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Comparative Investigation on Biological Activity of *Tilia Tomentosa* M. and *Matricaria Chamomilla* L. Water Extracts

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Running title: **Biological Activity of *Tilia tomentosa* M. and *Matricaria chamomilla* L.**

Abstract

Tilia tomentosa M. and *Matricaria chamomilla* L., widely used in the everyday life herbal plants, were the objects of the present research paper. In order to find a more effective method of extraction one-way ANOVA with post-hoc Tukey-Kramer's test was conducted to compare the effect of water extraction by infusion, decoction, and microwave-assisted extraction on the total phenolic content and *in vitro* antioxidant activity assays (DPPH, ABTS, FRAP and CUPRAC). The performed analyses show the prevalence of biological activity in the decocts referring to the both plant species. The highest TEAC values were established according the CUPRAC assay for the *T. tomentosa* decoct (272.96 ± 6.23 µMTE/g DW). The results indicate that the total phenolic content correlated well with the evaluated antioxidant potential. The outcomes of the research support the recommendations of frequently intake of the *T. tomentosa* and *M. chamomilla* water extracts and could confirm the health benefits of their consumption.

Practical applications

The determination of the most suitable method for water extracts obtaining contribute to the practical application of the *Tilia tomentosa* M. and *Matricaria chamomilla* L. The evaluation of the biological activity of both plant species especially in widely used in everyday life form like water extracts could contribute to the interpretation of their potential health benefit. Furthermore, the assessment of the total polyphenolic content and antioxidant activity of the investigated extracts expand the knowledge of those widely used in the phytotherapy plants.

Key words: antioxidant activity, *Matricaria chamomilla* L., *Tilia tomentosa* M., total polyphenolic content, water extracts

Introduction

Various research studies have shown relationship between the consumption of food rich in antioxidants and the frequency of various diseases (Harrison et al., 1999; Dhalla et al., 2000). Nowadays special attention is paid to diverse raw materials, mainly of plant origin, rich in antioxidant substances. In tea and herbal infusions the main antioxidants are polyphenols (Das et al., 1997; Majchrzak et al., 2004; Georgieva et al., 2015a).

The plant infusions are very popular drinks all over the world. They are consumed based of the various containing polyphenolic compounds, such as flavonoids and phenolic acids (Kohlmünzer, 2003; Pereira et al., 2014), and their content in the raw material affects the level of their antioxidant activity (Cai et al., 2004). The content of these compounds may differ not only due to the natural diversification of the raw material resulting from, i.e. different environmental growing conditions and various processing technologies (fermented teas, half-fermented, non-fermented), but also due to the method of their preparation (tea infusions, herbal infusions). As a consequence, a different level of their activity should be expected.

In ethnobotanical studies decoction is considered to be a preferred mode of use as it is the most common method of traditional drug preparation (Grønhaug et al., 2008; Simbo, 2010).

Chamomile has been used for centuries as a medicinal plant mostly for its anti-inflammatory, analgesic, antimicrobial, antispasmic and sedative properties (McKay & Blumberg, 2006). As a member of *Compositae* family, it is widely represented by two known varieties viz. German chamomile (*Matricaria chamomilla*) and Roman chamomile (*Chamaemelum nobile*). It is known to contain several classes of biologically active compounds including essential oils and several polyphenols.

Numerous reports are available on the identification of several phenolic compounds including apigenin, quercetin, and patuletin as glucosides and various acetylated derivatives (Avallone et al., 2000; Svehlikova et al., 2004). Infusion is one of the most popular methods and has been traditionally used as carminative and mild sedative to calm nerves and reduce anxiety, to treat hysteria, nightmares, insomnia and other sleep problems. Additionally, chamomile has been valued as a digestive relaxant and has been used to treat various gastrointestinal disturbances including flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea, and vomiting (Forster et al., 1980; Tyler, 1993). The widespread use and medicinal properties has made chamomile increasingly popular in the form of tea which is consumed at a rate of over one million cups per day. Apart from the existing traditional knowledge on its therapeutic efficacy more work has been conducted in recent years on chamomile to establish its antioxidant, hypocholesteroemic, anti-parasitic, anti-aging and anticancer properties (Lee & Shibamoto, 2002; Babenko & Shakhova, 2005; Skovgaard et al., 2006; Srivastava & Gupta, 2007; Di Giorgio et al., 2008). The aqueous and methanolic extracts obtained from chamomile were reported to exhibited anti-proliferative and apoptotic activity in various human cancer cells with minimal effect on normal cells (Srivastava & Gupta, 2007).

