Plasma Medicine, 6(3–4): 315–324 (2016)

Effect of Cold Plasma Processing on Botanicals and Their Essential Oils

Gerald J. Buonopane,[a] Cosimo Antonacci,[a] & Jose L. Lopez[b,*]

[a]Department of Chemistry & Biochemistry, Seton Hall University, South Orange, New Jersey; [b]Department of Physics, Seton Hall University, South Orange, New Jersey

*Address all correspondence to: Jose L. Lopez, Seton Hall University, Department of Physics, 400 South Orange Avenue, South Orange, New Jersey, 07079; Tel.: 973-761-9057; Fax: 973-761-9528, E-mail: jose.lopez1@shu.edu

ABSTRACT: Increased agricultural food production and the subsequent preservation of yielded food are matters of grave importance to all humanity. In this work, we studied methods for increasing the production yield of plants that produce essential oils. Essential oil plant extracts have demonstrated antibacterial effects and contain antioxidants that are important for food product preservation and quality. Cold plasma processing is a novel technique for increasing the yield of botanicals that are known to be key producers of essential oils. Cold plasma–treated plants were compared to control nontreated plants and to commercially available essential oils. On the basis of 2,2-diphenyl-1-picrylhydrazyl antioxidant assay results, some commercial essential oils and those from homegrown (greenhouse) plants were found to be effective antioxidants. This preliminary research points to the potential natural replacement of synthetic commercial preservatives, such as butylated hydroxytoluene and butylated hydroxyanisole, that have been demonstrated to be potentially harmful to human health. Furthermore, other studies investigating plasma treatment on seeds suggest that plasma treatment enhances growth and may increase phenolic antioxidant effects.

KEY WORDS: cold plasma processing, lipid autoxidation, antioxidant, essential oils, steam distillation, GC-MS, agricultural food production, natural preservative

I. INTRODUCTION

Current global population growth models estimate that the world will reach a population of 9.1 billion individuals by 2050. The Food and Agriculture Organization of the United Nations predicts that world food production must eventually rise by 70%, and food production in the developing world must double. Such projected increases in agricultural production will have to overcome rising global energy costs, growing depletion of fresh water sources and aquifers, continuing loss of farmable land due to urbanization, and subsequent increases in drought and flooding resulting from global climate change.

With existing global natural-resource limits, research into new methods and techniques that help plants to grow faster and produce more food are of paramount importance. A particularly promising approach for improving agriculture yield is through methods of cold plasma treatment. These methods have been shown to be rapid, economical, and pollution-free in their effort to improve plant seed performance and crop yield.[1] In particular, microplasma treatment techniques have demonstrated a role in a wide array of developmental and physiological processes in plants, including reducing

1947-5764/16/$35.00

detrimental bacterial and mold growth on plants and stimulating seed germination and seedling growth.[2,3] Researchers including have found that plasma processing, particularly with increases in plasma treatment, increases antioxidant properties.[4] When studying basmati rice flour, Thirumdas et al. found that increasing plasma power resulted in increases in reducing power in the flour (Fe^{3+} to Fe^{2+}) that may be due to then augmentation in total polyphenols.[5]

Once enhanced food production is achieved, the preservation and increased shelf life of food products is paramount. One common type of food preservative is the antioxidant, which helps to prevent or retard lipid oxidation. Lipid oxidation is a deteriorative process that causes oil or fat to turn rancid. The processes of lipid oxidation (autoxidation) and antioxidant protection are shown in Fig. 1, which depicts the major steps of autoxidation: initiation (H-atom abstraction from a lipid substrate [R] to yield an alkyl radical [R·]), propagation (an explosive process, beginning with the reaction of an alkyl radical with oxygen to form a peroxyl radical [ROO·] that attacks another molecule of the lipid substrate to yield a hydroperoxide [ROOH] and another radical, thus repeating the process), and termination (two radical species react with or quench each other to terminate the reaction). Alternatively, antioxidants (such as AH) slow or block autoxidation by competing with other radicals for the peroxyl radical $ROO^{\cdot}$. These sacrificial antioxidants react with peroxyl radicals more rapidly than with the oxidizable lipid substrate to form species that do not propagate the oxidation chain.[6]

