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ORIGINAL ARTICLE

Physicochemical evaluation, nutraceutical composition and HPLC-UV fingerprint of *Helicanthus elastica* (Desr.) Danser (Indian Mango Mistletoe)

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Background: *Helicanthus elastica* (Desr.) Danser (Loranthaceae) is less-known Indian medicinal mistletoe growing commonly on mango trees as hemiparsites. It is used to prevent abortion, in vescical calculi and kidney affections. These groups of plants are medicinally important as they are potential sources of anticancer, immunomodulatory, hepatoprotective, antimicrobial and antioxidant molecules. **Materials and Methods:** In the current study whole plant of *H. elastica* growing on mango trees is collected and subjected for physicochemical and nutraceutical analysis aiding standard methodology. The total ethanolic extract was fingerprinted with HPLC-UV. **Results:** Parameters like moisture content, total ash, water-soluble ash, acid-insoluble ash, alcohol-soluble and water-soluble extractive, successive extractive values by cold and hot extraction, heavy metals like arsenic, lead, cadmium and mercury, total bacterial count, total fungal count, presence of enterobacteriaceae, *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* were tested under the head physicochemical examination. Determination of carbohydrate, fat, protein and fiber, calorific value, vitamins like niacinamide, pyridoxine, riboflavin, thiamine and ascorbic acid, trace elements like iron and zinc were estimated. **Conclusion:** The physicochemical tests are used as parameters for routine analysis and quality control of the plant. The investigations revealed appreciable quantity of important vitamins and trace elements in the plant. The HPLC-UV fingerprint would be an efficient tool for the standardisation and quality control of the mistletoe extract.

Key words: *Helicanthus elastica*, hemiparsites, HPLC-UV fingerprint, mango mistletoe, nutraceutical composition

INTRODUCTION

Lack of standards is a major problem associated with herbal medicine. Correct identification of herbal materials and its active principles is an important step in proving the efficacy of medicinal herbs. The quality control of phytopharmaceuticals is a status of the drug, determined either by identity, purity, content and other chemical, physical or biological properties or by the manufacturing process.[1] Physicochemical evaluation is a part and parcel of experiments deciding the purity and strength of the herbal medicinal material. They are useful criteria for authentication and detection of adulteration in plant material. Limit tests for heavy metals and microbial load will indicate its safety.

Plant materials have been source of energy and nutritional supplements since ancient time. Human disorders attributed to nutritional deficiency are treated with plants having higher concentration of required nutritional supplement. All human beings require a number of complex organic compounds as added caloric requirements to meet the need for their muscular activities. They avail carbohydrates, fats and proteins form the major portion of the diet, while minerals and vitamins form comparatively smaller part. Plant materials form a major portion of the diet with high nutritive value.

HPLC-UV has been used for single or simultaneous determination of main components of plants used in medicine. In the present study, shade-dried whole plant is coarsely powdered and subjected to physicochemical and nutraceutical analysis with HPLC-UV fingerprinting of the ethanolic extract.

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MATERIAL AND METHODS

Plant Material

Fresh plants of the mistletoe growing on *Mangifera indica* were collected during flowering season in the month of August, 2009 from Kasaragod District of Kerala. Morphological features are noted referring to regional floras.[2,3] Photograph form natural habitat and plant parts were taken. It was authenticated by Dr. S. Amerjothy, retired Head, Department of Plant Biology and Biotechnology, Presidency College, Chennai. Voucher specimen of the plant collected was deposited at the Pharmacognosy Department of Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai [voucher specimen number 00637].

Analytical Parameters

The procedures recommended as per World Health Organization (WHO) guidelines[4,5] were followed to calculate the parameters like moisture content, total ash, water-soluble ash and acid-insoluble ash. The percentage of alcohol-soluble and water-soluble extractive was also determined. Successive extractive values by cold extraction were determined by percolation and hot extraction by Soxhlet method.

Heavy Metals

The arsenic, lead, cadmium and mercury concentration in the sample were estimated using atomic absorption spectrophotometer method.[6,7]

Arsenic and lead were estimated by using hydride generator technique, cadmium estimated by flame technique and mercury by cold vapour technique. Concentrations of each metal were obtained from standard curve prepared using standard solution of each metal.[6]

Microbial Contaminants

Determination of microbial load was carried out as per WHO guidelines.[5] Ten grams of the sample was suspended in lactose broth and diluted to 100 ml with the same medium. The pH was adjusted to 7. Total bacterial count, total fungal count, presence of enterobacteriaceae, *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* were tested.

