CHAPTER 1

INTRODUCTION

Herbal and natural products of folk medicine have been used for centuries in ever culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies. Folk medicine in different cultures has a long history of ancestors creating primitive medicines during their struggles against natural calamity and disease. Tea is one of the first Chinese herbs mentioned in ancient literature. Tea supposedly originated in China, and was discovered to be an antidote for poisonous herbs by a great herbalist, Shen Nong, about 4700 years ago, when tasting unknown herbs to find plants with medicine value. He is generally known as the "God of Agriculture" in China for his great achievements as both a pioneer and a leader in farming. His findings were compiled in a book in the Dong-Han dynasty (25-220 AD) called "Shen Nong' Herbs," which search still a classic herbal book today. While searching for food, the ancient found that some foods had specific properties of relieving or eliminating certain diseases, and maintaining good health. It was the beginning of herbal medicine [1].

Fermentation is the "slow decomposition process of organic substances induced by micro-organisms, or by complex nitrogenous substance (enzymes) of plant or animal origin" [2]. Fermentation has been used for several thousand years as an effective and low cost means to preserve the quality and safety of foods. Fermentation is one of the oldest forms of food preservation biotechnology in the world. Each nation has its own types of fermented food, representing the staple diet and the raw ingredients available in that particular place. Although the products are well known to the individual, they may not be associated with fermentation. Indeed, it is likely that the methods of producing many of the worlds fermented foods are unknown and came about by chance. Some of the more obvious fermented fruit and vegetable products are the alcoholic beverages - beers and wines. Fermenting fruits and vegetables can bring many benefits to people in developing countries.

Fermented foods play an important role in providing food security, enhancing livelihoods and improving the nutrition and social well being of millions of people around the world, particularly the marginalized and vulnerable.

The definition of fermentation is "breaking down into simpler components". Fermentation makes the food easier to digest and the nutrients easier to assimilate. In effect, much of the work of digestion is done for you. Since it doesn't use heat, fermentation also retains enzymes, vitamins, and other nutrients that are usually destroyed by food processing. The active cultures that pre-digest the food as part of the fermentation process actually generate nutrients. So there are more vitamins especially B-vitamins and minerals like iron are released from the chemical bonds that prevent them from begin assimilated. In effect, the nutritional value of a food goes up when it has been fermented.

Many fermented fruit juices may contain fat soluble vitamins e.g. vitamin A, vitamin E, β -carotene etc. and water soluble vitamin e.g. vitamin C, vitamin B etc. α -Tocopherol and beta-carotene, the fat soluble and water soluble vitamins, act as antioxidants primarily by scavenging active radicals to inhibit chain initiation and break chain propagation. There were widely experimental and epidemiological investigation about the protection activity to many disease states associated with increased oxidative metabolism. The low blood concentrations of α -tocopherol and vitamin c were found in patients suffering from many diseases, especially age-related diseases such as atherosclerosis.

There are some groups of fungi, yeast and bacteria with can produce vitamin B₂ during fermentation as shown in Table 1.1

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d bacteria can produce vitar	$\min \mathbf{B}_2[3]$
Yeast	
Anascosporogenous	Clos
Candida arborea	Cl. a

Table	1.1	Fungi,	yeast and	bacteria	can	produce	vitamin	B ₂ [3].
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Fungi	Fungi Yeast	
Ashbya gossypii	Anascosporogenous	Clostridium butylicum
Eremothecium ashbyii	Candida arborea	Cl. acetobutylicum
Aspergillus niger	C. krusei, C. flareri	Cl. felsineum,
Aspergillus flavus	C. tropicalis varrhaggi,	Cl. propylbutylicum
Penicillium chrysogenum	C. guilliermondia,	Cl. roseum,
Fusarium spp.	C. utilis, C. lactis, C. ghoshi,	Cl. aerogenes
Aspergillus terreus	C. chalmersi, C. lipolytica,	Cl. cloacae, Azotobacter
	C. olea, C. pulcherrima ;	Az.agile, Az.vinelandii
	C. solani ; C. melibiosis,	Lactobacillus spp.
	Torulopsis sp.	Arthrobacter sp.
	Rhodotorula sp.	Bacillus subtillis
	Asco-sporogenous	Methanobacterium
	Hansenula suaveolens	thermoautophicum
	Saccharomyces fragilis	

The art of traditional processes needs to be refined to incorporate objective methods of process control and to standardize quality of the final product without losing their desirable attributes such as improved keeping quality, test and nutritional qualities. Quality control procedures are essential for the production of safe products and contribute. It is important to carry out participative research to identify ways to improve the quality control procedure for fermented food product.

