ABSTRACT

Free radicals can be generated either from normal cell metabolisms which are generated during various metabolic reactions of the body. Antioxidants are an important property by which living organisms can neutralize the toxic and cell-damaging molecules called free radicals. Antioxidants have innumerable medical significances and absence of undesirable side-effects. Medicines and their scientific validation keep growing due to its complications such as heart attack, cancer, diabetes, malaria, and herbs are found to be used as medicine against many health conditions in rural India since ancient times including the northeastern (NE) region. In this part of India, several wild plants are traditionally used medicinal plants have been the source of health care system in rural India since ancient times including the northeastern (NE) region. Due to its pharmacological properties, the present study was aimed to investigate the phytochemical, antioxidant and heavy metal content of the plants.

INTRODUCTION

Traditionally used medicinal plants have been the source of health care system in rural India since ancient times including the northeastern (NE) region. In this part of India, several wild plants and herbs are found to be used as medicine against many health complications such as heart attack, cancer, diabetes, malaria, jaundice, wound healing, microbial infections, helminthic infections, inflammation, etc [1]. The use and attention towards ethno medicines and their scientific validation keep growing due to its innumerable medical significances and absence of undesirable side-effects. The various medicinal properties present in a plant can be attributed to the diversities of phytochemicals present in the plant. Antioxidant is an important property by which living organisms can neutralize the toxic and cell-damaging molecules called free radicals which are generated during various metabolic reactions of the body. Free radicals can generate either from normal cell metabolisms in situ or from external sources such as pollution, cigarette smoke, radiation, medication etc. In addition, a by-product of oxidative phosphorylation called reactive oxygen species (ROS) is also a free radical, the accumulation of which can lead to many chronic and degenerative diseases like cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [2]. Various antioxidant molecules or enzymes have the potential to exert their biological activity and scavenge ROS by quenching chain initiating catalysis, metal ion chelation, co-antioxidants, or by gene expression regulation [3, 4].

Mineral elements play vital functions in the body which include components of structural proteins, cofactors and activator of enzymes, regulators of nerve transmission, muscle contraction, osmotic pressure and salt-water balance etc. [5]. It has been reported that metals such as cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel, selenium, zinc, etc are essential nutrients required for various biochemical and physiological functions. Inadequate supply of these micro-nutrients results in a variety of deficiency diseases or syndromes [6]. Several scientific studies have also proved that plants are a good source of trace elements and micronutrients. However, the presence of certain heavy metals such as cadmium, lead etc. are toxic to human body affecting multiple health complications including kidney, bone, neurobehavioral and developmental disorders, high blood pressure and cancer [7, 8]. In view of these facts, several studies have been conducted around the world to ensure plants as an alternative and safe source of antioxidant and micronutrients. In a recent study, Acorus calamus Linn. and Hibiscus rosa-sinensis L. showed significant anthelmintic activity against the cestode parasite, Hymenolepis diminuta [9, 10]. Cynodon dactylon L. Pers and Elaeocarpus floribundus also showed anthelmintic efficacy against Hymenolepis diminuta, both in vitro and in vivo [11].

Acorus calamus Linn. (Acoraceae) commonly known as the sweet flag is a perennial herb possessing numerous medicinal properties and have been widely used against several health complications such as diarrhea, bronchial catarrh, fevers, cough, asthma etc [12, 13]. A plethora of literature revealed the biological properties of the plant such as antimicrobial, insecticidal etc. and several phytochemicals like α-asarone, (E)-methyl Isoeugenol, methylcucumenol, β-asarone, α-cedrene have been isolated [14, 15]. Hibiscus rosa-sinensis L. belonging to the family Malvaceae is a popular medicinal plant of NE region used against diseases like dysentery, diarrhea, fever, cough and several others [10, 16]. Cynodon dactylon (L.) Pers belonging to the family Poaceae, commonly known as Bermuda grass is known for several biological activities such as antimicrobial, antiviral, antioxidant, anti-diabetic, hypolipidemic and hepatoprotective effects and phytochemicals like apigenin, luteolin, 6-C-pentosyl-8-C-hexosyl apigenin etc. have been...
reported from the plant [17]. Elaeocarpus floribundus (Elaeocarpaceae) is a plant that grows well in lowland hills and mountains of India, Burma, Thailand, Vietnam, Malaysia and Indonesia [18]. Along with the traditional use of the plant for making pickles, it is also used as an anthelmintic against helminth infections in NE region. In view of medicinal properties, the present study has attempted to study the phytochemical, antioxidant and trace element of Acorus calamus, Hibiscus rosa-sinensis, Cynodon dactylon and Elaeocarpus floribundus.