Proximate Analysis

Determination of carbohydrate,[8] fat,[9] protein and fibre[10] were estimated by standard methodology. Calorific value was determined by the combination of four times of carbohydrate, nine times of fat and four times of protein (Carbohydrate x 4 + Fat x 9 + Protein × 4), multiplied with formula and after adding all the values, finally get the calorific value.[10]

Vitamins

Estimation of niacinamide, pyridoxine, riboflavin, thiamine and ascorbic acid were performed by HPLC method.[11]

Elemental Analysis

Estimation of iron[12] and zinc[13] were performed by standard methodology.

HPLC-UV fingerprinting of total ethanolic extract

Instrumental - An Agilent 1100 HPLC system (Agilent Technologies, USA) consisted of a quaternary solvent delivery system, an on-line degasser, an auto-sampler, a column temperature controller and ultraviolet detector coupled with an analytical workstation and a YMC pack, ODS A -C_{18} column, 5 µ, 150 mm × 4.6 mm i.d. were used in the HPLC-UV experiments. Flow rate was 1.0 ml/min and sample injection volume was 20 µl. Detection wavelength was set at 280 nm and the column temperature was at 30°C. Mobile phase A contained 1.0 ml of orthophosphoric acid in 1000 ml water and B was filtered and degassed acetonitrile. Diluent was methanol. Mobile phases A and B were used as per the following elution program. Zero to 5 min A = 95 (%v/v) and B = 5 (isocratic); 5 to 25 min, A = 95 to 10 and B = 5 to 95 (linear gradient); 25 to 30 min, A = 10 and B = 90 (isocratic); 3o to 35 min, A = 10 to 95 and B = 90 to 5 (linear gradient). Re-equilibrium was 5 min; the total run time was 35 min.[14,15]

RESULTS

The physicochemical constants of *H. elastica* are presented in Table 1. The heavy metals content of *H. elastica* is shown in Table 2. The Microbial contamination of the plant *H. elastica* is shown in Table 3. The nutraceuetical composition of *H. elastica* is shown in Tables 4 and 5. The percentage areas of the compounds are tabulated in Table 6. The HPLC fingerprint is depicted in Figure 1.

Table 1: Results of physicochemical parameters of *Helicanthus elastica*

Parameters	Results (*n*=3) ±S.D. (%w/w)	
Foreign matter	Nil	
Moisture content	7.87±03	
Ash	7.01±0.38	
Water-soluble ash	2.99±0.05	
Acid-insoluble ash	0.49±0.04	
Extractive values (successive)	Hot (soxhlet)	Cold (percolation)
Hexane	2.35	0.77
Chloroform	0.80	0.73
Ethyl acetate	5.49	2.34
Ethanol	11.57	10.61
Total	20.21	14.45
Solubility of extractives		
Ethanol	19.09±0.02	
Water	12.73±0.11	

S.D. – Standard deviation

DISCUSSION

The presence of soil, stones, sand, dust and other foreign inorganic matters are removed before medicinal plant materials are cut or ground for testing. The whole plant of *H. elastica* did not show any foreign matter content. Loss on drying which infers the presence of water and other volatile content is used as an important parameter to assess the moisture content in any plant material. Excess of moisture makes the material prone for mould growth. *H. elastica* retains 7.87 ± 03% w/w moisture after complete air drying. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash, which is derived from the plant tissue itself, and non-physiological ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. The total ash content of *H. elastica* was found to be 7.01 ± 0.38% w/w. Water-soluble ash, indicative of inorganic contents is the difference in weight between the total ash and the residue after treatment of the total ash with water. The content of water-soluble ash accounted to 2.99 ± 0.05% w/w. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially sand and siliceous earth. as *H. elastica* growing on *Mangifera indica* was used for the study, the amount of acid-insoluble ash was found to be as less as 0.49 ± 0.04% w/w indicating very low content of silica-like substances.

When extracted successively in hot condition by Soxhlet and in cold by percolation, difference in the yield of the extract to various solvents was found. *n*-Hexane yielded 2.35% under the influence of heat while only 0.77% in cold percolation. Chloroform yielded 0.80 and 0.73% in hot and cold condition, respectively. Similarly hot and cold extraction using ethyl acetate and alcohol yielded 5.49, 2.34, 11.57 and 10.51%, respectively. The total percentage yield of the successive extracts was 20.21 and 14.45% when extracted under hot and cold condition of the solvent. Hot extraction is found better for the extraction of *H. elastica*. The solubility of powdered plant material in ethanol and water was found to be 19.09 ± 0.02% w/w and 12.73 ± 0.11% w/w, respectively. Alcohol yielded higher percentage of extract in *H. elastica*.

Contamination of medicinal plant materials with arsenic and heavy metals are attributed to many causes including environmental pollution and traces of pesticides. All heavy metals were found within the prescribed limits as per WHO.