However, several more fermented fruit and vegetable products arise from lactic acid fermentation and are extremely important in meeting the nutritional requirements of a large proportion of the world's population.

1.1 Detail about medicinal plants used in this investigation

1.1.1 Morinda citrifolia Linn.

The genus *Morinda*, of the family *Rubiaceae*, has many common names such as indian mulberry (India), noni (Tahiti and Raratonga), polynesian bush fruit, painkiller tree (Caribbean islands), Mengkudo (Malaysia), grand morinda (Vietnam), cheesefruit (Australia), kura (Fiji) and bumbo (Africa) [4, 5,6].



Figure 1.1 Leave and fruit of M. citrifolia

Description

M. citrifolia is a large evergreen shrub or small tree to 6 m or more in height and 13 cm or more in stem diameter. Sapwood is yellow-brown and soft. The bark is gray or brown, smoothish to slightly rough. Twigs are light green and four-angled. The opposite leaves are attached by stout petioles 1 to 2 cm long. The blades are dark green and shiny, ovate or elliptic, 14 to 30 cm long by 8 to 18 cm broad, and have prominent veins. The white tubular flowers are grouped in globose heads at the leaf axils. The five-lobbed flower tubes are about 6 mm long. The greenish-white to pale-yellow, fleshy fruits are ovoid or globose syncarps 5 to 7 cm long. They have an unpleasant odor resembling cheese. They contain a number of seeds about 4 mm long [7].

The ancestors of Polynesians are believed to have brought many plant's with them, as food and medicine, when they migrated from Southeast Asia 2000 year ago [8]. Of the 12 most common medicinal plants they brought, Noni was the second most popular plant used in herbal remedies to treat various common diseases and to maintain overall good health. Noni is the common name for *M. citrifolia* and is also called Indian Mulberry, Ba Ji Tian, Nono or Nonu, Cheese Fruit, and Nhau in various cultures throughout the world. It has been reported to have a broad range of health benefits for cancer, infection, arthritis, diabetes, asthma, hypertension, and pain [9]. The Polynesians utilized the whole Noni plant in their medicinal remedies and dye for some of their traditional clothes. The roots, stems, bark, leaves, flowers, and fruits of the Noni plant are all involved in various combinations in almost 40 known and recorded herbal remedies [10]. Additionally, the roots were used to produce a yellow or red dye for tapa cloths and fala (mats), while the fruit was eaten for health and food.

M. citrifolia fruit has a long history of use as a food in tropical regions throughout the world. In 1943, Merril described *M. citrifolia* as an edible plant in a technical manual of edible and poisonous plants of the Pacific Islands, in which the leaves and fruits could be used as emergency food [11]. The medicinal history and accumulated scientific studies, to date, have revealed and confirmed the Polynesian's claim of the health benefits of Noni.

In Thailand "Yor" leaves cooked with curry give a wonderful and nutritious dish. "Yor" fruits can be prepared as a Thai traditional salad "Somtumyor". Dried fruits or leaves powder boiled with water produces a tea, which is used to relief blood pressure, muscle pains and vomiting [12].

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Major components

A number of major components have been identified in the Noni plant such as scopoletin, octoanic acid, potassium, vitamin C, terpenoid, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin-1methyl ether, anthraquinone glycoside), β -sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, alizarin, amino acids, acubin, *L*-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and a putative peroxeronine [13-24].

