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Research Article

Formulation Studies on Hibiscus Sabdariffa (HS) Aqueous Extract

Y.J. Oyeniyi[1*], F.B.O. Mojiminiyi[2]

[1]*Department of Pharmaceutics & Pharm. Microbiology, Faculty of Pharm. Sciences, Usmanu Danfodiyo University, Sokoto ,Nigeria,* [2]*Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto , Nigeria*

ABSTRACT

Background: Hibiscus sabdariffa L. family Malvaceae is a perennial herbaceous medicinal shrub widely cultivated for its proven anti-hypertensive property

Objectives: The focus of this investigation is to formulate the aqueous extract of HS as oral tablets for accurate dosing and to ease the administration of the aqueous extract.

Method: Five formulations of HS granules were prepared by wet granulation technique and thereafter the flow characteristics of the granules were assessed through, angle of repose, Hausner's ratio, density measurements, moisture content and compressibility index determinations. Heckel and Kawakita analyses were however employed to study HS granules compactability, while the United State Pharmacopeia 2012 (USP) quality control tests were used to investigate the tabletting properties.

Result: There were significant differences ($p \leq 0.05$) in the values obtained for the granules flow, compaction and tabletting properties. The ranking order was the same for the angle of repose and the Hausner's ratio; HS2 ≤ HS3 ≤ HS4 ≤ HS5 ≤ HS1, implying HS2 having the best desirable flow characteristics. All the formulations underwent significant volume reduction under applied pressure, of which the PY and PK values ranked HS1 ≤ HS2 ≤ HS4 ≤ HS5 ≤ HS3 and HS4 ≥ HS5 ≥ HS2 ≥ HS3 ≥ HS1 respectively. All formulations except HS1 passed the quality assessment tests. HS1 even though had the fastest onset of plastic deformation; it however had the least quantitative plasticity and this may be responsible for its poor friability.

Conclusion: All HS Formulations except HS1 investigated had excellent tabletting properties that fell within the specifications of the United State Pharmacopeia. The high friability of HS1 may not be unconnected to its lack of binding agent and low plasticity even though it onset of plastic deformation was the fastest.

Keywords: Hibiscus sabdariffa; Plastic deformation; tableting; Heckel and Kawakita analyses.

INTRODUCTION

Hibiscus sabdariffa L. family Malvaceae (HS) is a perennial herbaceous shrub grown widely in India, west and east Africa for its importance in folk medicine. It is a tropical plant native to Asia countries but now widely grown in both tropical and subtropical countries for its leaf, fleshy calyx, seed and fiber [1, 2]. HS calyx is widely used in production of non-alcohol drink popularly called Sobo enjoyed by all and sundry, [3]. HS is probably one of the most beautiful flowers in the whole world with long history of uses such as its fragrant flowers used in the production of deodorant spray, the young fresh leaves for production of salad, the fibers used as jute substitutes, while the fleshy red calyxes are used in the preparation of jellies, jams, teas and drink, [4, 5].In traditional medicine, HS aqueous extract is use in treating hypertensive and stroke patients. Interestingly there exist extensive research reports confirming the hypotensive properties of HS aqueous extract in both laboratory animals and in human subjects [6, 7, 8]. Other therapeutic properties of HS reported include, antioxidant [9], hypocholesterolemic [10, 11],

Correspondence to: Y.J. Oyeniyi, Department of Pharmaceutics & Pharm. Microbiology, Faculty of Pharm. Sciences, Usmanu Danfodiyo University, Sokoto1, Nigeria, Tel no: 2348033472945; E-mail: drdeyinkaoyeniyi@gmail.com

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anti-obesity [12], insulin resistance reduction, skin cancer chemo preventive [13], diuretic [14], antimicrobial, and cytotoxic properties [15]. Phytochemical screening revealed that HS calyx as good source of carbohydrate, dietary fiber, protein, vitamins, minerals and bioactive compounds (flavonoids mainly anthocyanins, phytosterols, polyphenols, cardiac glycosides, saponins, alkaloids and organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids), [16,17,18,19]. The Anthocyanin content is said to be responsible for improving the function of blood vessels and also strengthens protein collagen, [20].There are several reports of HS aqueous extract significantly decreasing both the systolic and diastolic blood pressure in human subjects at a dose of 15mg/kg/day [21, 22, 23, 24,25, 26].