Medicinal plant materials normally carry a great number of bacteria and moulds, often germinating in soil. While a large range of bacteria and fungi form the naturally occurring microflora of herbs, aerobic spore-forming bacteria frequently predominate. Current practices of harvesting, handling and production cause additional contamination and microbial growth. The determination of *Escherichia*

Table 2: Heavy metals content of *Helicanthus elastica*

Heavy metal	*H. elastica* ppm	API Limit ppm
Lead	0.45	10
Cadmium	ND	0.3
Mercury	0.0131	1
Arsenic	0.0450	3

API – Ayurvedic Pharmacopoeia of India; ND – Not detected

Table 3: Microbial load in *Helicanthus elastica*

Parameters	CFU/g	WHO Limit (IM) CFU/g
Total bacterial count	$<10^3$	$<10^5$
Total fungal count	$<10^3$	$<10^3$
Enterobacteria	$<10^1$	$<10^3$
E. coli	Absent	10
Salmonella sp	Absent	Absent
P. aeruginosa	Absent	Absent
S. aueus	Absent	Absent

IM – Internal medicine; CFU – Colony forming units; WHO – World health organization

Table 4: Proximate composition of *Helicanthus elastica*

Parameters	% w/w
Protein	22.96
Carbohydrate	9.05
Fat	2.92
Fibre	22.95

Table 5: Nutraceutical composition of *Helicanthus elastica*

Parameters	mg/g
Vitamin B1	0.3134
Vitamin B2	0.0241
Vitamin B6	0.1212
Niacinamide	0.0088
Folic acid	0.1121
Vitamin C	1.8027
Iron	0.624
Zinc	19.14

Table 6: Peaks detected in HPLC of ethanolic extract of *H. elastica*, R_T and % area

R_T	% area
2.089	14.23
4.237	1.77
12.450	7.87
12.467	5.68
12.658	2.92
13.363	7.74
13.445	19.98
13.683	8.01
13.719	14.40
13.850	2.59
13.909	14.84