Traditional use of M. citrifolia

The Polynesians utilized the whole Noni plant in various combinations for herbal remedies. The fruit juice was in high demand in alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems, and drug addiction. Scientific evidence of the benefits of the Noni fruit juice in limited but there was some anecdotal evidence for successful treatment of colds and influenza [25]. Allen reported some information on the ethnobotanical properties of Noni. He said that the fruit was used as deobstruent and emmenagogue. This is one of the earliest articles on the medicinal benefits of Noni [26]. Isabel Abbott, a former botanical chemist at University of Hawaii, stated that, "People were crazy about this plant. They used it for diabetes, high blood pressure, cancer, and many other illnesses" [27]. Bushnell reported that Noni was a traditional remedy used to treat broken bones, deep cut, bruises, sores and wounds [28].

Biological activities of M. citrifolia

Acubin, *L*-asperuloside, and alizarin in the Noni fruit, as well as some other anthraquinone compounds in Noni roots, were all proven antibacterial agents. These compounds have been shown to fight against infectious bacteria strains such as *Pseudomonas aeruginosa, Proteus morgaii, Staphylococcus aureus, Baciillis subtlis,* Escherichia coli, Salmonella, and Shigela. These antibacterial elements within Noni were responsible for the treatment of skin infections, colds, fevers, and other bacterial-caused health problems [29]. Bushnell reported on the antibacterial properties of some plants found in Hawaii, including Noni. He further reported that Noni was traditionally used to treat broken bones, deep cuts, bruises, sores and wounds. Extracts from the ripe noni fruit exhibited moderate antibacterial properties against Psaeruginosa, M pyrogenes and E coli, and were also shown to have moderate antibacterial properties against Salmonella typhosa, Salmonella Montevideo, Salmonella schottmuelleri, Shigella paradys, BH and Shigella paradys, III-Z [28]. Leach demonstrated that the acetone extracts obtained from Cycascircinalis, Morinda citrifolia, Bridelia penangiana, Tridax Procumbens, Hibiscus tiliaceus, and Hypericum papuanun showed antibacterial activity. The widespread medicinal use of these plants would suggest that they do contain pharmacologically active substances and alternative methods of extraction and screening should be utilized to find purpose of new drug development [30]. These plants were investigated for anti-viral, antifungal, and anti-bacterial activity in vitro. Their study using biological assays in vitro confirmed that some of the ethno botanical reports of Hawaiian medicinal plants have curative properties against infectious diseases.

Duncan demonstrated that scopoletin a health promotor in Noni, inhibits the activity of *E coli*, commonly associated with recent outbreaks resulting in hundreds of serious infections and even death. Noni also helps stomach ulcer through inhibition of the bacteria *H pylori* [31].

Umezawa and coworkers found a compound isolated from Noni roots named 1–methoxy-2-formyl-3-hydroxyanthraquinone suppressed the cytopathic effect of HIV infected MT-4 cells, without inhibiting cell growth [32].

In 1992, Hirazumi, a researcher at the University of Hawaii, reported anticancer activity from the alcohol-precipitate of Noni fruit juice (noni-ppt) on lung cancer in C57 B1/6 mice at the 83th Annual Meeting of American Association for Cancer Research. The noni-ppt was shown to significantly prolong the life of mice up to 75% with implanted Lewis lung carcinoma compared with the control group [33]. It was conculded that the noni-ppt seems to suppress tumor growth indirectly by stimulating the immune system [34]. Improved survival time and curative effects occurred when noni-ppt was combined with sub-optimal doses of the standard chemotherapeutic agents such as adriamycin (Adria), cisplatin (CDDP), 5-fluorouracil (5-FU), and cincristine (VCR), suggesting important clinical applications of noni-ppt as a supplemental agent in cancer treatment [35]. These results indicated that noni-ppt might enhance the therapeutic effect of anticancer drugs.

In 1993, Hiramatsu and collegeues reported that the effects of over 500 extracts from tropical plants on the K-Ras-NRK cells. damnacanthal, isolated from Noni root, is an inhibitor of Ras function. The *ras* oncogene is believed to be associated with the signal transduction in several human cancers such as lung, colon. pancreas, and leukemia [36].

