The curry tree (*Murraya koenigii*) is a tropical to sub-tropical tree in the family Rutaceae (the rue family, which includes rue, citrus, and satinwood), which is native to India and Sri Lanka. Its leaves are used in many dishes in India, Sri Lanka, and neighboring countries. Often used in curries, the leaves are generally called by the name 'curry leaf', although they are also literally 'sweet neem leaves' in most Indian languages (as opposed to ordinary neem leaves which are very bitter and in the family Meliaceae, not Rutaceae). Curry leaves have always been sought after for their unique flavor and usefulness in cooking, but there are also a number of health benefits that make them highly appealing.
This book aims to provide a brief and simple description of the background, agronomy aspects and physico-chemical properties of curry leaf. This book will provide readers a comprehensive aspects of pre-processing methods of curry leaf, and the potential of curry leaf as antimicrobial agents. Last but not least, this book also provide readers with a self-contained guide on the application of statistical analysis in curry leaf related research.

Siti Nuurul Huda and Huck Ywih Ch'ng are Senior Lecturers in Faculty of Agro-Based Industry, Universiti Malaysia Kelantan. Siti Nuurul Huda specialises in process and product design, Chemical Engineering while Huck Ywih Ch'ng specialises in Land Resources Management. They have published numerous quality scientific articles and books worldwide.

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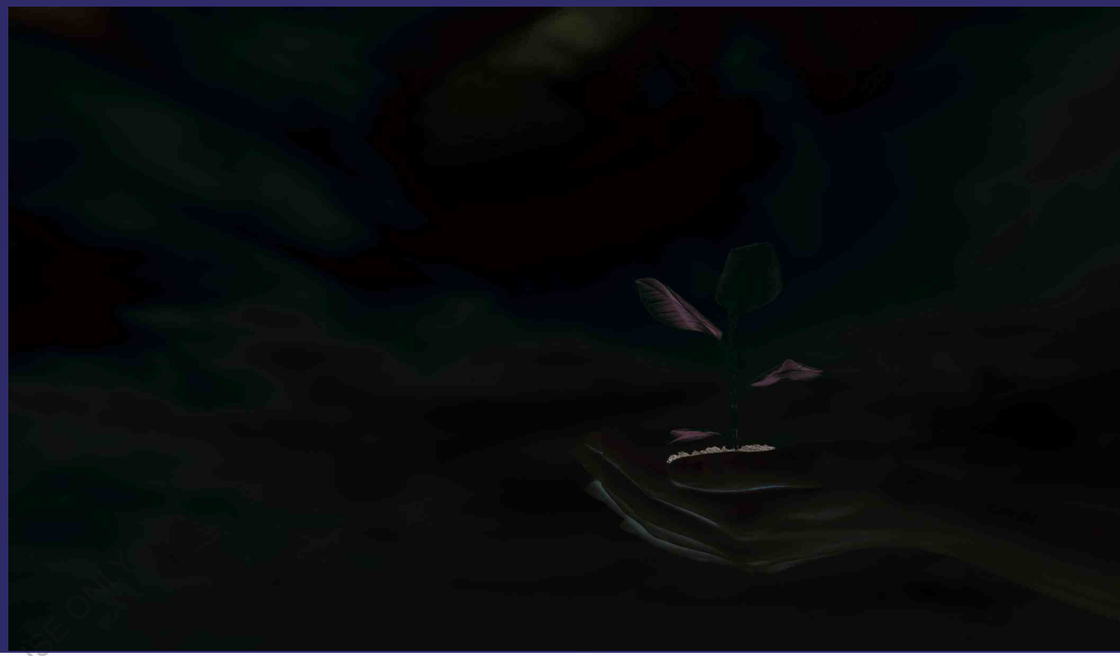

Siti Nuurul Huda Binti Mohammad Azmin
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The Story of Potential Miracle Plant

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Editors

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Contents

Preface

The curry tree (*Murraya koenigii*) is a tropical to sub-tropical tree in the family Rutaceae (the rue family, which includes rue, citrus, and satinwood), which is native to India and Sri Lanka. Its leaves are used in many dishes in India, Sri Lanka, and neighboring countries. Often used in curries, the leaves are generally called by the name 'curry leaf', although they are also literally 'sweet neem leaves' in most Indian languages (as opposed to ordinary neem leaves which are very bitter and in the family Meliaceae, not Rutaceae). Curry leaves have always been sought after for their unique flavor and usefulness in cooking, but there are also a number of health benefits that make them highly appealing.

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Therefore, this book is designed as a quick reference text, with the aim that researchers, students, academicians with little experience in curry leaf plant are able to grasp their understanding of the scientific aspects of the curry leaf plant. This book will also be of significant interest to those working or doing research in the applied sciences.

Siti Nuurul Huda Mohammad Azmin

Huck Ywih Ch'ng

1.0 Background of Curry leaf

Huck Ywih Ch'ng

Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan

The curry leaf tree is native to India, Sri Lanka, Bangladesh and the Andaman Islands. Curry leaf was later spread by Indian migrants, and they now grow in other areas of the world where Indian immigrants settled. Curry leaf is widely cultivated, and the leaves are particularly associated with South Indian cuisines. India is frequently known by enormous biodiversity of medicinal plants. Among them, curry leaf (*Murraya koenigii*) has a lots of bioactive principles and it has been proven as the medicinally important plant. However, less or no attention received by the scientist. Curry leaf is proven as the natural medicinal plant (Singh *et al.,* 2014).

1.1 Origins

Curry leaf trees are naturalized in forests and abandoned land throughout the Indian subcontinent except in the higher parts of the Himalayas. From the Ravi River in Pakistan its distribution extends eastwards towards Assam in India and Chittagong in Bangladesh, and southwards to Tamil Nadu in India. The plants spread to Malaysia, South Africa and Reunion Island with South Asian immigrants (Priyanka *et al.,* 2012). The botanical name of the curry leaves is *M. koenigii* (Ajay *et al.,* 2011). As literature exposed the species name commemorates the botanist called Johann Konig. The genus Murray commemorates Swedish physician and botanist Johann Andreas Murray who died in 1791.

1.2 History

The use of curry leaves as a flavoring for vegetables is described in early Tamil literature dating back to the 1st to 4th centuries AD. Curry leaves are still closely associated with South India where the word 'curry' originates from the Tamil 'kari' for spiced sauces (Parrota, 20010). An alternative name for curry leaf throughout India is kari-pattha. Today curry leaves are cultivated in India, Sri Lanka, Southeast Asia, Australia, Pacific Islands, and in Africa as a food flavoring (Bonde *et al.,* 2007; Prajapati *et al.,* 2003).

1.3 Synonym in Indian language

Curry Leaf (English), Karepaku (Andhra Pradesh), Narasingha (Assam); Barsanga, Kartaphulli (Bengal); Gorenimb (Gujrat); Mitha Neem (Himachal Pradesh); Kathnim, Mitha Neem, Kurry Patta (Hindi); Karibeva (Karnataka); Kariveppilei (Kerala); Gandhela, Gandla, Gani (Kumaon); Bhursanga (Orissa); Mahanimb (Sanskrit); Karivempu (Tamilnadu).

