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STABILITY OF PYRETHRINS IN DRYING METHODS OF PYRETHRUM FLOWERS

BY

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Thesis submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science in Analytical Chemistry of The University of Nairobi.

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for research. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

I dedicate this research to my siblings and my parents Elkana Owuor and Pamela Anyango for the foundation they started, nurturing and imparting values of hard work resilience and persistence in me.

I also dedicate this research to my well-wishers Susan Best and Michael Best who encouraged me to soldier on with the study despite many challenges.

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ABSTRACT

Pyrethrins are extracted from pyrethrum flowers and are classified into two groups; Pyrethrins I and Pyrethrins II. They are used as a broad spectrum natural insecticide active ingredient in agriculture and public health. Pyrethrins are degradable on exposure to air, moisture and high temperatures. The aim of this research was to investigate the optimum drying temperature, time, light intensity and moisture content of drying pyrethrum flowers for maximum yield of pyrethrins on extraction. Mature pyrethrum flowers from experimental farm, College of Agriculture and Veterinary Sciences, Kabete campus, University of Nairobi were harvested into brown paper bags, divided into sixteen portions and taken to the laboratory. The first six portions of the flowers were dried at varying temperatures of 30, 40, 50, 60, 70 and 80 ºC respectively to total dryness in an oven. The seventh to twelfth portions of flowers were dried in the oven at 30, 40, 50, 60, 70 and 80 ºC for a maximum period of 18 hrs. The thirteenth portion of the flowers were dried in darkness to a constant weight at temperatures below 50ºC. The fourteenth portion of the flowers were dried in sunlight to a constant weight at temperatures below 50ºC. The fifteenth and sixteenth portions were dried in darkness and in direct sunlight respectively for two weeks. The determination of moisture content of the flowers dried in the oven was carried out at given temperatures at one hour intervals. Hexane was used for solvent extraction after the flowers were ground into fine particles. Analysis of the extracts was carried out using Ultra High Performance Liquid Chromatography and Titrimetric method after refining the extracts. Maximum moisture loss of about 10% was achieved by the flowers at varying times and temperatures. The time recorded for drying at temperatures of 70 and 80 ºC was 18 hours. A percentage yield of 0.84 was obtained when the flowers were dried to constant weight at 30 ºC while drying for 18 hours yielded 0.75. Extractable Pyrethrins II were found to reduce by 8.6% when the drying temperature was raised from 50 to 60 ºC, by 11.3% from 60 to 70 ºC and by 8.5% when temperature was raised from 70 to 80 ºC for flowers dried for 18hrs. Extractable Pyrethrins I were found to reduce by 2.5% when the temperature was raised from 50 to 60 ºC, by 5.2% from 60 to 70 ºC and by 5.5% from 70 to 80 ºC when flowers were dried to constant weight. The percentage reduction of Pyrethrins II when the temperature was raised from 50 to 60 ºC during drying was 8.2%, 12.5% from 60 to 70 ºC and 12.2% from 70 to 80 ºC. Percentage reduction of Pyrethrins I when the drying temperature was raised from 50 to 60 ºC was 6.0%, 5.1% from 60 to 70 ºC and 9.5% from 70 to 80 ºC. Percentage of pyrethrins obtained from flowers dried at 50 ºC for 18 hours was 1.37% and 1.44% when drying was done at the same temperature to constant weight. Total pyrethrins obtained from the flowers dried in darkness was 1.38% and 1.02% when drying was done in direct sunlight. Moisture level for the flowers dried to a constant weight was below 10%. There was a variation in the ratio of Pyrethrins I : II over the temperature range of 30-80ºC. The optimum conditions for drying pyrethrum flowers were found to be at a temperature of 50 ºC, a duration of 21 hours in darkness to moisture level of less than 10%.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS/ACRONYMS

A.O.A.C - Association of Analytical Chemists

BCF- Bioconcentration Factor

ECD – Electron Capture Detector

GC – Gas Chromatography

GLPC – Gas Liquid Partition Chromatography

HPLC - High Pressure Liquid Chromatography

KARI – Kenya Agricultural Research Institute

K_p – Partition coefficient

LOD – Limit of detection

LOQ- Limit of quantitation

OSHA – Occupational Safety and Health Administration

PBK – Pyrethrum Board of Kenya

PI – Pyrethrins I

PII – Pyrethrins II

PHP - Potassium Hydrogen Phthalate.

RMM – Relative Molecular Mass

SGD – Sustainable Development Goals

TF – Titration Factor

TLC – Thin Layer Chromatography

UHPLC – Ultra High Performance Liquid Chromatography

WHO – World Health Organization

WSPE – World Standard Pyrethrum extract

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Pyrethrum is a plant from Compositae family and has been widely studied for its commercial importance as a source of insecticidal components known as Pyrethrins. There are two main species of pyrethrum; *Chrysanthemum cinerariifolium* and *Chrysanthemum coccineum*. *C. cinerariifolium* is also referred to as the Dalmatian chrysanthemum derived from the region of its origin, Dalmatia. It has white flowers, with yellow center that sprout from the stiff stems as shown in Fig. 1. The stems have blue-green leaves and can grow to about 46-100 cm tall. *C. coccineum* is known as the Persian chrysanthemum and it is a perennial species native to Caucasus. The species has large white, pink or red flowers as shown in Fig. 2 and grows to a height of about 30-60 cm with leaves resembling ferns. Insecticidal potency substances in its flowers are lower compared to those *C. cinerariifolium* (Motsoeneng *et al.,* 2015).

Figure 1: Mature flower of *Chrysanthemum cinerariifolium*

Source: Motsoeneng *et al.,* 2015.

Figure 2: Mature flower of *Chrysanthemum coccineum*.

Source: Motsoeneng *et al.,* 2015.

C. cinerariifolium is the most planted species in the world for its flower head. Countries producing this species for commercial purposes are: Uganda, Kenya, Ecuador, Japan, Rwanda, Tasmania, Papua New Guinea and Italy (Laugeray *et al.*, 2017).

Pyrethrum grow well in semi-arid conditions and cool winters deliver optimal pyrethrins production (Vadhana *et al.,* 2013). Cool temperatures, high rainfall of above 750mm and high altitude areas with ranges of about 1500-3000m favors the growth of pyrethrum. As the altitude increases, the concentration of pyrethrins increases. In addition, fertile soils that are well drained with favorable organic matter are ideal for pyrethrum flower production. The land should be well tilled to allow easy penetration by roots while all weeds should be removed and ploughing done during dry months to help destroy persistent weeds. Temperature change affects the content of pyrethrin in the flowers of the plant. Pyrethrum plants flowers continuously for about 10 months annually. The first harvesting of mature flowers is done 4 months after planting with follow up harvest every two (Wanjala *et al.,* 2008).

The active substance used as natural insecticide extracted from the flowers of *C. coccineum* or *C. cinerariifolium* are referred to as pyrethrins. Pyrethrins is a mixture of six closely related esters namely pyrethrin I and II, Jasmolin I and II and Cinerin I and II. The pyrethrins are used as a broad spectrum natural insecticide in agriculture and public health. They are biodegradable thus making them environmental friendly. The pyrethrin insecticides have been used for several decades with no resistance developed since they get degraded in the environment. Pyrethrins I have got a "knock down" effect while Pyrethrins II have the "kill" effect on insects. The efficacy

of pyrethrins is determined by the ratio of Pyrethrins I to Pyrethrins II. The pyrethrins are very efficient when the ratio is 1:1.

Growing of pyrethrum flowers in Kenya brings great economic value since it is an income generating activity for small scale farmers and also earns the country foreign exchange through the export. Pyrethrum growing areas in Kenya are; Kiambu, Nyandarua, Nakuru, Nyamira, Murang'a, Bomet, Kericho, Koibatek, Nandi, Nyeri, Laikipia and Kisii. These areas receive adequate rainfall amounts and the temperatures are cooler.

Processing the flowers to extract the pyrethrin is a process that varies from place to place. Processing starts with picking of the mature flowers that have attained horizontal petals positioning (Hughes *et al.,* 2016). The flowers are then dried to total dryness and then ground into fine particles before commencement of extraction. The dried flowers are then transported to the processing factories and sold for extraction of pyrethrins which are the active components (Ang'endu, 1994). In the pyrethrum industry, drying is necessary to remove the extra moisture. Drying of the flowers is done in open air shortly after picking to avoid fermentation and pyrethrins losses. A number of drying techniques have been employed which are time consuming and labor intensive. For instance, some farmers spread the flowers on polythene sheets in direct sunlight or in a shade for two to three weeks. Farmers growing pyrethrum on large scale use solar driers or roasters to hasten the drying process (Manna *et al.,* 2005). Heat causes rearrangements of the pyrethrins structure to form iso-pyrethrins which are insecticidally inactive. Exposure to air and direct sunlight also leads to degradation of the pyrethrins due to the presence of the UV in the sunlight (El Okda *et al.,* 2017). The described drying methods leads to degradation of pyrethrins in the flowers since drying conditions are not controlled.

Extraction of pyrethrins is done from *C. cinerariifolium* flowers grown as an agricultural commercial crop in the world. Farmers and suppliers of crude pyrethrum extract use procedures that have not changed since shortly after World War II (Costa, 2015). The flowers are harvested by hand on attaining maturity and full bloom to obtain maximum pyrethrins content then dried. Transportation of the dried flowers is done to the nearest processing facility where grinding is done and solvent extraction commences. Any undissolved substance is filtered from the ground pyrethrum flowers after dissolving using an organic solvent. The solvent used is removed

resulting in a crude oleoresin containing about 30% pyrethrins (Wylie *et al*., 2016). Components of the crude oleoresin (which is black and viscous) produced in a batch are: vegetable matter, waxes and pyrethrins. The produced oleoresin is then ready for refining.

Producers in Tasmania have genetically selected plants to fully bloom within a short span of about two weeks. Harvesting of the flowers is done mechanically rather than using labour intensive, hand-picking method used by farmers in other regions. The flowers are left to dry in the fields, cut from the plant and then separated from the stems. The dry flowers are ground, pelletized and extracted using hexane. Removal of the solvent is then done leaving a crude oleoresin that consistently has had an increased pyrethrins content greater than 35% (Singh, *et al*., 2012).

Crude oleoresin undergoes further processing to get rid of impurities from the plant matter, which are resins and waxes (Oda *et al.,* 2012). The refining step yields a clear, amber solution of pyrethrins normally diluted with kerosene to a standard concentration for marketing. Refined extract has low staining characteristics and a minimum level of inert insoluble substance (Skolarczyk *et al.,* 2017). Since the objective of refining operations being a light coloured extract, a high recovery of the pyrethrins is important. Major refineries operate batch processes based upon proprietary solvents for extraction to separate the pyrethrins from the unwanted vegetable waxes and resins. Despite the fairly simple solvent extraction operations concept, refiners have realized that the content of the extract vary with the type of solvent used. Research on determining the best solvent mixture should be done to achieve a high quality product with good pyrethrins recovery (Wang *et al.,* 2017). Dilution of the refined pyrethrins to a standard concentration is done based on the analysis method from the Association of Analytical Chemists (AOAC) and Butylated hydroxytoluene (BHT) is added as an antioxidant.

The standard concentration in United States has historically been 20% pyrethrins which has been marketed as a technical grade material. The material was eventually blended with synergists, emulsifiers and solvents to produce insecticide and formulations primarily for consumer product applications (Wylie *et al.,* 2016).

Kenya began to refine oleo resins in the 1970s which produced about 50-60% pyrethrins. Transportation costs for the highly concentrated product was lower compared to that of dilute material. In addition, the concentrated extract appealed pyrethrum users who preferred to

formulate technical grade material with a higher purity. The refined pyrethrum concentrate is still being marketed in Kenya today (Kumar *et al*., 2011). A pyrethrum refining process using carbon (IV) oxide in a super-critical-fluid extraction procedure has been investigated. This process is advantageous since it prevents exposure of the extract to heat when flashing off the solvents used in the conventional procedures operated by major primary pyrethrum refiners (Del Prado-Lu, 2015). Pyrethrum refiners must be able to recognize the differences and respond appropriately to process the material properly to achieve acceptable recoveries and a high quality product (Costa, 2015).

Different analytical methods such as; Gas Chromatography, Ultra High Performance Liquid Chromatography, Titrimetric method and High Performance Liquid Chromatography are used in the analysis of pyrethrins (Holynska *et al.*, 2018).

Pyrethrins content is determined using the listed analytical methods for pyrethrum flowers dried using different methods. Determining the pyrethrins content helps in selecting the optimum drying conditions.

1.2 Statement of the Problem

Drying methods of pyrethrum flowers used by farmers may lead to degradation of pyrethrins since they are biodegradable when exposed to air, direct sunlight, moisture and temperatures above their boiling points. Degradation of pyrethrins leads to losses of income for the pyrethrum farmers due to low quality and quantity of their produce. In addition, sun drying pyrethrum flowers is time consuming, labor intensive and lead to inconsistence of pyrethrins content per flower. Drying the flowers under sunlight is weather dependent and therefore consistent pyrethrin content would not be attained. Moreover, inadequate drying would lead to rotting and fermentation during storage process leading to economic losses. There is a threshold of moisture content beyond which leads to loss of the pyrethrins in the flowers. This research investigated the optimum conditions in relation to light, moisture content and temperature of drying pyrethrum flowers without losing the pyrethrin in them.

1.3 Objectives

General Objective

The main objective is to establish the optimum drying conditions of pyrethrum flowers to retain high pyrethrins content. To realize the main objective, the following specific objectives were undertaken:

I. To establish the threshold moisture content of dried pyrethrum flowers for pyrethrin extraction.
II. To validate the titrimetric method of analysis of pyrethrins from pyrethrum flowers.
III. To determine the effects of temperature on the content of pyrethrins in pyrethrum flowers.
IV. To determine the effects of drying pyrethrum flowers in direct sunlight.

1.4 Significance of the Study

Establishing the optimum drying conditions of pyrethrum flowers ensures minimum loss of pyrethrins. This leads to improved quality of the pyrethrum flowers thus increasing revenue from the produce since farmers are paid depending on its quantity and quality. Improved drying methods would also help save on time and work load required to dry the pyrethrum flowers. This would go a great mile in helping to actualize sustainable development goal (SDG) 1 on fighting poverty and SDG 8 on promoting economic growth. Availability of high pyrethrin content in the manufacture of insecticides will reduce the need to use pyrethroids which are toxic to human and animal life. Usage of pyrethrins in the manufacture of insecticides will help in ensuring a sustainable environment, good health and well-being hence realization of SDG 3. Pyrethrins are biodegradable in the environment and in mammalian tissues hence they do not last long in the environment. Pyrethrins being esters are not toxic to animals. This is because they are swiftly hydrolyzed into harmless products in the gut of animals which are subsequently excreted.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Pyrethrum

Pyrethrum seeds were first introduced to Kenya by Captain Gilbert Walker in 1928 from Yugoslavia and he established a commercial farm in Nakuru (Casida, 1973). A senior entomologist at the then Scott Agricultural Laboratories, Mr. Anderson obtained some seeds of *C. cinerariifolium* in 1929 from the ministry of Agriculture, Harpenden in England (Laugeray *et al.,* 2017). Production of the crop spread among Kenyan farmers in the highland regions such that Kenya became the leading world producer of Pyrethrum by 1945. Pyrethrum is regarded as a modern insecticide whose composition, methods of analysis, toxicology and biochemistry are documented (Hossain *et al.,* 2017). The application of pyrethrum plant extends beyond its insecticidal property. Pyrethrum in addition to having insecticidal properties that results in economic value, has some medicinal properties. The grist that remains after the extraction of pyrethrins from the flowers is used to deworm animals (Ranjkesh *et al.,* 2013).

2.2 Production of Pyrethrum

Farming pyrethrum flowers is done to obtain pyrethrins. Farmers earn their income based on the amount and quality of pyrethrins in the flowers. They therefore plant pyrethrum species or cultivars which can withstand the conditions within their regions. The cultivars are propagated in pyrethrum nurseries and farmers get them from such nurseries. The cultivars are relatively effective since they are highly resistant to pests and diseases (Nasuti *et al.,* 2013).

Pyrethrum should be spaced 2 feet inter-row and 1 foot intra-row. A table spoon of triple super phosphate fertilizer should be used per plant and mixed thoroughly with soil to avoid scorching. Pyrethrum should remain in the fields for 3-4 years after which it should be uprooted and transplanted in a new field (Hughes *et al.,* 2010).

Pyrethrins are used as remedy for lice in most countries in the world hence recommended in agricultural field as an organic insecticide. Farmers dry and grind flowers of pyrethrum then mix with water to make local insecticides (Shafer *et al.,* 2008).

2.3 Toxicity of Pyrethrins

Pyrethrins as a mixture of cinerin, pyrethrin and jasmolin causes irritation to human beings when it gets into contact with the eyes, tongue or skin. A legal limit for pyrethrum exposure to man according to the Occupational Safety and Health Administration (OSHA) is 5mg/m^3 over an 8 hour exposure (Costa, 2015). However, pyrethrins is dangerous to health at the levels of 5000 mg/m^3. People exposed to pyrethrins above the legal limit may experience symptoms such as dermatitis, itching, erythema, papules, sneezing, asthma and rhinorrhea. Precautions should be taken by people using or exposed to pyrethrins by wearing safety equipment. Due to the rising usage of pyrethrins in the consumer products and agriculture sector, the environmental fate of pyrethrins should be monitored by use of advanced analytical techniques (Dohlman *et al*., 2016). When pyrethrins and pyrethroids spray containing insect growth regulators e.g., methoprene are used on animals, their health deteriorates and it can take about 10 minutes of application for symptoms to develop in animals. Symptoms include ataxia, muscle fasciculation, salivation and inability to right themselves. Some animals have extreme sensitivity to pyrethrins and pyrethroids (Carcamo *et al.,* 2017). Dermal decontamination, diazepam for seizures and bathing in copious amounts of water are some of the treatment practices for pyrethrins and pyrethroids toxicity. Intoxication should be prevented by taking great care in keeping pyrethrin and pyrethroids sprays away from reptile's eye and mouth. Prognosis depends on the strength of the agent used, the duration of the exposure, and the size of the animal involved (Dohlman *et al.,* 2016).

2.4 Chemistry of Pyrethrins

Pyrethrins are six closely related esters derived from two acids and three alcohols. *Chrysanthemic* acid and pyrethric acid are the two acid moieties while jasmolone, cinerolone and pyrethrole are the three alcohol moieties. The chrysanthemates; pyrethrin 1, cinerin 1 and jasmoline 1 make up Pyrethrins I while the esters of pyrethric acid, pyrethrin 2, cinerin 2 and jasmolin 2 makes up Pyrethrins II. The six components contribute to knockdown and kill properties of the pyrethrum extracts. Pyrethrins have been fully isolated, characterized and their chemical structures differentiated as in Figure 3 (Sundaramoorthy *et al.,* 2016). Pyrethrins are viscous gums with high boiling points of about 250^{o}C. Pyrethrins are insoluble in water but readily dissolve in organic solvents such as alcohols (Njiru, 2006).