MATERIALS AND METHODS

Chemicals and drugs

Ascorbic acid; gallic acid; quercetin; aluminum chloride (AlCl3); ferric chloride (FeCl3); folin-Ciocalteu; bovine serum albumin (BSA); oxalic acid; 1,1-diphenyl-2-picrylhydrazyl (DPPH); thiobarbituric acid (TBA); trichloroacetic acid (TCA); 2,4,6-tripyridyl-s-triazine (TPTZ); sulphuric acid; hydrochloric acid; ammonium molybdate; potassium ferriyanide [K3Fe(CN)6]; chloroform and alcohols were purchased from Himedia and SRL (India). All chemicals used were analytical grade.

Collection, identification, and preparation of crude extract of plants

Acorus calamus Linn. (rhizomes), Hibiscus rosa-sinensis L. (leaves), Cynodon dactylon L. Pers (whole plant) and Elaeocarpus floribundus (leaves) were collected from three different districts of Tripura, India. Prior permission was taken from the local heads for the collection of the plants. The collected plant materials were washed with tap water and air dried at room temperature. All the dried plant materials were ground to powder and extracted using soxhlet equipment at 40 °C for 4-5 h. The process was repeated for three times and the filtrate obtained was evaporated in a rotary evaporator. Dry, semi-solid extracts (crude extract) were kept in plastic vials at 4 °C for further use.

Phytochemical study

Carbohydrate

The presence of total carbohydrate content of plant extract was estimated following the anthrone method as described by [19]. Values were expressed as µg/mg plant extract using the calibration curve of glucose.

Protein

The protein content of all the plant extracts was estimated following the method of [20]. Values were expressed as µg protein/mg plant extract using the calibration curve of BSA.

Total phenolic content (TPC)

The TPC of all the four plant extracts was estimated using folin-ciocalteu reagent and sodium carbonate [21]. The amount of TPC was calculated from a calibration curve of gallic acid and the results were expressed as µg gallic acid equivalent (GAE)/mg plant extract.

Total flavonoid content (TFC)

The flavonoid content of the plant was determined by the determination of yellow color using the AlCl3 method of [22]. The TFC was calculated from the standard curve of quercetin and the values were represented as µg quercetin equivalent (QE)/mg plant extract.

Antioxidant study

Total antioxidant capacity (TAC) assay

The TAC of the plant extract was done following the method of [23] using ammonium molybdate reagent and the absorbance was measured at 905 nm. TAC was expressed as µg ascorbic acid equivalent (AAE)/mg plant extract.

DPPH assay

The DPPH scavenging activity of the plant extracts was estimated using DPPH reagent and the absorbance read at 517 nm [24]. The scavenging activity of plant extract was calculated using the formula:

\[
\text{DPPH Scavenging activity (µg) = } \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]

Abs control = absorbance of DPPH and methanol.

Abs sample = absorbance of DPPH and plant extract or ascorbic acid.

FRAP assay

FRAP assay was performed using TPTZ and FeCl3 reagents following the method of Benzie and Strain 1996 with slight modifications as described by [25] and color developed was measured using spectrophotometer at 593 nm. The FRAP activity of plant extracts was compared with the standard ascorbic acid.

Heavy metal analysis

Five essential elements—copper (Cu), zinc (Zn), cobalt (Co), manganese (Mn) and iron (Fe) and three toxic elements such as lead (Pb), chromium (Cr) and cadmium (Cd) of all the four plants were analyzed using atomic absorption spectrophotometer (AAS) following the method of [26]. Briefly, 0.5 g of each plant sample was digested with conc. HNO3:HCl (3:1) at 85 °C and kept overnight. This was followed by heating the mixture until the NO3 fumes ceased. Then conc. HC1O4 (2 ml) was added. The solutions were filtered and diluted to 50 ml of distilled water. The blank solution was taken as the same procedure. The samples were then analyzed in AAS (Perkin Elmer 3110).

Statistical analysis

All data are presented as means±standard deviation (SD) for at least three replications for each experiment. Calibration curves were prepared using a linear curve. Statistical analysis was performed using one-sample t-test. The results are considered to be significant at P<0.05.