1.4 Synonym in other language

Burmese: Pindosine; Danish: Karrry bald; Dutch: Kerriebladeren; English: Curry leaves; French: Feuilles de cury; German: Curryblatter; Indonesian: Daun kari; Italian: Fogli de Cari; Spanish: Hoja.

1.5 Taxonomic status

a. Kingdom - Plantae

b. Sub-kingdom - Tracheobionta

c. Superdivision - Spermatophyta

d. Division - Magnoliophyta

e. Class - Magnoliospida

f. Subclass - Rosidae

g. Order - Sapindales

h. Family - Rutaceae

i. Genus - Murraya J. Koenig ex L

j. Species - *Murraya koenigii* L. Spreng.

1.6 Growing season

Curry Leaf plant to have flowers and vibrant green leaves throughout the spring, summer and in rain fall. The leaves drop off during its' resting period in the winter months. They like full sun, well-drained soil, which should be the dry side and they need fertilizer in the month of summer (Sindhu and Arora, 2012). The fruiting season was observed to continue from the end of June to the end of August, and the July is considered as the peak fruiting season. In India, harvesting of leaves started from 15 months after planting out and collection of leaves repeated in every 2 to 3 months (Adeleke *et al.,* 1997).

1.7 Plant description

The M. koenigii is semi deciduous, unarmed aromatic small spreading shrub or tree with strong woody stem but slender with the stem which is dark green to brownish in color the tree is 4–8.7m (13–31 feet) tall, with a trunk up to 81 cm diameter. The diameter of main stem is about 16cm (Ganesan *et al.,* 2013; Naggapan *et al.,* 2011).

The flowers of curry leaves are small, white fragrant and funnel shaped, regular, pentamerous, stalked, complete, ebracteate, hypogynous, persistent, inferior, green, corolla, polypetalous, androecium, polyandrous, lanceolate, stigma, bright, sticky, style,

short, ovary, inflorescence, a terminal cyme. The diameter of a flower is 1.12cm in the fully opened form. Each cluster bears approximately 60 to 90 flowers at a time, 5-lobed calyx, with 5 petals having length of 5 mm each. There are 10 stamen in small in size, which is approximately 4 mm. They are dorsifixed, arranged into circles, with long superior gynoecium with size of 5 to 6 mm. Curry tree flowers have a sweet fragrance, bisexual with self-pollinated for produce black berries in small size with shiny appearance containing a large visible seed (Handral *et al.,* 2012).

Curry leaves are aromatic in nature. The leaves are shiny and smooth with paler undersides. Leaves are pinnate, exstipulate, having reticulate venation and having ovate lanceolate with an oblique base, with 11-21 leaflets, in which each leaflet is 0.79–1.57 inch long and 0.39–0.79 inch broad. Leaflets are short stalked, alternate, gland dotted and having 0.5 cm long petiole. The leaf margins are irregularly serrate. The yield of a bush is approximately 480 g (Parrota, 2001). The stem of *M. koenigii* is brown to dark green in color, with dots on the bark like small node on it. When the bark is peeled off longitudinally, there is a white color wood underneath. The girth of the main stem is 16 cm, 6 meters in height, and 15 to 40 cm in diameter (Parrota, 2001).

Fruits of the *M. koenigii* occur in cluster form varies in 32 to 80 in number (Prabhu and Tamilanban, 2012). The fruits are in the ovoid form and small in size. The fruits are 1 to 1.2cm in the diameter with a length of 1.4 to 1.6 cm. They are purple black in color after ripening and they are edible. The fruit yields 0.76% of a yellow volatile oil (Sathaye *et al.,* 2012). Curry leaf fruit is 11 mm long and weigh about 445 mg fruits. The weight of pulp is 880 mg and the volume is 895 microliters (Parrota, 2001). The seeds of the *M. koenigii* are poisonous in nature and should not be consumed for any purpose.

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2.0 Pre-processing of Curry leaf Plant

Laila Naher[1,2]

[1]*Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan*

[2]*Institute of Food security and Sustainable Agriculture, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan*

2.1 Introduction

Curry plant (*Murraya koenigii L*) belongs to Rutaceae family which is a popular leaf-spice in food due to its distinct aroma by the presence of volatile compounds and ability to improve digestion (Singh *et al.,* 2014). The curry leaf is a form in small and narrow leaves (Figure 2.1) which little resemble to neem leaves. However, it can be distinguished by length, usually, curry leaf is shorter than neem leave. Curry leaf plant is native to India, Sri Lanka, Bangladesh, and Island of Andaman (Singh *et al.,* 2014). Nevertheless, in Southern Indian and Sri Lanka are extensively used curry leaves since 14 centuries AD (Singh *et al.,* 2014). By the time of progress, it ahead the growing area and using of curry leaf are spared in another area of the world by native curry leaves immigrants. Currently, curry leaves are commercially cultivated in India, Sri Lanka, Bangladesh, Southeast Asia, Australia, and in few countries of African (Singh *et al.,* 2014).

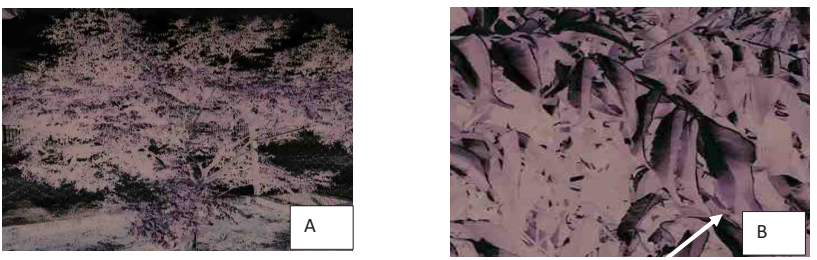

Figure 2.1: Curry leaf plant. 1A, mature plant; 1B, close view leaf of curry plant

2.2 Uses of curry leaves

Curry leaves are in aromatic group plant, and thus it uses as an herb. It mainly used in cooking to provide flavoring in cuisine including fish or meat curries, vegetables, fried rice, soup, pickles, egg omelet, scrambled egg, and curry powder as well. Besides, it used as the cuisine; curry leaves have used for centuries for biological activities in human health (Bhandari, 2012). In the eastern Asian community, curry leaves traditionally used in diet to improve appetite and digestion. As for folk medicine, curry leaf used for the management of diabetes mellitus, application of blades used on bruises, or bites for poisonous animals. The root and stem of curry plant also used for kidney pain disorders. While the stem branch used for gum and clean teeth. However, for use in whatever purpose, pre-processing is the essential element to get the proper function of the herbs which also need to follow in curry leaves as well. Thus, in sub-sequent para in this chapter can find the discussion for pre-processing of curry leaf.

2.3 Pre-processing of curry leaves for cuisine:

For cuisine purpose, most of the time curry leaves used as unprocessed form. Nevertheless, for trading it is required as good quality and quantity which need to process for international trade, profitability and tastiness (Bahat *et al.,* 2013). Harvesting of the plant on optimum time is the main key element to full fill the consumer acceptance.

