CORRELATION BETWEEN PENTACYCLIC TRITERPENOID LEVELS AND GELLING OF PYRETHRUM REFINED EXTRACT

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

Date 3/10/95

This thesis has been submitted with our approval as university supervisors

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SUMMARY

Pyrethrum, *Chrysanthemum cinerariaefolium* , flower head is the source of the most widespread botanical insecticide in use today for insect control.

The commercial basis of the pyrethrum industry is dependent on the insecticidal principles (pyrethrins content) of the extract. There are however other non-insecticidal constituents like pentacyclic triterpenoids, the most abundant being taraxasterol, present in the extract.

The demands in the public health area and use on human beings have necessitated development and inclusion of various refining processes to purify the crude extract into a dewaxed and decolourised (refined) extract. However, at times, this extract becomes gelatinous and semi-solid, thus presenting serious production and formulation problems that bring about loss to the industry and to the country.

The objective of this research is to identify possible causes of this gelling and to make quantitative assesment of the constituents in the extracts that induce the process. This work describes investigations of taraxasterol in relation to gelling of extracts.

This study entailed collection of flowers from various farming regions, their processing and analysis of extracted materials for pyrethrins and taraxasterol levels in relation to the quality of extract.

Variation of farming regions was found to influence the physical characteristics of the extract. Some regions yielded good quality extracts while others yielded poor quality extracts. This variation was ascribed to climatic conditions and soils which influence the synthesis of the various chemical components in the pyrethrum plant.

Taraxasterol levels were not the only factor influencing the physical state of extracts. Gelling of pyrethrum extract may be attributed to inter actions between taraxasterol and pyrethrins molecules possibly through hydrogen bonding. However, the investigation has shown that such interac tion, if present, are not the sole contibutors to gelling of the extracts.

Mixing of flowers in certain ratios based on the source of the flowers resulted in processing of flowers at higher, easily-maintained temperatures yielding non-gelling extracts.

It is therefore concluded that pentacyclic triterpenoids, specifically taraxasterol, are not the sole factor that induce gelling in pyrethrum extract. The modulation of physical characteristics of the extract suggests that thermodynamic parameters are also involved in the gelling of extract.

ACKNOWLEDGEMENTS

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Dr. W. A. Odinga, while she was the Chief Chemist at Pyrethrum Board of Kenya, recommended me to be sponsered by the Board for the project. For this, she deserves a lot of thanks. I am also indebted to Dr. and Mrs. Mahungu for material assistance in the course of this work.

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DEDICATED

TO

MY LATE BROTHER

AND

MY DEAR PARENTS

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1.0 INTRODUCTION

1.1 GENERAL OBJECTIVES:

The pyrethrum flower heads produced by the species *Chry santhemum cinerariaefolium* are the source of the most widespread botanical insecticide accepted for use in public health and veterinary services worldwide. The rapid paralytic action of pyrethrum on a large variety of insects and the factor it's practically non-toxic to mam mals, has contributed to its continued commercial success compared to synthetic insecticides. Its rapid photo-degradation and concomi tant rapid biodegradation are added advantages that promise attrac tive use profile and prolonged demand. Knowledge of the composi tion of pyrethrum extract, is however, of immense value in the under standing of the physico-chemical properties of the extracts to facili tate the devel-opment of end use-products of high quality and effec tiveness.

The best quality of refined pyrethrum extract suitable for for mulating products for use on humans and his environment ought to be a clear free flowing oil. Often, the extract becomes a gelatinous semi-solid presenting serious marketing and formulation problems.

Gelled extract therefore increases production time and formulation problems thus increasing related costs. This brings about financial loss to the industry, formulators and the country at large.

This thesis describes investigations on taraxasterol, a non-insecticidal constituent of pyrethrum extract which is pressumed to be a major cause of gelling of the extract. The levels of taraxasterol in relation to gelling of the extract were investigated.

It was found necessary to give an outline of the various aspects of agronomic practices in pyrethrum cultivation; the botany of the pyrethrum flower head; development of the pyrethrins within the flower head; processing of the flowers; the chemistry of pyrethrins; and the composition of the extract, in order to relate the investigation to the actual commercial industry in Kenya.

1.2 HISTORY AND AGRONOMIC PRACTICES IN PYRETHRUM CULTIVATION

The insecticidal activity of pyrethrum flowers is believed to have been accidentally discovered in the early 19th century by a German who picked flowers for beauty and threw them into a corner after they withered. Several weeks later the flowers were found surrounded by dead insects[1].

The death of the insects was attributed to the insecticidal property of the plant.

Pyrethrum in Kenya is grown by small scale farmers who depend entirely on the crop as the financial resource for their livelihood. The crop is also a major foreign exchange earner for the country, coming third after tea and coffee. The pyrethrum industry also provides gainful employment. The main areas in Kenya that produce the plant are: Kericho, Kiambu, Kisii, Meru, Nakuru, Nandi, Nyandarua, Nyeri, and Uasin-Gishu districts.

Pyrethrum is an agricultural product. Its availability is therefore determined by the crop acreage, the flower yields and the quantity of the active principles (pyrethrins content).

Pyrethrum grows well in deep well drained soils. A general decrease in the mean temperature during the rainy season is essential for flower bud initiation and satisfactory flower development[2]. In Kenya, such optimum conditions are met in the highlands (over 1800 meters above sea level) where rainfall is over 1000 mm a year.

The plants are established from both seedlings and vegetative plant divisions (splits). In the former method, each plant is genetically different and therefore varies greatly in their growth habit, flower production, resistance to disease and pyrethrins content. Selections from

seedlings based on these properties produces improved plants and is the basis of plant breeding methods. A selected plant can then be multiplied by splitting, thus resulting in genetically homogeneous plants and the planting material is then termed a "clone". The performance of a clone varies considerably with climatic conditions and they are therefore usu ally confined to specific altitude and geographical area.

1.3 BOTANICAL ASPECTS OF PYRETHRUM

The botanical structure of pyrethrum *(Chrysanthemum cinerari-aefolium* Vis) has been documented by Chandler[3]. The flower head (Figure 1) is typical of the Asteraceae (*Compositae*) and is a collection of small flowers (florets) set on a slightly convex receptacle, the undersur face of which is covered by the scales of the involucre. The disc florets (Figure 1c), which are sited over the centre of the receptacle are sur rounded by an outer ring of ray florets (petals) (Figure 1b). The disk and ray florets surmount ribbed achenes which are seated upon the recep tacle. The disc florets comprise a yellow tubular corolla which is sepa rated from the ovary by a small calyx. Five stamens arising from the base of the inner surface of the corolla each terminate in an elongated anther which are joined at their outer edges to form a cylinder. At the centre of the floret stands the style which rises from the ovary.

The style terminates in a bilobed stigma and is located within the cylin der formed by the anthers. In the unopened floret, the two lobes of the stigma are vertical and the inner receptive surfaces oppressed. As the corolla opens, the style elongates and when the stigma is exposed, the two lobes open outwards ready for pollination. The outer disc florets open first and the development proceeds progressively across the disc towards the centre. The ray florets differ in basic structure to the ray florets in that the corolla is enlarged forming the petals and the stamens are absent.

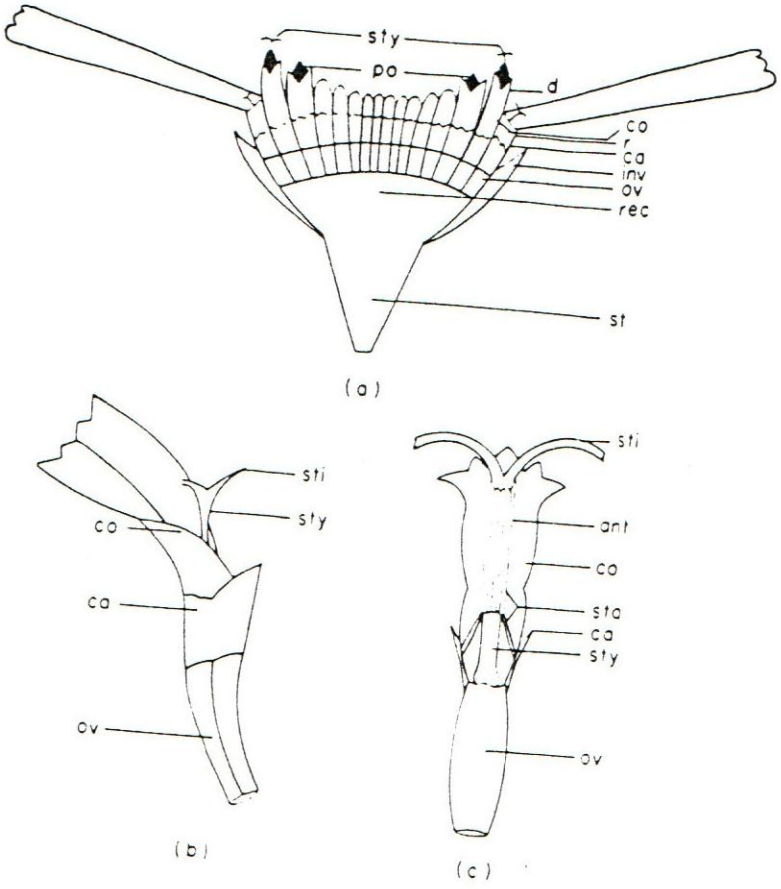
More than 90 % of the pyrethrins in the flower head are located within the oil glands and secretory ducts of the achenes. Chandler[3] showed that flower heads having a high pyrethrins content show a greater devel opment of these structures than those with low pyrethrins content.

Early studies on the distribution of the pyrethrins in the pyre thrum plant and their rate of development in the flower head were reviewed by Gnadinger[4]. Another investigation[5] using Gas Liquid Chro-matography (G.L.C.) for analysing the active constituents confirmed the presence of pyrethrins throughout the whole plant, the greatest concen-tration being located in the flower head.

Although the weight of pyrethrins in a single flower head increases with maturity, the pyrethrins content (% w/w in the dry flowers) is at optimum at stage 4 (Table 1)[1]. In practice, only flower heads with horizontal petals are harvested (stages 3 to 5 inclusive, Table 1).

FIGURE.1. THE STRUCTURE OF THE PYRETHRUM FLOWER HEAD[1]

(a), inflorescence; (b), ray floret; (c), disc floret; sty, style; po, pollen; d, disc floret; co, corolla; r, ray floret; ca, calyx; inv, involucre; ov, ovary; rec,receptacle; st stalk; ant, anthers; sti, stigma; sta, stamens.



The interval between picking is regulated so as to keep to a minimum the number of overblown flowers (stages 6 and 7 in Table 1) which tend to reduce the plants flower bearing capabilities and hence the pyrethrins yield.

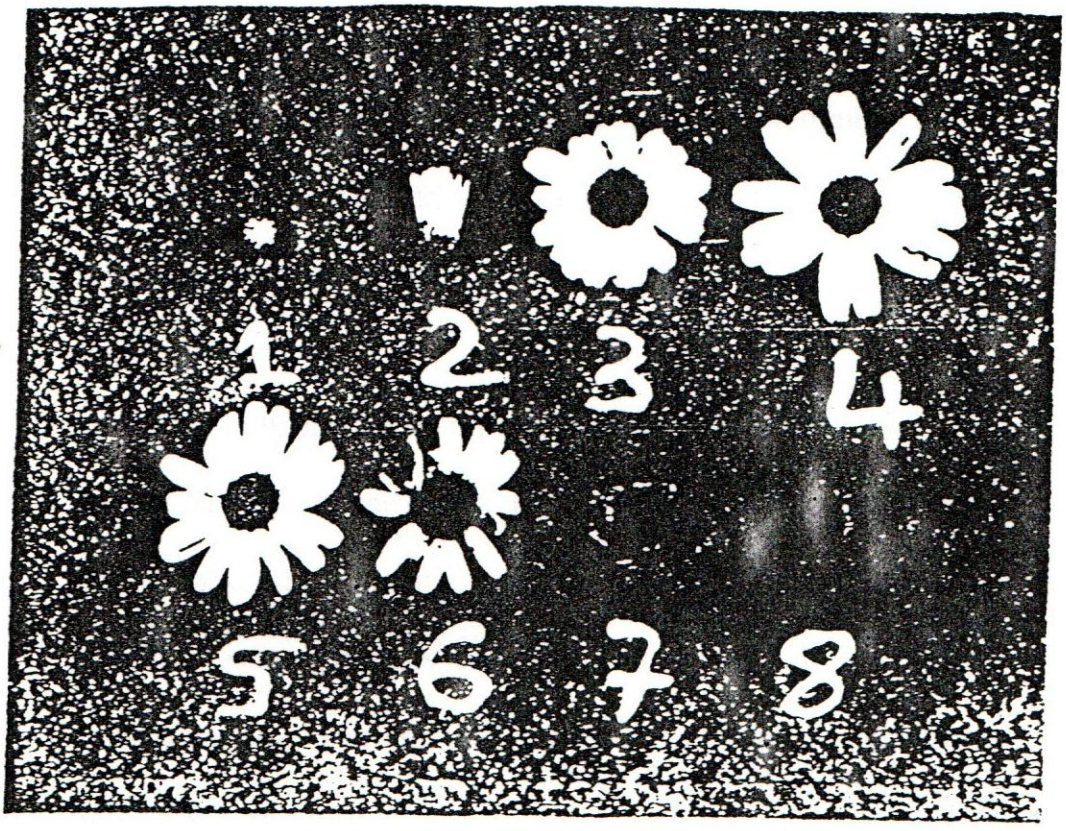
TABLE 1 : WEIGHTS OF FLOWER HEADS BEFORE AND AFTER DRYING AT DIFFERENT STAGES OF DEVELOPMENT[1]