Tilia is a genus in the family of *Tiliaceae* with about thirty species of trees, native throughout most of the temperate Northern Hemisphere, in Asia, Europe and eastern North America; it is absent in western North America. Only four of these species occur naturally in Europe, i.e. Caucasian lime *Tilia dasystyla* Stev., silver lime *Tilia tomentosa* Moench., small-leaved lime *Tilia cordata* Mill. and large- leaved lime *Tilia platyphyllos* Scop. (Radoglou et al., 2008). Moreover, *Tilia tomentosa* M. is mentioned in Scientific Opinion at EFSA Journal (2010) as to contribute to body defences against external agents.

Linden blossom is used in folk medicine mainly in the form of decoctions and infusions, as inflammation of the upper respiratory tract, due to its contents of essential oil, flavonoids, saponines, tannins etc. (Ivanov et al., 1972; Petkov, 1982; Stefanov et al., 1983; Ahtardzhiev & Benbasat, 1985).

However, the both investigated plant species - *Tilia tomentosa* M. and *Matricaria chamomilla* L. are widely used in the everyday life with various applications. Based on this, the purpose of the present study was to evaluate the biological activity of the water extracts and thus to compare the different techniques for extracts obtaining.

Materials and methods

Plant material

Tilia tomentosa M. and *Matricaria chamomilla* L. as dry herbs were obtained from a local pharmacy (Plovdiv, Bulgaria). After additional drying the plants parts – leaves and stems were roughly grounded and stored in air-tight dark containers until extraction.

Preparation of plant extracts

In order to explore the most suitable form of extract obtaining for everyday consumption by human, different extraction procedures with water were investigated.

Grounded plant mass of *Tilia tomentosa* M. and *Matricaria chamomilla* L. was extracted individually with water at ratio of solvent to raw material of 1:20 (v/v).

Three extraction procedures were carried out as follow:

• Infusion was conducted using boiling water and then pouring it over the plant material allowing it to steep in the liquid for 30 minutes;

• Decoction was conducted in round-bottomed flask with a lid by boiling the plant material with the solvent for 30 min;

• Microwave-assisted extraction (MAE) experiments were performed with a domestic microwave oven (LG MB4047C). The extraction was carried out at output power 800 W for 30 sec (with frequency of the waves 2450 MHz).

All obtained extracts were filtered after incubation and stored at 4 °C without adding any preservatives until analyses.

Antioxidant activity (AOA)

Total polyphenol content analysis (TPC)

The total polyphenol content was analyzed using the Folin-Ciocalteu method of Kujala et al. (2000) with some modifications. Each sample extract (1 cm^3) was mixed with 5 cm^3 of Folin-Ciocalteu`s phenol reagent and 4 cm^3 of 7.5 % Na_2CO_3. The mixture was vortexed well and left for 5 min at 50 °C. After incubation, the absorbance was measured at 765 nm.

The TPC in the extracts was expressed as mg gallic acid equivalent (GAE) per g dry weight.

DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge DPPH radical was determined by the slightly modified method of Brand-Williams et al. (1995). Freshly prepared $4x10^{-4}$M methanolic solution of DPPH was mixed with the samples in a ratio of 2:0.5 (v/v). The light absorption was measured at 517 nm at room temperature after 30 min incubation. The DPPH radical scavenging activity was presented as a function of the concentration of Trolox. Trolox equivalent antioxidant capacity (TEAC) and was defined as the concentration of Trolox having equivalent AOA expressed as the μM Trolox per g dw.

ABTS radical cation decolorization assay

The radicals scavenging activity of the extracts against radical cation ($ABTS^{\bullet+}$) was estimated according to a previously reported procedure with some modifications (Re et al., 1999). The results were expressed as TEAC value (μM TE/g dw).

