

Table 5: The content of free amino acids in *Orthosiphon stamineus* extracts.

| Components | OS-W nmol/mg | OS-E nmol/mg | OS-M nmol/mg | OS-EW nmol/mg | OS-MW nmol/mg |
|---------------------------------------|-----------------|-----------------|-----------------|------------------|------------------|
| L-aspartic acid | 0.93 ± 0.01 | 1.17 ± 0.03 | 1.11 ± 0.06 | 1.32 ± 0.07 | 1.07 ± 0.03 |
| L-Glutamic acid | 0.68 ± 0.04 | 3.53 ± 0.16 | 2.17 ± 0.10 | 4.01 ± 0.12 | 2.49 ± 0.12 |
| L-serine | 0.33 ± 0.02 | 0.12 ± 0.01 | 0.06 ± 0.01 | ND | ND |
| L-Histidine hydrochloride monohydrate | ND | 1.83 ± 0.05 | 1.47 ± 0.03 | 2.09 ± 0.04 | 1.60 ± 0.08 |
| Glycine | ND | ND | ND | ND | ND |
| L-Threonine | ND | 0.14 ± 0.03 | 0.06 ± 0.01 | 0.26 ± 0.02 | 0.19 ± 0.01 |
| L-arginine | ND | ND | ND | ND | ND |
| L-Alanine | ND | ND | ND | ND | ND |
| L-Tyrosine | ND | ND | ND | ND | ND |
| L-Cystine | ND | ND | ND | ND | ND |
| L-Valine | 0.53 ± 0.04 | 0.34 ± 0.02 | 0.33 ± 0.02 | 0.44 ± 0.03 | 0.46 ± 0.02 |
| L-Methionine | 0.40 ± 0.01 | ND | ND | 0.32 ± 0.03 | 0.37 ± 0.03 |
| L-Phenylalanine | ND | ND | ND | ND | ND |
| L-Isoleucine | ND | ND | ND | ND | ND |
| L-Leucine | ND | ND | ND | ND | ND |
| L-Lysine Hydrochloride | 0.62 ± 0.06 | 0.35 ± 0.04 | 0.28 ± 0.03 | 0.47 ± 0.06 | 0.52 ± 0.04 |
| L-Proline | ND | ND | ND | ND | ND |

