ORIGINAL ARTICLE

Nutraceutical evaluation of *Acalypha indica* L. - A potential wild edible plant

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Abstract

Aim: This study aims to evaluate the nutritional and nutraceutical properties of a traditional medicinal and wild edible plant *Acalypha indica* L. **Materials and Methods:** Qualitative phytochemical analysis was done by prescribed methods, nutritive contents, phenols, flavonoids, and alkaloids were estimated as per standard methods, quantification of mineral elements was done by inductively coupled plasma atomic emission spectroscopy (ICP-AES) technique, and high-performance thin-layer chromatography (HPTLC) was carried and compared with standard gallic acid and quercetin. **Results and Discussion:** Plant shows the good concentration of protein, carbohydrates, fats, and lipids. Phytochemical analysis revealed the presence of alkaloids, phenolics, saponins, flavonoids, tannins, and coumarins. HPTLC analysis was also carried out for the estimation of nutraceuticals and antioxidants. 25 elements were screened by ICP-AES technique, show the presence of Ca, Mg, Mn, Cu, Fe, K, Na, Al, B, Ba, and Sr in appreciable quantity. **Conclusion:** A study indicates the presence of remarkable concentration of nutritive content, mineral elements, and phytochemical which provides strong evidence of nutraceutical and antioxidant property of *A. indica* L. Further, elaborative investigation is needed to validate this plant for its daily consumption as vegetable.

Key words: *Acalypha indica* L., inductively coupled plasma atomic emission spectroscopy, nutraceutical, tribal communities, wild edibles

INTRODUCTION

Acalypha indica L., commonly called as khokli and kuppi, belongs to the family Euphorbiaceae occurs throughout tropical India. Whole plant is used for asthma, pneumonia, bronchitis, and rheumatism.[1] It is useful in the treatment of skin disease snakebite.[2,3] Leaves of *A. indica* are used in bed sores, as anthelmintic.[4,5] The leaf extract has been reported to possess various properties such as antimicrobial, antibacterial, antifungal, antioxidant, and antidiabetic activities.[6-10]

Leaves are used by the locals of Nandurbar district of Maharashtra as vegetable.[11] In West Africa, the leaves are cooked and eaten as a vegetable.[12] Leaves are also consumed by Irula tribe of Kotagiri hills.[13] Since *A. indica* L. is an important medicinal herb and has been significantly validated as excellent source of medicine, but its nutritional evaluation is not available.

Wild edible plants play a very important role in the diet of tribal communities. They are major source of food for tribes of forest area. Edible parts of wild plants are promising gift of nature to mankind, these are not only delicious and refreshing but also the chief source of vitamins, minerals, proteins, and other nutrients.

"Nutraceutical" the term coined in 1979. It is designed as a food or parts of food that provides medical or health benefits including the prevention and treatment of disease.[14] Nutraceutical may range from isolated nutrients, dietary supplements, herbal products, and processed products. Nutraceutical plays an important role in physiological benefits and provides protection against the diseases.[15]

The major nutraceutical ingredients in plant are phenolic compounds mainly flavonoids.[16] Gallic acid (3, 4, 5,-tryhydroxybenzoic acid) is naturally occurring

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Received: 18-01-2018
Revised: 15-09-2018
Accepted: 25-09-2018

polyphenolic compounds that possess astringent, antioxidative, and antimicrobial activity.[17-19] Quercetin is a phenolic compound that exhibits antiulcer, anti-inflammatory, antioxidant, antimicrobial, and antiallergic activity.[20-23] They have shown regulatory activity of hormones such as transport, metabolism, and action of thyroid hormones.[24] High-performance thin-layer chromatography (HPTLC) has emerged as a useful analytical method for qualitative and quantitative estimation of chemical constituents presents in plant materials.[25]

Present work mainly focused to explore nutritional and nutraceutical approach of this plant which includes the laboratory evidences for protective and health promoting phytochemical, quantification of elements by ICP-AES technique and estimation of important nutraceuticals and antioxidants like Gallic acid & Quercetin in Acalypha indica L. by HPTLC method.