The therapeutic index of the extract is high with low degree of toxicity as reported LD50 ranged between 2,000 to 5,000mg/kg/day) [27]

This present investigation is devoted to developing HS aqueous extract into oral tablets thus ensuring accurate dosing, patients' compliance as a result of ease of administration of HS extract.

METHODS

Extraction

The extraction protocol adopted in this study was a modified version described by Kunle et al., 2002. The fresh red calyces of HS were harvested from the botanical garden of the department of pharmacognosy and drug development, Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto. The identification and voucher numbering were carried out by the department of biological sciences, Usmanu Danfodiyo University Sokoto. The calyces were thereafter air dried and size reduced into fine powder from which about 500g were measured and suspended in 2.7 L of hot water (50 OC) maintained under constant agitation with the aid of a magnetic stirrer for about 18 Hrs and thereafter filtered to obtained a red colored extract which was evaporated into a jelly mass using an hot air oven (Gallenkamp, Model OV-335, Vindon Scientific Ltd, UK), maintained at 60 0C, [28, 29].

Production of HS granules

HS granules were produced using the wet granulation technique according to table 1. Wet granulation technique (WGT) is a combination of series of sequential steps commencing with the weighing and mixing of both the API (HS) and the diluents. Since our API is in semi-solid state; require quantities of the diluent was weighed directly into a known quantity of HS jell, mixed thoroughly using a rotary mixer (VM-5 V-shaped blender, Tokuju, Japan), and thereafter screened through sieve mesh size 1500. The wet granules were collected into a clean stainless steel tray, spread out evenly and thereafter dried using hot air operating at 45 0C for about 2hr. The dried granules were rescreened, weighed and kept in airtight container until needed, [30, 31, and 32].

Table1: Batch formulation of HS tablets

Materials (Mg/Tab.)	HS1	HS2	HS3	HS4	HS5
API	275.0	275.0	275.0	275.0	275.0
MCS	217.0	144.5	144.5	Nil	Nil
Starch	Nil	Nil	Nil	144.5	Nil
Lactose	Nil	Nil	Nil	Nil	144.5
Gelatin	Nil	72.5	Nil	Nil	72.5
Acacia	Nil	Nil	72.5	Nil	Nil
NG	Nil	Nil	Nil	72.5	Nil
Mg. Stearate	7.3	7.3	7.3	7.3	7.3
Talc	0.7	0.7	0.7	0.7	0.7
Total	500.0	500.0	500.0	500.0	500.0

Characterization of HS Granules

The obtained dried HS granules were subjected to the following tests;

• Particle size analysis

The mean granule sizes (MGS) of each of the formulation were determined with the aid of laboratory sieves of various pore sizes (1000 µm to < 75 µm (Pan) arranged in descending order. Known weight of sample was placed in the topmost sieve, belt of the shaker properly engaged and the Endecott sieves shaker operated for 10 minutes. Fraction of sample retained on each sieve was collected and weighed, [33].

The MGS values were calculated using Eq.1

$$Mean\ granule\ size = [\Sigma\ (\%\ retained) \times (sieve\ size)]/100 \ldots\ldots\ldots\ldots \text{Eq1}$$

• Moisture content analysis

The moisture content was determined using protocol described by Crouter and Brien 2013, [34]. Know weight of the sample was placed in a humidity chamber. The sample was re-weighted after operating the chamber for 48 hours. The weight gain/loss was determined as in Eq2.

$$MC = \left[{}^{W1 - W2}/_{W1} \right] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq2}$$

• Angle of repose

This was determined using a powder flowability tester, (HMKFLOW 329, Dandong, HMKTEST Instrument Co. Ltd, China.). A known weight of granules was allowed to flow through the orifice of the instrument funnel fixed at an angle and height of about 45 ◦ and 8 cm respectively. The dimensions of height and radius of conical heap of powder formed were noted. The angle of repose was computed using Eq3, [33].

$Tan\ \theta$ = height of cone/0.5 × diameter .....Eq3.

