Preparation and Physico-chemical Evaluation of an Anti-diarrhoeal *Safoof*

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Abstract

Diarrhoea is one of the global public health concerns now a days. In Unani System of Medicine (USM) various anti-diarrhoeal formulations are used for the treatment of diarrhoeal diseases. For the assurance of safety, efficacy and reproducibility, it is mandatory that every herbal product should pass the tests of identification, authentication and physico-chemical stanardization. In the present study, a Unani anti-diarrhoeal *Safoof* (ADS) was prepared and its quality standards were established. After preparation of powder dosage form (ADS), it was evaluated on pysico-chemical parameters which included organoleptic properties; alcohol soluble matter; water soluble matter; successive extractives; ash values; moisture content; loss of weight on drying; pH in 1% solution and 10% solution; angle of repose; bulk density; tapped density; carr's index; hausner's ratio; volatile oils; qualitative estimation; quantitative estimation and TLC finger printing. The results of standard parameters of ADS have shown alcohol soluble matter (23.964 ± 0.03%), water soluble matter (17.381 ± 0.06%), moisture content (3.661 ± 0.33%), loss of weight on drying (7.156 ± 0.04%), pH in 1% solution and 10% solution (6.83 ± 0.04 and 5.67 ± 0.03 respectively), angle of repose (51.3469 ± 0.50), bulk density (0.3216 ± 0.00 gm/ml), tapped density (0.4245 ± 0.00 gm/ml), carr's index (24.2452 ± 1.02), hausner's ratio (1.3205 ± 0.01), volatile oils (3.066 ± 0.13%) and tannins (2.54 ± 0.04%). Various chemical constituents were also found positive. The TLC finger printing showed five spots on silica gel plate. These developed physicochemical standards of ADS may be used for future reference.

Keywords: Anti-diarrhoeal, Physico-chemical standards, *Safoof*, Powder.

Introduction

The disease

Is'hal which strongly simulates with diarrhoea in symptoms and pathogenesis is a global public health concern. According to the World Health Organization (WHO) and UNICEF, about two billion cases of diarrheal disease occur worldwide every year, and 1.9 million children younger than five years of age perish from diarrhoea annually, mostly in developing countries. This amounts to 18% of all the deaths of children under the age of five years. 78% of all child deaths from diarrhoea occur in the African and South-East Asian regions (Anonymous, 2012). In Unani literature, there are number of powder formulations, which are used to treat diarrhoea such as *Safoofe Kharnoob, Safoofe Sumaq, Safoofe Ood* etc. However, a powdered drug (hence forth ADS – anti-diarrhoeal Safoof.) suggested

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by Azam Khan is commonly used in the management of diarrhoea of diverse orgin in patients of all age groups. It contains *Belgiri, Zeera Safaid, Kishneez Khushk, Zanjabeel, Badiyan* and *Raal safaid* and used by the physicians for the treatment of *Is'hal* (Khan, 2011) in common practice.

In view of the fact that there are reports of wide-spread use of adulterated raw material leading to the production of inferior medicines (both single and compound), there is dire need for developing their pharmacopoeial standards. Present study on anti-diarrhoeal drug 'Safoof' is based on this rationale and presents data on its physicochemical standards for future reference in an efforts to check adulteration in the finished product.

Materials and Methods

Materials

The ingredients of ADS *Belgiri, Zeera Safaid, Kishneez Khushk, Zanjabeel, Badiyan* and *Raal safaid* (Table 1) were procured from the authorised dealer at Bengaluru. Prof. K. Ravikumar of Centre for Repository of Medicinal Resources (C-RMR), Trans-Disciplinary University (TDU), Attur, Bengaluru authenticated the drug samples. The voucher specimens of all the ingredients have been deposited in the museum of Institute of Trans-Disciplinary Health Sciences and Technology, Bengaluru.