Hiwasa and coworkers demonstrated that damnacanthal, and anthraquinone compound, isolated from Noni roots, possessed a potent of inhibitory activity towards tyrosine kinases such as Lek, Src, Lyn, and EGF receptors. In his study, he examined the effects of damnacanthal on ultraviolet ray-in-duced apoptosis in ultraviolet-resistant human UVr-1 cells. Consequently, the ultraviolet light induced a concurrent increased in both phosphorylated extracellular signal-regulated kinases and stress-activated protein kinases. After pretreatment with damnacanthai, there was a stimulatory effect on ultraviolet-induced apoptosis [37].

An ethanol extract of the tender Noni leaves induced paralysis and death of the human parasitic nematode worm, *Ascaris Lumbricoides*, with in a day [38]. A botanist via Morton report that Noni has been used in the Philippines and Hawaii as an effective insecticide [39].

Joseph Betz reported that the Noni fruits possesses analgesic and tranquilizing activities [15]. A French research team led by Younos, tested the analgesic and sedative effects of extracts from the *Morinda citrifolia* plant. The extract did "show a significant, does-related, central analgesic activity in the treated mice." They stated that "these findings validate the traditional analgesic properties of this plant." The analgesic efficacy of the Noni extract is 75% as strong as morphine, yet non-addictive and side effect free [40].

Dang Van Ho demonstrated that a total extract of the Noni roots has a hypotensive effect [41]. Moorthy and coworkers found that an ethanol extract of the Noni roots lowered the blood pressure in an anesthetized dog [18]. Youngken's

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research team determined that a hot water extract of Noni roots lowered the blood pressure of an anesthetized dog [42, 43].

Asahina found that an alcohol extract of Noni fruit at various concentrations inhibited the production of tumor nercrosis factor-alpha (TNF- α), which is an endogenous tumor promotor. Therefore the alcohol extract may inhibit the tumor promoting effect of TNF- α [44]. Hirazumi found that noni-ppt contains a polysaccharide-rich substance that inhibited tumor growth. It did not exert significant cytotoxic effects in adapted culture of lung cancer cells, but could activate peritoneal exudates cells to impart profound toxicity when co-cultured with the tumor cells. This suggested the possibility that noni-ppt may suppress tumor growth through activation of the host immune system. Noni-ppt was also capable of stimulating the release of several mediators from murine effector cells, including TNF- α , interleukin-1 beta (IL-1 β), IL-10, IL-12, interferon-gamma (IFN- γ) and nitric oxide (NO) [35]. Hokama separated ripe noni fruit juice into 50% aqueous alcohol and precipitated fractions that stimulated the BALB/c thymus cells in the [³H]thymidine anlaysis. It is suggested that inhibition of Lewis lung tumors in mice, in part, may have been due to the stimulation of the T-cell immune response [44].

1.1.2 Phyllanthus emblica Linn.

The genus *Phyllanthus*, of the family *Euphorbiaceae*, has many common names such Emblic, Emblic myrobalan, Indian-gooseberry, Myrobalan emblic, Amblabaum, Amla, Aonla, Mirobalano and Neli [45].

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Figure 1.2 Leave and fruit of P. emblica

Description [46]

