The ash content of a crude drug biology essay

The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. There is a considerable difference varies within narrow limits in the case of the same individual drug. Hence an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. Ash standards have been established for a number of official drugs. Usually these standards get a maximum limit on the total ash or on the acid insoluble ash permitted.

The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.

The ash or residue yielded by an organic chemical compound is as a rule, a measure of the amount of inorganic matters present as impurity. In most cases, the inorganic matter is present in small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drugs in powder form.

Procedures given in Indian pharmacopoeia were used to determine the different ash values such as total ash and acid insoluble ash.

Total ash

Weighed accurately about 3 gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature

to make it dull red until free from carbon cooled and weighted and then calculated the percentage of total ash with reference to the air dried drug.

Acid insoluble ash

The ash obtained as directed under total ash above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on ash less filter paper, washed with hot water ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Water soluble ash

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450ËšC. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

b. EXTRACTIVE VALUES

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of alcohol soluble extractive value

5 gm of the air-dried coarse powder of Anogeissus latifolia wall (Roxb. ex. DC) was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18hours. Thereafter, it was filtered rapidly taking precautions against the

loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ËšC and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air- dried drug. The results are recorded in the table.

Determination of water soluble extractive value

Weigh accurately 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow to standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ËšC and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug. The results are given in the table.

c. LOSS ON DRYING

Loss on drying is the loss in weight in percentage w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Desiccators or hot air oven). If the sample is in the form of large crystals, then reduce the size by quick crushing to a powder.

Procedure

About 1. 5 gm of powdered drug was weighed accurately in a tarred porcelain dish which was previously dried at 105ËšC in hot air oven to constant weight and then weighed. From the difference in weight, the

percentage loss of drying with reference to the air dried substance was calculated.

d. FLUORESCENCE ANALYSIS [Kokate. C. K, 2002; Khandelwal KR 1996].

In the near-ultra region of the spectrum (3000-4000AËš) some of the phytoconstituents show more or less brilliant coloration when exposed to radiation. This phenomenon of emitting visible wavelengths as a result of being excited by radiation of a different wavelength is known as fluorescence. Sometimes the amount of ultra-violet light normally present with visible light is sufficient to produce the fluorescence, but often a more powerful source of ultra-violet is necessary, e. g. mercury vapour lamp. It is often possible to make use of this phenomenon for the qualitative examination of herbal drugs. A fluorescence characteristic of the powdered leaves of Anogeissus latifolia wall (Roxb. ex. DC) was observed in daylight and UV light. Also the fluorescent study was performed on treating the drug powder with different chemical reagents. The observed results are given in table.

e. FOAMING INDEX: [Divakar M. C., 1996]

Foaming index is mainly performed to determine the saponin content in an aqueous decoction of plant material.

Determination of foaming index:

Weighed accurately about 1g of coarsely powdered drug and transformed to 500ml conical flask containing 100ml of boiling water. Maintained at moderate boiling at 80-90Ëšc for about 30min. Cooled and added sufficient

water through the filter to make up the volume to 100ml (V1). Cleaned 10 stoppered test tube of uniform dimension were taken and transferred the successive portions of 1, 2, 3ml up to 10ml and adjusted the volume of the liquid in each test tube with water to 10ml. Stoppered the tubes and shaken them in a lengthwise motion for 15 sec uniformly and allowed to stand for 15min and measure the height of foam. If the height of the foam in every tube is less than 1cm, the foaming index is less than 100(not significant). Here the foam was more than 1cm height after dilution of plant material. If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of first decoction of plant material is measured and transferred to 100ml volumetric flask (V2) and volume is made to 100ml and followed the same procedure.

5. 1. 2. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extraction of plant material:-

Petroleum ether extraction:-

About 400gm of dry coarse leaf powder of the Anogeissus latifolia wall (Roxb. ex. DC) was extracted with petroleum ether 2500ml (40-600c) for 18 hrs by continuous hot percolation method. It was allowed to cool to 40oC and then filtered using whatman No. 1 filter paper. The filtrate was then concentrated in a rotary evaporator and the extract stored at 4°C until required. The extract yield (% w/w) from the plant material was recorded.