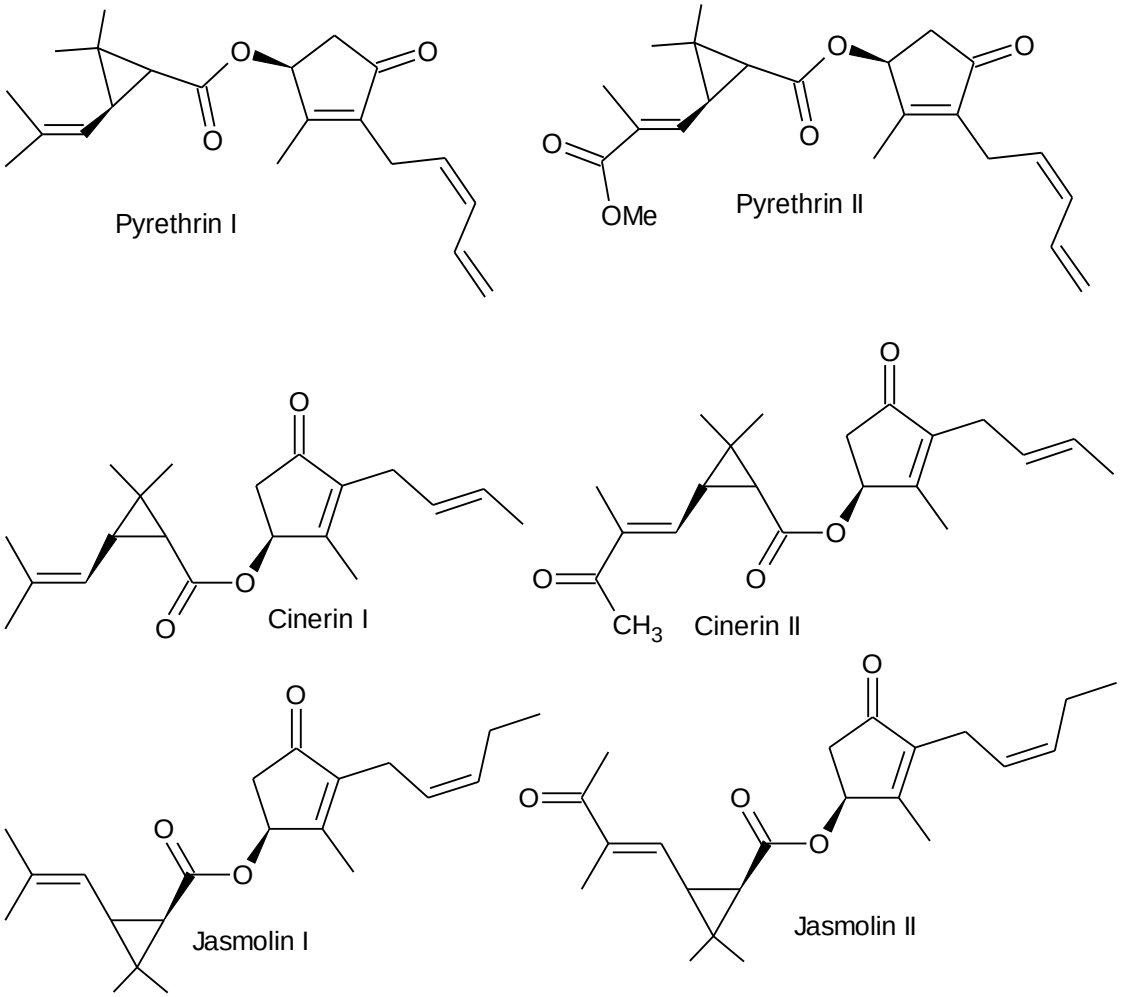


Figure 3: A Schematic Diagram of the Chemical Structures of Pyrethrins

Source: Casida and Quistad, 1995

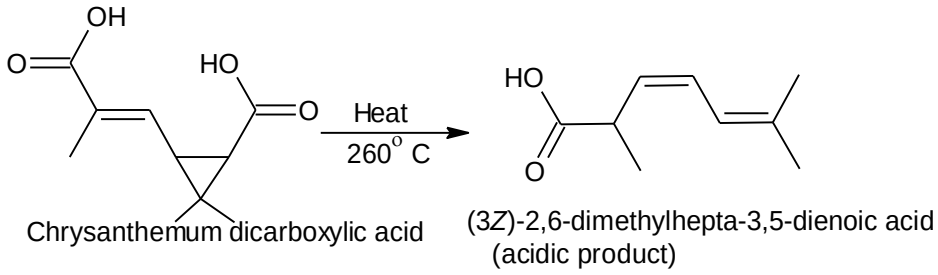
2.4.1 Properties of Natural Pyrethrins

Pyrethrins have a "knockdown" and "kill" effect on insects. The minimum dose required to 'knockdown' a mosquito and a housefly are 1.5 x 10 $^{-12}$g and 3.33x I0 $^{-9}$g per kilogram body weight respectively. Pyrethrins have a repellency power that makes insects to quickly get out of their hiding places (Kotila and Yon, 2015). They are biodegradable in the environment hence insects cannot develop resistance to them when used as an insecticide (Romero *et al.*, 2017). Pyrethrins being esters, are hydrolyzed into non-toxic products in the gut of animals which are then excreted. There are derivatives of pyrethrins known as pyrethroids which are also used as

insecticides. They are very stable compared to pyrethrins and have a long life when used. This causes insects to develop resistance to them since they adjust to the new environment with harsh conditions (Fedeli *et al.,* 2013). Properties of pyrethrins like its low mammalian toxicity, rapid paralysis of insects and environmental safety have contributed to the development of the pyrethrum industry (Gunduz *et al.,* 2015). Pyrethrins are therefore suitable for formulations of insecticides for household pest control and storage of grains. The advancement of agricultural production of pyrethrins is attributed to its use in horticultural crops for pest control (Chi *et al.,* 2014).

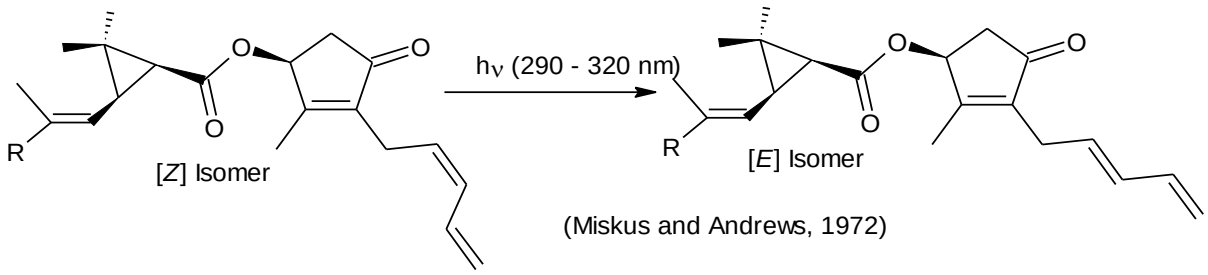
2.4.2 Thermochemistry and Photochemistry of Pyrethrins

Heat causes rearrangements of the pyrethrins structure to form iso-pyrethrins which are not efficacious against insects. Exposure to air and direct sunlight leads to degradation of the pyrethrins. Due to the presence of the UV in sunlight, the resonance conjugation of the unsaturated side chain with the cyclopropane ring encampasses the disappearance of the vital activated methylene next resulting to reduced biological activity of iso-pyrethrins (Wang *et al.,* 2017). Equation 1 shows the thermal degradation of pyrethrins.



Equation 1: Thermal Degradation of Pyrethrins

Pyrethrins undergo photochemical isomerization. Stereochemical change of pyrethrins II majorly occurs in the cinerins and jasmolins (Kawano et *al,* 1980). It is therefore necessary to evaluate the analytical techniques used to quantify these esters. Photochemical degradation of pyrethrins is illustrated in Equation 2.



Equation 2: Photochemical Degradation of Pyrethrins.

2.5 Environmental Fate of Pyrethrins

Approximately 200,000 kg of pyrethrins are used each year as an insecticide (WHO, 2016). The US Environmental agency are researching on the environmental fate of insecticides specifically made from pyrethroids for registration but not that of pyrethrins (Soderlund, 2012). The academic research of such data has also been slowed down by the difficulty of isolating and keeping the individual congeners. The dissolution, volatility, bio concentration and adsorption-desorption and environmental transformations of natural pyrethrins offer useful insights into toxic action, spray drift and persistence as well as practical applications in their extractions, storage and utility (WHO, 2014).

2.5.1 Solvent Partitioning

The basis of a substance's environmental transport is determined by its distribution in immiscible phases. A partition coefficient is the distribution of the substance between any two immiscible liquid solvents and is a constant.

$$K_p = \frac{\text{C organic solvent}}{\text{C water}}$$

The common organic solvent for environmental considerations in 1970s was 1-octanol and octanol-water partition coefficient is termed K_{ow}. Hansch and Leo (1979) found that K_{ow} was intimately related to chemical structures and that the contribution of each structural element or atom was additive. The formula of pyrethrin I is $C_{21}H_{28}O_3$ and the structure contains an ester, ketone, rings and unsaturation, therefore, the appropriate fragmentation constants can be added to provide an estimate of log $K_{ow.}$ The measured K_{ow} Pyrethrins has been reported by Worthing and Hance, (1991) and is identical to its calculated log K_{ow} confirming the fact that fragment additivity works in this case.

The K_{ow} values for Pyrethrins II series indicates that they are hydrophilic with the values averaging around 1600 (Kumar *et al.*, 2011).

The logarithms aqueous partition coefficients of pyrethrins that helps in the calculation of their aqueous solubility are as shown in Table 1.

Table 1: Aqueous Partition Coefficient of Pyrethrins.

Chemical	**Log K_{ow} (calc'd)**	**Log K_{ow} (Lit)**	**S (mg/l) (calc'd)**	**S (mg/l) (Lit)**	**Bioconcentration factor**
Bioallethrin	4.68	4.68[a]	4.45	4.6[a]	2100
Pyrethrin I	5.62	5.9[b]	0.35	0.2[b]	11000
Cinerin I	4.77	5.6[b]	3.62	30.6[b]	2500
Jasmolin I	5.42	-	0.60	-	4700
Pyrethrin II	3.56	4.3[b]	125.6	9.0[b]	300
Cinerin II	2.71	-	1038.0	-	70
Jasmolin II	3.37	-	214.8	-	210

Source: Casida and Quistad, 1995

a -Measured

b- Calculated

When log K_{ow} is specified, aqueous solubility can be calculated as in table 3. The solubility of Pyrethrins I is low compared to that of Pyrethrins II, with cinerin II having an aqueous solubility of more than a gram per litre, indicating that Pyrethrins I and Pyrethrins II may be separable by aqueous extraction alone (Glorennec *et al.*, 2017).

The chemical concentration ratio in an animal to that in its environment, the bioconcentration factor (BCF) is related to K_{ow}. Bioconcentration factor in aquatic animals is an important factor that has received considerable attention and a number of regression equations are available for its calculation (Hocine *et al.*, 2016).

2.6 Chemical Transformations of Pyrethrins

Pyrethrins undergo chemical reactions when exposed to various conditions hence converted to other chemical compounds. Natural pyrethrins; carboxylic esters, undergo hydrolysis, especially in alkaline waters. The rate of hydrolysis is low at 25^{o}C and pH 7 as the ester linkage is between alcohols and aliphatic acid (Kumar *et al.,* 2011).

Rapid and extensive reactions of the cyclopropane ring arise when ethyl chrysanthemate is treated with 50 % aqueous sulphuric acid at room temperature. Incase cis-*Chrysenthemic* acid is lactonized in the presence of a Lewis acid to dihydrochrysanthemolactone, the trans-*Chrysanthemic* acid is readily separated from the racemic cis/trans mixture of ethyl chrysanthemate (Sigh *et al.,* 2012). *Chrysanthemic* acid undergoes the same reaction at a temperature of 210 ^{o}C in the presence of an acid catalyst and thermal degradation of Pyrethrins II follows a similar trend at a temperature of 400^{o}C. These are rare "environmental" transformations as shown in Figure 4 (Casida, 1973). When pyrethrins are treated with sodium hydroxide in ethanol at room temperature, thermal elimination of chrysanthemic acid occurs. Simple chrysanthemic acid derivatives react with gaseous ozone at the olefince double bond to produce the corresponding ozonide (3), aldehyde (4), acid (5) and epoxide (6). With rethronoles, only the aldehydes associated with side-chain cleavage have been reported. The environmental ozonolysis of natural pyrethrins takes place in ambient air (WHO, 2015).

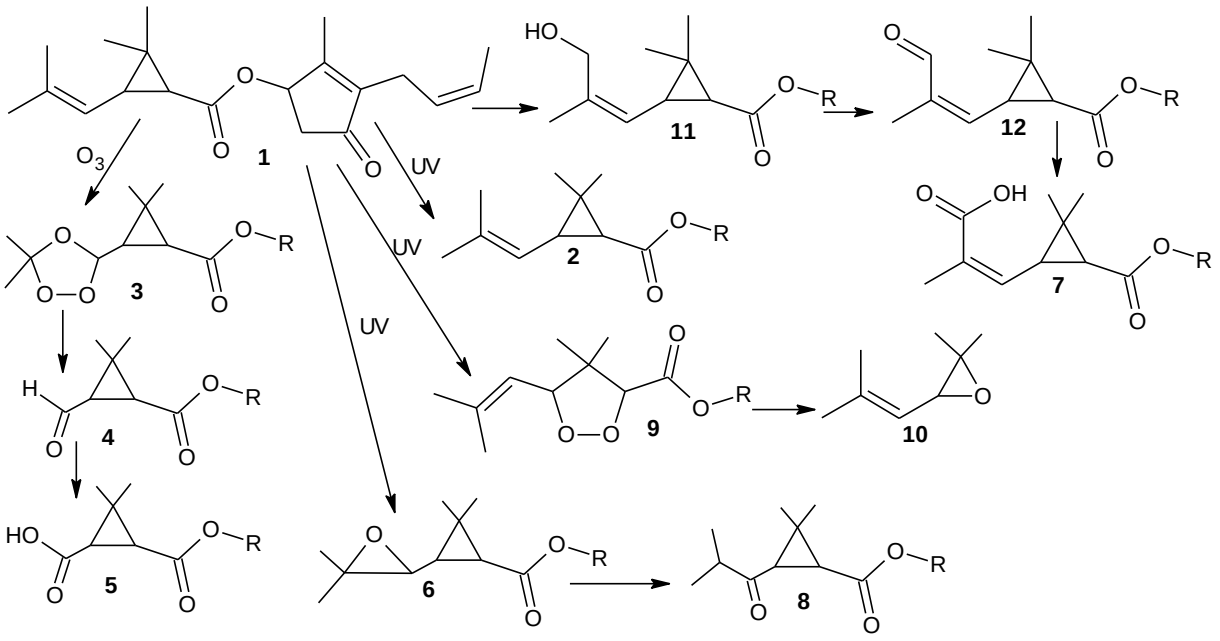


Figure 4: Photodegradation and Ozonolysis Pathways of Pyrethrins.

2.6.1 Effects of Pyrethrins on insects

Pyrethrins are botanically used as natural insecticide which are obtained from dried pyrethrum flowers (Hocine *et al.,* 2016). Parasites are affected by pyrethrins since the activity of sodium ion channels of the nerves are altered by the molecules. The sodium conductance period are prolonged by these poisons and they also prolong the action of depolarization resulting to death (Omotoso *et al.,* 2014). The host animals can be intoxicated by higher doses of these compounds. Immediately after the application of pyrethrins sprays on the animal, they should be thoroughly rinsed off using warm water due to the potential for transcutaneous absorption (WHO, 2017).

When the sprays are used on smaller animals, dilution should be done to the least effective dose to ensure no accidental intoxication from transcutaneous absorption (Hossain *et al.*, 2017).

2.7 Sampling of Pyrethrum Flowers

Flowers are randomly sampled from the nurseries in cultivar plots that are ready for picking. It is advisable to harvest the flowers early in the morning since it is easier to determine flower ready for picking. The flowers whose petals are horizontal early in the morning in the absence of external factors such as heat from the sun are the best for harvesting since they have fully

matured. Sampling should be done in bags with good aeration to prevent rotting of the flowers. The bags should also be able to prevent the flowers from exposure to direct sunlight (Njiru, 2006).

2.7.1 Methods of Drying Pyrethrum Flowers

Drying of the flowers is one of the most essential post-harvest activity that determines the content of pyrethrin retained in the dry flowers. Farmers dry the flowers on some material or bare ground in the fields as shown in Figure 5. Once the flowers are harvested on maturity, they are spread on the ground in direct sunlight and left for about 2- 3 weeks to dry depending on the weather during that drying period. This lead to soiling and fermentation of the flowers that reduces the quality of the flowers (Wanjala *et al.*, 2008).

Figure 5: Drying of Pyrethrum Flowers on the Ground.

Source: Wanjala et al., 2008.

Some farmers dry the flowers on raised racks to avoid soiling. The racks are made by constructing frames using wood in rectangular shape then making the surface where the flowers are spread using plywood as shown in Figure 6. The Kenya Agricultural Research Institute (KARI), improved the drying method covering the rack with a transparent polythene as shown in Figure 7. The rack is then moved to a place exposed to direct sunlight and the flowers are spread on the surface (Wanjala *et al.*, 2008).

Figure 6: Drying of Pyrethrum on a Rack.

Source: Wanjala *et al.,* 2008.

Figure 7: Drying Pyrethrum Flowers on a Rack Covered with a Polythene.

Source: Wanjala *et al.,* 2008.

In Japan, farmers use air drying method in drying pyrethrum flowers (Bai Chi Sun, 1994). Air drying flowers is one of the most traditional and more time-consuming, methods of drying the flowers. In this method, excess foliage are stripped from the flowers and stems of the plant are cut into desired lengths. The flowers are hung upside down individually or the stems are tied together using a rubber-band forming bouquet and hung. The hung flowers are then placed in a

dark, dry area with good air circulation. The flowers are left to dry for about two to three weeks (Singleton *et al.,* 2014).

Pyrethrins degrade when the flowers are exposed to direct sunlight and air. This is due to the presence of the UV in the sunlight. The resonance conjugation of the unsaturated side chain with the cyclopropane ring encampasses the disappearance of the vital activated methylene next to the ring and so reduced biological activity in iso-pyrethrins results (Pratera *et al.,* 2002). Moreover, when drying is done at high temperatures, it leads to loss of the pyrethrin (Costa *et al.,* 2013). Heat causes rearrangements of the pyrethrins structure to form iso-pyrethrins which are insecticidally inactive. Pyrethrins undergo photochemical reactions which are mainly the photochemical isomerization. An indication is made that the stereochemical change of pyrethrins II majorly occurs in the cinerins and jasmolins (Elwan *et al.,* 2006).

2.7.2 Extraction of Pyrethrins

The dried flowers are ground using suitable grinding machines and pyrethrins are extracted using cold extraction method in organic solvents or soxhlet extraction. Soxhlet extraction is used in case of small sample volume. The working mechanism and instrumentation of soxhlet extractor is described.

Instrumentation of Soxhlet extractor

The apparatus is used in extraction of lipids and other molecules from a solid material when the compound of interest has a limited solubility and impurities within the material are insoluble in the solvent used. Its operation reduces monitoring work and is very efficient since a small amount of the solvent is recycled to dissolve a larger amount of material. It has various components such as a reflux condenser used in condensing the solvent, a thimble holder with side tubes, a siphon device and a round bottomed flask connected by the thimble holder (Kim *et al.,* 2008). Figure 8 shows the diagram of a soxhlet extractor.

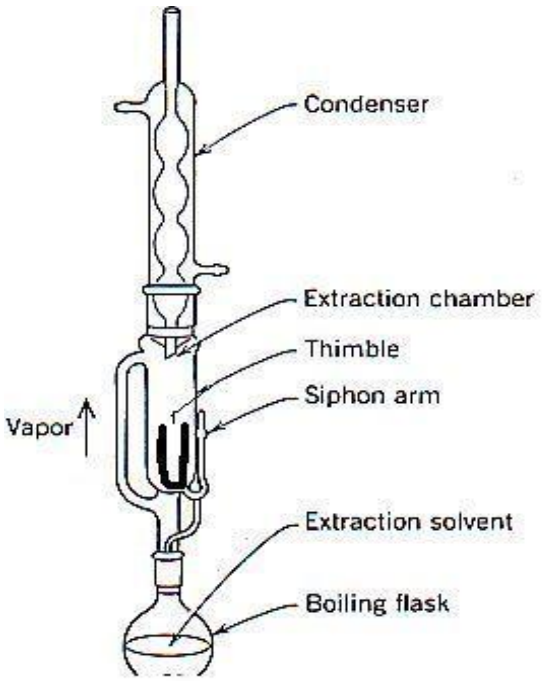


Figure 8: Soxhlet Extractor.

