

PHYTOCHEMICAL, ANTIOXIDANT, AND TRACE ELEMENT ANALYSIS OF ANTHELMINTIC PLANTS OF NORTH-EAST INDIA

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ABSTRACT

Objective: *Cynodon dactylon*, *Hibiscus rosa-sinensis*, *Elaeocarpus floribundus* and *Acorus calamus* are anthelmintic medicinal plants of north-east India. Due to its pharmacological properties, the present study was aimed to investigate the phytochemical, antioxidant and heavy metal content of the plants.

Methods: Preliminary phytochemical screening was carried out for the presence of phenolics, flavonoids, proteins, and carbohydrates following standard protocols. The antioxidant potential of plants was studied by phosphomolybdate, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay. The heavy metal content was estimated using atomic absorption spectrophotometer (Perkin Elmer 3110).

Results: The result showed that the alcoholic extracts of the plant sample contain high quantity of proteins, carbohydrates, phenolics, and flavonoids. Antioxidant assays revealed appreciable free radical scavenging potential. The total antioxidant activity was highest in *E. floribundus* (62.48±1.53 µg/mg extract) while *C. dactylon* showed lowest (34.00±0.32 µg/mg extract). Similarly, DPPH and FRAP study also showed high in *E. floribundus* and lowest in *H. rosa-sinensis* and *A. calamus*. Elemental analysis of the plant extracts showed a high content of Mn in all the plants while Co was found to be lowest. The tested plants showed low levels of toxic elements.

Conclusion: The high quantity of nutritional content and antioxidant property suggest that plants may be a good source of phytomedicine. Less quantity of toxic elements offers an opportunity to explore the medicinal application of the plants. However, further study needs to be carried out to know its mode of action.

Keywords: Anthelmintic plants, Phytochemicals, Antioxidant, Trace elements

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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 property of the plant such as antimicrobial, insecticidal etc. and several phytochemicals like α -asarone, (E)-methyl Isoeugenol, methyleugenol, 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, antidiabetic, 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 anthelmintics 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 ferricyanide [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 extractor 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 formation 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 695 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:

$$\text{DPPH Scavenging activity (\%)} = \left[\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right] \times 100$$

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 modification 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 NO₂ fumes ceased. Then conc. HClO₄ (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±9.68 µg/mg extract) followed by MeAc (122.41±8.58 µg/mg extract), MeHr (116.40±3.53 µg/mg extract) and MeCd (63.60±5.77 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

revealed higher activity in MeEf. MeHr showed lowest DPPH activity with IC_{50} 1750 μ g. An increasing trend of FRAP activity was observed in a concentration-dependent manner in all the plants (fig. 1). The reducing potential of the tested extracts was observed over a concentration range 25-100 μ g/ml. The trend of reducing potential

was significantly ($P < 0.05$) lower than the standard ascorbic acid. Like other antioxidant activity, FRAP result also showed high activity in MeEf. Many studies have investigated the reducing power activity of several plants which showed an increasing trend of reducing power with the increase of plant concentrations [33,34].

Table 1: Phytochemical content and IC_{50} value DPPH assays of plant extracts

Phytochemicals contents/ IC_{50} values	<i>C. dactylon</i>	<i>H. rosa-sinensis</i>	<i>E. floribundus</i>	<i>A. calamus</i>
Carbohydrates	357.54 \pm 23.75	386.22 \pm 6.44	296.54 \pm 8.65	331.68 \pm 28.20
Protein	213.60 \pm 9.14	301.33 \pm 7.85	310.40 \pm 11.44	246.93 \pm 1.36
TPC	63.62 \pm 5.77	116.4 \pm 3.53	245.66 \pm 9.68	122.41 \pm 8.58
TFC	6.84 \pm 0.92	14.62 \pm 0.58	12.77 \pm 0.33	5.86 \pm 0.21
TAA	34.00 \pm 0.32	45.41 \pm 1.95	62.48 \pm 1.53	39.50 \pm 0.73
DPPH, IC_{50} (μ g)	930	1750	123.50	1170

Values are expressed in mean \pm SD. All the calculations were represented as μ g/mg extract with three replications ($n = 3$) for each experiment. The IC_{50} of standard ascorbic acid is 6.85 μ g.

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 [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).

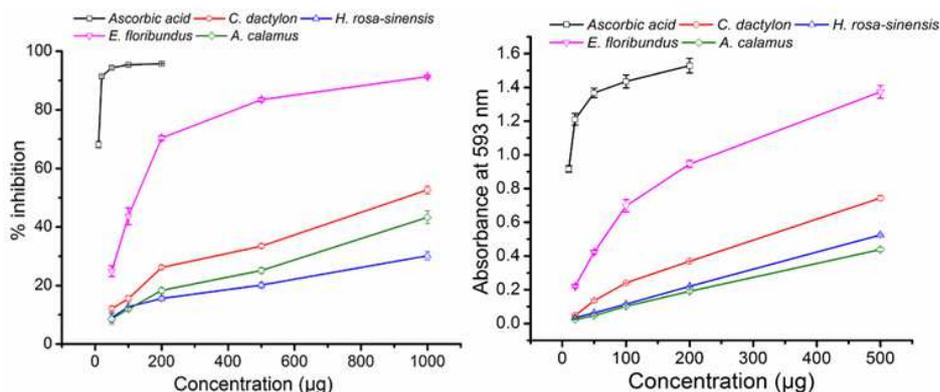


Fig. 1: DPPH activity of tested plants compared to (left) standard ascorbic acid and (right) increasing trend of FRAP activity with the increase of plant extract. All values are significantly different at $P < 0.05$ compared to standard ascorbic acid

