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Physicochemical and elemental studies of *Hydrocotyle javanica* Thunb. for standardization as herbal drug

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ABSTRACT

Objective: To explore the leaves of *Hydrocotyle javanica* Thunb. as a source of safe and effective antibacterial herbal medicine.

Methods: The standardization was validated by stepwise physicochemical studies, element analysis, determination of ash values, fluorescence analysis, assessment of moisture content, extractive values in different solvent systems and extraction methods. Heavy metal contents, mineral and element contents were analysed by atomic absorption spectrophotometry, inductively coupled plasma-mass spectrometer and CHNS/O analyser, respectively.

Results: The methanol extract of the folklore medicinal plant having antibacterial efficacy contained flavonoids and phenolic OH groups. The ICP multi standard indicated the presence of three major compounds with molecular mass of 161 190 and 221 Da. Heavy metals *viz*. lead, mercury and copper content were 4.38 ppm, < 0.05 ppm and 24.70 ppm, respectively. Minerals content of calcium, phosphorus, potassium and iron were 1 190.94 mg/100 g, 375.57 mg/100 g, 2 820 mg/100 g and 340.20 mg/100 g of plant sample, respectively. Elements like carbon, hydrogen, nitrogen and sulphur contents were 38.18%, 5.67%, 2.23% and 0.51%, respectively. Heavy metal profile of the tested plant was within the permissible limits of the regulatory authorities.

Conclusions: Hence the present physicochemical and elements studies reveals that the plant *Hydrocotyle javanica* Thunb. could be a potent source of herbal preparation as well as a safe and novel synthetic antibacterial drug.

1. Introduction

Traditional healing is the treatment of diseases by cumulative knowledge, skills and practices of the indigenous people of different cultures from ancestors to progenies to improve health, avoid and reduce diseases and its spread, or for complete cure of diseases and maintain physical, mental, social and spiritual well-being of an individual and the community as a whole [1]. Since time immemorial, ancient Indians, Egyptians, Chinese and people of other countries have employed a variety of plants and plant products for curing all kinds of ailments. Approximately 25 000 plant based formulations are available in the indigenous medical texts [2]. Also, the modern pharmacopoeias contain at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants [3]. In India, plants have been used as sources of medicine since the Vedic era. Ancient texts have reported about 500 plants with medicinal uses and various indigenous system of medicines use around 800 plants [4]. In developing as well as in developed countries, the demand of herbal plants is increasing day by day as drugs, food supplements (nutraceuticals), beverages and cosmetics [5]. About 31.4% population in industrialized societies, 42%–69% in United States, 71% Canadians and 90% British population are consuming dietary supplements or natural health products (vitamins, minerals, amino acids, essential fatty acids, herbal products, traditional

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Chinese medicines, homeopathic medicines, and probiotics) for treatment, including elimination of disease causing agents, avoidance of side effects and for getting quality life [6]. Herbal medicines being natural are preferred over synthetic remedies by a major section of the world. The herbal drugs are well established for their therapeutic benefits, safe with lesser adverse effects, economical, effective, and relatively less toxic and easily available in neighbourhood [7]. As the herbal medicines are conventionally being used directly from natural sources, so it may contain heavy metals, toxic substances, impurities, biological contaminants, hazardous foreign materials. Therefore, herbal medicines must be checked before consumption for theses parameters, otherwise it may cause serious health risks to the consumers [8].

