

This chapter deals with preliminary detection and extraction of alkaloids, flavonoids and crude extracts in different polar and non polar solvents were carried out from selected plants using well established methods.

MATERIALS AND METHODS

Plants Collection & storage

Selected medicinal plants parts viz: Leaves of *Aloe vera* L. , bulbs of *Allium cepa* L., bulbs of *Allium sativum* L., leaves of *Azadiracta indica* A Juss. and stem bark of *Mangifera indica* L. were collected from different localities of Jaipur and Bharatpur districts in the month of November and December, 2011. Voucher specimens were deposited in the 'Herbarium', Department of Botany, University of Rajasthan (UOR), Jaipur.

All the selected parts of plants were separated and washed thoroughly in running tap water, separately shade dried at room temperature. Dried plant materials were milled to make fine powder in a grinder. Each powdered sample was stored at room temperature in an air-tight polythene bag and were labeled to be used for extraction.

Preliminary Detection of Secondary Metabolites

Presence of secondary metabolites (alkaloids, flavonoids) in the powdered samples were determined using standard methods.

Chemicals & Reagents Used

Chemicals

HgCl₂, I₂, KI, Bismuth subnitrate, HCl, Picric acid, Ammonia solution, H₂SO₄, NaOH, Ethyl acetate, Magnesium ribbon and Acetic acid, Ethanol, NH₄OH, Methanol, Petroleum ether, Ethyl ether, Ethyl acetate, Sulphuric acid, and Benzene.

Reagents

Modified Mayer's reagent: Prepared by mixing 1.35 g HgCl₂ and 3.95 g KI in 100 ml Dw.

Wagner's reagent: Prepared by mixing 1.25 g I₂ and 2 g KI in 100 ml of Dw.

Dragendorff's reagent: Two stock solutions were prepared:

(A) 0.6 g Bismuth subnitrate in 2 ml conc. HCl and 10 ml DW.

(B) 6 g KI in 10 ml DW.

Both the solutions were mixed together and diluted with 400 ml DW.

Hager's reagent: Prepared by dissolving 1g Picric acid in 100 ml DW.

Detection of Alkaloids:

Presence of alkaloids in each extract was carried out as per the standard methods (Brain and Turner, 1975; Evans, 1996).

Test samples were acidified by 5 ml of 2% HCl at 60⁰C for 2 h and later cooled and filtered. Filtrates were used to test the presence of alkaloids.

- i) **Modified Mayer's Test:** On addition of Mayer's reagent to the filtrate, formation of yellow creamy ppt., indicated the presence of alkaloid.
- ii) **Wagner's Test:** On addition of Wagner's reagent to the filtrate, appearance of reddish brown colour indicated the presence of alkaloids.
- iii) **Dragendorff's Test:** Filtrate when treated with the Dragendorff's reagent, formation of red ppt. indicated the presence of alkaloids.
- iv) **Hager's Test:** Filtrates when treated with Hager's reagent, yellow coloured ppt. was formed which indicated the presence of

alkaloids.

Detection of Flavonoids

Presence of flavonoids in each extract was carried out as per the standard methods (Sofowara, 1993; Harborne, 1973).

Following methods were used to determine the presence of flavonoids in the test samples:

- (i) Ammonia solution Test:** Dilute ammonia solution (5 ml) was added to a portion of the aqueous extracts of each plant sample, followed by addition of concentrated H₂SO₄. Formation of yellow colour in each extract and the colour disappearance on standing indicated the presence of flavonoids.
- (ii) Alkaline solution Test:** Few drops of NaOH solution was added to the test extracts. Formation of yellow colour and the colour disappearance after addition of dilute acid indicated the presence of flavonoids in the samples.
- (iii) Ethyl acetate Test:** Ten ml of ethyl acetate was added to the powdered samples and were heated over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was

shaken with 1ml of dilute ammonia solution. A yellow coloration appeared, indicating a positive test for flavonoids.

Preparation of crude extracts in polar solvents

Dry plant materials were taken in round bottomed flask in different solvents, 30 g powder was taken in each flask and water, methanol, ethanol, acetone, toluene and petroleum ether added as solvent in each flask in the ratio of 1:10. Soxhlet extraction was carried out for 24 hours and filtered. The filtrates were subjected to evaporation to obtain dried extract which were weighed and calculated for each gram of plant material.

Extraction of Secondary Metabolites

(i) Alkaloids

Alkaloids were extracted from different parts of selected plants by the well established method (Harborne, 1984). Finely powdered samples (100 g) of plants parts were separately extracted with 10% acetic acid in ethanol (500 ml) for 4 hours. Filtered extracts were concentrated and were made alkaline by NH_4OH . Precipitate thus obtained was collected separately by centrifugation, washed with 1% NH_4OH , filtered, dried in *vacuo* and weighed. Extracts thus obtained were stored in glass vials at 4°C for further

use.

(ii) Flavonoids

Different parts of selected plants were subjected to flavonoid extraction following the method of Subramanian and Nagarjan, (1969). Hundred grams of finely powdered plant material each was Soxhlet extracted with hot 80% methanol (500 ml) on a water bath for 24 h and were filtered. Filtrate was re-extracted successively with petroleum ether, ethyl ether and ethyl acetate. Each step was carried out three times to ensure complete extraction. Petroleum ether fraction was discarded due to being rich in fatty substances and ethyl ether fractions (free flavonoids) were collected. Ethyl acetate fractions were analyzed for bound flavonoids. Each ethyl acetate fraction was hydrolyzed in 7% H₂SO₄ for 2 h. Resulting mixture was filtered and filtrate was again extracted with ethyl acetate. The ethyl acetate extract was washed with distilled water to neutrality and collected. The ethyl ether (free flavonoids) and ethyl acetate (bound flavonoids) fractions were dried in *vacuo*, weighed and stored in glass vials at 4 °C.

