3. PRELIMINARY PHYTOCHEMICAL SCREENING

3.1 INTRODUCTION

All the drugs- Ayurvedic, Unani and Herbal extracts were subjected to preliminary phytochemical screening to test the presence of alkaloids, carbohydrates and reducing sugars, glycosides, proteins and amino acids, steroids and triterpenoids, phenolic compounds and tannins, flavonoids, fixed oils and fats, volatile oils, gums and mucilages.\textsuperscript{[32,33,218]}

3.2 MATERIALS AND METHODS

Drugs tested were Panchagavya Ghrutham (PG), Hab-e-Jund (HJ), Kushmanda Lehyam (KL), Itrifal Kishneezi (IK), extracts of Cynodon dactylon (CD) and Barleria cristata (BC). All the chemicals and solvents used for analysis were obtained from sd-fine Chemicals, India and the reagents were freshly prepared.

For testing the presence of alkaloids, carbohydrates and reducing sugars, glycosides, phytosterols, saponins, phenolic compounds, tannins, gums and proteins, methanolic extracts of formulations were prepared by extracting 25g of the formulation with 100ml (4 x 25g) of methanol using soxhlet extractor followed by filtration and concentration. The extracts were placed in desiccators for future use.\textsuperscript{[219]}

For testing the presence of fixed oils and fats, small quantities of formulations and crude drugs were extracted with petroleum ether.
For testing the presence of volatile oils the formulations and crude drugs were used directly and hydro-distillated using volatile oil estimation apparatus and separated volatile oils if any were tested.

### 3.2.1 Tests for Alkaloids:

Small quantities of methanolic extracts of all the drugs were treated with few drops of diluted hydrochloric acid and filtered. The filtrates of each extract were divided into four portions and the following tests were carried out -

i) *Dragendorff’s test:* With Dragendorff’s reagent (solution of potassium bismuth iodide) formed orange brown precipitate.

ii) *Mayer’s test:* With Mayer’s reagent (potassiomercuric iodide solution) formed creamy precipitate.

iii) *Hager’s test:* With Hager’s reagent (saturated picric acid solution) formed yellow precipitate.

iv) *Wagner’s test:* With Wagner’s reagent (solution of iodine in potassium iodide) formed reddish-brown precipitate.

The formation of respective precipitates indicated the presence of alkaloids.

### 3.2.2 Tests for Carbohydrates and Reducing Sugars:

The extracts were dissolved in water and filtered. The filtrates were divided into several portions and were tested as follows:
i) *Molisch’s test:* To one portion of filtrates of various drugs’
extracts, few drops of α-naphthol solution in alcohol were added
and mixed well followed by concentrated sulphuric acid from
the sides. Purple ring at the junction of two liquids indicated the
presence of carbohydrates.

ii) *Benedict’s test:* To a set of filtrates of various drugs’ extracts,
added equal volumes of *Benedict’s reagent* and heated in boiling
water bath for 5min. The appearance of green, yellow or red
color indicated the presence of reducing sugars.

iii) *Fehling’s test:* One ml each of Fehling’s A and Fehling’s B were
mixed and heated for one minute and equal volumes of the
filtrates were added and heated for 5-10min on a water bath.
First yellow, then brick red precipitate indicated the presence of
reducing sugars.

### 3.2.3 Tests for Glycosides:

The following tests were carried out to detect the presence of
different types of glycosides.

i) *Legal’s test:* To the methanolic extracts, added pyridine and
sodium nitroprusside and development of pink or red color
indicated the presence of cardiac glycosides.

ii) *Borntrager’s test:* The methanolic extracts were boiled with dilute
sulphuric acid and filtered. To the cold filtrates equal volumes of
chloroform were added. After thorough shaking the organic
solvent layers were separated and ammonia solution was added.
The change of ammonia layer to pink or red color indicated the presence of anthraquinone glycosides.

iii) *Foam test:* Small quantities of drugs were shaken vigorously with water. Formation of persistent foam indicated the presence of saponin glycosides.

iv) *Guignard reaction or sodium picrate test:* Soaked filter paper strips first in 10% picric acid and then in 10% sodium carbonate and dried. Drugs were taken in small bottles and the strips were suspended from the mouth of the container and the lids were tightly closed with portion of the strip stuck in the lid. The strips did not turn brick red or maroon indicating the absence of cyanogenetic glycosides.

v) Extracts when made alkaline did not show blue or green fluorescence indicating the absence of coumarin glycosides.