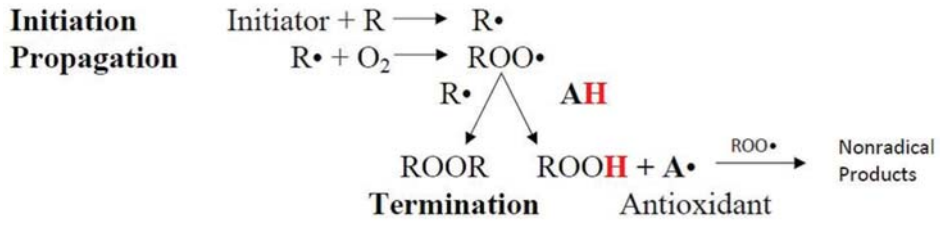


FIG. 1: Schematic of the quenching of lipid oxidation by antioxidant

Two synthetic (chemical) antioxidants commonly used by the food industry are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Although BHA and BHT are effective antioxidants, animal studies have shown that they are potentially harmful to human health, as reviewed by Amorati et al.[6] BHA has been shown to promote the action of some carcinogens, and BHT potentially causes lung damage.[7] Due to the safety concerns with these synthetic (artificial) antioxidants, food scientists have been seeking alternative natural compounds as substitute antioxidants. Among these alternatives are essential oils extracted from various plant species. Essential oils are aromatic oily liquids extracted from different parts of plants, including leaves, peels, barks, flowers, buds, seeds, and roots, that can be extracted by several methods; steam

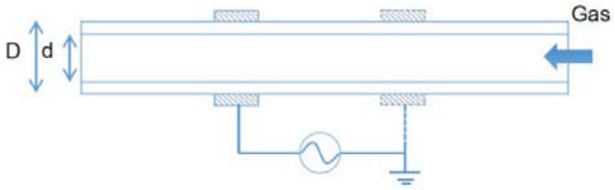
distillation is most widely used. Essential oils have been shown to demonstrate antibacterial and antioxidant potential for food products.[8] The antioxidant activity is largely due to the phenolic and polyphenolic content of the plant oils.

In this study, we focus on using cold plasma treatment to increase the growth production yield of plants that produce essential oils, components that would be used as additives for enhancing food preservation and product quality. The data presented herein show (1) antioxidant activity of a variety of essential oils, (2) plasma treatment of seedlings and seeds to yield larger plants, and (3) higher antioxidant potential of essential oils extracted from plasma-treated plants.

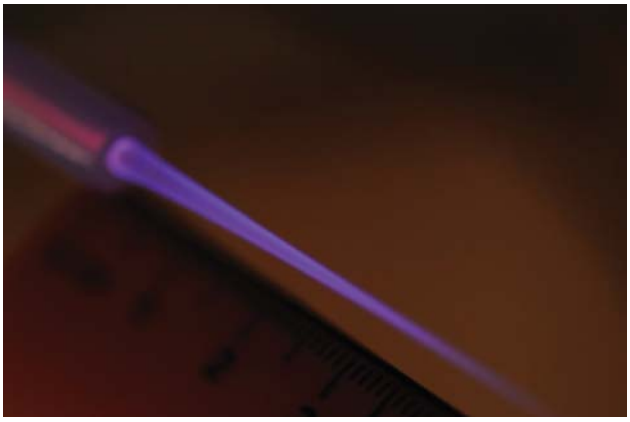
II. MATERIALS AND METHODS

A. Cold Plasma and Air/Helium Atmospheric Pressure Plasma Jet

A simple plasma jet made of Teflon tubing and external copper electrodes connected to a kilohertz-frequency high-voltage power supply and an air and helium gas mixture were used to treat botanicals. Figure 2(a) shows the schematic and Fig. 2(b) the image of the plasma jet. The created cold plasmas were used to treat the seeds and plant bodies. The