MATERIALS AND METHODS

Collection of Plant Material

The aerial parts of wild edible plant *A. indica* L. were collected from different areas of Nanded district and then properly shade dried in an airy place, crushed, powdered, and stored in dry glass bottle. Plant material was identified and authenticated from Botanical Survey of India (BSI), Pune, BSI/WRC/Cert./2015/459/SKU06.

Phytochemical Screening

Qualitative phytochemical analysis was carried out for iridoids, polyoses, flavonoids, phenolics, emodins, aucumbins, tannins, coumarins, phlobatannins, flavonols, flavononols, flavones, terpenoids, cardenolins, leucoanthocyanins, steroids, and saponins as per methods prescribed in various literature.[26,27]

Nutritional Evaluation

Proximate analysis including moisture content, ash value, crude lipid, proteins, carbohydrates, and reducing and non-reducing sugar was done using standard methods.[28,29] Energy value was finally determined by the following equation: [30]

Energy value (Kcal/100 g) = (4 × % protein) + (9 × % fat) + (4 × % total sugar).

Quantitative Elemental Analysis

Plant sample was hydrolyzed by strong acid and was analyzed by ICP-AES. All spectrometric measurements were performed with ICP spectrometer (Arcos from M/S Spectro, Germany). The software used was smart analyzer vision 5.01.0921. The detector was charge-coupled device. All the samples were analyzed in triplicate and mean value of concentrations for each element's is given along with standard deviation. The instrumental parameters and operating conditions for ICP-AES are given in Table 1.

Estimation of Alkaloids, Phenolics, and Flavonoids

Determination of alkaloids

5 g of ground sample was weighed into a 250 ml beaker, and 200 ml of 20% acetic acid in ethanol was added and was covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to evaporate one-quarter of the original volume. The concentrated ammonium solution was added

Table 1: The instrumental parameters and operating conditions for ICP-AES

ICP-AES parameter	Value
R.F. generator	1.6 KW, 27.12 MHz
Plasma power	1400 W
Pump speed	30 rpm
Coolant flow	12.00 l/min
Auxiliary flow	1.00 l/min
Nebulizer flow	0.80 l/ml

ICP-AES: Inductively coupled plasma atomic emission spectroscopy

Table 2: Phytochemical screening

Phytocompounds	Acalypha indica L.
Alkaloids	+++
Leucoanthocyanins	-
Iridoids	-
Emodins	-
Aucumbins	-
Polyoses	-
Polyurenoids	-
Phenolics	+++
Tannins	+++
Anthraquinone	-
Coumarins	+++
Phlobatannins	++
Flavonoids	+++
Terpenoids	++
Proteins	+
Cardenolins	+
Juglone	-
Saponins	++
Cardiac glycosides	++

+++: Strong, ++: Moderate, +: Weak, -: Absent

Table 3: Proximate analysis

Proximate content	Value (%)
Moisture content	76
Total ash	16.1
Water-insoluble ash	76
Acid-insoluble ash	28
1% NaOH solubility	0.31
1% HCL solubility	30.9
Hot water solubility	29.4
Cold water solubility	37.4
Total sugar	4
Reducing sugar	0.65
Non-reducing sugar	3.75
Proteins	17.5
Fat	5
Energy value	131*

*Kilocalories/100 g of dry weight

Table 4: Quantitative elemental analysis

Elements	Wavelength (nm)	Concentration in ppm (mean±SD)
Al	176.641	497.72±0.119
B	249.773	37.284±0.004
Ba	455.404	21.282±0.002
Ca	422.673	42316.93±3.695
Cd	214.438	ND
Cr	267.716	ND
Cu	324.754	10.08±0.001
Fe	259.941	597.51±0.051
In	325.609	ND
K	766.491	26797.19±2.633
Li	670.780	ND
Mg	279.079	3949.945±0.302
Mn	257.611	50.88±0.004
Na	589.592	599.91±0.094
Ni	231.604	ND
Pb	220.353	9.76±0.002
Sr	407.771	131.69±0.014
Zn	213.856	19.04±0.002
As	189.042	ND
Hg	184.95	ND
Se	196.09	ND
Mo	202.095	ND
Te	170	ND
V	292.464	ND
Th	401.913	ND

