**Elemental analysis of Plants using INAA and ICP-OES: Application of mathematical modelling**

Ammar M Ebrahim1,2, Hamid Bounouira3, Mohammed Abdassalam2, Elsadig Shiekheldin4,Akram Joda1 , Khalid Embarch3, Moussa Bounakhla2, Bernhard Michalke6

1 King Faisal University, Hofuf, Saudi Arabia

2 Sudan Atomic Energy Commission، Khartoum,

3 Sudan National Centre of Energy, Sciences and Nuclear Techniques (CNESTEN), Rabat, Morocco

4 Sudan Academy for Science, Khartoum, Sudan

6 Research Unit Analytical BioGeoChemistry, Helmholtz Center, Munich, German Research Center for Environmental Health, Neuherberg, Germany

Corresponding Author: Ammar M Ebrahim; ammar\_mubark@yahoo.com

**Abstract**

Fourteen plants were determined in parallel by INAA and ICP-OES to point out the possible integration of the two employed techniques. INAA technique correlates well with ICP-OES for measuring of Ca, K, Fe, Mn, Mg and Zn. One of the most important advantages of INAA is its non-destructive nature with preservation of the sample for further measurements, aside from ease of sample preparation. ICP-OES analysis needs prior (destructive) sample digestion, but this technique provided superior limits of detection (LOD). Therefore ICP-OES is the method of choice when element concentrations are < INAA's LOD and a better choice for Cr, Sr and Cu quantifications in plants, when considering accuracy. On the other hand, a simple mathematical model was developed for predicting the concentration of Cu and Cr that can be measured by INAA. The predicted values of such elements partly showed consistency to their corresponding certified values as well to the concentrations measured by ICP-OES, specifically for Cu or when concentrations showed values above 5 mg/kg.

**Keywords**

Trace elements, INAA, ICP-OES, plants, mathematical modelling

1. **Introduction**

 Trace elements are nutrients requisite in minute quantities in a number of physiological functions from the entire plant cells and human organ (Rajan et al., 2014). They are playing essential role in the formation of the active chemical constituents in medicinal plants (Ebrahim et al., 2012). The elemental contents of these plants are very important and need to be screened for their dosage control. Knowledge about elements concentrations in medicinal plants is important from the point of their nutritional requirements as well as their control to avoid risks associated with consumption (Raju et al., 2013, Devi et al., 2015). Metal loading in plants is therefore an important consideration when assessing potential health impacts where metals can be assimilated either in ionic form or as part of metabolites.

Instrumental Neutron Activation Analysis (INAA) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) are well suited for plant elemental analysis. The principles, implementation and applications of these techniques are varied and each has its own considerations.

Nuclear analytical techniques with their broad band of applicability to almost all matrix types are indispensable tool for environmental research (Sroor et al., 2003). They are specifically important as primary methods and as a reference within certification campaigns. However, they are not easily available since they require an access to nuclear facilities such as nuclear reactors or accelerators (Papaefthymiou and Papatheodorou, 2011, Kim et al., 2013). Instrumental neutron activation analysis (INAA) is a very common analytical method adopted for elements determination in herb samples (Nedjimi and Beladel, 2015). It has a nondestructive nature, multi-elemental capability and minimal sample preparation (Isam and Ebihra , 2012). INAA is less affected by the matrix of the sample because of the high penetrating power of neutrons and gamma rays, besides it is not affected by the chemical and physical states of the elements (Papaefthymiou and Papatheodorou, 2011, Kim et al., 2013). Detection sensitivity, however, is partly borderline for some elements, resulting in LOD being too high for low concentrated elements in various matrices. On the other hand, ICP-OES is a powerful tool for the determination of metals in a variety of different sample matrices. Noteworthy, ICP-OES requires rigorous extraction or sample destructing digestion procedures prior measurement (Ebrahim et al., 2014). Contrary to element analysis by INAA, ICP-OES can be operated in routine laboratories and can routinely determine most of the environmentally important elements (Papaefthymiou and Papatheodorou, 2011).

A number of studies showed that mathematical models have potential to predict elemental concentrations with a good accuracy. Neural network has been applied to obtain elemental concentrations of organic materials (muscle, liver and gills) (Correia et al. 2006), cement samples (Medhat, 2015), and soil (El Haddad et al. 2012,). These studies proved that, mathematical models can help to reasonably estimate elemental concentrations, therefore being mostly safe, typically inexpensive and a fast tool to estimate elemental concentration.

