**Quantification of macro, micro and trace elements, and antimicrobial activity of medicinal herbs and their products**

**Running Title:** Herbs and products multi-element analysis

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**Abstract**

The study describes the content of macro, micro, and trace essential and toxic elements in thirteen medicinal herbs and their products including *Acorus calamus*, *Blepharis edulis*, *Caesalpinia bonducella*, *Curculigo orchioides*, *Helicteres isora*, *Holarrhena pubescens*, *Pastinaca sativa*, *Pistacia integerrima*, *Quercus infectoria*, *Rauwolfia serpentina*, *Saussurea lappa*, *Teucrium stocksianum*, and *Xanthium strumarium* available in the local markets of Pakistan. The elemental content were analyzed with the techniques of inductively coupled plasma (ICP) optical emission spectroscopy (OES) and ICP-mass spectrometry (MS). Furthermore, their antibacterial and antifungal activities were evaluated against the selected microbial pathogens including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*, *Candida albicans*, *Candida krusei*, *Aspergillus flavus*, and *Trichophyton mentagrophytes*. Among macro elements, K and Ca showed the highest content, micro elements were in the order of Rb85/ Sr87 > Zn64/Cu63 > Ni60, and among essential trace elements, the content of Cr52/Cr53 and Co59 were high. The content of the analyzed toxic elements were lower than the permissible standard values. The antimicrobial activities against the subject strains were significant with inhibition zones of 7.0 mm to 19.0 mm in disc diffusion procedure, and 62.5 µg/mL to 1000 µg/mL in minimum inhibitory concentration method. Hence, the presence of nutritional elements at appreciable concentrations, toxic elements within permissible ranges, and significant antimicrobial potential assume the subject herbs as promising nutritional and therapeutic remedies.

**Keywords:** herbs; Pakistan; trace elements; inductively coupled plasma-optical emission spectroscopy, antimicrobial

Table S1. List of herbs, part used in medicine, scientific names, families, phytochemicals present, and medicinal and pharmacological properties

