

Original Article

H. SABDARIFFA CALYCES ASH: ANTIANXIETY AND ANTIDEPRESSANT ACTIVITY EVALUATION AND PREPARATION OF ITS TABLETS

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ABSTRACT

Objective: Ash and its preparations have been used in Ayurveda for the treatment of various ailments since 7th century AD. Numerous studies suggest that the elements present in ash have significant role in affective disorders. Thus, the present study aimed at evaluating the antianxiety and antidepressant activity of total ash of *H. sabdariffa* calyces, and also of the tablets of total ash.

Methods: Powdered calyces were taken in tared silica crucible, and were incinerated at a temperature not exceeding 450 °C. The resultant ash was cooled and weighed. This was evaluated for antianxiety and antidepressant activity using an elevated plus maze and Porsolt's swim test, respectively. Further, the ash samples were analysed through ICP-MS to know their composition. The ash was formulated into tablets using the wet granulation technique, using only organic excipients.

Results: Results indicated that the total ash of *H. sabdariffa* calyces exhibited significant ($p < 0.001$) antianxiety and antidepressant activity at 50 and 100 mg/kg, po, respectively. The activities were comparable to the standard drugs. ICP-MS analysis showed the presence of magnesium, phosphorous, potassium and calcium as major elements. Tablets of *H. sabdariffa* total ash were as effective ($p < 0.001$) as the total ash.

Conclusion: Magnesium, phosphorous, potassium and calcium have been reported to play a significant role in affective disorders, explaining, thereby, as to why ash of *H. sabdariffa* calyces exhibited anti-anxiety and antidepressant activity.

Keywords: Total ash, Antianxiety, Antidepressant, Elevated plus maze, Porsolt's despair test, Tablets, ICP-MS, Elements

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INTRODUCTION

Affective disorders, especially anxiety and depression are among the most common psychiatric problems globally, with prevalence rates of 3.6 % and 4.4 %, respectively [1]. Anxiety is a frequent negative emotional state, manifested as unsettling feeling of concern or worry that is displayed by worried facial expressions and bodily responses like muscle tension, restlessness, impaired concentration, sleep disturbances and irritability [2]. Depression is characterized by recurring episodes of dysphoria and negative thinking that is reflected in behaviour [3]. Over the years, relationship between anxiety and depression has been extensively examined, and studies show that anxiety often precedes depressive disorders. Reported percentage comorbidity for anxiety and depression is estimated to be 70 % [4]. A wide range of therapeutic drugs are available for treatment of anxiety and depression, ranging from specific drugs like barbiturates, benzodiazepines to atypical drugs like buspirone as well as antipsychotics all of which exhibit various types of side effects [5]. Given the issues with current treatment options, patients are inclining towards complementary and alternative medicines (CAM), i.e., herbal remedies, meditation and spiritual practices, acupuncture and dietary practices; with more than 80 % of the population worldwide relying on herbs for the treatment of specific disorders [6, 7].

Bhasmas/metallic preparations have been extensively employed in Ayurvedic and Unani systems of medicine for the treatment of various ailments. Bhasmas are essentially ashes obtained through incineration. Numerous literature reports have shown that essential elements present in bhasmas are involved in the neurobiological mechanisms of various diseases including anxiety and depression. Deficiencies of these essential elements are associated with the pathogenesis of anxiety and depression [8-10]. Thus, taking cue from the literature, the present study was designed to evaluate the antianxiety and antidepressant activity of total ash of *H. sabdariffa* calyces. Calyces of *H. sabdariffa* (Malvaceae), popularly known as Red Sorrel (English) or Lal Ambari (Hindi), have been used in folk

medicine in the management of nervous disorders, and also as sedative, diuretic, diaphoretic, choleric, laxative, antifungal, antibacterial and antitussive [11, 12]. The authors have already reported that the ethanol extract of *H. sabdariffa* calyces exhibits significant antianxiety and antidepressant activity in mice [13].