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie & Strain (1999). The FRAP reagent was prepared fresh daily and was warmed to 37 °C prior to use. The absorbance of the reaction mixture was recorded at 593 nm after incubation at 37 °C for 4 min. The results were expressed as μM TE/g dw.

Copper reduction assay (CUPRAC)

CUPRAC assay was performed according to the method of Ak & Gülçin (2008). To a test tube were added 1 cm^3 of $CuCl_2$ solution (1.0×10^{-2}M), 1 cm^3 of neocuproine methanolic solution (7.5×10^{-3}M), and 1 cm^3 NH_4Ac buffer solution (pH 7.0), and mixed; 0.1 cm^3 of herbal extract (sample) followed by 1 cm^3 of water were added (total volume of 4.1 cm^3), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of herbal extracts was measured as μM TE/g dw.

Statistical analysis

All experimental results were analyzed by one-way analysis of variance (ANOVA), using Tukey-Kramer's post-hoc test at a significance level of 5 %. The presented results are average from independent experiments carried out in triplicates. The results were expressed as mean ± SD, analyzed using MS Excel 2007 software.

Results

In the present study the total polyphenolic content and antioxidant activities of the most frequently consumed forms in the everyday life of *Tilia tomentosa* M. and *Matricaria chamomilla* L. were studied. One-way ANOVA with post-hoc Tukey-Kramer's test was conducted to compare the effect of extraction by infusion, decoction, and microwave on the TPC and AOA.

The results for total polyphenolic concentration of the water extracts from *T. tomentosa* and *M. chamomilla* (Chamomilla) are shown in Table 1. There was a statistically significant difference between the three extraction methods as determined by one-way ANOVA ($F(2,6)= 11327.0$, $p=0.05$ and $F(2,6)= 998.3$, $p=0.05$ for *T. tomentosa* and *M. chamomilla,* respectively). The highest phenolic content for both studied plants was measured for decoction extraction resulting in 11.59±0.13 and 8.36±0.30 mg GAE/g DW for *T. tomentosa* and *M. chamomilla,* respectively).

AOA was measured according to four different reliable methods: two stable radicals - $DPPH^{•}$ and $ABTS^{•+}$; FRAP and CUPRAC assays. The results are presented in Table 2. The established TEAC values according the DPPH assay were in range from 25.28 ± 1.58 to 288.93 ± 0.58 µM TE/g DW. The TEAC values according the ABTS assay were determined to be from 26.46 ± 0.46 to 222.14 ± 5.83 µM TE/g DW, which is in agreement with the evaluated polyphenolic content.

The FRAP values for the investigated extracts of *T. tomentosa* and *M. chamomilla* were in accordance with the above mentioned results as follows: from 33.61 ± 0.57 to 225.41 ±0.75 µM TE/g DW (Table 2). Using the CUPRAC assay the established results were from 36.95 ± 0.58 to 272.96 ± 6.23 µM TE/g DW for the three different types of water extracts.

It has to be noted, that the infusion technique seems more suitable for *M. chamomilla* rather than *T. tomentosa.* Regarding the other two extraction methods the obtained results were better for *T. tomentosa*.

Discussion

In order to investigate more properly the biological activity of the *Tilia tomentosa* M. and *Matricaria chamomilla* L. three different extraction procedures were investigated. The extractions were performed with water as the most frequently used in everyday life solvent.

Determination of total polyphenolic content (TPC)

Among all applied methods for extraction the decoction technique revealed as the most effective in terms of total polyphenolic substances. These findings correspond to the previously established by Georgieva et al. (2015b). The authors established 4.50 ± 0.69 and 18.49 ± 0.45 mg GAE/g DW for ethanol and methanol extract of *M. chamomilla*, resp. Other research groups (Rusaczonek et al., 2010) reported TPC for infusion of *M. chamomilla*– 44 ± 7.9 mg GAE/g dry tea. It has to be noted that the results were in prevalence of *T. tomentosa* excluding the infusion. This could be explained with the differences in the extraction techniques.