Preparation of *Safoof*

All crude drugs were cleaned manually from impurities and allowed to dry in shade. *Zeera safaid* was roasted in a pan on low flame separately. Then all crude drugs were ground in an electrical grinder and passed through mesh No. 80 (Said, 1997) separately. The required quantity of each powder drug was mixed

Table 1: Ingredients of ADS

S.No.	Unani Name	Scientific Name	Part used	Quantity
1.	Zeera safaid biryan	*Cuminum cyminum* L.	Roasted dried fruits	100gm
2.	Badiyan	*Foeniculum vulgare* Mills	Dried fruits	100gm
3.	Zanjabeel	*Zingiber officinale* Rosc.	Dried rhizome	100gm
4.	Belgiri	*Aegle marmelos* Corr.	Dried fruit pulp	100gm
5.	Kishneez khushk	*Coriandrum sativum* (L.) Corr.	Dried fruits	100gm
6.	Raal safaid	*Vateria indica* L.	Resin	100gm

together to get homogenous powder and stored in an air tight glass container at room temperature (Fig. 1).

Physico-chemical Evaluation

The physicochemical studies of this anti-diarrhoeal *Safoof* were carried out in the laboratory of dept. of Ilmul Saidla, NIUM, Bengaluru, which included; (a) organoleptic properties like appearance, colour, smell and taste; (b) alcohol soluble matter; (c) water soluble matter; (d) successive extractives; (e) ash values; (f) moisture content; (g) loss of weight on drying; (h) pH in 1% solution and 10% solution; (i) angle of repose; (j) bulk density; (k) tapped density; (l) carr's index; (m) hausner's ratio; (n) volatile oils; (o) qualitative estimation; (p) quantitative estimation and; (q) TLC finger printing.

Determination of organoleptic properties

- Appearance: Small quantity of ADS was taken and uniformity of particle size was observed.
- Colour: Five gram of ADS was taken into watch glass and placed against white background in white tube light. The colour of ADS was noted by using Pantone colour chart.
- Odour: A small quantity of the ADS was rubbed between thumb and index finger and inhaled. First, the strength of the odour like none, weak, distinct, strong etc was determined and then the odour sensation like aromatic, fruity, musty, mouldy, rancid, etc was evaluated.

Fig. 1: Prepared sample of ADS

- Taste: A pinch of ADS was examined for the taste on the upper surface of the tongue and one minute time was given to decide the taste.

Determination of alcohol-soluble matter

Five gm of anti-diarrhoeal *Safoof* was placed in a glass-stopper conical flask. Macerated with 100 ml of Ethyl alcohol for six hours shaking and then allow standing for 18 hours. Shake well and filtered rapidly through a dry filter paper. 25 ml of the filtrate was transferred to a previously weighed and tarred flat-bottom petridish and evaporate to dryness on a water bath. This filtrate was dried at 105°C for six hours, cooled in a desiccator for 30 minutes and weighed without delay. The percentage of alcohol soluble matter was calculated with reference to the amount of drug taken (Anonymous, 1998; 2009).

Determination of water-soluble matter

The percentage of water soluble matter was determined as above by using chloroform water instead of ethanol (Anonymous, 1998; 2009).

Determination of successive extractive values

The extractive values of ADS in different solvent viz. petroleum ether, chloroform and ethanol were carried out in soxhlet extractor. Five gm of *Safoof* was successively extracted with 150 ml of each solvent for 6 hours. The extracts were filtered with filter paper and transferred to a previously weighed and tarred flat-bottom petridish and evaporated for complete drying on a water bath. After complete drying, the successive extractive values were determined with reference to the weight of *Safoof* (% w/w) (Anonymous, 2009).

Determination of total ash

Two gm accurately weighed air dried *Safoof* was incinerated in tarred silica dishes at a temperature not exceeding 450°C until free from carbon. Thereafter proper cooling was done in desiccators and weighed. The percentage of total ash was calculated with reference to the air-dried *Safoof* (Anonymous, 1998; 2006).

Determination of acid-insoluble ash

Total ash of ADS was boiled with 25 ml of diluted hydrochloric acid for five minutes and filtered. The insoluble matter was collected on an ash less filter paper (Whatman), washed with hot water and ignited at a temperature not exceeding 450°C for one hour and weighed after cooling in desiccator. The percentage of acid-insoluble ash was calculated with reference to the air dried *Safoof* (Anonymous, 1998; 2006).