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Botanical Name** | **Local Name** | **Family** | **Part used** | **Phytochemicals** | **Folk uses and pharmacological activities** | **References** |
| 1 | *Acorus calamus* | Skhawaja, muskrat root, sweet flag | Acoraceae | Root | Alcohol, phenols, flavonoids, aldehyde, ketones, ester, furan, N-containing compounds | Abdominal pain, dyspepsia, dysentery, fat removal, hemorrhoids, colic pain, diabetes, bronchitis, skin diseases, improving speech and mental problems in children, insect pest, CNS depressant, anti-inflammatory, antidiabetic, anticancer, antimicrobial | Kim et al. 2009; Wu et al. 2009 |
| 2 | *Blepharis edulis* | Utangan | Acanthaceae | Seeds | Aromatics, flavonoids, tannins, saponins, phenolics | Diuretic, expectorant, cough, asthma, fever, throat swelling, ulcers, wounds, antimicrobial, anticancer, antispasmodic, antiplatelet aggregation | Mahboubi et al. 2013 |
| 3 | *Caesalpinia bonducella* | Karanjwa  (Bonduc nut or fever nut) | Fabaceae (Leguminosae) | Seeds | Alkaloids, triterpenoids, phenols, flavonoids, glycosides, saponins, tannins, essential oils | Inflammation, liver disorders, diarrhea, fever, antidiarrhoeal, antimicrobial, antidiabetic, antitumor, antipyretic, anti-inflammatory, antioxidant, insecticidal | Billah et al. 2013 |
| 4 | *Curculigo orchioides* | Musli syah | Amaryllidaceae | Rhizomes | Triterpenoids, sterols, flavonoids, phenolic glycoside, carbohydrates, saponins, tannins | Coolant, diuretic, jaundice, asthma, male sex dysfunction, bleeding, injuries, menstrual cycle, hepatoprotective, cytotoxic, anticonvulsive | Dall’Acqua et al. 2009; Nie et al. 2009 |
| 5 | *Helicteres isora* | Maror phali (screw tree) | Sterculiaceae | Fruit | Flavonoids, alkaloids, phenolics, glycosides, phytosterols, carotenoids, tannins, volatile oil | Diarrhea, snake biting, constipation, antioxidant, anticancer, antidiabetic, hepatoprotective, anthelmintic, antimicrobial, anti-inflammatory | Kumar & Singh, 2014 |
| 6 | *Holarrhena pubescens* | Andrajao talkh | Apocynaceae | Seeds, stem bark | Steroidal alkaloids; kurchine, kurchimine, regholarrhimine, kurchamine, holaphyllidine, conessidine, conkurchicine, holamine, holaromine, holarrhimine mitiphylline, holadysenterine, non-alkaloids; kurchinin kurchinicin, holarrhenol | Amoebic dysentery, spleen and chest infections, anti-helminthic, appetizing, antidiarrheal, astringent | Tuntiwachwuttikul et al. 2007; Yang et al. 2012 |
| 7 | *Pastinaca sativa* | Shaqaqul  (Parsnip) | Apiaceae | Root | Coumarins, furanocoumarins, essential oil | Tonic, skin allergies, carminative, anti-inflammatory | Waksmundzka-Hajnos et al. 2004 |
| 8 | *Pistacia integerrima* | Kakra singi (crabs claw, Zebrawood) | Anacardiaceae | Galls | Alkaloids, saponins, flavonoids, catechins, triterpenoids, tannins, hydroxydecanylarachidate, octadecan-9, 11-diol-7-one, pisticialanstenoic acid, pistagremic acid, essential oils | Cough, asthma, fever, wound healing, anticancer, anti-inflammatory, hepatoprotective, anti-gastrointestinal, antiasthmatic, analgesic, leishmanicidal | Ahmad et al. 2008; Rauf et al. 2014 |
| 9 | *Q. infectoria* | Mazoo, manjakani, gall oak, magic nut | Fagaceae | Galls, seeds | Tannins (50-70%), gallic acid,ellagic acid(2-4%), digallate, theogallin, gallic acid | Wound healing, digestive disorder, kidneys, dental, toothache, gum remedies, local anesthetic, vaginal tightening, antimicrobial, anti-oxidant, antidiabetic,and anti-inflammatory | Fan et al 2014; Kaur et al. 2004 |
| 10 | *Rauwolfia serpentina* | Sarpagandha | Apocynaceae | Roots, stem | Alkaloids, flavonoids, phenolics, saponins, tannins | Schizophrenia, cholera, colic, snake bite, antihypertensive, tranquilizing agent | Bharti et al. 2017 |
| 11 | *Saussurea lappa* | Kuth, Qasht-e-shireen | Asteraceae | Roots | Alkaloids, steroids, ﬂavonoids, resins | Skin allergies, asthma, toothache, dysentery, nausea, anthelmintic, listening problems, anti-trypanosomal, anti-cancer, anti-ulcer, anti-inﬂammatory, hypoglycemic, antidiarrheal | Robinson et al 2008; Julianti et al. 2011 |
| 12 | *Teucrium stocksianum* | Mastyara | Labiatae | Whole plant | alkaloids, tannins**,** flavonoids, saponins, steroids, terpenoids, anthraquinones, phlobatannins, and glycosides | Stomachic, diarrhea, diabetes, gastrointestinal ailments, inflammatory conditions, hypertension, sore throat, insect repellent, anti-inflammatory, analgesic, antispasmodic, cytotoxicity, phytotoxicity | Bakhtiari & Asgarpanah, 2015; Shah et al. 2015 |
| 13 | *Xanthium strumarium* | Jishkay | Asteraceae | Leaves, fruits, seeds | Phenolics, flavonoids xanthiazone, chlorogenic acid, ferulic acid, formononetin, ononin, VOCs | Asthma, nasal sinusitis, headache diuretic, prostate diseases, antifungal, anti-allergic, antimicrobial, anticancer, anti-hepatotoxic | Han et al. 2007; Peng et al. 2014 |