(a)



(b)

FIG. 2: (a) Schematic of helium and air mix atmospheric plasma jet source with a Teflon tube of an outer diameter [D] of 6.4 mm and inner diameter [d] of 3.2 mm. (b) Image of the produced plasma jet with a mixture of air and helium (4 slm flow).

seedlings were treated for 30 s on a once-a-week basis during the plants' growth cycle for a period of 1 mo. Seeds were exposed to a single 30-s treatment before planting.

B. Growth and Harvesting of Plants

Single seeds from sweet basil (*Ocimum basilicum*), thyme (*Thymus satureioides* Coss.), oregano (*Origanum vulgare*), lavender (*Lavandula angustifolia*), and St. John's wort (*Hypericum perforatum*) were planted and grown in Seton Hall University's McNulty Hall greenhouse. Once the seeds sprouted and there was visible evidence of seedlings, half of the plants were treated with cold plasma, and the other half was left untreated. All plant heights were monitored up to the time of harvesting. Once a critical mass was reached, plants were harvested for the next step.

C. Chemical Extraction of Essential Oils

Plant material was subjected to steam distillation, and the distillate was extracted with hexanes. The resulting organic layer was dried with sodium sulfate, filtered, and evaporated to yield the essential oils used in the study.

D. Antioxidant Assay

The antioxidant activity of commercial essential oils (purchased from New Directions Aromatics, Mississauga, Ontario, Canada) and oils extracted from in-house plants was determined by the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method. DPPH radical has been widely used to evaluate the antioxidant properties of natural products.[9]

We followed the variation of the DPPH method by Mensor et al.[10] Sample stock solutions of essential oils (1.0 mg/mL) were diluted to final concentrations of 250, 125, 50, 25, and 15 μg/mL in methanol. Dilutions were 5.0 mL and prepared in duplicate. A volume of 1.6 mL of a 0.3-mM DPPH methanol solution was added to 4.0 mL of the sample solutions in 10-mL glass cuvette tubes. Mixtures were gently vortexed for 15 s followed by incubation in the dark for 30 min at 25°C. After 30 min, absorbance values (Abs) were measured at 518 nm using a spectrophotometer 20D+ (Thermo Electron Corporation, Madison, WI). The percentage of antioxidant activity (AA%) for the essential oils was determined using the following formula:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\}.$$

Methanol (1.6 mL) plus plant extract solution (4.0 mL) was used as a blank, and DPPH solution (1.6 mL) plus methanol (4.0 mL) was used as a negative control.

Due to its odd electron, the DPPH radical, purple in color, has a strong absorption maximum at a wavelength of 517 nm. The study showed that the color turned from purple to yellow as the "molar absorptivity of the DPPH radical at 517 nm reduced from 9660 to 1640 when the odd electron of the DPPH radical becomes paired with

a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to the number of electrons captured."[11]

E. Gas Chromatography–Mass Spectrometry

A combination of plasma-treated and nontreated essential oil that was steam distilled from sweet basil grown in our greenhouse, commercial sweet basil oil, oregano oil, and oregano oil that was steam distilled from a plant purchased from a local nursery, all solvated in methanol (100 parts-per-million samples), were analyzed via gas chromatography (GC) and mass spectrometry (MS) using a Shimadzu TQ-8030 GC-MS. Instrumental conditions for the GC-MS are described in Table 1.

TABLE 1: Conditions for GC-MS analysis

Column	RTX-5 MS: 15 m × 0.25 mm × 0.25 μm
Inlet temperature	250°C
Oven ramp	50°C held for 1 min, increased by 10°C/min to 250°C, and held for 5 min
Ion source temperature	230°C
Transfer line temperature	25°C
m/z Range in full-scan mode	50–600
Solvent cut time	3 min
Helium flow	0.99 mL/min

GC-MS, Gas chromatography mass spectrometry; m/z, mass to charge ratio.

