ISSN 2278-3091
Volume 8, No.1.3, 2019
International Journal of Advanced Trends in Computer Science and Engineering
Available Online at http://www.warse.org/IJATCSE/static/pdf/file/ijatcse4781.32019.pdf
https://doi.org/10.30534/ijatcse/2019/4781.32019

Formulation and anti-aging evaluation of polyherbal tea

Nurul'ain Nadhirah Mohd Nasir [1], Mohd. Fadzelly Abu Bakar[1,2*], Nur Amalina Ismail[1,2]
[1]Faculty of Applied Sciences and Technology (FAST), Universiti Tun Hussein Onn Malaysia, KM 1, Kampus Pagoh, 84000, Muar, Johor, Malaysia, fadzelly@uthm.edu.my
[2] Centre of Research for Sustainable Uses of Natural Resources (CoR-SUNR), Faculty of Applied Sciences and Technology,
Universiti Tun Hussein Onn Malaysia, KM 1, Kampus Pagoh, 84000, Muar, Johor,Malaysia

ABSTRACT

Herbal tea has been shown to possess multiple health benefit. Current study was done to formulate polyherbal tea from antioxidant-rich medicinal herbs such as *Camellia sinensis, Hibiscus sabdariffa, Marantodes pumilum, Morinda citrifolia, Centella asiatica* and *Stevia rebaudiana* based on the sensory evaluation. Then, the chemical composition and bioactivities were investigated for the selected formulation. 5 formulations with different proportion of selected herbs were evaluated based on aroma, colour, taste, aftertaste, mouthfeel and overall acceptability. Formulation 348 containing 15% *M. pumilum*, 19% *M. citrifolia*, 10% *C. sinensis*, 10% *H. sabdariffa*, 45% *C. asiatica*, and 1% *S. rebaudiana* exhibited the highest mark for overall acceptibility. Single component of *S. rebaudiana* showed the highest total phenolic (TPC) and total flavonoid content (TFC). The mixture of six selected herbs consisted significant amount of TPC (198.18 ± 0.02 mg GAE/L) and TFC (192.07±0.03 mg RE/L infusion). Based on GCMS analysis, it was found that caffeine (69.54%) was the most abundant compound in the herbal mixture followed by quinic acid (9.95%) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (8.40%) which might contribute to its biological activities. For antioxidant activity, the single component of *C. sinensis* and *S. rebaudiana* showed higher DPPH scavenging and FRAP reducing ability compared to the herbal mixture. However, for ABTS assay, there were no significant differences in ABTS value between the single component and the herbal mixture. The herbal mixture displayed 16.46 ± 3.02 % inhibitions towards tyrosinase enzyme and 7.22% inhibition in collagenase inhibition assay. Further study is needed to explore the potential of the selected herbal formulation which could be developed into nutraceutical beverages.

Key words : Anti-aging; Antioxidant; Polyherbal tea; Tyrosinase inhibition; Collagenase inhibition; GCMS analysis

1. INTRODUCTION

Herbal tea or also called tisane has increased in popularity due to its biological properties and certainly can be complement to modern medicine. This beverage is made up of dried leaves, seeds, grasses, flowers, nuts or any botanical elements which originated from plant species other than widely consumed tea species, *Camellia sinensis* [1,2]. In spite of brewing single herb in hot water, the herbal tea also has been prepared in form of mixture. Polyherbal tea has been used in many traditions for centuries. The old culture, such as Ayuverda and Traditional Chinese Medicine (TCM) has formulated herbal mixtures for the treatment of various ailments [3]. The herbs were mixed based on the similarities of health benefit for individual species [4]. Current market has showed that most of herbal-based products have been shifted from using single herb to polyherbs which are believed to exert more pharmacological effects compared to the single herb [4].