The tree is a graceful ornamental, normally reaching a height of 60 ft (18 m) and, in rare instances, 100 ft (30 m). Its fairly smooth bark is a pale gravish-brown and peels off in thin flakes like that of the guava. While actually deciduous, shedding its branchlets as well as its leaves, it is seldom entirely bare and is therefore often cited as an evergreen. The miniature, oblong leaves, only 1/8 in (3 mm) wide and 1/2to 3/4 in (1.25-2 cm) long, distichously disposed on very slender branchlets, give a misleading impression of finely pinnate foliage. Small, inconspicuous, greenishyellow flowers are borne in compact clusters in the axils of the lower leaves. Usually, male flowers occur at the lower end of a growing branchlet, with the female flowers above them, but occasional trees are dioecious. The nearly stemless fruit is round or oblate, indented at the base, and smooth, though 6 to 8 pale lines, sometimes faintly evident as ridges, extending from the base to the apex, give it the appearance of being divided into segments or lobes. Light-green at first, the fruit becomes whitish or a dull, greenish-yellow, or, more rarely, brick-red as it matures. It is hard and unyielding to the touch. The skin is thin, translucent and adherent to the very crisp, juicy, concolorous flesh. Tightly embedded in the center of the flesh is a slightly hexagonal stone containing 6 small seeds. Fruits collected in South Florida vary from 1 to 1 1/4 in (2.5-3.2 cm) in diameter but choice types in India approach 2 in (5 cm) in width. Ripe fruits are astringent, extremely acid, and some are distinctly bitter.

Chemical Composition [46]

They contain proteolytic and lipolytic enzymes, lignans (e.g., phyllanthine and hypophyllanthine), alkaloids, and bioflavonoids (e.g., quercetin),phosphatides and a small amount of essential oil. Approximately 16% consists of a brownish-yellow fixed oil.

Major components

The *P. emblica* tree contains the different classes of constituents listed in Table 1.2.

Table 1.2 The classes of chemical constituents reported in *Phyllanthus emblica* L.(Euphorbiaceae).

Class	Compound	Occurrence	Reference
Alkaloids	phyllantine	leaves, fruit and	[47]
	phyllantidine	tissued cultures	
	zeatin	leaves	[48]
	zeatin mucleotide	fruit	
	zeatin riboside		
Benzenoids	chebulic aicd	leaves	[49,50]
0	chebulinic acid	- d	2
lans	chebulagic acid	1919198	OIKU
	gallic acid		[49,50]
oyright	S by Chian	g Mai Un	IVE [51] IV
	ellagic acid		[49, 52, 53]
	amlic acid	fruit	[49, 54]
	coriligin	fruit	[55]
	3-6-di-O-galloy-glucose		
	ethyl gallate		

Table 1.2 (continued)

Class	Compound	Occurrence	Reference
Benzenoids	β-glucogallin	leaves	[49]
	ANEKA	fruit	[55]
a	1,6-di-O-galloyl-β-D-glucose	fruit	[56]
	1-di-O-galloy- β-D-glucose	5	
	Putranjivain A digallic acid	. 31	
	phyllemblic acid	fruit	[57]
	emblicol		
	music (galactaric) acid		[51]
Furanolactones	ascorbic acid	fruit	[58, 59, 60]
		leaves 9	[51]
Diterpenes	gibberellin A-1		[61]
	gibberellin A-3		
	gibberellin A-4	6 9	
	gibberellin A-7		
	gibberellin A-9		
Triterpenes	lupeol	fruit	[54]
	AI UNIVE	leaves	[52]
Flavonoids	leucodelphinidin	leaves	[62]
	kaempherol		[53]
	kaempherol-3-glucoside		2
	rutin	BOIRI	[63]
	quercetin		[63]
	kaempherol-3-O-β-D-glucoside	fruit	[56]
	quercetin-3-O-β-D-glucoside	eser	ved
Sterols	β-sitosterol	leaves	[52]
Carbohydrates	acidic and neutral		
	polysaccharides fruit		
	glucose	leaves	[49]