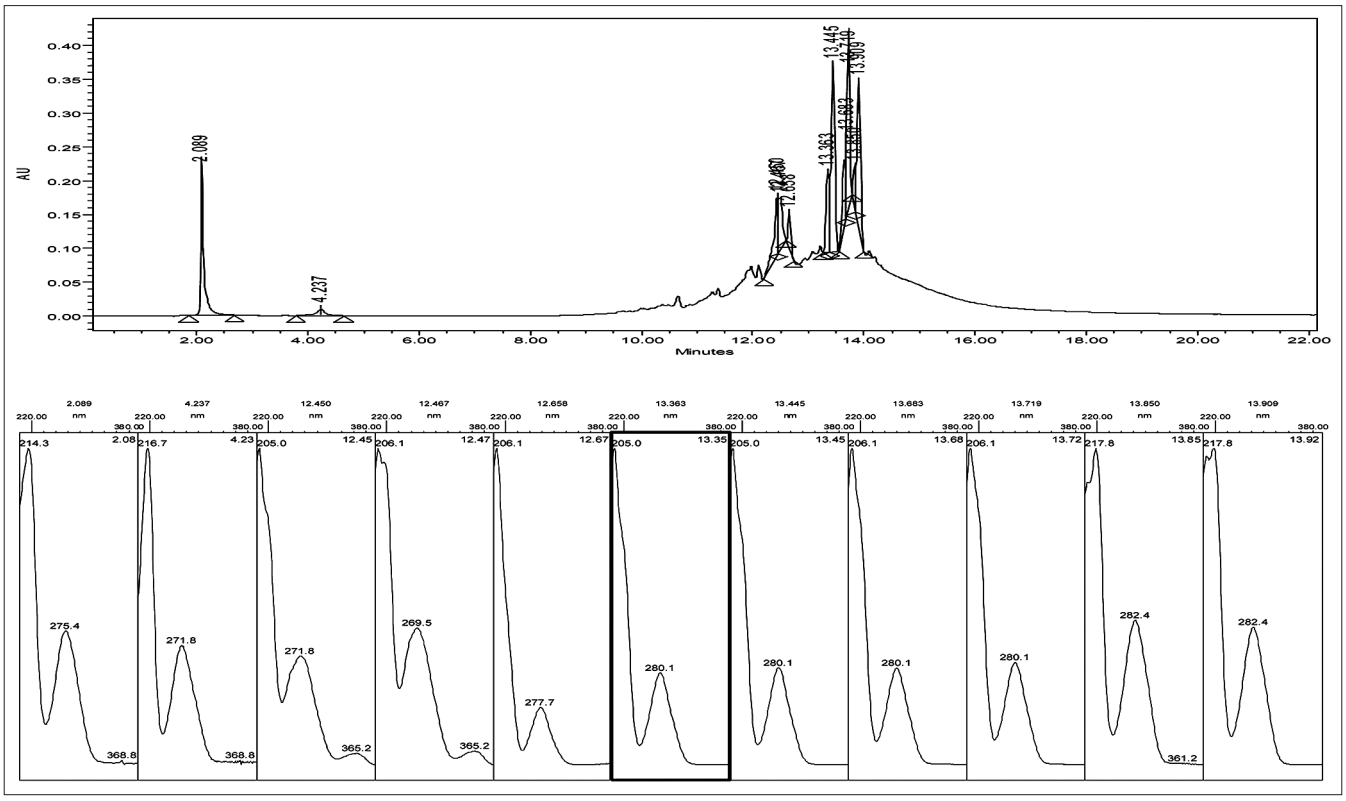

Figure 1: HPLC fingerprint of ethanolic extract of H. elastica, (a) HPLC chromatogram of ethanolic extract, (b) Absorption maxima at 280 nm of compounds eluted

coli and moulds may indicate the quality of production and harvesting practices. The microbial load is found well within the permitted limit.

The high value of protein and carbohydrate suggest its nutritional quality. Protein, fat and carbohydrate are sources of energy in diet and the amount was found to be 22.96%, 2.92%, 9.05%, respectively. Hence calorific value was estimated as (9.05 × 4) + (2.92 × 9) + (22.96 × 4) =36.2 + 26.28 + 91.84 = 154.32 Kcal.

Although formally not proposed essential macro-nutrient, dietary fibre is important for diet. It alters the contents of the gastrointestinal tract, by influencing the absorption of nutrients and chemicals through bulking and viscosity.[16] Some types of soluble fibres lower cholesterol levels in the blood by binding the bile acids.[17] Insoluble fibre reduce diabetes risk through unknown mechanism of action.[18] The total fibre content of *H. elastica* was found to be 22.95%. Thiamine (vitamin B_1) is needed for functioning of nervous system and it helps in releasing energy from carbohydrates.[19] *H. elastica* was found to contain 0.3134 mg/g of thiamine. Riboflavin (vitamin B_2) helps release energy from foods and is essential for healthy eyes, skin, nails and hair.[20] *h. elastica* contains 0.0241 mg/g of riboflavin. Nicotinamide demonstrated anti-inflammatory actions that benefits patients with inflammatory skin conditions, *H. elastica* was found to contain 0.0088 mg/g of nicotinamide.[21] *h. elastica* was found to contain 0.1212 mg/g of pyridoxine (vitamin B_6). It helps to form red blood cells and is needed for metabolism, normal reproductive process and healthy pregnancies.[22] The amount of ascorbic acid (vitamin c) was found to be 1.8027 mg/g. Ascorbic acid is necessary for healthy teeth, gums and bones and is essential for proper functioning of adrenal and thyroid glands. Also, ascorbic acid is an anti-oxidant and as such acts as a general de-toxicant.[23] The amount of folic acid (Vitamin B9) in *H. elastica* was found to be 0.1121 mg/g. It is essential to numerous bodily functions. The human body needs folate to synthesise, repair and methylate DNA and act as a cofactor in biological reactions involving folate. Its aiding quality in rapid cell division and growth proved advantageous in infancy and pregnancy. Both, children and adults require folic acid to produce healthy red blood cells and prevent anaemia.[24] Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats.[25] Iron is an energiser. But excess of it can cause fatigue. If collected from natural sources it may hardly get excess in supply.[26] The amount of iron was found to be 0.624 mg/g in *H. elastica*. Zinc is a component of many metalloenzymes, including some enzymes which play a central role in nucleic acid metabolism. In addition, Zinc is a membrane stabiliser and a stimulator of the immune response. Its deficiency leads to impaired growth and malnutrition.[27] The content of zinc in *H. elastica* was found to be 19.14 mg/g.

HPLC was used for the analysis of components in ethanolic extract. UV absorption of 11 peaks was obtained by HPLC-UV. The HPLC chromatogram is shown in Figure 1.1. R_T and % area of the peaks are shown in Table 6. The UV absorption of detected peak at 280 nm is shown in Figure 1.2.

CONCLUSIONS

The physicochemical constants of *H. elastica* were standardised as a check for purity of medicinal plant materials outlined by WHO. The heavy metal content and microbial load were found to be well within the limit indicating the safety of the material for medicinal applications. *H. elastica* was scientifically validated for nutraceutical composition. It contains fat, protein, carbohydrates, vitamins like thiamine, nicotinamide, riboflavin, pyridoxine, ascorbic and folic acid and elements like zinc and iron. The HPLC-UV fingerprint is an efficient tool for the standardisation and quality control of the mistletoe extract.

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