SD: Standard deviation, ND: Not detected

dropwise to the extract until the precipitation was completed. The entire solution was allowed to settle and the precipitate was collected by filtration, after which it was weighed.[31]

Determination of phenolics

2 g of the sample were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 h. The fat-free sample was boiled with a 50 ml of ether for 14 min. 5 ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated ethyl alcohol were also added. The sample was made up to mark and left to react for 30 min for color development. The absorbance of the solution was read using visible spectrophotometer at 505 nm wavelength.[29]

Determination of flavonoids

5 g of the ground plant sample was weighed in a 250 ml titration flask, and 100 ml of the 80% aqueous methanol was added at room temperature and shaken for 4 h in an electric shaker. The entire solution was filtered through Whatman filter paper no. 1 and again this process was repeated. The filtrate as a whole was later transferred into a crucible and evaporated to dryness over a water bath and weighed.[32]

Estimation of Gallic Acid and Quercetin by HPTLC Technique

Preparation of extract for HPTLC

Edible parts of plants were shade dried and made into coarse powder and then extracted with methanol by Soxhlet apparatus and concentrated.

Reagents and other materials

Gallic acid and quercetin (Sigma-Aldrich) and silica gel F_{254} TLC aluminum plates (E-Merck) were used. Solvent system used for gallic acid was toluene: ethyl acetate: formic acid: methanol (3:3:8:2, v/v/v/v/v) and solvent system for quercetin was ethyl acetate: formic acid: glacial acetic acid: water (10:0.5:0.5:1.3 v/v/v/v/v) used as mobile phase[33] (all reagents of analytical grade, E-Merck).

Preparation of standard and sample solutions

Gallic acid and quercetin 10 mg were accurately weighed into 10 mL volumetric flask dissolved in 10 mL of methanol (1 mg/mL). The 100 mg of extract was dissolved in methanol (10 mL) and solution was filtered through Whatman filter paper No. 42.

The sample was spotted in the form of bands with microliter syringe on precoated silica gel plates F_{254} (10 cm × 10 cm with 0.2 mm thickness) using CAMAG Linomat 5 applicator, automatic sample spotter of bandwidth 6 mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min.

The distance traveled was 8 cm. Subsequent to the scanning, TLC plates were air dried and scanning was performed on a CAMAG TLC scanner in absorbance at 254 nm and operated with winCATS Planar Chromatography Manager.