III. RESULTS AND DISCUSSION

A. Effect of Cold Plasma on Botanical Growth

Cold plasma processing has been found to increase the growth of various plant species.[12] To investigate this effect, we attempted to grow sweet basil, thyme, oregano, lavender, and St. John's wort. However, only sweet basil grew to any extent of maturity that allowed for sufficient harvesting and subsequent chemical analysis. Thus, plasma processing enhanced only the growth of sweet basil. This effect can be seen in Figs. 3 and 4, where plasma-treated basil grew significantly larger than nontreated basil. During treatments, the plasma-treated seedlings were observed to be under duress, demonstrated by the withering of their leaves during the initial period of plasma exposure compared to the nontreated sweet basil. However, once plasma treatment ceased, the treated plants grew more robust and larger than the nontreated controls. For this reason, it is thought that the plasma treatment triggered abiotic stress responses that eventually allowed the plant to rebound and surpass the growth of the nontreated controls. This phenomenon of abiotic stress response might be likened to comparable models in nature such as muscle building through intense exercise, where muscle fibers damage

FIG. 3: Comparison of plasma-treated sweet basil (left) and the control, nontreated sweet basil (right)

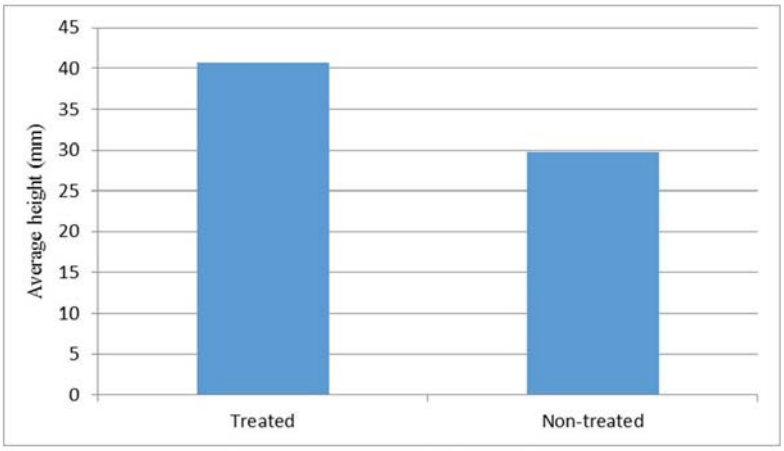


FIG. 4: (a) Graph demonstrating average final height of 12 treated and nontreated sweet basil plants after a month of growth from seeds.

and repair in a refractory period following exercise. It might be analogous to the case of the plasma-treated seedlings that plasma damage to plant cells initiated a recovery response that eventually left the plant more resilient. In addition, it has been established that certain abiotic stressors have the ability to enhance a plant's response to other environmental challenges.[13]

Several investigators have found that early seed germination can be achieved with plasma.[12] It is speculated that active particles penetrate the seed coat and directly influence underlying cells. The hypothesized mechanism involves increased transmission of oxygen and moisture through the seed coat, resulting in increased germination.[12] Our research further investigated the effect of plasma on basil seeds, and we also observed

that these seeds yielded plants that were significantly larger and more robust than those of nontreated plants (Fig. 5).