Marantodes pumilum (Blume) Kuntze or locally known as Kacip Fatimah is among the popular herbs in Malaysia. *M. pumilum* (Family : Myrsinaceae) possess wide range of bioactivities such as phytoestrogenic, anti-inflammatory, antioxidant, anti-aging, anti-microbial and anti-carcinogenic activities [5]. Other tropical medicinal plants such as *Morinda citrifolia* L. (family: Rubiaceae), *Hibiscus sabdariffa* L. (family: Malvaceae) and *Centella asiatica* (L.) Urb (family: Apiaceae) have been used in traditional medicine and currently been consumed in the form of infusion or added as flavor in the drinks.

Current study aims to develop new polyherbal tea comprise of the well-known green tea, from *C. sinensis* and also antioxidant-rich tropical medicinal plants (*M. pumilum*, *M. citrifolia*, *H. sabdariffa*, and *Centella asiatica*) with addition of Stevia *rebaudiana* as sweetener. Other than health benefit displayed by herbal tea, the sensory characteristic (i.e taste, aroma, and colour) always become major factors in selecting the herbal beverages by consumers [6]. Thus, the herbal mixture was formulated based on sensory evaluation. Subsequently, the chemical composition, antioxidant, anti-aging (anti-collagenase) and anti-tyrosinase activities were evaluated.

2. EXPERIMENTAL PROCEDURE

2.1. Plant materials and infusion preparation

The dried leaves of *Camellia sinensis* leaves, *Hibiscus sabdariffa* flowers, *Marantodes pumilum* leaves, *Morinda citrifolia* leaves, *Centella asiatica* leaves and *Stevia rebaudiana* leaves were obtained from herbal supplier at

Melaka, Malaysia. The dried samples were stored in an air-tight container with the aid of silica gel to ensure the moisture content of the herbs is maintained and the shelf life can be extended.

The herbal tea was freshly prepared by mimicking domestic brewing technique [7]. About 1.0 g of the single herb/herbal mixture was infused in 100 mL of boiling water (100°C). The mixture was stirred constantly by using magnetic stirrer for 3 minutes and the sample was left to cool for 3 to 5 minutes. The infusion was then filtered by using filter paper and the filtrate was used for the assay. Five formulations of herbal tea from six plant species were prepared based on manual testing in preliminary studies (data not shown). The formulations were randomly labelled with 3 numbers. The percentage composition of each herb in the coded formulations was displayed in Table 1.

Table 1 : Percentage composition of herbs for each formulations

Formulation	348	529	847	253	482
M. pumilum	15	20	10	25	20
M. citrifolia	19	20	25	25	15
C. sinensis	10	15	10	15	20
H. sabdariffa	10	15	10	15	10
C. asiatica	45	29	39	18.5	33.5
S. rebaudiana	1	1	1	1.5	1.5

2.2. Sensory evaluation

The best formulation was selected based on sensory evaluation. A total of 30 semi-trained panelists were involved and the evaluation was carried out at Sensory Analysis Laboratory, Universiti Tun Hussein Onn Malaysia. The evaluation was done by asking the panelists to score the infusions with respect to colour, taste, appearance, odour, flavor and overall quality by using 9-point hedonic scale (varied from dislike extremely or like extremely) in prepared questionnaire.

2.3. Chemical characterization

2.3.1 Total phenolic and flavonoid contents

The total phenolic content (TPC) of samples was determined by using Folin-Ciocalteu method [8] and the result was presented as milligram gallic acid equivalent in 1 litre of infusion (mg GAE/L). The total flavonoid content (TFC) was determined by using aluminium chloride colorimetric method [9] and the result obtained was expressed as milligram catechin equivalent in 1 litre of infusion (mg CE/L).

2.3.2 GCMS analysis

The chemical composition of the best herbal tea formulation was determined by using Gas Chromatography coupled with Mass Spectrometry (GCMS). Freeze dried infusion was reconstituted with methanol and was injected into column. The spectrum obtained was compared with NIST 11 Mass Spectral Library.

2.4. Antioxidant activites

Three antioxidant assays were used in current study, which were 2,2-diphenyl-1-pycryl-hydrazyl (DPPH) free radical scavenging assay [10], ferric reducing/antioxidant power (FRAP) [11] and 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) decolourization assay [12]. The DPPH scavenging activity of selected formulation was presented in the form of percentage inhibition. The final result for FRAP was presented as the concentration of antioxidant having a ferric reducing ability. The result for ABTS decolourization activity was expressed as milligram ascorbic acid equivalent antioxidant capacity in 1 litre of infusion (mg AEAC/L).

