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CONTROL ANALYSIS OF A MULTICOMPONENT HERBAL TEA

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ABSTRACT

Herbal tea preparations have been subjected to precise quality control analysis including physical properties, physicochemical tests, microscopical identification of the diagnostic elements of the mixture as well as TLC identification, finger print of the different active constituents, GLC fingerprint of the volatile constituents and their quantitative determination were carried out. Quantitative estimation of the main active constituent by spectrophotometric and HPLC, was carried out. Potential contaminants; Toxic botanicals, pesticide residue, heavy metals, microorganisms and aflatoxins were subjected to analysis, too.

INTRODUCTION

Recently the medicinal use of plants as remedy has been widely progressed. It can be assumed that the success of this trend depends mainly on setting up the correct standardization methods for right dosage of these plants. Several methods for qualitative identification of plants in herbal teas have been proposed. While, their quantitative determination has received less investigations[1-5].

On the other hand, potential contaminants have be taken into account in the quality control of herbal medicine as recommended by WHO and FDA[6]. In fact, plant drugs, during their growth are exposed to multitude of environmental pollutants like attack by microorganisms (bacteria, moulds), contamination by heavy metals e.g lead from motor exhausts), and pesticides. Also, during the drying of plant parts for drugs manufacture, residues of the formentioned pollutants, are present and this requires special attention of hygienic and toxicological grounds. Obviously, toxic botanicals (Adulteration of commercial herbal products), are the direct toxicological implications for the user[7]. Also, pesticide residues are known to induce liver, thyroid and lung tumors[8]. Furthermore, toxic metals such as lead, cadmium and arsenic are contributing factors to chronic toxicity problems. Meanwhile, microorganisms and aflatoxins induce pathological manifestation and cancer[7-9], thus their determination is an important control work.

As far as medicinal plants are concerned, these questions have only started to arise in the last few years.

In the light of these guidelines, studying and standardization of the "RheumaR" tea herbal mixture was carried out. This herbal tea has been previously evaluated as antiinflammatory, analgesic and antipyretic(10). Thus, this work was undertaken to provide an ample justification for most of the raised questions.

EXPERIMENTAL

Materials:

"Rheuma" tea: Batch No. HS 342, Man. date 03/1993, Exp. date 30/1996 is a preparation in the Egyptian Market produced by SEKEM Phytopharmaceuticals, Cairo, Egypt and has the following composition:

Salix bark 30 g, Taraxacum leaves 15 g, Peppermint herb 10 g, Chamomile flowers 10 g, Urtica leaves 20 g, Parsley fruits 10 g, Ambrosia herb 5 g.

Standards:

A standard herbal mixture was prepared by the

authors from the above plants provided by SEKEM Phytopharmaceuticals Company, Cairo the plants were authenticated and standardised according to DAB and BHP[11,12], in the same proportion as "Rheuma" herbal tea, and assured to be free from pathogenic microorganisms, insects, rodents and other pollutants.

Pure salicin (TLC grade and HPLC grade) was provided by sarsynthese (avenue President J. F. kennedy, B. P. 100 F-33701, Merignac, France).

Volatile oil standards:. Chamazulene, Apiole, Menthone, Myristicin, Bisabolol, Menthol, Bisabolol oxide.

Flavonoids standards: Apigenin 7-0-glucoside, Luteoline-7-0-glucoside.

Coumarin Standard: Scopoletin.

All the above standards were provided by Carl. Roth GmbH + Co. D7500 karlsruhe 21 Germany.

Sesquiterpene lactones standards: Damsin, Ambrosin, were obtained from Faculty of Pharmacy, Assiut University.

Pesticide standards:

Organochlorine pesticide standards: (α, β, γ-hexachlorocyclohexane (HCH), DDE, DDT (both o, p), Aldrin, Dieldrin, Heptachlor, Heptachlorepoxide and Endrin].

Organophosphorus pesticide standards: [Dimethoate, malathion, 'Chlorpyrifos- methyl, Chlorpyrifos, Pirimiphos- methyl, Pirimiphos- ethyl, Diazinon, Triazophos, Fenitrothion].

All the above pesticide standards were provided by the National Food Administration, Food Research Department, Uppsala Sweden and were prepared in hexane.

Heavy metals standards solutions are nitrate salts of: (Lead, Chromium, Cadmium, Zinc, manganese, Copper and Iron) from Merck (Merck, Darmstadt, Germany) concentration 1 ppm.