Determination of water-soluble ash

The total ash was boiled with 25 ml of distilled water for five minutes and filtered. The insoluble matter was collected on an ash less filter paper (Whatman), washed with hot water and ignited at a temperature not exceeding 450°C for one hour. The weight of insoluble ash was subtracted from the weight of total ash, giving the weight of the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried *Safoof* (Anonymous, 1998; 2006).

Determination of moisture content

The moisture content of *Safoof* was determined by Toluene Distillation method. 10 gm of *Safoof* was taken in a flask and 75 ml of Toluene was added to it. Distillation was carried out for 5 hours. The volume of water collected in the receiver tube (graduated in ml) was noted and the percentage of moisture was calculated with reference to the weight of the air dried *Safoof* (Afaq, 1994).

Determination of loss on drying (gravimetric determination)

Four gm of *Safoof* was taken, spread uniformly and thinly in a shallow petridish. It was heated at a regulated temperature of 105°C for five hours, cooled in desiccator and weighed. The process was repeated many times till two consecutive weights were constant. The percent loss in weight was calculated with reference to initial weight of *Safoof* (Anonymous, 1998).

Determination of pH

pH value of 1% solution

One gm of *Safoof* was dissolved in accurately measured 100 ml of distilled water, filtered and pH measured with digital pH meter (Anonymous, 2006).

pH value of 10% solution

Ten gm of *Safoof* was dissolved in accurately measured 100 ml of distilled water, filtered and pH measured with digital pH meter (Anonymous, 2006).

Determination of angle of repose

The angle of repose was determined by using fixed funnel method. The height of the tip of funnel was fixed two cm above the horizontal surface. A graph paper was placed below the funnel on the table. The *Safoof* was allowed to flow through the funnel freely on to the surface until the apex of the conical pile just touches

the tip of the funnel. The diameter of the powder cone base was measured and the angle of repose was calculated by using this formula: (Anonymous, 2007).

$$\tan \theta = \frac{\text{height of funnel}}{0.5 \text{ base}}$$

Determination of bulk density

An accurately weigh ADS was introduced into a dry 100 ml graduated glass cylinder. The test sample was carefully levelled without compacting and the unsettled apparent – volume (Vo), was observed. The bulk density was calculated by using the following formula:

$$\rho b = \frac{M}{V_o} \text{ gm/ml}$$

Where; ρb = Apparent bulk density

M = Weight of sample

V_o = Apparent (untapped) volume of sample (Anonymous, 2006 and Gupta, 2013).

Determination of tapped density

After carrying out the bulk density by above procedure, sample containing graduated cylinder was tapped firstly for 500 times, followed by an additional taps of 750 and 1250 times in Tapped Density Apparatus (TD 1025) Lab India, until the difference between the two succeeding measurement is less than 2% and then tapped volume (Vf), was measured, to the nearest graduated unit. The tapped density was calculated, in gm per ml, using the following formula:

$$\rho tap = \frac{M}{Vf} \text{ gm/ml}$$

Where; ρtap = Tapped density

M = Weight of sample

Vf = Tapped volume of sample (Anonymous, 2006 and Gupta, 2013).

Determination of Carr's index

Carr's index was calculated by using the following formula:

$$\text{Compressibility index} = \left[\frac{\text{Tapped Density } (\rho tap) - \text{Bulk Density } (\rho b)}{\text{Tapped Density } (\rho tap)} \right] \times 100$$

Where; ρb = Bulk Density

ρtap = Tapped Density (Anonymous, 2006; Gupta, 2013).

Determination of Hausner's ratio

Hausner's ratio is an indirect index of ease of powder flow. It was calculated by the following formula (Anonymous, 2006; Gupta, 2013):

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density } (\rho tap)}{\text{Bulk Density } (\rho b)}$$

Qualitative Estimation

Alkaloids

Mayer's test: One ml of acidic aqueous extract of ADS was taken in a test tube and few drops of Mayer's reagent were added. If pale yellow precipitate is formed then it is the indication of presence of alkaloids (Anonymous, 2006).

Resin

Small quantity of ethanolic extract of ADS was dissolved in five ml of acetic anhydride solution and gently heated. After cooling, 0.05 ml of sulphuric acid was added. If bright purplish red colour rapidly changed into violet then it is indication of presence of resin (Anonymous, 2006).