2.5. Collagenase inhibition assay

The assay was performed by using drug discovery kits (MMP-1 Colorimetric Protocols) by Enzo Life Science. Positive controls were performed with NNGH whereas negative controls were performed without any inhibitor. The absorbance was read at 412 nm. The slope remaining activity was calculated as below (1)-(3).

Inhibitor activity remaining (%) = ($V_{inhibitor/}$ $V_{control}$) x 100 (1)

Velocity reaction of inhibitor, $V_{inhibitor}$ = Absorbance / min (2)

Velocity reaction of control,$V_{control}$= $Absorbance_{control}$ / $min_{control}$ (3)

The percentage of inhibition was obtained by subtracting the obtained value from 100.

2.6. Tyrosinase inhibitory activity

The tyrosinase inhibitory activity was conducted according to method by Atta-ur-Rahman *et al.*[13]. Kojic acid was used as standard. Percentage of tyrosinase inhibition was calculated as follows (4):

Tyrosinase activity (%) = [(A-B)/(C-D)] x 100 (4)

Where, A = absorbance of reaction mixture containing test sample and mushroom tyrosinase; B = absorbance of blank sample containing test sample but without mushroom tyrosinase; C = absorbance of reaction mixture without test sample and with mushroom tyrosinase; D = absorbance of the well without both test sample and mushroom tyrosinase (L-DOPA alone)

2.7. Statistical analysis

All experiments were carried out in triplicate in 3 independent experiments. The results were presented as mean ± standard deviation. The data was statistically analysed by using one-way ANOVA with a significant value of $p < 0.05$ to test

the significant difference between the samples. Pearson's correlation was used to determine the relationship between phytochemicals and antioxidant activity.

3. RESULTS AND DISCUSSION

3.1. Sensory evaluation of herbal tea

The average score for aroma, colour, taste, aftertaste, mouthfeel and overall acceptability varied between 3.17 and 7.20. This indicates that the panelists have their own preferences for the herbal tea. For all attributes, the most preferred formulation was formulation 348 (Figure 1). This formulation contains 15% *Marantodes pumilum*, 19% *Morinda citrifolia*, 10% *Camellia sinensis*, 10% *Hibiscus sabdariffa*, 45% *Centella asiatica* and 1% *Stevia rebaudiana*. The colour of formulation 348 is greenish brown, which obtained the highest score among the panelist. The small proportion of *M. pumilum* and *C. sinensis* in formulation 348 and 847 might be the reason of the higher taste acceptance. Individual *M. pumilum* has woody-like taste, which undesirable for consumer acceptance. *C. sinensis* has bitter and astringent taste [14], but has sweet aftertaste. The undesirable taste of the formulation 348 has been masked by the sweetness of *S. rebaudiana* which estimated to be 110 to 270 times sweeter than sucrose [15]. Since formulation 348 obtained the highest score for sensory evaluation, it has been selected for further evaluation of chemical composition, antioxidant and anti-aging activities.