2.4 Harvesting time of curry leaf

Curry leaf plant is a medium spreading shrub, which about 2.5- 6 meters tall. Generally, the terminal bud cut off when plant reach in 1 meter height and usually 5-6 branches maintain per plant. The first harvesting can be done from 10-12 months old plant and continue to harvesting 5 years onwards. After harvesting of the leaves, it should be keep in room temperature for to pre-cooling the leaves which will help to prolong the self-life during storage. After the pre-cooling the harvested leaves should be wash with clean water and drain the all water, this step can be repeat at least 2-3 times. Finally the leaves now can go further processing based on the using.

2.5 Processing of curry leaf

The using of curry leaf in many ways including food, medicine and folk treatment thus it has mainly three different process as fresh, dried, powder and cooked in Figure 2.2 (Singh et al., 2012).

Figure 2.2: Three main pre-processing of curry leaf

A. Fresh leaf processing

Fresh leaf mainly prefer for cooking as it release aroma in the food. Thus, in the cook fresh leaf can be used just after harvest. However, the leaf can be package in vacuum packed in plastic bags. For technique of vacuum pack, the air form needs to remove from the packaging packet before sealing. The method can be done by manual or automatic. The clean and pre-cooled leaves first need to put in the film and then the air need to removes by pumping vacuum or manual syringe vacuum. The packet can be kept in freezer for 2 months.

B. Drying process of curry leaves

Drying process is one of important element because it influence nutrition, mineral and volatile compounds in the leaf. Mainly fresh Curry leaf used for cosines purpose,

nevertheless, sometimes used dried or dehydrate leaves in case not available the fresh or some recipes require dried leaves instated of fresh leaves. Precise technique can help to dry the leaves without losing its aromatic flavor, nutrient content. Drying methods help to become light weight for transportation and long storage. The first thing for drying method, the plant leaf need to harvest at mid-morning when sunlight is mild and dry weather. After harvest leaf need to keep in room temperature for few minutes and then rinse the leaves with running tap water. The leaf need to dry by using paper towel to remove excess water, then can use the leaf for drying. There are many methods can be used to dry or dehydrate the leaves as stated in Figure 2.3.

Figure 2.3: Methods of drying process of curry leaves

1. Shade

Shade drying is traditional method which has long been used for food staff drying. It is good not use direct sun light for leaf drying. Drying under direct sun light may degrade useful molecule in leaf. Dry the leaf under shade is good practice for processing of curry leaf. Usually shade drying should be carried out for 10-15 days at temperature 22°C-27°C. After drying vacuum packaging can be used for long storage at 4-10 °C.

2. Hot air

Hot air means the air provide from an electric device named hot air oven. This is very common method in food industry (Mazandarani *et al.,* 2014). Generally in oven dry heat operate from 50°C-300°C (122°F to 527°F), however the capacity of air to remove moisture from the food staff depends on temperature. The leaf need to put on the plate then heat will transfer to surface of the plate to leads the vapor for vaporization of the water from the leaves and drying process continue with uniformly that means removing and absorbing of moisture with precisely in leaf (Mazandarani *et al.,* 2014).

3. Microwave

Microwave is one of the modern technology to use drying process of agriculture products. Microwaves are electromagnetic wave consist with beams which need high frequency electricity like 2450 MHZ frequency (Mazandarani *et al.,* 2014; Zhang *et al,* 2006 Aghajani, 2006; Seiiedlou *et al,* 2010). When food texture passing through microwave the polar molecules such as water and salt continuously vibrating and this phenomenon results into heat production in foodstuffs to absorb the moisture and dry the foodstuff including leaf.

4. Combo (Oven Wall and Microwave)

Combo is the combination of oven wall and microwave. It has been design the browning on the outside of food stuff by oven wall and cooking by microwave. Thus, combo technique can be used for leaves drying where browning on the outside will be carried out by oven wall heat circulation and moisture of water will absorbed by microwaves at 100 ºC for 5 minutes. In same microwave can function for microwave and combo dry.

5. Inert gas

An inert gas is a material which does not undergo chemical reactions under a set of given conditions. The noble gases and nitrogen often do not react with many substances. Inert gases are used generally to avoid unwanted chemical reactions degrading a sample. These undesirable chemical reactions are often oxidation and hydrolysis with the oxygen and moisture in air. The term inert gas is context dependent because nitrogen gas and several of the noble gases can be made to react under certain conditions. The Inert gas dryer (Model: Bry-Air FFB-170, Alpha, Chennai), inert gas (nitrogen) circulation, capacity; 10kg wet product per batch, tray size: 20 x 20 x 1.75 (l x w x h, inches) optimized at 100ºC, 2 hours. The leaves can be kept for six months using inert gas packaging.

6. Freeze

Freeze drying is one of old and cosmopolitan method for preserving leaf for longer shelf life. Freeze drying called lyophilization or cryodesiccation which is the process to of removing water from the product/food stuff like leaf. Thus the physical sate of the leaf will be changed when energy will be taken out through cooling freezing temperature generally -18°C to below (Fennema *et al*., 1973). The cooling temperature retards growth of microorganism and slow the chemical function in leaf that cause to spoil and reduce quality. The leaves can be store for one year in freeze temperature.

C. Processing of powder

Instead of curry leaves sometimes used curry leaves powder as ingredient in food. Nevertheless, curry leaves powder confused with curry mix powder, actually curry mix powder is the curry powder mix with other species powder. For processing of curry leaves powder, sun/solar/shade dried leaves can be transformed in powder, however, in cabinet drier at 50°C for 3 hours is best timing for drying the curry leaves and also in this treated leaves can be easily do powder form (Figure 2.4). In literature found that nutritional value was higher in processed curry leaves powder then fresh curry leaves

(http://agritech.tnau.ac.in/postharvest/). The powder can keep in air tide pack until two months at room temperature and for six month in freezer.at 10-12°C.

Figure 2.4: Curry leaves (A) and powder form of curry leaves (B). Source Dr. P. Vennila, Professor (Food Science and Nutrition TNAU, Coimbatore)

2.6 Conclusion

Curry leaf plant is a leafy herbal plant which is useful in various sector. The various notable use of curry leaf as ingredient in food spice, pharmaceutical activity and also for cosmetic purpose. Since curry leaf has both as culinary and pharmaceutical using thus it is potential to enhance entrepreneur to local people for trade. The knowledge of pre-processing can be value added to the local community for development of small trade and also will be act in functional food.

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3.0 Review on Extraction Techniques of *Murraya koenigii*

Norhafizah Md. Zain[1,2]

[1]*Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan*

[2]*Institute of Food security and Sustainable Agriculture, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan*

3.1 Introduction

Currently, extraction and isolation of plant metabolites for medicinal, nutraceutical properties, cosmetic uses and insecticide purpose has become main focus of bioactive compounds research. Generally, selective solvents used in the extraction process to separate the active plant tissues from the inactive constituents (Amita and Shalini, 2014), so various solvents with different polarities must be used to extract different active compounds from plants with a high degree of accuracy (Wong and Kitts, 2006). There are two groups of extraction techniques called conventional and green techniques. Conventional extraction was done by using cheaper equipment with high amount of solvent and extraction time is long, meanwhile green techniques uses elevated pressure and/or temperatures, costly equipment with short extracting time. Next after secondary metabolites extraction, a step of purification and isolation are required using chromatographic or non-chromatographic techniques.