Tannins

Five ml of aqueous extract of ADS was taken in a test tube and few drops of 1% solution of lead acetate were added. If yellow precipitate is formed then it is indication of presence of tannins (Anonymous, 2006).

Flavonoids

0.5 ml of alcoholic extract of ADS was taken in a test tube, 5-10 drops of diluted hydrochloric acid was added, after that small piece of zinc was added in this solution. This solution was boiled for few minutes. If pink colour is produced then it is indication of presence of flavonoids (Anonymous, 2006).

Glycosides

Small amount of alcoholic extract of ADS was dissolved in one ml of water, and sodium hydroxide solution was added in it. Yellow colour is produced in presence of glycosides (Anonymous, 2006).

Phenols

A small quantity of alcoholic extract of *Safoof* was dissolved in five ml of distilled water, and 5-8 drops of 1% solution of lead acetate was added. If yellow precipitate is formed then it is indication of presence of phenols (Anonymous, 2006).

Steroids

Salkowski Reaction: One ml of conc. sulphuric acid was added in two ml of chloroform extract of ADS carefully from the side of test tube. Red colour is produced in chloroform layer, in the presence of steroids (Anonymous, 2006).

Terpenoids

Five ml of aqueous extract of ADS was taken in a test tube and two ml chloroform was added, then three ml conc. sulphuric acid was mixed in solution. If reddish brown interface is formed then it is indication of presence of terpenoids (Pandey and Mishra, 2011).

Quantitative Estimation

Determination of volatile oils

50 gm of ADS was mixed with the 30 ml of glycerol and 300 ml of water in the flask and few pieces of earthenware was added. Clavenger's apparatus was attached to the flask and heated on heating mantle with frequent agitation for five hours. The flask was rotated occasionally to wash down the adhering material to the upper part of the flask wall. After five hours, distillation was discontinued. After five minutes of cooling, volume of volatile oil was observed in Clavenger's apparatus graduated tube. This process was repeated few times for obtaining constant reading (Anonymous, 2006; 2009 and Afaq, 1994).

Tannin Assay

Preparation of drug infusion: Three gm of *Safoof* was extracted in 250 ml DD water for four hours at room temperature and then filtered.

Quantitative estimation of Tannin:

For the analysis of tannin content in test drugs, 25 ml of drug infusion, prepared by the method given above, was taken into one litre conical flask, then 25 ml of indigo solution and 750 ml distilled deionised water were added. 0.1 N aqueous solution of $KMnO_4$ was used for titration until the blue coloured solution changed

to green colour. Then few drops at time were added until solution turned into golden yellow colour. The volume of 0.1 N KMnO4 solution required for titration was recorded. For blank test (without drug infusion), the mixture of 25 ml Indigo carmine solution and 750 ml DD H_2O was titrated with 0.1 N KMnO4 solution and the volume required for titration until solution turned into golden yellow colour was recorded. All samples were analyzed in triplicates.

Calculations

The tannin content (T %) in the sample is calculated as follows:

$$T\ (\%) = \frac{(V - V_0) \times 0.004157 \times 250 \times 100}{g \times 25}$$

Where;

T (%) = Tannin quantity in percentage

V = Volume of 0.1 N aq. solution of $KMnO_4$ for the titration of the test sample in ml.

V_0 = Volume of 0.1 N aq. solution of $KMnO_4$ for the titration of the blank sample in ml.

0.004157 = Tannin equivalent in 1 ml of 0.1 N aqueous solution of $KMnO_4$

g = Mass of the sample taken for the analysis in gm

250 = Volume of the volumetric flask in ml. (Atanassova *et al.*, 2009)

TLC finger printing

For the separation of different phytochemical constituents in the ADS, the methanolic extract was spotted manually using a capillary tube on pre-coated silicagel TLC plates 60 F 254 (layer thickness 0.25). The spotted plates were put into a solvent system- Toluene : Ethyl acetate : Formic acid (6 : 3 : 1). After the separation of phytochemical constituents, the plate was taken out and placed in oven at 110°C for drying. After drying, iodine vapours were used to visualize the spots. The colour of the spots were noted and R_f values were calculated by using the following formula (Karthika *et al.*, 2014):

$$\text{Retention time } (R_f) = \frac{\text{Distance travelled by spot}}{\text{Distance travelled by solvent}}$$