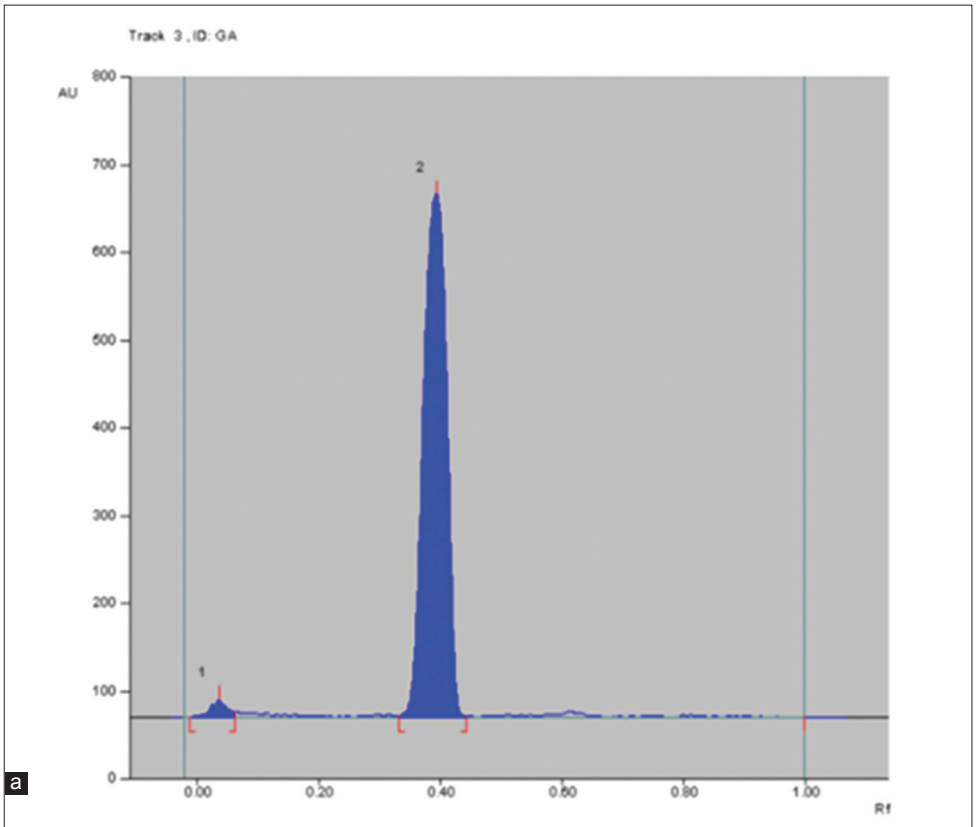
RESULTS AND DISCUSSION

Phytochemical screening

Screening tests of *A. indica* L. for various phytochemicals show positive results for alkaloids, flavonoids, tannins, phenolics, coumarins, phlobatannins, flavonols, flavononols, flavones, terpenoids, cardenolins, and saponins [Table 2]. Presences of these phytocompounds highlight its nutraceutical value. Flavonoids and tannins are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the *A. indica*, it might be responsible for its potent antioxidant capacity. Saponins have hypotensive and cardiodepressant[34] properties. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia.[35] The presence of phytoconstituents makes the plant useful for treating different ailments and has a potential of providing useful drugs of human use.