Stage number	Description	Approximate time for development (days)	Average fresh Weight (mg)	Average dry Weight. (mg)	Total pys. (mg)	dry Weight (%)
1.	Well developed closed buds	0	178.0	52.0	0.40	0.76
2.	Ray florets vertical	12	418.0	119.0	1.78	1.49
3.	Ray florets horizontal; first row of disc florets open	16	480.0	126.0	2.48	1.97
4.	Approximately three rows of disc florets open	19	615.0	164.0	3.45	2.10
5.	All disc florets open; fully mature	21	689.0	195..0	3.89	2.00
6.	Early overblown condition; colour of disc florets diminishing but ray florets still intact	31	716.0	253.0	3.92	1.55
7.	Late overblown condition; little colour remaining in disc, florets dried out	43	666.0	347.0	4.02	1.16
8.	Disc forets fallen, stems dry 1/2 in below head - suitable for collection for seed	60	321.0	280.0	x	x

x = not analysed

pys = pyrethrins

FIGURE 2: THE PYRETHRUM FLOWER HEAD AT DIFFERENT STAGES OF DEVELOPMENT[1]



1.4 FLOWER PREPARATION AND PROCESSING

At picking time the flowers have a moisture content of aproximately 80 % (w/v)[1]. This is reduced to about 10 % by either sun drying or, for large flower quantities, by forced artificial heat draught units. Provided the flowers are dried with a good flow of air and drying temperatures do not exceed 82° C, there would be little degradation of the active constituents[6].

The bulk of the crop is processed to yield a liquid concentrate. At the processing plant, the ground flowers are first extracted with a light petroleum solvent (n-hexane) using a counter current extraction procedure. The resulting primary extract is concentrated to yield a dark greenish-brown viscous oleoresin (OR concentrate) containing approximately 30 % (w/w) pyrethrins.

The demand for use of pyrethrum on humans and consequent formulation in the aerosol industry has resulted in development and use of various processes that convert the dark viscous oleoresin concentrate into a decolourised and dewaxed extract termed "pale extract". The ini tial step in this secondary refining process involve extraction of oleoresin concentrate with methanol. The methanol insoluble residue material is known as pyrethrum residue or "sludge".

solvent (shellsol-T) and decolourised with activated carbon to give the final pale and oily product referred to as pale extract.

1.5 STRUCTURE OF PYRETHRINS

The first major contribution to the chemistry of pyrethrins was the tentative elucidation of the structures of the two major constituents of pyrethrum namely: pyrethrin I and pyrethrin II by Staundinger and Ruzicka[7] . Investigations[8,9] carried out later led to some modification of the pyrethrin structures and resulted in the currently accepted struc tures.

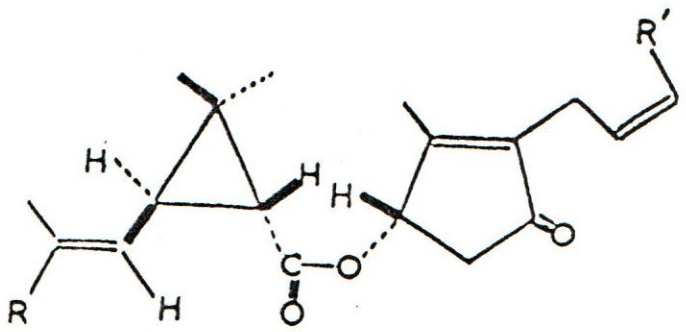
Pyrethrin I (1) and pyrethrin II (2) (see Figure 3) are pyrethrolone (3) (see Figure 4) esters of chrysanthemic acid (4) and pyrethric acid (5) [the monomethyl ester of chrysanthemum dicarboxylic acid (6)] respectively.

In 1945, LaForge and Barthel[10] isolated a second alcohol, cinerolone (7), also present as esters of the same chrysanthemum acids which were termed cinerin I (8) and cinerin II (9). Gas Liquid Chromatographic analysis[11] revealed the presence of a further two (minor) components, jasmolin I (10) and jasmolin II (11) which were shown by Godin *et al* [12] to be jasmololone (12) esters of the two chrysanthemum acids respectively.

The work leading to the identification of the four major active constituents (pyrethrin I, pyrethrin II, cinerin I and cinerin II) has been reviewed both by Harper[13] and West[14] and has confirmed these structures.

Pyrethrin I, cinerin I and jasmolin I are collectively referred to as **pyrethrins I fraction** while pyrethrin II, cinerin II and jasmolin II are collectively referred to as **pyrethrins II fraction.**

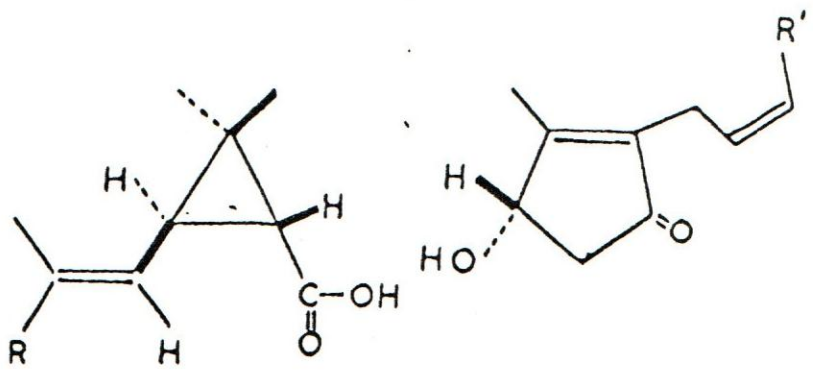
FIGURE 3: STRUCTURE OF THE NATURAL ESTERS



Structure ref.	Name	R	R'
1	pyrethrin I	Me	$CH=CH_2$
10	jasmolin I	Me	CH_2CH_3
8	cinerin I	Me	Me
2	pyrethrin II	COOMe	$CH=CH_2$
11	jasmolin II	COOMe	CH_2CH_3
9	cinerin II	COOMe	Me

Me = CH_3 (methyl)

FIGURE 4 : STRUCTURES OF THE ACID AND KETONE MOIETIES THAT MAKE UP THE NATURAL ESTERS



Structure Ref.	Name	R	R'
4	chrysanthemum monocarboxylic acid (chrysanthemic acid, chrysanthemumic acid)	Me	
6	chrysanthemum dicarboxylic acid	COOH	
5	pyrethric acid	COOMe	
3	pyrethrolone		$CH=CH_2$
12	jasmololone		CH_2CH_3
7	cinerolone		Me

1.6 ANALYSIS OF PYRETHRINS

Two methods accepted in commercial transactions for determining pyrethrins are: the Association of Official Agricultural Chemists (A.O.A.C.)[15] and the Pyrethrum Board of Kenya (P.B.K.)[16] analytical procedures.

In both cases, pyrethrins are hydrolysed, and the liberated chrysanthemic acid (4) and chrysanthemum dicaboxylic acid (6) separated by utilising the solubility of their barium salts from long chain aliphatic acids. The solution of the barium salts of the chrysanthemic acid and chrysanthemum dicarboxylic acid is then acidified with either (a) hydrochloric acid in the P.B.K. method or (b) sulphuric acid in the A.O.A.C. method, this being the major difference between the two methods. In the A.O.A.C. technique, there is precipitation of excess barium as barium sulphate while in the P.B.K. method, the salt of barium chloride formed is soluble. The mono and dicarboxylic acids are then separated by solvent partition and estimated separately using Deniges reagent (mercurous sulphate) and alkali titration respectively. Both the A.O.A.C. and P.B.K. analytical methods use Deniges reagent and have come to be known as mercury reduction methods.

Several more rapid methods of analysis are currently available for the determination of total pyrethrins, the most commonplace is the absorption of the pyrethrins by an ultraviolet (U.V.) spectrophotometric procedure[1]. The U.V. absorption method is calibrated using a standard pyrethrins extract predetermined using A.O.A.C. method.

This investigation used the A.O.A.C. and the U.V. absorption techniques to determine the pyrethrins.

1.7 NON-INSECTICIDAL CONSTITUENTS OF PYRTHRUM EXTRACT

The compounds which have been reported to be present in pyrethrum extract in addition to the pyrethrins include: taraxasterol (pyrethrol)[17,18,19,20,21,22,23] β-amyrin and related triterpene[23,24], ceryl alcohol [24], carotenoids[25,26,27], alkanes[23,24,28] and fatty acids [23,29,30,31].

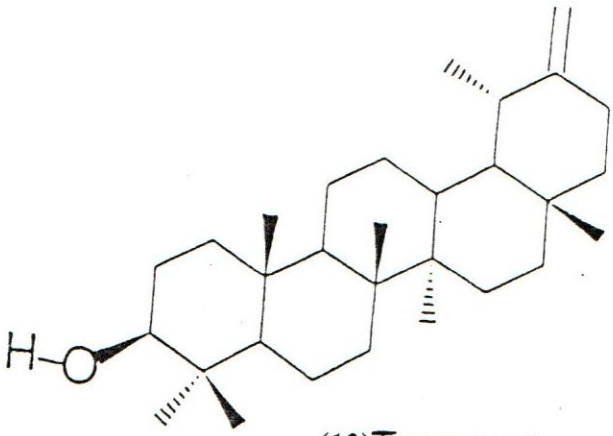
Detailed investigations of the non-insecticidal constituents have been confined to the relatively insoluble by-products of the extraction process. This investigation has confirmed the presence of taraxasterol in refined extract. Taraxasterol (pyrethrol) was first isolated from pyrethrum flowers by Fujitani in 1909[18]. It's presence in pyrethrum extract was noted later by Staundinger and Ruzicka in 1924[7], but it was not until 1966 that Hertz and Millington[20] established its identity as taraxasterol (13). This identity has been confirmed in two other publications by different workers[21,22]. Work[33] on pyrethrum extract has also indicated the presence of sterol like compounds, namely βamyrin (14) and α-amyrin (15) in addition to taraxasterol which is the most abundant triterpenol.

Crystalline insolubles precipitated from the refined extract have been examined and found to be mainly pentacyclic triterpenols[34].

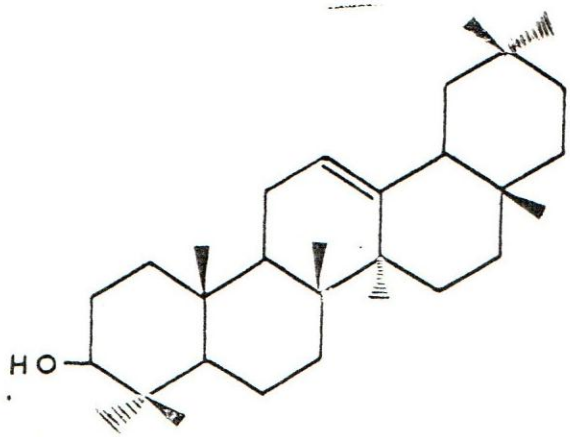
Members of this series are waxy crystalline solids which have poor solubility in iso-alkanes and cold partitioning solvents used in the refining stages of pyrethrum extract. If temperatures are permitted to rise, larger quantities of these materials are carried through to the final refined extract where they can later cause formulation problems.

In 1952, Fukushi[24] investigated the composition of the unsaponifiable matter present in a wax-like residue from commercial extract production. The unsaponifiable matter was crystallised from alcohol and the alcohol soluble portion treated with hot acetone. A substance which was thought to be ceryl alcohol ($C_{26}H_{53}OH$) was isolated from the insoluble portion whilst the acetone soluble portion yielded a sterol that was identified as β- amyrin (14).