Results

All the plant samples showed positive result for the presence

of alkaloids and flavonoids in preliminary tests carried out for the purpose.

Crude extracts in polar solvents, Alkaloids and total flavonoids were extracted from each of the selected plant parts and quantity for each gram of dried plant material was calculated.

Polarity of different solvents is shown in Table 1.1. Quantity of content obtained from each gram of dried of plant material is shown in Tables (1.2 to Table 1.6) and Figures (1.1 to Figure 1.5).

Maximum amount of extracts was recorded in different polar and non polar solvents to be maximum by using water as solvent followed by ethanol, acetone, methanol, toluene and pet ether in all plants collected from both districts.

Quantity of extracts of plants collected from Jaipur district:

Aloe vera L.

Water (102.25 mg/g.d.w.), ethanol (98.80 mg/g.d.w.), acetone (97.04 mg/g.d.w.), methanol (71.96 mg/g.d.w.), toluene (32.52 mg/g.d.w.) and pet ether (26.87 mg/g.d.w.).

Alkaloid content was recorderd to be 17.01 mg/g.d.w. whereas

flavonoid content was recorded to be 8.8 mg/g.d.w. in leaves of plant.

***Allium cepa* L.**

Water (83.28 mg/g.d.w.), ethanol (72.56 mg/g.d.w.), acetone (71.23 mg/g.d.w.), methanol (64.59 mg/g.d.w.), toluene (22.17 mg/g.d.w.) and pet ether (18.26 mg/g.d.w.).

Alkaloid content was recorderd as 28.60 mg/g.d.w. wheareas flavonoid content was recorded as 2.30 mg/g.d.w. in bulbs of plant.

***Allium sativum* L.**

Water (62.60 mg/g.d.w.), ethanol (59.02 mg/g.d.w.), acetone (55.6 1 mg/g.d.w.), ethanol (29.56 mg/g.d.w.), toluene (14.54 mg/g.d.w.) and pet ether (12.13 mg/g.d.w.).

Alkaloid content was recorderd as 34.40 mg/g.d.w. wheareas flavonoid content was recorded as 5.0 mg/g.d.w. in bulbs of plant.

***Azadiracta indica* A. Juss**

Water (106.56 mg/g.d.w.), ethanol (92.38 mg/g.d.w.), acetone (88.45 mg/g.d.w.), ethanol (65.07 mg/g.d.w.), toluene (36.72 mg/g.d.w.) and pet ether (28.13 mg/g.d.w.).

Alkaloid content was recorded as 14.20 mg/g.d.w. whereas flavonoid content was recorded as 6.60 mg/g.d.w. in leaves of plant.

Mangifera indica L.

Water (98.48 mg/g.d.w.) ethanol (88.14 mg/g.d.w.), acetone (86.35 mg/g.d.w.), methanol (82.39 mg/g.d.w.), toluene (34.58 mg/g.d.w.) and pet ether (26.17 mg/g.d.w.).

Alkaloid content was recorderd as 6.6 mg/g.d.w. wheareas flavonoid content was recorded as 7.2 mg/g.d.w. in stem bark of plant.

Quantity of extracts in plants of Bharatpur district:

Aloe vera L.

Water (98.13 mg/g.d.w.), ethanol (96.76 mg/g.d.w.), acetone (95.25 mg/g.d.w.), methanol (68.58 mg/g.d.w.), toluene (31.29 mg/g.d.w.) and pet ether (25.54 mg/g.d.w.).

Alkaloid content was recorded as 16.53 mg/g.d.w. wheareas flavonoid content was recorded as 7.30 mg/g.d.w. in leaves of plant.

Allium cepa L.

Water (81.19 mg/g.d.w.), ethanol (72.14 mg/g.d.w.), acetone (70.20

mg/g.d.w.), methanol (63.56 mg/g.d.w.), toluene (21.23 mg/g.d.w.) and pet ether (17.53 mg/g.d.w.).

Alkaloid content was recorded to be 26.53 mg/g.d.w. whereas flavonoid content was recorded to be 2.10 mg/g.d.w. in bulbs of plant.

Allium sativum L.

Water (61.83 mg/g.d.w.), ethanol (59.42 mg/g.d.w.), acetone (56.71 mg/g.d.w.), ethanol (27.43 mg/g.d.w.), toluene (13.16 mg/g.d.w.) and pet ether (11.29 mg/g.d.w.).

Alkaloid content was recorded to be 32.21 mg/g.d.w. whereas flavonoid content was recorded to be 4.65 mg/g.d.w. in bulbs of plant.

Azadiracta indica A Juss.

Water (111.54 mg/g.d.w.), ethanol (95.46 mg/g.d.w.), acetone (91.53 mg/g.d.w.), methanol (68.17 mg/g.d.w.), toluene (37.53 mg/g.d.w.) and pet ether (30.19 mg/g.d.w.).

Alkaloid content was recorded to be 15.93 mg/g.d.w. whereas flavonoid content was recorded to be 7.81 mg/g.d.w. in leaves of plant.

Mangifera indica L.

Water (108.58 mg/g.d.w.), acetone (91.23 mg/g.d.w.), ethanol (89.43 mg/g.d.w.), methanol (86.28 mg/g.d.w.), pet ether (37.17 mg/g.d.w.) and toluene (29.54 mg/g.d.w.).

Alkaloid content was recorded to be 7.93 mg/g.d.w. whereas flavonoid content was recorded to be 8.74 mg/g.d.w. in stem bark of plant.

Significant difference was observed in the content extracted in different polar solvents in plants collected from both the districts but the difference in the content of plants collected from both districts were not very significant.