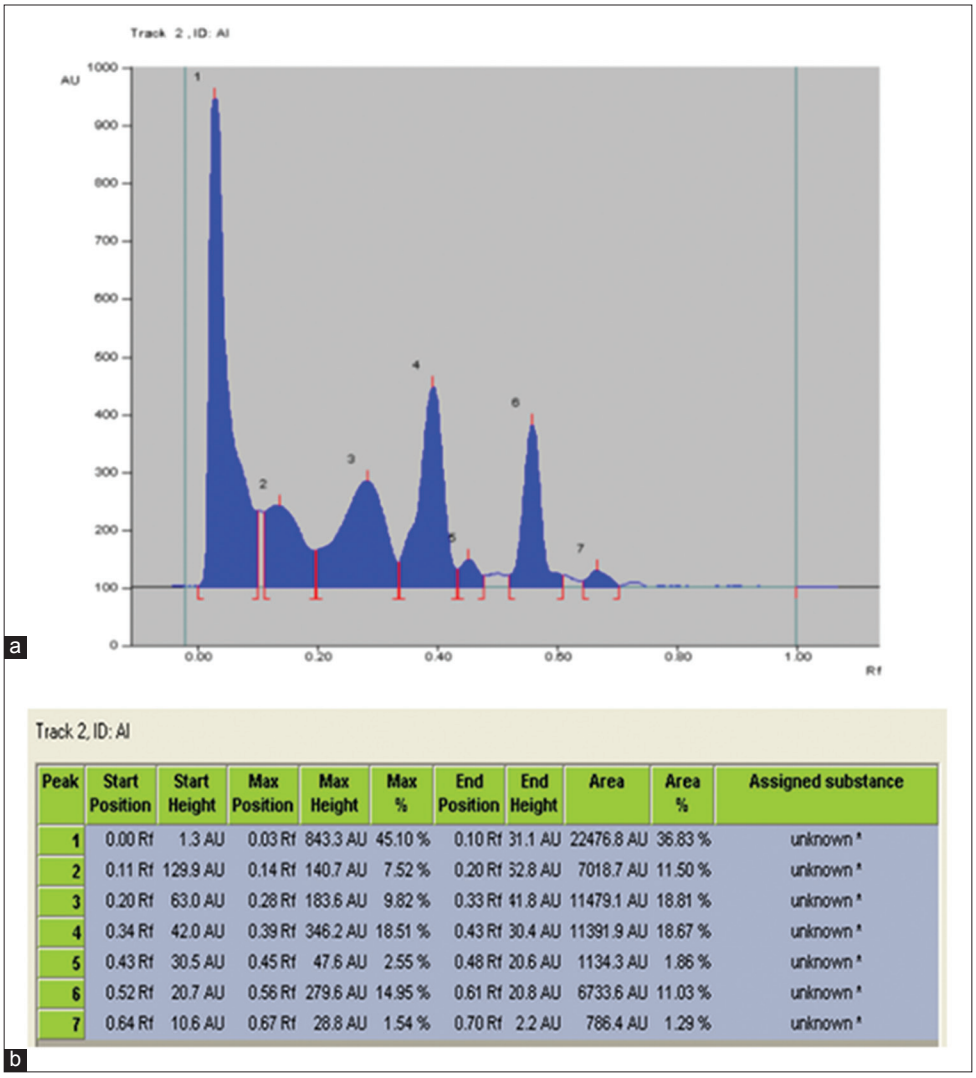
Nutritional Evaluation

Moisture content of *A. indica* leaves was found 76%, solubility of samples was found in 1% NaOH (0.31%), 1% HCl (30.9%), hot water (29.4%), and cold water (37.4%). Leaves show high moisture content (76%) and this is within the reported range (70.5–92.3%) in some Indian green leafy vegetables. Ash content, which is an index of mineral contents in plants, is 16.1% of total dry weight and is more soluble in acid than water. Crude protein content is 17.5% (175 mg/1 g) of total fresh sample. Plant food that provides more than 12% of its calorific value from protein is considered a good source of protein.[36] Therefore, the protein content



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.01 Rf	0.0 AU	0.04 Rf	20.5 AU	3.32 %	0.06 Rf	5.8 AU	438.7 AU	2.28 %	unknown *
2	0.33 Rf	2.2 AU	0.39 Rf	596.8 AU	96.68 %	0.44 Rf	0.9 AU	18780.5 AU	97.72 %	unknown *

Figure 1: (a) High-performance thin-layer chromatography profile for gallic acid standard. (b) Rf values for Gallic acid standard



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	1.3 AU	0.03 Rf	843.3 AU	45.10 %	0.10 Rf	31.1 AU	22476.8 AU	36.83 %	unknown *
2	0.11 Rf	129.9 AU	0.14 Rf	140.7 AU	7.52 %	0.20 Rf	32.8 AU	7018.7 AU	11.50 %	unknown *
3	0.20 Rf	63.0 AU	0.28 Rf	183.6 AU	9.82 %	0.33 Rf	41.8 AU	11479.1 AU	18.81 %	unknown *
4	0.34 Rf	42.0 AU	0.39 Rf	346.2 AU	18.51 %	0.43 Rf	30.4 AU	11391.9 AU	18.67 %	unknown *
5	0.43 Rf	30.5 AU	0.45 Rf	47.6 AU	2.55 %	0.48 Rf	20.6 AU	1134.3 AU	1.86 %	unknown *
6	0.52 Rf	20.7 AU	0.56 Rf	279.6 AU	14.95 %	0.61 Rf	20.8 AU	6733.6 AU	11.03 %	unknown *
7	0.64 Rf	10.6 AU	0.67 Rf	28.8 AU	1.54 %	0.70 Rf	2.2 AU	786.4 AU	1.29 %	unknown *

Figure 2: (a) High-performance thin-layer chromatography profile for Acalypha indica L. for gallic acid. (b) Rf values for for *Acalypha indica* L. leaf extract for Gallic acid