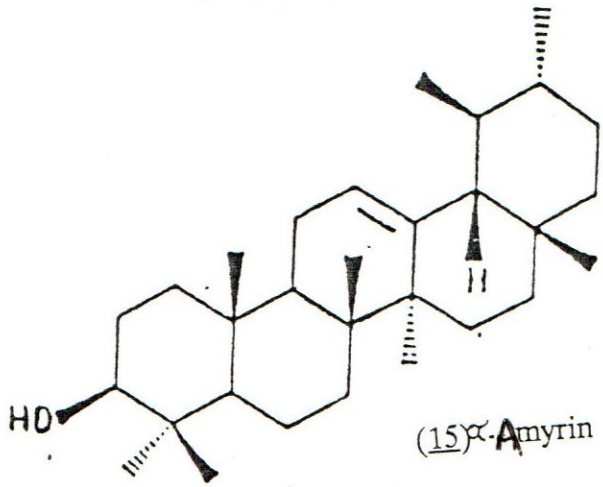
Steam distillate from pyrethrum flowers was examined by Merrit and West [32]. A hydrocarbon $C_{19}H_{40}$, m.p. 53 - 4° C was isolated from the distillate of Dalmatian and English flowers but not from flowers of Kenya origin[32]. The oils had a high acid value and their odour indicated the presence of furfural but which could not be confirmed. A volatile oil of factory origin was shown to contain naphthalene although this was not detected in the steam distillate. This study relates taraxasterol to gelling of pyrethrum refined extract.



(13)Taraxasterol



(14)β-Amyrin



(15)α-Amyrin

1.8 BIOSYNTHESIS

It is important to produce flowers with high insecticidal constitu ents and low non-insecticidal constituents, especially those that impair the quality of extract. An outline of the biosynthetic pathways of con stituents of interest may provide the basis for improved quality of ex tract by addition of suitable stimulants.

The distribution of constituents in the plant may be related to the sites of biosynthesis and / or to the sites of localization, if any. Levels of constituents differ in flowers from different locations suggesting that large variations occur in the activity of enzymes or availability of precursors for the various biosythetic steps[1].

1.8.1 BIOSYTHESIS OF PYRETHRINS

The biosynthetic pathway of the acid moieties of pyrethrins I and II have been documented (Figure 5)[1]. However, only a speculative route is given for the alcohol moieties (Figure 6).

FIGURE.5 BIOSYNTHETIC PATHWAY FOR THE ACID MOIETIES OF PYRETHRINS I AND II [1]

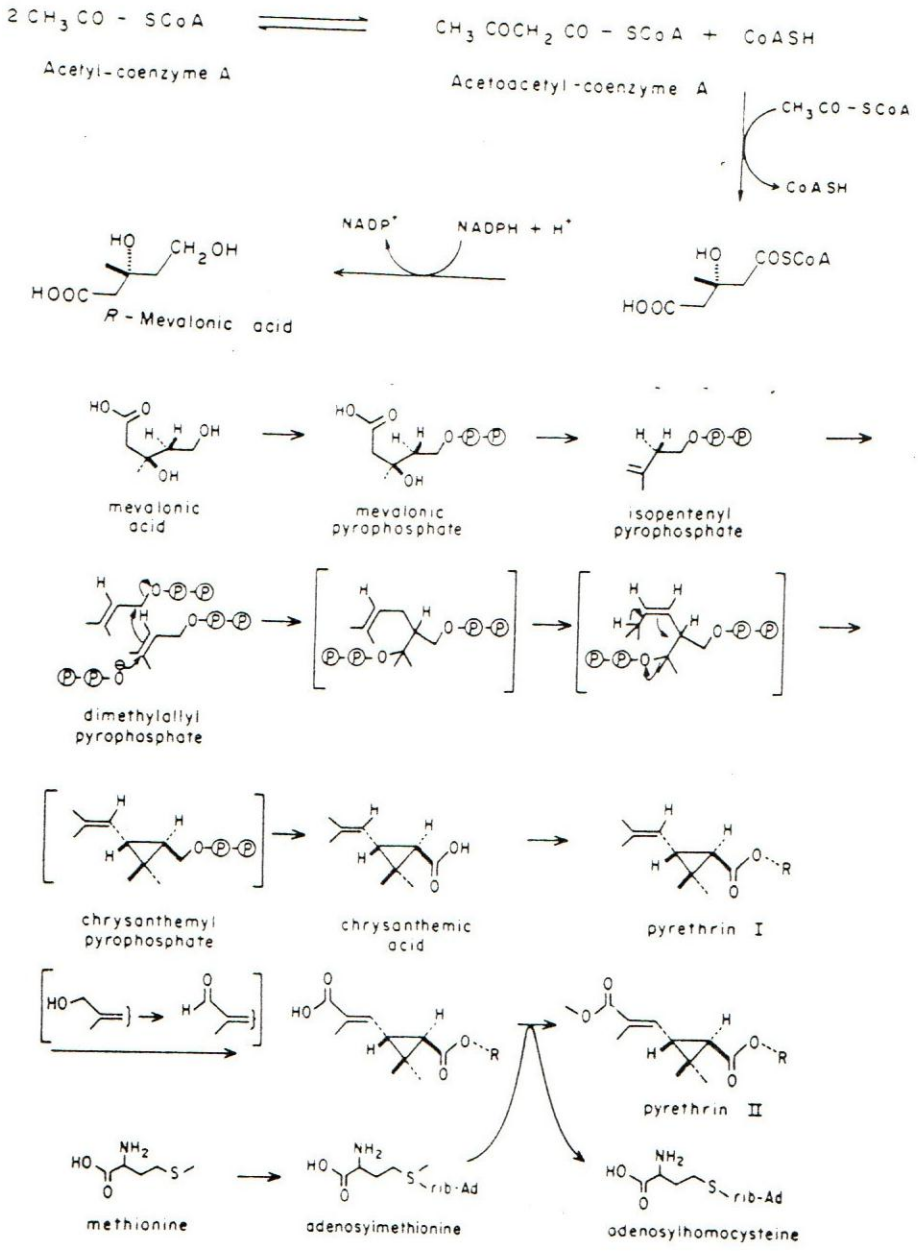
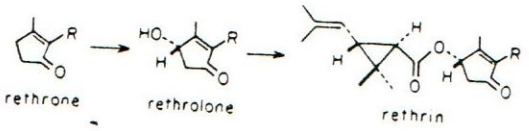


FIGURE.6: POSSIBLE PARTIAL BIOSYTHETIC PATHWAY FOR THE ALCOHOL MOIETY OF RETHRINS[1]



1.8.2 BIOSYNTHESIS OF TRITERPENOIDS

Terpenoids are synthesised by the plant for physiological function and as an alternative strategy to temporarily switching off metabolic pathways[35] (Figure 7).

FIGURE 7: BIOSYTHETIC PATHWAY OF TRITERPENOIDS [35]

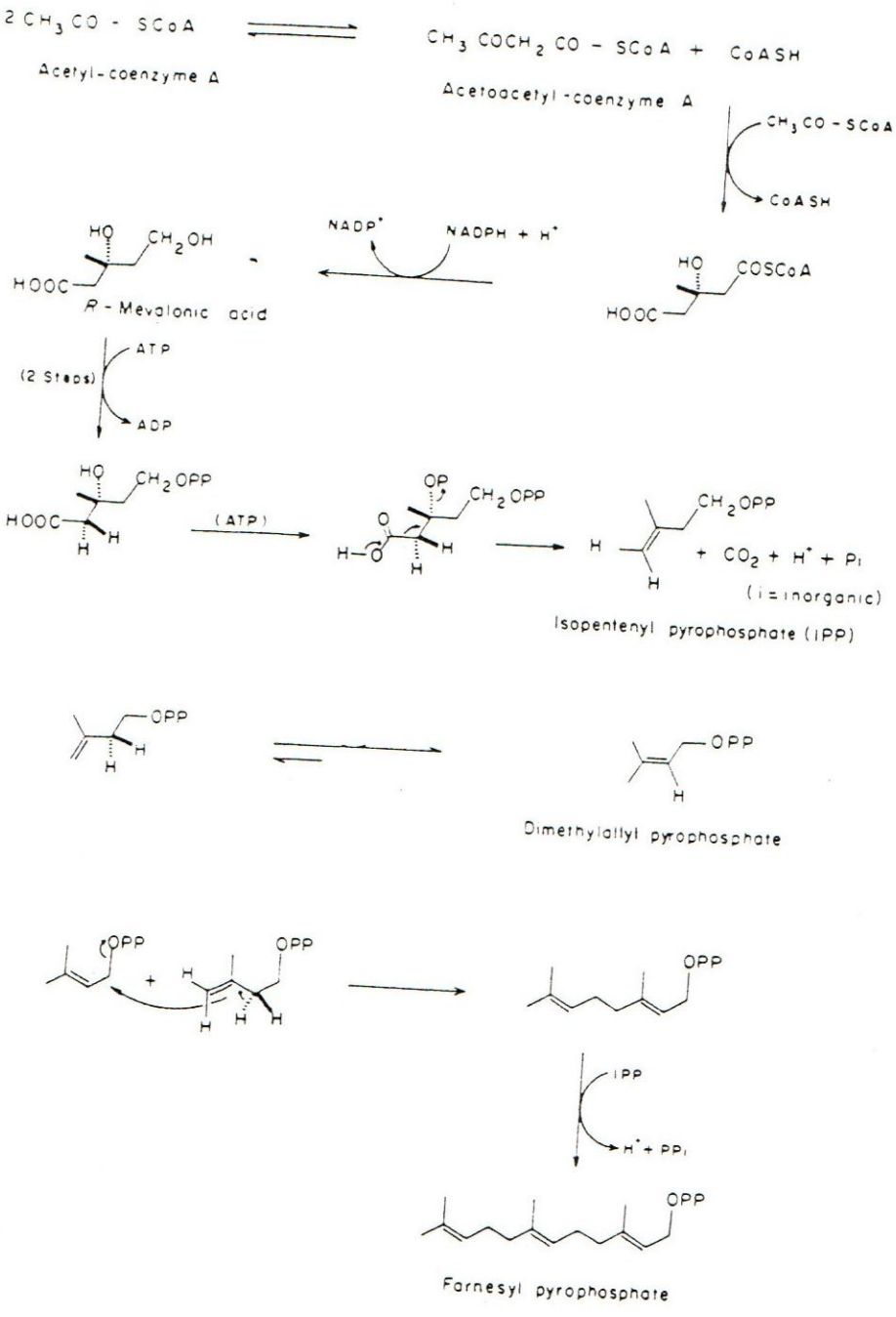
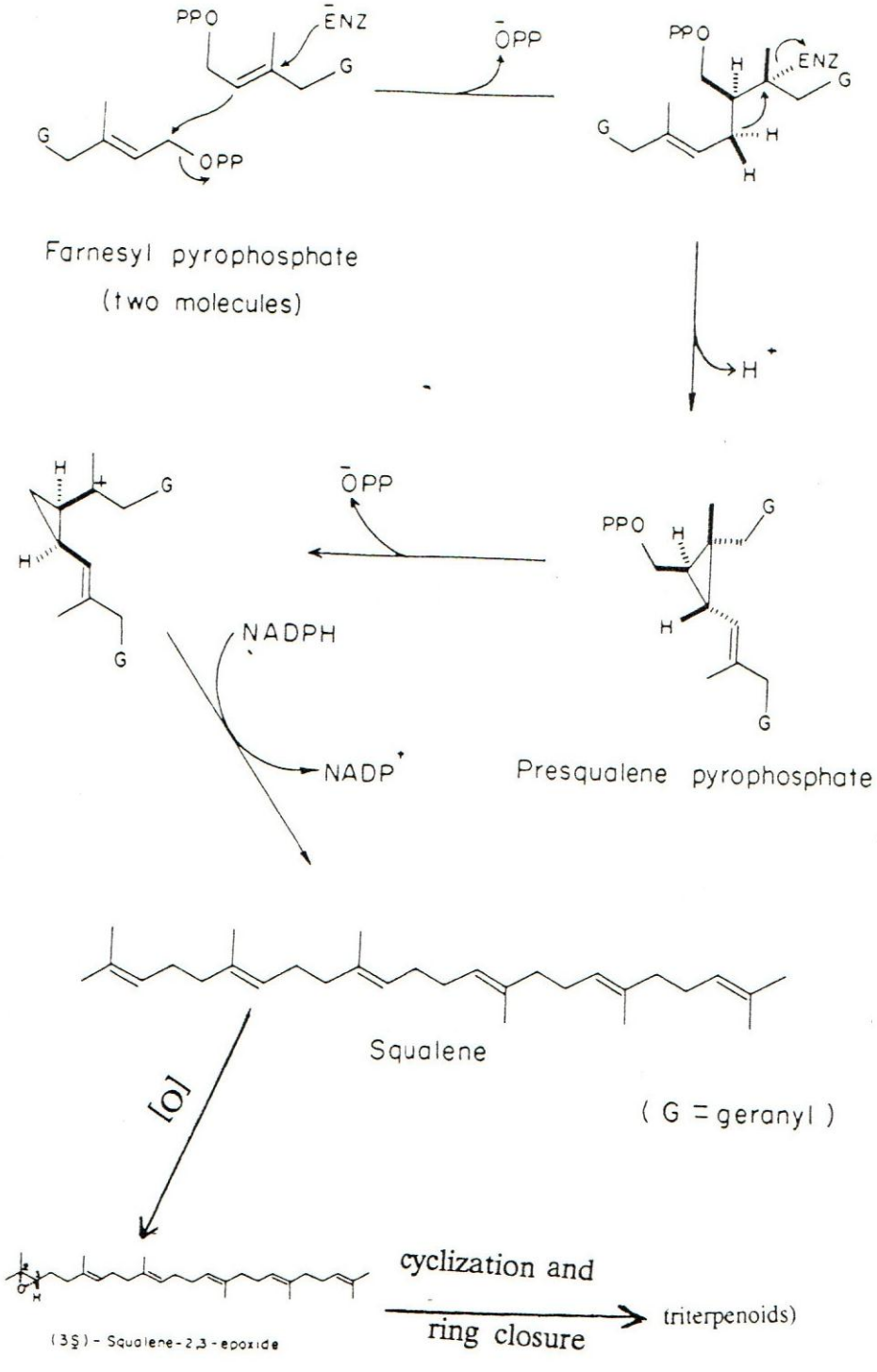


FIGURE 7: CONT.



