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
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.

| 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                    |

*d 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.

| Ingredients        | Quantity per tablet (mg) |
|--------------------|--------------------------|
| Ash                | Placebo *H. sabdariffa* total ash tablets |
| Steric 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            | Inference | H. sabdariffa total-ash tablets | 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                        | Inference | H. sabdariffa tablets           | Inference |
|-------|----------------------|--------------------------------|-----------|---------------------------------|-----------|
| 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/cm²              |           | 6.04±0.005 kg/cm²              |           |
| 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                |           | 3.50±0.005 sec                |           |
| 7.    | Drug Content Estimation** | 0±0.00 mg                  |           | 1±0.00 mg                      |           |

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.

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; \(^{\ast}p<0.001\) vs diazepam; \(^{\ast\ast}p<0.001\) 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; \(^{\ast}p<0.001\) vs control; \(^{\ast\ast}p<0.001\) 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; \(^{\ast}p<0.001\) vs imipramine; \(^{\ast\ast}p<0.001\) 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; *p<0.001 vs diazepam; *p<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; *p<0.001 vs imipramine; *p<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.*
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*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

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CONFLICT OF INTERESTS

None

REFERENCES

1. Depression and other common mental disorders: global health estimates, World Health Organization; 2020.
2. Nuss P. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. Neuropsychiatr Dis Treat 2015;11:165-75.
3. Fried EI, Epskamp S, Nesse RM, Tueterlinckx F, Borsboom D. What are ‘good’ mental health symptoms? Comparing the centrality of DSM and non-DSM symptoms of depression in a network analysis. J Affect Disord 2016;189:314-20.
4. Zhiguo WU, Yiru FANG. Comorbidity of depressive and anxiety disorders: challenges in diagnosis and assessment. Shanghai Arch Psychiatry 2014;26:271-37.
5. Guzman Gutierrez SL, Chilpa RR, Jaime HB. Medicinal plants for the treatment of nervios, anxiety, and depression in mexican traditional medicine. Rev Bras Farmacogn 2014;24:591-608.
6. Patricia M, Barnes MA, Barbara Bloom MPA. National health statistics reports; no 12. National center for Health Statistics. USA, Hyattsville; 2008.
7. Chaikelis AS. The thiamine content of herbs and medicinal plants. J Am Pharm Assoc 1946;35:343-6.
8. Pal S. The ayurvedic bhasma: the ancient science of nanomedicine. Recent Patents Nanomed 2014:5:12-8.
9. Młyniec K, Davies CL, de Aguerio SIG, Pytka K, Budziszewska B, Nowak G. Essential elements in depression and anxiety. Part 1. Pharmacological Reports 2014;66:534-44.
10. Młyniec K, Gaweł M, Doboszewska U, Starowicz G, Pytka K, Davies CL, et al. Essential elements in depression and anxiety. Pharmacol Reports 2015;67:187-94.
11. Sastri BN. The wealth of India, a dictionary of Indian raw material and industrial products. Vol. 3. CSIR, New Delhi; 1952.
12. Amos S, Bindal L, Chindo BA, Tsjea A, Odutola AA, Wambebe C, et al. Neuropharmacological effects of *Hibiscus sabdariffa* aqueous extract. Pharm Biol 2003;41:325-9.
13. Pansar G, Kumar A, Sharma A. *In vivo* antianxiety and antidepressant activity of calyx extracts. J Pharm Res 2017;11:962-6.
14. Ismail Z, Malaysia KK, Ismail N, Lassa J. Malaysian herbal monograph; 1999.
15. The United States Pharmacopoeia. Vol. 2. United Book Press, Inc; 2014.
16. OECD Guidelines for testing of chemicals: Acute oral toxicity-Acute toxic class method. Test no. 423, OECD, Paris; 2001.
17. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149-67.
18. Vogel HG, Vogel WH. Drug discovery and evaluation: pharmacological assays. 1st ed. Springer Berlin Heidelberg; 2013.
19. Porsolt RD, Antony G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379-91.

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