Hydrocotyle javanica (*H. javanica*) Thunb. is a naturally growing prostrate herb found throughout the Himalayas and Assam Hills, Nilgiri Hills and Western Ghats of India at altitudes of 2 000 to 8 000 ft [9]. The plant belongs to the family Apiaceae (formerly known as Umbelliferae) and the subfamily is Hydrocotyloideae [10]. Traditionally, the fresh plant parts of *H. javanica* Thunb. are used as crushed and ingested to cure sore throats and lungs. Leaf juice is often used as eye drops to cure eye infection, leaf paste is used in dressing of wounds to reduce swelling and juice of shoots is used to treat gastritis and constipation [11]. Ethnomedicinally the leaves of the plant *H. javanica* Thunb. are used by various ethnic communities of Darjeeling Hills of West Bengal, India (27°3′15.88″N and 88°15′28.10″E, elevation 2 034 m) to treat several diseases. Traditionally the leaves of *H. javanica* Thunb. are also used as liniment on rashes in China and Thailand [12]. *H. javanica* Thunb. also exhibits antioxidant with high DNA protective properties [13]. Ninty-five percent methanolic extract of *H. javanica* Thunb. leaves has antihaemolytic and snake venom neutralizing effect against the deadly *Naja* spp. [14]. The extract of *H. javanica* Thunb. was also reported to have larvicidal activity against *Culex* spp. (Diptera: Culicidae) [15]. Lingaraju *et al.* [16] reported that the whole plant of *H. javanica* Thunb. crushed in butter milk is taken orally to cure dysentery and the leaves of this plant along with the *Adiantum* (a fern) leaves crushed in butter milk is taken orally to cure menorrhagia. This plant has been reported to use orally against fever, and to be applied externally in skin diseases [17]. Kurichiya tribes of Kerala (India) apply the juice of the whole plant on the chest to cure asthma and convulsions [18]. Pharmacologically it had been proved that the methanolic fraction of the plant showed antibacterial activity against some human pathogenic bacteria which had been published in our earlier publication [19]. Therefore, it is very pertinent and important to standardize the plant material as herbal medicine. The process of standardization can be achieved by stepwise pharmacognostic studies, phytochemical studies, element analysis and toxicity studies [20]. These studies help in safety and authenticity of the herbal plant consumption in various ailments. Evaluation of physicochemical properties of the crude material such as determination of extractive values, moisture content, different types of ash values, fluorescence analysis, foreign matter content, trace elements, heavy metals and active components (saponin, alkaloids, essential oil *etc*.) plays a significant role for standardization of the indigenous crude drugs [21].

Physicochemical standardization of a crude plant material provides information on the quality and purity of the sample. The solvent based extraction from any crude drug can yield a mixture of compounds. The extractive values in different solvents are significantly important to optimize the per unit yield of active constituents from plant sample extracted with a particular solvent [22]. Ash values of herbal sample indicate the presence of natural impurities like carbonate, oxalate and silicate. The water-soluble ash value indicates the quantity of inorganic compounds present in crude drug sample while the acid insoluble ash indicates impurity in the form of silica and other earthy material. Moisture content provides information on loss on drying and presence of excess water in the crude material. It is also important because an excessive amount of moisture in a biological material encourages microbial growth in it and causes deterioration on hydrolysis [23]. Medicinal plant materials should be free from any visible signs of contamination by moulds which may produce aflatoxins or insects and other animals which may produce contamination in the form of their excreta [24].

Quality of some crude drugs is often assessed by their fluorescence behaviour in both visible and ultraviolet (UV) light. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. It is an important parameter which gives a definite idea on the chemical nature of the compositions [25]. Contamination by heavy metals is also an important parameter and should be checked before use of the crude drug. According to the physiological roles, heavy metals are classified into two main categories: essential and non-essential heavy metals. Chromium (Cr), iron (Fe), cobalt, copper (Cu), zinc *etc*. are essential in very little concentration for our enzyme systems, vitamin synthesis and haemoglobin formation and are needed for the growth and development of our body. On the other hand, non-essential heavy metals *viz*. cadmium (Cd), arsenic, mercury (Hg), lead (Pb) *etc*. are not needed to perform specific function and they have harmful effects even at very low concentrations in the body [26,27].

Plants absorb various elements from soil and water, and the rate of absorption is variable in case of different elements. Most significantly the heavy metals like Cd, thallium and zinc have higher transfer coefficients and are readily taken up by plants, whereas Pb, Cr, cobalt and Cu show minimum transfer ability *i.e.* a lower transfer coefficient to plants from soil as they remain stably bound to the soil system [28]. Therefore, quantification of heavy metal content within the crude plant sample is necessary due to the variability of habitat selection. Very little information is available about the potential influence of metals on pharmacological activity of natural drugs obtained from medicinal plants from varied location especially from the polluted sites. Consumption of raw herbal drugs from the medicinal plants grown in such environment can cause serious health problems in human body. Higher levels of As, Hg and Pb are carcinogenic and they severely affect the central nervous system: Cd, Hg, Pb and Cu cause kidney damage and liver dysfunction, toxic to skin; Cd, Cr, nickel and Cu damage bones and teeth. Heavy metals are also reported to have adverse effects on memory and reproductive potency of human being [29].