• True, Bulk and Tapped densities

The loose volume (Vb) of a known weight (30g) of sample was noted in a measuring cylinder, while Vt was the volume of the same sample after 100 tapping of the measuring cylinder affording the sample particle rearrangement and realignment. The bulk (BD) and tapped TD) densities as well as other flow characteristics such as compressibility index (CI) and Hausner ratio(HR) of the samples were obtained using the expression in Eq 4-8. The true of densities (ρт) of the samples were determined using the pycnometeric technique with n- Hexane as displacement fluid and 4g of sample.

$$\rho_T = {4g \times 0.661}/{Weight\ of\ displaced\ n-Hexane} \quad \text{.....Eq 4.}$$

Where ρт is the true density of sample and 0.661 is the density of n-Hexane

$$BD = {30g}/{Vb} \quad \text{.....Eq5.}$$

$$TD = {30g}/{Vt} \quad \text{.....Eq6.}$$

$$HR = {Vb}/{Vt} \quad \text{.....Eq7.}$$

$$CI = 100 \times \frac{Vb-Vt}{Vb} \quad \text{.....Eq8.}$$

• Compaction studies on HS granules

Tablets weighing 500mg were produced using a Carver hydraulic press (model C, Carver inc. Menomonee Falls, WI) fitted with 12.5mm die and flat faced punches lubricated with a 1% dispersion of Magnesium Stearate in acetone before each compression. A total of twenty five (25) tablets were produced per formulation at varying pressure, (50,100,150,200 and 250 MPa), [35, 36]. All tablets were properly label and kept in a desiccator charged with silica gel for 24 h to allow for elastic recovery. And for each of the formulation the mean of tablet weight, diameter and thickness after three reading were noted. The compacts were evaluated using Heckel and Kawakita equations, [37, 38, 39]

$In\ (1/1-D) = KP + A$ .....Eq 9 (Heckel)

Where D is the relative density of the sample (calculated using Eq 9), K is constant (relating the plasticity of the sample), P is the applied pressure and A is related to the die filling and particle rearrangement before deformation and bonding of the discrete particles obtained from the intercept of Heckel plot on y-axis.

$$D = {weight}/{Volume \times \rho T} \quad \text{.....Eq 10}$$

$$\frac{P}{C} = \frac{P}{a} + \frac{1}{ab} \quad \text{.....Eq11 (Kawakita)}$$

The values of P represents the applied pressure, C is the degree of volume reduction, a & b are constants relating to the total degree of powder volume reduction and the yield strength of particles, respectively.

Tablet production and tabletting properties of HS

Using a single punch tabletting machine, (Type EKO, Erweka, Apparatebau-GmbH,

Germany) , HS tablets were produced as per the batch formulary (table 1). The tablets were properly labeled and kept in a desiccator for about 24 h for elastic recovery. The mean tablet weight, diameter and thickness were determined using digital weighing balance (EX 224, Ohaus, Switzerland) and a digital vernier caliper (model 500 Copley Scientific Nottingham UK). The properties of the resulting tablets were determined through the various tests prescribed by United States Pharmacopeia convention (USP2012).

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• Tablet crushing strength

The force required to diametrically break ten tablets from each batch were noted using a digital tablet hardness tester (porTAB 01, ToroTech, Canada). The mean of three determinations was recorded and taken as tablet crushing strength for the batch.

• Tablet weight lost

The weight lost recorded by twenty representative sample randomly picked per formulation using a Roche Friabilator, (Erweka TAR120, GmbH, Heusenstamm, Germany) operated at 25rpm for 4 min. The weight lost for each batch due to abrasion were computed and expressed as percentage of the initial weight of tablets, (Eq12)

$$F = {weight\ lost}/{Initial\ weight} \times 100 \quad \text{.....Eq12}$$

• Tablet disintegration time test

This was conducted us a BP disintegrating apparatus (model BJ-3, Ningbo Hinotek Instrument Co., Ltd, China). The mean time for six tablets placed in disintegrating basket to break into pieces and passed through the screen mesh at the bottom of the

basket was noted for each formulation. The temperature of the deionized water used as the disintegrating medium was maintained 37 OC and all other experimental conditions were as prescribed by USP 2020 [40].