Results and Discussion

The organoleptic properties of ADS were found to be greenish yellow in colour, amorphous uniform particles with aromatic pungent taste (Table 2). These are

Table 2: Organoleptic Properties of ADS

Appearance	Amorphous powder
Colour	Goldenrod 3 (greenish yellow)
Odour	Aromatic pungent
Taste	Spicy

very important for drug identification and quality assurance and also necessary for patient compliance; the acceptance among the consumers automatically gets increased if these properties are good.

The alcohol and water soluble matter of ADS was found to be 23.964 ± 0.03 and 17.381 ± 0.06 respectively (Table 3). This shows that the constituents of the drug are more soluble in alcohol than water (Fig. 2).

Table 3: Physicochemical parameters of ADS

Parameters	**Samples**			**Mean ± SEM**
	1	**2**	**3**	
Alcohol soluble matter (%)	23.936	24.026	23.930	23.964 ± 0.03
Water soluble matter (%)	17.265	17.388	17.492	17.381 ± 0.06
Successive Extractive Values:				
Petroleum ether (%)	27.540	26.220	28.280	27.346 ± 0.60
Chloroform (%)	1.14	1.34	1.26	1.246 ± 0.05
Ethyl alcohol (%)	4.86	5.48	5.34	5.226 ± 0.18
Ash Values:				
Total ash (%)	6.036	5.950	5.966	5.984 ± 0.02
Acid insoluble ash (%)	1.484	1.623	1.380	1.495 ± 0.07
Water soluble ash (%)	1.949	2.171	2.178	2.099 ± 0.07
Moisture content (%)	2.999	3.997	3.989	3.661 ± 0.33
Loss of weight on drying (%)	7.217	7.178	7.073	7.156 ± 0.04
pH Value:				
1% solution	6.75	6.83	6.92	6.83 ± 0.04
10% solution	5.63	5.65	5.73	5.67 ± 0.03
Angle of repose	52.2238	50.4768	51.3401	51.3469 ± 0.50
Bulk density (gm/ml)	0.3191	0.3125	0.3333	0.3216 ± 0.00
Tapped density (gm/ml)	0.4285	0.4166	0.4285	0.4245 ± 0.00
Carr's index	25.5309	24.9879	22.2170	24.2452 ± 1.02
Hausner's ratio	1.3428	1.3331	1.2856	1.3205 ± 0.01

The mean percentage of successive extractive values of ADS were found to be 27.346 ± 0.60, 1.246 ± 0.05 and 5.226 ± 0.18 in petroleum ether, chloroform and ethanol respectively (Table 3). This is an important parameter to evaluate the quality, purity and adulteration of the drugs. The chemical constituents of the drugs are extractable in different solvent systems. These values are high in particular solvent in which the drug constituents are maximally soluble (Fig. 3).

The mean percentage of total ash, acid insoluble ash and water soluble ash were found to be 5.984 ± 0.02, 1.495 ± 0.07 and 2.099 ± 0.07 respectively (Table 3) (Fig. 4). Total ash includes both "physiological ash", which is derived from the plant tissue itself and "non-physiological ash" which is residue of the extraneous matter adhering to the plant surface. Acid insoluble ash includes the amount of silica present, especially as sand and siliceous earth (Bele *et al.*, 2011).

The mean percentage of moisture content by toluene distillation was found to be 3.661 ± 0.33 (Table 3) (Fig. 5). Low moisture content is always desirable for higher stability of drugs (Bhargava *et al.*, 2013). An excess of water in medicinal plant materials provide good media for microbial growth, and deterioration following hydrolysis.

The mean percentage of loss of weight on drying was found to be 7.156 ± 0.04 (Table 3) (Fig. 5). Higher value than the moisture content by toluene method shows the presence of volatile oil in the ADS. It indicates the loss of volatile substances along with the water.