Medicinal plants are not only used in folk herbal preparations but also as food supplements. Many of them are utilized as vegetables and as other food preparations like condiments. It is therefore very necessary to assess the heavy metals level of medicinal plants before they are utilized as medicines and condiments as these metals have lethal physiological effects in a higher threshold level [30]. World Health Organization/Food and

Agriculture Organization in their guidelines also has put forward this critical issue and recommended the determination of heavy metal content in the herbal medicines [31]. The present study was designed to investigate the levels of three heavy metals (Hg, Pb and Cu) and four minerals [calcium (Ca), Fe, phosphorus (P) and potassium(K)] and elemental analysis (CHNS) of the whole plant (leaf and petiole) of the medicinal plant *H. javanica* Thunb. which has been locally used by the people of traditional medicine to treat various illnesses for decades. The other member of the family Apiaceae like leaves of *Centella asiatica* and *Foeniculum vulgare* and the fruits of *Carum carvi* and *Pimpinella anisum* have been reported regarding their physicochemical and elemental standardization. To the best of our knowledge, the ethnomedicinal plant *H. javanica* Thunb. remains unexplored for its physicochemical and elemental standardization for potent source of herbal preparation as well as safe and novel synthetic antibacterial drug.

2. Materials and methods

2.1. Plant collection and identification

The aerial plant parts of *H. javanica* Thunb. were collected from Darjeeling Hill areas of West Bengal, India. It is a prostrate, perennial aquatic or semi-aquatic plant with long creeping stems that often form dense mats, often in and near ponds, lakes, rivers, marshes and some species in coastal areas of the sea. Formerly the plant has been classified in the family Apiaceae, now it has been placed in the family Araliaceae. The prepared herbarium specimen of the plant was identified by Central National Herbarium, Indian Botanic Garden, Shibpur, Kolkata, India and deposited there (voucher specimen No. DGC/SP-02) as described earlier [19]. It is commonly known as 'Java pennywort' in English. *Hydrocotyle nepalensis* Hook. is a synonym of *H. javanica* Thunb.

2.2. Sample preparation for ash content

Water soluble ash, acid insoluble ash, sulphated ash and total ash of the plant powder of *H. javanica* Thunb. were determined following the protocol of Indian Pharmacopoeia, 2007 [32]. The methods have been elaborated in each section of ash parameter estimation. For ash estimation, a platinum crucible was heated to redness for 10 min, allowed to be cooled in desiccators and to be weighed. Samples for different ash parameters were prepared as follows.

2.3. Estimation of total ash

1 g of the plant powder under examination was taken in the platinum crucible and dried at 100–105 °C for 1 h and ignited to constant weight in a muffle furnace at (600 ± 25) °C. After prolonged ignition, a carbon-free ash obtained from the charred mass, was washed with hot water and the residue was washed on an ash-less filter paper; the residue and filter paper was incinerated until the ash was white or nearly white; the filtrate was added to the dish and evaporated to dryness and ignited at a temperature not exceeding 450 °C. The percentage of ash was calculated on the dried drug basis, as follows:

$$\%\ \text{Total ash value} = \frac{\text{Weight of ash}}{\text{Weight of drug}} \times 100$$

2.4. Estimation of water soluble ash

The ash (total ash) was boiled for 5 min with 25 mL of water; the insoluble matter was taken in a Gooch crucible and washed with hot water, and ignited for 15 min at a temperature not exceeding 450 °C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated on the dried basis.

2.5. Estimation of acid-insoluble ash

The ash (total ash) was boiled with 25 mL of 2 M hydrochloric acid for 5 min; the insoluble matter was collected in an ash less filter paper, washed with hot water, ignited, cooled in a desiccator and weighed. The percentage of acid-insoluble ash was collected on the dried drug basis.

2.6. Estimation of sulphated ash

A total of 1 g of the substance under examination was taken in the crucible and ignited gently in a place protected from air currents until the substance was thoroughly charred. The residue was mixed with 1 mL of sulphuric acid on cooling and moistening and heated gently until the white fumes were no longer evolved. It was ignited at (800 ± 25) °C until all black particles disappeared. After allowing to be cooled, a few drops of sulphuric acid were poured and ignited as before. After cooling and weighing of the crucible, the operation was repeated until two successive weights did not differ by more than 0.5 mg.