Aflatoxins standard B_1, B_2, G_1, G_2 (Sigma chemical company, USA).

Apparatus:

Microscope (MEOPTA, Czechoslovakia).

A Beckman UV-7 spectrophotometer with 1 cm matched glass cells was used for colorimentric assay of salicin. HPLC: binary LC pump 250; Detector: UV/Vis. LC 290 (Perkin Elmer); integrator: LC_1/100 Lab computing integrator, column, reversed phase (spherisorb OS II) C-18 column (ID X L) = 0.46 x 3 cm).

GLC (For organochlorine compounds): Pye Unicam 610 Ni^{63} EC-Detector; Columm: Capillary DB 17; column temp: Initial temp. 100°, Initial time 2 min, 1st ramp 2 °C/min up to 240 °C 15 min, 2nd ramp 10°C/min up to 260 °C 7min, injector: 280 °C, detector: 300°C. Gas flow: N_2: 30 ml/min.

GLC-Pye Unicam 104 Ni^{63} EC-Detector, column: glass 9 Ft x 4 mm packed with 10% DC-200. Temp: column 210°C, injector 220°C, detector 220°C. Gas flow: 8 ml/min. (Confirmatory test of organochlorine compounds).

GLC Philips: 4500 FI-Detector; column: glass 7 Ft x 2mm packed with 4% SE-30+6% OV 210 on Gas Chrom. Q 80 -100 mesh; Temp: column 220°C; injector 235 °C; detector 250°C, Gas flow N_2 = 60 ml/min, O_2 = 30 ml/min, air = 30 ml/min. (For organophosphorus compounds). Atomic absorption spectrophotometer: Perkin Elmer (2380) USA.

GLC (For volatile oil of "Rheuma" tea): pye Unicam gas chromatograph equipped with dual flame detectors. The chromatograph was fitted with a coiled glass column (1.5m x 4mm) packed with Diatomite C (100 - 200 mesh) and coated with 10% PEGA. The oven temperature was programmed at 4°C/min from 60°C to 180°C and was held at 180°C for 15 min. Detector and injector temperatures were 220°C for 15 min. Detector and injector temperatures were 220°C and 300°C respectively. Gas flow rates for N_2 , H_2 and air were 30, 33 and 330 ml/min, respectively.

Methodology:

Study of the pharmacognostic characteristics of the "Rheuma" tea alongside with the standard:

A. Macroscopical characters of the powdered mixture, preliminary chemical tests, microscopical tests, Pharmacopoeial constants, and determination of % of volatile oil were carried out according to E. P. 1984[13], alongside with that of the standard

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herbal mixture prepared by the authors (Table 1).

B. Microscopical identification; Diagnostic elements of each plant of the mixture is illustrated in Fig. 1.

The main key elements of "Rheuma" tea herbal mixture are the following:-

1. Stinging hairs with long conical unicellular head having swollen tip "Stimuli" and multicellular enlarged basal part (urtica).
2. Long, mostly collapsed, thin walled glandular trichomes (Taraxacum).
3. Lignified fibres, surrounded by a crystal sheath containing prisms of calcium oxalate (Salix).
4. parenchymatous cells containing calcium oxalate clusters (Parsley seeds).
5. Labiaceous glandular trichomes (Peppermint).
6. Non glandular trichomes uniseriate multicellular 2-8 cells covered with warty cuticle (Peppermint).
7. Glandular hairs multicellular biseriate head (Chamomile).
8. Spiny pollen grains (Chamomile).
9. Spheroidal pollen grains (Ambrosia).
10. Beaded parenchymatous cells containing large clusters of calcium oxalate (Salix).
11. Epidermal cells, with striated cuticle and anomocytic stomata containing clusters of calcium oxalate (Parseley).
12. Fragments of vittae (Parseley).
13. Fragments of endocarp and the inner most layer of mesocarp (Parseley).
14. Surface preparation containing cystolithes and non glandular hairs, unicellular with warty cuticle and glandular hairs with multicellular head (urtica).
15. Pitted parenchyma (Parseley, Taraxacum).

TLC Identification of the different constituents of "Rheuma" TEA herbal mixture.