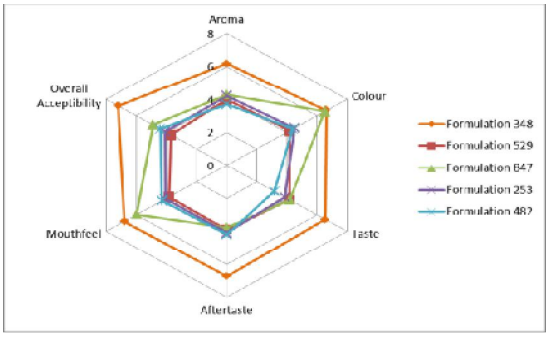

Figure 1: Radar plot of sensory evaluation for all formulations

3.2. Chemical characterization

3.2.1 Total phenolic and flavonoid content

The total phenolic content (TPC) and total flavonoid content (TFC) of the formulation 348 is compared with its single component (Table 2). In terms of single herb, *S. rebaudiana* was recognized to contain high TPC (490.15 ± 0.05 mg GAE/L) and TFC (621.27±0.10 mg RE/L) followed by green tea (*C. sinensis*), *C. asiatica*, *H. sabdariffa*, *M. citrifolia* and *M. pumilum*. Formulation 348 contain significant amount of TPC and TFC. Eventhough *S. rebaudiana* contain the highest TPC and TFC, the contribution of this herb is only 1% to the mixture. Previous research on herbal infusions showed that green tea contains higher TPC, TFC and non-flavonoid component compared to stevia and roselle [16]. However, current study indicates that *S. rebaudiana* contain higher TPC and TFC followed by green tea and roselle. The differences might due to the distinction in the way of preparation, varieties and cultivation characteristic [16]. The major phenolic in *Centella asiatica* is asiatic acid and asiaticoside [17]. A strong correlation ($r=0.964$, $p<0.05$) existed between phenolic and flavonoid, which indicate flavonoid may contribute to most of the phenolic in the tea which in agreement with previous study [18] on various herbal tea.

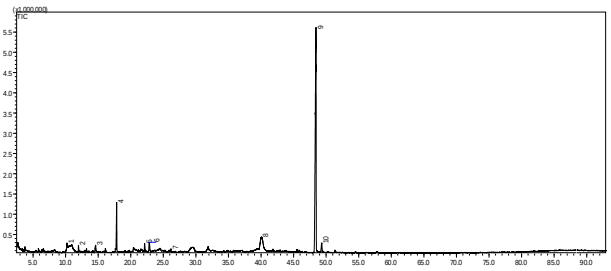

Figure 2 : A typical gas chromatogram of the chemical constituents of formulation 348 extract

Table 2 : TPC and TFC of formulations and single herb.

Sample	TPC (mg GAE/L)	TFC (mg RE/L)
Formulation 348	198.18 ± 0.02^{c}	$192.07 \pm 0.03^{c,d}$
M. pumilum	72.80 ± 0.02^{e}	16.73 ± 0.01^{g}
M. citrifolia	77.84 ± 0.02^{e}	$164.20 \pm 0.02^{d,f}$
C. sinensis	258.18 ± 0.04^{b}	341.27 ± 0.02^{b}
H. sabdariffa	146.56 ± 0.08^{d}	118.20 ± 0.01^{e}
C. asiatica	148.86 ± 0.13^{d}	$197.93 \pm 0.02^{c,f}$
S. rebaudiana	490.15 ± 0.05^{a}	621.27 ± 0.10^{a}

Values are presented as mean ± standard deviation (SD) (n = 3) which, with different letters (within column), are significantly different at $p < 0.05$.

3.2.2 GCMS analysis

The chromatogram of chemical compounds for formulation 348 has been displayed in Figure 2. The major peak with the highest concentration as can be seen in Figure 2, is peak 9 that was represented by caffeine. Quinic acid (9.95%) showed second highest concentration followed by 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (8.4%) (Table 3). Quinic acid is among the phenolic acid that could be found in *Centella asiatica* extract [19]. As *C. asiatica* make up 45% of the formulation, the presence of quinic acid might contribute by *C. asiatica*. Caffeine is the common compound found in green and black tea which exhibited the antioxidant activities and showed stimulatory effects on the nervous system [20]. The high concentration of caffeine might be contributed by the 10% of green tea in the polyherbal formulation. According

to Okello *et al.* [21], caffeine exhibited strong inhibitory activities against acetylcholinesterase enzyme. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- which also known as DDMP also have been found in *Bougainvillea x buttiana* [22] and *Pyrus pyrifolia* which contribute to the ABTS radical scavenging activity [23]. The major compound in the formulation might be responsible for the bioactivities of the selected formulation.

Table 3: Chemical composition of formulation 348.

