, 1.862% and 16.8% respectively were also rather high, indicated the presence of sugars and resins etc. The qualitative chemical examination of the petroleum ether extract, chloroform extract, alcohol extract and water extract, obtained by successive solvent extraction of the fruits, indicated the presence of alkaloids, fixed oils and fats, resins, traces of glycosides, proteins and aminoacids, tannins, reducing sugars and sterols and absence of saponins, gums and mucilages. The results of the tests applied are tabulated in Table 1 to 4. Thin-layer chromatography was carried out using Toluene: ethyl acetate (8: 2) as solvent system and visualized the spots in day light by spraying the plate with anisaldehyde - sulphuric acid reagent followed by heating at 120° for 10 minutes. One of the yellowish green spot having hRf - value 29 revealed the presence of diosgenin by Co-chromatography using authentic sample while other yellowish green spots having hRf- values 13 and 84, prominent violet spots having hRf- values 91, 53, 43, 34 and 21 and a dark blue spot having hRf- value 14 were also observed in the extract solution as shown in Table 5. The successive solvent extracts of the fruits of *T. terrestris* with petroleum ether, benzene, chloroform, ethanol and water were
scanned at 366 nm by HPTLC using solvent system Toluene: ethyl acetate (8:2). indicated the presence of 5,6,4,4 and 2 components respectively as shown in table 6.

The macroscopic characters (colour, odour, taste, size, shape and surface) of the fruits were observed by naked eyes (Fig. 10). Transverse section of the fruit and its powder characteristics were observed under microscope (Fig.s. 11,12 and 13).

Chicory, cichorium intybus Linn. (Fig. 2), belongs to the compositae family is locally known as ‘Kasni’, Hakims use seeds, roots and leaves of the plant for the treatment of various ailments.

The proximate analysis of the seeds of the C. intybus was carried out to lay certain standards for the air dried drug. The high value of total ash 13.03% indicated the presence of considerable amount of inorganic constituents in the seeds. The petroleum ether-soluble extractive value 4.18% was also rather than high, indicated the presence of fixed oils and fats and sterols etc. The qualitative chemical examination of the petroleum ether extract, alcohol extract and water extract, obtained by successive solvent extraction of the seeds revealed the presence of carbohydrates, phytosterols, proteins and amino acids, tannins, fixed oils and fats and absence of alkaloids, glycosides, saponins, resins, gums and mucilages. The results of the tests applied are tabulated in Table 7 to 10. Thin-layer chromatography of the alcoholic extract of seeds was carried out using chloroform: methanol: formamide (80:19:1) as solvent system and visualized the spots by spraying the plate with sulphuric acid followed by drying at 75\(^0\) for 3 minutes and their Rf-values are recorded in Table 11. Three substances having Rf-values 0.90, 0.86 and 0.83 gave positive Libermann-Burchard test, revealed the presence of three different sterols and other three spots having Rf-values 0.36, 0.05 and 0.00 gave positive Molisch’s test revealed the presence of three different sugars. Further studies require the
identification of different five phytoconstituents. The successive solvent extracts of
the seeds of *C. intybus* with petroleum ether, benzene, chloroform, ethanol and water
were scanned by HPTLC using solvent system chloroform : methanol : formamide
(80:19:1) indicated the presence of 3,3,3,11 and 10 components respectively as shown
in Table 12.

The morphological characters (colour, odour, taste, size, shape and surface) of
the seeds were observed by naked eyes (Fig. 21). Transverse section of the seed and
its powder characteristics were observed under microscope (Figs. 22 and 30).

**Horse gram, Dolichos biflorus** Linn. (Fig. 3), belongs to the Leguminosae
(Papilionaceae) family is popularly known as ‘Kulthi’. It is extensively cultivated and
used either as human food (beans or seeds) or as animal fodder (leaves and stem).
The seeds have been used in the indigenous system of medicine for a long time as
astringent, anthelmintic, nerve tonic, diuretic, aphrodisiac and antipyretic.