2.0 EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES

2.1 PLANT MATERIAL (*Chrysanthemum cinerariaefolium* Vis)

Pyrethrum flowers were collected from different farming areas namely: Kisii, Eldoret, Bahati, Bomet, Molo, Meru, Nyahururu and Nyeri. The flowers were air dried till they contained less than 10 % (w/v) moisture content. The flowers were then ground to fine particles (grist) using Willey mills.

2.2 MOISTURE CONTENT DETERMINATION

Moisture content (% w/v) of flowers was determined using Dean Stark apparatus. Approximately 30 g of flowers were weighed into a 250 mL conical flask. Benzene (200 mL) and a few anti-bumping chips were added. The apparatus was connected to the conical flask and fitted with a condenser. The contents of the flask were refluxed for four hours and the volume of water collected read. Moisture content (% w/v) was calculated as shown below[36] giving the results shown in Table 2.

Calculation:

Assuming that the density of water = 1.0 g / cm^3

Assuming that the density of benzene = 0.878 g / cm^3

percentage (w/w) of water in analytical reagent benzene = 0.05 %

Moisture content (% w/w) = { [moisture content in water (g) -

moisture content in benzene (g)] /

weight of sample} x 100 %

% moisture content = $\{[v - (4.39 \times 10^{-4} \times v_T)] / w\} \times 100$.

where:

v = volume (mL) of water collected

v_T = total volume (mL) of benzene used

w = weight (g) of flowers.

TABLE 2: MOISTURE CONTENT OF FLOWERS COLLECTED FROM VARIOUS FARMING REGIONS

Farming region	Wt. of flowers (g)	Vol. of H_2O collected (mL)	Moisture content (% w/w)
Kisii	30.0283	2.9	9.37
Nyahururu	30.0624	2.9	9.35
Bomet	30.0977	2.9	9.34
Bahati	30.0338	3.1	10.03
Molo	30.0434	2.7	8.69
Nyeri	30.0260	2.8	9.03
Eldoret	30.0587	3.0	9.69
Meru	30.0934	2.1	6.69

2.3 EXTRACTION AND SEPARATION PROCEDURES

2.3.1 EXTRACTION AND SEPARATION PROCEDURES FOR PYRETHRINS DETERMINATION IN FLOWERS

The grist was weighed in duplicate (8.0 g and 9.0 g) and subjected to soxhlet extraction using n-hexane (200 mL) as solvent for 7.5 hours. The solvent was evaporated using a water bath at 60° C to reduce the sample volumes to 50 mL and the flasks were stoppered and kept in a refrigerator at -5° C overnight.

The cold extracts were filtered through cotton wool to remove the waxes. Alcoholic 0.5M NaOH (20 mL) was added to the filtrate samples and refluxed for 1.5 hours. The resulting samples were then made to 200 mL with distilled water. The alcohol was removed by evaporating the solution on a hot plate to 150 mL and the solutions cooled to room temperature. Filter cel (diatomaceous earth) (1 g) to remove waxes, and 10% barium chloride ($BaCl_2$) solution (10 mL) to remove fatty acids, were added and the samples made up to 250 mL with distilled water.

The samples were manually shaken vigorously to facilitate removal of the fatty acids. The samples were filtered and a few drops of phenol-phthalein indicator added to 200 mL of the samples. The solutions were neutralised with excess 20% (v/v) sulphuric acid (H_2SO_4).

The white precipitate of barium sulphate ($BaSO_4$) was filtered through filter paper coated lightly with a suspension of filter cel in water (to adsorb any remaining waxes) on a buchner funnel and washed several times with distilled water. The filtrates were transfered to separating funnels and extracted using hexane (2 x 50 mL). The hexane extracts were then transferred to separating funnels and washed with distilled water (2 x 5 mL) to remove traces of aqueous layer. The aqueous extracts contained pyrethrins II while the hexane extracts contained pyrethrins I[15,16]. These were then analysed as in section 2.6.1.

2.3.2 EXTRACTION AND SEPARATION PROCEDURES FOR PYRETHRINS AND TARAXASTEROL DETERMINATION IN EXTRACTS

Ground flowers (1 kg) were extracted using n-hexane (7.5 litres) in a column (40cm x 13cm i.d.). The combined hexane extracts were concentrated using rotary evaporator to a dark greenish-brown, viscous crude extract (oleoresin (OR)). A small amount of the oleoresin was examined for pyrethrins and taraxasterol content as in sections 2.6.2. and 2.6.3 respectively to give results shown in Tables 11 and 17 (OR).

2.3.3 REFINING OF THE CRUDE EXTRACT

2.3.3.1 REMOVAL OF SLUDGES (WAXES)

The crude extract was divided into three portions and placed in 1 litre conical flasks. Methanol was added to the crude extracts in the ratio 5:1 (v/v) and stirred with a magnetic stirrer to maintain homogeneity. The resulting solutions were kept at the various investigation temperatures (0, -10 and -30° C) for 36 hours. Sludge settled at the bottom of the flasks and was filtered off, whereas the resulting methanol micella was concentrated using a rotary evaporator. Aliquots of the concentrates were then analysed for pyrethrins and taraxasterol contentas in section 2.6.2. and 2.6.3 respectively to give results shown in Tables 11 and 17 (MeOH).

2.3.3.2 REMOVAL OF GREEN OILS

Shellsol-T (SST) was added to the concentrates in section 2.3.3.1, in the ratio 5:1 (v/v) and stirred with a magnetic stirrer to maintain homogeneity. The solutions (SST micella) were kept at the various investigation temperatures (0, -10 and -30° C) in the same order as in section 2.3.3.1 for 36 hours. Green oils settled at the bottom of the flasks and were filtered off.

Activated carbon was then added in the ratio of 1 litre : 5g carbon to the SST micella recovered above, and shaken in a mechanical shaker for 1 hour. The carbon was then filtered off. The resulting solutions were concentrated using a rotary evaporator to yield refined pale extracts which were later analysed for taraxasterol and pyrethrins content (see section 2.6.2 and 2.6.3) to give results given in Tables 8, 9, 10, 11, 12, 13, 14 and 15 (pale)

2.4 PREPARATION OF COMPOSITE FLOWER EXTRACTS

A method based on colour, physical state (gelled or not gelled), taraxasterol and pyrethrins contents of refined extracts from the farming regions was used. Flowers from regions yielding gelled (poor quality) extract were mixed with those from regions yielding non-gelled (good qual ity) extract in two different ratios (1:1 and 1:3) and the composites proc essed as in section 2.3.2 to 2.3.3 to give results given in Tables 12 and 18.

Table 3: MIXING RATIOS OF FLOWERS

Gelled	non-gelled	Ratio (gelled:non-gelled)
Kisii	Bahati	1:1 1:3
Nyahururu	Meru	1:1 1:3
Bomet	Molo	1:1 1:3

2.5 DETERMINATION OF LEVELS OF TARAXASTEROL CAUSING GELLING IN REFINED PYRETHRUM EXTRACT

Crude taraxasterol obtained from Pyrethrum Board of Kenya laboratories was washed several times with hexane. The taraxasterol was then recrystallised from acetone and its purity determined using Gas Liquid Chromatograph as follows: The taraxasterol (0.5085 g) was dissolved in n-butanol (250 mL). Chromatograms of the resulting solution were run in duplicate and % purity of the taraxasterol calculated as follows:

% purity = (peak height of taraxasterol / total peak height of the sample)

x 100

= (1034448 / 1146714) x 100

= 90.2 %

Varying amounts of the taraxasterol (90 % purity) in acetone were added to refined extract (20 mL) [obtained from Pyrethrum Board of Kenya and determined to contain 3.65 % taraxasterol and 49.43 % pyrethrins]. The acetone was then removed using a rotary evaporator and the resulting mixtures monitered for gelling over a period of 6 months. The results are shown in Tables 13 and 19 and were determined using U.V. and G.L.C. methods outlined in sections 2.6.2 and 2.6.3 respectively.

2.6 ANALYTICAL PROCEDURES

2.6.1. CHEMICAL ANALYSIS OF PYRETHRINS [ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (A.O.A.C.) METHOD]

The extracts from section 2.3.1 were analysed for pyrethrins content using A.O.A.C. methods[15,16].

2.6.1.1 DETERMINATION OF PYRETHRINS I

The hexane extract from section 2.3.1 was extracted using 0.1M NaOH solution(2 x 5 mL) and the hexane layer discarded. Deniges reagent (10 mL) was added to the sodium hydroxide (NaOH) extract in a 100 mL beaker and kept in a dark water-bath at room temperature for 1 hour. On removing from the dark water-bath, 3 mL of saturated sodium chloride (NaCl) were added immediately followed by 20 mL of amyl alcohol (mixture of ca. 60-70 % n-pentanol and ca. 30-40 % 2-Methylbutanol). The saturated sodium chloride (NaCl) solution precipitates mercurous chloride (HgCl). The solution was warmed to 60° C and filtered through filter paper with all the precipitate being transferred to the filter paper. The residue on the filter paper was rinsed with hot amyl alcohol (10 mL) and the filter paper and the residue placed in a conical flask.

The beaker containing the original NaOH extract was washed with HCl:H_2O (3:1) solution and the sides of the beaker wiped with cotton wool on a glass rod before emptying both the hydrochloric acid solution and the piece of cotton wool into the conical flask containing the filter paper and the residue. Chloroform (20 mL) was added to the residue followed by iodine monochloride indicator (1 mL) and titrated with 0.01M KIO_3 solution with constant shaking until the pinkish colour of indicator in chloroform disap peared. The % pyrethrins I was determined using the following formula[36]:

Derivation of % (w/w) Pyrethrins I formula:

Experiments have shown that 1 mL of 0.01M KIO_3 is equivalent to 0.0057 g of Pyrethrins I, Initially the sample was made to 200 mL of which only 200 mL was used for analysis.

Let the weight of sample = w, Let the volume of 0.01M KIO_3 used = v

Therefore amount of pyrethrins I in the 200 mL taken = (v x 0.0057) g

Amount of Pyrethrins I in the original 250 mL = (250/200 x v x 0.0057)

therefore % Pyrethrins I = (250/200 x v/w x 0.0057 x 100)

$$= 0.7125 \times v/w$$

where:

v = volume of 0.01M KIO_3 used

w = weight of sample

The results are shown in Table 5.

2.6.1.2 DETERMINATION OF PYRETHRINS II

The aqueous extract obtained in section 2.3.1 was evaporated (hot plate) to 50 mL and cooled to room temperature. The aqueous extract was then added to the saturated solution of sodium chloride (NaCl) prepared by adding hydrochloric acid solution (10 mL) to a separating funnel containing solid NaCl. The sodium chloride supersaturates the aqueous extract and reduces the solubility of pyrethrins II in the aqueous layer. The aqueous layer was extracted with diethyl ether (3 x 50 mL) to remove traces of hydrochloric acid. The ether was evaporated off in a water bath and the remaining sample placed in an oven at 100°C for 10 minutes to vapourise any traces of hydrochloric acid. On removing from the oven, a current of compressed air was blown through the sample to remove hydrochloric acid fumes. The sample was then dissolved in 3 mL of neutral ethanol and 20 mL of distilled water added. Phenolphthalein indicator (2 drops) was added and titrated with 0.02M NaOH. The % w/w pyrethrins II was determined using the following formula[36].

$$\% \text{ pys II} = (0.4675 \times v_s/w_s) \times T.F$$

Where:

v_s = volume of 0.02M NaOH used

w_s = weight of sample T.F = titration factor

The results are shown in Table 6.