A. Identification of the volatile oil content[13] :

The oil was prepared by hydrodistillation of 100 g "Rheuma" tea. About 20 µl of the essential oil was dissolved in 1 ml toluene.

Reference solutions were prepared by dissolving 5 mg or 10 µl of each standard compound in 10 ml toluene. Adsorbent; Silica gel 60 F_{254} plates (Merck, Darmstadt); developed with; dichloromethane; Detected by: spray with p-anisaldehyde H_2SO_4 reagent[5] and heated at 100-150° for 5-10 min. (Table 2):

B. Identification of the flavonoid content[5]

Powdered drug (2 g) was extracted with 10ml methanol for 5 min. on a water bath at about 60°C and filtered. The clear filtrate was used for TLC.

Reference solutions: 0.05% solutions in methanol; Adsorbed on: Silica gel 60 F_{254} precoated TLC plates (merck, Darmstadt); developed by; Ethyl acetate; Formic acid: Glacial acetic acid; Water (100: 11: 11: 27); Detected: by spraying with Natural Products-Polyethyleneglycol reagent NP/PEG[5] (Table 3):

C. Identification of the sesquiterpene lactones content[5]:

2 g of the powdered drug was refluxed with 50 ml dichloromethane and filtered, then concentrated to small volume.

Reference solutions: 1mg of both in 10ml $CHCl_3$; on: Silica gel 60 F_{254} plates (merck Darmstadt); developed by: Chloroform: benzene (9 :1); detected by: Spraying with Drangendorff's reagent[5]. The R_f of Ambrosin and Damsin are 0.64 and 0.45, respectively corresponding to that of standard Ambrosia and appearing in the visible light as orange spots.

D. Identification of the coumarin content[5] About 2 g powdered drug was extracted with 10 ml methanol for 5 min on water bath at 60°C and filtered. Reference solution 1 mg scopoletin in 10 ml methanol, Adsorbed on:- silica gel 60 F_{254} Plates (Merck Darmstadt), developed with Toluene, Detected under UV light at 365 nm. The Scopoletin was identified alongside with Urtica standard as Blue fluorescent spot in UV 366 nm at R_f 0.73.

Identification of salicin content[5] :

2 g powdered drug was refluxed with 50 ml methanol, filtered and then cooled. The filtrate was shaken for 2-3 minutes with 0.5 g polyamide powder, again filtered, evaporated to dryness and the residue was dissolved in 3 ml methanol.

Reference solution 2 mg salicin in 1 ml methanol; water (1: 1). Adsorbed on silica gel 60 F_{254} plates (Merck, Darmstadt), developed by Ethyl acetate; anhydrous formic acid: water (80: 13: 7), Detected (After drying at 100-105°C for 2 min) by spraying with a mixture of 19 ml 0.5% ethanolic thymol and 1 ml conc. sulphuric acid then heated at 120°C for 2-3 min and examined at once in day light[14]. Salicin was detected in the extract as red brown spot at R_f 0.54 alongside with the standard in Salix.

RESULTS

All the above finger prints are confirmed and matched with the finger prints of the standard herbal mixture prepared by the authors.

GLC fingerprint and estimation of the main volatile constituents in the volatile oil:

Steam distillation of the "Rheuma" tea gave a bluish green oil (0.3%). This oil was analysed by GC against available standards: (Chamazulene, bisabolol, menthol, menthone) and revealed the presence of 69% menthone, 0.2% α-bisabolol, 23% menthol, and 7.2% chamazulene. These results are comparable with the results of the standard herbal tea prepared by the authors.

Quantitative estimation of salicin by two different methods.

"Rheuma" tea is formulated to contain 30% salix powder, therefore any given weight of "Rheuma" tea should contain an amount of salicin equal to that present in 30% its weight of salix powder. The salicin content of *Salix alba* was previously standardised by the authors to be 0.6% (see standards) which is below the limit required by the pharmacopeas (1%)[14].

A. Assay of salicin by chromatographic- spectrophotometric method:

1. Preparation of "Rheuma" tea sample[14,15]:

Exactly, 6 g. of powdered "Rheuma" tea were mixed with 30 ml methanol and heated under reflux for 10 min. The extract was cooled, filtered and the combined filtrate and washings were evaporated under vaccum. The produced residue was suspended in 2 ml of methanol.

