**PHYTOCHEMICAL QUALITATIVE ANALYSIS**

**Test for anthraquinones:** Five ml of the extract solution was hydrolysed with diluted Conc. H2SO4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

**Test for alkaloids**

1.Mayer‟s test (Evans, 1997): To a few ml of the filtrates, a drop of Mayer‟s reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

2.Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff‟s reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

**Test for Polyphenols**

1.Ethanol (10.0 ml) was added to each extracts and the resulting solution (3.0 ml) was transferred in test tubes and warmed in a water bath (15 minutes). Three drops of freshly prepared ferric cyanide solution were added to the extract solution. Formation of a blue green colour indicated the presence of polyphenols.

2.Yellow precipitates were obtained by the addition of 3 drops of lead acetate solution (5%) indicated the phenolic compounds.

3.3 ml of 0.1% of gelatin solution was added to 5ml of ethanolic extract. Precipitation indicated.

**Test for Tannins:** About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

**Test for Phlobatannins:** Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

**Test for Saponin:** About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

**Test for Flavonoids:**

1.Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H2SO4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

2.Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

3.A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

**Test for Terpenoids (Salkowski test):** Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

**Test for triterpenoids:** Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H2SO4. Formation of reddish violet colour indicates the presence of triterpenoids.

**Test for Cardiac glycosides (Keller-Killani test):** Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

**Test for Anthocyanins:** 2 ml of aqueous extract is added to 2 ml of 2N HCl and ammonia. The appearance of pink-red turns blue-violet indicates the presence of anthocyanins.