MATERIALS AND METHODS

Plant material

Dried calyces of *H. sabdariffa* were procured from Earth Expo Company, Gujarat, India. These were authenticated by comparing their morphological and microscopical characters with those reported in the literature [14]. Voucher specimen (Number 252) of the calyces has been deposited in Herbarium-cum-Museum of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh.

Preparation of ash and determination of ash values

Powdered plant material (10 g) was taken in tared silica crucible, and incinerated in muffle furnace at a temperature not exceeding 450 °C until free from carbon. The resultant ash was cooled and weighed. Ash values (total, acid-insoluble and water-soluble) of the plant material were determined, in triplicate, following the procedures prescribed in USP [15].

Experimental animals

Lacca mice (either sex), housed at the Central Animal House, Panjab University, were allowed standard pellet diet (Ashirwad, Chandigarh) and water *ad libitum*. Groups of 6 mice (20-30 g) were used in all sets of experiments. The animals were fasted for 12 h before use. Approval (PU/IAEC/S/16/112) from the Institutional Animal Ethical Committee of Panjab University, Chandigarh was taken before carrying out biological studies. Standard group received diazepam (Jawa Pharmaceuticals Pvt. Ltd., Gurugram) or imipramine (Torrent Pharmaceuticals, Solan, India).

Dosing and treatment

Tween 80 (5%) in aqueous carboxymethylcellulose (CMC 0.5% w/w) was used as a vehicle for preparing the suspension of total ash. Doses were prepared by suspending appropriate quantities in the vehicle so as to administer these to mice in volumes ranging between 0.20-0.30 ml per oral route. Mice were randomly divided into control, test and standard drug groups, each comprising 6 animals. Control group was administered vehicle only. Test groups received the suspension of total ash or tablets of total ash of *H. sabdariffa* calyces.

Acute toxicity studies of the total ash

Acute toxicity studies of total ash of *H. sabdariffa* calyces were carried out on mice as per OECD 423 guidelines [16]. After 12 h of fasting, different groups of mice were administered a single oral dose (500, 1000 or 2000 mg/kg) of the total ash. Immediately after dosing, animals were observed for signs of toxicity during the first 0.5, 1, 2, 4, 8 and 12 h, and at every 24 h for 14 d. Behavioral parameters, tremors, lethargy, death, amount of water and feed taken were observed.

Antianxiety activity evaluation: elevated plus-maze model

Antianxiety activity was evaluated using elevated plus maze (EPM) model [17, 18]. The apparatus consisted of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof. It was kept elevated (25 cm) from the floor for evaluating the anxiolytic behavior. Doses were administered orally using tuberculin syringe fitted with an oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the EPM 60 min after the administration of the vehicle, diazepam or the test substance. Each mouse was placed at the centre of EPM with its head facing towards the open arm. During 5 min duration of the experiment, the behavior of the mouse was recorded as (a) the number of entries into the open arms and (b) mean time spent by the mouse in open arms. The arms of EPM were cleaned using ethanol-soaked cotton swab every time the animal was used for recording the results.

Antidepressant activity evaluation: porsolt's forced swim test

Forced swim test (FST) was used to evaluate antidepressant activity [18, 19]. Mice were forced to swim in a vertical Plexiglas jar (25×12×25 cm) containing water upto a height of 15 cm at room temperature (25±1°C). Mice were administered standard antidepressant imipramine or the test substance 60 min before the evaluation. After an initial period of vigorous activity to escape, the animals assumed a typical immobile posture (ceased to struggle with minimal limb movements just sufficient to keep their head above the level of water). Total immobility period during 6 min session was noted.

Antianxiety and antidepressant activity evaluation of total ash of *H. sabdariffa* calyces

The total ash of *H. sabdariffa* calyces was evaluated for antianxiety and antidepressant activity using EPM and FST, respectively at the dose of 25, 50, 100 and 200 mg/kg, po.