Most commonly used herbs for extraction are oregano, pepper, chamomile, neem, tulsi, turmeric, cinnamon, cardamom, curry leaves and saffron. Plant based natural compounds can be drawn from plant parts like flowers, stems, bark, roots, leaves, fruits and seeds (Ammar *et al*., 2017). Curry leaves, bark, root and fruits of this plant has higher constituent of secondary metabolites where, the leaves of curry tree (*Murraya koenigii)* contain proteins, carbohydrate, fibre, minerals, carotene, nicotinic acid, Vitamin C, Vitamin A,

calcium and oxalic acid. It has been reported that this plant fruits, leaves and roots has Carbazole alkaloids which has shown tremendous medical benefits such as anticancer, antidiabetic, antibacterial, antioxidant and anti-nociceptive activities (Prasan, 2019). Carbazole alkaloids are murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline and xynthyletin.

3.2 Extraction and solvents selection

Extraction of raw materials is the first crucial step to separate the desired natural compound from other insoluble compounds in the plant. Extraction is difficult because secondary metabolites are often bound to or dissolved in other compounds in the plant (Amita and Shalini, 2014). The extraction method is the most important step in the study of phytochemical activity in *M. koenigii* because it affects the feasibility, quality and quantity of the products (Table 3.1).

Table 3.1: Domain questions for extraction process
(Source: Amita and Shalini, 2014).

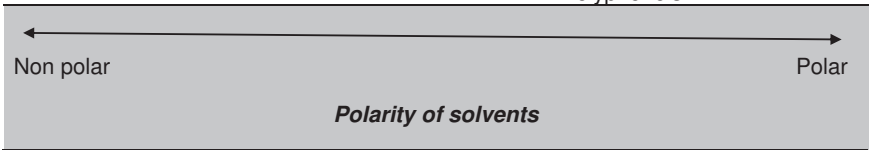
Extraction methods	Extract quality	Phytochemical	Secondary metabolite composition
• Extraction period • Solvent • pH of the solvent • Temperature • Particle size of plant tissues • Solvent-to-sample ratio	• Plant material • Solvent • Extraction procedure	• The nature of the plant material • Degree of processing • Moisture content • Particle size	• Type of extraction • Time of extraction • Temperature • Nature of solvent • Solvent concentration • Polarity

The type of solvent used in the extraction is the key success to detect and obtain desired biologically active compounds from plant material of *M. koenigii*. In order to succeed in the isolation of phytochemical, the solvent must dissolve all metabolites of interest with little or zero contaminants (Simon *et al*., 2015). Acidic and neutral compounds can be extracted by using acetone, methanol or water. Water is used as extractant to determine active compounds concentrations available to other plants in soil, and to determine phenolics content, organic solvents commonly used (Khoddami *et al*., 2013). Solvents such as water, ethanol, chloroform, ethyl acetate and methanol are frequently used in allelopathic studies. Phenolic compounds represent the primary allelopathic agents in weeds and other plants (Table 3.2).

Table 3.2: Solvents used for active component extraction

(Source: Amita and Shalini, 2014).

Ether	Chloroform	Acetone	Ethanol	Methanol	Water
Alkaloids	Terpenoids	Phenol	Tannins	Anthocyanins	Anthocyanins
Terpenoids	Flavonoids	Flavonols	Polyphenols	Terpenoids	Starches
Coumarins			Polyacetylenes	Saponins	Tannins
Fatty acids			Flavonols	Tannins	Saponins
			Terpenoids	Xanthoxyllines	Terpenoids
			Sterols	Totarol	Polypeptides
			Alkaloids	Quassinoids	Lectins
				Lactones	
				Flavones	
				Phenones	
				Polyphenols	



3.3 Plant materials

Natural compounds can be derived from any part of the plant like leaves, bark, roots, flowers, fruits, stems and seeds that contain active constituents. The preparation of plant sample is crucial to preserve the biomolecules in the plants prior to extraction. The preservation of phytochemicals in the final extracts depends on the grinding and drying process of the plant materials (Azwanida, 2015).

Plant tissue homogenization in solvent allows the compound to be separated from the plants efficiently. Fresh plant parts of *M. koenigii* are washed with clean tap water to remove dust particles. The air dried leaves are powdered to fine particles. Solvent is poured into conical flasks that previously filled with *M. koenigii* powder and then shook continuously for 24h at 200 rpm at 25 ºC on an orbital shaker (Yehia, 2016). The supernatants obtained are filtered using a double layer of Muslin cloth and the filtrate is dried under lower pressure or re-dissolved in the solvent to find out the phytoactive compounds present in the plant (Figure 3.1).

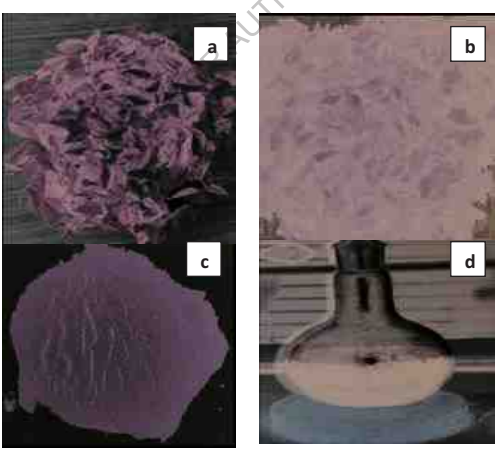


Figure 3.1: Fresh (a), dried (b), powder (c) and methanol extract (d) of *M. koenigii* (Source: Manisha et al., 2018).

3.4 Extraction methods

Phytochemicals study begins with both the pre-extraction and extraction procedures to process bioactive constituents from plant parts, where two existing extraction method such as conventional and novel method has been commonly adopted. Conventional extraction methods such as percolation, maceration and soxhlet extraction methods are prominently used in phytochemical screening studies. Significant advances have been practiced in processing medicinal plants such as microwave assisted (MAE), supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE) and accelerated solvent extraction (Azwanida, 2015). A brief summary of the other various extraction methods used for medicinal plants including *M. koenigii* is shown in Table 3.3.

Table 3.3: Extraction methods for plant or natural products (Source: Zhang *et al*., 2018).