Source: Maalej *et al.*, 2017**.**

A solid sample is placed in a thimble since liquid materials can percolate through and dissolve the analytes of interest. The thimble containing the material is then placed in the Soxhlet extractor. Heating of the organic material is then done at reflux. Its vapor rises up as it boils and reaches the condenser where they are condensed. The condensed materials then trickles back and the thimble is filled up. Once the thimble is filled with the organic solvent, it automatically siphons back into the round bottomed flask. The process is continuous and this maximizes the chances of dissolving the analytes completely into the organic solvent (Mader and Nancy, 2012).

2.8 Analysis of Pyrethrins

Different analysis methods such as: Gas Chromatography-Electron Capture Detector (GC-ECD), Mercury reduction, High Performance Liquid Chromatography (HPLC) and Ultra High Performance Liquid Chromatography (UHPLC) can be used in pyrethrins analysis. Each of these methods has its own limitations or shortcomings ranging from high cost, low sensitivity to low speed (Abdel *et al.*, 2015).

2.8.1 Working Mechanism and Instrumentation of High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) has been the most valuable method for pyrethrins analysis. A method of analyzing pyrethrins based on HPLC uses a Zorbax ODS column of 25 cm × 4.6 um i.d. using acetonitrile: water (7:3) as the eluent, a flow rate of 1 ml/minute and detection of 240 nm (Assoc, 1985). Separation of the six types of pyrethrins and quantification can also be done by HPLC using a mobile phase of a mixture of anhydrous dichloromethane and water saturated dichloromethane in the ratio 1:1 and a double u-porasil column (Otieno, 1982). Normal-phase liquid chromatography can be used in the analysis of pyrethrins (Debon, 1989). Samples are dissolved in tetrahydrofuran and analyzed on a 5 um amino column of 25 cm × 4.5 um i.d. with a mobile phase of hexane: tetrahydrofuran (9:1). A flow rate of 1.5ml/minute. High performance liquid chromatography has been used in determining trace levels of pyrethrins down to 0.1 µg/litre in tap water. A 5um ODS C_{18} 25 cm × 4.6 um i.d. is used in this method. The mobile phase is methanol/water 90/10, v/v (helium degassed), flow rate 0.8 ml/minute (Njiru, 2006).

An enhanced type of liquid chromatography is the High Performance Liquid Chromatography. The principle of its chromatographic partition works under basic standard; dispassion of particles into their fundamental parts in view of the difference in the relative affinities of various particles for the mobile phase and the stationary phase used as the part of division (Bordoni *et al.*, 2015).

The different types of HPLC, are:

Normal Phase HPLC- Analytes are isolated on the principle of extremity. The mobile phase is non-polar while the stationary phase is polar. Consequently, silica is the general stationary phase and hexane, diethyl ether, methylene chloride, chloroform or their blends are the mobile phases. Materials with hydrophilic particles are held in the stationary phase since they press longer than the less polar materials.

Reverse Phase HPLC- Blends of water and acetonitrile or methanol which are polar are used in the mobile phase while non-polar substances are used in the stationary phase. The rule or

principle used is the hydrophobic collaborations rule hence materials which are more non-polar will be held more.

Size-exclusion HPLC- Materials packed in the column have controlled pore sizes thus the isolation of particles is based on their atomic sizes. Particles that are washed faster through the column are those with large atomic sizes since particles with small atomic sizes enter into the pores of the pressing units and eluted last.

Ion-Exchange HPLC- Composition of the stationary phase are charged surfaces which are ionic and of opposite charge to the particles of interest. Particles suitable for this method are ionizable particles or those that are ionic. The rate at which the grounded particles will be pulled into the ionic surface is directly proportional to how grounded the charge of those particles are and the time taken by the particles to elute. Ionic quality and pH of the fluid support in the mobile phase are used to control the elution time (Carloni *et al.*, 2017).

High performance liquid chromatography instrumentation includes a pump, injector, column and detector. The column is the basic and an important part in the instrumentation where division occurs. Figure 9 illustrates the instrumentation of High Performance Liquid Chromatography.

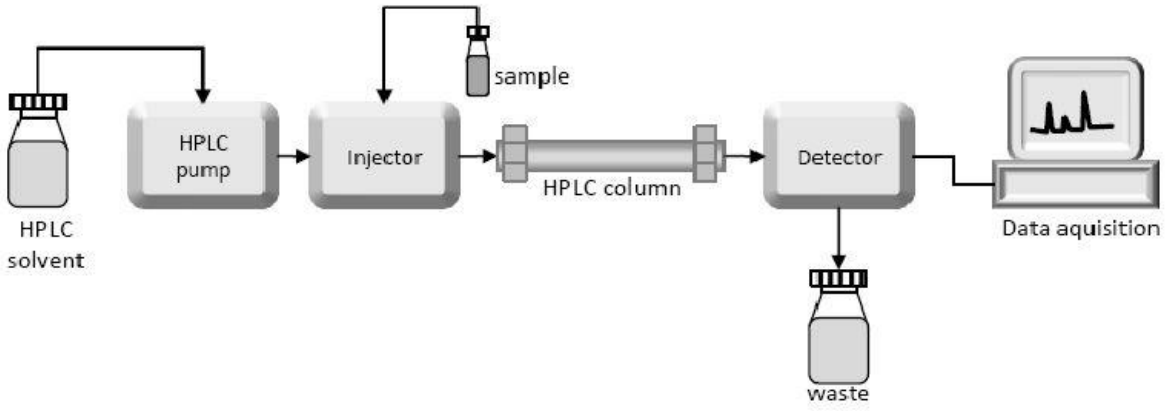


Figure 9: Block Diagram of High Performance Liquid Chromatography.

Source: El Okda et al., 2017.

Solvent reservoir has the solvent in which the sample is transported throughout the system of HPLC. Sensitivity of the components are ensured by removing particles through filtration with

an inlet solvent filter. The pump is used in impelling the solvent through the system which may include, pump seals that breaks down slowly over a given period. The presence of an inline solvent filter ensures that the particles released when the seals break down do not cause any damage to the post-pump components. The sample injector or injection valve ensures reproducible introduction of sample into the flow path since it has sample loops of suitable size for the performed analysis. Valve and column damage are prevented by ensuring the usage of a sample filter or pre-column filter since sample may have some particulate matter. The primary sample separation takes place in the column due to the difference in attraction of components of the sample for the packing material within the column and the solvent. The main column is protected from sample components that would foul it by including a sacrificial guard column just before the analytical column that chemically removes them.

The separated components just before passing into the waste reservoir, they pass through a detector flow cell. The detector gives an electrical response due to the presence of the components in the flow cell which are digitized and sent to a recorder that analyzes and interprets the data. Immediately after the detector, installation of a back pressure regulator is done as an ultimate improvement of the system. The regulator helps in the prevention of bubble formation by the solvent until it goes through the detector completely. The detection of sample components can be interfered with by formation of bubbles in a flow cell. Installation of an inert sparging system can be an alternative to remove dissolved gases out of the solvent that is kept in the solvent reservoir (Ding *et al.,* 2017).

2.8.2 Working Mechanism and Instrumentation of Gas Chromatography

Analytical chromatographic techniques have been developed for both qualitative and quantitative analysis of Pyrethrins. The separation of Pyrethrins by GLC using the extremely sensitive electron capture detector (ECD) was developed in 1959 and was taken to be one of the most valuable advances in the analysis of Pyrethrins by that time (Sundaramoorthy *et al.,* 2016). Lately GLC coupled with mass spectrometry (GLC/MS) is gaining ground in order to identify and broadly quantify Pyrethrins (Soderlund, 2012). The GLC results have been found to be generally lower than AOAC results may be due to the possible degradation of pyrethrin 2 in the GLC

columns. Pyrethrins are usually analyzed using an electron capture detector (ECD), while flame ionization detector (FID) is used for the non-Pyrethrins constituents of pyrethrum extract.

Gas Liquid Chromatography (GLC) is basically used for separation and analysis of compounds that can be vaporized without decomposition. It is used to test for the purity of a substance, identifying a compound, separation of various components of a mixture and determining the amounts of the components (Gabbianelli *et al.,* 2009).

The mobile phase is in gaseous form and the frequently used gasesuj are helium or nitrogen. Helium remains the most commonly used carrier gas in most instruments although nitrogen is preferred for improved separations. The mobile phase inside a column is composed of a liquid on an inert solid support or a microscopic layer of polymer. The compounds being analyzed always have an interaction with the walls of the column. The compounds elute at different time referred to as the retention time of the compounds. The analytical usefulness of a GC is attributed to by the comparison of the retention times (Hughes *et al.,* 2010). There are notable differences between a GC, HPLC and Thin Layer Chromatography (TLC). In GC, separation of compounds in a mixture is carried out between a solid or liquid stationary phase and a gas mobile phase. The column of a GC is situated where temperature can be controlled in an oven. Lastly, the compound's concentration in the mobile phase is exclusively a function of the vapor pressure of the gas (Holynska *et al.,* 2018). Gas Chromatography has alternative names used in scientific literature as Gas-liquid partition chromatography (GLPC) or Vapor- phase chromatography (VPC) (Johnson *et al.,* 2010).

2.8.2.1 Gas Chromatography Analysis

Chemical constituents of a sample pass in a carrier gas at different rates due to their physical and chemical properties and their interaction with the stationary phase in the column. The chemicals are identified and detected electronically as they exit the column. Components are separated by the stationary phase in the column causing each component to exit the column at different time, retention time. Temperature, carrier gas flow rate and column length are parameters that are used to alter the order or retention time (Khalatbary *et al.,* 2015). The schematic diagram of a gas chromatograph is as shown in Figure 10.

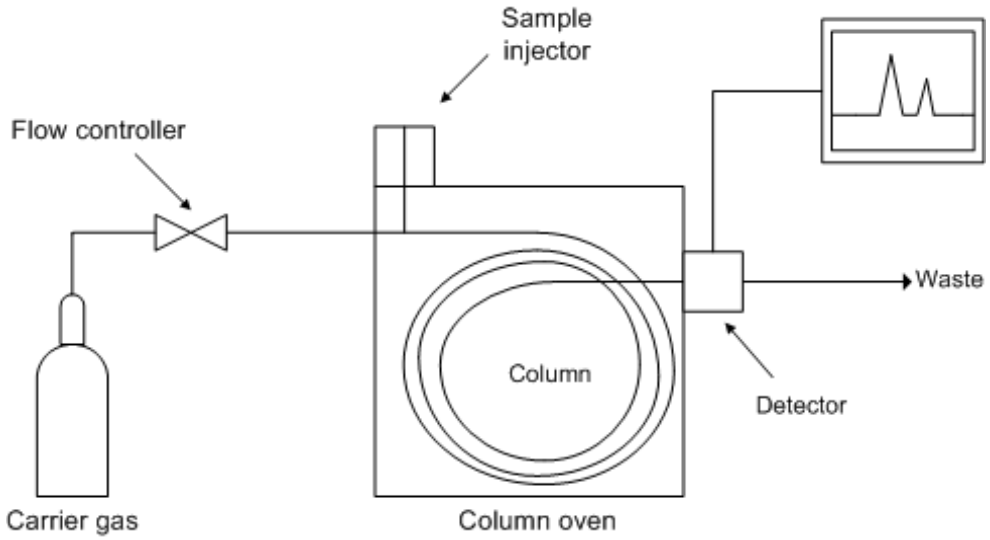


Figure 10: Diagram of a Gas Chromatograph

Source: Lutnicka *et al.,* 2009.

Gaseous or liquid analyte of known volume is injected into the entrance of the column using a microsyringe, microextraction fibers or a gas source during a GC analysis. As the analyte molecules are swept through the column by a carrier gas, the motion is inhibited by the analyte molecules' adsorption onto packing materials in the column or onto the column walls. The strength of adsorption depends on the type of molecules and the material on the stationary and this determines the progression rate of the molecules along the column. Separation of the different components of the analyte mixture happens as they move along the column and reaches the column end at different times due to the different rate of progression of each molecule. The outlet stream from the column is checked by a detector thus time taken by the component to reach the exit and the quantity of the component can be determined. The substances are generally qualitatively identified by the order in which elute emerge from the column and the retention time of the analyte (Motsoeneng *et al.,* 2015).

The physical components of a gas chromatography include:

Auto-samplers- Helps in provision of the means through which a sample is automatically introduced into the inlets. Manual insertion is not common since automatic insertion has the advantages of providing better reproducibility and time optimization.

Inlets- It is always attached to the column head and helps in provision of a means through which a sample is introduced into a continuous flow of carrier gas.

Detector- Used in detection of components of the mixture being eluted off the column. The two general types of detectors are destructive and non-destructive. Destructive detectors perform continuous transformation of the column eluent; evaporation, burning or mixing with reagents with ensuing measurement of some physical property of the resulting material, plasma and aerosol or reaction mixture. The measurement of some property of the column eluent is directly done by the non-destructive detector and thus affords greater analyte recovery (Nasuti *et al.*, 2014).

2.8.2.2 Gas Chromatograph with Electron Capture Detector

Halogenated compounds are analyzed using this technique which is basically used in the pharmaceutical, environmental and forensic markets. The current measured is reduced when given molecules capture electrons in the sample as they pass through the detector and the compensation for this reduction is recorded as a positive peak (Carloni *et al.*, 2012).

The carrier gas should be void of low oxygen and water impurities because they can cause problems when they react with the stationary phase. The problems that arise due to the interaction of the impurities with the stationary phase include, high baseline and column bleed in the output gas chromatogram which reduces analyzer sensitivity and decreases column lifetime. The radioactive Nickel source can be oxidized by water and oxygen impurities and the detector response reduced. The electron capture detector is sensitive to halocarbons hence the carrier gas should have low level of the compounds (Caldwell *et al.*, 2013).

Incase Helium is used as the carrier gas, ECD needs a make-up gas because this helps in the provision of electrons which are not contained within the Helium. Routine calibration of the analyzer using calibration mixture is frequently done.

Gas Chromatography- Electron capture detector is a valuable method for analysis of pyrethrins. Electron capture detector (ECD) is used since the pyrethrins respond to it and the sensitivity helps in provision of a method suitable for residues determination. Pyrethrins response to the detector (ECD) is proportional to that in flame ionization detector. The electron capture detector is preferred for its specificity. Its linear range is quite limited and it is also very sensitive to changes in the operating conditions. Due to the reasons given, a lot of care has to be taken when used in quantitative measurements. A detector that does not suffer the above defects is the hydrogen flame ionization detector and it allows the use of temperature programming (Head, 1966) but it is non-specific.

2.8.3 Mercury Reduction Method

This method is based on the saponification of esters. The reduction of mercuric sulphate or chloride by chrysanthemic acid to mercurous (+1 oxidation state) (Equation 4) is accompanied by a series of characteristic colour changes: Pink -Purple - Blue – Blue green.

Reduction of mercuric oxide by chrysanthemic acid

$HgO(s) + H_20 + 2HC1 \longrightarrow HgCl_2 + 2H_20$---------- Equation 3

$2HgCl_2$ + Chrysanthemic acid $\longrightarrow$ 2HgCl(s) + 2HC1---------- Equation 4

$2HgCl(s) + 2IC1 \longrightarrow 2HgCl_2\,(aq) + I_2$ ----------Equation 5

$2\ I_2(s) + K\ I\ O_3\,(aq) + 6HC1 \longrightarrow 5\ IC1 + KC1 + 3H_2O$ ---------- Equation 6

Pyrethrins I are calculated by a titrimetric method using iodine monochloride as the indicator base. Pyrethrins II are determined using normal acid-base titration using standardized Sodium Hydroxide as the base. The process is composed of alkaline hydrolysis of the mixed pyrethrin esters to give chrysanthemum mono-carboxylic acids and di-carboxylic acids. Fatty acids that cause interference are eliminated as barium salts. The mono-carboxylic acid is extracted by

petroleum ether. The acid reacts with Dinige's mercury reagent. The quantity of monocarboxylic acid is accurately determined by Iodometric titration of the mercurous salts formed. The dicarboxylic acid is insoluble in petroleum ether hence is extracted by diethyl ether. It is then subsequently determined alkalimetrically. The amount of these two acids gives the content of the pyrethrins since they are expressed as pyrethrin I and pyrethrin II (Ang'endu, 1994).

The percentage w/w pyrethrins are calculated using Equations 7 and 8

The % (w/w) Pyrethrins I is calculated using Equation 7,

Percentage of Pyrethrins I = $0.7125 \times \frac{V_1}{W_1}$ …….. Equation 7

Where V1- Titre volume (volume of 0.01M KIO_3 used) (cm^3)

W1- Weight of sample (g)

0.7125 - Stoichiometric factor for pyrethrins I

2.8.3.1 Derivatization of the Pyrethrins I Stoichiometric Factor.

Experiments have shown that:

1 ml of 0.01M KIO_3 = 0.0057g of Pyrethrins I

Let the weight of the sample extracted at step l = W_1 (g)

Let the volume of 0.01M KI03 used on titration = V_1 (cm3)

The amount of Pyrethrins I in the 200 mls = (V_1 x 0.0057) g

For the total sample of 250ml the amount of Pyrethrins I

250/200 x $V_1/W_1 \times 0.0057$

% pyrethrins I = $(250/200 \times V_1/W_1 \times 0.0057 \times 100)$ %

= $0.7125 \times V_1/W_1$

Calculation of Pyrethrins II concentration is done using Equation 8.

The percentage (w/w) Pyrethrins II = 0.4675 x $\frac{V_2}{W_2}$ x TF ……. Equation 8

V_2= Volume of 0.02M NaOH (cm^3)

W_2 = Weight of Sample extracted (g)

TF = Titration factor due to the hygroscopic alkali base NaOH

0.4675 = Stoichiometric factor for Pyrethrins II

2.8.3.2 Derivatization of Pyrethrins II Stoichiometric Factor

A volume of 1ml of 0.02M NaOH is equivalent to 0.00374g of Pyrethrins II. During sample clean-up, 200ml of the 250ml total extract are analyzed. 250/200 factor is to be applied in total

Pyrethrins II concentration determination.

Let weight of Sample = W_2 (g)

Let Volume of 0.02N NaOH used = V_2,

Therefore, amount of Pyrethrins II in 200ml = (V_2 x 0.00374) g

Total Pyrethrins II in 250ml= (250/200 x V_2 x 0.00374)

% Pyrethrins II = 250/200 x V_2/W_2× 0.00374 X 100) % ……Equation 9

Incorporating the titration factor (TF) explained below:

% Pyrethrins II = 250/200 x V_2/W_2 × 0.00374 X 100 x TF) % ……..Equation 10

% Pyrethrins II = (0.467 5 x V_2/W_2 x TF) % ……..Equation 11

2.8.3.3 Standards used in calculation of the Titration Factor

Sodium hydroxide and potassium hydroxide are highly hygroscopic bases. A certain amount of alkali carbonate and water are present in these reagents on standing for some time. This implies that exact results cannot be obtained in titrimetric determinations when using these bases and hence the necessity to standardize these alkalis just before use. Potassium hydrogen phthalate (PHP) is one of the primary standards used in standardization of Sodium hydroxide. PHP is a substance of high purity (99.9%) (Rjeibia *et al.*, 2016). Drying at 120°c for 2 hours before use is

recommended. Other recommended primary standards in addition to potassium hydrogen phthalate are Benzoic acid ($C_6H_5C0_2H$; RMM 122.12), Succinic acid $(CH_2C0_2H)_2$; RMM = 118.09), Potassium hydrogen iodate $(KH(I0_3)_2$; RMM = 118.09) and sulphamic acid (NH_2S0_3H ; RMM = 97.09) (Ang'endu, 1994). The titration factor explained above is an expression of change of basicity of sodium hydroxide with respect to potassium hydrogen phthalate.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Flower Sampling

Flowers were randomly sampled from the nurseries in cultivar plots that were ready for picking. All the plots from which samples were taken had been subjected to uniform breeding treatment. Sampling was done at an experimental farm in the College of Agriculture and Veterinary Sciences, University of Nairobi in Kenya.