Preparation of tablets

The total ash of *H. sabdariffa* calyces was formulated as tablets (50 mg) using wet granulation technique. Ash of *H. sabdariffa* was mixed with lactose and dry starch in a polythene bag. This was followed by slow addition of 10 % starch paste to form a uniform mass which was passed through sieve (#16) and dried in hot air oven at a temperature not exceeding 50 °C. Dried granules were mixed with stearic acid and passed through a sieve (#20). The granules were compressed using direct compression tablet punching machine. Before compression, the dies and punches were lubricated with stearic acid. Three batches, each of hundred tablets, were prepared and stored in an airtight container at room temperature for further studies. Placebo was prepared in the similar manner. While preparing the tablets, it was ensured to use only organic excipients to prevent any elevation or diminution of activity due to the inorganic composition of the excipients. Both the placebo and *H. sabdariffa* total ash tablets were subjected to antianxiety and antidepressant activity evaluation using EPM and FST, respectively.

Evaluation of Pre-formulation and Post-formulation parameters

The tablet granules were subjected to pre-formulation parameters' evaluation (angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index) prior to compression. The prepared tablets were subjected to post-formulation parameters' (visual inspection, hardness, friability, uniformity in weight and disintegration time). The ash content uniformity of the tablets was determined gravimetrically by incinerating 20 tablets as per the procedure for preparing the total ash. The content determination was performed in triplicate. Both pre and post-formulation parameters were evaluated following the procedures prescribed in USP, 2014 [15].

Antianxiety and antidepressant activity evaluation of tablets of total ash of *H. sabdariffa* calyces

Placebo and *H. sabdariffa* total ash tablets were evaluated for antianxiety and antidepressant activity at dose of 50 and 100 mg/kg, respectively.

Elemental analysis of total-ash: ICP-MS technique

The total-ash of *H. sabdariffa* calyces was analyzed for quantitative estimation of different elements using inductively coupled plasma-mass spectrometer (Agilent 7900, IIT Delhi).

Antianxiety and antidepressant activity evaluation of major elements in total ash of *H. sabdariffa* calyces

Salts of the major elements (Mg, K and Ca) present in the total ash of *H. sabdariffa* calyces were evaluated for antianxiety and antidepressant activity using EPM and FST, respectively. Doses of the salts (MgSO₄, K₂SO₄ and CaCO₃) were calculated according to the percentage concentration of each element present in the total ash.

Statistical analysis

The data have been expressed as mean±standard error of mean (SEM). Significant differences among the groups were assessed by one way analysis of variance (ANOVA) using GraphPad Prism 5. The test was followed by Tukey's multiple range test; p values less than 0.001 were considered as significant.

RESULTS

Determination of ash values of *H. sabdariffa* calyces

Table 1 shows the results of ash value determination of *H. sabdariffa* calyces.

Table 1: Ash values of the *H. sabdariffa* calyces

Ash	Ash values* (% w/w) (Mean#±SEM)
Total-ash	7.19±0.008
Acid insoluble ash	0.53±0.005
Water-soluble ash	1.20±0.005

*dry weight basis, #n=3

Acute toxicity studies

No toxic effects were observed in the total-ash of *H. sabdariffa* calyces up to a dose of 2000 mg/kg.

Preparation of tablets

Table 2 shows the formula for placebo and *H. sabdariffa* total ash tablets.

Table 2: Formula of placebo and *H. sabdariffa* total ash tablets

Ingredients	Quantity per tablet (mg)	
	Placebo	<i>H. sabdariffa</i> total ash tablets
Ash	-	1
Stearic acid	2	2
Lactose	29	26
Starch dry	8	6
Starch paste	q. s.	q. s

Pre formulation and post formulation parameters' evaluation

The results of pre and post formulation parameters' evaluation are depicted in table 3 and 4, respectively.