ID	Chemical Compounds	Concentration (%)	Retention Time (min)
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1.44	10.21
2	1,2,5,6-Tetrahydropyridin-2-one, 5-methyl-	1.17	11.98
3	4H-Pyran-4-one, 2-hydroxy-3-methyl- (CAS) 2-Hydroxy-3-methyl-4-pyrone	2.07	14.61
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.40	17.84
5	Hydroxymethylfurfural	1.92	22.14
6	1,2,3-Propanetriol, 1-acetate	2.75	22.86
8	Quinic acid	9.95	40.07
9	Caffeine	69.54	48.43
10	Theobromine	2.10	49.31

3.3. Antioxidant activities

Antioxidant play roles by donating a hydrogen atom or electron to reactive species (i.e reactive oxygen species, reactive nitrogen species), which deactivate the activity of the reactive species [24]. In DPPH inhibition assay, formulation 348 and its single components showed inhibition towards DPPH radicals *in vitro* (Table 4). No significant difference existed between formulation 348 with *C. sinensis* and *S. rebaudiana*. Eventhough *C. asiatica*, *M. citrifolia* and *M. pumilum* individual infusion exhibit low antioxidant activity, it did not influence the high antioxidant activity of green tea when it is mixed. This indicate that formulation 348 exhibit high DPPH scavenging activity in comparable with antioxidant-rich green tea. Jain *et al.* [25] has found out that the the mixture of *Vitis vinifera, Phyllanthus emblica, Punica granatum, Cinnamomum cassia, Ginkgo biloba* and *Camellia sinensis* produced higher antioxidant properties compared to the individual species which in line with current research. In ABTS assay, statistically, there is no significant difference between the herbal mixture and single component (Table 4). Meanwhile, in FRAP assay, *S. rebaudiana* and *C. sinensis* exhibit high antioxidant potential followed by formulation 348, *C. asiatica, H. sabdariffa, M. pumilum,* and *M. citrifolia.* In correlation analysis, it was found that TPC have significant positive correlation with DPPH and FRAP ($r=0.771$, $p< 0.05$; $r=0.845$, $p<0.05$). ABTS did not show any significant correlation with TFC. Meanwhile, TFC is positively correlate with FRAP ($r=0.817$, $p<0.05$). Among the antioxidant activities, DPPH and FRAP has been found to be highly correlated with each other ($r= 0.968$; $p<0.05$). The same relationship has been found by Wong *et al.* [26] on the antioxidant activities of aqueous infusion including *Centella asiatica* and *Hibiscus sabdariffa*. This indicates that the compound presented in the infusion is able to donate hydrogen atom, at the same time able to reduce ferric ion via single electron donation [26]. Meanwhile, no significant correlation has been observed between ABTS with DPPH and FRAP.

Table 4 : Comparison of antioxidant activity of selected formulation and single herb

Sample	DPPH (%)	ABTS (mg AEAC/L)	FRAP (mM/L)
Formulation 348	87.72 ± 0.04[a]	38.10±0.04[a]	3700 ± 410[b]
M. pumilum	25.57 ± 0.01[c]	40.02±0.02[a]	560 ± 170[e]
M. citrifolia	9.43 ± 0.05[e]	36.11±0.01[a]	290 ± 90[e]
C. sinensis	92.10 ± 0.01[a]	40.27±0.01[a]	5600 ± 110[a]
H. sabdariffa	21.76 ± 0.03[d]	30.66±0.16[a]	920 ± 800[d]
C. asiatica	34.95 ± 0.01[b]	36.03±0.11[a]	1600 ± 180[c]
S. rebaudiana	90.12 ± 0.03[a]	40.41±0.01[a]	5400 ± 10[a]

Values are presented as mean ± SD (n = 3) which, with different letters (within column), are significantly different at $p < 0.05$.