REFERENCES

- Ahamed MBK, Aisha AF, Nassar ZD, Siddiqui JM, Ismail Z, Omari S, *et al.* Cat's whiskers tea (*Orthosiphon stamineus*) extract inhibits growth of colon tumor in nude mice and angiogenesis in endothelial cells via suppressing VEGFR phosphorylation. *Nutr Cancer* 2012;64:89-99.
- Tezuka Y, Stampoulis P, Banskota AH, Awale S, Tran KQ, Saiki I, *et al.* Constituents of the Vietnamese medicinal plant *Orthosiphon stamineus*. *Chem Pharm Bull* 2000;48:1711-9.
- Awale S, Tezuka Y, Banskota AH, Kouda K, Tun KM, Kadota S. Five novel highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar. *J Nat Prod* 2001;64:592-6.
- Awale S, Tezuka Y, Banskota AH, Shimoji S, Taira K, Kadota S. Norstaminane- and isopimarane-type diterpenes of *Orthosiphon stamineus* from Okinawa. *Tetrahedron* 2002;58:5503-12.
- Hossain MA, Mizanur Rahman SM. Isolation and characterisation of flavonoids from the leaves of medicinal plant *Orthosiphon stamineus*. *Arabian J Chem* 2015;8:218-21.
- Akwuah G, Zhari I, Norhayati I, Sadikun A, Khamseh S. Sinensetin eupatorin 3'-hydroxy-5,6,7 4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. *Food Chem* 2004;87:559-66.
- Sahib HB, Aisha AF, Yam MF, Asmawi MZ, Ismail Z, Salhimi SM, *et al.* Anti-angiogenic and antioxidant properties of *Orthosiphon stamineus* Benth methanolic leaves extract. *Int J Pharm* 2009;5:162-7.
- Arafat OM, Tham SY, Sadikun A, Zhari I, Haughton PJ, Asmawi MZ. Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats. *J Ethnopharmacol* 2008;118:354-60.
- Ohashi K, Bohgaki T, Shibuya H. Antihypertensive substance in the leaves of kumis kucing (*Orthosiphon aristatus*) in Java Island. *Yakugaku Zasshi* 2000;120:474-82.
- Siddiqui M, Hafizoh S, Ismail Z, Sahib H, Helal M. Analysis of total proteins, polysaccharides and glycosaponins contents of *Orthosiphon stamineus* benth in spray and freeze dried methanol:water (1:1) extract and its contribution to cytotoxic and antiangiogenic activities. *Pharmacog Res* 2009;1:320-6.
- Gomez L, Faurobert M. Contribution of vegetative storage proteins to seasonal nitrogen variations in the young shoots of peach trees (*Prunus persica* L. Batsch). *J Exp Bot* 2002;53:2431-9.
- Shimizu MM, Mazzafera P. Compositional changes of proteins and amino acids in germinating coffee seeds. *Braz Arch Biol Technol* 2000;43:259-65.
- Hara Y, Luo S, Wickremasinghe R, Yamanishi T. Special issue on tea. *Food Rev Int* 1995;11:371-542.
- Kimura O, Ozeki M, Juneja LR, Ohira H. L-Theanine reduces psychological and physiological stress responses. *Biol Psychol* 2007;74:39-45.
- Sugiyama T, Sadzuka Y. Theanine and glutamate transporter inhibitors enhance the antitumor efficacy of chemotherapeutic agents. *BBA Rev Can* 2003;1653:47-59.
- Yamada T, Terashima T, Kawano S, Furuno R, Okubo T, Juneja L, *et al.* Theanine, γ -glutamylethylamide, a unique amino acid in tea leaves, modulates neurotransmitter concentrations in the brain striatum interstitium in conscious rats. *J Amino Acids* 2009;36:21-7.
- Chen L, Chen Q, Zhang Z, Wan X. A novel colorimetric determination of free amino acids content in tea infusions with 2,4-dinitrofluorobenzene. *J Food Compos Anal* 2009;22:137-41.
- Wang L, Xu R, Hu B, Li W, Sun Y, Tu Y, *et al.* Analysis of free amino acids in Chinese teas and flower of tea plant by high performance liquid chromatography combined with solid-phase extraction. *Food Chem* 2010;123:1259-66.
- Alcazar A, Ballesteros O, Jurado J, Pablos F, Martin M, Vilches J, *et al.* Differentiation of green, white, black, Oolong, and Pu-erh teas according to their free amino acids content. *J Agric Food Chem* 2007;55:5960-5.
- Aucamp J, Hara Y, Apostolides Z. Simultaneous analysis of tea catechins, caffeine, gallic acid, theanine and ascorbic acid by micellar electrokinetic capillary chromatography. *J Chromatogr A* 2000;876:235-42.
- Ding Y, Yu H, Mou S. Direct determination of free amino acids and sugars in green tea by anion-exchange chromatography with integrated pulsed amperometric detection. *J Chromatogr A* 2002;982:237-44.
- Pongsuwan W, Bamba T, Harada K, Yonetani T, Kobayashi A, Fukusaki E. High-throughput technique for comprehensive analysis of Japanese green tea quality assessment using ultra-performance liquid chromatography with time-of-flight mass spectrometry (UPLC/TOF MS). *J Agric Food Chem* 2008;56:10705-8.
- del Álamo M, Casado L, Hernández V, Jiménez JJ. Determination of free molecular phenolics and catechins in wine by solid phase extraction on polymeric cartridges and liquid chromatography with diode array detection. *J Chromatogr A* 2004;1049:97-105.
- Pérez-Magariño S, Ortega-Heras M, Cano-Mozo E. Optimization of a solid-phase extraction method using copolymer sorbents for isolation of phenolic compounds in red wines and quantification by HPLC. *J Agric Food Chem* 2008;56:11560-70.
- Henderson J, Brooks A. Improved amino acid methods using Agilent ZORBAX Eclipse Plus C18 columns for a variety of Agilent LC instrumentation and separation goals. <https://www.agilent.com/cs/library/applications/5990-4547EN.pdf>.
- Shahir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J Chromatogr A* 2003;987:57-66.
- Mao H, Chen B, Wang W, Zhuang P, Zong M, Xu Z. Simultaneous analysis of citrulline and arginine in serum and tissue. *Microchem J* 2011;97:291-5.