Statistical analysis

The observed differences in both the compaction behavior and the tabletting properties among the different formulations were subjected to statistical analysis (ANOVA) using the student's t-test as a statistical tool. A 95% confidence interval ($p \leq 0.05$) was considered significant.

RESULTS AND DISCUSSION

Table2: HS granules characterizations

Granules Characteristics	HS1	HS2	HS3	HS4	HS5
Angle of repose(0)	48.82±0.13	29.1±0.02	30.6±0.03	31.6±0.06	31.8±0.06
True density (g/mL)	1.71±0.11	1.89±0.01	1.85±0.18	1.75±0.16	1.78±0.19
Bulk density (g/mL)	0.83±0.09	0.79±0.15	0.75±0.11	0.68±0.08	0.66±0.07
Tapped density (g/mL)	1.91±0.11	0.98±0.09	0.98±0.15	0.99±0.05	1.05±0.10
MGS (µM)	500.89±1.29	428±0.27	430.6±0.29	465±0.77	481±1.87
MC (%)	0.31±1.1	0.11±0.09	0.14±0.19	0.20±0.08	0.22±0.18
HR	2.3±0.01	1.24±0.09	1.31±0.16	1.46±0.05	1.59±0.05
CI (%)	56.5±0.14	19.4±0.06	23.5±0.16	31.3±0.11	37.1±0.24

The MGS and MC values differ from batch to batch and were ranged 428 -500 (µM) and 0.11- 0.31(%) respectively. These differences were significant, ($p \leq 0.05$) even though they were similarly ranked HS1≥HS5 ≥ HS4 ≥ HS3 ≥ HS2. Both MGS and MC values are dependent on the nature of the different polymeric material used as diluents and binder in the formulations, [41,42]. Careful selection of materials is therefore advocated. There were significant differences ($p \leq 0.05$) in the values of the true, bulk and tapped densities, (table 2). While the true density of a material is the density of the material without void, which go a long way to influence critical unit operations such mixing, granulation process, compression and compactability of granules [39], the bulk and tapped densities however control both the fluidity and the flow of granules during tablet compression and as such optimization of the granulation process is critical to achieving compacts of desire quality attributes [43, 44].

During tabletting operations, the granules are expected to flow freely from the hopper into the die cavity to guaranty uniformity of tablets weight, diameter and thickness. The flow of granules represent a critical parameter that must be control in order to produce tablets of uniform micrometrics as well as to prevent dose variation that may lead to drug therapy failure. The angle of repose is use to assess the ability of materials to flow freely and uniformly under gravity from the hopper to the die wall during tablet manufacturing process. Materials with angle of repose values below 25 are classified as excellent, values ranging from 26 to 40 are moderate, while values above 40 are generally poor flowing material requiring addition of flow imparting substances (glidants). The values of the angle of repose obtained differ significantly ($p \leq 0.05$), from batch to batch and are ranked HS2 ≤ HS3 ≤ HS4 ≤ HS5 ≤ HS1.

The ranking order for Ø above was basically the same for HR and CI.

CI is a measurement of material resistance to flow. [45, 46, 47]. Generally materials with high CI values have poor flow characteristics since they easily bond together as a result of high cohesive forces in play within the powder bed. HS1 had the highest values for CI, Ø and HR (table 2) indicating extremely poor flowing granules and may not be suitable for compression on a rotary press. There is therefore the need to consistently strike a balance between powder cohesiveness and the flow. [48, 49]

Table3: Tabletting properties of HS

Parameters	HS1	HS2	HS3	HS4	HS5
Av.Tab. Weight (g)	0.5±0.07	0.5±0.27	0.49±0.32	0.49±0.32	0.49±0.38
Friability (%)	≥ 1.00	0.20±0.32	0.22±0.22	0.34±0.32	0.34±0.38
Crushing strength (KgF)	4.5±2.05	5.5±0.32	5.5±0.32	4.5±2.05	4.5±2.05
Disintegration time test (Min)	9.4±2.35	6.0±1.32	6.0±1.32	6.6±1.32	6.6±1.32

There were significant batch to batch variations ($p \leq 0.05$) among HS tablets produced. (Table 3). The mean tablet weights and the crushing strengths ranged from 0.49±0.32 to 0.5±0.07 and 4.5±2.05 to 5.5±0.32 indicating total compliance with the official (USP 2012) specifications for tablet weight uniformity and tablets hardness, which guaranty both dose uniformity and resistance to breakage during transportation and handling.