3.4 . Collagenase inhibition activity

Collagenase inhibition activity was determined by analysing the inhibition ability of plant infusion towards Matrix Metalloproteinase-1 (MMP-1) enzyme. MMP-1 is one of the key collagenase which involved in physiological and pathological of connective tissues [27]. Table 5 shows the percentage inhibition of MMP-1 enzyme by formulation 348 in comparison with the standard (NNGH Inhibitor). The percentage inhibition of the collagenase activity was very low for herbal tea sample as compared to the NNGH (N-Isobutyl-N-(4-methoxyphenylsulfonyl) glycylhydroxamic acid) inhibitor. Based on previous research, *C. sinensis*, *M. pumilum* and *C. asiatica* extracts have demonstrate the ability to restore collagen synthesis and reduce the MMP-1 expression [28–30].

Sample	MMP-1 Inhibition (%)
Formulation 348	7.22
NNGH Inhibitor (1.3 µM)	86.11

Table 5 : Collagenase inhibition activity

3.5. Tyrosinase inhibition activity

Tyrosinase is an enzyme that responsible for melanin production which has protective function in human skin. However, the accumulation of melanin will caused undesirable skin condition such as pigmented patches [31,32]. The search for natural tyrosinase inhibitors from plants in replacement with synthetic tyrosinase inhibitor is still on-going [32]. Current study showed that tyrosinase inhibition by formulation 348 increased with increasing concentration (Table 6). However, the percentage inhibition is lower than kojic acid which act as standard. In previous research, individual *Morinda citrifolia* leaf extract [33], *C. sinensis* [34] and *M. pumilum* [5] has showed the protection against melanin in *in vitro* and *in vivo* assay which might contribute to the tyrosinase inhibition activity in current study.

Table 6 : Tyrosinase inhibitory activity

Sample/Standard	0.5 mg/mL	1.0 mg/mL
Formulation 348	7.56 ± 0.17	14.72 ± 0.39
Kojic acid	85.72±0.34	89.24 ± 0.82

4. CONCLUSION

The selected formulation 348 is the most acceptable formulation based on sensory evaluation. By comparing the mixture of herb with individual herb, the mixture formulation exhibit significant phytochemical and antioxidant activities. The evaluation on anti-aging potential by using tyrosinase and collagenase inhibition assays exhibit considerable inhibition activity. Further study is needed to explore the potential of the selected herbal formulation which could be developed into nutraceutical beverages.

ACKNOWLEDGEMENT

The authors would like to acknowledge Ministry of Higher Education (MOHE) and Universiti Tun Hussein Onn Malaysia (UTHM) for financial assistance under the Fundamental Research Grant Scheme (FRGS) (Project No: 1560). The authors also would like to thanks Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Faculty of Medicine and Health Science and Institute of Bioscience, Universiti Putra Malaysia for the permission to use laboratory equipment and technical assistance.