Method	Solvent	Temperature	Pressure	Time	Volume of organic solvent used	Polarity of natural products
Maceration	Water, aqueous & non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Depend on the solvent
Percolation	Water, aqueous & non-aqueous solvents	Room temperature/ under heat	Atmospheric	Long	Large	Depend on the solvent
Decoction	Water	Under heat	Atmospheric	Medium	None	Polar compounds
Reflux extraction	Aqueous & non-aqueous solvents	Under heat	Atmospheric	Medium	Medium	Depend on the solvent
Soxhlet extraction	Organic solvents	Under heat	Atmospheric	Long	Medium	Depend on the solvent
Pressurized liquid extraction	Water, aqueous & non-aqueous solvents	Under heat	High	Short	Small	Depend on the solvent
Supercritical fluid extraction	Supercritical fluid (usually S-CO_2)/ modifier	Near room temperature	High	Short	None or small	Nonpolar to moderate polar compound
Microwave assisted extraction	Water, aqueous & non-aqueous solvents	Room temperature	Atmospheric	Short	None/ Medium	Depend on the solvent
Ultrasound assisted extraction	Water, aqueous & non-aqueous solvents	Room temperature/ under heat	Atmospheric	Short	Medium	Depend on the solvent

Pulsed electric field extraction	Water, aqueous & non-aqueous solvents	Room temperature/ under heat	Atmospheric	Short	Medium	Depend on the solvent
Hydro distillation and steam distillation	Water	Under heat	Atmospheric	Long	None	Essential oil (usually non-polar)
Enzyme assisted extraction	Water, aqueous & non-aqueous solvents	Room temperature/ heated after enzyme treatment	Atmospheric	Medium	Medium	Depend on the solvent

3.5 Conclusion

Plant materials are very complex and it has many different tissues that serve different purposes. Hence, proper plant part, solvents and extraction method selection requires more attention to ensure repeatable and efficient extraction. Till now, conventional extraction methods are extensively practiced and new extraction methods is increasing sturdily. New extraction method often offers lower environmental impact, better extraction efficiency, simpler and shorter in time. A reliable and continuous yield through standard extraction process is needed for consistent chemical profile and biological activity for quality assurance in production and manufacturing of *M. koenigii* herbal formulations.

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4.0 Physico-chemical Properties of Curry leaf

Siti Nuurul Huda Mohammad Azmin[1]

Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan

4.1 Introduction

The interaction of a compound with its physical environment indicates the physicochemical properties of plant materials. Chemical structure of each compound in a plant determines the physicochemical properties which influence the biological activities of the plant. As an example, the sweetness of fruit comes from the reaction of enzyme in mouth with the polysaccharides (starch) in the fructose and other shorter water-soluble molecules. Thus, this topic explains on the physicochemical properties of curry leaves and its plant which has important uses in traditional system of medicine in eastern Asia (Giday *et al.,* 2009).

4.2 Ethnobotanical uses of curry leaf

Figure 4.1 shows the morphology of curry leaves including the whole plant, flowers, leaves, fruits and bark. Each part of this plant has its own benefits. Antimicrobial, antioxidant, anticancer and renal protective effects of the plant had been reported (Ghasemzadeh *et al., 2014;* Gill and Sharma, *2014;* Punuru *et al., 2014;* Rajendran *et al., 2014;* Shekar *et al.,* 2016). They contain several medicinal properties such as anti-diabetic, antioxidant, antimicrobial, anti-fungal, anti-inflammatory, anticarcinogenic and hepatoprotective properties (Al Harbi *et al.,* 2016).

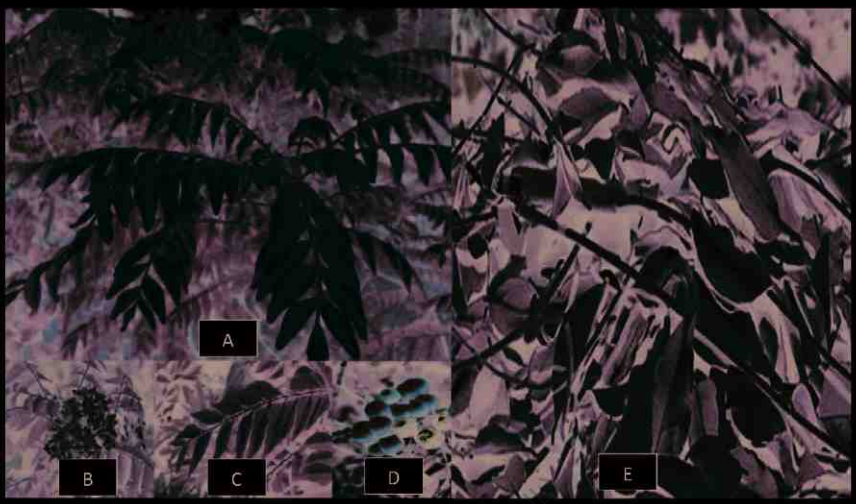

Figure 4.1: Morphology of curry leaves (A- Whole plant, B- Flowers, C- Leaves, D- Fruits, E-bark) (Source: Akula et al., 2016; Kumari, 2018)

Curry leaves have slightly pungent, bitter and feebly acidic taste. It retains the flavour and other qualities even after drying process. Curry leaves are a popular leaf-spice and used in very small quantities for their distinct aroma due to the presence of volatile oil and their ability to improve digestion (Azad et al., 2016; Singh et al., 2014). Leaves are widely used in Indian cookery for flavouring foodstuffs (Singh et al., 2014). It is also the sources of Vitamin A, Vitamin B, Vitamin C, Vitamin B2, Calcium and iron. Curry leaves are beneficial in for women who suffer from calcium deficiency such as osteoporosis, where an ideal natural calcium supplement can be found in curry leaves. Traditionally, it is used as antiemetic, antidiarrheal, dysentery, febrifuge, blood purifier, tonic, stomachic, antipyretic, antidiabetic, anti-obesity, flavouring agent in curries and chutneys. Besides, the oil has been applied externally for bruises, eruption, in soap and perfume industry (Gahlawat et al., 2014; Jain et al., 2012).

The fruits are known to have high nutritional values with many medicinal properties. The fruit of this plant also contains antioxidant and can trigger lipid-lowering, antidiabetic and

analgesic activities. Branches of the plant have been employed to strengthen gums and teeth (Akula *et al.,* 2016). Besides, the branches have also been used as an antiperiodic. The seed of the plant is poisonous and is not used for the culinary purpose (Trimen and Alston, 1893).

4.3 Research on physicho-chemical properties of curry leaves

The dried leaves powder has been mixed with honey and juice of betel nut was recommended in the Ayurveda of medicine (Mhaskar et al., 2000). Table 4.1 lists the other benefits of curry leave parts as found by Kumari (2018).

Table 4.1: Various Ethnobotanical uses of *curry leaves*

(Source: Kumari, 2018)

Part of plant	**Benefits**
Bark and roots	• Cure eruptions and bites of poisonous animals.
Juice of root	• Cure kidney pain and preventing the premature greying of hair.
Green Leaves	• Cure dysentery and diarrhoea • Infusion of the washed leaves stops vomiting • Blood purifier and tonic.
Fresh juice of curry leaves, with lime juice and sugar	• Treatment of morning sickness, nausea and indigestion. • Treatment of renal diseases • Dropped into the eyes for the prevention of cataracts.
Leaves and roots	• Curing piles, inflammation, itching • useful in leukoderma and blood disorders.
Tea of curry leaves	• Treat fever.
A paste made of curry leaves	• Applied on a burn, bruises, boils and skin eruption • Applied on gums to avoid Pieria
A paste made of leaves of *M. koenigii* and *Psidium guajava* with flowers of *Catharanthus roseus*	• Maintain sugar level
Fresh leaves chewed	• Reduce the body weight gain

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5.0 Curry Leaf as Potential Antimicrobial Agents

Khomaizon A.K. Pahirulzaman

Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan

5.1 Introduction

Out of more than 300,000 plant species on the earth, there are only about 2 percent of plants were analysed for their antimicrobial properties. The plants, which have potential antimicrobial properties, are more than 157 plant families. It considered as natural antimicrobial agents, a chemical compound or substance that derived from natural sources. They are effective against a range of yeast, molds and bacterial such as *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*. For centuries, the plant extracts have been used in disease treatment, pharmaceutical, foods, detergent, cosmetics and traditional medicine. Interest towards screening of plant extract for discovery of new effective compounds that potentially is used in treatment of bacterial infection. Although effectiveness of natural antimicrobial agents against microorganism generally lower than that of synthetic or artificial antimicrobial agents, these natural antimicrobial agents have little or no harmful effects due to their natural origin. They are safer to use compare to chemical or artificial preservatives.