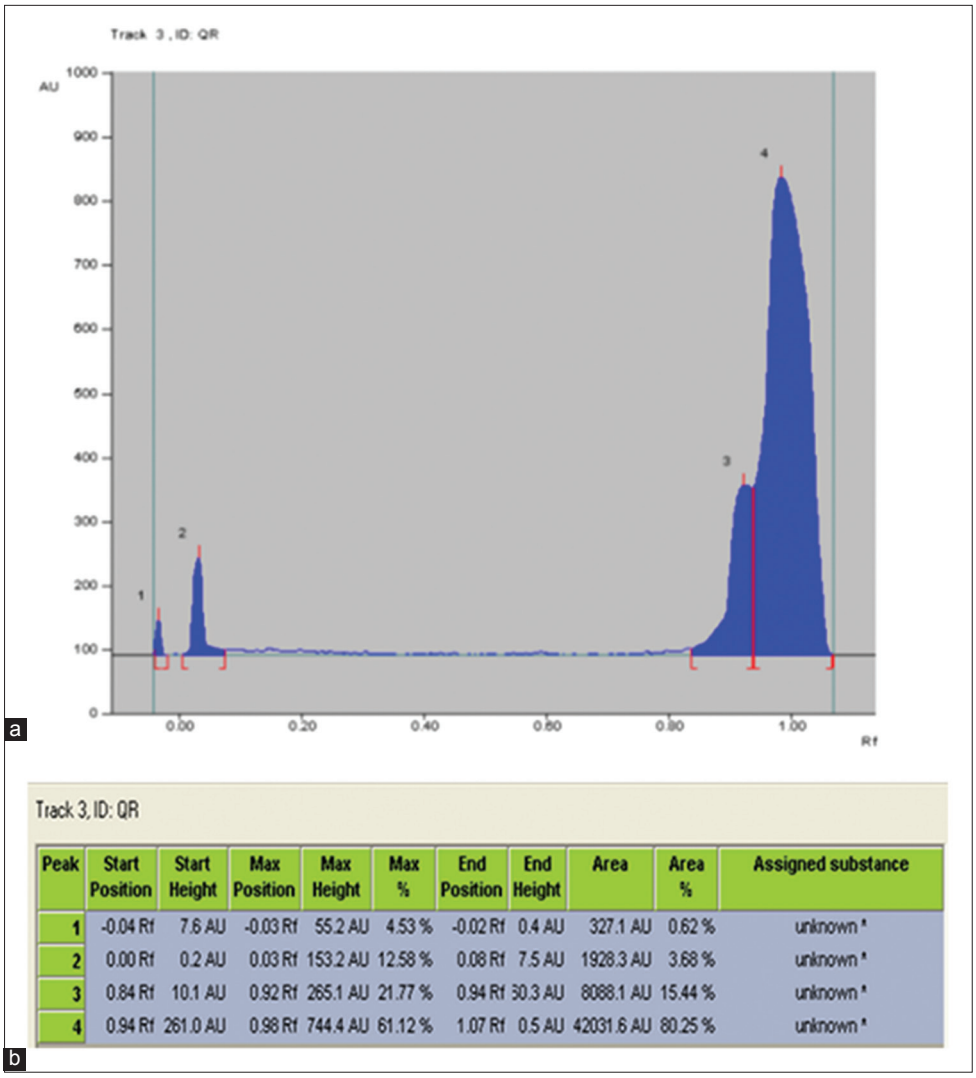
Table 5: Estimation of phenolics, alkaloids, flavonoids, and ascorbic acid

Phytoconstitutes	Concentration (mg/g)
Phenolics	0.12
Alkaloids	38
Flavonoids	148

of the leaves of the investigated plants will go a long way in meeting the protein requirement of the local people. Total sugar in this plant is found 40 mg/1 g (4%) of plant sample which is an appreciable amount in vegetables. Reducing and non-reducing sugar were estimated to 0.65 mg/1 g and 37.5 mg/1 g, respectively, fat value that is petroleum ether extract and is found to be 50 mg/1 g (5%) of plant sample. Leaves of this plant contain good concentration of proteins (17.5%) and fats (5%), in comparison to T. foenum-graecum leaves which having Protein 4.4% and fat 1%[38]. Energy value was finally determined as 131 Kcal/100gm of sample [Table 3].