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

	Carbohydrate	Protein	TPC	TFC	TAA	DPPH (IC_{50})
Carbohydrate	1	-0.206	-0.775	0.145	-0.632	0.909
Protein	-0.206	1	0.775	0.891	0.865	-0.129
TPC	-0.775	0.775	1	0.498	0.974*	-0.693
TFC	0.145	0.891	0.498	1	0.673	0.063
TAA	-0.632	0.865	0.974*	0.673	1	-0.608
DPPH (IC_{50})	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 \pm 0.02 μ g/g powder) in *H. rosa-sinensis* followed by Fe

($1.71 \pm 0.09 \mu\text{g/g}$ powder) and Cu ($0.51 \pm 0.22 \mu\text{g/g}$) in *A. calamus*, respectively. The presence of cobalt was detected to be lowest ($0.06 \pm 0.01 \mu\text{g/g}$ powder) in *A. calamus* among all the tested plants. Cu was found to be highest in *A. calamus* while Zn and Co showed the highest concentration in *C. dactylon*. Subsequently, the content of Mn and Fe were highest in *H. rosa-sinensis* and *E. floribundus*. And the content of Cu ranged from a minimum of $0.04 \mu\text{g/g}$ to $0.244 \mu\text{g/g}$ in *H. rosa-sinensis* and for *A. calamus* the range was 0.12 to 1.809 mg/g . Cu is an essential enzymatic element for normal plant growth and development, but it can also be toxic at excessive levels [35]. Likewise, Zn ranged from a minimum 0.035 to $0.139 \mu\text{g/g}$ in *A. calamus* to a maximum 0.519 to $0.906 \mu\text{g/g}$ in *C. dactylon*. Zn is also an essential trace element for plant growth and it also plays an important role in various cell processes, including normal growth, brain development, behavioral response, bone formation and wound healing [35]. The dietary limit of Zn has been set as 100 ppm . The presence of metallic compounds at a certain concentration is

beneficial to plants and animals as well [36]. Pb was found to be highest with $0.60 \pm 0.11 \mu\text{g/g}$ of plant powder in *H. rosa-sinensis*. Similarly, Cr and Cd were found to be highest in *C. dactylon* and *A. calamus*, respectively. Cr was found to be lowest among all the tested elements. All the tested plants showed a significant difference in the concentration of the metal contents among the plants. However, statistically, no significant differences were detected in the concentration of Cr among all the four anthelmintic plants (table 3). Like many biological molecules metals serve either as cofactors and activator of enzyme reactions forming enzymes/substrate metal complex, exert a catalytic property such as a prosthetic group in metalloproteins or regulators of nerve transmission, muscle contraction, osmotic pressure and salt-water balance [37]. On the other hand, heavy metals like arsenic, cadmium, chromium, lead, and mercury do not have any beneficial biological role and are known to be systemic toxicant that induces multiple organ damages [8].

Table 3: Trace element concentration (in $\mu\text{g/g}$) of four anthelmintic plants

Elements ($\mu\text{g/g}$ extract)	Essential elements				Toxic metals			
	Cu	Zn	Co	Mn	Fe	Pb	Cr	Cd
<i>A. calamus</i> ^a	$0.51 \pm 0.22^{b,c,d}$	$0.10 \pm 0.01^{b,c,d}$	$0.06 \pm 0.01^{b,c,d}$	$0.37 \pm 0.10^{b,c,d}$	$0.53 \pm 0.10^{b,c,d}$	0.06 ± 0.10^b	0.02 ± 0.01	$0.35 \pm 0.09^{b,c,d}$
<i>H. rosa-sinensis</i> ^b	$0.17 \pm 0.02^{a,c,d}$	$0.42 \pm 0.02^{a,c,d}$	$0.10 \pm 0.01^{a,c,d}$	$1.87 \pm 0.02^{a,c,d}$	$0.81 \pm 0.04^{a,c,d}$	$0.60 \pm 0.11^{a,c,d}$	0.01 ± 0.01	0.02 ± 0.01^a
<i>C. dactylon</i> ^c	$0.13 \pm 0.03^{a,b,d}$	$0.77 \pm 0.024^{a,b,d}$	$0.16 \pm 0.01^{a,b,d}$	$0.95 \pm 0.03^{a,b,d}$	$0.17 \pm 0.03^{a,b,d}$	0.07 ± 0.02^b	0.03 ± 0.01	0.02 ± 0.09^a
<i>E. floribundus</i> ^d	$0.15 \pm 0.03^{a,b,c}$	$0.38 \pm 0.16^{a,b,c}$	$0.05 \pm 0.02^{a,b,c}$	$0.73 \pm 0.23^{a,b,c}$	$1.71 \pm 0.09^{a,b,c}$	0.03 ± 0.02^b	0.02 ± 0.01	0.06 ± 0.02^a

Data are expressed as mean \pm 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 a good source of phytonutrients. The presence of high phenolic, 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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