2.7. Fluorescence analyses

These analyses were carried out as per the standard procedures [33]. In the present study, the powdered was treated with various chemical reagents like aqueous 1(N) sodium hydroxide, alcoholic 1(N) sodium hydroxide, 1(N) hydrochloric acid, 50% sulphuric acid and concentrated nitric acid, ferric chloride, *etc*. and their extracts were subjected to fluorescence analysis in day light and UV light (254 nm and 365 nm).

2.8. Moisture content

Moist sample was weighed immediately and recorded as 'wet weight of sample'. The wet sample was dried at a temperature not exceeding 115 °C using hot air oven. The sample was allowed to be cooled and to be weighed again after 1, 2 and 3 h and was recorded as the 'dry weight of sample', successively. The amount of moisture was calculated using the following formula:

$$\%\ \text{Moisture content (wb)} = \frac{\text{Ww} - \text{Wd}}{\text{Ww}} \times 100$$

Ww: wet weight of sample; Wd: dry weight of sample.

2.9. Different extraction methods

Shaded dried and powdered leaf samples were extracted in Soxhlet, microwave, maceration and infusion methods. The extracts were filtered and concentrated using rotary vacuum evaporator and weight.

2.10. Different solvents used in extraction

Plant samples were successively extracted with *n*-hexane, chloroform, 80% methanol and water according to ascending polarity indices.

2.11. Element analyses

Reagents and chemicals used: Milli-Q (Millipore Elix) water was used all through the testing procedure (10 MΩ). The standard stock solutions contained 1 000 ppm multi-element standard solution of inductively coupled plasma-mass spectrometer mix certified from Merck (Germany), nitric acid (J.T. Baker, 69% purity), hydrogen peroxide (30% purity).

Sample preparation and analysis of mineral by atomic absorption spectrophotometry through microwave assisted wet digestion method: a suitable quantity of sample was weighed accurately and transferred into a clean teflon digestion tube. Then 7–8 mL of concentration nitric acid (69%) and 1–2 mL of hydrogen peroxide (30%) was added into it and the tube was closed with cap after 10 min. The tube was kept in microwave tube stand and then kept in microwave digester (CEM Corp., USA). After completion of digestion the digester was switched off and allowed to cool the system, then the tube was removed and opened; the content was filtered using Whatman No. 42 filter paper. The filtrate was collected in any graduated vessel and diluted suitably with Milli-Q water [34].

Sample preparation and analysis of heavy metals by inductively coupled plasma-mass spectrometer: 0.5 g (dry mass) of plant sample was weighed into the digestion vessels. Then 5.0 mL of concentrated HNO_3 and 1.0 mL of 30% H_2O_2 were added to each sample. Samples were decomposed overnight (16 h) under a fume hood at room temperature. A temperature sensor was placed in one vessel to control temperature during the entire pre-digestion process. The plant samples were digested according to the following optimized program (power in w/time in min): 1 000/28, ventilation 20 min. The internal temperature was limited to 240 °C during the last step and ventilation. After cooling the entire digest was transferred into 60 mL plastic bottles and diluted to 50 g with double deionized water. Reagent blanks were prepared similarly to the samples. All sample solutions were cleared and diluted 10 times before analysis [35].

Instrument condition for CHNS analysis by CHNS/O analyser: The total carbon, hydrogen, nitrogen and sulphur was determined using a CHNS analyzer, (Model FLASH 2000 CHNS/O Analyzers Thermo Fisher Scientific Instruments). For the CHNS analysis, freeze-dried and crushed samples were weighed (5–10 mg) and mixed with an oxidizer (vanadium pentoxide) in a tin capsule, which is then combusted in a reactor at 1 000 °C. The sample and container melt, and the tin promote a violent reaction (flash combustion) in a temporarily enriched oxygen atmosphere. The combustion products CO_2, SO_2, and NO_2 were carried by a constant flow of carrier gas (helium) that passes through a glass column packed with an oxidation catalyst of tungsten trioxide and a copper reducer, both kept at 1 000 °C. At this temperature, the nitrogen oxides reduced to N_2. The N_2, CO_2, and SO_2 were then transported by the helium to and separated by, a 2-m-long packed column (Poropak Q/S50/80 mesh) and quantified with a TCD (set at 290 °C) [36].