The samples were picked in the morning into brown paper bags and weighed immediately to get their fresh weights. The samples were then transported on the same day to the laboratory in The University of Nairobi, department of chemistry for drying and analysis. Transportation was done on the same day to ensure that the quality of the flowers were never compromised by rotting.

3.2 Reagents, Instrumentation and Apparatus

3.2.1 Chemicals and Reagents

Acetone, Ethyl Acetate, Silica gel, Filter-celite, Chloroform, Activated Charcoal, Methanol, Deionized water, Mercuric Oxide, Potassium Iodide, Potassium Iodate, 1 Normal Sodium Hydroxide, Barium Chloride, Sulphuric Acid, Hexane, Diethyl Ether, Petroleum Ether, Sodium Chloride, 50% w/w pyrethrin standard from Sigma-Aldrich.

3.2.2 Instrumentation and Apparatus

A Beckmann Gradient Liquid Chromatograph Model 332, Model 210 sample injector, a varian micropack ODS C18 analytical UHPLC column, Soxhlet extractor, Gas chromatograph, Oven, Hot Plate, Gas Chromatograph, Volumetric flasks, Measuring cylinders, Pipettes, Watch glass, Filter papers, Spatulas, Amber vials and thermometers.

3.3 Drying of Flowers

The flowers were divided into sixteen portions and dried under different conditions. The first six portions of the flowers were dried at regulated temperatures in an oven. The flowers were put in the oven and the temperatures were regulated between 30-80^{o}C at intervals of 10 and the flowers dried to a constant weight.

The next six portions were placed on trays and put in the oven. The seventh, eighth, ninth, tenth, eleventh and twelfth portions were dried at a temperature of 30, 40, 50, 60, 70 and 80^{o}C respectively for 18 hours.

The thirteenth portion of the flowers were spread on a large polythene sheet and exposed to direct sunlight. The flowers were turned daily for two weeks of the drying period. The flowers were kept away from other factors that would alter the results as rainfall or exposure to water. The fourteenth portion were spread on a large polythene sheet and exposed to direct sunlight. The flowers were dried monitoring the change in weight until a constant weight was achieved. The fifteenth portion of flowers were spread on a large polythene sheet and dried in darkness for two weeks. The sixteenth portion were spread on a large polythene sheet and put in darkness. The flowers were dried monitoring the change in weight until a constant weight was achieved. The temperature within Chiromo Campus and its surrounding during the drying period was between 16-29^{o}C which was confirmed with the Meteorology department at The University of Nairobi. The moisture contents of the dry flowers were tested and percentage weight loss was calculated using Equation 12.

$$\text{Percentage weight loss} = \frac{\textbf{Weight of wet flowers} - \textbf{Weight of dry flowers}}{\textbf{Weight of wet flowers}} \times 100\% \quad \ldots\ldots\ldots \text{ Equation 12}$$

3.4 Grinding of Dry Pyrethrum Flowers

The dried flowers were kept in the laboratory for 2 days. The flowers were ground at room temperature using a pestle and a motor. Sieving of the crushed flowers was done using a BS 410 mesh and the dust obtained stored in brown paper bags awaiting extraction.

3.5 Cleaning and Sterilization of Glassware

All glassware used in the research were cleaned and later soaked for 12 hours in 50% Nitric Acid. Deionized water was used in rinsing the glassware and in order to leach off adsorbed nitric acid, they were soaked in deionized water for 6 hours. They were then dried in open racks.

3.6 Preparation of Working Solutions

Diniges reagent – Mass of 5 g yellow mercuric oxide was mixed with 40 ml of water and while stirring slowly 20 ml sulphuric acid was added then additional 40 ml water was added and stirred

until all dissolved. The absence of mercurous Hg was tested by adding few drops of iodine monochloride solution to 10 ml and titrating with potassium iodate standard solution.

Iodine monochloride solution – Masses of 10 g of KI and 6.44 g of KIO_3 were dissolved in 75 ml water in glass stoppered bottle. Volumes of 75 ml HCl and 5 ml $CHCl_3$ were added. The solution was kept in dark.

0.01 M Potassium iodate standard solution – Mass of 2.14 g pure KIO_3 previously dried at 105^0, was dissolved in water and diluted to 1 Litre. 1 ml is equivalent to 0.0057 g pyrethrin.

1M Sodium Hydroxide – 40 grams of Sodium Hydroxide in 1 Litre of deionized water.

3.7 Extraction of Pyrethrins Using Soxhlet Extraction apparatus

10g of the already ground flowers was placed in an extraction thimble made of cellulose and put in a soxhlet extractor. Round bottomed flask that had 1000 ml of hexane was connected to the soxhlet and boiling chips were added. A condenser was attached to the soxhlet after it had already been connected to a source of water. The glassware used had quick fit joints. The extraction was done at a temperature of 50^{o}C. Filtration of the resultant solution was done using cotton plug and hexane present was evaporated using a rotary evaporator. The yield of the extract was determined after it had been left to cool and weighed. They were placed in amber bottles and stored in a refrigerator after labelling to await clean-up before analysis.

3.7.1 Sample Clean-Up for Analysis Using Mercury Reduction Method

Hexane was added to the extracts in three different flasks in the ratio of 3:1 v/v. The flasks were kept at a temperature of 2^{o}C for a period of 12hours. After 12hours, the extracts were filtered through cotton wool into another conical flask. The cotton wool was carefully rinsed with hexane. Hexane was evaporated from the resultant solution in a rotary evaporator. A volume of 20 ml alcoholic NaOH was added into the conical flask containing the dry extracts and refluxed for 1 hour to hydrolyze the esters. The extracts were transferred into 250 ml beakers after saponification. Deionized water was added to the samples to a volume of 200 ml. The solutions were evaporated using a hot plate up to a volume of 150 ml to remove all the alcohol. The solution was then cooled to room temperature using tap water. The solutions transferred into 250 ml volumetric flasks and 1g of filter-Celite was added to each solution. A volume of 10 ml of 10%

$BaCl_2$ was added to the volumetric flasks containing the samples and topped up to the mark with distilled water. The mixtures were shaken vigorously until yellowish-orange precipitate was formed. Aliquots of 200 ml of the extracts were filtered into 250 ml beakers and three drops of phenolphthalein indicator were added. The filtrates were neutralized with 20% sulphuric acid. An excess of 1 ml sulphuric acid was added to precipitate the excess $BaCl_2$. White precipitate of $BaSO_4$ formed was then filtered off through whatmann filter paper no. 1 coated with a suspension of filter-Celite in a Buchner funnel aspirator. The precipitate was washed with 10 ml distilled water.

3.7.2 Separation of Chrysanthemic Acid and Pyrethric Acid

The filtrates were transferred into a 500 ml separating funnel and extracted twice with two 50 ml portions of petroleum ether. The petroleum ether layers were transferred into a 250 ml separating funnel and washed twice with 5 ml of deionized water to remove traces of aqueous layer. The aqueous layer was concentrated by evaporation to 50 ml for 1 hour. The mixture was cooled to room temperature using tap water.

3.7.3 Mercury Reduction Method for Analysis of Pyrethrins I

Petroleum ether layers were extracted twice using 5 ml 0.1M NaOH solution. The extracts were then placed in 100 ml beakers and the petroleum ether layers were discarded. A volume of 10 ml of Dinige's reagent was added to the basic extract. The 100 ml beaker and its contents were then kept for one hour in a water-bath at 25^{o}C. The mixture was removed from the water bath and immediately 3 ml of saturated NaCl solution followed by 20 ml amyl alcohol added. The saturated NaCl precipitated Mercurous Chloride. The precipitate was briefly boiled and filtered through a small filter paper carefully transferring all the precipitate to the filter paper. A volume of 10 ml amyl alcohol was added to the empty beaker and boiled briefly again to precipitate HgCl. The white precipitate of HgCl was filtered through the same filter paper. The filter paper was washed twice with 10 ml of chloroform using the same 100 ml beaker above. The filter paper was placed in a 200 ml conical flask. The beaker was washed with 50 ml of 60% HC1 aqueous solution and the sides of the beaker wiped thoroughly with cotton wool on a small glass rod before emptying both the HCl solution and the piece of cotton wool into the 200 ml conical flask-containing the filter paper and HgCl(s). An aliquot of 20 ml of $CHC1_3$ was put into the same 100

ml beaker above and added to the 200 ml conical flask contents. 1ml of iodine mono chloride indicator was added and titration with 0.01M KIO_3 solution carried out. The titration was done with constant shaking until the pink colour in the chloroform phase just disappeared. The percentage of Pyrethrins I was calculated using Equation 7.

3.7.4 Determination of Pyrethrins II

The aqueous layer containing Pyrethrins II acid moiety was transferred into a 50 ml separating funnel containing 20 g NaCl and 10ml concentrated HCl such that a saturated solution of NaCl was formed. The aqueous layer was then extracted three times with diethyl ether washing twice with 10ml portions of saturated NaCl solution to remove traces of HC1. The total diethyl ether layer was filtered to remove undissolved excess NaCl through a cotton plug and residue washed with additional 10ml of diethyl ether. The total diethyl ether extract in a 500ml conical flask was evaporated in a water-bath designed to recover the solvent. The sample was put in an oven at 60^{o}C for ten minutes. On removing the sample from the oven, a current of compressed air was blown into the flask to remove HCl fumes. The sample was dissolved in 2ml of neutral alcohol and added 20 ml of acid free water. Both the neutral alcohol and acid free water were prepared by neutralization of absolute alcohol and deionized water using dilute sodium hydroxide to make pH value to 7. Two drops of phenolphthalein indicator were then added to the conical flask containing the sample and titrated with 0.02M NaOH. The percentage of Pyrethrins II was calculated using Equation 8.

To determine the titration factor using PHP the following steps were followed: 0.8g of PHP (Wp) were weighed exactly in duplicate (A and B) and dissolved in acid free water in 200ml volumetric flasks and volumes made up to the mark. Taking one of the two samples to explain further, 10ml of the solution was pippeted into a conical flask and titrated with the prepared 0.02N NaOH solution using phenolphthalein indicator. The calculation of the titration factor was done as follows:

Let the average titre volume 0.02M NaOH = Vp (cm^3)

Let the weight of PHP = Wp (g)

Concentration of Sodium hydroxide = 0.02M

Number of moles of NaOH in Vp (cm^3)

0.02 x V/1000 moles

Number of moles in PHP in

10ml = (0.02 x Vp) / 1000

Exact weight of PHP in

10 ml = (0.02 x Vp /1000 x 204.22)

The expected weight of PHP if the concentration of sodium hydroxide is exactly 0.02N is given by

Wp/200 gml^{-1} × 10 ml = Wp/20g

Titration Factor (TF)

Expected weight of PHP calculated/ exact weight PHP used

TF= Wp X 1000/ 0.02 × Vp × 204.22 × 20…….Equation 13

TF = 12.2417 Wp/Vp

Where Wp = weight of PHP used (g)

Vp = Volume of 0.02 NaOH used on 10ml PHP (cm^3)

3.8 Sample Preparation for Analysis of Pyrethrins Content Using Ultra High Performance Liquid Chromatography

A volume of 30ml ethyl acetate was used to dissolve 10 mg of the extract in a 50 ml beaker. Filter-celite was packed in a glass column which was 5 inches long and cotton wool used to block the tapering end. The filter-celite was made wet by passing ethyl acetate through it before clean-up could begin. A volume of 5 ml ethyl acetate containing the extract was passed through the column and collected in a pear-shaped flask. To prevent the filter-celite from drying up, a lot of care was taken when handling it. After all the solution in the beaker had passed through the

column, the beaker was rinsed twice using 20 ml ethyl acetate which was then allowed to pass through the column. A volume of 2 ml ethyl acetate was used to rinse the column twice to remove pyrethrins that may have remained on the filter-celite. Ethyl acetate was completely evaporated using a rotary evaporator.

Pyrethrins from the pear-shaped flask were dissolved using methanol: water, 90:10 v/v in a 50 ml volumetric flask and the volume topped up to the mark using the same solvent, methanol: water. The solution was subjected to vigorous shaking then cotton wool used for filtration. A small amount of the filtrate was transferred into 5 ml vials and stored in a refrigerator after labelling to await UHPLC analysis.

3.8.1 Analysis of Pyrethrins Using Ultra High Performance Liquid Chromatography

Weights of 1g of the prepared filtrate were extracted in triplicates using hexane as the solvent in 50 ml volumetric flasks. Volumes were made up to the mark of the 50 ml volumetric flasks with the same solvent. The extracts were left to settle for 3 hours before injection into the instrument. This procedure was repeated the following day with the same instrument thus checking for reproducibility.

Methanol was always used to keep the UHPLC column. Before the analysis began, pumping of methanol through the system was done for about an hour with the detector on till stability was achieved. Introduction of methanol and water on a linear gradient was done in three minutes until a ratio of 90: 10, v/v of methanol: water was achieved. The UHPLC machine was left for 30 minutes to stabilize again with a flow rate of 2 ml/minute when the detector was still on. The flow rate was then reduced to 0.8 ml/minute after stability was achieved before the analysis began. After stability was achieved, the detector was zeroed which meant that the pen of the chart recorder could start at the baseline.

Aliquots of 20 microlitres were injected into the injection pot for all samples analyzed. The sample loop was connected to the column through the sample injection system. Each compound in the mixture interacted otherwise with the adsorbent material in the column which led to variation in the flow rates for the components. Separation is enhanced as the components exit the

column. Once all the pyrethrins had passed through the column i.e. elution was complete, the operation and that of the data collection by a computer was synchronized.

Data was collected after every analysis and passed through "smoothing" routine to ensure no random electronic noise and recorded as digital spectrum. Identification of the peaks of interest was done by comparison of their retention time with those of known standards. Spiking the samples with known standards was done in cases of ambiguity. The identity was confirmed by an increase in peak height. Three injections were made for each of the prepared samples and average peak areas were calculated which was used in calculation of the percentage concentrations.

Percentage pyrethrins content was calculated using Equation 14,

The percentage of Pyrethrins (w/w) = $H_s \times \frac{C_{std}}{H_{std}} \times C_s \times$ % Purity of pyrethrin standard …Equation 14

Where Hs = Average peak height of sample

H std = Average peak height of WSPE

Cs = Concentration of sample (gm/L)

Cstd = Concentration of pyrethrin standard

The experiments were done in triplicates and the average value that was used was the arithmetic mean as in Equation 15 which is the sum of all the measurements $\sum x_i$, divided by the number of measurements, n.

Mean = $x = \frac{\sum_{i=1}^{n} Xi}{n}$ …….Equation 15

A more useful measure that utilizes all the values known as the standard deviation was also calculated as stated in Equation 16.

S = $\sqrt{\frac{\sum(Xi-X)^2}{n-1}}$ …….Equation 16

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Percentage Moisture Loss during Drying of Pyrethrum Flowers

Percentage weight loss was calculated using Equation 12 after drying pyrethrum flowers using different methods. The results obtained were tabulated and analyzed.

4.1.1 Percentage Moisture Lost during Drying in an Oven to Constant Weight.

The percentage weight lost when flowers were dried to a constant weight are presented in Table 2.

Table 2: Drying of Flowers to a Constant Weight.

S/No.	Temperature (^{O}C)	Time Taken (HRS)	Percentage Moisture Lost	Log of % Moisture Lost
1	30	33	70.80	1.85
2	40	26	71.20	1.85
3	50	21	75.30	1.88
4	60	19	75.80	1.88
5	70	18	77.10	1.89
6	80	18	79.40	1.90

Pyrethrum flowers were dried to a constant weight at varying temperatures of 30, 40, 50, 60, 70 and 80 ºC. The percentage moisture content loss was between 70.80% and 79.40%. Drying of the flowers took 33 hours at a temperature of 30^{o}C and as the temperature was increased at an interval of 10^{o}C, time taken to dry the flowers reduced.

4.1.2 Percentage moisture lost during drying in an oven at constant time of 18hours.

The percentage moisture lost when flowers were dried in an oven for 18 hours were presented in Table 3.

Table 3: Percentage Moisture Lost During Drying in an Oven at Constant Time of 18hours.

S/No.	Temperature (°C)	Time Taken (Hours)	Percentage Moisture Lost	Log of Percentage Moisture Lost
1	30	18	46.74	1.67
2	40	18	51.33	1.71
3	50	18	70.65	1.85
4	60	18	79.16	1.90
5	70	18	79.39	1.90
6	80	18	80.54	1.91

Percentage moisture lost was between 46.74% and 80.54%. The percentage moisture lost increased as temperature was increasing from 30 to 80°C. The rate of moisture loss was directly proportional to the drying temperature since drying period was held constant.

The logarithm of percentage weight lost at given temperatures was plotted against time as shown in Figure 11.

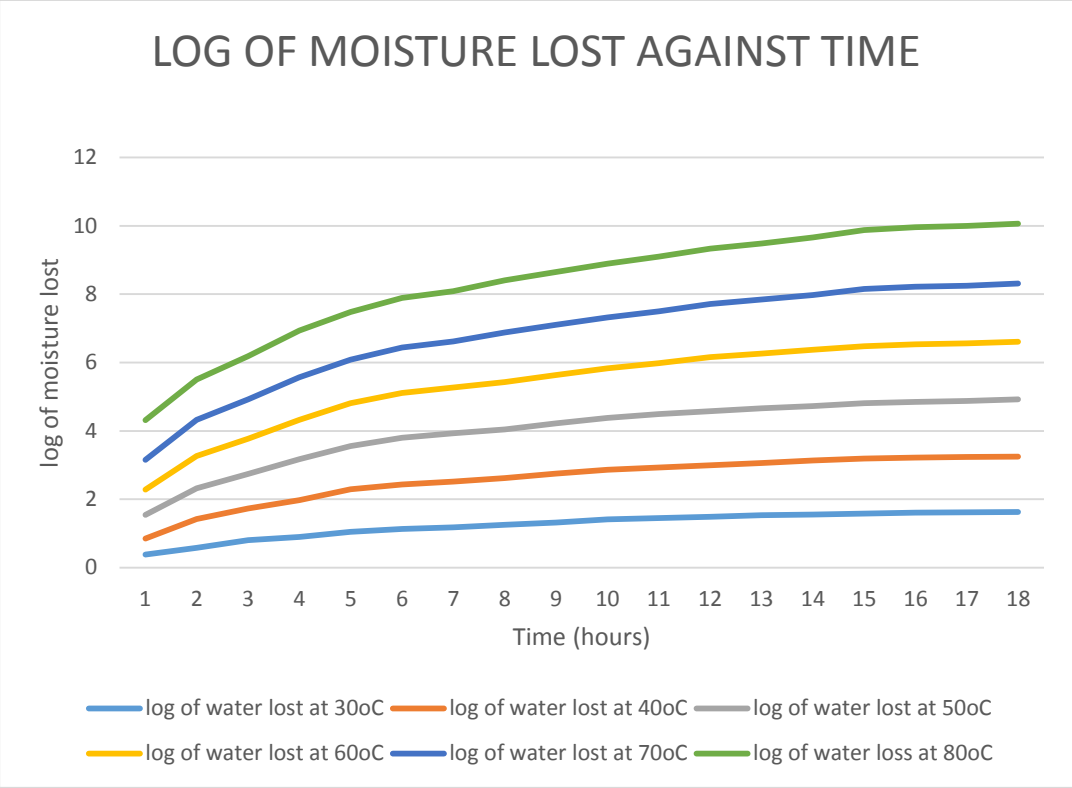


Figure 11: Graphical Representation of the Weight Lost During Drying.