Methanolic extraction:-

About 400g of air dried coarse powdered material was taken in 1000ml soxhlet apparatus and soaked with petroleum ether for 2 days. At the end of

second day the powder was taken out and it was dried. After drying it was again packed and extracted by using methanol (Changshu yangyuan chemicals, China) as solvent, till colour disappeared. The temperature was maintained at 55°C-65°C. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield (% w/w) of methanolic extract were noted.

S. No.

Name of extract

Colour

Consistency

Yield% W/W

1

2

Methanolic extract

Petroleum ether extract

Blackish brown

Blackish green

Non Sticky mass

sticky oily mass

16. 75

1. 60Table: 1. Nature and colour of extract of Anogeissu latifolia wall (Roxb. ex. DC).

5. 1. 3 CHEMICAL TESTS:

A) Test for carbohydrates

1. Molisch Test: It consists of treating the compounds of a-naphthol and concentrated sulphuric acid along the sides of the test tube.

Purple colour or reddish violet colour was produced at the junction between two liquids. (Kokate, C. K et al, 2000)

2. Fehling’s Test: Equal quantity of Fehling’s solution A and B is added. Heat gently, brick red precipitate is obtained.

3. Benedict’s test: To the 5ml of Benedict’s reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained.

4. Barfoed’s test: To the 5ml of the Barfoed’s solution add 0. 5ml of solution under examination, heat to boiling, formation of red precipitate of copper oxide is obtained.

B) Test for Alkaloids

1. Dragendroff’s Test: To the extract, add 1ml of Dragendroff’s reagent Orange red precipitate is produced.

2. Wagner’s test: To the extract add Wagner reagent. Reddish brown precipitate is produced.

3. Mayer’s Test: To the extract add 1ml or 2ml of Mayer’s reagent. Dull white precipitate is produced.

4. Hager’s Test: To the extract add 3ml of Hager’s reagent yellow Precipitate is produced.

C) Test for Steroids and Sterols

1. Liebermann Burchard test: Dissolve the test sample in 2ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

2. Salkowski test: Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides

1. Legal's test: Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red colour is produced.

2. Baljet test: To the drug sample, sodium picrate solution is added. Yellow to orange colour is produced.

3. Borntrager test: Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

4. Killer Killani test: Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes. 1 cm layer of foam indicates the presence of saponins.

F) Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid is added. Red colour is produced.

G) Test for Tri-terpenoids

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H) Tests for Tannins and Phenolic Compounds:

To 2-3 ml of extract, add few drops of following reagents:

a). 5% FeCl3 solution: deep blue-black color.

b). Lead acetate solution: white precipitate.

c). Gelatin solution: white precipitate

d). Bromine water: decolouration of bromine water.

e). Acetic acid solution: red color solution

f). Dilute iodine solution: transient red color.

g). Dilute HNO3: reddish to yellow color.

I) Test for Fixed Oils and Fatty acids

a). Spot test:

Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

b). Saponification test:

Few drops of 0. 5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated on water bath for 1-2 hours soap formation indicates the presence of fixed oils and fats in the extracts.

J) Test for Gums and Mucilage:

a). Ruthenium red test:

Small quantities of extract are diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilage.

K) Test for Proteins and Amino acids

Biuret test: Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.

Ninhydrin test: Add 2 drops of freshly prepared 0. 2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid.

5. 1. 4. TOXICOLOGICAL EVALUATION

Determination LD50 value of Anogeissus latifolia (Roxb. ex. DC). wall. Gull&perr

Acute Oral Toxicity Study

The procedure was followed by using OECD guidelines 423 (Acute toxic class method)

Animals:

Adult albino rats (Wister strain) of either sex with weighing 150 – 180gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from in house ethical committee has been taken to carry out (Reference no. JKKMMRF/2010/009) and complete this study.

Procedure:

Twelve animals (Wister Albino rats, 150-200gm) were selected for studies. The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000 mg / kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized system (GHS) for the classification of chemical which cause acute toxicity.

Most of the crude extracts possess LD50 value more than 2000 mg. /kg of the body weight of animal used.

Dose volume was administered 0. 1 ml / 100 gm body weight to the animal

by orally after giving the dose the toxic signs were observed within 3-4 hours.