Determination of antioxidant activity (AOA) – DPPH, ABTS, FRAP and CUPRAC assays

The reason for using different methods for assessment of the antioxidant potential was to avoid errors associated with the *in vitro* methods.

By evaluation of the antioxidant capacity of the studied extracts higher TEAC value indicates that a sample has stronger AOA. Toward $DPPH^{•}$ Georgieva et al. (2015a) evaluated TEAC in ethanol and methanol extracts of *M. chamomilla* - 6.01 ± 0.57 and 4.65 ± 0.55 µMTE/g DW, resp. In addition, Rusaczonek et al. (2010) and Ivanova et al. (2005) established TEAC toward $ABTS^{•+}$ for infusions of *Chamomilla* of 180 ± 10.2 µmol of Trolox/g and 0.86 ± 0.11 mM, respectively.

Among all extracts the established results were in prevalence of decoctions. This method of extraction performed with water revealed as more effective in respect of biological activity.

In summary, the results of the total antioxidant capacity assays (Table 2) showed that the investigated extracts possessed AOA, which for the decoctions was higher than the other investigated type of extracts. The established total phenolic content confirmed the effectiveness of the decoction approach.

Interestingly, the highest AOA values were evaluated by the CUPRAC assay. The probable reason for a slight difference among the results of the applied assays – DPPH, ABTS, FRAP and CUPRAC could be the unique mechanism and the unequal sensitivity of each method performed. Therefore, the authors suggested that, when analyzing the antioxidant activity of samples, it is better to use at least two methods due to the differences between the test systems (Ou et al., 2001).

From the results it can be concluded that the decoction technique is more efficient compared to the microwave-assisted extraction and the infusion method in order to obtain extract rich in biologically active substances in terms of antioxidant potential. It should be noted that the results of microwave extracts were also relatively higher compared to traditional form - infusions. Thus nowadays the microwave extraction is increasingly used in everyday life, it can be concluded that such type of herbal extract of the studied plants *T. tomentosa* and *M. chamomilla* is reasonable to be recommended in terms of total polyphenolic content in addition to the decoction technique.

Conclusions

The present investigation dealt with biological activity evaluation of different water extracts of *Tilia tomentosa* and *Matricaria chamomilla.*

The results supported the recommendations of frequently intake of both plants in water extracts and could confirm the health benefits of their consumption.

However, the decoction technique revealed the best results in terms of total phenolic content and antioxidant activity in prevalence for the water extract of *M. tomentosa*.

References

Ahtardzhiev, H., Y. Benbasat (1985). Pharmacognosy, *Medicina i fizkultura*, Sofia. (In Bg)

Ak, T., I. Gülçin (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*, **174**(1): 27–37. DOI: 10.1016/j.cbi.2008.05.003

Apak, R., K. Guclu, M. Ozyurek, S. E. Karademir (2004). Novel total antioxidant capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method. *Food Chemistry,* **52**(26): 7970-7981. DOI: 10.1021/jf048741x

Avallone, R., P. Zanoli, G. Puia, M. Kleinschnitz, P. Schreier, M. Baraldi (2000). Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. *Biochemical Pharmacology,* **59**(11): 1387–1394. DOI: 10.1016/S0006-2952(00)00264-1

Babenko, N. O., O. H. Shakhova (2005). Age-dependent effects of flavonoids on secretory function of the rat liver. *Fiziolohichnyĭ zhurnal,* **51**(4): 65–69.

Benzie, I. F. F., J. J. Strain (1999). Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, **299**: 15–23. DOI: 10.1016/S0076-6879(99)99005-5 ·

Brand-Williams, W., M.E. Cuvelier, C. Berset (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, **28**(1): 25-30. DOI: 0.1016/S0023-6438(95)80008-5

Cai Y., Q. Luo, M. Sun, H. Corke (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, **74**(17): 2157–2184. DOI: 10.1016/j.lfs.2003.09.047

Das, M., J. R. Vedasiromoni, S. P. S. Chauhan, D. K. Ganguly (1997). Effect of green tea (*Camellia sinensis*) extract on the rat diaphragm. *Journal of Ethnopharmacology*, **57**(3): 197–201. DOI: 0.1016/S0378-8741(97)00069-X

Dhalla, N. S., R. M. Temsah, T. Netticadan (2000). Role of oxidative stress in cardiovascular diseases. *Journal of Hypertension*, **18**(6): 655–673.