Derivation of % Pyrethrins II formula:

Experiments have shown that 1 mL of 0.02M NaOH is equivalent to 0.00374 g of pyrethrins II. Initially the sample was made to 250 mL of which only 200 mL were used for analysis.

Let the weight of sample = w_s

Let the volume of 0.02M NaOH used on the sample = v_s

Amount of Pyrethrins II in the 200 mL used = $(v_s \times 0.00374)$g

Therefore amount of Pyrethrins II in the original 250 mL

$$= (250 / 200 \times v_s \times 0.00374)\ g$$

Therefore , % Pyrethrins II $= (250 /200 \times v_s / w_s \times 0.00374 \times 100)$.

This formula requires a titration factor (T.F) which is the change of basisity of sodium hydroxide with respect to potassium hydrogen phthalate. The modified formula thus becomes:

% Pyrethrins II $= (250 /200 \times v_s / w_s \times 0.00374 \times 100 \times T.F)$

$$= (0.4675 \times v_s / w_s) \times T.F.$$

Determination of Pyrethrins II Titration Factor (T.F)

Potassium hydrogen phthalate (0.8 g) was dissolved in distilled water (200 mL). The resulting solution (10 mL) was titrated against 0.02M NaOH solution. Titration factor was calculated using the following formula[16]:

$T.F = A/2 \times 1/0.40844 \times 1/T \times 10 \times 0.4675$

Where A = weight of potassium hydrogen phthalate used

T = mean titre

0.40844 and 0.4675 are fundamental constants

The results are shown below

Table 4: TITRATION FACTOR FOR PYRETHRINS II DETERMINATION

Weight of potassium hydrogen phthalate (g)	titre (mL)	mean titre (mL)	T.F
0.799	9.40 9.85	9.63	0.4748
0.801	9.75 9.50	9.63	0.4759
0.801	9.45 10.05	9.75	0.4700
0.801	9.50 9.55	9.53	0.4810
0.802	9.65 9.70	9.68	0.4740

TABLE 5: PERCENTAGE PYRETHRINS I CONTENT OF FLOWERS FROM VARIOUS FARMING AREAS

Farming area	Wt. of sample (g)		Vol. of KIO_3 used v (cm_3)		% Pys I		Mean %Pys I
	A	B	A	B	A	B	
Kisii	8	9	5.05	5.55	o.450	0.439	0.448
	8	9	5.10	7.15*	0.454	0.566*	
Nyahururu	8	9	7.95	8.85	0.708	0.701	0.703
	8	9	7.65	9.10	0.681	0.720	
Bomet	8	9	12.20	13.90	1.087	1.100	1.110
	8	9	13.00	13.85	1.158	1.096	
Bahati	8	9	15.75	17.55	1.403	1.389	1.436
	8	9	16.55	18.65	1.474	1.476	
Molo	8	9	10.35	11.55	0.922	0.914	0.918
Nyeri	8	9	6.90	7.80	0.615	0.618	0.606
	8	9	6.85	7.35	0.610	0.582	
Meru	8	9	15.45	18.45	1.376	1.461	1.177
	8	9	10.45	11.85	0.931	0.938	
Eldoret	8	9	10.45	11.65	0.931	0.922	0.924
	8	9	10.30	11.70	0.917	0.926	

* values not used (there was an overshoot of KIO_3 during titration)

TABLE 6: PERCENTAGE PYRETHRINS II CONTENT OF FLOWERS FROM VARIOUS FARMING AREAS

Farming area	Wt. of sample w_s (cm_3)		Vol. of NaOHused v_s (cm_3)		T.F	% Pys		Mean %Pys II
	A	B	A	B		A	B	
Kisii	8	9	9.15	10.45	0.4748	0.254	0.258	0.259
	8	9	9.40	10.65		0.261	0.263	
Nyahururu	8	9	15.60	17.55	0.4748	0.433	0.433	0.428
	8	9	15.15	17.25		0.420	0.425	
Bomet	8	9	9.60	10.75	0.4759	0.267	0.266	0.280
	8	9	10.20	10.95		0.284	0.304	
Bahati	8	9	15.90	16.75	0.4700	0.437	0.409	0.423
	8	9	15.95	16.75		0.438	0.409	
Molo	8	9	18.45	22.10	0.4810	0.519	0.552	0.536
Nyeri	8	9	15.70	17.00	0.4748	0.436	0.419	0.420
	8	9	15.15	16.35		0.420	0.403	
Meru	8	9	9.50	11.60	0.4740	0.263	0.286	0.316
	8	9	13.30	14.05		0.368	0.346	
Eldoret	8	9	14.15	14.45		0.398	0.361	
	8	9	14.45	16.00	0.4810	0.406	0.400	0.391

Total % pyrethrins content of flowers was calculated as shown below[36]:

Total% w/w pys = % pys I + % pys II

The results are shown in Table 7

TABLE 7: PERCENTAGE PYRETHRINS CONTENT OF FLOWERS FROM VARIOUS FARMING AREAS

Farming Area	Total pyrethrins
Kisii	0.707
Nyahururu	1.131
Bomet	1.390
Bahati	1.859
Molo	1.454
Nyeri	1.026
Meru	1.493
Eldoret	1.315

2.6.2 PYRETHRINS CONTENT ANALYSIS IN EXTRACT BY ULTRAVIOLET SPECTROPHOTOMETRY[36]

Ultraviolet (U.V) analyses were performed on DMS-80 UV/Visible spectrophotometer fitted with a UV lamp. A 1 nm spectral band width was used and the wavelength was set at 225 nm.

The pyrethrum extracts at various stages were weighed within the set weight limits (0.10 - 0.12) in 100 mL volumetric flasks in duplicate. The same was done for pyrethrum extract standards. Both the sample extracts and the standards were made to the 100 mL mark with distilled hexane. The solutions were then diluted 10 times with hexane. Absorbances of the samples and the standard solutions were determined using the UV spectrophotometer at 225 nm and ratios calculated as shown below[36]:

Ratio = absorbance / weight taken.

The factor for the standard was calculated as follows[36]:

Standard Factor = concentration of std. / ratio of std.

The pyrethrins content (% w/w) of the samples were calculated as follows[36]:

% w/w pyrethrins of sample = ratio of sample x std factor .

The obtained results were recorded (Tables 8, 9, 10, 11, 12 and 13).

TABLE 8: PERCENTAGE PYRETHRINS CONTENT FOR PALE EXTRACTS PROCESSED AT 0° C

Farming area	Absorbance		Weight (g)		Ratio		conc. of Std. (%)	% Pys
	sample	std.	sample	std.	sample	std.		
Kisii	0.459	0.613	0.1125	0.1096	4.080	5.593	52.91	38.73
	0.436	0.634	0.1061	0.1133	4.109	5.596		
Nyahururu	0.473	0.613	0.1054	0.1096	4.488	5.593	52.91	42.49
	0.488	0.634	0.1085	0.1133	4.498	5.596		
Bomet	0.578	0.613	0.1099	0.1096	5.259	5.593	52.91	49.69
	0.570	0.634	0.1086	0.1133	5.249	5.596		
Bahati	0.683	0.583	0.1094	0.1030	6.243	5.660	54.05	59.65
	0.696	0.567	0.1119	0.1007	6.220	5.631		
Molo	0.538	0.667	0.1015	0.1165	5.182	5.622	54.05	49.90
	0.586	0.580	0.1107	0.1013	5.185	5.607		
Nyeri	0.553	0.613	0.1083	0.1096	5.106	5.593	52.91	48.15
	0.565	0.634	0.1113	0.1133	5.076	5.596		
Meru	0.700	0.613	0.1188	0.1096	5.892	5.593	52.91	55.73
	0.637	0.634	0.1081	0.1133	5.893	5.596		
Eldoret	0.598	0.590	0.1168	0.1048	5.120	5.630	54.05	49.05
	0.599	0.575	0.1187	0.1033	5.046	5.566		

TABLE 9: PERCENTAGE PYRETHRINS CONTENT FOR PALE EXTRACTS PROCESSED AT -10° C

Farming area	Absorbance		Weight (g)		Ratio		conc. of Std. (%)	% Pys
	sample	std.	sample	std.	sample	std.		
Kisii	0.409	0.603	0.1028	0.1002	3.979	6.018	52.91	35.75
	0.427	0.589	0.1060	0.1010	4.028	5.832		
Nyahururu	0.567	0.602	0.1209	0.1071	4.690	5.621	52.91	43.45
	0.551	0.649	0.1155	0.1100	4.771	5.900		
Bomet	0.620	0.603	0.1037	0.1002	5.979	6.018	52.91	53.31
	0.624	0.589	0.1047	0.1010	5.960	5.832		
Bahati	0.630	0.583	0.1012	0.1030	6.225	5.660	54.05	60.11
	0.665	0.567	0.1050	0.1007	6.333	5.631		
Molo	0.466	0.667	0.1032	0.1165	4.399	5.622	54.05	42.35
	0.452	0.580	0.1000	0.1013	4.400	5.607		
Nyeri	0.630	0.602	0.1170	0.1071	5.385	5.621	52.91	50.06
	0.590	0.649	0.1070	0.1100	5.514	5.900		
Meru	0.714	0.602	0.1115	0.1071	6.404	5.621	52.91	58.88
	0.760	0.649	0.1188	0.1100	6.397	5.900		
Eldoret	0.547	0.590	0.1064	0.1048	5.141	5.630	54.05	49.88
	0.554	0.575	0.1066	0.1033	5.197	5.566		

TABLE 10: PERCENTAGE PYRETHRINS CONTENT FOR PALE EXTRACTS PROCESSED AT -30° C

Farming area	Absorbance		Weight (g)		Ratio		conc. of Std. (%)	% Pys
	sample	std.	sample	std.	sample	std.		
Kisii	0.447	0.702	0.1023	0.1149	4.370	6.110	52.91	38.35
	0.474	0.682	0.1066	0.1126	4.447	6.057		
Nyahururu	0.594	0.702	0.1209	0.1149	4.913	6.110	52.91	44.14
	0.563	0.682	0.1075	0.1126	5.237	6.057		
Bomet	0.477	0.603	0.1002	0.1002	4.760	6.018	52.91	42.57
	0.486	0.589	0.1018	0.1010	4.774	5.832		
Bahati	0.662	0.583	0.1049	0.1030	6.310	5.660	54.05	60.40
	0.636	0.567	0.1008	0.1007	6.310	5.631		
Molo	0.491	0.667	0.1042	0.1165	4.597	5.622	54.05	44.38
	0.477	0.580	0.1006	0.1013	4.622	5.607		
Nyeri	0.566	0.648	0.1060	0.1057	5.340	6.131	52.91	47.77
	0.619	0.630	0.1107	0.1054	5.592	5.977		
Meru	0.726	0.702	0.1163	0.1149	6.242	6.110	52.91	54.95
	0.741	0.682	0.1159	0.1126	6.393	6.057		
Eldoret	0.504	0.590	0.1014	0.1048	4.970	5.630	54.05	47.54
	0.545	0.575	0.1116	0.1033	4.884	5.566		

Pyrethrins content of the other extracts (OR and MeOH) were calculated in the same manner as for pale extracts and a summary Table 11 constructed.