2. Column chromatographic purification:

The methanolic test solution was quantitatively transferred to a silica gel column (1 x 25 cm) in petroleum ether. The column was first eluted with chloroform till complete disappearance of the green colour. The elution was continued with chloroform-methanol (1: 1), till complete purification of the extract. Finally, the column was eluted with 100 ml of methanol. The methanolic eluent was evaporated under vacuum till dryness. The residue was dissolved in 10 ml 50% methanol, completed to 50 ml with methanol (test solution).

3. TLC separation of salicin:

Preparation of standard:

5 mg accurately weighed standard salicin was dissolved in 10 ml 50% methanol, completed to 50 ml with methanol (standard solution). 300 µl from each of the standard and test solutions were applied separately against blank of pure methanol on TLC silica gel plate 60 F_{254} (E. merck), then developed in ethyl acetate: methanol: water (100: 13.5 :10). Salicin bands in both test and standard solutions were detected under U.V. light, scraped from silica gel plate and eluted twice with 10 ml methanol. Each was evaporated till dryness under vacuum and the produced residue was dissolved in 5 ml methanol. Each of the two solutions was treated with 5 ml Thymol- sulphuric acid and the colour produced was measured spectrophotometrically at 420 nm against a blank. Results indicated the presence of 0.08% salicin in the analyzed sample.

B. Quantitative determination by HPLC[16-18]:

HPLC analysis was carried out in the Laboratories of Faculty of Science, Cairo University.

1. Standard salicin: 1mg of salicin standard was dissolved in 3 ml methanol + 3 ml water.

2. Preparation of "Rheuma" tea sample.

1 g of fine & accurately weighed powder was mixed with 200 ml boiling water, filter, the filtrate was lyophilized and then chromatographed.

A rapid quantitative measurement was done against a standard using mobile phase (35%

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Table 1 :- Pharmacognostic characteristics of the "Rheuma" tea herbal mixture and the standard.

Items	"Rheuma" tea herbal mixture	Standard Prepased by authors
Macroscopical description:-	heterogeneous dry coarse powder, yellowish green colour, aromatic odour, slightly bitter taste and gritty touch.	
Preliminary Chemical tests :-		
* Fixed and volatile oils.	both positive	both positive
* Saponins (Froth test).	- ve	- ve
* Sulphuric acid 66%.	- ve	- ve
* Flavonoid test (Potassium hydroxide test).	yellow colour	
* Tannin test ($Fecl_3$).	bluish green colour	
* Alkaloid test (Mayer's Reagent).	no white ppt.	
Microscopical tests :-		
* Iodine solution (starch)	blue spots	
* Sudan III (volatile oil)	red globule	
* Millon's (protein)	no red colour	
* Ruthenium Red (mucilage)	no red patches	
Pharmacopoeial constants*		
* Moisture content.	8.5 %	8-9%
* Foreign matter.	- ve	- ve
* Ash.	12%	11-13%
* Acid - insoluble Ash.	2.3-3%	3%
* Water - soluble extractives.	35%	37%
* Volatile oil.	0.3%	0.3-0.4%

Results of Determinations : are the average of 3 Determinations.

methanol in 1% o-phosphoric acid). UV detector was used at 270 nm (Perkin Elmer LC_{290}) on reversed phase (spherisorb OS II) C-18 column = (0.46 ID x 3 cm L), the elution was begun after 3 min. Results of HPLC revealed 0.172% Salicin which was comparable to that of the standard mixture.

Determination of pesticide residues in Rheuma herbal tea:

The pesticide residual analysis was carried out by GLC, in the Central Agricultural Pesticide Laboratory, Department of Residue Analysis and Environmental pollution Dokki, Giza. The determination of the organochlorine and organophosphorus pesticide residue were undertaken according to previously published methods[18-22] (Tables 4 & 5).

Determination of heavy metals:

Determination of the heavy metals was carried out in National Research Center Dokki, Giza by atomic absorption[24,25] and results are presented in Table (4).

Microbiological assays:

The microbiological counts and tests were done in the Microbiology Department, Faculty of Pharmacy, Cairo University according to supplement to DAB 9 (1989)[14] for category of herbal drugs which undergo a diminution of germ count before used (i.e. by making a tea). Results are presented in Table (5).

Aflatoxin test:

This was carried out according to previously published methods[7,26] and results are found to be negative against four standards aflatoxins spots B_1, B_2, G_1, G_2, at R_f 0.55, 0.53, 0.5, 0.47 respectively, giving purple blue (B_1, B_2) and blue green colour (G_1, G_2) under UV 366 nm.