The graphs in Figure 11 shows that much moisture is lost in the first one hour of drying across all the drying temperatures. The rate of moisture loss was different for the temperatures used. Temperature of 80^{o}C recorded the highest rate of moisture loss while 30^{o}C was the lowest. The rate of moisture loss after 15 hours for 70 and 80^{o}C was very low since the curved was flattening. The curves showing the rate of moisture loss for 30, 40, 50 and 60^{o}C showed that when drying was done for longer hours, much moisture could still be lost.

4.1.3 Percentage Moisture Loss of Flowers after Drying in Direct Sunlight and in the Dark

The percentage moisture loss of flowers dried in the dark and of those dried in direct sunlight are presented in Table 4.

Table 4: Percentage Moisture Loss of Flowers after Drying in Direct Sunlight and in the Dark

Drying Condition	Dried for 2 weeks	Dried to Constant Weight
Dried in direct sunlight	70.93%	71.23%
Dried in the dark	56.34%	69.45%

Percentage moisture loss for flowers dried in direct sunlight for two weeks was 70.93% while that of flowers dried to a constant weight was 71.23%. Percentage moisture loss for flowers dried in the dark for two weeks was 56.34% while that of flowers dried to a constant weight was 69.45%. The difference in percentage weight loss from flowers dried in darkness for 2 weeks was 14.59 while the difference in percentage moisture loss in drying to a constant weight was 1.78. The difference in percentage weight loss maybe due to temperature variations.

4.2 Results Obtained from Pyrethrins Analysis Using Mercury Reduction Method

Volumes obtained from titration of *Chrysanthemic* acid against a base and the percentage of Pyrethrins I are shown in Table 5.

Table 5: Titrimetric Volumes in Determining Pyrethrins I

	1	2	3
Final volume(cm^3)	10.67	21.25	31.83
Initial volume(cm^3)	0.00	10.67	21.25
Titre volume(cm^3)	10.67	10.58	10.58
Percentage pyrethrins (w/w)	0.76	0.75	0.75

The experiment was done in triplicates as shown in Table 5. Percentage of pyrethrins for each titration was calculated using equation 10. The average percentage of pyrethrins was calculated by summing up all the three percentages calculated and dividing by three i.e. 0.76 + 0.75 + 0.75 = 2.26

Average = 2.26/3 = 0.75. The percentage of Pyrethrins I for this sample was calculated as 0.75%.

Volumes obtained from titration of Pyrethric acid against a base and the percentage of Pyrethrins II are shown in Table 6.

Table 6: Titrimetric Volumes in Determining Pyrethrins II

	1	2	3
Final volume(cm^3)	10.67	20.85	31.24
Initial volume(cm^3)	0.00	10.46	20.85
Titre volume(cm^3)	10.46	10.39	10.39
Percentage pyrethrins (w/w)	0.51	0.51	0.51

The experiment was done in triplicates as shown in Table 6. Percentage of pyrethrins for each titration was calculated using Equation 11. The average percentage of pyrethrins was calculated by summing up all the three percentages calculated and dividing by three i.e. 0.51 + 0.51 + 0.51 = 0.51

Average = 0.51/3 = 0.51. The percentage of Pyrethrins II for this sample was calculated as 0.51%.

Percentage Pyrethrins I was calculated using equation 7 and Pyrethrins II were calculated using Equation 8.

4.2.1 Percentage of Pyrethrins on Drying for 18 hours in an Oven

Percentage of pyrethrins from flowers dried in the oven for 18 hours are presented in Table 7 as shown.

Table 7: Percentage of Pyrethrins on Drying for 18 hours in an Oven

S/No.	Drying Temperature (ºC)	Pyrethrins I	Pyrethrins II	Total Pyrethrins	Ratio (PI:PII)
1	30	0.44	0.31	0.75 ± 0.16%	1:0.70
2	40	0.46	0.33	0.79 ± 0.18%	1:0.72
3	50	0.79	0.58	1.37 ± 0.22%	1:0.73
4	60	0.77	0.53	1.30 ± 0.04%	1:0.69
5	70	0.73	0.47	1.20 ± 0.36%	1:0.64
6	80	0.69	0.43	1.12 ± 0.24%	1:0.62

Total pyrethrins content were found to vary with change in drying temperatures as shown in Table 7. At 40 ºC, the pyrethrins content was recorded as 0.79 ± 0.18% and at 50 ºC was 1.37 ± 0.22%. However, the total pyrethrins content as well as the Pyrethrins I: II ratio was found to reduce with increase in temperature beyond 50 ºC. Total pyrethrins were 0.75 ± 0.16% and 0.79 ± 0.18% at temperatures of 30 and 40ºC respectively due to hydrolysis of pyrethrins due to the presence of moisture in the flowers. Pyrethrins were degrading at temperatures beyond 50ºC since heat causes rearrangement of pyrethrin structures to form iso-pyrethrins.

4.2.2 Percentage of Pyrethrins on Drying to a Constant Weight in an Oven

The percentages of pyrethrins from flowers dried in an oven to a constant weight are presented in Table 8.

Table 8: Percentage of Pyrethrins on Drying to a Constant Weight in an Oven

S/No	Temperature (ºC)	Pyrethrins I	Pyrethrins II	Total Pyrethrins	Ratio (PI:PII)
1	30	0.51	0.33	0.84 ± 0.18%	1: 0.65
2	40	0.53	0.37	0.90 ± 0.21%	1: 0.70
3	50	0.83	0.61	1.44 ± 0.26%	1: 0.73
4	60	0.78	0.56	1.34 ± 0.31%	1: 0.72
5	70	0.74	0.49	1.23 ± 0.20%	1: 0.66
6	80	0.67	0.43	1.10 ± 0.22%	1: 0.64

Total pyrethrins content was found to vary with change in drying temperature, with a peak at 50 ºC and the least at 30 ºC as shown in Table 8. Total pyrethrins were 0.84 ± 0.18% and 0.90 ± 0.21% at temperatures of 30 and 40ºC respectively due to hydrolysis of pyrethrins due to the presence of moisture in the flowers. Pyrethrins were degrading at temperatures beyond 50ºC since heat causes rearrangement of pyrethrin structures to form iso-pyrethrins. Beyond 50 ºC, both extractable pyrethrins I and II were found to reduce, hence affecting the ratio and total pyrethrin content. Extractable pyrethrins II were found to reduce by 8.2% when the drying temperature was raised from 50 to 60 ºC, by 12.5% from 60 to 70 ºC and by 12.2% from 70 to 80ºC. Extractable pyrethrins I were found to reduce by 6% when the drying temperature was raised from 50 to 60 ºC, by 5% from 60 to 70 ºC and by 9.5% from 70 to 80ºC. Extractable pyrethrins II were found to reduce at a higher rate with increase in temperature beyond 50 ºC compared to pyrethrins I, thereby affecting the ratio.

4.2.3 Percentage of Pyrethrins from Flowers Dried in Direct Sunlight and in Darkness to a Constant Weight.

Results obtained from the analysis of pyrethrins from flowers dried in darkness and in direct sunlight are as shown in Table 9.

Table 9: Percentage of Pyrethrins on Drying to a Constant Weight

Drying Condition	Pyrethrins I	Pyrethrins II	Total Pyrethrins	Ratio (PI:PII)
Dried in the dark	0.81	0.57	1.38 ± 0.04%	1: 0.70
Dried in direct sunlight	0.63	0.39	1.02 ± 0.01%	1: 0.62

Total pyrethrins obtained from the flowers dried in darkness was 1.38 ± 0.04% while that of from flowers dried in direct sunlight was 1.02 ± 0.01% as shown in Table 9. Pyrethrins content obtained from flowers dried in the dark exceeded that from the flowers dried in direct sunlight by 0.36. This shows that drying flowers in the dark helps in retaining a lot of extractable pyrethrins content. This is due to the presence of the UV in the sunlight. The resonance conjugation of the unsaturated side chain with the cyclopropane ring encampasses the disappearance of the vital activated methylene next to the ring and so reduced biological activity in iso-pyrethrins results.

4.2.4 Percentage of Pyrethrins from Flowers Dried for Two Weeks

Percentage of pyrethrins from flowers died in darkness and those dried in direct sunlight for two weeks are as presented in Table 10.

Table 10: Percentage of Pyrethrins from Flowers Dried for Two Weeks

Drying Condition	Pyrethrins I	Pyrethrins II	Total Pyrethrins	Ratio (PI:PII)
Dried in the dark	0.59	0.42	1.01 ± 0.02%	1: 0.71
Dried in direct sunlight	0.50	0.36	0.86 ± 0.01%	1: 0.72

Total pyrethrins obtained from the flowers dried in darkness was 1.01 ± 0.02% while that of from flowers dried in direct sunlight was 0.86 ± 0.01% as presented in Table 10. Pyrethrins content obtained from flowers dried in the dark exceeded that from the flowers dried in direct sunlight

by 0.15. The percentage of pyrethrins from flowers dried for two weeks were lower than those from flowers dried to a constant weight. This was due to the presence of moisture in the flowers dried for two weeks which may have hydrolyzed pyrethrins giving lower yields.

4.3 Comparison of the Percentage of Pyrethrins I and Pyrethrins II Concentrations on Drying for 18 hours in the Oven.

Figure 12 shows the comparison of Pyrethrins I and Pyrethrins II from the flowers dried in the oven for 18 hours.

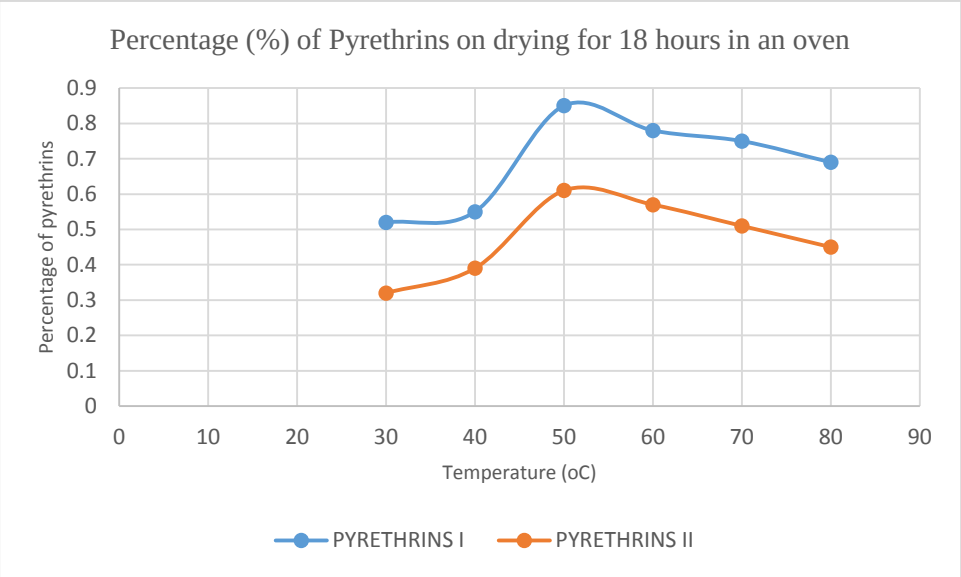


Figure 12: Percentage of Pyrethrins on Drying the Flowers for 18 hours in an Oven

4.3.1 Comparison of the Percentage of Pyrethrins I and Pyrethrins II Concentrations.

The concentration of Pyrethrins I and Pyrethrins II as analyzed by the titrimetric method were compared as shown in Figure 13.

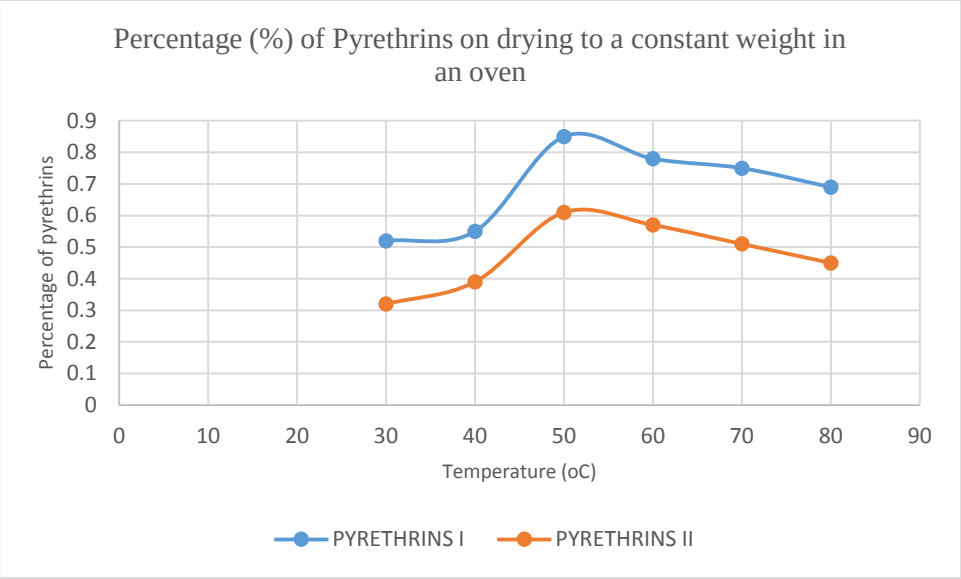


Figure 13: Comparison of the Percentage of Pyrethrins I and Pyrethrins II Concentrations Analyzed Using Titrimetric Method.

The concentration of Pyrethrins I was above that of Pyrethrins II as shown in Figure 12 and Figure 13. The concentrations of pyrethrins rose with an increase in temperature from 30 and reached a peak at 50ºC. The concentration of pyrethrins decreased past a temperature of 50 up to 80ºC.

4.4 Results Obtained from Pyrethrins Analysis Using Ultra-High Performance Liquid Chromatographic Method

Ultra-High Performance Liquid Chromatographic analyses was carried out on the pyrethrin extracts. The illustrative chromatograms attained are presented in Figures 14 and Figure 15.

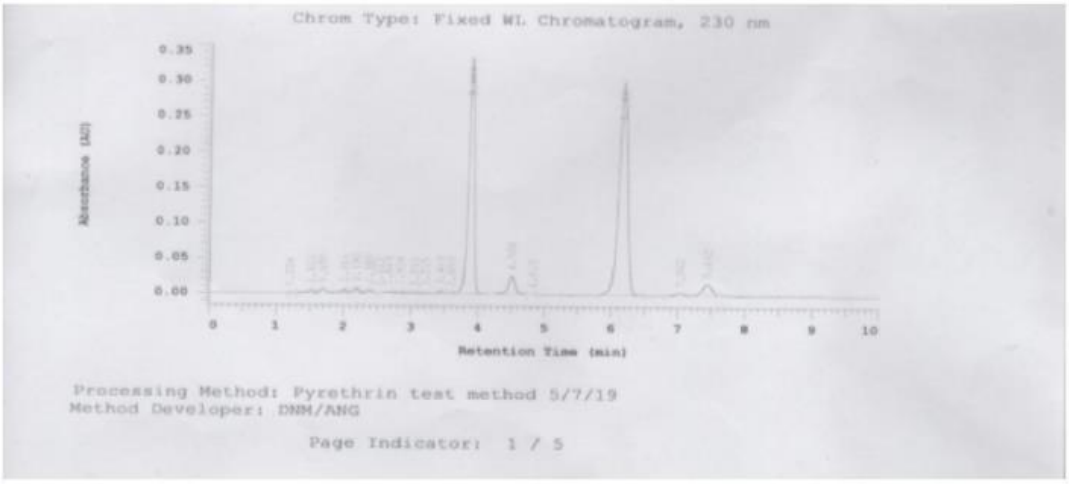


Figure 14: Chromatograms of 50% w/w Pyrethrins Standard

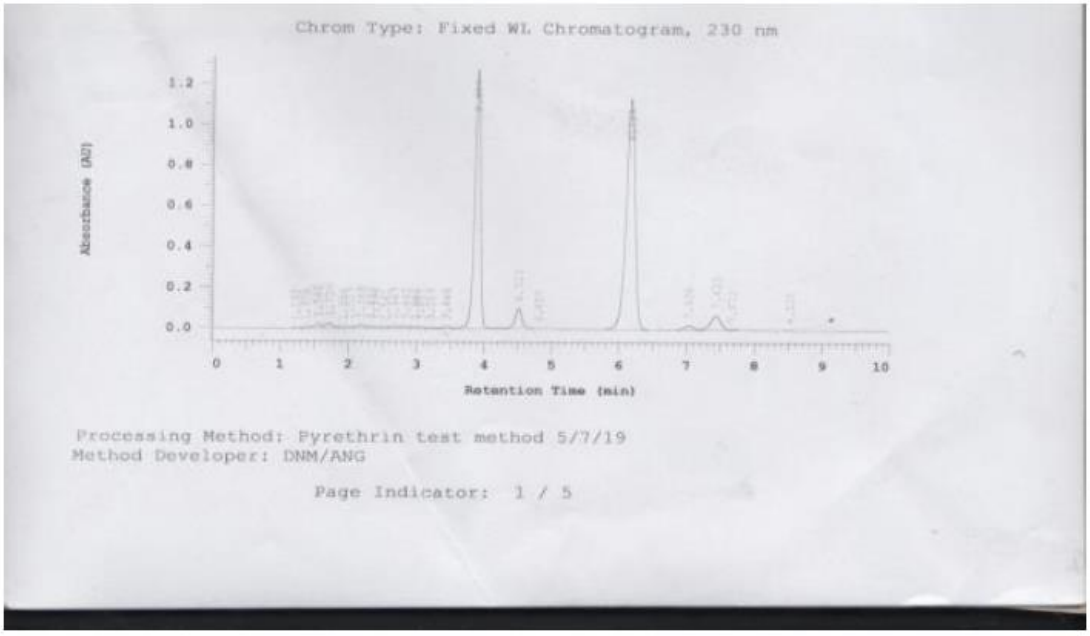


Figure 15: Chromatogram of Sample Dried at 50˚C to Constant Weight.

Percentage of pyrethrins on drying to constant weight obtained from Ultra High Performance Liquid Chromatography are presented in Table 11.

Table 11: Percentage of Pyrethrins Obtained from Ultra High Performance Liquid Chromatography Analysis

S/No.	Temperature(ºC)	Pyrethrins I	Pyrethrins II	Total Pyrethrins	Ratio(PI:PII)
1	30	0.52	0.32	0.84 ± 0.14%	1:0.62
2	40	0.55	0.39	0.94 ± 0.18%	1:0.71
3	50	0.85	0.61	1.46 ± 0.26%	1:0.72
4	60	0.78	0.57	1.35 ± 0.36%	1:0.73
5	70	0.75	0.51	1.26 ± 0.15%	1:0.68
6	80	0.69	0.45	1.14 ± 0.26%	1:0.65

Total pyrethrins content was found to vary with change in drying temperature, with a peak at 50 ºC and least at 30 ºC as in Table 11. At 30 ºC, the pyrethrins content was found to be 0.84 ± 0.14% and 1.46 ± 0.26% at 50 ºC. Thus, there were more pyrethrins I and II when flowers were dried at 50 than 30 ºC. However, beyond 50 ºC, both extractable pyrethrins I and II were found to reduce, hence affecting the ratio and total pyrethrin content. Extractable pyrethrins II were found to reduce at a higher rate with increase in temperature beyond 50 ºC compared to pyrethrins I, thereby affecting the ratio.