Di Giorgio, C., F. Delmas, M. Tueni, E. Cheble. T. Khalil, G. Balansard (2008). Alternative and complementary antileishmanial treatments: assessment of the antileishmanial activity of 27 Lebanese plants, including 11 endemic species. *Journal of Alternative and Complementary Medicine*, **14**(2): 157–162. DOI: 10.1089/acm.2007.7041.

Forster, H. B., H. Niklas, S. Lutz (1980). Antispasmodic effects of some medicinal plants. *Planta Medica*, **40**(4): 309–319. DOI: 10.1055/s-2008-1074977

Gardiner, P. (2007). Complementary, holistic, and integrative medicine: chamomile. Pediatrics in Review, **28**(4): 16–18. DOI: 10.1542/pir.28-4-e16

Georgieva L., A. Gadjalova, D. Mihaylova, A. Pavlov (2015a). *Achillea millefolium* L.- phytochemical profile and *in vitro* antioxidant activity, *International Food Research Journal*, **22**(4): 1347-1352.

Georgieva L., D. Mihaylova (2015b). Screening of total phenolic content and radical scavenging capacity of Bulgarian plant species, *International Food Research Journal*, **22**(1): 240-245.

Grønhaug T. E., S. Glaeserud, M. Skogsrud, N. Ballo, S. Bah,D. Diallo, B. S. Paulsen (2008). Ethnopharmacological survey of six medicinal plants from Mali, West-Africa. *Journal of Ethnobiology and Ethnomedicine,* **4**(26). DOI: 0.1186/1746-4269-4-26

Harrison, D. G., Z. Galis, S. Parthasarathy, K. Griendling (1999) Oxidative Stress and Blood Pressure. 2nd ed., Lippincott Williams & Wilkinson, Baltimore, pp. 163–166.

Ivanov, I., I. Landzhev, G. Neshev (1971). The herbs in Bulgaria and their application, *Zemizdat*, Sofia, 126-127. (In Bg)

Ivanova, D., D. Gerova, T. Chervenkov, T. Yankova (2005). Polyphenols nd antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology*, **96**(1-2): 145-150. DOI: 10.1016/j.jep.2004.08.033

Kohlmünzer S. (eds.), Farmakognozja. (2003). 5th ed., PZWL, Warszawa, pp. 475, 550–555, 577–579. (in Polish)

Kujala, T. S., J. M. Loponen, K. D. Klika, K. Pihlaja (2000). Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds. *Journal of Agricultural and Food Chemistry*, **48**(11): 5338-5342. DOI: 0.1021/jf000523q

Lee, K. G., T. Shibamoto (2002). Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *Journal of Agricultural and Food Chemistry,* **50**(17): 4947–4952. DOI: 10.1021/jf0255681

Majchrzak, D., S. Mitter, I. Elmadfa (2004). The effect of ascorbic acid on total antioxidant activity of black and green teas. *Food Chemistry*, **88**(3): 447–451. DOI: 0.1016/j.foodchem.2004.01.058

McKay, D. L., J. B. Blumberg (2006). A review of the bioactivity and potential health ben efits of chamomile tea (*Matricaria recutita* L.). *Phytotherapy Research*, **20**(7): 519–530. DOI: 10.1002/ptr.1900

Pereira, V. P, F. J. Knor, J. C. R. Vellosa, F. L. Beltrame (2014). Determination of phenolic compounds and antioxidant activity of green, black and white teas of *Camellia sinensis* (L.) Kuntze, Theaceae. *Revista Brasileira de Plantas Medicinais,* **16**(3), 490-498. DOI: 10.1590/1983-084X/13_061

Petkov, V. (1982). Modern phytotherapy, *Medicina i fizkultura*, Sofia. (In Bg)

Radoglou, K., D. Dobrowolska, G.. Spyroglou, V.N. Nicolescu (2008). A review on the ecology and silviculture of limes (*Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia tomentosa* Moench.) in Europe. 29 pp. http://www.valbro.uni-freiburg.de/

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. A. Rice-Evans (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**(9-10): 1231-1237. DOI: 10.1016/S0891-5849(98)00315-3

Scientific Opinion on the substantiation of health claims related to various food(s)/food constituents(s) and “immune function/immune system” (2010). *EFSA Journal*, **8**(10): 1799.