DISCUSSION

"Rheuma" herbal tea is an antirheumatic formula which consists of mixture of herbs. This may be classified[27] according to the purpose of their mixing, as principal drugs: i.e of major revelance for the treatment of the condition concerned as Salix, containing salicin the main active constituent and Chamomile containing chamazulene, bisabolol and matricin.

Also Urtica which contains β- sitosterol. These types of compounds are antiphlogistic and acts in a synergistic way as lipooxygenase and cyclooxygenase inhibitors and represents about 60% of the total constituents.

The second type is the supplementary drugs (10% of the total constituents) ambrosin and damsin in Ambrosia; apigenin & luteolin 7-glucoside in Taraxacum, and Parsley. Volatile oils Apiol and Myristicin in Parsely fruits, listed according to their importance, for the treatment and they act mainly as diuretics which is necessary in the condition concerned[28].

Table 2 :- TLC identification of the volatile oil content.
Solvent system :- Dichloromethane.
Spray reagent : - P. anisaldehyde - Sulphuric acid.

Authentic samples	R_f of the coresponding spot in the tea	Colour of the spot	Standard plant
Chamazulene	0.95	Red-violet	Chamomile
Apiole	0.75	Brown violet	Parsley
Menthone	0.7	Blue green	Peppermint
Myristicin	0.8	Brown violet	Parsley
Bisabolol	0.35	Violet	Chamomile
Menthol	0.3	Blue	Peppermint
Bisabolol Oxide	0.2	Yellow green	Chamomile

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Table 3 :- TLC identification of the flavonoid content.
Solvent system :- Ethylacetate : Formic acid : Glacial acetic acid : Water
100 : 11 : 11 : 27
Spray reagent :- Natural Product Polyethyleneglycol reagent

Authentic Samples	R_f of the coresponding spot in the tea	Colour of the spot under U.V	Standard plant
Apigenin 7-0- glucoside	0.80	Green-yellow	Salix, Chamomile, Peppermint
Luteolin 7-0- glucoside	0.65	Orange	Salix, Chamomile
Rutin	0.35	Orange	Peppermint, Parsley

The adjunct drugs (10% of the total composition) as peppermint in Rheuma tea is added to improve the appearance of the tea, its taste and aroma. This classification may help as a guide, in which direction the analysis should be carried in such multicomponent herbal tea.

The macroscopical examination is important in recognizing if the herbs are genuine and not contaminated. It is essential also to identify by microscopy the most diagnostic features of the tea to avoid the complaints which appear in the literature about the substitution of teas by toxic botanicals [30,31]. Beside the microscopy. TLC plays a very important role in the identification as a qualitative tool in the quality control of the drugs for the three types of classes present in the mixture.

GLC as a fingerprint for the volatile oil of the tea, represents a helpful tool for qualitative and quantitative analysis of the "Rheuma" tea.

Salicin identification and quantitative determination is a must due to its major therapeutic role in the mixture[32].

The effective dose required daily is 60-120 µg salicin[14]. By calculation. 6 g of daily dose of Rheuma tea is equivalent to 6 x 0.172 x 1000 (mg)/100 = 10.32 µg which is less than the daily requirement. However. the efficacy of the tea may be explained by the more bioavailabilty of salicin when present in combined form than single purely isolated compound.

The pesticidal residue analysis in "Rheuma" tea revealed the presence of 0.014 mg (per kg of the tea) of DDE, PP,. This compund is a metabolite of DDT. the use of which was prohibited many years ago but still existing adsorbed in the agricultural soil.

The acceptable daily intake (ADI) of DDE, PP is 0.02 mg/kg body weight i.e 20 µg/kg body weight so by calculation: when the herb is usually consumed in doses up to 6g per day, it can be calculated that a patient with an average body weight 60 kg is maximally exposed to 0.084 µg which is far less than the permisible limit and hence does not involve any serious health risk.

The organophosphorus compound chloripyrifos-methyl was detected in the tea mixture in very low concentration, at a level of 0.04 µg/kg of the tea, so when the tea is consumed in doses of 6 g, daily the user of 60 kg body weight is exposed to 0.24 µg which is far less than the permisible limit, compared to the ADI 10 µg/kg body weight. Moreover the level of pesticide residue is reduced by extraction of the tea with boiling water, as only a fraction of pesticide (about 10% percent) will get into the infusion[14].