4.4.1 Percentage of Pyrethrins on Drying to a Constant Weight in an Oven Analyzed Using Ultra High Performance Liquid Chromatography.

Percentage of pyrethrins obtained from flowers dried to a constant weight in an oven and analyzed using Ultra High Performance Liquid Chromatography is shown in Figure 16.

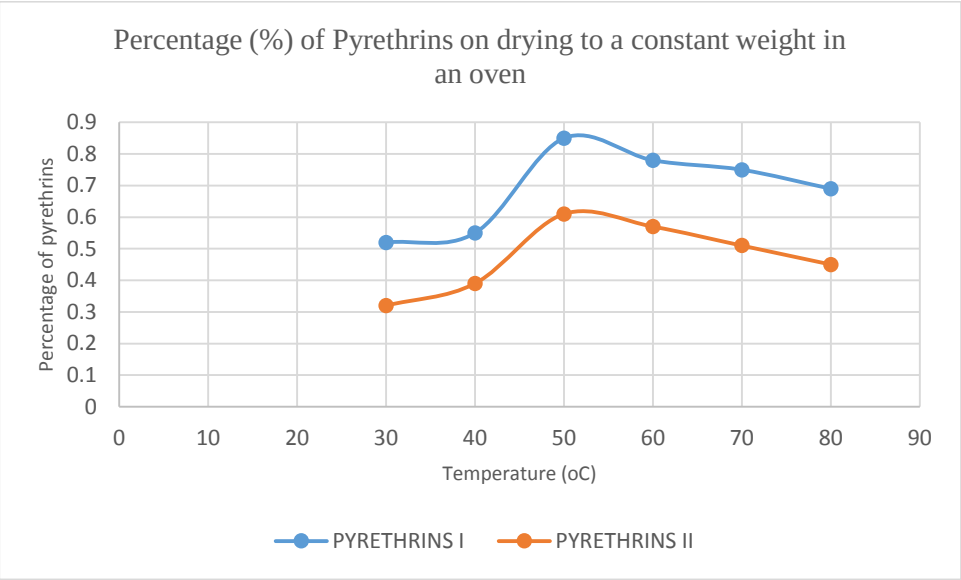


Figure 16: Percentage of Pyrethrins on Drying to a Constant Weight in an Oven Analyzed Using Ultra High Performance Liquid Chromatography.

Concentration of Pyrethrins I is more than that of Pyrethrins II as shown in Figure 16. This confirms that the results obtained from both methods are comparable since similar trend was observed. In this case, the average ratio of PI:PII was found to be 1:0.68. The standard value of the PI:PII ratio cited in literature is 1:0.70 indicating that the two methods of analysis used in this research were within the cited range.

Table 12: Percentage of Pyrethrins on Drying to a Constant Weight

Drying Condition	Pyrethrins I	Pyrethrins II	Total Pyrethrins	Ratio (PI:PII)
Dried in the dark	0.83	0.58	1.41 ± 0.03%	1: 0.70
Dried in direct sunlight	0.65	0.41	1.06 ± 0.12%	1: 0.63

Total pyrethrins obtained from the flowers dried in the dark was 1.41 ± 0.03% while that of from flowers dried in direct sunlight was 1.06 ± 0.12% as shown in Table 12. Pyrethrins content obtained from flowers dried in the dark exceeded that from the flowers dried in direct sunlight by 0.35. This shows that drying flowers in the dark helps in retaining extractable pyrethrins content.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The percentage of pyrethrins extracts from flowers dried for 21 hours in an oven at 50^{O}C was 1.44% and 1.37% for 18 hours. Drying pyrethrum flowers at 30^{O}C for 18 hours yielded 0.75% of pyrethrins extract. Percentage pyrethrins extracts were found to be marginally increase to 0.84% when flowers were dried at 30^{O}C for 33 hours to a moisture content of 8%. Drying at 50^{O}C for 21 hours achieved moisture content of 8% and 13% in 18 hours. A moisture content of 27% was achieved when pyrethrum flowers were dried at 30^{O}C for 18 hours which yielded 0.75% pyrethrin extracts. Pyrethrum flowers dried to a moisture content of 8%, yielded a higher concentration of pyrethrins extract of 0.07% compared to those of 13%.

Titrimetric method was found to be 98% accurate when referenced to the Ultra High Performance Chromatography. The titrimetric method reported 0.83% of Pyrethrins I while Ultra High Performance Liquid Chromatography 0.85%. This indicates that titrimetric method of analysis is as accurate and can be used for routine pyrethrum flowers quality assessment with Ultra High Performance Liquid Chromatography as a confirmatory test. The amount of Pyrethrins I were found to be higher than Pyrethrins II with an average ratio PI: PII being 25:17.

The total pyrethrins content were found to decrease with increase in drying temperature beyond 50^{O}C. An increase by 30^{O}C corresponded with a decrease of 22% of pyrethrins content. The respective pyrethrins content were found to decrease at varying percentage with Pyrethrins II being most affected with 8.2% reduction when drying temperature was raised from 50 to 60^{O}C and Pyrethrins I by 2.5%. This shows that an increase in temperature beyond 50^{O}C affects the content of extractable pyrethrins, the PI:PII ratio and hence the efficacy.

Pyrethrum flowers dried in darkness at temperatures below 50^{O}C yielded a pyrethrins extract of 1.41% compared to 1.06% from those under direct sunlight. Total pyrethrins extracts obtained from the flowers dried at controlled temperatures in the oven was 0.42% more than from those under direct sunlight. Pyrethrum flowers should therefore be dried in darkness at a temperature of 50^{O}C to a constant moisture content of 8% for retention of maximum extractable pyrethrins.

5.2 RECOMMENDATION

Based on the findings and discussions from this research, the following recommendations are proposed to retain maximum extractable pyrethrins content and improve their efficacy:

I. The current drying methods of pyrethrum flowers should be reviewed such that a temperature of $50^{O}C$ is used until a moisture content of 8% is achieved for retention of maximum extractable pyrethrins content.

II. Pyrethrum flowers should not be exposed to sunlight during drying.

III. Grinding of the flowers is to be in a room where drying was done to prevent pyrethrins loss due to maintenance of its concentration within the location.

IV. Researchers should improve on the extraction methods to retain more of Pyrethrins II such that the ratio of Pyrethrins I: Pyrethrins II is 1:1.

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APPENDICES

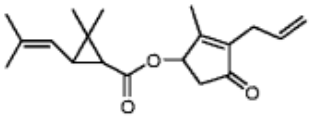
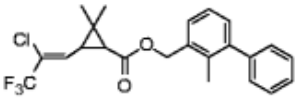
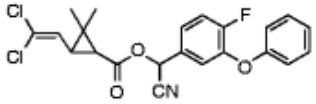
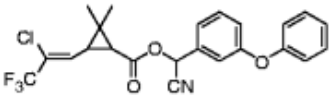
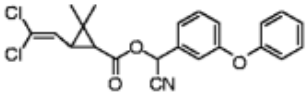
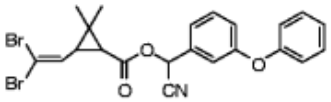
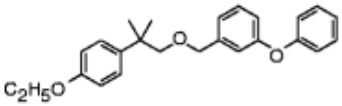
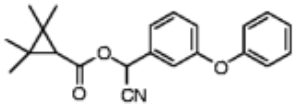
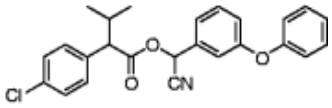
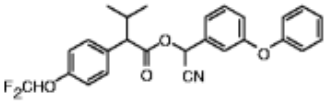
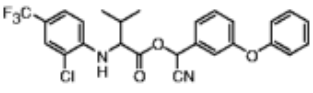
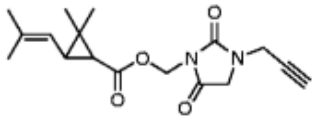
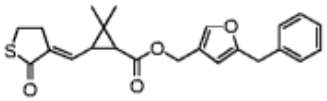
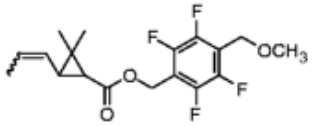
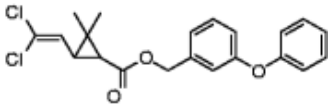
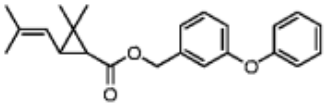
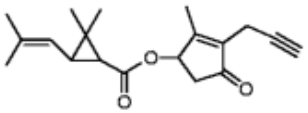
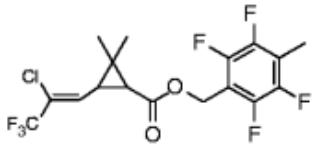
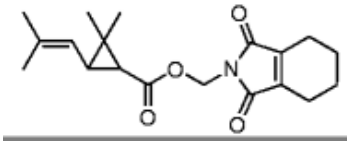
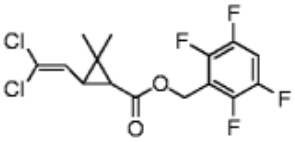
Appendix 1: Tables Showing Moisture lost by drying pyrethrum flowers at constant at different temperatures.

TIME (HRS)	40^{O}C(moisture lost in g)	log of water lost at 40OC	50^{O}C(moisture lost in g)	log of moisture lost at 50OC	60^{O}C(moisture lost in g)	log of moisture lost at 60OC	70^{O}C(moisture lost in g)	log of moisture lost at 70OC
1	2.96	0.47	4.89	0.69	5.54	0.74	7.48	0.87
2	6.99	0.84	7.98	0.9	8.95	0.95	11.14	1.05
3	8.56	0.93	10.27	1.01	10.61	1.03	14.03	1.15
4	11.68	1.07	15.77	1.2	14.16	1.15	17.59	1.25
5	17.39	1.24	18.73	1.27	17.94	1.25	18.81	1.27
6	20.17	1.3	23.27	1.37	20.28	1.31	21.46	1.33
7	22.11	1.34	25.6	1.41	22.09	1.34	22.54	1.35
8	23.26	1.37	26.55	1.42	24.61	1.39	28.03	1.45
9	27.19	1.43	29.41	1.47	25.74	1.41	29.83	1.47
10	27.9	1.45	32.91	1.52	28.26	1.45	30.72	1.49
11	29.88	1.48	36.59	1.56	30.83	1.49	33.48	1.52
12	31.55	1.5	38.81	1.59	37.63	1.58	35.34	1.55
13	33.88	1.53	39.91	1.6	39.5	1.6	37.7	1.58
14	38.07	1.58	40.18	1.6	43.38	1.64	40.66	1.61
15	40.77	1.61	41.59	1.62	46.95	1.67	46.94	1.67
16	40.99	1.61	42.84	1.63	47.72	1.68	48.44	1.69
17	41.22	1.62	43.97	1.64	48.31	1.68	49.34	1.69
18	41.38	1.62	46.69	1,67	48.73	1.69	50.13	1.7

TIME (HOURS)			TEMPERATURES						
	30	40	50	60	70	80			
1	2.97	3.65	4.79	6.47	7.92	8.81			
2	5.92	7.11	7.93	10.01	11.37	12.11			
3	7.61	10.24	11.07	13.27	14.23	16.3			
4	9.17	11.97	13.63	16.43	17.36	19.57			
5	11.25	14.26	17.99	18.13	20.17	22.12			
6	13.41	18.01	19.67	20.31	23.61	26.44			
7	16.73	20.43	22.82	23.66	25.33	29.36			
8	19.11	23.16	25.41	25.92	28.43	32.19			
9	23.68	25.75	27.79	28.13	31.92	35.72			
10	25.37	28.28	30.63	31.29	35.62	39.17			
11	27.83	32.29	33.22	33.94	39.01	45.01			
12	31.61	36.17	37.01	37.22	42.36	49.62			
13	33.48	39.31	40.77	41.36	49.17	53.17			
14	35.98	42.65	43.03	43.72	51.29	55.66			
15	38.12	44.18	46.23	47.63	53.92	57.31			
16	41.25	46.61	47.38	48.31	54.07	58.61			
17	43.71	47.37	48.61	48.94	54.11	58.93			
18	45.29	48.09	49.27	49.89	54.12	58.95			
19	45.73	48.97	49.69	50.11					
20	46.01	49.13	49.92						
21	46.33	49.43	49.97						
22	46.67	49.67	49.97						
23	46.92	49.86							
24	47.33	49.91							
25	47.78	49.96							
26	47.95	49.97							
27	48.36								
28	48.39								
29	48.61								
30	48.73								
31	48.79								
32	48.81								
33	48.83								

TIME (HOURS)	log of water lost at 30oC	log of water lost at 40oC	log of water lost at 50oC	log of water lost at 60oC	log of water lost at 70oC	log of water at 80oC
	0.47	0.56	0.68	0.81	0.9	0.94
1	0.77	0.85	0.9	1	1.06	1.08
2	0.88	1.01	1.04	1.12	1.15	1.21
3	0.96	1.08	1.13	1.22	1.24	1.29
4	1.05	1.15	1.26	1.26	1.3	1.34
5	1.13	1.26	1.29	1.31	1.37	1.42
6	1.22	1.31	1.36	1.37	1.4	1.47
7	1.28	1.36	1.41	1.41	1.45	1.51
8	1.37	1.41	1.44	1.45	1.5	1.55
9	1.4	1.45	1.49	1.5	1.55	1.59
10	1.44	1.51	1.52	1.53	1.59	1.65
11	1.5	1.56	1.57	1.57	1.63	1.7
12	1.52	1.59	1.61	1.62	1.69	1.73
13	1.56	1.63	1.63	1.64	1.71	1.75
14	1.58	1.65	1.66	1.68	1.73	1.76
15	1.62	1.67	1.68	1.68	1.73	1.77
16	1.64	1.68	1.68	1.69	1.73	1.77
17	1.66	1.68	1.69	1.7	1.73	1.77
18	1.66	1.69	1.7	1.7		
19	1.66	1.69	1.7			
20	1.67	1.69	1.7			
21	1.67	1.7	1.7			
22	1.67	1.7				
23	1.68	1.7				
24	1.68	1.7				
25	1.68	1.7				
26	1.68					
27	1.68					
28	1.69					
29	1.69					
30	1.69					
31	1.69					
32	1.69					
33						

Appendix 2: Table Showing Chemical Structures of Synthetic Pyrethroids

1) allethrin; racemic 	**2)** bifenthrin; (*Z*)-(1*RS*)-cis 	**3)** cyfluthrin; racemic 
4) cyhalothrin; (*Z*)-(1*RS*)-cis, (*αRS*) 	**5)** cypermethrin; racemic 	**6)** deltamethrin; (1*R*)-cis, (*αS*) 
7) etofenprox 	**8)** fenpropathrin; racemic 	**9)** fenvalerate; racemic 
10) flucythrinate; (2*S*),(*αRS*) 	**11)** fluvalinate; (2*R*),(*αRS*) 	**12)** imiprothrin; (1*R*)-*trans*/*cis* (4:1) 
13) kadethrin; (*E*)-(1*S*)-*cis* 	**14)** metofluthrin; racemic, (*Z*)-rich 	**15)** permethrin; racemic 
16) phenothrin; racemic 	**17)** prallethrin; racemic 	**18)** tefluthrin; (*Z*)-(1*RS*)-*cis* 
19) tetramethrin; racemic 	**20)** transfluthrin; (1*S*)-*trans* 	

Appendix 4: Chromatograms showing concentrations of pyrethrins.

Flowers dried at 30ºC

CSM: Samples Series: 0101 Report Name: original System: Sys 1

ChromasterUltra Rs System Manager Report

Analyzed Date and Time: 02/26/2020 04:31:24 PM Reported Date and Time: 02/27/2020 02:50:06 PM

Processed Date and Time: 02/26/2020 04:41:29 PM

Data Path: C:\Win32app\ChromasterRs\samples\DATA\0101\

Processing Method: Pyrethrin test method 5/7/19

System (acquisition): Sys 1 Series: 0101

Application(data): Samples Vial Number: 32

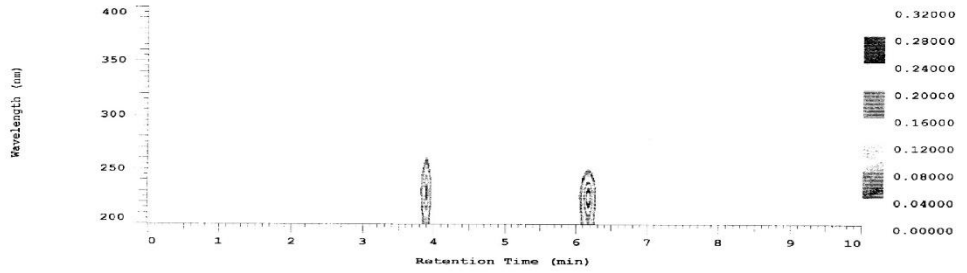
Sample Name: 30 Deg HPLC Vial Type: UNK

Injection from this vial: 1 of 1 Volume: 10.0 ul

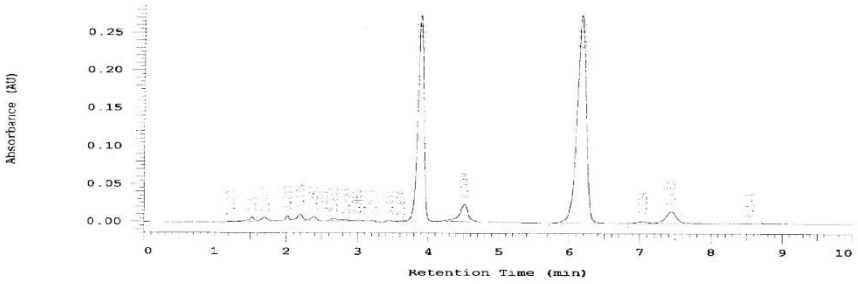
Sample Description: 30 Deg HPLC

Absorbance Mode: NORMAL(2.0 AU) Absorbance Scale: Auto

Spectral Bandwidth: 4 nm Spectral Interval: 50 ms



Chrom Type: Fixed WL Chromatogram, 230 nm



Processing Method: Pyrethrin test method 5/7/19

Method Developer: DNM/ANG

Page Indicator: 1 / 5

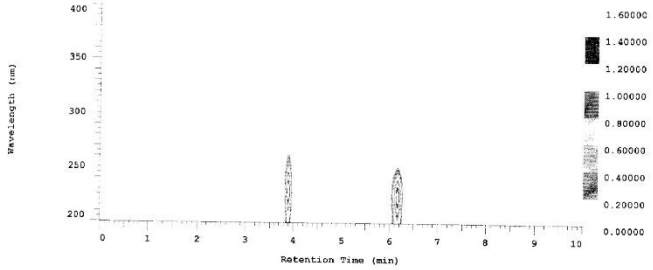
Flowers dried at 40ºC

CSM: Samples Series: 0096 Report Name: original System: Sys 1

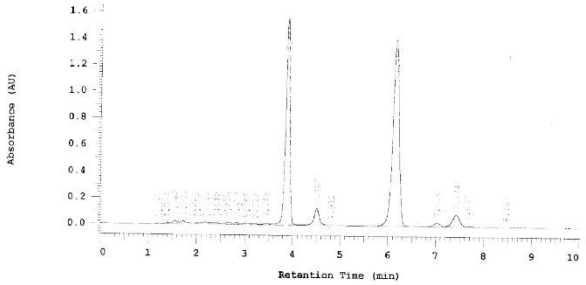
ChromasterUltra Rs System Manager Report