Qualitative and Quantitative Elemental Analysis

Elements play a key role in various complex functions of the body to keep healthy. Calcium has major function in the formation of bones and teeth, control on nerve impulses, muscle concentration, and blood clotting; in the present study *A. indica* leaves show higher concentration of calcium 42316.91 ppm. Other essential elements such as potassium, sodium, magnesium, zinc, copper, and iron were recorded and found 26797.91 ppm, 599.91 ppm, 3949.94 ppm, 19.04 ppm, 10.08 ppm, and 597.51 ppm. The order of concentration is Ca>K>Mg>Na>Fe>Al>Sr>Mn>B>Ba>Cu. Some trace elements which having key role in metabolism have also detected aluminum (497.72 ppm), boron (37.284 ppm), barium (21.282 ppm), and stromium (131.69 ppm), heavy elements such as arsenic and mercury were not detected except lead. Lead is poisonous element for consumption, it causes serious health problems but according to the WHO guideline daily intake of Pb for 3–4 μg/kg of body weight is permissible for all age groups, and it was not associated with an increase in blood lead



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	7.6 AU	-0.03 Rf	55.2 AU	4.53 %	-0.02 Rf	0.4 AU	327.1 AU	0.62 %	unknown *
2	0.00 Rf	0.2 AU	0.03 Rf	153.2 AU	12.58 %	0.08 Rf	7.5 AU	1928.3 AU	3.68 %	unknown *
3	0.84 Rf	10.1 AU	0.92 Rf	265.1 AU	21.77 %	0.94 Rf	30.3 AU	8088.1 AU	15.44 %	unknown *
4	0.94 Rf	261.0 AU	0.98 Rf	744.4 AU	61.12 %	1.07 Rf	0.5 AU	42031.6 AU	80.25 %	unknown *

Figure 3: (a) High-performance thin-layer chromatography profile for quercetin standard. (b) Rf values for for quercetin standard.

levels or the body burden of lead.[38] Moreover, there should be a traditional method of pre-cooking such as boiling and soaking used by tribes who consume such vegetables to remove heavy elements from food so that intake of anti-nutritional factors is not allowed in body though it is present in row vegetable. Elemental analysis from this plant [Table 4] shows good results for most of essential elements which have great significance in diet.

Estimation of Phenolics, Alkaloids, and Flavonoids

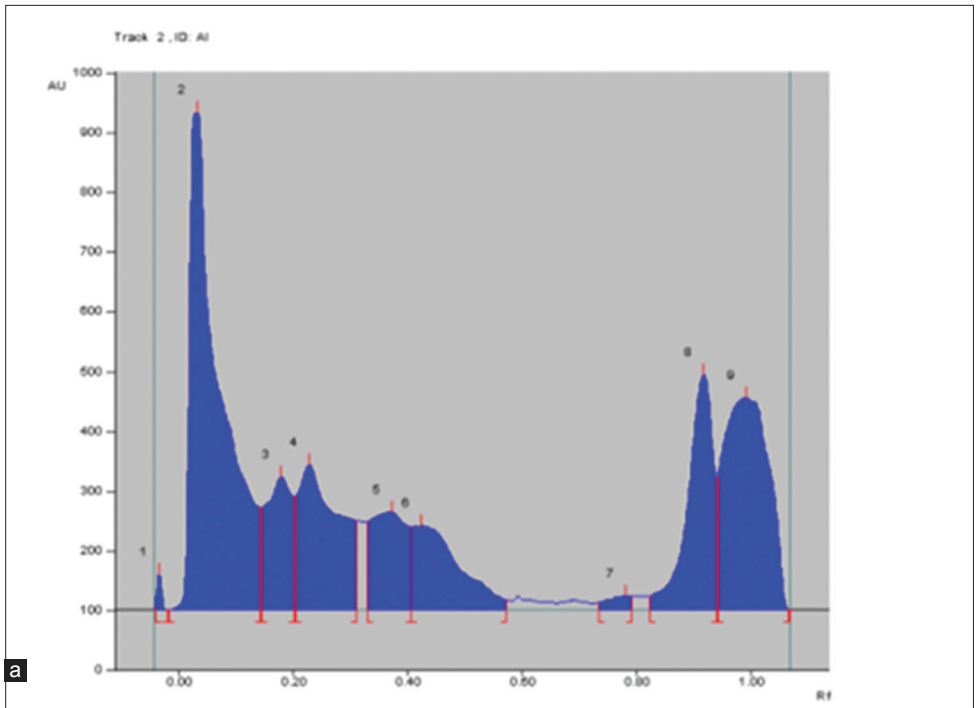
Phenolics, alkaloids, and flavonoids are the major nutraceutical and antioxidative ingredients. A study on *A. indica* shows appreciable amount of these nutraceuticals. They are natural antioxidants and shown regulatory activity of hormones such as transport and metabolism [Table 5].[39]