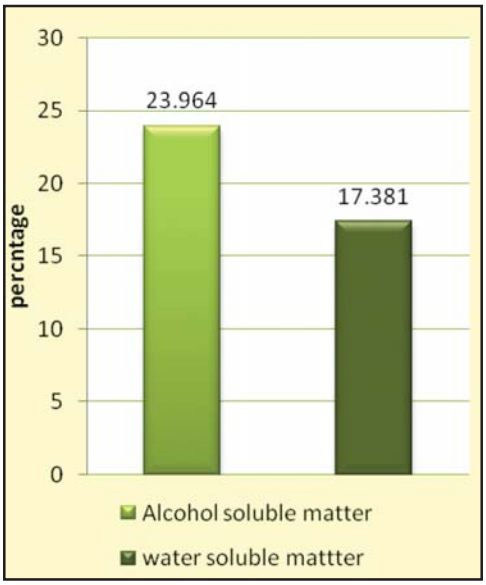


Fig. 2: Alcohol and Water soluble matter of ADS

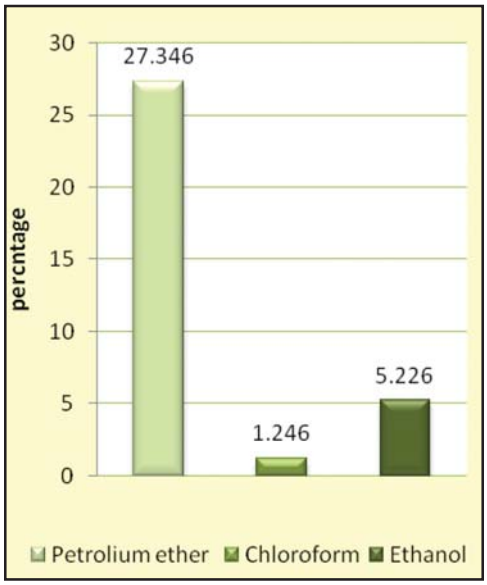


Fig. 3: Successive extractive values of ADS

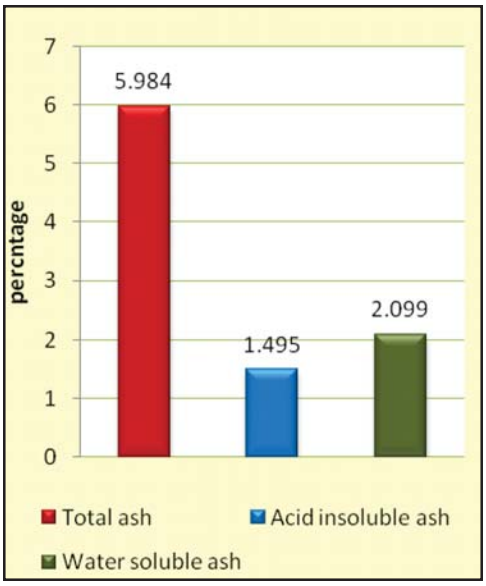


Fig. 4: Ash values of ADS

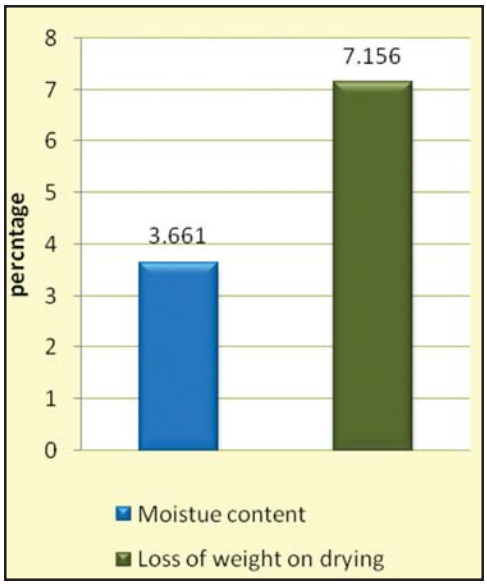


Fig. 5: Moisture content & Loss of wt. on drying of ADS

Angle of repose was found to be 51.3469 ± 0.50 (table 3). The result shows that inter-particular friction of particles of ADS is very high which shows its low flow ability.