Table S2. Analytical methods validation parameters for ICP-OES and ICP-MS in the macro, micro, and trace elements

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Element | Correlation coefficient  (*R2*) | Limits of detection  (ng/g) | Limits of quantification  (ng/g) | Coefficient  of variance  (CV%) | Spike recovery† (%) |
| Macro elements | | | | | |
| Al | 0.9999 | 8.50 | 28.1 | 1.21 | 103 |
| Ca | 0.9974 | 9.12 | 30.1 | 1.42 | 94 |
| Fe | 0.9998 | 0.448 | 1.48 | 1.15 | 94 |
| K | 0.9978 | 12.2 | 40.3 | 1.19 | 94 |
| Mg | 0.9974 | 8.11 | 26.8 | 1.14 | 95 |
| Na | 0.9986 | 13.0 | 42.9 | 2.19 | 95 |
| P | 0.9999 | 7.57 | 25.0 | 1.19 | 98 |
| S | 0.9999 | 11.1 | 36.7 | 2.15 | 94 |
| Micro elements | | | | | |
| Cu | 0.9996 | 0.054 | 0.169 | 1.16 | 100 |
| Ni | 0.9998 | 0.250 | 0.835 | 1.03 | 102 |
| Rb | 0.9999 | 0.055 | 0.191 | 1.14 | 99 |
| Sr | 0.9999 | 0.478 | 1.564 | 1.17 | 97 |
| Zn | 0.9999 | 0.214 | 0.709 | 1.37 | 98 |
| Trace essential elements | | | | | |
| Co | 0.9997 | 0.049 | 0.145 | 0.890 | 96 |
| Cr | 0.9999 | 0.093 | 0.326 | 0.984 | 98 |
| Se | 0.9999 | 0.248 | 0.796 | 1.16 | 103 |
| V | 0.9999 | 0.030 | 0.105 | 1.66 | 98 |
| Trace non-toxic element | | | | | |
| Ba | 0.9999 | 0.079 | 0.251 | 0.930 | 99 |
| Be | 0.9999 | 0.073 | 0.222 | 0.562 | 97 |
| Ga | 0.9999 | 0.048 | 0.158 | 0.519 | 95 |
| Li | 0.9999 | 0.078 | 0.234 | 0.981 | 99 |
| Trace toxic elements | | | | | |
| As | 0.9999 | 0.067 | 0.222 | 1.41 | 98 |
| Cd | 0.9999 | 0.080 | 0.273 | 1.10 | 103 |
| In | 0.9999 | 0.041 | 0.126 | 0.938 | 96 |
| Pb | 0.9999 | 0.058 | 0.172 | 0.889 | 104 |
| Tl | 0.9999 | 0.039 | 0.122 | 1.12 | 98 |
| U | 0.9999 | 0.011 | 0.011 | 1.53 | 102 |

†Macro elements were spiked at 3,000 µg/kg, while micro and trace elements were spiked at 50 µg/kg

Table S3. Accuracy determination of ICP-OES and ICP-MS techniques by analyzing standard reference material (NIST SRM-1573a), tomato leaves

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Instrument | Element | Certified value  (mg/kg) | Measured value (mg/kg) | Recovery (%) |
| ICP-MS | Cr | 1.98 ± 0.05 | 2.04 ± 0.05 | 103 |
| Co | 0.58 ± 0.01 | 0.56 ± 0.01 | 97 |
| Cu | 4.66 ± 0.14 | 4.36 ± 0.43 | 94 |
| Se | 0.056 ± 0.003 | 0.060 ± 0.001 | 107 |
| Zn | 30.3 ± 0.7 | 30.9 ± 1.01 | 102 |
| Cd | 1.50 ± 0.04 | 1.45 ± 0.02 | 97 |
| As | 0.117 ± 0.004 | 0.121 ± 0.013 | 103 |
| ICP-OES | Al | 573.0 ± 12.0 | 549.0 ± 55.0 | 96 |