2.12. Chemical standardization

The chemical standardization of the methanolic extract of *H. javanica* Thunb. has been done by Fourier transform infrared spectrometer (FT-IR) and LC–MS chromatogram analysis. The methodology details are mentioned in the following sections.

FT-IR chromatogram: The FT-IR spectrum of the samples was determined using a FT-IR (Perkin Elimer Spectrum II, 450–4 000 cm^{-1}). Approximately 2 mg of the semi-solid methanol extract of *H. javanica* Thunb. sample was grounded with 200 mg of dried potassium bromide (KBr) to form a very homogeny fine powder and the powder was compressed into a thin pellet by using 15 tons hydraulic press. KBr pellet held in KBr pellet holder was then recorded for FT-IR measurement in the wave number ranging from 450 to 4 000 cm^{-1} using 16 scans.

LC–MS chromatogram: The mass of the methanol extract of *H. javanica* Thunb. was analysed by LC–MS mass spectrometer (Model AB SCIEX 4000 QTRAP triple quadrupole linear ion trap) with a suitable mobile phase system with water (0.1% v/v formic acid) and acetonitrile. A total of 1 mg of the methanol fraction was dissolved in 10 mL of HPLC grade methanol to yield 100 ppm. The solution was sonicated for 15 min, followed by filtration through 0.2 μm membrane.

3. Results

3.1. Ash values and moisture contents of the plant sample

In the present study, *H. javanica* Thunb. had 22% total ash value, 12% sulphated ash and more water-soluble ash (4%) than acid insoluble ash (3%). The moisture content analysis showed a very low amount of weight loss on drying *i.e.* 8.9%. It was significant to mention that the plant sample does carry no foreign organic matter.

3.2. Florescence analysis

In the present study, the powder sample of *H. javanica* Thunb. exhibited a green colouration under day light, brown under short UV (254 nm) and black in long UV (365 nm) light. The colour appearance of the sample in different reagents was shown in Table 1.

Table 1
Fluorescence analysis of powdered sample of *H. javanica* Thunb.

Experiments	Visible/day light	UV light	
		254 nm	365 nm
Drug powder as such	Green	Brown	Black
Powder + 1 N NaOH (aqueous)	Dark yellow	Green	Black
Powder + 1 N NaOH (alcohol)	Yellow	Brown	Black
Powder + 1 N HCl	Yellow	Light brown	Black
Powder + 50% H_2SO_4	Light Brown	Brown	Black
Powder + nitric acid	Light brown	Dark brown	Black
Powder + ferric chloride	Green	Dark green	Black
Powder + Na_2HPO_4	Yellow	Brown	Black

Table 2
Extractive value in different extraction methods of *H. javanica* Thunb.

Extraction method	Extraction solvent	Extractive value (%)
Microwave	Chloroform	2.900 ± 0.145
	80% Methanol	4.400 ± 0.220
Infusion	Chloroform	7.700 ± 0.380
	80% Methanol	12.600 ± 0.630
Soxhlet	Chloroform	11.200 ± 0.560
	80% Methanol	15.300 ± 0.760
Maceration	Chloroform	5.500 ± 0.275
	80% Methanol	9.400 ± 0.470

3.3. Extractive values

The extractive values of *H. javanica* Thunb. in different solvent indicated that 80% methanol [(2.60 ± 0.13)%] extract showed the highest extractive value when compared to other solvents [(0.60 ± 0.03)% for *n*-hexane, (1.00 ± 0.05)% for chloroform, and (5.40 ± 0.27)% for water]. Table 2 shows the extractible values in different extraction methods. The Soxhlet method was the most effective regarding yield.

3.4. Heavy metals and minerals content

Pb, Hg and Cu contents in the plant sample were 4.38 ppm, <0.05 ppm, and 24.70 ppm, respectively. The tests were carried out in triplicate and analysed statistically. The content of heavy metals was found to be within the prescribed limit. The plant powder of *H. javanica* Thumb. contained trace metal Cu and non-essential heavy metals like Pb and Hg which were present within the recommended limit. The study reflects that *H. javanica* Thunb. contains a few times greater amount of iron than any other plants. In the present study Ca, Pand K content