Analyzed Date and Time: 02/26/2020 03:32:56 PM
Reported Date and Time: 02/27/2020 02:48:18 PM
Processed Date and Time: 02/26/2020 03:43:03 PM
Data Path: C:\Win32app\ChromasterRs\samples\DATA\0096\
Processing Method: Pyrethrin test method 5/7/19
System (acquisition): Sys 1
Application(data): Samples
Sample Name: 40 Deg HPLC
Injection from this vial: 1 of 1
Sample Description: 40 Deg HPLC
Series: 0096
Vial Number: 27
Vial Type: UNK
Volume: 10.0 ul

Absorbance Mode: NORMAL(2.0 AU)
Spectral Bandwidth: 4 nm
Absorbance Scale: Auto
Spectral Interval: 50 ms



Chrom Type: Fixed WL Chromatogram, 230 nm



Processing Method: Pyrethrin test method 5/7/19
Method Developer: DNM/ANG

Page Indicator: 1 / 5

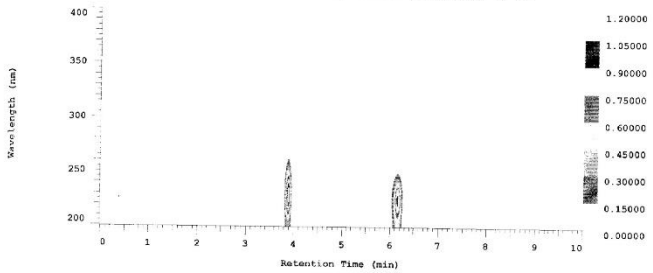
Flowers dried at 60ºC

CSM: Samples Series: 0099 Report Name: original System: Sys 1

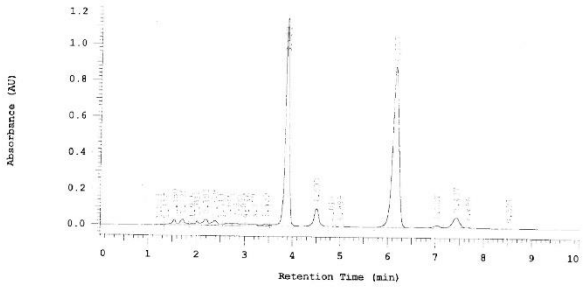
ChromasterUltra Rs System Manager Report

Analyzed Date and Time: 02/26/2020 04:07:58 PM
Reported Date and Time: 02/27/2020 02:49:33 PM
Processed Date and Time: 02/26/2020 04:18:07 PM
Data Path: C:\Win32app\ChromasterRs\samples\DATA\0099\
Processing Method: Pyrethrin test method 5/7/19
System (acquisition): Sys 1
Series: 0099
Application(data): Samples
Vial Number: 30
Sample Name: 60 Deg HPLC
Vial Type: UNK
Injection from this vial: 1 of 1
Volume: 10.0 ul
Sample Description: 60 Deg HPLC

Absorbance Mode: NORMAL(2.0 AU)
Absorbance Scale: Auto
Spectral Bandwidth: 4 nm
Spectral Interval: 50 ms



Chrom Type: Fixed WL Chromatogram, 230 nm



Processing Method: Pyrethrin test method 5/7/19
Method Developer: DNM/ANG

Page Indicator: 1 / 5

Flowers dried at 70ºC

CSM: Samples Series: 0092 Report Name: original System: Sys 1

ChromasterUltra Rs System Manager Report

Analyzed Date and Time: 02/26/2020 02:46:10 PM
Reported Date and Time: 02/27/2020 02:46:51 PM
Processed Date and Time: 02/26/2020 02:56:20 PM

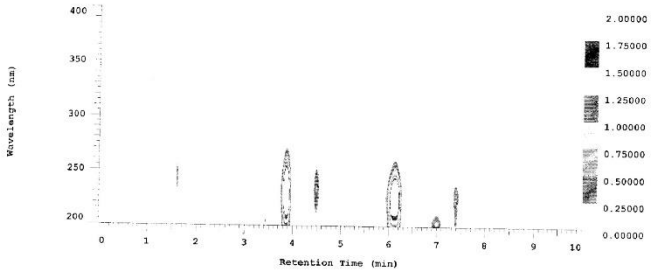
Data Path: C:\Win32app\ChromasterRs\samples\DATA\0092\
Processing Method: Pyrethrin test method 5/7/19

System (acquisition): Sys 1
Application(data): Samples
Sample Name: 70 Deg HPLC
Injection from this vial: 1 of 1
Sample Description: 70 Deg HPLC

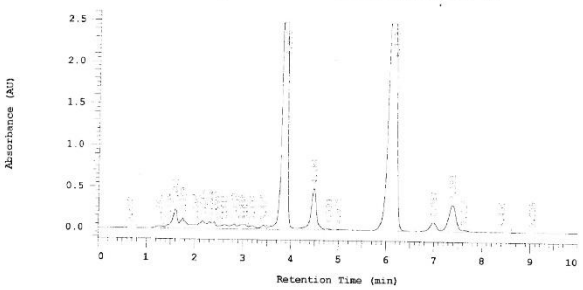
Series: 0092
Vial Number: 23
Vial Type: UNK
Volume: 10.0 ul

Absorbance Mode: NORMAL(2.0 AU)
Spectral Bandwidth: 4 nm

Absorbance Scale: Auto
Spectral Interval: 50 ms



Chrom Type: Fixed WL Chromatogram, 230 nm



Processing Method: Pyrethrin test method 5/7/19
Method Developer: DNM/ANG

Page Indicator: 1 / 5

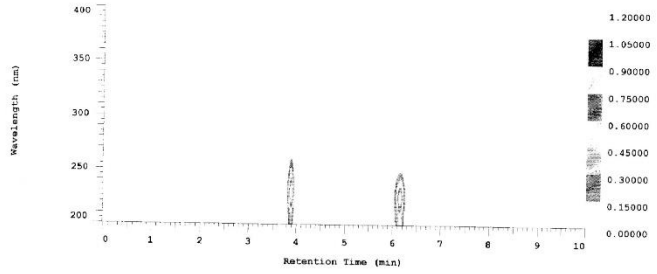
Flowers dried at 80ºC

CSM: Samples Series: 0094 Report Name: original System: Sys 1

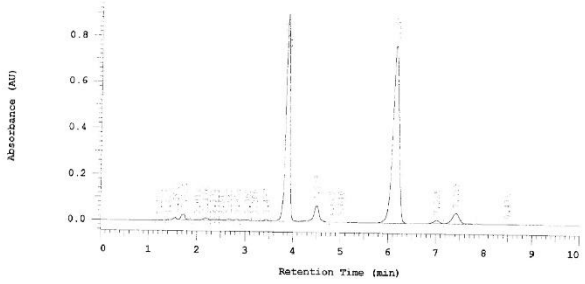
ChromasterUltra Rs System Manager Report

Analyzed Date and Time: 02/26/2020 03:09:34 PM
Reported Date and Time: 02/27/2020 02:47:38 PM
Processed Date and Time: 02/26/2020 03:19:41 PM
Data Path: C:\Win32app\ChromasterRs\samples\DATA\0094\
Processing Method: Pyrethrin test method 5/7/19
System (Acquisition): Sys 1
Application(data): Samples
Sample Name: 80 Deg HPLC
Injection from this vial: 1 of 1
Sample Description: 80 Deg HPLC
Series: 0094
Vial Number: 25
Vial Type: UNK
Volume: 10.0 ul

Absorbance Mode: NORMAL(2.0 AU)
Spectral Bandwidth: 4 nm
Absorbance Scale: Auto
Spectral Interval: 50 ms



Chrom Type: Fixed WL Chromatogram, 230 nm



Processing Method: Pyrethrin test method 5/7/19
Method Developer: DNM/ANG

Page Indicator: 1 / 5

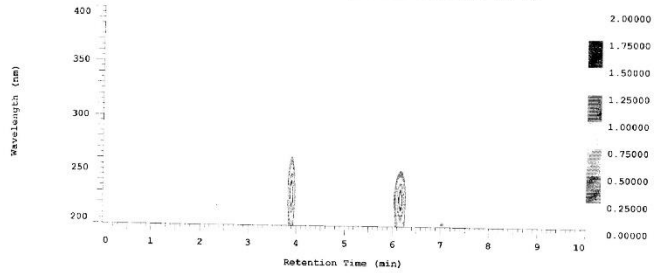
Flowers dried in the dark

CSM: Samples Series: 0093 Report Name: original System: Sys 1

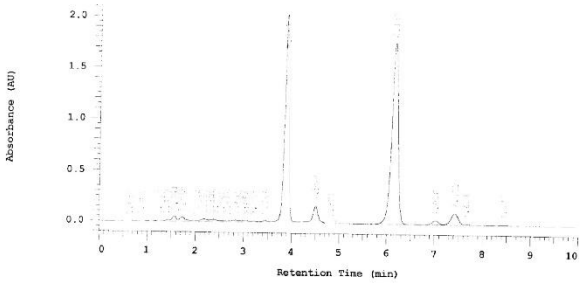
ChromasterUltra Rs System Manager Report

Analyzed Date and Time: 02/26/2020 02:57:53 PM
Reported Date and Time: 02/27/2020 02:47:17 PM
Processed Date and Time: 02/26/2020 03:08:00 PM
Data Path: C:\Win32app\ChromasterRs\samples\DATA\0093\
Processing Method: Pyrethrin test method 5/7/19
System (acquisition): Sys 1
Application(data): Samples
Sample Name: Darkness HPLC
Injection from this vial: 1 of 1
Sample Description: Darkness HPLC
Series: 0093
Vial Number: 24
Vial Type: UNK
Volume: 10.0 ul

Absorbance Mode: NORMAL(2.0 AU)
Spectral Bandwidth: 4 nm
Absorbance Scale: Auto
Spectral Interval: 50 ms



Chrom Type: Fixed WL Chromatogram, 230 nm



Processing Method: Pyrethrin test method 5/7/19
Method Developer: DNM/ANG

Page Indicator: 1 / 5

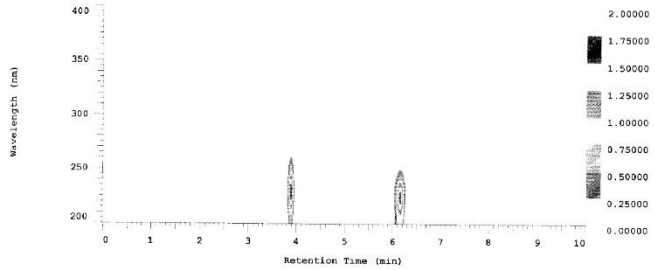
Flowers dried in direct sunlight

CSM: Samples Series: C095 Report Name: original System: Sys 1

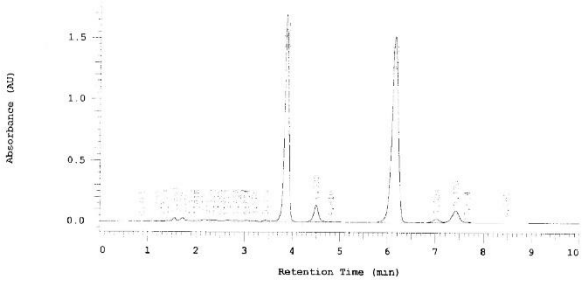
ChromasterUltra Rs System Manager Report

Analyzed Date and Time: 02/26/2020 03:21:15 PM
Reported Date and Time: 02/27/2020 02:48:01 PM
Processed Date and Time: 02/26/2020 03:31:21 PM
Data Path: C:\Win32app\ChromasterRs\samples\DATA\C095\
Processing Method: Pyrethrin test method 5/7/19
System (acquisition): Sys 1
Series: C095
Application(data): Samples
Vial Number: 26
Sample Name: Direct Sunlight HPLC
Vial Type: UNK
Injection from this vial: 1 of 1
Volume: 10.0 ul
Sample Description: Direct Sunlight HPLC

Absorbance Mode: NORMAL(2.0 AU)
Absorbance Scale: Auto
Spectral Bandwidth: 4 nm
Spectral Interval: 50 ms



Chrom Type: Fixed WL Chromatogram, 230 nm



Processing Method: Pyrethrin test method 5/7/19
Method Developer: DKM/ANG

Page Indicator: 1 / 5

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Pyrethrum (*Chrysanthemum cinerariifolium)* Flowers' Drying Conditions for Optimum Extractable Pyrethrins Content

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Abstract

Pyrethrum flowers of the genus *Chrysanthemum cinerariifolium* are grown in Kenya by small scale farmers for extraction of pyrethrins, a natural insecticide's active ingredient. Pyrethrins are classified in two groups, Pyrethrins I and II, and are degradable when exposed to air, moisture and high temperatures. The contents and ratio of Pyrethrins I:II determine the efficacy of the insecticide. Therefore, drying of the pyrethrum flowers should be optimized in order to attain maximum extractable pyrethrins content and optimum ratio. The aim of this research was to optimize the drying temperatures, time and moisture content of pyrethrum flowers. The flowers were harvested and dried at varying temperatures of 40, 50, 60 and 70 ℃ to total dryness. Another set of flowers were harvested and dried in the oven at the same temperatures for a maximum period of 18 hrs. Moisture content was determined at each temperature, at intervals of one hour. The dried flowers were then ground into fine powder and extracted using Soxhlet extraction method with hexane. The extracts were refined and analyzed by Mercury reduction and High Performance Liquid Chromatographic methods. Pyrethrum flowers were found to achieve maximum moisture loss, at varying times and temperature with 70 ℃ recording the shortest time of 18 hrs. The yield of pyrethrins obtained on drying the flowers to constant weight at 40 ℃ was 0.90% while drying for 18 hrs yielded 0.79%. Extractable Pyrethrins II were found to reduce by 8.6% when the drying temperature was raised from 50 to 60 ℃ and by 11.3% from 60 to 70 ℃. Extractable Pyrethrins I were found to reduce by 6% when the drying temperature was raised from 50 to 60 ℃ and by 5% from 60 to 70 ℃. The total pyrethrins obtained from the flowers dried at 50 ℃ were found to be 1.37% at 18 hrs and 1.44% to constant weight drying. The pyrethrins I:II ratio was found to vary over the temperature range 40-70^{O}C.The optimum temperature and time for drying pyrethrum flowers was found to be 50 ℃ for 21 hrs.

Keywords: *Chrysanthemum cinerariifolium*, pyrethrins I:II ratio, drying, temperature, time

1. Introduction

Pyrethrum is a plant genus classified as *Chrysanthemum* or *Tanacetum* and is commercially cultivated for its flowers that are used to produce natural insecticides. The natural insecticide extracted from the dried flowers of *C. cinerariifolium* or *C. coccineum*are referred to as pyrethrins. *Chrysanthemum cinerariifolium* is the genus grown in Kenya and has white flowers, with yellow center that sprout from the stiff stems. The stems have bluish-green leaves and can grow to about 46-100 cm tall (Elliott, 2007). The growing of pyrethrum flowers in Kenya is of great economic value as it is an income generating activity for small scale farmers and also earns the country foreign exchange through the export of pyrethrins. Processing the flowers to extract pyrethrins is a long process involving many actors, such as agronomists, farmers, chemical engineers, chemists and entomologists. Processing starts with picking of the mature flowers with horizontal petals. The flowers are then dried at optimum conditions to total dryness and then ground into fine particles before extraction. Finely ground flower powder is better suited for use as an insecticide but the coarsely ground have long shelf life and deteriorates less. The dried flowers are then transported to the processing factories for extraction of pyrethrins which are the active components and sold as oleoresin (Ang'endu, 1994).

Great interest was taken in the various methods used in drying the flowers and the thermal effect on the stability of the active components of the flowers. Small scale farmers always spread the flowers on polythene sheets in direct sunlight or in a shade for about two weeks. More advanced farmers always use solar driers or roasters to hasten the drying process due to the quantity of the produce. In Japan, the flowers are hung upside down to dry

which increases pyrethrin concentration. In the recent past due to the technological developments, use of oven was adopted as a faster and more efficient method of drying the flowers. During this process, drying temperature and moisture content of the flowers are of great significance and should be monitored. Research shows that pyrethrins are thermally and photochemically unstable. Heat causes rearrangements of the pyrethrins structure to form iso-pyrethrins which are insecticidally inactive. There are high chances that the resonance conjugation of the unsaturated side chain with the cyclopropane ring encampasses the disappearance of the vital activated methylene next to the ring and so reduces biological activity in iso-pyrethrins results (Wang, 2017). Pyrethrins are classified in two groups, Pyrethrins I and II. Pyrethrins I is composed of pyrethrin 1, jasmolin 1 and cinerin 1 while Pyrethrins II is composed of pyrethrin 2, jasmolin 2 and cinerin 2. The structures of the pyrethrins are shown in Figure 1.

Pyrethrins "knockdown" and "kill" insects by delaying the closure of voltage gated sodium ion channels in the nerve cells. Insecticidal and insect repellant properties of these compounds have been known for millennia and *chrysanthemum* species have long been cultivated for this purpose. Pyrethrins I are responsible for the knockdown and pyrethrins II for killing the insects. The knowledge of the ratio of pyrethrins I to pyrethrins II is of great importance since it helps in determining the efficacy of the insecticide. The highest efficacy is achieved with a pyrethrins I: II ratio of 1:1 (Wagner, 2000).

Pyrethrins as an insecticide is always applied in places where animals and human are present and therefore their toxicity must be considered. Pyrethrum as a mixture of cinerin, pyrethrin and jasmolin can be inhaled by people in their workplaces, it can get into the eye or the skin or it can be swallowed. A legal limit for pyrethrum exposure to humans has been set as 5mg/m^3 over an 8 hours workday (Costa, 2015). Pyrethrum becomes dangerous to health at the levels of 5000 mg/m^3. Symptoms as pruritus (itching), dermatitis, papules, erythema, rhinorrhea, sneezing and asthma can be experienced by people exposed to pyrethrum. Safety equipment should be put on by people using pyrethrum (Mader, 2012). Due to the increasing use of pyrethins in agricultural and consumer products, there is a need for improved analytical techniques both to assure product quality and to monitor the fate of pyrethrins in the environment. The degradation of pyrethrins in pyrethrum flowers with respect to the moisture content and drying rates is also of great importance. Pyrethrins degrade when exposed to the environment hence insects are unable to develop resistance to the insecticide. This partly explains the insecticide's continuous use and efficacy for more than a century. It does not pose threat to human beings and animals since it does not persist in the environment (Power et al, 2007).