The mean values of bulk density and tapped density of ADS were found to be 0.3216 ± 0.00 and 0.4245 ± 0.00 respectively (Table 3) (Fig. 7). Bulk and tapped density are very important while deciding the size of containers needed for handling, shipping, and storage of raw material and blend.

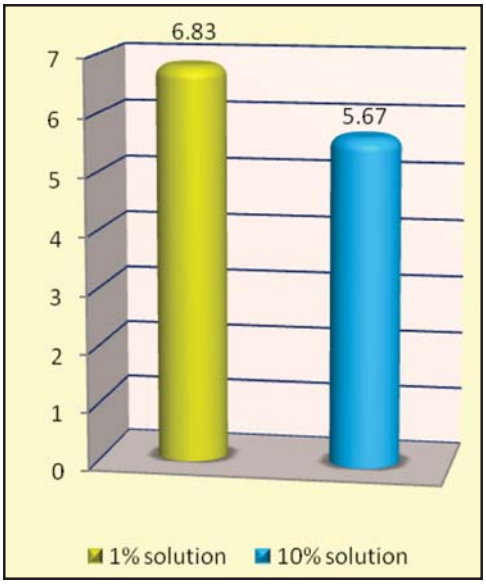


Fig. 6: pH values of ADS

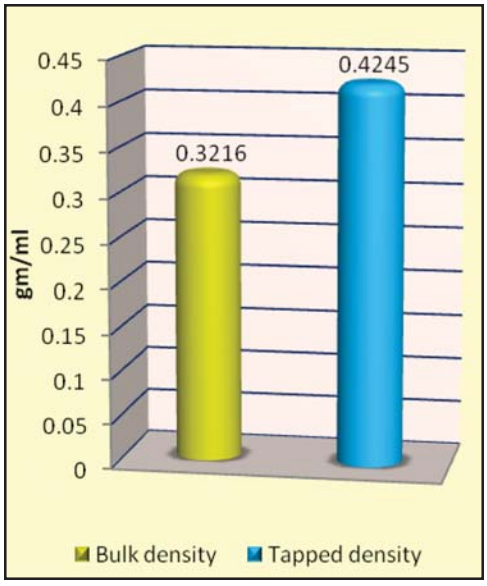


Fig. 7: Bulk density and Tapped density of ADS

The mean values of carr's index and hausner's ratio were found to be 24.2452 ± 1.02 and 1.3205 ± 0.01 respectively (Table 3) (Fig. 8) which is graded as passable flow ability. In theory, the less compressible a material the more flowable it is (Anonymous, 2006).

In qualitative estimation of ADS; alkaloids, resin, trannins, flavonoids, glycosides, phenols, steroids and terpenoids were examine for their presence which are responsible for the therapeutic effects and these were found to be positive (Table 4).

Volatile oils are plant's secondary metabolites that are known for their fragrance and food flavour properties. They consist of a complex mixture of mono and sesqui-terpenes, phenyl propanoids and oxygenated compounds (Bazaid *et al*, 2013). The mean percentage of volatile oil was found to be 3.066 ± 0.13 (Table 5) (Fig. 9).

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is that which causes the dry and puckery feeling in the mouth on consumption. Tannins may be employed medicinally in anti-diarrheal, haemostatic, and anti-haemorrhoidal compounds. Tannins have been used for immediate relief of sore throats, diarrhoea, dysentery, haemorrhage, and skin ulcers (Ashok, 2012). The mean percentage of tannin was found to be 2.54 ± 0.04 (Table 5) (Fig. 9).

