SECTION V

Summary
and
Conclusions
In recent years, consumers’ demands for reduced-additive and more natural foods have increased due to increased awareness over the nutritional value and safety of natural food ingredients as well as concerns about the toxic and carcinogenic effects of synthetic food additives. Phytochemicals as naturally occurring plant components with discrete bioactivities are being widely examined for their ability to provide health benefits. They have attracted a great deal of attention because of their role in preventing oxidative stress related diseases. Lipid oxidation caused by free radicals is a natural phenomenon in food and biological systems. Phytochemicals can act as antioxidants and free radical scavengers. Dietary intake of phytochemical antioxidants such as tocopherols, vitamin C, carotenoids, and phenolic compounds can increase the plasma antioxidant capacity resulting in preventing diseases caused by oxidative stress. Crude extracts of plant materials rich in phenolic compounds are increasingly of interest in the food industry because they retard oxidative deterioration of lipids and thereby improve the quality and nutritional quality of foods. A number of studies report the antioxidant potential of isolated constituents and different extracts of plants in food and biological systems. The importance of plant-based antioxidants in foods is appreciated for preserving foods against oxidative deterioration as well as supplying the essential antioxidants in vivo. Epidemiological studies report a significant positive association between consumption of fruits, vegetables, herbs and spices, and reduced risk of cardiovascular disorders, cancers, and other degenerative diseases besides aging.
Plants are important sources of naturally occurring antimicrobials. The use of plants, their extracts, essential oils, and active components constitutes an alternative approach to chemical substances for controlling microbial growth in food materials as well as treating/preventing infectious diseases. The potential antimicrobial activity of many plants and their extracts has been reported.

Recently, the role of some dietary components from plant sources as antithrombotic and anti-atherosclerotic has been reported. These components may inhibit platelet aggregation that is one of the risk factors of atherosclerosis leading to cardiovascular diseases.

The present research work was carried out to study the nutritional and pharmacological properties of extracts obtained from the leaves of three plants, namely, *Moringa oleifera* (drumstick), *Morus indica* (mulberry), and *Mentha spicata* (mint). Various solvent extracts of selected samples were screened for their antioxidant properties using four different methods (reducing power, total antioxidant capacity, DPPH radical scavenging activity, and *in vitro* inhibition of lipid peroxidation). The effect of processing (pH, heat, and storage) on the antioxidant activity of extracts was evaluated. The physicochemical characteristics of phenolic extracts were studied and their efficacy as preservative in pineapple juice was assessed. The extracts were examined for their antimicrobial and anti-platelet activities. The protective effect of extracts on lipid peroxidation was evaluated in model food and biological systems. The antioxidant activity of individual components of drumstick leaves was determined and the most active component was characterized using various spectroscopic methods.
The salient findings of this investigation are:

"Antioxidative properties of various solvent extracts of selected plants":

- The selected samples were good sources of antioxidant components including, β-carotene, ascorbic acid, α-tocopherol and glutathione. Drumstick leaves were a rich source of ascorbic acid. Mulberry leaves had the highest content of α-tocopherol and glutathione.

- Methanolic extract of each sample had the maximum yield of total soluble substances and the highest TPC followed by acetone extract and water extract.

- Various solvent extracts of each sample showed varying degree of reducing power (RP), total antioxidant capacity (TAOC), DPPH-radical scavenging activity (DPPH-RSA), and antioxidant activity (AOA) in linseed oil, in a dose-dependent manner. They were inferior to ascorbic acid and BHT in their antioxidant activities.

- Methanolic extract of each sample containing the highest TPC was the most potent antioxidant in all the assays used.

- Methanolic extract of mint leaves with the highest TPC was the most active extract in RP, TAOC and DPPH-RSA assays.

- Methanolic extract of mulberry leaves showed the highest antioxidant activity (81.80%) in linseed oil, followed by that of drumstick leaves (78.84%) and mint leaves (73.74%).

- Correlation coefficients of 0.80**, 0.63*, 0.85**, and 0.73** were found between TPC and RP, TPC and TAOC, and TPC and DPPH-RSA, TPC and AOA, respectively.
Correlation coefficients of 0.85**, 0.74**, and 0.58* were found between RP and TAOC, RP and DPPH-RSA, and TAOC and DPPH-RSA, respectively.

Correlation coefficients of 0.91**, 0.86**, and 0.79** were found between AOA and TAOC, AOA and RP, and AOA and DPPH-RSA, respectively.

Methanolic extract of drumstick leaves showed a relatively high antioxidative stability to pH and heat treatments.

The antioxidant activity of methanolic extract of mulberry decreased on heat treatment, while the activity of mint extract increased.

The optimum pH for antioxidant activity of methanolic extract of mulberry and mint were 7 and 3, respectively.

The three extracts were potent antioxidants even after 90 days of storage at 4 °C.

"Physicochemical characteristics and food preservative efficacy of phenolic extracts"

The leaves of drumstick and mint had higher total phenolic content (TPC) than mulberry leaves.

The prominent group of phenolic compounds in the leaves of drumstick and mulberry was flavonoids, while both phenolic acids and flavonoids were prominent in mint leaves.

The three phenolic extracts were stable to acid and neutral pH, but not to alkaline pH. The spectral changes of drumstick extract in alkaline media were less pronounced compared to those of other samples.
Gallic acid, protocatechic acid, and catechin in drumstick leaves, gallic acid, protocatechic acid, epicatechin, and gentisic acid in mulberry leaves, and gallic acid, gentisic acid, and caffeic acid in mint leaves were identified by RP-HPLC.