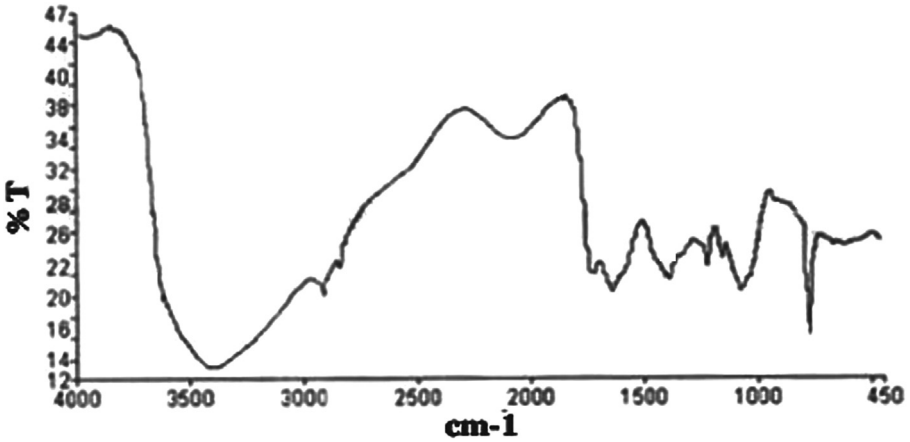

Figure 1. FT-IR analysis of methanol extract of *H. javanica* Thunb.

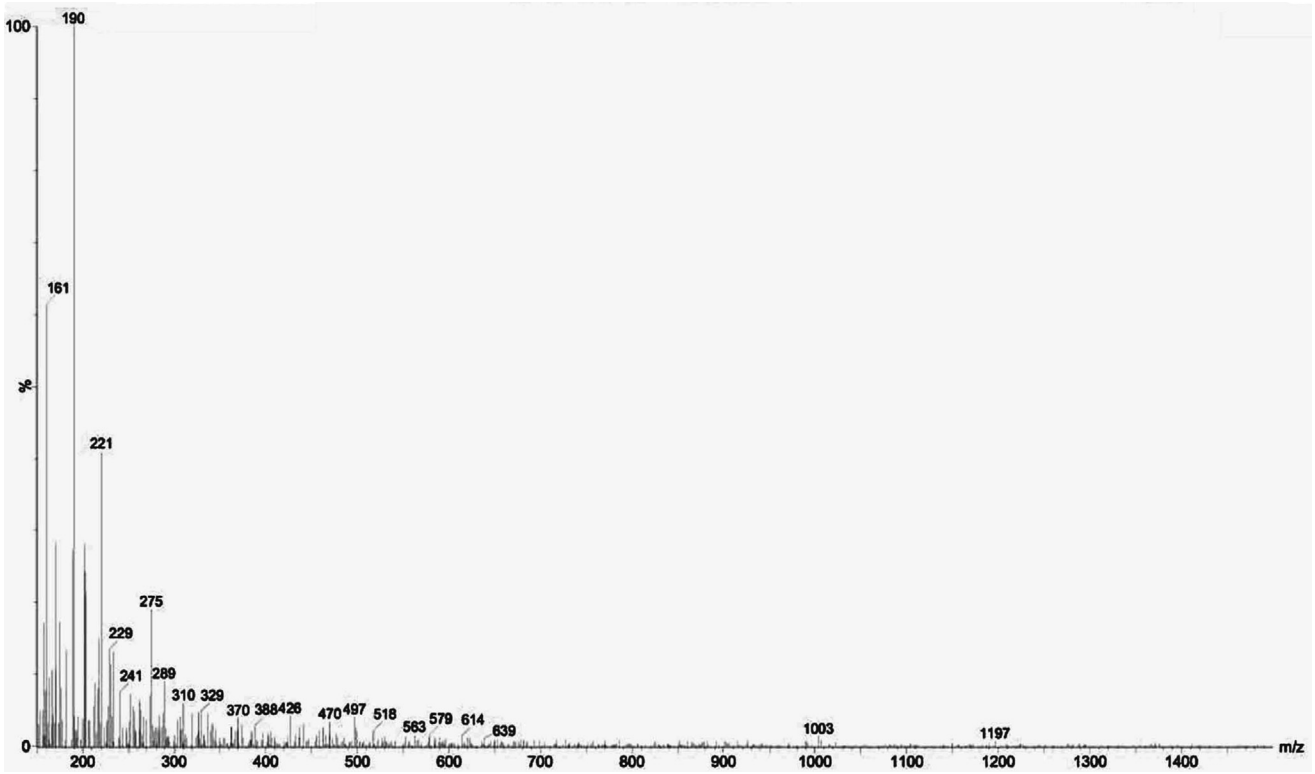

Figure 2. LC–MS analysis of methanol extract of *H. javanica* Thunb.

was detected as 1 190.94 mg/100 g, 375.57 mg/100 g and 2 820 mg/100 g of plant sample. In this study, the Fe content was detected 340.20 mg/100 g of plant sample.