Body weight of animals before and after administration, onset of toxicity and signs of toxicity like changes in skin and fur, eyes, and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern, signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was also to be noted, if any , was observed.

Observation

No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD 50 of the Anogeissus latifolia wall (Roxb. ex. DC), as per OECD guidelines-423 is greater than 2000mg/kg (LD50 > 2000mg/kg).

Hence, the biological dose was fixed at 200, 400 and 600mg/kg of body weight for the extract.

PHARMACOLOGICAL EVALUATION

5. 2. 1 Evaluation of Anti-ulcer Activity:-

Animals used:

Adult albino rats (Wister strain) of either sex with weighing 150 – 180gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from in house ethical committee has been taken to carry out (Reference no. JKKMMRF/2010/009) and complete this study.

5. 2. 2 Experimental procedure

Ethanol induced ulcer:-

Male albino-Wistar rats were divided in to five groups of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy. Six groups were made as below

Group I – animals served as normal controls.

Group II – received 1% CMC (1. 0ml/kg p. o) as vehicle control.

Group III – received 200mg/kg, p. o methanolic extract of Anogeissus latifolia.

Group IV – received 400mg/kg, p. o methanolic extract of Anogeissus latifolia.

Group V – received 100mg/kg, Sucralfate as standard

One hour after the drug treatment the animals were treated with absolute ethanol [5ml/kg] to induce ulcers. The animals were sacrificed after 1hrs and stomach was opened and percentage inhibition of ulcer was determined. (Mozafar khazaei et al., 2006, Paul V. et al 2002, Paul V. et al., 2000)

Aspirin induced ulcer:-

Male albino-Wistar rats were divided in to five groups of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy. Six groups were made as below

Group I – animals served as normal controls.

Group II – received 1% CMC (1. 0ml/kg p. o) as vehicle control.

Group III – received 200mg/kg, p. o methanolic extract of Anogeissus latifolia.

Group IV – received 400mg/kg, p. o methanolic extract of Anogeissus latifolia.

Group V – received 100mg/kg, Sucralfate as standard

One hour after the drug treatment the animals were treated with aspirin [200 mg/kg] to induce ulcers. The animals were sacrificed after 1hrs and

stomach was opened and percentage inhibition of ulcer was determined. (Mozafar khazaei et al., 2006, Paul V. et al 2002, Paul V. et al., 2000)

5. 2. 3 BIOCHEMICAL PARAMETERS:-

The stomach was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The gastric contents were analyzed for gastric juice volume, pH, free and total acidity.

5. 2. 4 Measurement of gastric juice volume and pH:-

Gastric juice was collected from ethanol induced ulcer rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100g body weight. The pH of the supernatant was measured using digital pH meter. (Canmon DC. et al., 1969, Kannappan et al., 2008, Patil K. S. et al., 2008, Paul V. et al., 2000)

5. 2. 5 Determination of free and total acidity:-

An aliquot of 1. 0 ml of gastric juice was pipette out in to a 50 ml conical flask and 2/3 drops of Topfers reagent was added to it and titrated with 0. 01N NaOH until all traces of the red colour disappeared and the colour of the solution turned yellowish orange. The volume of 0. 01N NaOH was noted which corresponds to free acidity. Then 2/3 drops of phenolphthalein was added and titration was continued until a permanent pink colour was developed. The volume of total alkali consumed was noted which corresponds to total acidity. The free acidity and total acidity was determined using the formula and values are expressed as mEq/l 100g. (Kannappanetal. 2008, Rajkapoor et al., 2002).

Acidity = Volume of NaOH X Normality of NaOH X 100 (mEq/L per 100g)

0. 01

5. 2. 6 Ulcer index (UI):-

The mucosa was flushed with saline and stomach was pinned on frog board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined. (Malairajan et al., 2007)

0 – Normal coloured stomach

0. 5 – Red colouration

1 – Spot ulceration

1. 5 – Haemorrhagic streak

2 – ulcers

3 – Perforations

5. 2. 7 Percentage inhibition:

Percentage inhibition was calculated using the following formula. (Malairajan et al., 2007)

UI ulcer control – UI ulcer treated

% inhibition = X 100

UI ulcer control

5. 2. 8. Statistical Analysis:

All the values are expressed as mean ± S. E. M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison tests. The values are statistically significant at three levels, ***p <0. 001. **p <0. 01, *p <0. 05 But ns if p > 0. 05.