The heavy metal detection picture revealed that no harm if they are present in just concentration, necessary for plant or animal nutrition (Copper, Iron, Manganese, Zinc, Arsenic....) and not in large

Table 4 :- Results of the organochlorine pesticidal residue analysis in "Rheuma" tea herbal mixture.

Pesticide residue type		Concentration level mg/kg "Rheuma" tea	(ADI)
D D T O, P	DDT	ND	
D D T P, P	DDT	ND	0.02
D D E P, P	DDT	0.014	
D D E P, P	DDT	ND	
H C H alpha	HCH	ND	0.02
H C H bata	HCH	ND	
H C H gama	HCH	ND	0.0001
Aldrin		ND	0.0001
Dieldrin		ND	0.0005
Heptachlor		ND	0.0005
Heptachlorepoxide		ND	0.0002
Endrin		ND	

ND : not detected
ADI : acceptable daily intake mg/kg bw
BW : body weight

concentrations where these metals may become toxic to plants and animals and affect the quality of foodstuffs for human consumption[34].

The main sources of metal contaminants in soils are from metalliferous mining and smelting activities other industrial emissions and effluents, urban development vehicle emissions, dumped waste materials, contaminated dusts and rainfall, sewage sludge, composted town refuse, fertilizers, soil ameliorants and pesticides. Analysis of "Rheuma" tea revealed the presence of these metals in the allowed concentrations. Also, the absence of toxic metals viz Mercury, Arsenic, Thallium, was observed.

The Microbiological assays are very important because the richer a plant part in nutrients and the slower the drying process, the higher the bacterial count of the resulting drug. The total germ count is of relatively little significance because it varies enormously from drug to drug[14] and the human being themselves are not sterile (buccal flora 10^9-10^{12} anaerobes/g and 10^5 - 10^{11} aerobes/g saliva, human skin 10^5 - 10^6 microbes/cm^2)[14]. On the other hand, it is important to ensure the absence of pathogenic germs and to limit the number of enterobacteria which seems to be satisfiying in the results of "Rheuma" tea. Finally, aflatoxins determination was an easy task by thin layer chromatography but the challenges inherent in aflatoxin analysis are sampling, subsampling and sample extractin methods. Results indicated that, the sample of "Rheuma" tea under investigation was found free from aflatoxins B_1, B_2, G_1, and G_2 which are routinely monitored in foods for market.

Recommendations:

In analysing a multicomponent herbal tea, attention should be paid to:

1. The amount of drug in a single dose and the amount of water used in preparing the tea.

2. The extent of comminution of the drug: There is a large influence of the degree of comminution on the liberation of active principles during preparation of the tea. In mixed herbal teas, attention must be given to ensure that the particle size ranges have rightly been criticized. Particle size must be uniform as possible, otherwise separation will occur just on storage and to a great extent during transport.

3. Method of extraction (temperature, time); the amount of the constituents increase and the highest yields are obtained with powdered drugs.

4. Stability must be taken into consideration especially for the essential oil containing drugs which can be calculated from the initial value (content at the time of packaging) and the percentage decrease per unit time.

5. In the present study we didn't put into consideration, the release and actual quantity of active ingredients in the infusion (i.e. hot water extract) because salicin which is the main active constituent is readily soluble in water. In any other

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Table 5. Results of the organophosphorus pesticidal residue in "Rheuma" tea herbal mixture.

Pesticide residue type	Concentration level mg/kg "Rheuma" tea	(ADI)
Dimethoate	ND	0.01
Malathion	ND	0.02
Chloropyrifos-methyl	0.04	0.01
Pirimiphos - methyl	ND	0.01
Pirimiphos - ethyl	ND	0.01
Diazinone	ND	0.02
Triazophos	ND	0.002
Fenitrothion	ND	0.005

Table 6 :- Results of heavy metal analysis of in "Rheuma" tea herbal mixture

Heavy metal	Concentration level mg/kg "Rheuma" tea	MAL
Cadmium (Cd)	0.25	0.1
Copper (Cu)	6.18	
Ferrous (Fe)	171.8	
Manganese (Mn)	37.2	
Lead (Pb)	- ve	0.02
Zinc (Zn)	41.01	
Chromium (Cr)	1.3	
Mercury (Hg)	- ve	0.05
Arsenic (As)	- ve	
Thallium (Th)	- ve	

MAL : Maximum allowed level.