Estimation of Nutraceuticals by HPTLC Technique

The Rf value of standard gallic acid was found to be 0.39 and the peak area 18780.5 (Figure 1a and b]. Methanolic extract of *A. indica* L. showed seven peaks [Figure 2a and b], the third peak of Rf value 0.41 was coinciding with standard Rf values and its peak area was 11391.9. The Rf values of standard quercetin were found to be 0.98 and peak area was 42031.6 [Figure 3a and b]. Methanolic extract of plant showed eight peaks, the seventh peak of Rf values 0.99 was coinciding with standard Rf value, peak area was found to be 23220.4 for quercetin [Figure 4a and b].

CONCLUSION

A. indica L., a traditional medicinal plant, has been significantly validated as an excellent source of medicine. The presence of phytochemical such as alkaloids, flavonoids, cardenolins, phenolics, tannins, terpenoids, coumarins, and saponins is significantly enriched its use in pharmaceuticals as health-promoting phytocompounds. Plant contains essential elements as well as trace elements in good concentration. It can be a good calcium-rich food and encourage its use as mineral supplements. It has appreciable concentration of nutrients, highly antioxidative phenolics such as gallic acid, quercetin, flavonoids, and alkaloids were found to present in



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Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	6.6 AU	-0.03 Rf	59.2 AU	2.44 %	-0.02 Rf	0.0 AU	356.8 AU	0.31 %	unknown *
2	-0.02 Rf	0.0 AU	0.03 Rf	831.2 AU	34.28 %	0.14 Rf	70.3 AU	36269.0 AU	31.35 %	unknown *
3	0.15 Rf	170.4 AU	0.18 Rf	221.6 AU	9.14 %	0.20 Rf	38.5 AU	8200.5 AU	7.09 %	unknown *
4	0.20 Rf	188.9 AU	0.23 Rf	241.9 AU	9.98 %	0.31 Rf	48.6 AU	14486.2 AU	12.52 %	unknown *
5	0.33 Rf	146.8 AU	0.37 Rf	162.7 AU	6.71 %	0.41 Rf	38.1 AU	8559.1 AU	7.40 %	unknown *
6	0.41 Rf	138.0 AU	0.43 Rf	139.3 AU	5.75 %	0.57 Rf	16.2 AU	9590.2 AU	8.29 %	unknown *
7	0.74 Rf	11.3 AU	0.78 Rf	22.4 AU	0.93 %	0.79 Rf	21.4 AU	745.2 AU	0.64 %	unknown *
8	0.82 Rf	22.1 AU	0.92 Rf	392.3 AU	16.18 %	0.94 Rf	19.7 AU	14246.3 AU	12.32 %	unknown *
9	0.94 Rf	221.9 AU	0.99 Rf	354.0 AU	14.60 %	1.07 Rf	1.0 AU	23220.4 AU	20.07 %	unknown *

b

Figure 4: (a) High-performance thin-layer chromatography profile of *Acalypha indica* L. for quercetin. (b) Rf values for for Acalypha indica L. leaf extract for quercetin

remarkable concentration which provides strong evidence of nutraceutical and antioxidant property of *A. indica* L. Further, elaborative investigation is needed to validate this plant for its daily consumption as vegetable.

ACKNOWLEDGMENT

The authors are thankful to Principal, N.E.S. Science College, Nanded, for providing Central Instrumentation Laboratory facilities for HPTLC analysis and SAIF, IIT Mumbai, for their cooperation in ICP-AES analysis.

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Source of Support: Nil. **Conflict of Interest:** None declared.