Essential oils contain a number of phenolic compounds and flavonoids that are potent antioxidants. For example, *O. basilicum* contains cinnamic, caffeic, sinapic, and ferulic acids that, as antioxidants, function as free radical scavengers and metal chelators.[14] Research ha revealed that some commercial essential oils are effective antioxidants, as shown by their percent of effectiveness as inhibitors of the synthetic free radical DPPH. These include holy basil and clove leaf (Table 2), two essential oils that compared favorably with synthetic and effective antioxidant BHT. Commercial sweet basil was not an effective antioxidant; however, a combination of oil extracted from our homegrown basil plants that were treated and nontreated with plasma proved to have higher antioxidant activity. The ineffectiveness of the commercial sweet basil agrees with literature results,[6] as does the effectiveness of clove leaf and holy basil.[6,15] Eugenol, a phenylpropanoid, is a main component of clove leaf that is largely responsible for its antioxidant effect.[6]

After observation of the enhanced antioxidant effect of the combined plasma-treated and nontreated extracted sweet basil oil (Table 2, row 5), a subsequent study was con-

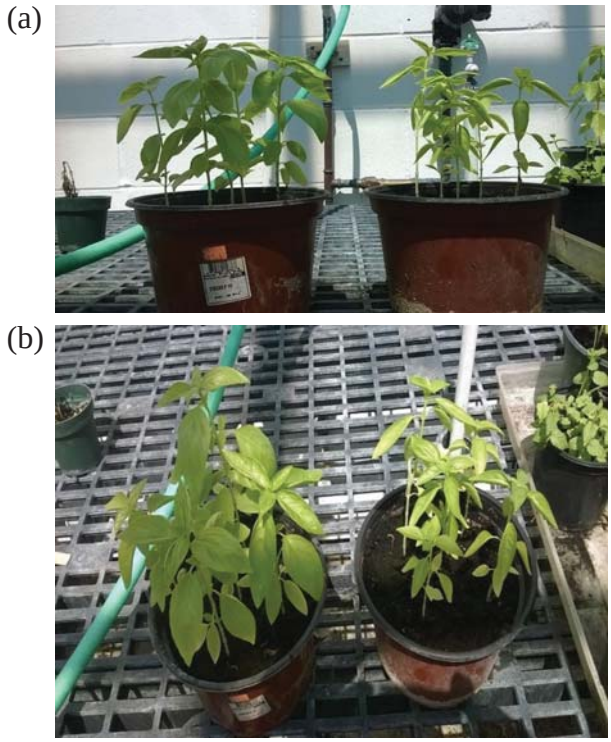


FIG. 5: (a) Side view of basil seedlings grown from plasma-treated seeds (left) and untreated seeds (right). (b) Top view of basil seedlings grown from plasma-treated seeds (left) and untreated seeds (right).

TABLE 2: Essential oil AA% of commercial and extracted homegrown herbs

Antioxidant/concentration	15 µg/mL (%)	25 µg/mL (%)	50 µg/mL (%)	125 µg/mL (%)	250 µg/mL (%)
BHT	40.20	64.32	77.24	83.34	100.0
Holy basil (commercial)	63.32	79.69	83.21	98.13	98.96
Sweet basil (commercial)	–6.16	–4.83	–4.21	–1.34	1.38
Sweet basil[a] (extracted)	59.79	73.16	86.53	93.16	95.34
Clove leaf	73.01	86.42	96.26	97.39	97.32

AA%, Percentage of antioxidant activity; BHT, butylated hydroxytoluene

[a]Sweet basil oil was collected from homegrown plants that were treated and untreated with plasma.

ducted to parse out the contribution of plasma treatment and extraction. Unlike the prior study, plasma-treated and nontreated sweet basil were separately steam distilled and extracted to obtain each respective essential oil. It was observed that plasma-treated sweet basil produced more pronounced antioxidant activity at concentrations of <250 µg/mL, as shown in Table 3.