Curry leaves are among the well-known plant-derived natural antimicrobial agents that possess antimicrobial activity and have been used as preservative. The antimicrobial effects are different and not always specific throughout the plants, as some may act at microbial cell wall while others may focus on cytoplasmic membrane. The efficacy of plants extract as antimicrobial agent also varies from one plant to another. The extracts were prepared from fresh or dried plant material using various extraction methods such as maceration, Soxhlet extraction or percolation. By using suitable solvents with similar

polarity, compounds inside the plant material will be solubilized, and finally concentrated to get the plant active compounds. These active compounds are mostly secondary metabolites such as terpenoids, alkaloids, phenolics, and so on (Figure 5.1).

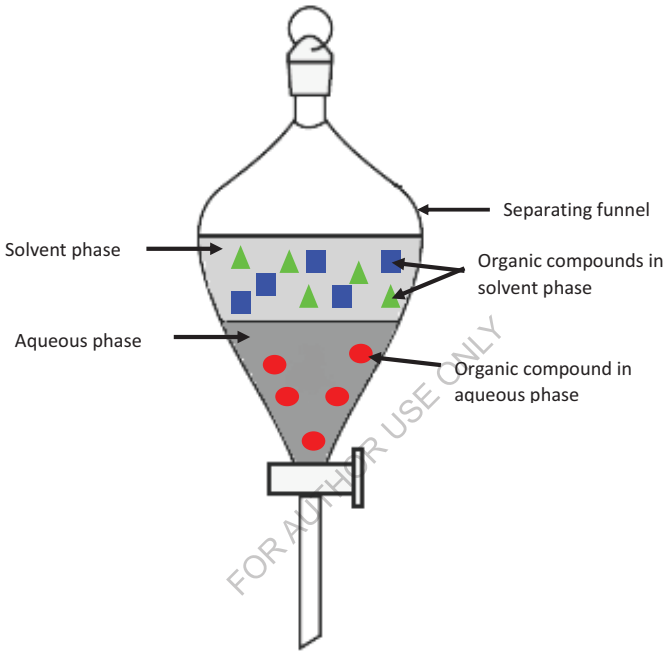

Figure 5.1: Method to separate components (compounds) of a mixture, using a separating funnel. The success of this method is depends upon the difference in solubility of a compound in various solvents

5.2 Antimicrobial assay

Antimicrobial assay is the measurement of the activity of compounds and its relative potency by determining the amount required to produce a specific, defined effect on a test microorganism such as bacteria, fungi and yeasts under standard conditions. At low concentrations, the antimicrobial compounds extracted from plants exert an action and

exhibit therapeutic toxicity towards the microorganisms. These substances that are from nature or chemically synthesize can be used to kill the test microorganisms. The antimicrobial activity of plant extracts can be assessed by several methods, such as the disc diffusion method and agar plug method (Table 5.1).

The disc diffusion method is used to test the sensitivity/resistant of the test microorganisms based on the diameter of the inhibitory zone of cultural growth. It provides a simple and reliable test in routine clinical bacteriology in order to find out the effect of particular substances on specific bacterium. The plant extracts will be placed in contact with the test microorganism. Impregnating a 6 mm filter paper disc with a known concentration of plant extract does this. The disc is then placed on solid growth medium containing test microorganism inoculum. The plant extract will diffuse into the surrounding agar and, inhibitory area will be observed throughout the incubation period. This method is widely used in many laboratories due to the low cost, easy result interpretation and can be used to test on various microorganisms.

The rate of plant extract diffuse into agar is also much faster in disc compared to agar plug. Other than that, the molecular weight of the plant extract also affect the rate of diffusion, in which, larger molecules diffused at lower rate compared to the smaller molecules. Growth media preparation should be standardized in order to minimize the error because the depth of the agar influences the inhibitory zone; a shallow layer agar will produce larger inhibitory zone than a deeper layer.

Table 5.1: Methods for antimicrobial activity.

Assay Method	Procedure, principle, advantages and disadvantages
Disc diffusion	**Procedure:** Agar plates are inoculated with test microorganism. Plant extract is then impregnated on sterile paper disc, before placed onto the inoculated agar surface. Plates are incubated at suitable conditions. Diameters of inhibition zones are measured. **Principle:** antimicrobial agent diffuses into the agar and inhibit the growth of the test microorganism. **Advantages:** Simple and inexpensive method. Easy to interpret the result. **Disadvantages:** Not suitable in determining the minimum inhibitory concentration (MIC)
Agar well diffusion	**Procedure:** Agar plates are inoculated with test microorganism. Using a cork borer, a hole (6 – 8 mm diameter) is aseptically punched on the inoculated agar plates. Plant extract at desired concentration is added into the well. Plates are incubated at suitable conditions. Diameters of inhibition zones are measured. **Principle:** Antimicrobial agent diffuses into the agar and inhibit the growth of the test microorganism. **Advantages:** Simple and inexpensive method. Easy to interpret the result. MIC can be calculated. **Disadvantages:** Some molecules in plant extract are not diffused into agar media.
Agar plug diffusion	**Procedure:** Agar plates are inoculated with test microorganism. Desired microbial strain is grow as a lawn. Using a cork borer, an agar plug is aseptically cut from the lawn and placed on the agar surface of the test microorganism. Plates are incubated at suitable conditions. Diameters of inhibition zones are measured. **Principle:** Based on antagonistic properties of microorganism. Antimicrobial agent diffuses from the agar plug and inhibit the growth of test microorganism.

	Advantages: Simple and inexpensive method. **Disadvantages:** Some molecules in plant extract are not diffused into agar media.

5.3 Antibacterial assay on curry leaf extract

Antimicrobial activity of curry leaves extract was determined by disc diffusion method that consists of impregnating a filter paper disc with various concentration of substance. The discs were placed on plates containing bacterial inoculum to be tested. The assay plates were subjected to incubation and, the degree of sensitivity was determined by measuring the inhibition zone produced by the diffusion of the active compound in the plant extract from the disc into the surrounding medium. In this study, curry leaves were extracted with ethanol using Soxhlet for 10 hours. The ethanol was evaporated at 45°C for 30 minutes to yield the crude extract. Gram-negative *E. coli* and Gram-positive *S. aureus* were used as test microorganisms on the crude curry leaves extract. Both are commonly used due to the speedy growth and easy to handle. *E. coli* can be pathogenic and cause various diseases like vomiting and diarrhea, whereas *S. aureus* is known to cause food poisoning and skin infections.