The solubility of phenolic extracts of drumstick and mulberry in pineapple juice was more than that of mint extract.

The extracts of mulberry and drumstick were relatively stable in the acid pH of pineapple juice during storage (4 °C, 2 weeks).

The juice containing 0.25% (w/v) of phenolic extract of mulberry was well acceptable in terms of sensory attributes, including flavour, colour, taste, appearance and overall quality.

Addition of mulberry extract (0.25%, w/v) in the pineapple juice significantly reduced the titrable acidity, and retained the sensory characteristics of the product during 8 weeks of storage at 4 °C.

"Effect of plant extracts on human platelet aggregation"

Aqueous extracts of mulberry and drumstick leaves showed a dose-dependent inhibitory activity against human platelet aggregation induced by collagen, ADP, and epinephrine *In vitro.*

The degree of inhibitory activity of each extract varied depending on the agonist used. The epinephrine-induced aggregation showed a higher sensitivity to the extract of mulberry leaves.

Prolonged incubation of platelets with the extracts improved the inhibitory activity against platelet aggregation.
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* The extracts significantly reduced the amount of malonaldehayde (MA) formed in agonist-challenged platelets.
* Heat treatment reduced the antiaggregatory activity of drumstick leaves against all the agonists used.
* The extract of drumstick (raw) was the most active in inhibiting platelet aggregation and reducing the formation of MA in agonist-challenged platelets.

"Effect of plant extracts on oxidative damage of human erythrocyte membrane"

* Phenolic extracts of selected samples dose-dependently reduced the formation of TBARS in human erythrocyte membrane oxidized by Fe²⁺, ADP, and ascorbic acid.
* The extracts enhanced the antioxidative status of membrane by protecting reduced glutathione (GSH) against oxidation.
* The extract of drumstick was the most potent agent in inhibiting lipid peroxidation and enhancing the level of GSH in erythrocyte membrane.

"Antimicrobial activity of plant extracts"

- Phenolic extracts of three samples exhibited antibacterial activity against both G (+) and G (-) bacteria, in a dose-dependent manner.
- The extract of drumstick showed a higher activity against G (-) bacteria. The extract of mulberry showed the highest activity against E. coli, and the lowest against S. aureus, while the activity of mint extract was the highest against S. aureus and lowest against S. typhimurium.
• MIC values showed that *E. coli* was more sensitive to extract of mulberry, *S. aureus* to that of mint, and *S. typhimurium* and *L. monocytogenes* to that of drumstick.

• The extract of drumstick leaves showed antifungal activity against *Aspergillus flavus* and *A. niger*.

"Antioxidative efficacy of plant extracts in model food systems"

◆ Addition of methanolic extract of drumstick leaves (MEDL) in soybean oil significantly reduced the formation of peroxides and TBARS during accelerated storage (70 °C, 10 days), even at level of 200 ppm.

◆ Addition of MEDL at 200, 500, and 1000 ppm prolonged the onset of rancidity of the oil to 2.5, 4.0, and 4.5 days, respectively.

◆ Formation of dimmers, trimmers, and TBARS in soybean oil at 180 °C significantly decreased on addition of MEDL.

◆ The antioxidant efficacy of MEDL (500 and 1000 ppm) was superior to that of synthetic antioxidant, BHA, in both storage and heating tests.

◆ The antioxidant efficacy of MEDL in the oil indicated a high thermal stability of antioxidant components present in the extract.

◆ Addition of phenolic extracts of mulberry (1.5%) and mint (1.0%) did not impart undesirable changes on the sensory and colour characteristics of biscuits.

◆ The biscuits containing extracts of mulberry and mint had lower PV and TBARS values than control during storage of 90 days at room
temperature. These samples were well acceptable in terms of sensory parameters during storage period.

- The antioxidant efficiency of extracts in biscuit was comparable to that of synthetic antioxidant, BHA.

"Characterization of antioxidant components of drumstick leaves"

- All the fractions separated from methanolic extract of drumstick leaves showed antioxidant activity in the TAOC assay.
- Some of the fractions showed DPPH radical scavenging activity.
- The fraction with the highest Rf value (F-1) showed the highest antioxidant activity.
- F-1 was found as an aliphatic compound belonging to esters and a molecular formula of C$_{31}$H$_{56}$O$_2$ was suggested.

From these observations, it can be concluded that:

- The antioxidant efficacy of various solvent extracts of drumstick, mulberry, and mint leaves in different test systems revealed the presence of antioxidant constituents in the samples and suggested their potential as natural antioxidants in food and biological systems.
- Phenolic compounds of selected samples have potential to be utilized as natural antioxidant and antimicrobial agents as alternative to synthetic ones.
- It is important to consider the optimum technological conditions and factors influencing the activity and bioavailability of plant products when applied as natural preservatives in food and biological systems. In
addition, their potential exploitable beneficial effects and safety in humans need to be proven in clinical trials.

✓ Highly nutritious drumstick leaves in the form of extracts exhibited potent antioxidant activity in both food and biological systems. They exhibited high antioxidative stability, antibacterial and antifungal activity, and potent inhibitory activity against platelet aggregation. By combining the results of these in vitro bioactivities and suitable in vivo experiments, it can be shown that the extracts or active constituents of drumstick leaves may have potential to be incorporated into food supplements as “nutraceuticals” or into “pharmaceuticals” for improving the health of human population.

✓ Regular and increased consumption of the leaves of drumstick, mulberry, and mint is recommended in order to exploit their nutritional and beneficial health effects such as their capacity as inhibitors of oxidative damage of cellular components, e.g. erythrocyte membrane, and antiplatelet agents.