This study compares trace element concentrations (Ca, K, Zn, Mn, Mg, Sr, Fe, Cu, Co and Cr) in 14 plants determined in parallel by INAA and ICP-OES. The aim is to compare the performance of such methods in plant matrices and different element concentrations. It would be valuable to point out the possible integration of these techniques. This might exclude the expected insufficiency and limitation of each employed technique. The present work also apply a simple mathematical model for estimating the concentration of elements that showed difficulties to be measured using INAA.

1. **Material and Methods**

**2.1. Sample collection**

Fourteen popular medicinal plants were collected from a local market in Sudan. Botanical identification and authentication of the collected species with deposition of herbarium specimens have been done by the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan. Common and scientific names as well as collection areas of studies medicinal plants are given in Table 1.

Table 1

* 1. **Sample drying and homogenizing**

Samples were dried at room temperature before transportation to the laboratory. There, freeze drying was applied at 10oC sample temperature (Christ-Heraeus Beta freeze dryer, Osterode, Germany) for four days until weight constancy was reached. Aliquots of the plant material were cut into small pieces using a ceramic knife and subsequently ground and homogenized using an agate ball mill. The resulting fine powder was used for analysis.

* 1. **ICP-OES analysis**
		1. **Sample preparation**

The dried and homogenized samples were properly weighed into quartz vessels (around 100 mg). Subsequently, 1 mL of supra-pure, sub-boiling distilled HNO3, (Merck, Darmstadt) was added. The vessels were closed and introduced into a pressure digestion system (Seif, Unterschleissheim, Germany) for 10 h at 170oC. The resulting clear solutions were diluted to 10 mL with Milli-Q H2O.

* + 1. **ICP-OES measurements**

An ICP-OES “Spectro Ciros Vision” system (SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, Germany) was used for measurement in digested samples. Sample introduction was carried out using a peristaltic pump equipped with an antipulse-head (SPETEC, Erding, Germany), connected to a Meinhard nebulizer with a cyclon spray chamber. The measured spectral element lines were Ca 317.933 nm, Cr 267.716 nm, Cu 324.754 nm, Fe 259.939 nm, Mg 279.079 nm, Mn 257.611 nm, Ni 231.604 nm and Zn 213.956 nm. The RF power was set to 1400 W, and the plasma gas was 13 L Ar/min, whereas the nebulizer gas was 0.6 L Ar/min. For quality control, standard reference materials, rye grass (ERM-CD281) and milk powder (ERM-BB150), were digested in parallel with samples and analyzed together with the samples by ICP-OES.

* 1. **INAA measurements**

**2.4.1. Sample irradiation**

0.1-0.2 g of dried and homogenized plant samples, were individually packed into polyethylene irradiation vials. Irradiation of plant samples were carried out at neutron activation analysis laboratory at the Maamoura Nuclear Research Center (MNRC) of the National Center for Energy, Sciences and Nuclear Techniques (CNESTEN- Morocco) directly connected to the Moroccan Triga mark II research reactor. Two types of irradiation have been demonstrated according to the period of elements to be measured (irradiations of 30 to 60 s for the elements with short periods and irradiation of 4 h for elements with long periods). For short irradiations, each sample was prepared with flux monitor (approximately 30 mg of pure foil of zinc). Both sample and flux monitor are then locked in a polyethylene rabbit, which were transferred to the reactor core via a pneumatic transfer system that allows irradiation sample by sample.

In the case of long irradiations, rabbits prepared were then transferred to the rotary specimen rack system containing 40 irradiation positions. In order to take into account for the flux variation in that system, flux monitors (pippeted zinc on filter) were placed in the same rabbit.

 **2.4.2. Data acquisition**

The relevant nuclear data for the examined radionuclides is compiled in Table 2. Counting of emitted gamma rays was performed using a Hyper Pur germanium detector (HPGe). This count can identify the emitting isotopes and calculate the concentration of the elements of interest (Bounouira et al., 2014). Data acquisition was performed using Genie 2000 software. Concentration calculations were made by the K0-IAEA software.