The phytochemical studies revealed that there was a high value of total ash
4.07% , indicated the presence of inorganic constituents in the seeds and water-
soluble extractive 2.97% , was also high indicated the presence of sugars . The
qualitative chemical examination of the petroleum ether extract, alcohol extract and
water extract obtained by successive solvent extraction of the seeds revealed the
presence of carbohydrates, sterols, proteins and amino acids, fixed oils and fats and
absence of alkaloids, glycosides, saponins, tannins, resins, gums and mucilages.
The results of the tests applied are tabulated in Tables 13 to 16. Thin-layer
chromatography of amino acids of seeds of *Dolichos biflorus* was carried out using
n-butanol: acetic acid: water (8:2:2) and 96% Ethanol: water (7:3) as solvent system
and visualized the spots by spraying the plate with ninhydrin (0.1% w/v) in butanol.
The *Rf*-values of the spots are recorded in Table 17. Eight different amino acids viz.
alanine, histidine, cystine, aspartic acid, leucine, glycine, serine and lysine were identified by Co-chromatography using authentic sample. Thin-layer chromatography of carbohydrates of the seeds was also carried out using Chloroform: methanol (6: 4) and Acetone: water (9: 1) as solvent system and aniline hydrogen phthalate as spraying agent. The Rf-values of the spots are recorded in Table 18. Five different sugars viz. rhamnose, arabinose, fructose, galactose and glucose were identified by Co-chromatography using authentic sample. The successive solvent extracts of the seeds of *D. biflorus* with petroleum ether, benzene, chloroform, ethanol and water along with authentic amino acids and sugars were scanned under UV light using n-butanol : acetic acid : water (8:2:2), 96% ethanol : water (7:3), chloroform : methanol (6:4) and acetone : water (9:1) as solvent systems by HPTLC as shown in Tables 19 and 20.

The morphological characters (colour, odour, taste, size, shape and surface) of the seeds were observed by naked eyes (Fig. 55). Transverse section of the seed and its powder characteristics were observed under microscope (Figs. 56 and 57).

**ANTIMICROBIAL ACTIVITY**

Results of screening of antibacterial activity and antifungal activity of *Tribulus terrestris* fruit extract are summerised in Table 21 and 22 respectively. It is evident from the results that petroleum ether (60 - 80\(^0\)) and ethanol (50%) extracts of the fruits showed significant antibacterial activity against the tested bacterial organisms, i.e., *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity of both the extracts was compared with Chloramphenicol as antibacterial standard. The petroleum ether (60-80\(^0\)) and ethanol (50%) extracts of the fruits also showed significant antifungal activity against *Candida albicans*. The antifungal activity of both the

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extracts was compared with Nystatin as antifungal standard.

From these results, it can be concluded that the fruits of *Tribulus terrestris* can be regarded as antimicrobial agent. Further phytochemical studies are needed to identify active constituent(s) responsible for the antimicrobial activity of the fruits.

Antimicrobial activity of petroleum ether and ethanolic extracts of *cichorium intybus* seeds is summerised in Table 23. It is evident from the results that the petroleum ether and ethanolic extracts exhibited moderate to significant antifungal activity against all the tested fungal organisms, i.e., *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Fusarium oxysporum* at a concentration of 30 µg and 60 µg. The antifungal activity of both the extracts was compared with Miconazole as antifungal standard at a concentration of 10 µg but none of the extracts was active against all the tested bacterial organisms, i.e., *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

From these results, it can be concluded that the seeds of *cichorium intybus* can be regarded as antifungal agent. Further studies require the detailed chemical nature of the active constituent(s) responsible for the antifungal activity of the seeds.

Antimicrobial activity of petroleum ether and ethanolic extracts of *Dolichos biflorus* seeds is summerised in Table 24. It is evident from the results that the ethanolic extract exhibited significant antibacterial activity against all the tested bacterial organisms, i.e., *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* at the concentration of 25 µg and 50 µg while the petroleum ether extract at the concentration 50 µg showed a slight antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial activity of both the extracts was compared with Ceftazidime as antibacterial standard
at a concentration of 25 μg but none of the extracts was found active against the tested fungal organisms, i.e., *Aspergillus niger* and *Candida albicans*.

From these results, it can be concluded that the seeds of *Dolichos biflorus* can be regarded as antibacterial agent. Further phytochemical studies are needed to identify active constituent(s) responsible for the antibacterial activity of the seeds.