TABLE 3: Essential oil AA% of plasma-treated, nontreated, and extracted homegrown sweet basil

Antioxidant/concentration	15 µg/mL (%)	25 µg/mL (%)	50 µg/mL (%)	125 µg/mL (%)	250 µg/mL (%)
Plasma-treated basil	48.00	62.55	81.55	90.55	94.82
Nontreated basil	19.55	26.91	46.36	78.27	90.64

AA%, Percentage of antioxidant activity

B. GC-MS Analysis of Essential Oils

Chromatograms and mass spectra were provided for two samples: commercial basil and extracted basil (combined plasma treated and nontreated). Using mass spectrometry, estragole was found to be the main component in commercial sweet basil. This agrees with literature reports, because sweet basil oil is mainly estragole.[15] The predominant component found in extracted homegrown basil (plasma treated and control) was eugenol. Figure 6(a) shows an overlay of commercial and extracted basil oil gas chromatograms, followed by the mass spectrum of eugenol fraction for extracted basil oil in Fig. 6(b). It is likely that eugenol in the homegrown samples is the predominant reason for enhanced antioxidant effect. The data in Table 3 suggest that the increased antioxidant effect (eugenol) is due to plasma treatment.

IV. CONCLUSIONS

On the basis of the research presented herein, we conclude that food production, preservation, and essential oil production may all benefit from cold plasma processing ap-

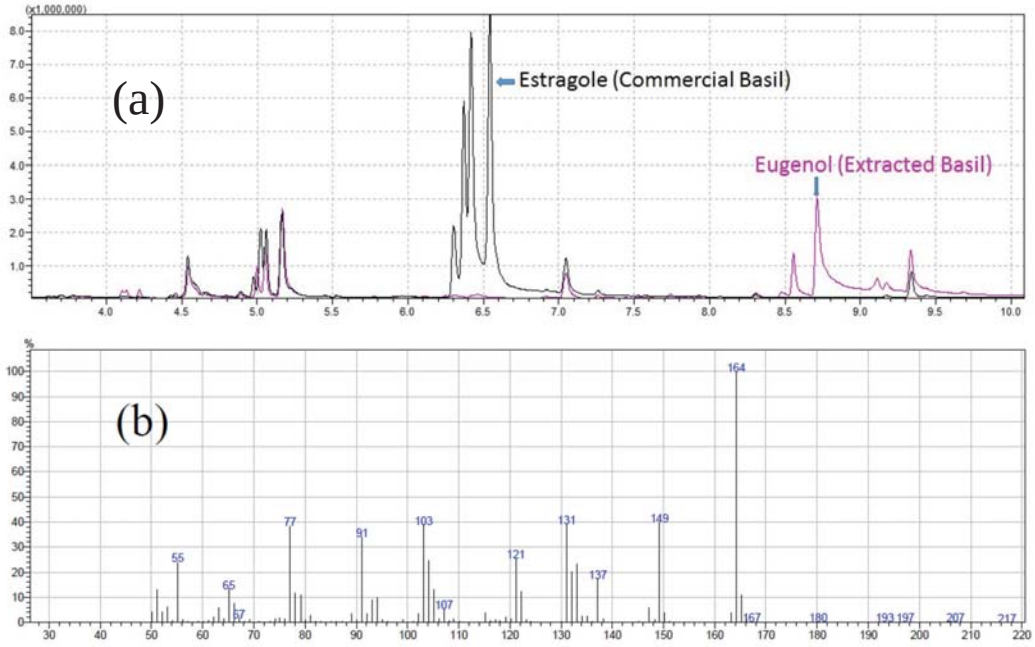


FIG. 6: (a) The gas chromatogram of commercial (darker trace) and extracted (lighter trace) basil oil. (b) The mass spectrum of eugenol fraction seen only in the extracted basil oil.

plications. Our experiments show that plasma processing increased the physical growth of sweet basil seedlings and seeds. The data are consistent with prior observations in the literature, stating that abiotic stress increases the resilience of plants. The essential oils extracted from homegrown sweet basil by our plasma treatment method clearly showed a greater concentration of eugenol compared to that found in commercial samples. This is reflected in the DPPH assay, which demonstrated that commercial basil oil had reduced antioxidant activity when compared to its homegrown counterpart. It should be noted that sweet basil oil has not traditionally shown significant antioxidant activity. However, our antioxidant studies show the potential for greater antioxidant activity in basil arising from plasma processing. In this case, it is possible that eugenol production within the basil plant increased after exposure to the plasma, which would explain the concomitant antioxidant activity of treated samples. Future studies may be directed at understanding the aforementioned effect and validating that the sweet basil oil obtained using our methods is a potential natural replacement of the synthetic commercial preservatives BHT and BHA, both classified to be possible carcinogens.