Table 3: Results of pre-formulation parameters' evaluation

S. No.	Parameter	Placebo		<i>H. sabdariffa</i> total-ash tablets	
		Observation	Inference	Observation	Inference
1.	Angle of repose	25.26 °	Excellent	25.52 °	Excellent
2.	Bulk density	0.47 g/ml	-	0.49 g/ml	-
3.	Tapped density	0.54 g/ml	-	0.56 g/ml	-
4.	Hausner's ratio	1.14	Good	1.14	Good
5.	Carr's index	12.96 %	Good	12.50 %	Good

Table 4: Mean[±]SEM values of post-formulation parameters' evaluation

S. No.	Evaluation parameter	Placebo	<i>H. sabdariffa</i> tablets
1.	Diameter	5.08±0.005 mm	5.06±0.005 mm
2.	Thickness	2.17±0.005 mm	2.20±0.005 mm
3.	Hardness	5.60±0.005 kg/cm ²	6.04±0.005 kg/cm ²
4.	Friability*	0.17±0.005 %	0.19±0.005 %
5.	% weight variation [#]	5.30±0.005 %	4.39±0.005 %
6.	Disintegration time ^{\$}	5.40±0.005 sec	35.00±0.005 sec
7.	Drug Content Estimation ^{and}	0±0.00 mg	1±0.00 mg

**n=3, USP, 2014 prescribed limits. *: ≤ 1 %; #: ±10 %; \$: ≤ 15 min; and: ±10 %

Antianxiety and antidepressant activity evaluation of total ash and its tablets of *H. sabdariffa* calyces

Administration of diazepam (2 mg/kg) significantly increased the number of entries and the time spent in the open arms compared to the control group. Both the total ash of *H. sabdariffa* calyces and its

tablets thereof exhibited significant antianxiety activity at a dose of 50 mg/kg (fig. 1 and 2). Similarly, at a dose of 100 mg/kg, these demonstrated a statistically significant diminution of immobility time when the animals were subjected to FST (fig. 3 and 4). Results of imipramine (10 mg/kg) were similar to results of those observed with the total ash.

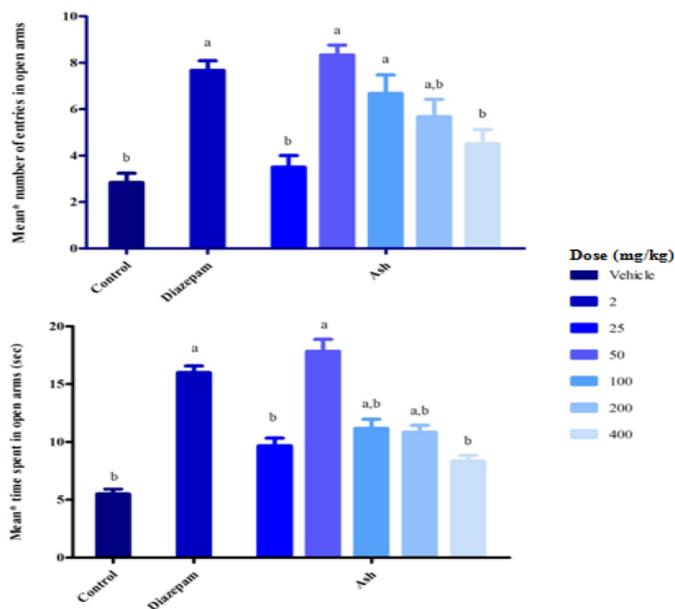


Fig. 1: Antianxiety activity profile of total-ash of *H. sabdariffa* calyces using EPM. The data is expressed as mean±SEM; *n=6; ^ap<0.001 vs control; ^bp<0.001 vs diazepam; one way ANOVA followed by Tukey's multiple range tests

Table 5: Results of quantitative estimation of elements present in total ash of *H. sabdariffa* calyces by ICP-MS technique

Element	% Concentration	Element	% Concentration
Ag	0.000004	Mg	0.316655
Al	0.029504	Mn	0.029390
As	0.000010	Ni	0.000339
Au	0.000001	P	0.020696
Ca	0.297205	Pb	0.000061
Cd	0.000009	Pt	0.000000
Co	0.000037	Se	0.000016
Cu	0.001341	Sn	0.000046
Fe	0.029330	Zn	0.004037
K	1.471199	-	-