A single colony of test microorganism was inoculated into Tryptic Soy Broth (TSB) and incubated for 24 hours at 37 °C. The inoculum was then spread onto Tryptic Soy Agar (TSA) plates using a sterile swab. 10 µL of the 5 mg/mL and 10 mg/mL curry leaves extract was pipetted onto a 6 mm diameter sterile disc. The disc containing the extract was air-dried before placed onto the inoculated plates. The discs were let to adhere well on surface of the agar for few minutes before incubated at 37 °C for 24 hours. Trimethoprim (50µg/mL) and sterile distilled water were used as positive and negative controls, respectively. The diameter of inhibitory zones surrounded the discs were measured and recorded. All tests were carried out in triplicate and mean value was calculated.

Results show that curry leaves extract at concentration of 10 mg/mL inhibit the growth of both *E. coli* and *S. aureus* with inhibitory zones of 3.2 mm and 5.9 mm respectively (Table 5.2). The inhibitory zone on *S. aureus* assay plate showed larger than on *E. coli* assay plate indicating that the bacteria are more sensitive to the curry leaves extract in the disc (Figure 5.2). Previous research also reported similar results (Hanan et al. (2016) and Prathyusha et al. (2016)). However, no inhibition zones were observed on both *E. coli* and *S. aureus* assay plates when tested with a lower concentration of curry leaves extract (5 mg/mL). This may due to the lower concentration of plant extract used.

Table 5.2: Average inhibition zone (mm) of curry leaves extract against test microorganisms.

Test Microorganism	Concentration of *curry* leaves extract		Trimethoprim	Negative control
	5 mg/mL	10 mg/mL		
E. coli	-	3.20	18.50	-
S. aureus	-	5.90	17.55	-

Note: (-) indicates no zone of inhibition.

Figure 5.2: Inhibition zone (red arrow) showed by different concentration of curry leaves extract against *E. coli* (left) and *S. aureus* (right)

The common secondary metabolites in plants include alkaloid, flavonoids, terpenoids and many more. Previous research indicates that curry leaves contain carbozole alkaloids, a secondary metabolite with antibacterial activity against a range of pathogenic bacteria. It is also reported that the curry leaves extract inhibits the growth of *Pseudomonas aeroginosa*, *C. albicans*, *Streptococcus gordonii* and *Streptococcus mutans*.

The variation in the antimicrobial activity of curry leaves extracts may due to the climatic difference of the plants origin itself. The medicinal value of curry leaves is due to the active constituents. It is generally advantages to extract the active constituents to formulate a control doses form of that active constituents rather than using the bulk quantity. Extraction method and solvent used are also contribute to the difference in the results obtained. For example, using ethanol as solvent results a greater inhibitory zones compared with using water. This shows that, ethanol proved to have more activity against the test microorganism used than water. Polarity of the solvent may affect the solubility of the extracted compounds. Only the active, desired constituents should be extracted from the plant material, which means that a high selectivity is required in selecting the best solvent.

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6.0 Statistical Analysis for Curry Leaf Related Research

Jeng Young Liew[1] and Leony Tham Yew Seng[1,2]

[1]Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan

[2]Institute of Food security and Sustainable Agriculture, Universiti Malaysia Kelantan, 17600, Jeli, Kelantan

6.1 Introduction

To many, statistics is no doubt anathema (Petrie and Watson, 2006). Many of us think that the concepts of statistics are hard to grasp. It is, nevertheless, undisputedly a crucial tool for each and every of us, especially those involved in disciplines such as herbal medicine, business studies, economics, and engineering. In the literature of herbal medicine, introductory text on statistics analysis are relatively limited. This book chapter is developed to give guide to statistics/ statistical analysis related to the study of herbal medicine.

In general, statistics is a discipline that deals with data collection, data organization, data displaying, data analysis, data interpretation and lastly, data validation. Statistical data analysis is defined as the systematic procedures of carrying out statistical operations. It is in fact, a type of quantitative research, which aims to quantify the data, and in most cases, employs several forms of statistical analysis. By layman's term, the statistical data analysis helps us to find significant meaning to the meaningless numbers. To wit, it is about the process of turning raw data into data structures that is used in generating us useful and cogent information. Descriptive data, for example survey data and observational data belongs to quantitative data. Data analysts may compute descriptive measures, such as means and standard deviation, etc. for descriptive purposes. Standard deviation is the variability within a data set around the mean.

Having an improper understanding of statistical knowledge, one cannot perform the statistical data analysis as it involves some form of statistical tools. Over the years, a variety of software packages are suggested to help us to conduct statistical data analysis. Statistical Analysis System (SAS), Statistical Package for the Social Sciences (SPSS), Minitab, JMP, Stata, to mention a few, are some of the popular software packages that have achieved widespread applications in most key sectors in today's world.

In regards to statistical data analysis, data is usually made up of variable(s) and herbal data makes no exception. Data can either be univariate or multivariate in most real-world situation. Different statistical methods need to be performed by data analysts, and this is subjected to the number of variables present in the data. Note that univariate calls for the analysis of a single variable, or commonly known as univariate statistical analysis that includes t test for significance, analysis of variance (ANOVA) and so on. On the other hand, multivariate analysis involves at least two different variables in an analysis. Therefore, multivariate statistical analysis, such as multivariate analysis of covariance (MANCOVA), multivariate analysis of variance (MANOVA), multivariate regression etc. must be carried out. Since multivariate analysis is typically dealing with techniques with the greater complexity, presenting statistical results in an understandable and systematic format is of particular importance. Tables and charts, for example pie charts, line charts, scatter plots are especially useful in helping decision makers to understand the statistical results.

There are two kinds of data in the statistical data analysis. This includes continuous data and discrete data. The continuous data belongs to data that cannot be counted, rather it can take any value within a range. For instance, weight of an herbs flower can be measured but is unable to be counted. The discrete data, on the other hand, is the one that can only take certain value. This implies that discrete data is countable. For example, the number of herb plants in an herb garden. We can count the number of herb plants. However, we cannot have half an herb plant.

In statistical data analysis, the continuous data is dealing with continuous distribution function, in which it is named as the probability density function. Meanwhile, the discrete data in statistical data analysis falls under the discrete distribution function. Discrete distribution function is also called the probability mass function. Poisson distribution, binomial distribution are examples of discrete distribution function while normal distribution, F-distribution are categorized under the probability density function.

Statistical inference is of particular importance in statistical data analysis. It helps data analyst to analyse the data properly. Statistical inference refers to a process of drawing conclusions about an underlying population based on a sample or subset of the data. Statistical inference is employed in everything from science and academic research to business and economic. The statistical inference consists of two areas, namely the (1) estimation and (2) tests of hypothesis. The goal of estimation is to describe an unknown aspect of population. It is concerned with the process of making estimation based on a value or range of values for a certain population parameter. Tests of hypothesis deals with the process of either rejecting or accepting a hypothesis that has been formed about the parameter. This is performed by computing a test statistic in accordance to the sample(s) from that population and testing it against an ideal standard value. Typically, a risk of a making wrong conclusion is established in advanced. This is referred as Type I error, or false positive, the probability of rejecting the original hypothesis when it is a correct one. Here, the basic hypothesis about the parameter is known as the null hypothesis (H_0) and an alternative hypothesis (H_1) is also established at concurrently.