TABLE 11: PERCENTAGE PYRETHRINS CONTENT OF EXTRACTS FROM FLOWERS AT DIFFERENT STAGES OF PROCESSING AND AT DIFFERENT OPERATIONAL TEMPERATURES (SUMMARY)

	Temperature (° C)						
Extracts	0			-10		-30	
Production region	OR	MeOH	Pale	MeOH	Pale	MeOH	Pale
Kisii	27.20	40.04	38.73	38.71	35.75	37.95	38.35
Nyahururu	29.99	45.49	42.49	45.06	43.45	46.32	44.14
Bomet	38.78	48.31	49.69	48.39	53.31	50.46	42.57
Bahati	38.26	47.46	59.65	49.90	60.11	47.77	60.40
Molo	37.27	45.38	49.90	48.49	42.35	49.01	44.38
Nyeri	38.34	53.36	48.15	53.02	50.02	57.68	47.77
Meru	46.53	57.46	55.73	57.60	58.88	59.84	54.95
Eldoret	39.85	43.19	49.09	44.23	49.88	41.75	47.54

TABLE 12: PERCENTAGE PYRETHRINS CONTENT OF PALE EXTRACTS FOR COMPOSITES PROCESSED AT -10^{O} C

Composite	Gelled: non-gelled	Absorbance	Wt. of sample (g)	Ratio of sample	% Pys	Physical state
Kisii/ Bahati	1:1	0.494	0.1016	4.862	45.82	non-gelled
		0.492	0.1009	4.876		
	1:3	0.404	0.1029	3.930	37.36	non-gelled
		0.430	0.1072	4.011		
Nyahururu / Meru	1:1	0.568	0.1014	5.602	52.79	non-gelled
		0.572	0.1018	5.619		
	1:3	0.570	0.1069	5.332	49.81	non-gelled
		0.577	0.1098	5.255		
Bomet / Molo	1:1	0.543	0.1019	5.329	49.89	gelled
		0.547	0.1037	5.275		
	1:3	0.477	0.1043	4.573	42.56	gelled
		0.453	0.1013	4.472		
Std. sample		0.622	0.1103	5.639		
		0.620	0.1106	5.606		

concentration of the std. = 52.91% pys

Pys = Pyrethrins

TABLE 13: PYRETHRINS CONTENT OF REFINED EXTRACTS IN WHICH TARAXASTEROL WAS ADDED

No.	Weight of tara added (g)	% Pys	Physical state
1	0.0000	50.12	non-gelled
2	0.0504	49.94	non-gelled
3	0.0994	49.97	non-gelled
4	0.1499	49.23	non-gelled
5	0.2000	49.98	non-gelled
6	0.2254	50.09	gelled
7	0.2507	49.87	gelled
8	0.2509	49.61	gelled
9	0.3002	49.68	gelled
10	0.3506	49.31	gelled
11	0.4000	48.65	gelled
12	0.4503	48.80	gelled

2.6.3 TARAXASTEROL CONTENT DETERMINATION IN EXTRACT BY GAS LIQUID CHROMATOGRAPH (G.L.C.)

The G.L.C. analyses were performed using a PU 4410 chromatograph fitted with a Flame Ionisation Detector (F.I.D.) and glass column (1.5m x 4mm i.d.) packed with 1% methylphenylsilicon (e) (50:50) (OV 17) on chromosorb W AW mesh100/120 treated with dimethyldichlorosilane (DMSC). Temperature was maintained at 280°C - oven, 330°C - detector, 300°C - injection port for the duration of analysis while nitrogen was used as the carrier gas at a flow rate of 60 mL/min.The pyrethrum extracts at various stages (OR, methanol and refined concentrates) were analysed for taraxasterol content using Gas Liquid Chromatograph. The extracts and standards were weighed within the set weight limits (0.5 + or - 0.005) in 100 mL volumetric flasks in duplicate and made to the mark with n-butanol.

Chromatograms of the standard and sample solutions were run in duplicate and using peak height (Ht), the % taraxasterol content was calculated as shown below[36]:

% w/w tara = {conc. of std. (g/mL) / conc. of sample (g/mL)} x {Ht of sample / Ht of std.} x % purity of std.

The obtained results were recorded (see Tables 14, 15, 16, 17, 18 and 19).

TABLE 14: PERCENTAGE TARAXASTEROL CONTENT OF PALE EXTRACTS PROCESSED AT 0° C

Farming area	Wt. (g) sample	std.	Conc. (g/mL) sample	std.	Ht (%) sample	std	% Tara
Kisii	0.5058	0.5	5.058×10^{-3}	3.8×10^{-4}	64407	60959	7.32
	0.5016		5.016×10^{-3}		62838		
Nyahururu	0.5082	0.5	5.082×10^{-3}	3.2×10^{-4}	47080	47125	5.93
	0.5075		5.075×10^{-3}		48374		
Bomet	0.5011	0.5	5.011×10^{-3}	3.2×10^{-4}	45803	47712	5.71
	0.5010		5.010×10^{-3}		45880		
Bahati	0.5111	0.5	5.111×10^{-3}	2.5×10^{-4}	37457	34333	4.93
	0.5176		5.176×10^{-3}		37476		
Molo	0.5055	0.5	5.055×10^{-3}	2.6×10^{-4}	39908	41100	4.64
	0.5063		5.065×10^{-3}		39966		
Nyeri	0.4985	0.5	4.985×10^{-3}	2.3×10^{-4}	34346	34368	4.24
	0.4964		4.964×10^{-3}		33311		
Meru	0.4977	0.5	4.977×10^{-3}	2.0×10^{-4}	30803	30975	3.74
	0.4966		4.966×10^{-3}		31111		
Eldoret	0.5056	0.5	5.056×10^{-3}	2.5×10^{-4}	36524	38348	4.40
	0.5076		5.076×10^{-3}		36900		

% purity of standard = 93 (predetermined)

TABLE 15: PERCENTAGE TARAXASTEROL CONTENT OF PALE EXTRACTS PROCESSED AT-10° C

Farming area	Wt. (g) sample	std.	Conc. (g/mL) sample	std.	Ht (%) sample	std	% Tara
Kisii	0.4917	0.5	4.917×10^{-3}	4.4×10^{-4}	67876	68246	8.27
	0.4919		4.919×10^{-3}		67731		
Nyahururu	0.4979	0.5	4.979×10^{-3}	3.8×10^{-4}	57013	59897	6.90
	0.4947		4.947×10^{-3}		57293		
Bomet	0.4985	0.5	4.985×10^{-3}	3.0×10^{-4}	49759	47439	5.90
	0.4974		4.974×10^{-3}		50287		
Bahati	0.5155	0.5	5.155×10^{-3}	3.0×10^{-4}	38005	39314	5.27
	0.5019		5.019×10^{-3}		37449		
Molo	0.4990	0.5	4.990×10^{-3}	1.9×10^{-4}	28822	28528	3.59
	0.5009		5.009×10^{-3}		29098		
Nyeri	0.4957	0.5	4.957×10^{-3}	2.6×10^{-4}	38987	40099	4.79
	0.4967		4.967×10^{-3}		39890		
Meru	0.4969	0.5	4.969×10^{-3}	2.2×10^{-4}	32960	33320	4.08
	0.4981		4.981×10^{-3}		33108		
Eldoret	0.4951	0.5	4.951×10^{-3}	1.9×10^{-4}	28204	28370	3.51
	0.4957		4.957×10^{-3}		27544		

% purity of standard = 93 (predetermined)

TABLE 16: PERCENTAGE TARAXASTEROL CONTENT OF PALE EXTRACTS PROCESSED AT-30° C

Farming area	Wt. (g) sample	std.	Conc. (g/mL) sample	std.	Ht (%) sample	std	% Tara
Kisii	0.4916	0.5	4.916×10^{-3}	2.4×10^{-4}	29279	29295	4.56
	0.4927		4.927×10^{-3}		29599		
Nyahururu	0.5077	0.5	5.077×10^{-3}	2.2×10^{-4}	36120	35956	4.12
	0.5043		5.043×10^{-3}		37012		
Bomet	0.5026	0.5	5.026×10^{-3}	1.2×10^{-4}	18296	16779	2.41
	0.5038		5.038×10^{-3}		18144		
Bahati	0.5009	0.5	5.009×10^{-3}	2.1×10^{-4}	27195	28809	3.72
	0.5079		5.079x 10-3		28057		
Molo	0.5018	0.5	5.018×10^{-3}	1.1×10^{-4}	15624	16499	1.92
	0.5038		5.038×10^{-3}		15462		
Nyeri	0.5040	0.5	5.040×10^{-3}	2.5×10^{-4}	36164	34490	4.88
	0.5023		5.023×10^{-3}		36631		
Meru	0.4971	0.5	4.971×10^{-3}	2.3×10^{-4}	37376	36917	4.33
	0.4957		4.957×10^{-3}		36800		
Eldoret	0.4968	0.5	4.968×10^{-3}	1.3×10^{-4}	19042	18582	2.51
	0.4959		4.959×10^{-3}		19220		

% purity of standard = 93 (predetermined)

Taraxasterol content of the other extracts (OR and MeOH) were calculated in the same manner as for pale extracts and a summary Table 17 constructed.

Table17: PERCENTAGE TARAXASTEROL CONTENT OF EXTRACTS FROM FLOWERS AT DIFFERENT STAGES OF PROCESSING AND DIFFERENT OPERATIONAL TEMPERATURE (SUMMARY).

		Temperature (oC)					
Extracts		0		-10		-30	
Production region	OR	MeOH	Pale	MeOH	Pale	MeOH	Pale
Kisii	7.42	6.71	7.32	7.25	8.27	3.21	4.56
Nyahururu	5.48	5.40	5.93	5.43	6.90	3.12	4.12
Bomet	5.98	5.80	5.71	5.70	5.90	2.60	2.41
Bahati	-	4.24	4.93	4.12	5.27	3.13	3.72
Molo	4.99	4.45	4.64	4.39	3.59	2.27	1.92
Nyeri	3.24	3.63	4.24	3.64	4.79	3.67	4.88
Meru	2.68	2.69	3.74	2.80	4.08	2.68	4.33
Eldoret	3.81	3.67	4.40	3.49	3.51	2.56	2.51

TABLE 18: PERCENTAGE TARAXASTEROL CONTENT OF PALE EXTRACTS FOR COMPOSITES PROCESSED AT -10^{O} C

Composite	Ratio (gelled:non-gelled)	Wt. (g) sample	Wt. (g) std.	Conc. (g/mL) sample	Conc. (g/mL) std	Ht. (%) sample	Ht. (%) std	% Tara	Physical state
Kisii / Bahati	1:1	0.5040 0.5072	0.5	5.040×10^{-3} 5.072×10^{-3}	3.0×10^{-4}	4984 4995	5008	5.50	non-gelled
	1:3	0.5004 0.5163	0.5	5.004×10^{-3} 5.163×10^{-3}	2.2×10^{-4}	3656 3681	3653	4.05	non-gelled
Nyahururu / Meru	1:1	0.5016 0.5029	0.5	5.016×10^{-3} 5.029×10^{-3}	3.0×10^{-4}	4352 4433	4668	5.23	non-gelled
	1:3	0.5148 0.5225	0.5	5.148×10^{-3} 5.225×10^{-3}	3.0×10^{-4}	4733 5023	5032	5.21	non-gelled
Bomet / Molo	1:1	0.5005 0.5033	0.5	5.005×10^{-3} 5.033×10^{-3}	3.8×10^{-4}	5966 5946	6259	6.70	gelled
	1:3	0.4993 0.5320	0.5	5.993×10^{-3} 5.320×10^{-3}	3.8×10^{-4}	5925 6639	6653	6.47	gelled

% purity of standard = 93 (predetermined)

TABLE 19: PERCENTAGE TARAXASTEROL CONTENT OF REFINED EXTRACTS IN WHICH TARAXASTEROL WAS ADDED

No.	Weight of tara added (g)	% Tara	Physical state
1	0.0000	3.65	non-gelled
2	0.0504	3.90	non-gelled
3	0.0994	4.15	non-gelled
4	0.1499	4.34	non-gelled
5	0.2000	4.65	non-gelled
6	0.2254	4.78	gelled
7	0.2507	4.90	gelled
8	0.2509	4.90	gelled
9	0.3002	5.15	gelled
10	0.3506	5.40	gelled
11	0.4000	5.65	gelled
12	0.4503	5.90	gelled

3.0 RESULTS AND DISCUSSION

3.1 EFFECT OF DIFFERENT FARMING REGIONS ON PHYSICAL CHARACTERISTICS OF EXTRACT

Pyrethrum is grown extensively in Kenya. Successful cultivation of pyrethrum flowers is dependent upon climatic conditions, type of soil, and also upon the methods of cultivation used and care taken during the life cycle of the plant. The regions investigated for taraxasterol and pyrethrins content were Eldoret, Kisii, Meru, Molo, Bahati, Nyahururu, Nyeri and Bomet.

Air dried pyrethrum flowers (< 10 % w/v moisture content, see Table 2) were processed as detailed in the experimental section to give a liquid concentrate. Different farming regions gave extracts with different physical characteristics in terms of physical state, taraxasterol content, and pyrethrins content (see Table 20).

Some regions, namely, Bahati, Nyeri, Molo, Meru and Eldoret gave good quality extract (i.e. high pyrethrins content, low taraxasterol content and non-gelled extract), while the other regions, namely, Kisii, Bomet and Nyahururu gave low quality extract (i.e. low pyrethrins content, high taraxasterol content and gelled extract) see Table 20.

TABLE 20: PHYSICAL CHARACTERISTICS OF EXTRACT FROM DIFFERENT FARMING REGIONS, PROCESSED AT DIFFERENT TEMPERATURES

	Temperature °C								
Extracts	0			-10			-30		
Farming region	PS	%pys	%tara	PS	%pys	%tara	PS	%py	% tara
*Kisii	G	38.73	7.32	G	35.75	8.27	N	38.35	4.56
*Nyahururu	G	42.49	5.93	G	43.45	6.90	N	44.14	4.12
*Bomet	G	49.69	5.71	G	53.31	5.90	N	42.57	2.41
oBahati	N	59.65	4.93	N	60.11	5.24	N	60.40	3.72
oMolo	N	49.90	4.64	N	42.35	3.59	N	44.38	1.92
oNyeri	N	48.15	4.24	N	50.06	4.79	N	47.77	4.88
oMeru	N	55.73	3.74	N	58.88	4.08	N	54.95	4.33
oEldoret	N	49.04	4.40	N	49.88	3.51	N	47.54	2.51

PS = Physical state G = Gelled N = Non-gelled Tara = Taraxasterol Pys = Pyrethrins

Values were obtained by using formulae on pages 54 and 61, with % values of pys extracted from Table 21 and % values of tara extraced from Table 27.