REFERENCES

1. Lasekan O & Lasekan A. **Flavour chemistry of mate and some common herbal teas.** *Trends in Food Science & Technology* 27(1), 37–46,2012. https://doi.org/10.1016/j.tifs.2012.05.004
2. Zhao J, Deng JW, Chen YW, Li SP. **Advanced phytochemical analysis of herbal tea in China**. *Journal of Chromatography A* 1313, 2–23,2013. https://doi.org/10.1016/j.chroma.2013.07.039
3. Kong D-X, Li X-J & Zhang H-Y. **Where is the hope for drug discovery? Let history tell the future.** *Drug Discovery Today* 14(3/4), 115–119,2009. https://doi.org/10.1016/j.drudis.2008.07.002
4. Guimaraes R, Barros L, Duenas M, Calhelha RC, Carvalho AM, Santos-Buelga C, Queiroz MJRP & Ferreira ICFR. I**nfusion and decoction of wild German chamomile: Bioactivity and characterization of organic acids and phenolic compounds.** *Food Chemistry* 136 (2), 947–954, 2013. https://doi.org/10.1016/j.foodchem.2012.09.007
5. Chua LS, Lee SY, Abdullah N & Sarmidi MR. **Review on *Labisia pumila* (Kacip Fatimah): Bioactive phytochemicals and skin collagen synthesis promoting herb.** *Fitoterapia* 83(8), 1322–1335,2012. https://doi.org/10.1016/j.fitote.2012.04.002
6. Kraujalyte V, Pelvan E & Alasalvar C. **Volatile compounds and sensory characteristics of various instant teas produced from black tea.** *Food Chemistry* 194, 864–872, 2016. https://doi.org/10.1016/j.foodchem.2015.08.051
7. Lusia Barek M, Hasmadi M, Zaleha AZ & Mohd Fadzelly AB. **Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah snake grass (*Clinacanthus nutans* Lind.) leaves.** *International Food Research Journal* 22 (2), 661–70,2015.
8. Velioglu YS, Mazza G, Gao L & Oomah BD. A**ntioxidant activity and total phenolics in selected fruits, vegetables, and grain products.** *Journal of Agricultural and Food Chemistry* 46 (10), 4113–4117,1998. https://doi.org/10.1021/jf9801973
9. Zhishen J, Tang M & Wu J. **The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals.** *Food Chemistry* 64, 555–559,1999. https://doi.org/10.1016/S0308-8146(98)00102-2
10. Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TC dos, Coube CS & Leitao SG. **Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method.** *Phytotherapy Research* 15, 127–130,2001. https://doi.org/10.1002/ptr.687
11. Benzie IFF & Strain JJ. **The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": The FRAP Assay.** *Analytical Biochemistry* 239(1), 70–76,1996. https://doi.org/10.1006/abio.1996.0292
12. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M & Rice-Evans C. **Antioxidant activity applying an improved ABTS radical cation decolorization assay.** *Free Radical Biology and Medicine* 26(9),1231–1237,1999.