Table 7 :- Results of microbiological detection in "Rheuma" tea herbal mixture

Microorganism	"Rheuma" tea	Limits
Aerobial bacteria	3×10^6 / g	max 10^7 / g
Yeasts and moulds	3×10^3 / g	max 10^4 / g
Escherchia coli	- ve	max 10^2 / g
Enterobacteria	$< 10^2$ / g	max 10^2 / g
Salmonella type	- ve	- ve
Staphylococus auerus.	- ve	- ve

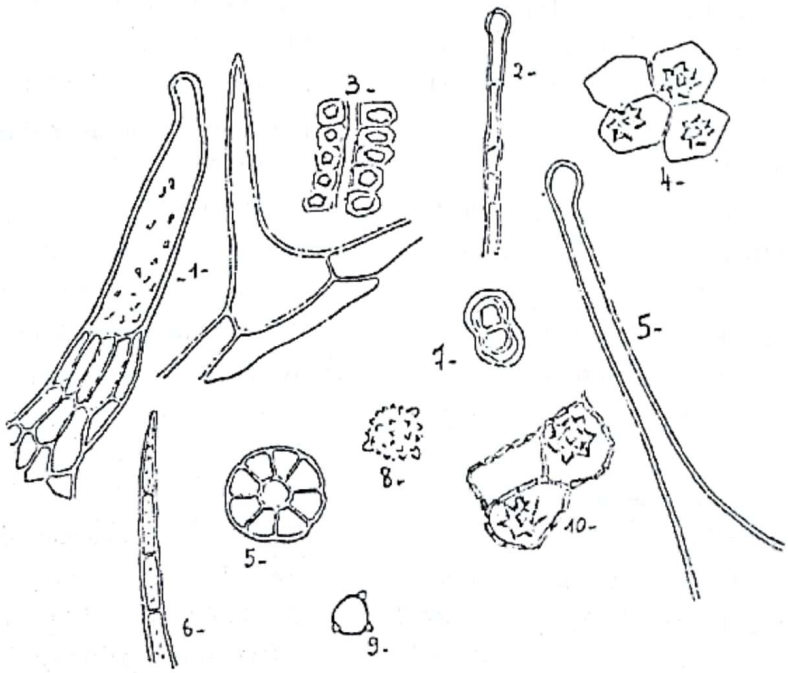

Fig I : Main diagnostic elements of "Rheuma" tea herbal mixture. (X = 360)

control analysis, the quantity of active constituents released in the infusion should be put into consideration and which mainly depends on:

1. The particle size.
2. The extraction time.
3. The extraction temperature.
4. The extraction solvent.
5. The No. of herbs in the tea (single, two, three).

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التحليل الرقابى لشاى طبى يحتوى على خليط من الاعشاب

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كثيرا ما تستعمل الأعشاب الطبية فى صورة شاى والتى تحتوى على خليط من الأعشاب وتباع بالصيدليات بدون تذكرة طبية لهذا يجب أن تخضع لتحليل رقابى دقيق حتى تقيم كدواء آمن وفعال.

وفى هذا البحث تم التقييم الكيفى والكمى لمكونات خليط من الأعشاب الطبية (شاى للروماتيزم) ويباع بالأسواق تحت اسم «رويا» من شركة سيكيم المصرية.

ويشتمل هذا التحليل على:-

الصفات الطبيعية - الاختبارات الفيزوكميائية - الفحص الميكروسكوبى- كروماتوجرافيا الطبقة الرقيقة للتعرف على المواد الفعالة - التحليل الكمى للمادة الفعالة الرئيسية فى الشاى الساليسين بطريقتين: الطيفية وكروماتوجرافيا السائل ذو الضغط العالى.

كما تم ايضا التحليل الكمى للزيوت الطيارة فى الخليط باستعمال كروماتوجرافيا الغاز السائل. وقد تم تحليل كل الملوثات المتوقعة فى الشاى مثل متبقيات المبيدات والمعادن الثقيلة والميكروبات والافلاتوكسين واحتمالات وجود بعض النباتات السامة بطريق الصدفة أو عدم الدقة فى جمع النباتات من مزارعها.