* Low quality extract (% pys < 45, % tara > 5) ; (o) High quality extract (% pys > 45, % tara < 5)

NB: The temperatures were controlled at methanol and SST stages

The variations in levels of constituents and physical state of pyrethrum extract can be explained in the light of the biosythetic pathways of constituents. Figures 5 and 7 show that some steps (from acetyl coenzyme A to dimethylallyl pyrophosphate) in the biosynthetic pathways of both pyrethrins and triterpenoids are similar.

Synthesis of the constituents is controlled by the activity of enzymes and the availability of precursors and therefore if conditions are not favour able, there is a possibility of inhibiting the synthesis of some components while promoting synthesis of the others[37].

Factors like enzymes and their precursors that influence the biosynthesis of constituents are modulated by such conditions as temperature, soil, precipitation, and quantity of sunlight[37]. This suggests that variations in physical characteristics of extract could be as a result of variation in climatic conditions which in turn possibly influence the synthesis of the components in the pyrethrum plant.

3.2 EFFECT OF OPERATIONAL TEMPERATURES DURING REFINING PROCESS ON THE PHYSICAL STATE OF EXTRACTS

Gels were observed in extracts of flowers from Kisii, Bomet, and Nyahururu that were processed at higher temperatures (0° C and -10° C). No gels were seen at lower temperatures(-30° C). Flowers from Bahati, Nyeri, Molo, Meru and Eldoret gave extracts which were not gelled even at higher temperatures (Table 20).

This suggests that operational temperatures do not necessarily govern the physical characteristics of extract. However, knowledge of the type of flowers (gelling or non-gelling) would help in selecting suitable operational temperatures which would result in non-gelled extracts.

3.3 CORRELATION BETWEEN TARAXASTEROL LEVELS AND GELLING OF REFINED EXTRACT

Formation of a gel from solution requires the gelling agent to separate into finely dispersed colloidal particles that join together to form a continous coherent framework throughout the fluid volume[39]. A group of steroidal molecules capable of both donating and accepting hydrogen bonds has been found to form very stable gels with cyclohexane[39]. The hydroxyl group in the taraxasterol molecule can facilitate hydrogen bond formation in the system which could lead to gelling.

Extracts were analysed for taraxasterol using G.L.C. and grouped according to their physical state (gelled or non-gelled, Table 21)

TABLE 21: TARAXASTEROL LEVELS AND PHYSICAL STATE OF EXTRACT

% taraxasterol	
Gelled	Non-gelled
8.27	5.50
7.32	5.24
6.90	5.23
6.70	5.21
6.47	4.93
5.93	4.64
5.90	4.56
5.71	4.40
5.65	4.33
5.40	4.12
5.15	4.08
4.90	4.05
4.78	3.72
	3.59
	3.51
	2.51
	2.41
	1.92

Data extracted from Tables 20, 18 and 19

NB. Entries in columns are for different samples and therefore need not be the same.

From Table 21, there is an overlap of data at 5.50 and 4.78 % taraxasterol values for gelled and non-gelled extracts. Therefore levels of taraxasterol at which gelling occurs cannot be ascertained.

Earlier work done by Pyrethrum Board of Kenya gave taraxasterol levels in extracts with the extracts being grouped according to their physical state (Table 22).

TABLE22: TARAXASTEROL LEVELS AND PHYSICAL STATE OF EXTRACTS

% Taraxasterol	
gelled	non gelled
4.78	3.81
4.58	3.68
4.52	3.66
4.45	3.58
4.40	3.57
4.30	3.56
4.26	3.55
4.16	3.53
4.11	3.50
4.10	3.46
4.09	3.43
4.07	3.40
4.01	3.39
3.99	3.38
3.95	3.37
3.94	3.35
3.92	3.33
3.91	3.30
3.80	3.29
3.73	3.21
3.71	3.18
3.70	3.13
3.69	3.07
3.65	3.03
3.57	2.99
3.56	2.97
3.55	2.94
3.54	2.90
3.53	2.87
3.50	2.85
	2.69
	2.63

data obtained from P.B.K[40]

NB: Entries in columns are for different samples and therefore need not be same.

From Table22, there is an overlap of data at 3.81 and 3.50 %taraxasterol values fof gelled and non-gelled extracts.

It is deduced from Tables 21 and 22 that gelling of pyrethrum extract is dependent on the sample and not necessarily taraxasterol content. This is apparent from the wide range of overlap of data for gelled and non-gelled extracts in Tables 21 and 22, and also from lack of correlation between investigation data (Table 21) and data obtained from Pyrethrum Board of Kenya (Table 22).

2.4 RELATIONSHIP BETWEEN TARAXASTEROL AND PYRETHRINS LEVELS IN EXTRACTS (GELLED AND NON-GELLED)

Taraxasterol measurements alone do not correlate with the physical state of extract (section 3.3). This necessitated other relationships to be investigated.

Pyrethrum extract gel is usually reversible to the sol state by thermal agitation and may only reappear after remaining undisturbed for some time. Taraxasterol will not cause gelling if dissolved in isoalkanes which are the usual diluents and thus the gelling of extract is not due to taraxasterol-solvent interactions.

By virtue of their structural pattern, pyrethrins are capable of interacting with pentacyclic triterpenols (taraxasterol) possibly through hydrogen bonding between the keto group of the pyrethrins and the exocyclic hydroxyl group of the pentacyclic triterpenols (Figure 8).

Gels are intermediate state of existence of matter that contain both solid and liquid components[41]. Theoretical models of the gel phase consider the solid component as a three dimensional network of interconnected molecular aggregates embedded in a liquid dispersion medium. One way of classifying gels is according to cohesion energies between the molecules that form the solid part of the gel.

This classification distinguishes chemical gels involving covalent bonds from physical gels which involve low energy bonds. In the related sol phase, which is liquid, aggregates and individual molecules are no longer interconnected as in the solid gel network but are dispersed throughout the solution. The sol-gel transition will be reversible if the cohesion of the gel network involves bonds such as hydrogen bonds, ionic or Van der Waals interactions of energy comparable to the thermal agitation[41]. This is similar to the case of physical gels in which pyrethrum extract gel falls.

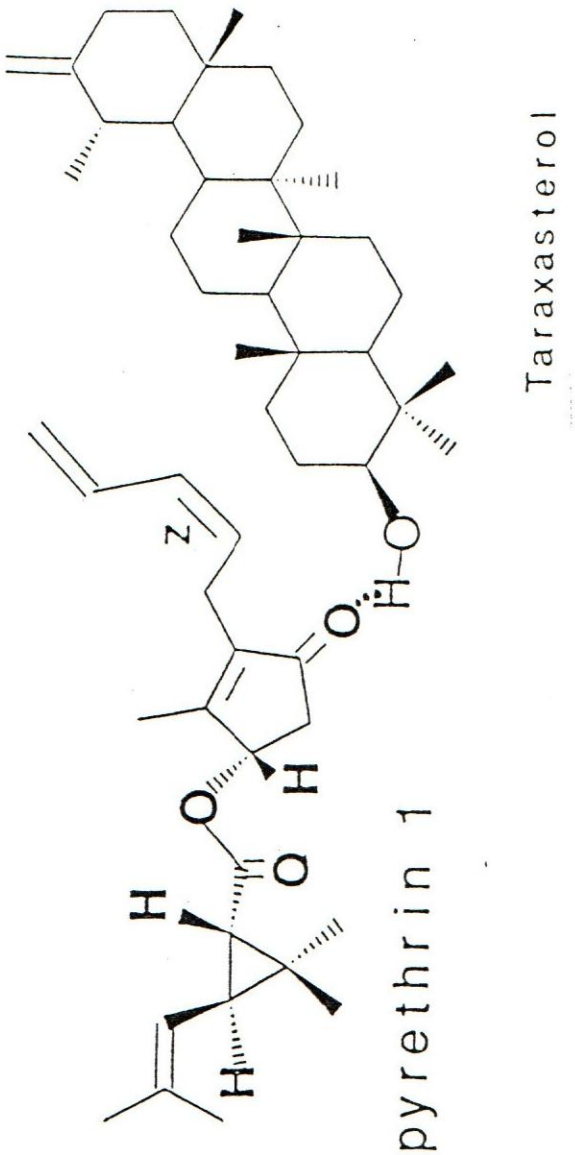


FIGURE 8: ..... Possible hydrogen bonding between taraxasterol and pyrethrins

In pyrethrum extract, there are constituents of crystalline structure among which taraxasterol is the most abundant[33]. These constituents can be considered to be the ones acting as centres of gelation. Considering taraxasterol (and related molecules) and pyrethrins molecules in the system, the following interactions through hydrogen bonds are possible: taraxasterol-taraxastrerol (Figure 9) and taraxasterol-pyrethrins (Figure 8) interactions.

Ratios of pyrethrins to taraxasterol levels in extracts were calculated to establish a relationship between the two parameters and the physical state of extracts.

TABLE 23: PYRETHRINS TO TARAXASTEROL RATIOS FOR GELLED EXTRACTS

% pys	% tara	pys / tara
50.09	4.78	10.78
49.87	4.90	10.17
49.61	4.90	10.12
49.68	5.15	9.65
49.31	5.40	9.13
53.31	5.90	9.04
49.69	5.71	8.70
48.65	5.65	8.61
48.80	5.90	8.27
49.89	6.70	7.45
42.49	5.93	7.17
42.56	6.47	6.57
43.45	6.90	6.30
38.73	7.32	5.29
35.75	8.27	4.32

Data extracted from tables 11, 12, 13, 17, 18 and 19

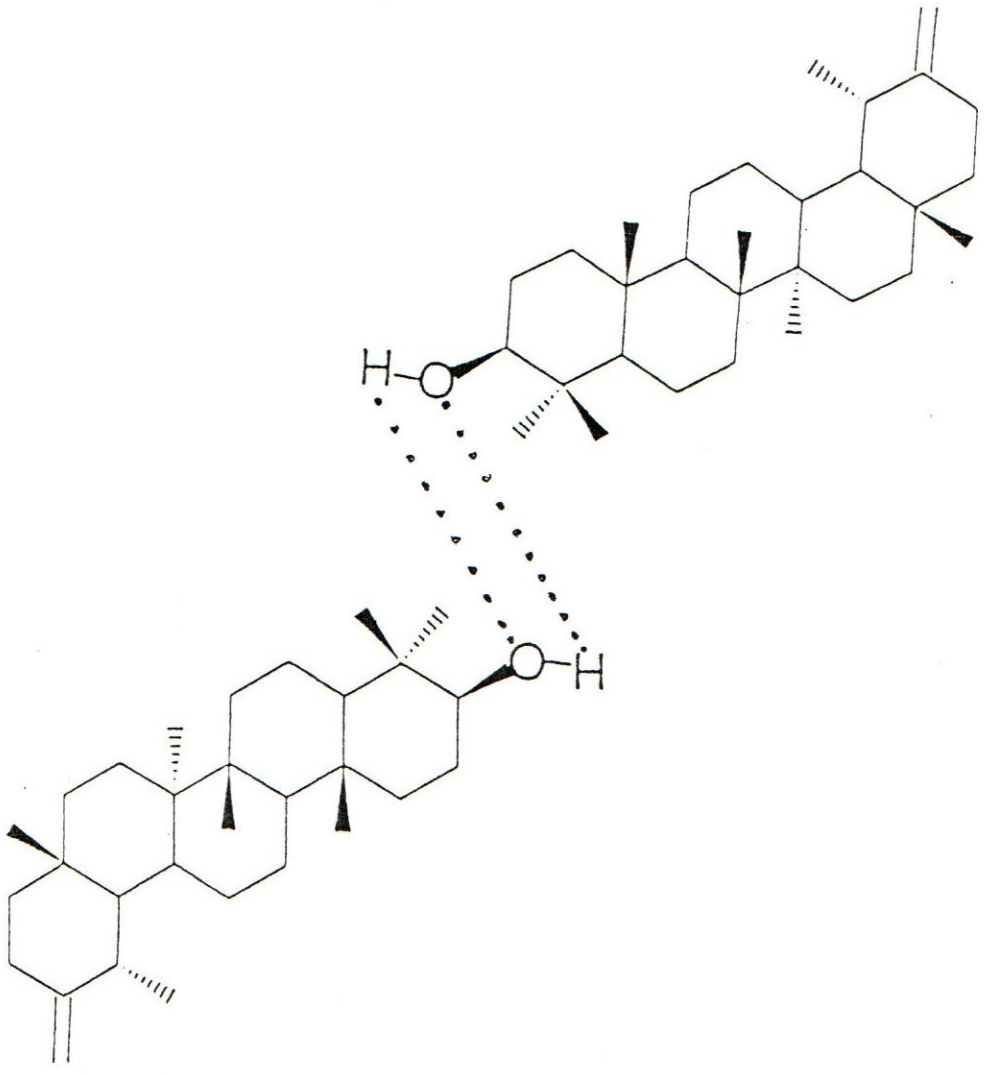


FIGURE 9: .....Possible hydrogen bonding between taraxasterol molecules

TABLE 24: PYRETHRINS TO TARAXASTEROL RATIOS FOR NON-GELLED EXTRACTS

Pys	tara	pys / tara
44.38	1.92	23.11
47.54	2.51	18.94
42.57	2.41	17.66
60.40	3.72	16.24
55.73	3.74	14.90
58.88	4.08	14.43
49.88	3.51	14.21
50.12	3.65	13.73
50.94	3.90	13.06
54.95	4.33	12.69
59.65	4.93	12.10
49.97	4.15	12.04
42.35	3.59	11.80
60.11	5.24	11.47
48.15	4.24	11.36
49.23	4.34	11.34
49.04	4.40	11.15
49.90	4.64	10.75
49.98	4.65	10.74
44.14	4.12	10.71
50.06	4.79	10.45
52.79	5.23	10.09
47.77	4.88	9.79
49.81	5.21	9.56
37.36	4.05	9.22
38.35	4.56	8.41
45.82	5.50	8.33