Table 4: Qualitative estimation of ADS

S.No.	Medicinal constituents	Presence
1.	Alkaloids	+
2.	Flavonoids	+
3.	Glycosides	+
4.	Phenols	+
5.	Resin	+
6.	Steroids	+
7.	Tannins	+
8.	Terpenoids	+

Table 5: Volatile oil and Tannins of ADS

S.No.	Volatile oil (%)	Tannins (%)
1.	3.2	2.50
2.	2.8	2.63
3.	3.2	2.50
Mean ± SEM	3.066 ± 0.13	2.54 ± 0.04

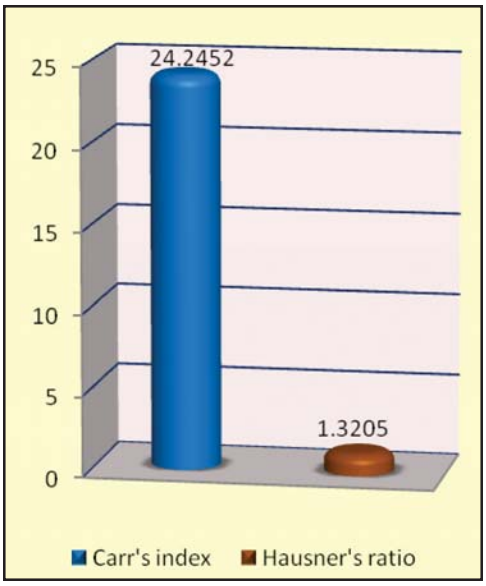


Fig. 8: Carr's index and Hausner's ratio of ADS

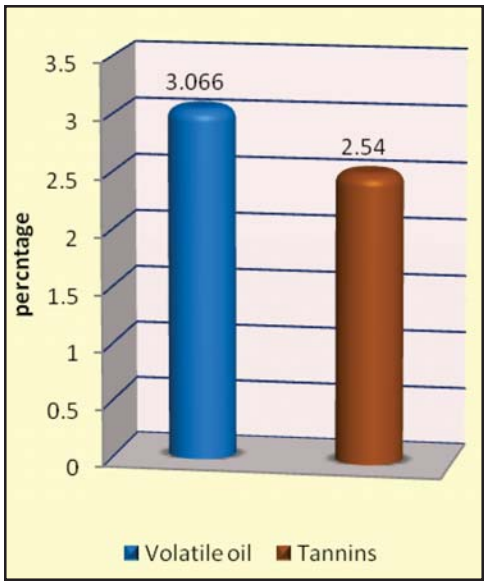
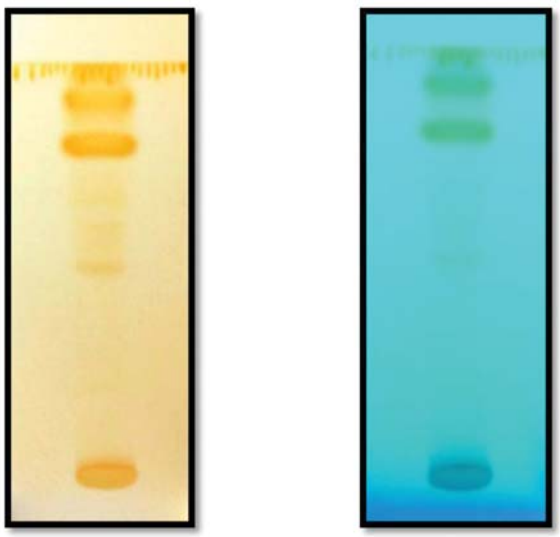


Fig. 9: Volatile oil and Tannins of ADS

TLC is one of the important parameters used for detecting the adulteration for evaluating the quality of drugs. If the drug is adulterated there might be appearance of the other compounds present in adulterant, in turn may increase the number of spots. On the other hand the exhausted or deteriorated drugs may lose the component and the number of spots appeared might be less. Five spots were found on TLC silica plate with the methanolic extract of ADS. The R_f values of five spots were found to be 0.50, 0.58, 0.66, 0.80 and 0.86 and the colour of spots were brown, light brown, light yellow, dark yellow and dark yellow respectively (Table 6) (Fig. 10).



Under visible light Under UV light

Fig. 10: TLC of ADS

Table 6: TLC of ADS

Extract	Solvent	No. of spots	R_f Value	Colour
Methanol	Toluene: Ethyl acetate: Formic acid (6 : 3 : 1)	5	0.50, 0.58, 0.66, 0.80 and 0.86	Brown, light brown, light yellow, dark yellow and dark yellow

Conclusion

The developed physicochemical standards of ADS may be used for future reference to check adulteration (if any) in the drug studied.

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