6.2 Steps in statistical data analysis

There are a few imperative steps involved in the journey of statistical data analysis including those herbal data. The steps are listed in the following:

Step 1: Decide on the objectives

Step 2: What to Measure and How to Measures

Step 3: Data Collection

Step 4: Data Cleaning

Step 5: Summarizing Data

Step 6: Data Analysis and Modelling

Step 7: Optimization and Repetition

Step 1: Decide on the objectives

Setting up objectives is the first step of the statistical data analysis pipeline. Significant data collection and analysis are deemed indispensable in achieving these objectives.

Step 2: What to Measure and How to Measures

Measurement is a process of allocating numbers to show distinctive value of variables. To be more detailed, if your research is to determine whether there is a correlation between weight and height of a herb, says curry leaves, it would be make sense to measure the height and weight of the herb using a scale, thread and ruler.

Step 3: Data Collection

The types of data involved in your statistical study need to be fixed in this step. There are two data sources, namely primary and secondary data. Primary data are collected by the investigator oneself who conducting the research. Secondary data in contrast, are data which is collected by someone who is someone other than the user. Note that it is vital for you to know where and how the data was obtained if you opt for secondary data as this will enable you to perform a proper data analysis. It is worthy to highlight here that having sufficient data will help you to achieve a better correlations while building better model for your study.

Step 4: Data Cleaning

Unwanted data or better known as junk data may deteriorate your work by causing you to generate inappropriate results. Missing values are among the junk data. Data cleaning is an essential step to enhance data quality. You need to handle missing values and get rid of useless information if in need.

Step 5: Summarizing and Visualizing Data

Summarizing and visualizing data help us to understand the data better. This can be accomplished by performing the descriptive statistical analysis. Of note, visual display like pie charts, etc. is one of the useful tools in the descriptive statistical analysis. Using visual display as a means of describing data surely helps us to have a reasonable “picture’ of the data. Quite often, one will supplement the visual display with the suitable numerical measures including the measure of location and measure of dispersion.

Step 6: Data Analysis and Modelling

Advanced statistical operations should be performed to examine patterns in data at this step. In the nutshell, whether there is an association between variables, or if certain groups are more likely to display particular attributes are the common questions that should be addressed when performing data analysis. Next, forming a model which correlates the data with your outcomes are the key to success. It helps you to predict your outcomes.

Step 7: Optimization and Repetition

The statistical data analysis is a repeatable process. Repetition and optimization leads to continuous improvements to the data value chain itself.

6.3 Selecting a test statistic

A few important factors must be taken into account when selecting a certain test statistics to be used in the statistical data analysis procedure (Awang, 2012). These factors are listed as follows:

1. The number of groups in a study. Are your study dealing with only a group, two groups or beyond two groups.
2. Independency. If your study deals with more than a single group, are they independent?
3. Types of data involved. There are four scales of measurement: nominal, ordinal, interval or ratio.

Choosing the right statistical test is of particular importance in analysing the data. Some common statistical tests used in statistical data analysis for herbal data is given in Table 6.1.

Table 6.1: Common statistical tests used in statistical data analysis

Name of Test	Explanation	Test Type
Pearson's Correlation	Test for the strength of the association between two continuous variables	**Correlational Test** Determining an association between variables
Paired T-test/Dependent sample T-test	A statistical procedure used to check whether two samples are statistically different from each other	**Mean Comparisons** Examining the difference between the means of variables
Independent T-test	A statistical procedure used to check for difference between two independent variables	
One-way ANOVA	A hypothesis-based statistical test that compares variance in the group means within a sample by involving only an independent variable (called factor)	

Two-way ANOVA	Like one-way ANOVA, two-way ANOVA is a hypothesis-based statistical test that helps one to test the difference between group means in which two independent variables (called factor) are considered	
MANOVA	Test for the difference in at least two vectors of means	
Simple Regression	Test how change in the predictor variable predicts the level of change in the outcome variable	**Regression** A statistical technique that attempts to explore and model the relationship between two or more variables.
Multiple Regression	Test how change in the combination of two or more predictor variables predict the level of change in the outcome variable	
Wilcoxon rank-sum test	A statistical tests for difference between two independent variables - takes into account magnitude and direction of difference	**Non-parametric** Non-parametric tests are considered when the data fail to meet the assumptions required for those parametric tests.
Wilcoxon signed-rank test	Tests for difference between two related variables -takes into account magnitude and direction of difference	
Sign test	Tests if two related variables are different –only takes into account direction but the magnitude of change is ignored	
Spearman Correlation	It is a non-parametric test used to measure the strength and direction of the association between two ordinal variables (assumption of normal distributed data is unimportant here)	
Chi-square	Tests for relationship between categorical variables	

6.4 Statistical analysis for curry leaves: A review

Curry leaves are useful as natural preservatives to increase the shelf-life of products. Najeeb *et al.* (2015) applied curry leaves to extend the shelf-life of meat products. They used the general 7 steps in statistical data analysis to determine the achievement of their primary objective, as shown in Table 6.2:

Table 6.2: 7 steps statistical data analysis used in a study by Najeeb *at al.* (2015) involving curry leaves

Step 1	Decide on the objectives	This study was to determine the efficacy of curry leaves powder as natural preservatives in extending the shelf life of the chicken meat in terms of physico-chemical, microbiological and sensory attributes of the chicken meat		
Step 2	What to Measure and How to Measures	Physico-chemical cooking yield (unit in %)	Microbiological standard plate count (unit in CFU/g)	Sensory evaluation appearance, flavour, juiciness, texture and overall acceptability (without unit)
Step 3	Data Collection	Weigh of restructured meat blocks recorded before and after	Yeast and mould counts for 1st, 6th, 10thm 14th, 17th, 20th storage days	10 panels preference
Step 4	Data Cleaning	Not available	Not available	Not available
Step 5	Summarizing Data	Table, treatments vs cooking yield	Table, treatments vs storage days	Table, treatments vs preferences
Step 6	Data Analysis and Modelling	one-way ANOVA	two-way ANOVA	two-way ANOVA
Step 7	Optimization and Repetition	Each experiment was	Each experiment was	Each experiment was

		replicated thrice and each parameter was analysed in duplicate	replicated thrice and each parameter was analysed in duplicate	replicated thrice and each parameter was analysed in duplicate

6.5 Conclusion

Data can be transformed into systematic information. A research findings become worthless if its data are improperly presented, analysed and interpreted. Therefore, using the right statistical methods in analysing data, for example data regarding curry leaves is deemed indispensable as it helps us to gain interesting and useful information.

References

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3. Najeeb, A.P., Mandal, P.K., Pal, U.K. (2013). Efficacy of Leaves (Drumstick, Mint and Curry Leaves) Powder as Natural Preservatives in Restructured Chicken Block. *Journal of Food Science and Technology*, 52(5): 3129-3133.