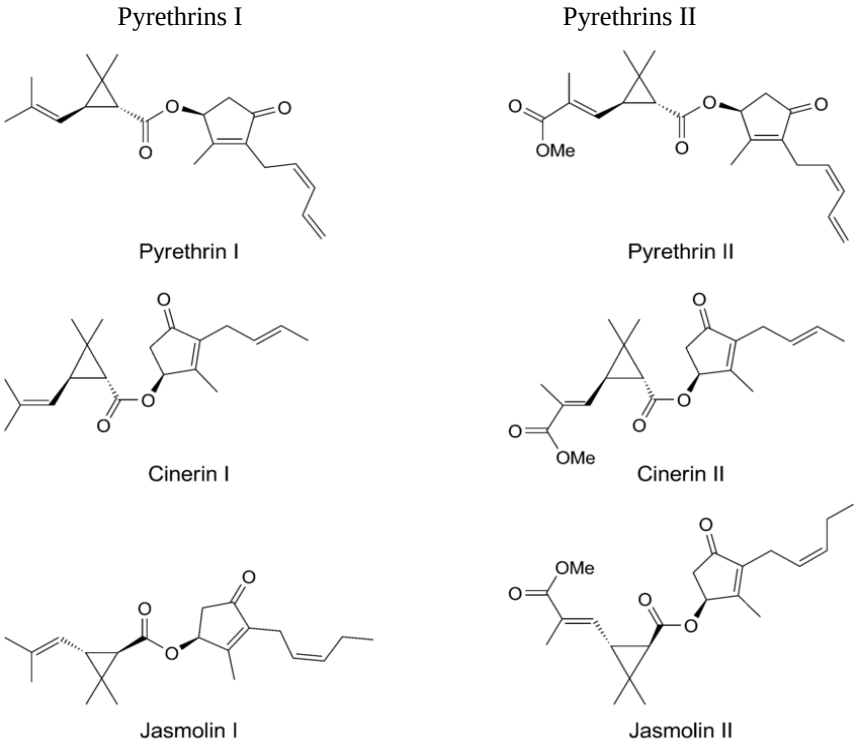

Figure 1. Structures of pyrethrins

Source: Casida, 1995

Processing of pyrethrins begins with the picking and drying of pyrethrum flowers. Mature pyrethrum flowers that are ready for picking are those that have petals lying horizontally. Flowers are picked and stored in dry, well ventilated bags for transport to drying sites or rooms. Drying of the flowers is an essential post-harvest activity that determines the extractable pyrethrins content. Farmers dry the flowers in the open, on materials such as mats, polythene sheet spread on the ground for a minimum of 4 days depending on sunlight (Wang, 2017).

2. Materials and Methods

Sampling

Once the flowers had horizontal petals, they were taken to have matured. The flowers were hand- picked ensuring that no part of the flower stock is taken. The flowers were picked in aerated baskets to avoid accumulation of heat generated by the picked flowers that may have led to rotting. The fresh flowers were weighed and then taken to the oven for drying.

Drying of pyrethrum flowers at regulated known temperatures

The harvested flowers were put on trays and then the trays were put in the oven. The temperatures of the oven were regulated between 30-70 ℃ at intervals of 10 ℃ but at constant time of 18 hrs. The moisture contents of the dry flowers were then tested and the flowers were then taken for analysis.

Grinding

The dried flowers were kept in the room for two days at ambient temperature before grinding, using a pestle and mortar. After grinding, the powder was sieved using a BS 410 mesh and put in labeled brown paper bags awaiting extraction.

Reagents

Dinige's reagent, potassium iodate standard solution, iodine mono-chloride all prepared using AOAC method 936.05, n-hexane, sodium hydroxide, barium chloride and methanol.

Extraction Procedures

Step 1 Extraction

The dried flowers were then placed into an extraction thimble. Extraction at varying temperatures was done using soxhlet extraction for 10 hours using 1000 ml n-hexane in a 2000 ml Erlenmeyer conical flask containing a few anti-bumping chips. After extraction, the n-hexane was removed through a rotor vapour to reduce extracts volume to about 50 ml.

Step 2 Dewaxing

Normal Hexane was added to the sample extracts in a flask at a ratio of 3:1 v/v solvent to sample. The extracts were then kept in the refrigerator at 2 ℃ for a period of 12 hrs for dewaxing. After 12 hrs, the extracts were filtered through cotton wool into conical flasks. The extracts were then evaporated to dryness using a rotor vapour.

Step 3 Saponification

A volume of 20 ml alcoholic NaOH was then added into extracts and refluxed for 1 hour for saponification of the fatty acids. The extracts were then transferred into 250 ml beakers after saponification.

Step 4 Evaporation

Distilled water was added to the extracts to a volume of 200 ml and evaporated on a hot plate up to a volume of 150 ml to remove the alcohol. The solution was then cooled to room temperature using tap water.

Step 5 Fatty acid removal

After cooling the solutions, they were transferred into 250 ml volumetric flasks and 1.0g of filter-Celite was added to each solution. A volume of 10 ml of 10% $BaCl_2$ was added to the extracts and topped up to the mark with distilled water. The mixtures were then shaken vigorously to ensure that the fatty acids were removed by the $BaCl_2$. Yellowish-orange Barium fatty acid salt precipitate was then formed. 200 ml of the extracts were then filtered into 250 ml beakers and three drops of phenolphthalein indicator was added. The filtrates were then neutralized with excess 20% sulphuric acid to precipitate the remaining $BaCl_2$. White precipitate of $BaSO_4$ formed was then filtered off through whatmann filter paper no. 1 coated lightly with a suspension of filter-Celite on a Buchner funnel aspirator. The precipitates were then washed severally with distilled water.

Step 6 Separation of Chrysanthemic and Pyrethric acid

The filtrates obtained in step 5 above were then transferred into a 500 ml separating funnel and extracted twice with two 50 ml portions of petroleum ether. The petroleum ether layers were transferred into a 250 ml separating funnel and washed twice with 5 ml of distilled water to remove aqueous portions that were emptied into 250 ml beakers. The *Chrysanthemic* acid is less polar than the *pyrethric* acid formed and therefore , the pyrethrins I are contained in the petroleum ether layer while the aqueous layer contain pyrethrins II. The main goal achieved in this step was the separation of the two acids subsequently used in quantifying the pyrethrins I and pyrethrins II. The aqueous layer was then evaporated to 50 ml for 1 hour. The mixture was then cooled to room temperature using tap water.

Analytical Methods

Titrimetric Method of Determination of Pyrethrins I

The petroleum ether layers were extracted twice using 5 ml 0.1M NaOH solution. *Chrysanthemic* acid was mixed with 0.1M NaOH and placed in 100 ml beakers formed a basic extract, while the petroleum ether layers were discarded.

Mercury Reduction

A volume of 10 ml of Dinige's reagent was added to the basic extract then kept in the dark for one hour in a water-bath at 25 ℃. The colour of the solution changed gradually to purple-light blue-deep blue-green which is characteristic of *Chrysanthemic* acid on adding Dinige's reagent. The intensity of these colours depend on the concentration of the acid. The mixture was then removed from the dark and immediately 3 ml of saturated NaCl solution was added followed by 20 ml amyl alcohol. The saturated NaCl precipitates Mercurous Chloride (HgCl). The precipitate was boiled, filtered and a further 10 ml amyl alcohol added to precipitate remaining HgCl. The white precipitate of HgCl was filtered and the filter paper washed twice with 10 ml of distilled chloroform, so as to remove the alcohol. The beaker was washed with 50 ml of 60% HC1 aqueous solution and the piece of cotton wool into the 200 ml conical flask-containing the filter paper and $HgCl_{(s)}$. Further 20 ml of $CHC1_3$ was put into the 100 ml beaker and added to the 200 ml conical flask contents. Indicator iodine mono chloride (IC1) 1 ml, was added into the solution and titrated with 0.01M KIO_3 solution carried out. The titration was carried out with constant shaking until the pink colour in the chloroform phase just disappeared.

Calculations of Pyrethrins I concentration

The % (w/w) Pyrethrins I was calculated using equation 1,

$$\% \text{ Pyrethrins I} = 0.7125 \times \frac{V_1}{W_1} \quad (1)$$

V_1- Titre volume (volume of 0.01M KIO_3 used) (cm3)

W_1- Weight of sample (g)

0.7125 - Stoichiometric factor for pyrethrins I

Determination of Pyrethrins II

After evaporating the aqueous layer containing Pyrethrins II in Step 6 above to about 50 ml and cooling to 20 ℃, the solution was transferred into a separating funnel containing a saturated solution of NaCl. The sodium chloride solution supersaturates the aqueous layer and reduces the solubility of pyrethric acid in the aqueous layer. The aqueous layer was then extracted three times with diethyl ether and 10 ml portions of saturated NaCl solution to remove traces of HC1. The diethyl ether layer was then filtered through a cotton plug and the filtrate evaporated to dryness before putting it in an oven at 100 ℃ for ten minutes. On removing the aliquot from the oven, a current of compressed air was blown into the flask to remove HCl fumes. The aliquot was then dissolved in 2 ml of neutral alcohol and 20 ml of water was added. The neutral alcohol and water were prepared by neutralizing both with dilute sodium hydroxide to pH 7. The aliquot was titrated against with 0.02N NaOH with phenolphthalein indicator.

Calculation of Pyrethrins II concentration.

$$\text{The percentage (w/w) Pyrethrins II} = 0.4675 \text{ x } \frac{V_2}{W_2} \text{ x TF} \quad (2)$$

V_2= Volume of 0.02N NaOH (cm^3)

W_2 = Weight of Sample extracted (g)

TF = Titration factor due to the hygroscopic alkali base NaOH

0.4675 = Stoichiometric factor for Pyrethrins II

Ultra-High Performance Liquid Chromatography (UHPLC) Analysis

Samples

One gram sample of the grist was each extracted in triplicates using n-hexane in volumetric flasks. The extract was then left to settle for three hours before injection into the UHPLC instrument. This procedure was repeated the following day for reproducibility.

Instrument

A Varian Model 5000 liquid chromatograph equipped with a UV-100 detector, a flow cell 4.5µl and an integrator model 4400 was used.

Operational conditions

Detector settings – lambda max 230nm; Absorbance range = 0.5Auf

High pressure pump: multi-head reciprocating type capable of minimizing pulsation

Flow rate – 0.8ml/min

Integrator settings: chart speed = 1cm/min

Attenuation = 8; Peak threshold = 6

Pressure: 14-16 atmospheres

Syringe; 10 µl Hamilton syringe

Solvents: Acetonitrile – HPLC grade, water: methanol (10:90).

Column: a Varian micropak CN-5 nitrile, 30cm × 4mm id and ambient column temperature.

Aliquots of 20 microliters were injected into the pot to be analyzed which is connected to the column through the sample injection system. The compounds in the aliquot mixture exhibit different flow rates through the adsorbent material in the column resulting in separation and quantification. The stationary phase is made of silica adsorbent material while the mobile phase is the pressurized mixture of solvents, water, methanol and acetonitrile. At the end of each analysis, the data recorded in the computer was passed through a "smoothing" routine to remove random electronic noise and then saved as a digital spectrum. The system was then ready for the next analysis.

The peaks of interest were identified by comparing with those of known standards and where there was ambiguity internal standardization was carried out.

Analysis was carried out in triplicates and the average peak area worked out. All the analyses were done at a wavelength of 230 nm.

Percentage pyrethrins content was calculated as follows:

$$\text{The percentage of Pyrethrins (w/w)} = H_s \times \frac{C_{std}}{H_{std}} \text{x } C_s \times \% \text{ Purity of pyrethrin standard} \quad (3)$$

Where H_s = Average peak height of sample

H_{std} = Average peak height of pyrethrin standard

C_s = Concentration of sample (gm/1)

C_{std} = Concentration of pyrethrin standard

3. Results and Discussion

Drying at different temperatures at a constant time

The logarithm of moisture content lost at given temperatures was plotted against time.

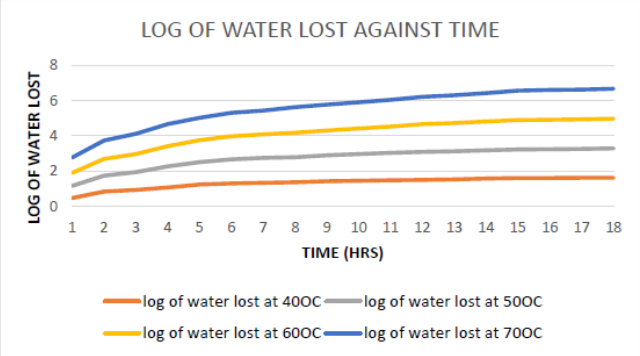

Figure 2. Graphical representation of the weight loss during drying

The graphs above show that much water is lost in the first one hour of drying across all the drying temperatures. After drying for about 12 hrs, there is insignificant change in moisture content loss. This shows that once the moisture content of the flowers is below 10%, high quantity of pyrethrins is obtained. However, when the drying is done for a longer period at temperatures above 60 ^{O}C, this leads to a negative change in the quantity of the pyrethrins due degradation.

Drying to total dryness at different temperatures and time

When the flowers were dried to total dryness at different temperatures, they dried at different times.

Table 1. Drying to total dryness at different temperatures and time

Temperature (^{O}C)	Time (Hrs)	Weight Lost (%)	Log Weight Lost
40	26	71.2	1.85
50	21	75.3	1.88
60	19.5	75.8	1.88
70	18	77.1	1.89

Pyrethrum flowers were dried to total dryness at varying temperatures of 40, 50, 60 and 70 ℃. The logarithm of loss of moisture content was found to marginally increase over the temperature range. The moisture content loss was stable at 50-60 ℃ range.

Table 2. Percentage (%) of Pyrethrins on drying at different temperatures for 18 hours

Temperature (^{O}c)	Pyrethrins I	Pyrethrins II	Total Pyrethrins	Ratio (PI:PII)
40	0.46	0.33	0.79	1:0.72
50	0.79	0.58	1.37	1:0.73
60	0.77	0.53	1.30	1:0.69
70	0.73	0.47	1.20	1:0.64

Total extractable pyrethrins content were found to vary with drying temperature. At 40 ℃, the extractable pyrethrins content was recorded as 0.79% and 1.37% at 50 ℃. However, the total extractable pyrethrins content as well as the Pyrethrins I : II ratio was found to reduce with increase in temperature beyond 50 ℃.

Table 3. Percentage of Pyrethrins on drying to a constant weight

Temperature(^{O}c)	Pyrethrins I	Pyrethrins II	Total Pyrethrins II	Ratio (PI:PII)
40	0.53	0.37	0.90	1: 0.70
50	0.83	0.61	1.44	1:0.73
60	0.78	0.56	1.34	1:0.72
70	0.74	0.49	1.23	1:0.66

Total extractable pyrethrins content was found to vary with drying temperature, with a peak at 50 ℃ and lowest at 70 ℃. At 40 ℃, the pyrethrins content was found to be 0.90% and 1.44% at 50 ℃, implying that, there was loss of pyrethrins at low temperatures. Thus, there were more extractable pyrethrins I and II when flowers were dried at 50 than 40 ℃. However, beyond 50 ℃, both extractable pyrethrins I and II were found to reduce, hence

affecting the ratio and total pyrethrin content. Extractable pyrethrins II were found to reduce by 8.6% when the drying temperature was raised from 50 to 60 ℃ and by 11.3% from 60 to 70 ℃. Extractable pyrethrins I were found to reduce by 6% when the drying temperature was raised from 50 to 60 ℃ and by 5% from 60 to 70 ℃. Extractable pyrethrins II were found to reduce at a higher rate with increase in temperature beyond 50 ℃ compared to pyrethrins I, thereby affecting the ratio.

High Performance Liquid Chromatographic analyses was carried out on the pyrethrin extracts. The chromatograms attained are presented in Figures 3 and 4. Figure 4 show chromatograms for pyrethrins dried at 50 ℃ to total dryness.

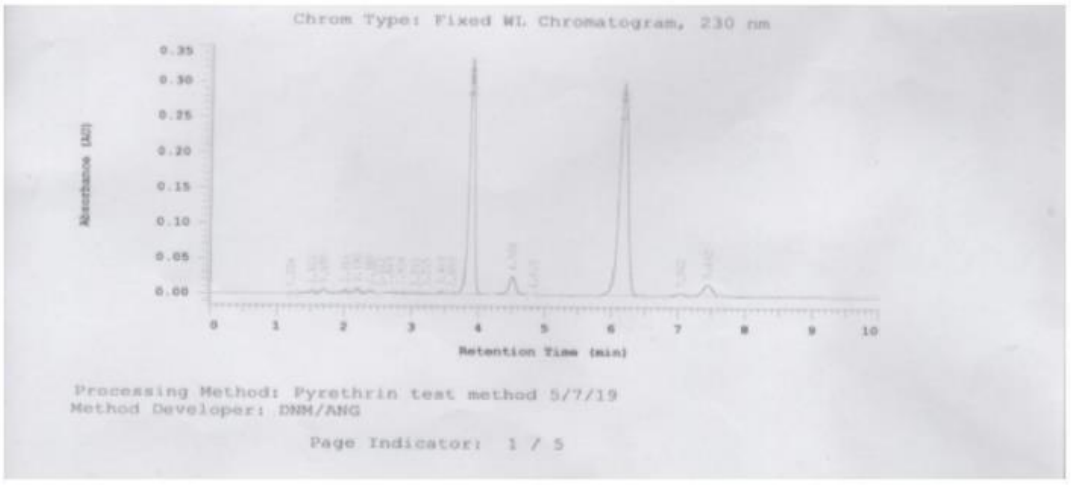

Figure 3. Chromatograms of the standard

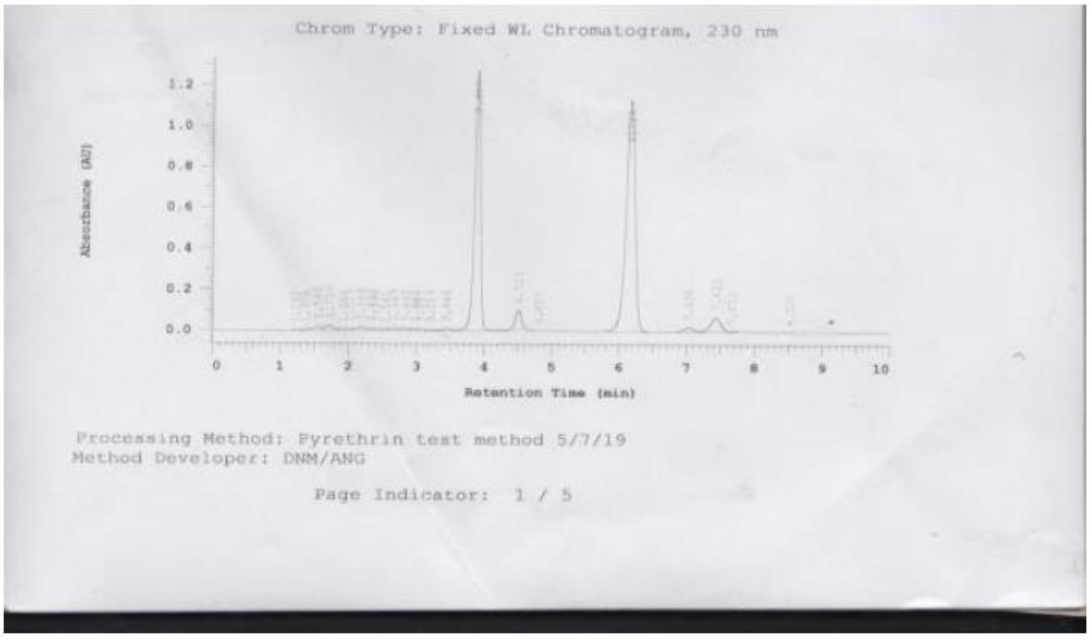

Figure 4. Chromatograms for the sample dried at 50 ℃ to total dryness

Results obtained from flowers dried at 50 ℃ to constant weight are presented in table 4.

Table 4. % Pyrethrins obtained using HPLC

Temperature(^{O}c)	Pyrethrins I (%)	Pyrethrins II (%)	Total Pyrethrins (%)	Ratio (PI:PII)
50	0.85	0.61	1.46	1:072

4. Conclusion

Total extractable pyrethrins content from pyrethrum flowers was highest at 1.44% and pyrethrins I:II ratio of 1:0.73, when dried at 50 ℃ at a time of 21 hrs, thereby presenting the optimum drying temperature and time. The flowers were dried to a constant moisture content of less than 10%. Drying flowers at a higher temperature of 70 ℃ resulted to lower yields of 1.20% and pyrethrins I:II ratio of 1: 0.64 while at 40 ℃ resulted in 0.79% which is 0.65% lower compared to that of 50 ℃.

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