3.5. Elemental compositions

In the present study the values of nitrogen and sulphur were the 2.23% and 0.51% respectively. So in the tested sample nitrogen to sulphur ratio was as high as 4:1. In this study the content of carbon and hydrogen detected in the crude material of the plant sample were 38.18% and 5.67% respectively. The carbon to hydrogen ratio of the present tested sample was 7:1. The carbon to nitrogen ratio of the present tested sample was 17:1.

3.6. FT-IR and LC–MS analyses

The FT-IR analysis showed (Figure 1) intensive band approximately at 1 724 cm^{-1} indicates C═O stretch of flavinoids as detected in the photochemical screening of methanol extract. The band at 771 cm^{-1} which indicates the presence of N−H secondary bond stretch while the peak at 1 219 cm^{-1} indicates CN stretch of aliphatic moieties. The broad peak at 3 625 cm^{-1} correspond the OH stretch of phenolic OH groups. The mass spectrum indicates the presence of compounds having molecular mass ranging from 161 to 221 *m/z*. Molecular ion (M^+) peak at 161, 190 and 221 *m/z* in the present study indicates the molecular weights of the three compounds with a basic peak (*m/z*) at 190 which corresponds to the molecular mass of the major compound of methanol fraction (Figure 2).

4. Discussion

Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug and is generally the index of the purity as well as identity of the drug. The total ash is particularly important in the evaluation of purity of drugs, *i.e.* the presence or absence of foreign inorganic matter such as metallic salts and/or silica [37]. The ash value therefore, using *H. javanica* Thunb. as crude drug, these values would be a standard index for this plant in future and thus the degree of purity will be assessed. Therefore, the amount of drug extraction from either wet or dry sample will not vary significantly. It was significant to mention that the plant sample does carry any foreign organic matter which indicates its no-health risk factor due to foreign organic matter content.

Presence or absence of different phytoconstituents exhibits varied colour response when reacting with specific chemicals. The fluorescence is specific for a particular compound. Many phytocompounds fluoresce when suitably illuminated with proper light. A non-fluorescent compound mixed with florescent impurities may also fluoresce. Therefore, fluorescence method is also very much necessary to standardize a crude drug material physically [38].

Extractive values can serve as a valuable source of information on the effectiveness of a solvent to extract active constituents from the plant sample and provide suitable data base to determine the quality of plant material for future investigators.

According to WHO and Pure Food and Drug Administration, the permissible limit of Cu was set to be 40 ppm in edible plants. Additionally, China and Singapore have set the permissible limits for Cu in medicinal plants to be 20 and 150 ppm respectively [39–41]. Levels of Pb beyond the permissible limits or long term use of these contaminated plant could lead to toxicity, characterized by colic, anaemia, chronic, nephritis, headache, convulsions, brain damage and central nervous system disorders [42,43]. In the present study, Pb was detected as 4.38 ppm which was identical with WHO [44] prescribed maximum limit and much lower than the maximum permissible limit (10 ppm) of Pb for raw herb recommended by Ayurvedic pharmacopeia of India [45]. Cu is considered to be an essential element for various metabolic processes, such as a cofactor for many critical enzymes and plays a very important role in metabolism. Cu mainly helps in maintaining the strength of blood vessels, epithelial and connective tissues, and skin in the human body. However, high concentrations of Cu can cause metal fume fever with flu-like symptoms, hair and skin discolouration, dermatitis, irritation of the upper respiratory tract and nausea [46,47]. Exposure to high levels of Hg in metallic, inorganic, or organic form results in permanent brain damage and impairs kidney functions. According to the guidelines of WHO (2007) [44], the permissible limit of Hg concentration was 0.5 ppm and according to the AYUSH (2005, 2011) [48,49], permissible limit of Hg in India is 1 ppm. In the present study, Hg concentration was detected as < 0.05 ppm, which is within the permissible limit recommended by both agencies. As Hg and Cu content of the tested plant sample is much lower than the highest permissible limit so it can be used as the crude drug without any risk of heavy metal toxicity. But the Pb content in the plant sample is equal to the maximum permissible limit of WHO so it has to be either neutralized or minimized by some remedial strategies or it should be checked before formulating the herbal as well as pharmaceutical drugs [34].