https://doi.org/10.1016/S0891-5849(98)00315-3

13. Atta-ur-Rahman, Choudhary MI & Thomsen WJ **Bioassay techniques for drug development**, pp. 128-130,2005.
14. Zhang YN, Yin JF, Chen JX, Wang F, Du QZ, Jiang YW, & Xu YQ. **Improving the sweet aftertaste of green tea infusion with tannase.** *Food Chemistry* 192, 470–476,2016. https://doi.org/10.1016/j.foodchem.2015.07.046
15. Massin CB, Giuliano S, Alletto L, Dayde J & Berger M. **Nitrogen limitation alters biomass production but enhances steviol glycoside concentration in *Stevia rebaudiana* Bertoni.** *Plos One* 10(7), 16 pages,2015. https://doi.org/10.1371/journal.pone.0133067
16. Deetae P, Parichanon P, Trakunleewatthana P & Chanseetis C. **Antioxidant and anti-glycation properties of Thai herbal teas in comparison with conventional teas**. *Food Chemistry* 133(3), 953–959,2012. https://doi.org/10.1016/j.foodchem.2012.02.012
17. Chan EWC, Lim YY, Chong KL, Tan JBL & Wong SK, **Antioxidant properties of tropical and temperate herbal teas.** *Journal of Food Composition and Analysis* 23(2),185–189,2010. https://doi.org/10.1016/j.jfca.2009.10.002
18. Ho SC, Wu SP, Lin SM & Tang YL. **Comparison of anti-glycation capacities of several herbal infusions with that of green tea**. *Food Chemistry* 122(3), 768–774,2010. https://doi.org/10.1016/j.foodchem.2010.03.051
19. Satake T, Kamiya K, Yin A, Oishi T & Yamamoto J. **The anti-thrombotic active constituents from *Centella asiatica*.** *Biological and Pharmaceutical Bulletin* 30(5), 935–940,2007. https://doi.org/10.1248/bpb.30.935
20. Cimpoiu C, Hosu A & Miclaus V. **Analysis of antioxidant compounds in different types of tea. Tea in Health and Disease Prevention**. *Elsevier Inc*. pp. 79–89,2013. https://doi.org/10.1016/B978-0-12-384937-3.00007-0
21. Okello EJ, Leylabi R & McDougall GJ. **Inhibition of acetylcholinesterase by green and white tea and their simulated intestinal metabolites.** *Food & function* 3(6), 651–661,2012. https://doi.org/10.1039/c2fo10174b
22. Abarca-vargas R, Malacara CFP & Petricevich VL. **Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea x buttiana* Holttum and Standl**, (var . Rose) extracts. *Antioxidants* 5(45), 11 pages,2016. https://doi.org/10.3390/antiox5040045
23. Hwang IG, Kim HY, Woo KS, Lee SH, Lee J & Jeong HS. **Isolation and identification of the antioxidant DDMP from heated pear (*Pyrus pyrifolia* Nakai).** *Preventive Nutrition and Food Science* 18(1), 76–79,2013. https://doi.org/10.3746/pnf.2013.18.1.076
24. Apak R, Ozyurek M, Guclu K & Capanoglu E. **Antioxidant activity/capacity measurement: II. Hydrogen atom transfer (HAT)-based, mixed-mode (Electron Transfer (ET)/HAT), and lipid peroxidation assays.** *Journal of Agricultural and Food Chemistry* 64(5), 1028–1045,2016. https://doi.org/10.1021/acs.jafc.5b04743
25. Jain DP, Pancholi SS & Patel R. **Synergistic antioxidant activity of green tea with some herbs.** *Journal of Advanced Pharmaceutical Technology & Research* 2(3), 177-183,2011. https://doi.org/10.4103/2231-4040.85538
26. Wong SP, Leong LP & Koh JW. **Antioxidant activities of aqueous extracts of selected plants.** *Journal of Food Chemistry*, 775–783,2006. https://doi.org/10.1016/j.foodchem.2005.07.058
27. Mukherjee PK, Maity N, Nema NK & Sarkar BK. **Natural matrix metalloproteinase inhibitors: Leads from herbal resources.** Studies in Natural Products Chemistry, Vol. 39, 1st ed. *Elsevier B.V.* pp. 91–113,2013. https://doi.org/10.1016/B978-0-444-62615-8.00003-5
28. Choi HK, Kim DH, Kim JW, Ngadiran S, Sarmidi MR & Park CS. ***Labisia pumila* extract protects skin cells from photoaging caused by UVB irradiation.** *Journal of Bioscience and Bioengineering* 109 (3), 291–296,2010. https://doi.org/10.1016/j.jbiosc.2009.08.478
29. Nema NK, Maity N, Sarkar BK & Mukherjee PK. **Matrix metalloproteinase, hyaluronidase and elastase inhibitory potential of standardized extract of *Centella asiatica***. *Pharmaceutical Biology* 51(9), 1182–1187,2013. https://doi.org/10.3109/13880209.2013.782505
30. Dzialo M, Mierziak J, Korzun U, Preisner M, Szopa J & Kulma A. **The potential of plant phenolics in prevention and therapy of skin disorders.** *International Journal of Molecular Sciences* 17 (160), 41 pages,2013. https://doi.org/10.3390/ijms17020160
31. Chang TS. **An updated review of tyrosinase inhibitors.** *International Journal of Molecular Sciences* 10(6), 2440–2475,2009. https://doi.org/10.3390/ijms10062440
32. Zengin G, Uysal S, Ceylan R & Aktumsek A. **Phenolic constituent, antioxidative and tyrosinase inhibitory activity of *Ornithogalum narbonense* L. from Turkey: A phytochemical study.** *Industrial Crops and Products* 70, 1–6,2015. https://doi.org/10.1016/j.indcrop.2015.03.012
33. Masuda M, Murata K, Fukuhama A, Naruto S, Fujita T, Uwaya A, Isami F & Matsuda H. **Inhibitory effects of constituents of *Morinda citrifolia* seeds on elastase and tyrosinase.** *Journal of Natural Medicines* 63(3), 267–273,2009. https://doi.org/10.1007/s11418-009-0328-6
34. Chen C-H, Chan H-C, Chu Y-T, Ho H-Y, Chen P-Y, Lee T-H, & Lee C-K. **Antioxidant activity of some plant extracts towards xanthine oxidase, lipoxygenase and tyrosinase.** *Molecules* 14(8), 2947–2958,2009. https://doi.org/10.3390/molecules14082947