Traditional use of P. emblica

The emblic is of great importance in Asiatic medicine, not only as an antiscorbutic, but in the treatment of diverse ailments, especially those associated with the digestive organs. For such use, the fruit juice is prepared in the form of a sherbet or is fermented. In the latter state, it is prescribed in jaundice, dyspepsia and coughs. The dried chips of flesh are dispensed by apothecaries and often are mixed with grape juice and honey for dosage. The fruit is considered diuretic and laxative. Triphala, a decoction of emblic with Terminalia chebula Retz. and T. bellerica Roxb. is given for chronic dysentery, biliousness, hemorrhoids, enlarged liver, and other disorders. A powder prepared from the dried fruit is an effective expectorant as it stimulates the bronchial glands. The juice that exudes when the fruit is scored while still on the tree is valued as an eyewash and an application for inflamed eyes. An infusion made by steeping dried fruit overnight in water also serves as an eyewash, as does an infusion of the seeds. A liquor made from the fermented fruits is prescribed as a treatment for indigestion, anemia, jaundice, some cardiac problems, nasal congestion and retention of urine. Emblic leaves, too, are taken internally for indigestion and diarrhea or dysentery, especially in combination with buttermilk, sour milk or fenugreek. The milky sap of the tree is applied on foul sores. The plant is considered as an effective antiseptic in cleaning wounds, and it is also one of the many plant as palliatives for snakebite and scorpion stings. A decoction of the leaves is used as a mouthwash and as a lotion for sore eyes. The flowers, considered refrigerant and aperient, and roots, emetic, are also variously employed. The root bark, mixed with honey, is applied to inflammations of the mouth. The bark is strongly astringent and used in the treatment of diarrhea and as a stomachic for elephants. The juice of the fresh bark is mixed with honey and turmeric and given in cases of gonorrhea. It is clear that the majority of the applications of the fruit and other parts are based on the astringent action of the tannins they contain. The short-term effects of tannins appear beneficial, but habitual indulgence can be highly detrimental, inasmuch as tannin is antinutrient and carcinogenic. An ointment made from the burnt seeds and oil is applied to skin afflictions. The seeds are used in treating asthma, bronchitis, diabetes and fevers. This plant use to treat jaundice, gonorrhea, frequent menstruation, and diabetes and using it topically as a poultice for skin ulcers, sores, swelling, and itchiness. The young shoots of the plant are administered in the form of an infusion for the treatment of chronic dysentery.

P. emblica has been used for anti-inflammatory and antipyretic treatments by rural populations in its growing areas. Malays use a decoction of its leaves to treat fever [64]. In Indonesia, the pulp of the fruit in smeared on the head to dispel headache and dizziness caused by excessive heat [65]. The earlier chemical findings and biological activities have since been confirmed with more advanced techniques. Active principles or extracts of *P. emblica* have been shown to possess several pharmacological action, e.g. analgesic, anti-inflammatory, antioxidant, chemoprotective, hypolipidaemic and anti-HIV-I (Human immunodeficience virus-1) activities.

Biological activities of *P. emblica*

 Table 1.3 Recently reported biological effects of P. emblica

Biological effect	References
Antagonistic activity against genotoxic	[66, 67, 68, 69, 70]
chemicals, anticlastogenicityin vitro	
Antimicrobial activity, in vitro	[71]
Antioxidant activity in vitro	[72, 73, 74]
Anti-inflammatory activity, in vivo and	[75, 76, 77]
DD in vivo nt Dy Chiang	
Hepatoprotective activity, prevention of	[78, 79, 80, 81]
hepatocarcinogenesis, in vitro and in vivo	

Table 1.3 (Continue)

Biological effect	References
Hypolipidaemic, in vivo and in vitro	[82, 83, 84, 85]
Enhancer of natural killer cell (NK)	[86]
activity, in vitro	
Inhibition of human immunodeficiency	[87]
virus-1 (HIV-1) reverse transcriptase,	[88]
in vitro	
Prevention of experimental acute	[89]
pancreatitis, in vivo	
Protection against radiation-induced	[90]
chromosome damage, in vitro	

Phyllanthus blocks DNA polymerase, the enzyme needed for the hepatitis B virus to reproduce. Fifty-nine percent of those infected with chronic viral hepatitis B lost one of the major blood markers of HBV infection (e.g., hepatitis B surface antigen) after using *Phyllanthus* for thirty days [46].

1.2 Research Aims

The main purposes of this research are as follows:

1. To determine vitamin C, thiamine HCl, riboflavin, nicotinamide, pyridoxine HCl, α -tocopherol and β -carotene in fruits of *M. citrifolia* and *P. emblica* and their fermented juices.

2. To study the stability of vitamin C and vitamins B in fermented juices of *M. citrifolia* and *P. emblica*