Table 2

Standard Reference Material (NIST1547-Peach leaves) was used for the quality control purposes, mainly the determination of elemental concentration, inter-comparison purposes and also to assess the precision of the technique used.

**2.4.3. Development of a mathematical equation**

A mathematical equation was developed based on measured concentration of K, Zn, Fe, Mg and Mn by INNA to predict the concentration of Cu and Cr. These elements were found to be very difficult to measure by INAA. The elements selection was based on the intersection of elements that determined effectively by both INAA and ICP-OES techniques (table7). The developed equation has the form:

$$F=a∙K+b∙Zn+c∙Mn+d∙Mg+e∙Fe$$

where a, b, c, d and e are the equation constants and F can be the concentration either of Cu or Cr. Different constants were found for each target element. The inputs of the equation were twelve different plant samples including four plant standard reference materials, and the targets were the measured concentrations of Cu and Cr measured by ICP-OES. The equation’s parameters were obtained using the least squares method (LSM) by minimizing the sum of squared errors between the predicted and measured concentration of Cu and Cr:

$$min\left(\sum\_{i=1}^{N}\left(F\_{i,predicted} - F\_{i, measured}\right)^{2}\right)$$

Where N is number of plants $F\_{i,predicted}$ is predicted concentration of Cu or Cr and $F\_{i, measured}$ is the measured concentration of value of Cu or Cr.

1. **Results and discussion**

**3.1. Analysis of reference materials**

Certified reference materials namely ERM-CD 281 (rye grass) and CRM 1547 (peach leaves) were analyzed using ICP-OES and INAA, respectively.

As shown in table 3 INAA technique tended to overestimate most of analyzed elements with the exception of K, Ca and Mn, which are negatively shifted from their certified values. However, good agreement between measured and certified values was achieved for other elements. Unlikely, Cr showed dramatic elevation with a relative error of 63%. In spite of its low concentration, Zn showed consistency to its certified value with a relative error of 4.4%.

Table 3

 On the other hand, the analytical procedure consisting of pressure digestion prior to ICP-OES measurement underestimated in average across all elements by 6.4% in the rye grass CRM. This CRM is supposed to contain a higher mass fraction of metals as oxides or silicates, the latter being not dissolved by the applied digestion procedure (table 4) and consequently would not be monitored by ICP-OES. In conclusion, both techniques were within the confidence intervals of certified values and thus were in agreement with certified values, suggesting the accurate working of the non-destructive INAA and destructive ICP-OES for plant elemental analysis.

Table 4

**3.2. Analysis of plant samples**

A comparison between the two different analytical methods was conducted to determine if a real difference in the determined concentrations of these elements can be observed. As can be seen in tables 5 and 6 most of the elements were detected by ICP-OES while the results from INAA showed that the technique either failed to determine some elements in several plant samples, (e.g. Cu and Sr) or determined values showed unsatisfactory precision with very high relative standard deviation (RSD) reaching up even to several thousand % (e.g. Co and Cr). Concentrations of such elements were apparently below the detection limit.

INAA was able to determine Ca and Mg in each of the fourteen plant samples (K in 13 plants) with very high precision having low median RSDs between 0.01% - 0.3%. This may be due to the high concentrations of these elements in all samples (c.f. table 5). Elements with lower concentrations in some samples were either not found or showed inacceptable high RSD values with more than 50 % RSD, partly even up to several hundred or thousand %. For keeping results and related conclusions reliable values with RSD > 50% were not considered. Therefore, aside from using all results from Ca, K, and Mg, only eight iron concentrations, seven zinc, six manganese and five strontium results were taken for comparison with ICP-OES results, whereas no results for Cu, Co and Cr were available (c.f. table 5)

Other than INAA, ICP-OES could determine most elements in all samples above LoD with low RSD. Median RSD ranged between 0.8 – 6.8 % proving high precision. However, only two results for Co and four for Cr were obtained above LoD (c.f. table 6).

Each employed technique has its own considerations and a different approach. However, in order to rely on both techniques their results should have some correlations. Furthermore, the slopes of such correlations ideally should be close to 1.0, indicating identical results by both techniques. Therefore, a comparison between ten elements (Ca, K, Fe, Mn, Mg, Sr, and Zn ) has been conducted.