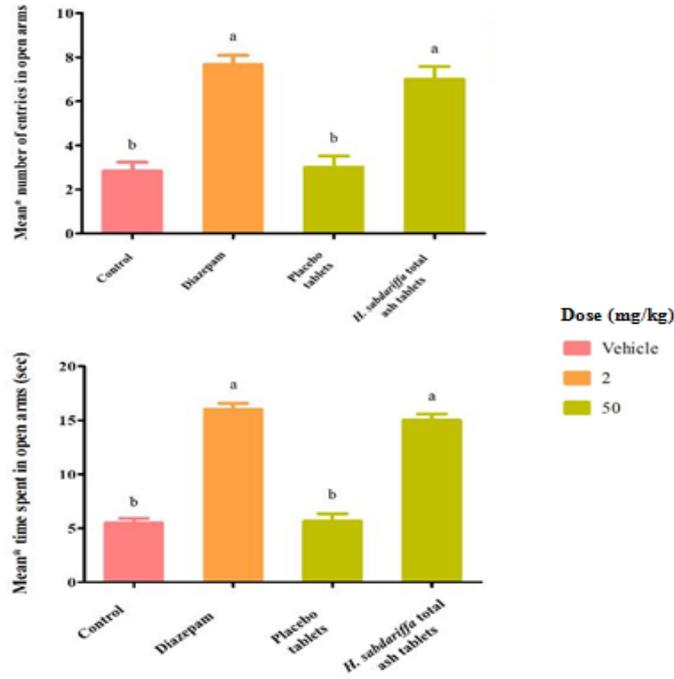


Fig. 2: Antianxiety activity profile of Placebo and *H. sabdariffa* total ash tablets using EPM. The data is expressed as mean±SEM; *n=6; ^ap<0.001 vs diazepam; ^bp<0.001 vs control; one way ANOVA followed by Tukey’s multiple range test

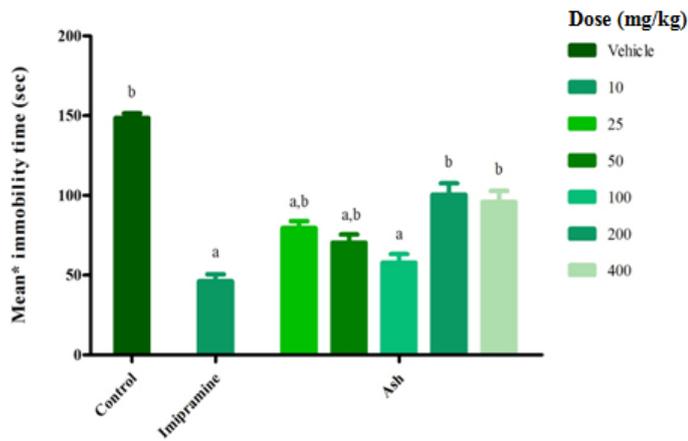


Fig. 3: Antidepressant activity profile of total ash of *H. sabdariffa* calyces using FST. The data is expressed as mean±SEM; *n=6; ^ap<0.001 vs control; ^bp<0.001 vs imipramine; one way ANOVA followed by Tukey’s multiple range test

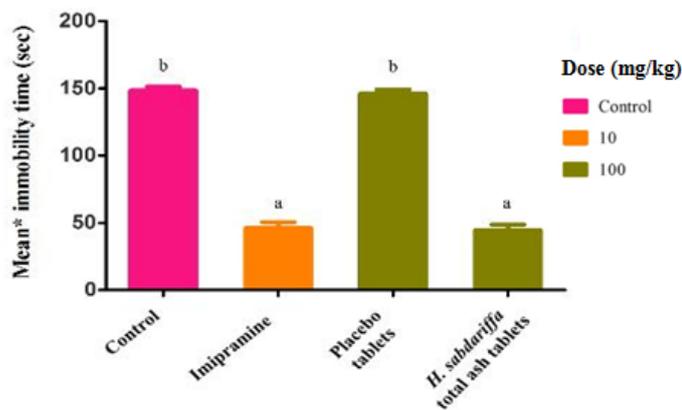


Fig. 4: Antidepressant activity profile of placebo and *H. sabdariffa* total ash tablet using FST. The data is expressed as mean±SEM; *n=6; ^ap<0.001 vs imipramine; ^bp<0.001 vs control; one way ANOVA followed by Tukey’s multiple range test