Data extracted from tables 11, 12, 13, 17, 18 and 19

There is an overlap of data in the range of 8.33 and 10.78 (pys / tara values) in gelled and non-gelled extracts (Tables 23 and 24). This sug gests that gelling of pyrethrum extract is not dependent on the ratio of pyrethrins and taraxasterol in extract.

Data obtained from Pyrethrum Board of Kenya[40] gave pys / tara ratios for gelled and non-gelled extracts (Tables 25 and 26)

TABLE 25: PYRETHRINS TO TARAXASTEROL RATIOS FOR GELLED EXTRACTS

% pys	% tara	pys / tara
53.45	3.53	15.14
51.45	3.55	14.49
54.63	3.80	14.38
51.30	3.57	14.37
49.88	3.50	14.25
51.59	3.71	13.91
51.63	3.73	13.84
49.89	3.70	13.48
49.17	3.65	13.47
53.92	4.01	13.45
54.50	4.09	13.33
48.79	3.69	13.22
52 07	3.95	13.18
51.50	3.92	13.14
53.74	4.10	13.11
51.56	3.99	12.92
53.05	4.11	12.91
49.99	3.91	12.79
48.79	3.94	12.38
52.95	4.30	12.31
51.07	4.40	11.61
52.20	4.52	11.55
50.91	4.45	11.44
47.92	4.26	11.24
51.01	4.58	11.14
45.59	4.72	9.66

Data obtained from Pyrethrum Board of Kenya[41]

TABLE 26: PYRETHRINS TO TARAXASTEROL RATIOS FOR NON-GELLED EXTRACTS

% pys	% tara	pys / tara
54.73	2.90	18.87
49.37	2.63	18.77
49.89	2.69	18.55
49.87	2.87	17.38
53.72	3.13	17.16
48.47	2.85	17.00
49.65	2.94	16.89
50.17	2.99	16.78
50.47	3.03	16.66
52.23	3.18	16.42
52.33	3.21	16.30
55.39	3.40	16.29
54.55	3.38	16.14
47.75	2.97	16.08
49.33	3.07	16.07
53.33	3.33	16.02
54.13	3.39	15.97
55.57	3.55	15.65
51.81	3.37	15.37
52.36	3.46	15.13
53.24	3.56	14.96
51.99	3.50	14.85
50.47	3.43	14.71
55.96	3.81	14.68
51.30	3.57	14.37
46.92	3.35	14.00
49.52	3.58	13.83
50.26	3.66	13.73
48.39	3.68	13.15

Data obtained from Pyrethrum Board of Kenya[40]

There is an overlap of data in the range of 13.15 to 15.14 in gelled and non-gelled extracts (pys / tara values, Tables25 and 26).It is apparent that the physical state of the extract is not dependent on the ratio of pyrethrins and taraxasterol in the extract. This is deduced from the wide range of overlap of pys / tara values for gelled and non-gelled extracts (Tables 23, 24, 25 and 26). Also, there is no correlation between investigation data (Tables 23 and 24) and P.B.K. data (Tables 25 and 26).

The above observations suggest that gelling of pyrethrum extract is dependent on the sample and not taraxasterol or pyrethrins levels.

From these findings, it can be deduced that interactions between pyrethrins and taraxasterol, if present, do not relate to gelling of extract and therefore taraxasterol levels in relation to pyrethrins levels in extracts can not be used as a measure of the physical state of extract.

3.5 EFFECT OF OPERATIONAL TEMPERATURES DURING REFINING PROCESS ON LEVELS OF TARAXASTEROL IN EXTRACTS

Pentacyclic triterpenoids, of which taraxasterol is a member, are crystalline solids which have poor solubility in partitioning solvents used in refining process of pyrethrum extract[34]. If temperatures are allowed to rise, these components can be carried to the final product and hence increased levels left in refined extract. The refining process involved removal of the extraneous constituents of the crude extract through differe ential solubility of the constituents by partioning in a solvent. Since temperature is a factor affecting solubility, specific operational temperatures (0°C, -10°C and -30°C) were employed at the methanol and SST stages of purification. The final extracts were then analysed for taraxasterol (Table 27).

TABLE 27: TARAXASTEROL LEVELS (%) IN EXTRACTS PROCESSED AT DIFFERENT TEMPERATURES

Source of flower	Temperature		
	0° C	-10° C	- 30° C
Kisii	7.32	8.27	4.56
Nyahururu	5.93	6.90	4.12
Bomet	5.71	5.90	2.41
Bahati	4.93	5.24	3.72
Molo	4.64	3.59	1.92
Nyeri	4.24	4.79	4.88
Meru	3.74	4.08	4.33
Eldoret	4.40	3.51	2.50

Data extracted from Table 17

Taraxasterol content was reviewed against temperature in order to describe the effect of temperature on levels of taraxasterol in extracts for the different regions of farming. There was no uniformity in levels of taraxasterol with temperature. As temperature was varied from high (0° C) to low (-30° C) taraxasterol levels varied, with Kisii, Bomet, Nyahururu, and Bahati showing a trend of low - high - low taraxasterol values, Nyeri and Meru showed a trend of low - high taraxasterol values, while Molo and Eldoret showed high - low taraxasterol values (see Table 27).

These observations suggest that taraxasterol levels in extract are not depedent on temperature and therefore do not directly relate to the gelling phenomenon of extracts. The findings show that there could be other factors related to the source of flowers that induce gel formation in extract.

3.6 EFFECT OF MIXING FLOWERS FROM DIFFERENT FARMING REGIONS ON PHYSICAL CHARACTERISTICS OF EXTRACTS

A method which permits processing operation at higher temperatures and gives extracts with desirable characteristics would offer great economic advantage to the industry since gels are undesirable, and operating at very low temperatures is very expensive and impractical.

From the previous sections (3.1, 3.2, 3.3, 3.4 and 3.5), it is evident that flowers from different regions and processing conditions yield extracts with varied formation characteristics. Some regions yield gelled (poor quality) extracts while others yield non-gelled (good quality) extracts. It was also observed that operational temperatures do not strictly govern the physical characteristics of the extract since it was possible to obtain several non-gelled extracts even at higher temperatures.

Flower composites were made by mixing gelling type with non-gelling type of flowers in different ratios and analysis conducted in the usual manner to give results presented in Table 28. The results were compared with those obtained from extracts of individual regions of pro duction (see Table 29).

TABLE 28: PHYSICAL CHARACTERISTICS OF COMPOSITE EXTRACTS PROCESSED AT -10^{O}C

Composite	Gelled: non-gelled	% Tara	% Pys	Physical state
Kisii/ / Bahati	1:1	5.50	45.82	non-gelled
	1:3	4.05	37.36	non-gelled
Nyahururu / Meru	1:1	5.23	52.79	non-gelled
	1:3	5.21	49.81	non-gelled
Bomet / Molo	1:1	6.70	49.89	gelled
	1:3	6.47	42.56	gelled

Tara = Taraxasterol Pys = Pyrethrins

TABLE 29: PHYSICAL CHARACTERISTICS OF EXTRACTS OF FLOWERS FROM THE INDIVIDUAL REGIONS OF FARMING PROCESSED AT -10^{O}C

Region	% Tara	% Pys	Physical state
Kisii	8.27	35.75	gelled
Nyahururu	6.90	43.45	gelled
Bomet	5.90	53.31	gelled
Bahati	5.24	60.11	non-gelled
Meru	4.08	58.88	non gelled
Molo	3.59	42.35	non gelled

Tara = taraxasterol Pys = pyrethrins

Data extracted from Table 20

The results (Tables 28 and 29) show that mixing of flowers could improve the physical state of extracts with the ratio 1:3 yielding extracts with lower taraxasterol levels and 1:1 ratio yielding extracts with higher taraxasterol levels. From this observation, it is deduced that quality of extracts can be improved by working out suitable mixing ratios of gelling to non-gelling type of flowers. This would allow for processing at higher temperatures which are economic and easy to attain.

For Bomet / Molo composite, both ratios (1:1 and 1:3) gave gelled extracts. This can be interpreted to mean that factors inducing gelling are more prominent in Bomet region.

3.7 CONCLUSION

Taraxasterol has been implicated in the gelling phenomenon of pyrethrum extract. This investigation revealed that taraxasterol meas urements alone do not, however, correlate with gelling and therefore high levels do not necessarily indicate gelling of extract.

It has been suspected that gelling of pyrethrum extract is due to interactions between taraxasterol and pyrethrins molecules. Investigations have shown that such interactions, if present, do not cause gelling of extract as there was no correlation between pys / tara ratios and the physical state of extracts. This suggests that taraxasterol levels in relation to pyrethrins levels in extract cannot be used as a measure of the physical state of the extracts.

Changes in temperature during refining process were found to influence the physical state of extract in some flower growing areas. How ever, for most regions, variations in processing temperatures did not give extracts with varied physical states. This suggests that operational temperatures cannot be used as as a criteria for improved quality of extract.

Different farming regions gave extracts with varied physical characteristics. Knowledge of the type of flowers (based on the farming region) would therefore help in selecting suitable mixing ratios and op erational temperatures which would allow for refining of pyrethrins at higher easily-maintained temperatures and economic operation.

From the investigations, there appeared to be other factors, other than taraxasterol, related to the source of flower that induce gel formation. It can therefore be concluded that gelling of pyrethrum extract is characteristic of flower source and not necessarily taraxasterol levels.

3.8 RECOMMENDATIONS

It has been established that taraxasterol levels alone do not influence gelling of extracts. There is an indication that the growing region of flower has an effect on the physical characteristics of extract. There is therefore need for further investigations to be carried out to establish other possible causes in relation to growing areas for example soil types that may cause gelling.

The extraction process involves use of different solvents at various stages of processing. In this study, n-hexane, methanol and SST. were used as solvents. It is appropriate to establish solvent systems that have solvent-gelling agent interactions thus expanding the gel from its unperturbed dimensions (in poor solvents, the interractions are fewer and expansions are restricted).

TABLE 30: PHYSICAL PROPERTIES OF SOLVENTS[42]

Solvent	Formula	M.Wt.	Evaporation. diethyl ether = 1	Vapour pressure mm Hg	Viscosity at 20° C Centpoise	Dielectric constant e
n-Hexane	C_6H_{14}	86.2	1.4	120	0.32	1.90
Benzene	C_6H_6	78.1	3.0	75	0.65	23.00
Methanol	CH_4O	32.0	6.3	96	0.55	3.60
Ethanol	C_2H_6O	46.1	8.3	44	1.22	25.00
Acetone	C_3H_6O	58.1	2.1	175	0.32	21.40
Diethyl ether	$C_4H_{10}O$	74.1	1.0	440	0.24	4.30
Furfural	$C_5H_4O_2$	96.1	1.1	75	1.54	41.70
Chloroform	$CHCl_3$	119.4	2.5	160	0.57	4.80
Carbon tetra-chloride	CC_{14}	153.8	0.4	91	0.97	2.24

The modulation of physical characteristics of the extract with tem perature suggests that thermodynamic parameters are involved in the gelling of extracts. There is therefore need to delve further into this process.

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