Table 5

Table 6

The correlations between the results obtained for the above-mentioned elements were examined using the correlation coefficient (R2) and the slope values. From figures 1-4, significant correlations were observed in all elements with high concentrations, e.g. Ca (R2 = 0.9214), K (R2 = 0.969) and Mn (R2 = 0.977). For K dependence was observed for concentration. Above ca. 18000 mg/kg ICP-OES provided slightly lower results compared to INAA, which could be explained probably by an overload of the digestion system. For concentrations below 18000 mg/kg slope b = 1.119 and R2 = 0.9923. Although Zn was found only in low amounts, good agreement between the two different techniques was noticed with R2 = 0.964, implying the ability of both techniques to detect this element effectively even at trace amounts.

Mg showed a low slope value (0.77) and correlation coefficient (0.648). Both techniques revealed equivalent ability to measure this element at similar level in the most analyzed samples, however surprisingly at higher concentration than 3000 mg/kg an unsystematic variation was observed in few samples, which might be the explanation for the lower slope and r2 value. It was striking that at concentrations below 3000 mg/kg R2 and slope “b” showed considerably improved values, i.e. R2 = 0.9278 and b= 0.8217 indicating acceptable comparability between both methods in this concentration range. Regarding Fe (figure 6), significant correlation (R2 = 0.9928) was reported, however the value of the slope was 0.723. Slope value below 1 may be due to either an overestimation by INAA as monitored for the reference material (c.f. table 3) or to loss during pressure digestion/ incomplete digestion for ICP-OES. Thus this finding indicates the variation between the results obtained by the two implemented techniques for the same samples.

The results obtained for Cr by INAA showed extremely high RSD values and were therefore not considered for comparison with ICP-OES, which in turn provided only results above LoD for three samples, however, with good precision. Furthermore, related to observations with CRM INAA tended to overestimate Cr concentrations as discussed above. Moreover, plant Cr contents measured by INAA were not confirmed by ICP-OES, as the most values from later technique are below or closed to LOD, which is in average 0.5 mg/kg. To avoid misleading results, this matter is recommended to be considered since Cr is discussed to have beneficial health effects e.g. in diabetes mellitus (Ebrahim et al., 2012). In this study, Cu and Sr were not detected by INAA in the most studied plants (below 3 mg/kg for Cu and 78 mg/kg for Sr). For this reason, the correlations between INAA and ICP-OES results for both elements were not conducted.

**Development of Mathematical Modelling**

Mathematical modelling has become a reliable tool in quantitative analysis in nuclear physics. Previous studies focused on using Artificial Neural Network **(**ANN) however, in this work a simple linear mathematical model was developed to estimate concentration of some elements.

Using the least square method (LSM) correlations were investigated for the predicted and measured concentration of Cu and Cr. Table 7 shows the parameters of equation (1) for each element. The equation was validated using three different WEAL standard plant reference materials. The predicted concentration of Cu and Cr calculated by the equation, showed in part acceptable consistency to their corresponding certified values as well to the ICP-OES measurements (table 7). Copper concentrations were estimated in general in the certified, low mg/kg range. At concentrations above 5 mg/kg estimated values differed less than 24% or even less than -13% above 7.5 mg/kg. Thismight be still an acceptable estimation when the analytical system cannot provide results above LoD. Cr- values were irregularly estimated too high or too low by at least 34 %. Consequently, we consider the modelling as not sufficiently reliable for this element. Overall, this simple modelling is far from being perfect but when Cu or Cr concentrations are below LOD of INAA and no alternative determination system is at hand it could help to roughly estimate Cu concentrations and for Cr at least a crude indication of the concentration range could be derived from measured concentration of K, Zn , Mn, Mg, and Fe .

**Conclusion**

As a conclusion, INAA is a non-destructive reference method which is equally competitive to ICP-OES for the measurements of Ca, K, Mg and mostly also Fe, in medicinal plants. For elements Zn, Mn, Sr, Cu and Cr the concentrations in medicinal plants were often too low for INAA determination (<LoD) or results were flawed by very high RSD. Due to its non-destructive feature and hence avoidance of sample preparation, INAA needs no consumption of chemicals compared to ICP-OES that require sample digestion procedure prior to analysis. In turn, ICP-OES seems to be the technique of choice when measuring Cu and Cr and Sr in plants, and in general, when element concentrations are below the limit of detection of INAA. Alternatively Cu and Cr levels can be roughly estimated by applying the developed equation at the obtainability of K, Zn , Mn, Mg, Fe concentrations in plant samples.