Simbo D. J. (2010). An ethnobotanical survey of medicinal plants in Babungo, Northwest Region, Cameroon. *Journal of Ethnobiology and Ethnomedicine*, **6**(8). DOI: 10.1186/1746-4269-6-8

Skovgaard, G. R., A. S. Jensen, M. L Sigler (2006). Effect of a novel dietary supplement on skin aging in post-menopausal women. *European Journal of Clinical Nutrition,* **60**(10): 1201–1206. DOI: 10.1038/sj.ejcn.1602438

Srivastava, J. K., S. Gupta (2007). Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. *Journal of Agricultural and Food Chemistry,* **55**(23): 9470–9478. DOI: 10.1021/jf071953k

Stefanov, G., M. Matev, I. Chakyrski, I. Angelov (1982). A clinical study of herbal tea Carthage in the treatment of patients with hypertonic, *Problems of Internal Medicine*, **11**(3): 25-30. (In Bg)

Svehlikova, V., R. N. Bennett, F. A. Mellon, P. W. Needs, S. Piacente , P. A. Kroon, Y. Bao (2004). Isolation, identification and stability of acylated derivatives of apigenin 7-O-glucoside from chamomile (*Chamomilla recutita* (L) *auschert*). *Phytochemistry*, **65**(16): 2323–2332. DOI: 10.1016/j.phytochem.2004.07.011

Tyler, V. E. (1993). The honest herbal: A Sensible Guide to the Use of Herbs and Related Remedies. 3rd ed., *Pharmaceutical Products Press*, New York.

Table 1. Total polyphenolic content (TPC) of *T. tomentosa* and *M. chamomilla* water extracts

Plants	Extracts	TPC, mg GAE/g DW
T. tomentosa	infusion	1.69±0.06
	decoction	11.59±0.13[a]
	MAE	3.71±0.02[a, b]
M. chamomilla	infusion	7.21±0.03
	decoction	8.36±0.30[c]
	MAE	2.36±0.05[c, d]

[a] = different from infusion method for *T. tomentosa*, [b] = different from decoction method for *T. tomentosa*, [c] = different from infusion method for *M. chamomilla*, [d] = different from decoction method for *M. chamomilla*, $p < 0.05$, Tukey-Kramer's test.

Table 2. Antioxidant activity of *T. tomentosa* and *M. chamomilla* water extracts (μM TE/g DW)

Plants	Extracts/Assays	$\mathbf{TEAC_{DPPH}}$	$\mathbf{TEAC_{ABTS}}$	$\mathbf{TEAC_{FRAP}}$	$\mathbf{TEAC_{CUPRAC}}$
T. tomentosa	infusion	30.10 ± 0.14	32.77 ± 0.15	33.61 ± 0.57	36.95 ± 0.58
	decoction	288.93 ± 0.58[a]	222.14 ± 5.83[a]	225.41 ± 0.75[a]	272.96 ± 6.23[a]
	MAE	80.65 ± 2.46[a, b]	70.11 ± 3.12[a, b]	47.10±0.81[a, b]	60.71 ± 0.27[a, b]
M. chamomilla	infusion	58.14 ± 0.80	72.08 ± 3.08	107.87 ± 3.52	148.02 ± 0.93
	decoction	177.66 ± 0.46[c]	111.03 ± 2.44[c]	187.03 ± 1.22[c]	245.66 ± 9.58[c]
	MAE	25.28 ± 1.58[c, d]	26.46 ± 0.46[c, d]	35.47 ± 0.75[c, d]	40.27 ± 0.95[c, d]

[a] = different from infusion method for *T. tomentosa*, [b] = different from decoction method for *T. tomentosa*, [c] = different from infusion method for *M. chamomilla*, [d] = different from decoction method for *M. chamomilla*, $p < 0.05$, Tukey-Kramer's test.