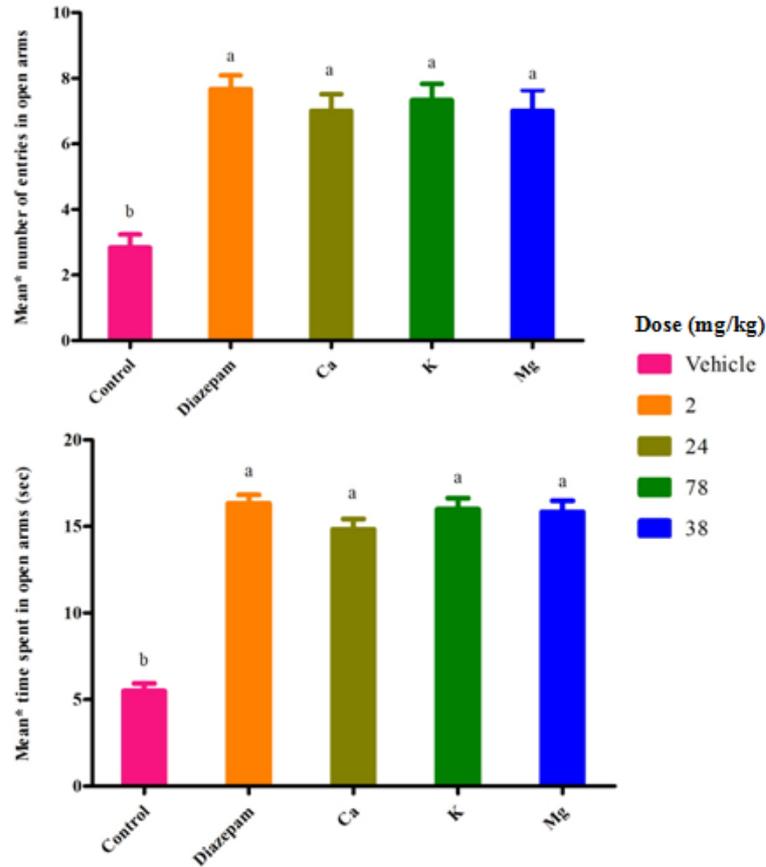


Fig. 5: Antianxiety activity profile of major elements present in the total ash of *H. sabdariffa* calyces, using EPM. The data is expressed as mean±SEM; *n=6; ^ap<0.001 vs diazepam; ^bp<0.001 vs control; one way ANOVA followed by Tukey's multiple range test

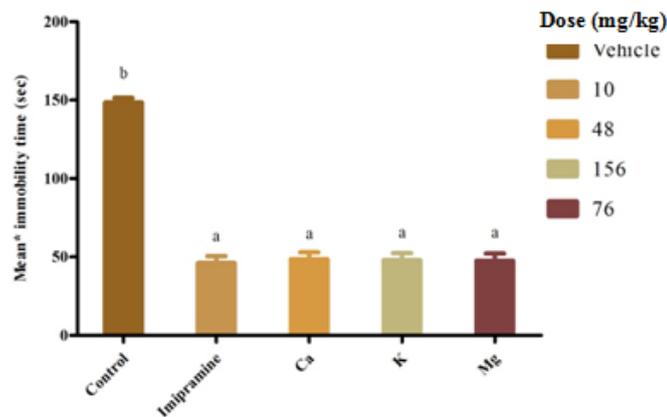


Fig. 6: Antidepressant activity profile of major elements present in the total ash of *H. sabdariffa* calyces, using FST. The data is expressed as mean±SEM; *n=6; ^ap<0.001 vs imipramine; ^bp<0.001 vs control; one way ANOVA followed by Tukey's multiple range test

Elemental analysis of total-ash: ICP-MS technique

Results of the ICP-MS of total ash of *H. sabdariffa* calyces evinced K, Mg and Ca to be the elements with maximum % concentration. Table 5 shows the percentage concentration of different elements in the total-ash of *H. sabdariffa* calyces.