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**Table captions**

**Table 1** Some basic information on the examined plants

**Table 2** Relevant nuclear data for the examined radionuclides

**Table 3** Elemental concentration (Mean ± SD mg/kg) of SRM 1547 (peach leaves) using INAA

**Table 4** Elemental concentration (Mean ± SD mg/kg) of reference ERM-CD 281 (rye grass) using ICP-OES

**Table 5** Element concentration (Mean ± SD mg/kg) in Sudanese medicinal plants obtained by INAA

**Table 6** Element concentration (Mean ± SD mg/kg) in Sudanese medicinal plants obtained by ICP-OES

**Table 7** Predicted concentrations (mg/kg) of Copper and chromium determined in WEPAL plant reference material

**Table 1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Botanical name** | **Local name** | **Family** | **Part used** | **Collection area** |
| Acacia nilotica (L.) Willd. Ex Del.  | Garad | Mimosaceae | Fruits | Khartoum Nile bank |
| Acacia Senegal (L.) Willd. | Hashab  | Fabaceae-Mimosoidae | Exudates and Woods | Al-Obeid |
| Balanitesaegyptiaca (L.) Del | Laloub  | Balanitaceae | Fruits and Seeds | Gadarif |
| Cajanuscajan (L.)Millsp. | Aladsia | Fabaceae-Papilionoidae | Seeds | Al-Faw |
| Capsicum frutescens L. | Shatta | Solanaceae | Fruits | Khartoum Nile bank |
| Citrulluscolocynthis (L.) Schrad. | Handal | Cucurbitaceae | Fruits and Seeds | West Omdurman |
| Cucurbita maxima L. | Garah-Asali | Cucurbitaceae | Seeds | Sinnar |
| CymbopogonproximusStapf | Maharaib | Poaceae | Leaves | Al-Butana |
| Foeniculumvulgare P. Mill. | Shamar | Apiaceae | Whole plants | Dongola |
| GuierasenegalensisJ.F.Gmel. | Gubeish | Combretaceae | Leaves | Tandalti |
| Haplophylumtuberculata | Haza | Rutaceae | Stems | Abu-Hamed |
| Lawsoniainermis L. | Henna  | Lythraceae | Leaves | Khartoum local market |
| Nigella sativa L. | Kamoon-Alasod | Ranunculaceae | Seeds | Khartoum local market |
| Salvadorapersica L. | Aarak | Salvadoraceae | Leaves | Kassala |

**Table 2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Nuclide analyzed** | **Radionuclide detected** | **Half-life T1/2** | **Gamma ray energy (keV)** | **Branching ratio (%)** |
| 26Mg | 27Mg | 9.45 min | 1014.44 | 28.00 |
| 41K | 42K | 12.36 h | 1524.60 | 18.08 |
| 50Cr | 51Cr | 27.7 d | 320.080 | 10.08 |
| 55Mn | 56Mn | 2.58 h | 846.760 | 98.85 |
| 58Fe | 59Fe | 44.5 d | 1099.25 | 56.50 |
| 59Co | 60Co | 5.27 Y | 1173.24 | 99.90 |
| 63Cu | 64Cu | 12.70 h | 1345.77 | 39.00 |
| 64Zn | 65Zn | 243.9 d | 1115.55 | 50.70 |
| 48Ca | 49Ca | 8.7 min | 3084.40 | 91.70 |
| 84Sr | 85Sr | 64.8 d | 1065.00 | 33.01 |

**Table 3**

|  |  |  |  |
| --- | --- | --- | --- |
| **Element** | **Measured value** | **Certified value** | **Relative Error %** |
| K | 23028±22 | 24300 | -5.23 |
| Mg | 4403±80 | 4320 | 1.9 |
| Ca | 13594±57 | 15600 | -12.86 |
| Fe | 239±17 | 218 | 9.63 |
| Zn | 18.68±3 | 17.9 | 4.4 |
| Mn | 91.87±16 | 98 | -6.25 |
| Cr | 1.63±0.9 | 1.0 | 63 |
| Co | 0.075±0.03 | 0.07 | 7.14 |