However, HS1 tablets were highly friable even though it passed the official test for tablet hardness. This poor friability may be as

result of high MGS values which present less contact and bonding force within tablets [50]. All the batches of HS formulations investigated in this study passed the disintegration time test as the mean disintegration time test were all below 15 minutes. The differences observed for disintegration time test were significant (P≤0.05) and may not be unconnected with differences in the intrinsic properties of the polymeric materials employed as excipients in the batch formulary. [51]

Table4: Heckel and Kawakita Compaction properties of HS granules

		Heckel			Kawakita		
Formulations	Py (Mpa)	DA	DO	DB	a	b	PK
HS1	66.47	0.75	0.49	0.26	03.00	0.2500	4.00
HS2	166.7	0.86	0.41	0.45	22.00	0.0227	44.05
HS3	800.00	0.80	0.41	0.39	18.00	0.0277	36.10
HS4	210.53	0.83	0.39	0.44	38.00	0.0132	75.00
HS5	400.00	0.94	0.37	0.57	35.00	0.0143	69.93

The compaction parameters,(compactability) of HS granules were assessed through the Heckel and Kawakita plots, (Figure 1 & 2).

While Heckel plot relates the HS granules relative density, (natural log) to the compaction pressure, Kawakita plot expressed the degree of volume reduction of HS granule to the compaction pressure.

All HS formulations under investigation were compactable, but with different significant (p≤ 0.05) degree of compactability under applied pressure,(table 4).

Individual onsets of plastic deformation (Py) of HS formulations were obtained from the inverse proportion of the slop of Heckel plots and were ranked HS1 ≤ HS2 ≤ HS4 ≤ HS5 ≤ HS3.(table 4) .

High value of Py means that it take longer time for the material to deformed plastically which is an undesirable situation in tablet manufacturing processes since the resident time of the granules to form compacts in the rotary press are low.