Antianxiety and antidepressant activity evaluation of salts of major elements present in total ash of *H. sabdariffa* calyces

The salts of major elements exhibited significant antianxiety and antidepressant activity, comparable to the standard anxiolytic and

antidepressant agents, respectively. The results of antianxiety and antidepressant activity are depicted in fig. 5 and 6, respectively.

DISCUSSION

Results of the present study indicate that the total ash of *H. sabdariffa* calyces exhibit significant antianxiety and antidepressant activity at 50 and 100 mg/kg, respectively. The activities were comparable to the standard antianxiety and antidepressant drugs diazepam and imipramine, respectively. However, the major concern associated with powder formulations is ingestion of exact dose and low palatability. Thus, to overcome this problem, the total ash of *H.*

sabdariffa calyces was formulated into tablets using organic excipients to prevent any elevation or diminution of activity due to inorganic excipients. Prior to the formulation of tablets, various pre-formulation parameters (angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index) were evaluated for determining the physical properties of active ingredients and excipients. Both the placebo and *H. sabdariffa* total ash tablets conformed to the prescribed norms for the pre-formulation studies. Further, the post formulation parameters were evaluated for the placebo and *H. sabdariffa* total ash tablets to check any defects in the structural integrity of the tablets, and to determine the uniformity of content of the tablets. Both placebo and *H. sabdariffa* total ash tablets were observed to conform to the laid down standards. The tablets were subjected to antianxiety and antidepressant activity evaluation to compare its activity with that of the total ash, and to observe if there were any changes due to the excipients added in the tablets. Tablet of *H. sabdariffa* total ash exhibited the same level of antianxiety and antidepressant activity as that of the total ash. However, as expected, placebo tablets were devoid of these activities.

Results of the ICP-MS analysis evinced K, Mg and Ca to be the major elements in the total ash of *H. sabdariffa* calyces. Numerous studies have indicated that Mg, K and Ca play significant role in the pathogenesis of anxiety and depression and deficiencies of these are known to trigger the symptoms of anxiety or depression [9, 10]. Thus, to ascertain their role in anxiety and depression, the antianxiety and antidepressant activity of commonly available salts of Mg, K and Ca, i.e., MgSO₄, K₂SO₄ and CaCO₃ were evaluated. The dose of various elements was selected based on the relative % concentration of the three major elements (Mg, K and Ca) in the total ash of the plant material. The three salts exhibited significant antianxiety and antidepressant activity at their respective doses. Thus, the antianxiety and antidepressant activity of *H. sabdariffa* total ash may be attributed to high concentrations of Mg, K and Ca; which are known to have a significant role in affective disorders [9, 10].

CONCLUSION

The present study indicates that total ash of *H. sabdariffa* calyces exhibits significant antianxiety and antidepressant activity in mice on EPM and FST, respectively. Also, the antianxiety and antidepressant activity of the total ash may be attributed to the elements, majorly magnesium, potassium and calcium, which are known to have significant role in pathogenesis of affective disorders. Thus, the results substantiate the role of elements in affective disorders.

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CONTRIBUTION OF AUTHORS

Findings reported in this manuscript are a part of PhD project. The project has been conceived by Professor Anupam Sharma and Dr

Ashwani Kumar who are the research supervisors of Ms Gulshleen, SRF. Latter has carried out all the laboratory work.

CONFLICT OF INTERESTS

None

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