ACKNOWLEDGMENTS

The authors thank Professor Nicholas Snow and graduate student Ms. Anumeha Muthal of the Department of Chemistry and Biochemistry, Seton Hall University, for provid-

ing GC-MS analysis. We are thankful for funding provided by a grant from the Seton Hall Priest Community and the Fund of Professional Development. We are grateful to the following students who performed laboratory analysis: Samuel Seckler, Jack Bowman, Khanh Tran, Sandra Cojocaru (Seton Hall undergraduate students), Oscar Cosme, Leonsteen Sterling, and Amrita Singh (high school students participating in the Summer 2016 Project SEED program of the American Chemical Society).

REFERENCES

1. Tong JY, He R, Zhang XL, Zhan RT, Chen WW, Yang SZ. Effects of atmospheric pressure air plasma pretreatment on the seed germination and early growth of Andrographis paniculata. Plasma Sci Technol. 2014;16:260–6.
2. Zhou ZW, Huang YF, Yang SZ, Chen W. Introduction of a new atmospheric pressure plasma device and application on tomato seeds. Agri Sci. 2011;2:23–7.
3. Selcuk M, Oksuz L, Basaran P. Decontamination of grains and legumes infected with Aspergillus spp. and Penicillum spp. by cold plasma treatment. Biores Technol. 2008;99:5104–9.
4. Yousif AN, Scaman CH, Durance TD, Girard B. Flavor volatiles and physical properties of vacuum-microwave-air-dried sweet basil (Ocimum basilicum L.). J Agr Food Chem. 1999;47:4777–81.
5. Thirumdas R, Deshmukh RR, Annapure US. Effect of low temperature plasma on the functional properties of basmati rice flour. J Food Sci Technol. 2016;53(6):2742–51.
6. Amorati R, Foti MC, Valgimigli L. Antioxidant activity of essential oils. J Agr Food Chem. 2013;61(1):10835−47.
7. Lim ASL, Rabeta MS. Proximate analysis, mineral content and antioxidant capacity of milk apple, malay apple and water apple. Int Food Res J. 2013;20(2):673–9.
8. Valgimigli L. Essential oils: an overview on origins, chemistry, properties and uses. In: Valgimigli L, editor. Essential oils as natural food additives. New York: Nova Science Publishers; 2012. pp. 1−24.
9. Bendary E, Francis RR, HMG Ali, Sarwat MI, El Hady S. Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. Ann of Agri Sci. 2013;58(2):173–81.
10. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001;15:127–30.
11. Prakash A, Rigelhof F, Miller E. Antioxidant activity. Medallion Laboratories Analytical Progress. [Internet]. Available from: http://www.medallionlabs.com/Downloads/Antiox_acti_.pdf. 2007.
12. Thirumdas R, Sarangapani C, Annapure US. Cold plasma: a novel non-thermal technology for food processing. Food Biophys. 2015;10:1–11.
13. Ben Rejeb I, Pastor V, Mauch-Mani B. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plants. 2014;3:458–75.
14. Jayasinghe C, Gotoh N, Aoki T, Wada S. Phenolics composition and antioxidant activity of sweet basil (Ocimum basilicum L.). J Agr Food Chem. 2003;51:4442–9.
15. Juntachote T, Berghofer E. Antioxidative properties and stability of ethanolic extracts of holy basil and galangal. Food Chem. 2005; 92(2):193–202.