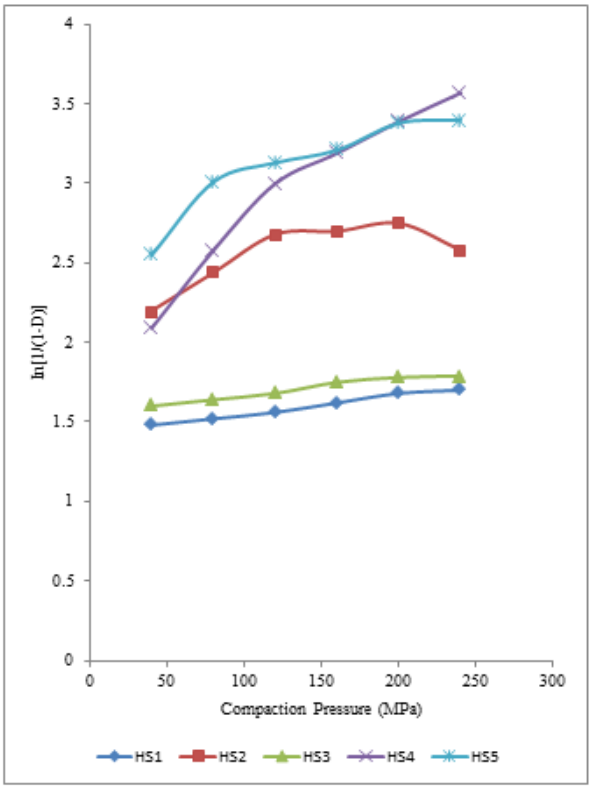

Figure1: Heckel plot for 5 batches of HS granules

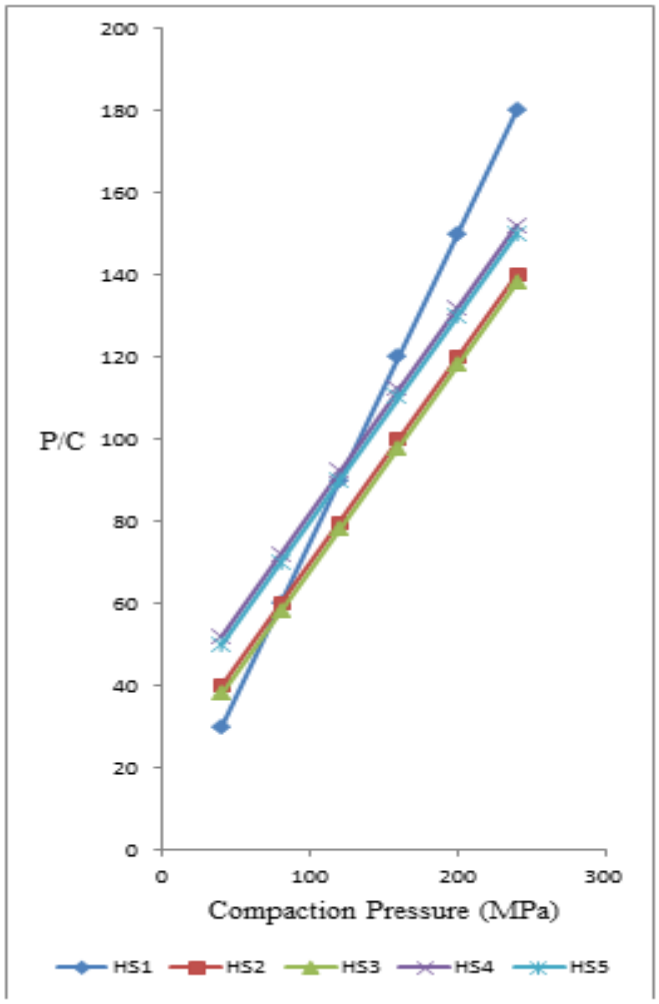

Figure2: Kawakita Plot

And as such a judicious selection of both the excipient and the granulation conditions is advocated. The degree of densification at the initial phase of compaction (DA) were obtained mathematically and were ranked, HS5 ≥ HS2 ≥ HS4 ≥ HS3 ≥ HS1. The low initial densification of HS1 may be connected to its lack of binder, revealing the important of binding agent in tablet formulation. The trend/order of consolidation due to particle in motion i.e. rearrangement at the initial stage DO and that due to fragmentation DB remain unchanged, (table 4).

The obtained data were equally fitted to Kawakita model with 0.99 correlations. All HS granules underwent volume reduction under compaction pressure to various degrees of densification (Figure2). The linear relationship between volume reduction and compaction pressure makes it possible to derive values for three key parameters, a, the minimum granules porosity just before compaction and its equal the y- axis intercept. The parameter b is a good measure of material plasticity and its reciprocal (PK) is the pressure that cause volume reduction by one-half (50%).The differences in the values of a and b are significant (p≤ 0.05) showing the effects of diluents and binders on the compaction behavior of HS granules. There is therefore the need for optimization and quantification of the formulations. The PK values ranged from 4.00 to 75.00 and were ranked HS1 ≤ HS3 ≤ HS2 ≤ HS5 ≤ HS4. There is a direct proportionality between PK values and the total plasticity of a material, therefore formulation HS1 underwent the least plastic deformation while HS4 had highest plastic deformation. The differences observed among the HS formulations may not be unconnected with different binders and diluents used and therefore there is a need to optimize the formulations through a critical evaluation of the contributing effects of nature and the concentration of various excipients on the compression and compaction of HS formulations.

CONCLUSION

HS aqueous extract was successful formulated into oral tablets which will ensure accurate dosing and ease its administration as adjuvant in the management of essential hypertension. Among the five formulations investigated in this study, only one of the formulations (HS1) failed the friability test conducted according to USP 2012 even though its onset of plastic deformation was the quickest judging by its least value of the obtained mean yield pressure. HS1 gave the lowest value for plastic deformation (PK) which may responsible for its failure of friability test and the fact that the formulation lack binder showing the critical role of binder in tablet formulations.

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