RESULTS AND DISCUSSION

Phytochemical analysis

Plants are the natural source of biomolecules having numerous biological properties as medicine or nutrition. In fact, the various biological properties can be ascribed to the presence of various phytochemicals such as phenolics, terpenoids or alkaloids, etc [27]. Phytonutrients such as carbohydrates, fats, proteins etc. are the primary metabolites from which secondary metabolites such as phenolics, flavonoids etc. are derived. Several studies have associated the presence of secondary metabolites to the pharmacological potential of the plants [28].

Flavonoids are important secondary metabolite that possesses tremendous medicinal property such as antioxidant, anti-inflammatory, anticancer, antibacterial, anti-viral activity etc [29]. The phytochemicals screening of methanolic extracts of A. calamus (MeAc), H. rosa-sinensis (MeHr), C. dactylon (MeCd) and E. floribundus (MeEf) showed high content of carbohydrates and proteins (table 1). Among all the plants, MeHr showed the highest quantity of carbohydrate (386.22±6.44 µg/mg extract). Similarly, MeEf showed higher protein content (310.40±11.44 µg/mg extract) among all the tested plants. Recent studies by Swargiary et al. [30] and Narzary et al. [31] also revealed a similar range of carbohydrate and protein content in the wild edible plants. Quantitative study of TPC showed highest concentration in MeEf (245.66±6.68 µg/mg extract) followed by MeAc (122.41±8.58 µg/mg extract), MeCd (116.40±3.53 µg/mg/mg extract) and MeCd (6.36±0.57 µg/mg extract), respectively. Flavonoid contents also showed higher in MeHr followed by MeEf, MeCd, and MeAc, respectively.

Antioxidant system is an important mechanism by which plants or animals can protect themselves from free radicals such as reactive oxygen species (ROS). Many diseases including diabetes, cancers etc. are directly or indirectly linked to ROS [32]. Our body has an innate capacity to neutralize most of the ROS called antioxidant property. The most significant pathway is the scavenging of free radicals in which free radical chain reactions are interrupted by phenolic compounds through its redox properties. In the present study, TPC, antioxidant activity by phosphomolybdate, DPPH and FRAP assay
Trace elements are a group of naturally occurring elements that are required for various biochemical and physiological functions. Inadequate supply of these micro-nutrients results in a variety of deficiency diseases or syndromes [6,8]. Our study revealed the presence of various essential and toxic elements in the test samples. In the present study, various essential elements like Cu, Zn, Co, Mn, and Fe were also found to be present in the samples of plant. Elemental analysis and the contents of all the four test plants were found to be within the World Health Organization permissible limits [6]. Elemental analysis and the contents of all the four test plants were found to be within the World Health Organization permissible limits [6].

Table 1: Phytochemical content and IC50 value DPPH assays of plant extracts

| Phytochemical contents/IC50 values | C. dactylon | H. rosa-sinensis | E. floribundus | A. calamus |
|-----------------------------------|-------------|-----------------|---------------|------------|
| Carbohydrates                     | 357.54±23.75| 386.22±6.44     | 296.54±8.65   | 331.68±28.20|
| Protein                           | 213.60±19.14| 301.33±7.85     | 310.40±11.44  | 246.92±11.36|
| TPC                              | 63.62±5.77  | 116.4±3.53      | 245.6±9.68    | 122.4±8.58  |
| TFC                              | 6.84±0.92   | 14.6±0.58       | 12.77±0.33    | 5.86±0.21   |
| TAA                              | 34.00±0.32  | 45.4±1.95       | 62.4±1.53     | 39.50±0.73  |
| DPPH, IC50 (µg)                   | 930         | 1750            | 123.50        | 1170        |

Values are expressed in mean±SD. All the calculations were represented as µg/mg extract with three replications (n = 3) for each experiment. The IC50 of standard ascorbic acid is 6.85 µg.

It is presumed that the reducing capacity of the extracts may function as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom. Studies have revealed the presence of a strong relationship between TPC and TFC with antioxidant activity [33,34]. In the present study, we have also observed that the phenolic content have a significant correlation with the overall antioxidant properties of all the tested plants. However, the presence of total phenolic content showed poor correlation with the antioxidant property of plants.

Pearson correlation studies among the tested plants and their phytochemical contents, antioxidant activities showed both positive as well as negative correlation (table 2). The phytochemical contents i.e., phenolics and flavonoids showed good correlation with the antioxidant activities. A significant correlation was found between the TPC and TAA that indicates a strong dependence of overall antioxidant activity on the phenolic contents of the plant. Our result showed there is no significant correlation between the TPC and TFC with DPPH (negative correlation).