Mineral composition and content is also very much important for herbal drugs. Maximum concentration of Ca, Fe and K in edible plants is much more effective for jaundice remedies [50]. Ca is very essential mineral for healthy bones, teeth, blood muscles and nerves [51,52]. It is needed for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine and for the activation of enzymes such as the pancreatic lypase. Ca helps to regulate the activity of skeletal and heart muscles and also the functions of many other tissues. The recommended daily dietary intake allowance of Ca is between 500 and 1 000 mg for children and 800 mg for adults. In the present study, Ca content was detected as 1 190.94 mg/100 g of plant sample. Higher Ca content in the tested plant suggests its use to overcome deficiency of Ca. Increased dietary intake of Ca is currently recommended for the general population to lower the risk of hypertension and osteoporosis. Dietary supplementation of Ca also lowers serum cholesterol [53]. Hence, the plant may also have hypolipidemic properties due to the good content of Ca. P is the second to Ca found in the human body. It plays an important role in filtering of wastes through the kidneys, in energy storage and its usage. It is also very essential for the growth, maintenance and repair of all tissues and cells of the body. As the P is present in highest amount in the tested plant so it becomes effective in P deficiency disease in children and adult. K is important in human physiology as it takes part in ionic balance of human body and maintains tissue excitability. K is the principal intracellular cation and also considered as a very important constituent of the extracellular fluids because it influences muscle activity,

particularly the cardiac muscle. Potassium ions are concerned with the transmission of electrical impulse in the nerve cells, maintaining the fluid balance of the body and can play a crucial role in curing renal disorder and diarrhoea [54]. K deficiency causes nervous disorders, diabetes, and poor muscular control resulting in paralysis. In the present study, K content was detected as 2 820 mg/100 g of plant sample. Presence of higher concentration of K in the medicinal plants is very effective for the K deficiency disease and formulation of diuretic drugs [50]. Fe is an essential trace element and along with proteins plays a vital role in metabolism in living organisms. It is also an essential component of the oxygen carrying metalloprotein haemoglobin. It is also needed for healthy immune system and energy production [55]. In this study, the Fe content was detected 340.20 mg/100 g of plant sample and hence it is beneficial for consumers.

The estimation of different element contents which are present in a plant material is useful for those who consume them. Carbon, hydrogen and nitrogen act as the major elements and sulphur is also very important for plants growth as it performs a number of functions in enzyme reactions and protein synthesis, it is a constituent of many bioactive compounds like biotin and other vitamins, co-enzymes and amino acids [56–58]. Sulphur acts in formation of collagen protein of connective tissues, keratin protein of the skin, hair, and nails which give strength, shape, and hardness to the tissues. Nitrogen is reported to help in food digestion also [59]. As nitrogen and sulphur constitutes many proteins and vitamins, the higher percentage of nitrogen and sulphur indicates that the plant is also a potent source of proteins and vitamins which are very important for health and may be a good source of nutraceutic products [60]. The carbon in the form of carbonate and bicarbonate ions plays a key role in pH regulation and maintains acid balance in different parts of human body [61]. It indicates that the herb is a source of hydrocarbons which are the building blocks of many primary as well as secondary metabolites. A higher carbon to nitrogen ratio in an organic material is indicative to be more carbonaceous in the form of carbohydrates and proteins which increases the strength to the cell wall [62].

In conclusion, WHO has emphasized the need to ensure quality control of herbal formulations by using modern techniques and by applying suitable parameters. The low moisture content of the plant indicates a very low amount of weight loss in drying. The physic-chemical analysis of the plant reflects its essential elements richness with remarkably much lower amount of heavy metals. Thus, the present physicochemical and elements study revealed that the plant *H. javanica* Thunb. could be a potent antibacterial source of herbal preparation as well as a source of safe and novel antibacterial synthetic drug.

Conflicts of interest statement

We declare that we have no conflict of interest.

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