**Table** 4

|  |  |  |  |
| --- | --- | --- | --- |
| **Element** | **Measured value** | **Certified value** | **Relative error (%)** |
| B | 5.5 ± 0.2 | 5.5 ± 0.5 | 0.0 |
| Cr | 22.7 ± 0.4 | 24.8 ± 1.3 | -8.5 |
| Cu | 9.7 ± 0.2 | 10.2 ± 0.5 | -4.9 |
| Mn | 77.5 ± 1.9 | 82 ± 4 | -5.5 |
| Ni | 13.9 ± 0.3 | 15.2 ± 0.6 | -8.6 |
| Zn | 28.3 ± 0.3 | 30.5 ± 1.1 | -7.2 |

**Table 5**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plant** | **Ca** | **K** | **Zn** | **Mn** | **Mg** | **Sr** | **Fe** | **Cu** | **Co** | **Cr** |
|  Acacia nilotica (L.) Wild. Ex Del. | 6955±5.7 | 12080±1.6 | 10.28±11.2 | 13.24±44.7 | 649.3±13.7 | 120.1±13.3 | 334.7±8.1 | ND | 0.2309±16.5 | 2.354±10.9 |
| Acacia Senegal (L.) Wild | 8039±2.9 | 7872±1.5 | ND\* | ND | 1282±3.5 | 78.05±5.5 | ND | 2.957±30.8 | ND | 4.51±7.6 |
| Balanitesaegyptiaca (L.) Del | 751.4±6.7 | 19100±1.4 | ND | ND | 433.4±11.4 | ND | 36.72±34.7 | ND | ND | 5.464±9.4 |
| Cajanuscajan (L.) Millsp | 748.2±5.2 | 13990±1.5 | 31.07±6.4 | 13.57±16 | 1026±4 | ND | ND | 13.58±8.6 | ND | 2.839±17.1 |
| Capsicum frutescens L. | 874.7±5.8 | 27430±1.5 | 19.07±20.8 | 14.95±35.3 | 1795±4.7 | ND | 218.4±11.3 | 10.79±27.5 | 0.6218±8.7 | 7.227±6.8 |
| CitrulluscolocynthisL.Schrad. | 602.4±10 | 5624±1.6 | 25.72±16.5 | 15.37±28.9 | 2043±6 | ND | 53.01±28.2 | 11.64 | 0.1303±24.1 | 5.273±7.9 |
| Cucurbita maxima L. | 371.9±6.9 | 7772±1.5 | 73.03±3.6 | 16.23±5 | 1901±2.8 | ND | 328.2±8.2 | 7.178±9.8 | 0.3685±9.7 | 4.847±6.5 |
| Cymbopogon ProximusStapf. | 1263±7 | 6354±1.5 | 11.66±12 | 145.5±4.8 | 975.8±10.5 | ND | 283.8±8.4 | ND | 0.1738±18.7 | 5.475±9.6 |
| FoeniculumvulgareP.Mill. | 13670±5.1 | 26440±1.6 | 27.92±8.1 | 83.5±10.1 | 5419±6.3 | 134.8±14.1 | 2887±3.3 | ND | 1.506±5.8 | 12.72±15.6 |
| GuierasenegalensisJ.F.Gmel. | 13360±3.3 | 9082±1.5 | 26.85±8 | 556.4±3.2 | 2571±5.8 | ND | 618.5±6.5 | ND | 2.602±7.6 | 9.025±8.8 |
| Haplophyllum tuber-culatum | 6586±4.3 | 14270±1.6 | 8.57±25.9 | ND | 1022±10.5 | 95.65±18.1 | ND | ND | ND | 4.369±8.3 |
| Lawsoniainermis L. | 10880±3 | ND | 13.93 | 41.71±7 | 2913±3.2 | 174.2±5.6 | 208.9±10.9 | ND | 0.522±9.7 | 7.151±8.4 |
| Nigella sativa L. | 6712±5 | 8921±1.7 | 45.6±6.2 | 39.2±15.1 | 3806±5 | ND | 1018±5 | 16.73±25.2 | 0.5236±9.8 | 6.222±9.2 |
| Salvadorapersica L. | 8018±5.6 | 13740±1.6 | ND | ND | 679.2±11.7 | 200±11 | ND | ND | ND | 4.048±7.8 |

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