Table 2: Pearson correlation study of phytochemical and antioxidant activities of four plants

|                      | Carbohydrate | Protein | TPC   | TFC   | TAA   | DPPH (IC50) |
|----------------------|--------------|---------|-------|-------|-------|-------------|
| Carbohydrate         | 1            | -0.206  | -0.775| 0.145 | -0.632| 0.909       |
| Protein              | -0.206       | 1       | 0.775 | 0.891 | 0.498 | -0.693      |
| TPC                  | -0.775       | 0.775   | 1     | 0.974*| 0.673 | 0.063       |
| TFC                  | 0.145        | 0.891   | 0.498 | 1     | 0.673 | 0.608       |
| TAA                  | -0.632       | 0.865   | 0.974*| 0.673 | 1     | -0.608      |
| DPPH (IC50)          | 0.909        | -0.129  | -0.693| 0.063 | -0.608| 1           |

*Correlation is significant at the P<0.05 level (n = 4 numbers of plants).

Elemental analysis

Trace elements are a group of naturally occurring elements that are required for various biochemical and physiological functions. Inadequate supply of these micro-nutrients results in a variety of deficiency diseases or syndromes [6,8]. Our study revealed the presence of various essential and toxic elements in the test samples. In the present study, various essential elements like Cu, Zn, Co, Mn, and Fe were also found to be present in the samples of plant collected from different sites, however, all the toxic elements were found to be within the World Health Organization permissible limits [6]. Elemental analysis and the contents of all the four test plants namely, A. calamus, H. rosa-sinensis, C. dactylon and E. floribundus are given in table 2. Concentrations of various essential elements (Cu, Zn, Co, Mn and Fe) and toxic elements (Pb, Cd, and Cr) were analyzed by AAS. Out of the five essential elements such as Cu, Zn, Co, Mn, and Fe, the highest elemental content was found to be Mn (1.87±0.02 µg/g powder) in H. rosa-sinensis followed by Fe.
The presence of phytochemicals such as total proteins, carbohydrates, phenolics and flavonoids in *A. calamus*, *H. rosa-sinensis*, *C. dactylon* and *E. floribundus* provides scientific evidence for the pharmacological significance of the plants and also accounts for the good source of phytonutrients. The presence of high phytonic, flavonoid compounds, and vit-C could be attributed to its pharmacological activity associated with free radical scavenging activity. Furthermore, antioxidant study by total antioxidant activity, DPPH, FRAP, and TBARS also showed the high potential of scavenging free radicals. The presence of high quantities of essential trace elements and cofactors and activator of enzyme reactions forming multiple organ damages. 

### Data and results

**Table 3: Trace element concentration (in µg/g) of four anthelmintic plants**

| Elements             | Cu    | Zn    | Co    | Mn    | Fe    | Pb    | Cr    | Cd    |
|----------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| *A. calamus*         | 0.51±0.02 |
| *H. rosa-sinensis*   | 0.17±0.02 |
| *C. dactylon*        | 0.13±0.03 |
| *E. floribundus*     | 0.15±0.03 |

**Toxic metals**

| Cu    | Zn    | Co    | Mn    | Fe    | Pb    | Cr    | Cd    |
|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.10±0.03 |
| 0.10±0.01 |
| 0.13±0.01 |
| 0.07±0.02 |

**Data are expressed as mean±SEM, values are significantly different at P<0.05, superscript a, b, c and d indicates the statistical significance of the plants. All experiments were carried out in triplicates (n = 3).**

### CONCLUSION

The presence of phytochemicals such as total proteins, carbohydrates, phenolics and flavonoids in *A. calamus*, *H. rosa-sinensis*, *C. dactylon* and *E. floribundus* provides scientific evidence for the pharmacological significance of the plants and also accounts for the good source of phytonutrients. The presence of high phytonic, flavonoid compounds, and vit-C could be attributed to its pharmacological activity associated with free radical scavenging activity. Furthermore, antioxidant study by total antioxidant activity, DPPH, FRAP, and TBARS also showed the high potential of scavenging free radicals. The presence of high quantities of essential trace elements and below the permissible range of toxic elements offers a better possibility of the use of the tested plants as medicines. The present data, therefore, would certainly help to ascertain the potency of the tested parts of the plants for medicinal use and functional food and nutraceuticals applications as well. However, in-depth investigations need to be carried out to isolate and identify the active components of the plants and to explore its mechanisms of action as well as their potential role in biological activity and antioxidant activities as well.

### CONFLICT OF INTERESTS

Declared none

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