### 3.2.4 Tests for Proteins and Amino acids:

i) *Biuret test:* To the methanolic extracts, 4% sodium hydroxide and 1% copper sulfate solution were added and formation of violet or pink color indicated the presence of proteins.

ii) *Million’s test:* To the methanolic extracts, Million’s reagent (mercury in nitric acid) was added. Formation of white precipitate which turned red on heating indicated the presence of proteins.

iii) *Ninhydrin test:* The extracts were heated with 5% Ninhydrin (in butanol) solution in boiling water bath for 10min and development of purple or bluish color indicated the presence of amino acids.
3.2.5 Tests for Steroids and Triterpenoids:

i) *Salkowski reaction:* To the methanolic extracts, chloroform and concentrated sulfuric acid were added and shook well. The appearance of reddish-blue color in the chloroform layer and green fluorescence in acid layer indicated the presence of steroids.

ii) *Liebermann-Burchard reaction:* To the methanolic extracts added chloroform, mixed and then added acetic anhydride followed by concentrated sulfuric acid from the sides of the tubes. Appearance of first red, then blue and finally green color indicated the presence of steroids and triterpenoids.

3.2.6 Tests for Phenolic compounds and Tannins:

Small quantities of the methanolic extracts were treated with the following reagents and the appearance of corresponding endpoints indicated the presence of phenolic compounds and tannins.

i) *With 5% Ferric chloride solution:* Deep blue-black color.

ii) *With 10% Lead acetate solution:* White precipitate.

iii) *With 10% Potassium dichromate solution:* Red precipitate.

3.2.7 Tests for Flavonoids:

i) *Shinoda test:* Methanolic extracts were extracted with 95% ethanol and hydrolyzed by concentrated hydrochloric acid. Pink color appeared after adding the magnesium turnings. Formation of yellow precipitates when lead acetate was added to the residues indicated the presence of flavonoids.
3.2.8 Tests for Fixed oils and Fats:

i) *Oil Stain test:* Petroleum ether extracts were pressed between two filter papers. Oil stains on the papers indicated the presence of fixed oils.

ii) *Saponification test:* To the petroleum ether extracts, few drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein were added and heated on a water bath for 1-2 hours. Formation of soap and/or partial neutralization of alkali indicated the presence of fixed oils and fats.

3.2.9 Tests for Volatile oils:

After hydro-distillation, characteristic odor of the distillates and their non permanent staining of filter papers indicated the presence of volatile oils.

3.2.10 Tests for Gums and Mucilages:

The aqueous solutions of extracts were mixed with absolute alcohol and dried in air and the residues were tested for swelling properties and for the presence of carbohydrates.

3.3 RESULTS:

The results of all the above tests were summarized and shown in the following Table 3.1.
Table 3.1 Preliminary Phytochemical Screening of the Drugs

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Tests performed for the presence of</th>
<th>PG</th>
<th>HJ</th>
<th>CD</th>
<th>KL</th>
<th>IK</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates and Reducing sugars</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Proteins and Amino acids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Steroids and Triterpenoids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Phenolic compounds and Tannins</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoids</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>8.</td>
<td>Fixed oils and Fats</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Volatile oils</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Gums and Mucilages</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>--</td>
</tr>
</tbody>
</table>


3.4 DISCUSSION

All the drugs showed the presence of phenolic compounds and flavonoids, gums and mucilages were not found in any of the above drugs.

PG showed the presence of all the constituents except gums and mucilages. Though it had several ingredients containing volatile oils, but only traces could be found. The method of preparation of heating for a prolonged period could explain to some extent, the loss of volatile oils in the final product. PG was rich in flavonoids, proteins and fats.
HJ showed all the constituents in moderation, except gums and mucilages, carbohydrates and fats which were absent.

CD was rich in flavonoids and phenolic constituents.

KL was rich in sugars, fats, flavonoids, fixed oils and fats. It also contained volatile oil.

IK was rich in tannins and flavonoids due to the presence of its main ingredients. It had good volatile oil content also.

BC did not show the presence of proteins, fixed oils, fats, volatile oils and gums. But it too tested positively for flavonoids.

3.5 CONCLUSION

All the drugs showed the presence of phenolic compounds and flavonoids which encouraged for further study of antioxidant activity of these drugs.