5. 3. EVALUATION OF DIURETIC ACTIVITY

Animals used:

Adult albino rats (Wister strain) of either sex with weighing 150 – 180gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals had access to laboratory chow and water. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from in house ethical committee has been taken to carry out (Reference no. JKKMMRF/2010/009) and complete this study.

Experimental procedure

The method of (Lipchitz et. al., 1943) was employed for the evaluation of diuretic activity. The Male Albino-Wistar rats were divided into four groups of six rats in each as mentioned below.

Group I – received Normal saline (25mg/kg, p. o) as control.

Group II – received (400mg/kg, p. o) methanolic extract of Anogeissus latifolia.

Group III- received (600mg/kg, p. o) methanolic extract of Anogeissus latifolia.

Group IV – received Furosemide (20mg/kg, p. o) as standard.

The animals were fasted and deprived of food and water for 18hour prior to the experiment. On the day of experiment, the group I animals serving as control, received normal saline (25ml/kg, p. o), the group II animals received methanolic extract of Anogeissus latifolia wall (Roxb. ex. DC) leaves (400mg/kg, p. o) and group III animals also received methanolic extract (600mg/kg, p. o), the group IV animals received Furosemide (20mg/kg, p. o), respectively, in normal saline. Immediately after the administration the animals were kept in metabolic cages (three per cage) specially designed to separate urine and fecal matter and kept at room temperature of 25 ± 0. 5° C throughout the experiment. The total volume of urine was collected at the end of 5hrs after dosing. During this period no water and food was made available to the animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na+ , K+ and Cl- in the urine. The Na+ and K+ were measured by flame photometry and Cl- concentration was estimated by titration with silver nitrate (N/50) using three drop of 5% potassium chromate solution as indicator . the results are reported as mean ±SD, the test of significance (P <0. 05) compared to control group.

5. 3. 1. Statistical analysis:

All the values are expressed as mean ± S. E. M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison tests. The values are statistically significant at three levels, ***p <0. 001. **p <0. 01 *p <0. 05 But ns if p > 0. 05.

5. 4 EVALUATION OF ANALGESIC ACTIVITY

Animals used:

Adult albino rats (Wister strain) of either sex with weighing 150 – 180gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from in house ethical committee has been taken to carry out (Reference no. JKKMMRF/2010/009) and complete this study.

Procedures:

Eddy's hot plate method:

The Male Albino-Wistar rats were divided into four groups of six rats in each as mentioned below.

Group I – received 1% CMC (3ml/kg, p. o) as control.

Group II – received (400mg/kg, p. o) methanolic extract of Anogeissus latifolia.

Group III- received (600mg/kg, p. o) methanolic extract of Anogeissus latifolia.

Group IV – received pentazocine (5mg/kg, p. o) as standard

Analgesic activity was performed by using Eddy’s hot plate (Inco, India) maintained at a temperature of 55±1°c. The basal reaction time of all animals towards thermal heat was recorded. The animals which showed forepaw licking or jumping response within 6-8 seconds were selected for the study. Male Albino rats were divided into 5 groups having 6 animals each and they were divided into 5 groups having 6 animals each and they were fasted overnight during the experiment free access to water. Group first received 1 % CMC (3ml/kg, p. o). Group second, third and fourth received methanolic extract of Anogeissus latifolia (Roxb. ex DC.) wall. Gull & perr leaves of dose 400mg/kg and 600mg/kg, orally as a suspension in 1%CMC solution respectively Group five received Pentazocine (5mg/kg, p. o) as reference drug .

60 mins after the administration of test and reference compounds, the animals in all the six groups were individually exposed to the plate maintained at 55°c and observations were recorded for 3 hours. The time taken in seconds for fore paw licking or jumping was taken as reaction time. A cut off period of 15 seconds is observed to avoid damage to the paws. The percentage protection was calculated using the formula,

Percentage protection = (T/C-1) Ã-100 where, T is the reaction time of treated group and C the reaction time of control group.