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Standardization of medicinal herbal means of hemostatic action

5A510501– "Pharmaceutical chemistry and pharmacognosy"

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ANNOTATION

ЎЗБЕКИСТОН РЕСПУБЛИКАСИ
СОҒЛИҚНИ САҚЛАШ ВАЗИРЛИГИ
ТОШКЕНТ ФАРМАЦЕВТИКА ИНСТИТУТИ

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МАГИСТРЛИК ДИССЕРТАЦИЯ АННОТАЦИЯСИ

Мавзунинг долзарблиги. Доривор ўсимликлардан олинган экстрактлар ва улар асосидаги препаратлар ҳозирги вақтда кенг спектрдаги касалликларни даволаш имконини бермоқда. Захиралари етарли маҳаллий хомашё бўлган икки уйли газанда, куштарон, аччиктарон қадимдан халқ табобатида турли хилдаги касалликларда ишлатилади. Илмий тиббиётда эса бу таркибдаги дори воситалар кон тухтатувчи сифатида қўлланилади. Маълумки, кўпчилик гемостатик таъсирдаги дори препаратлари асосан синтетик дори моддаларидан тайёрланиб, улар терапевтик самара кўрсатиши билан бир қаторда, ножўя таъсирларга ҳам эгадир. Доривор ўсимликлар асосида тайёрланган фармацевтик маҳсулотнинг организмга ножўя таъсири йўқлигини ҳисобга олиб, доривор хомашё икки уйли газанда, куш тарон, аччик тарон ўсимликларидан илмий асосланган гемостатик суртма ва шамча дори воситалари технологиясини ишлаб чиқиш долзарб вазифалардан бири ҳисобланади.

Ишнинг мақсади ва вазифалари. Икки уйли газанда, куш тарон, аччик таррон биофаол моддаларни экстракция йўли билан ажратиб олиш усулини танлаш, олинган экстракт асосида суртма ва шамча дори турлари таркиби ва технологиясини ишлаб чиқиш.

Тадқиқот объекти ва предмети. Икки уйли газанда, куш тарон, аччик таррон, ўсимликлари қуруқ экстрактлари, предмети ўсимликдан қуруқ экстракт олиш усули, суртма ва шамча дори тури таркиби, технологиясини ишлаб чиқиш, сон кўрсаткичлари, стандартлаш, стабиллигини аниқлаш.

Тадқиқот услубияти ва услублари. Икки уйли газанда, аччиктаррон, куштарон ўсимликларидан қуруқ экстракт олиш. суртма ва шамча дори тури таркиби ва технологиясини ишлаб чиқиш. Сифат кўрсаткичларини аниқлаш мақсадида фойдаланиладиган физик-кимёвий усуллар.

Тадқиқот натижаларининг илмий жиҳатдан янгилик даражаси. Биринчи марта икки уйли газанда, куш тарон, аччик таррон ўсимликлари қуруқ экстрактидан гемостатик таъсирли суртма ва шамча дори тури таркиби ва технологиясини ишлаб чиқилади.

Тадқиқот натижаларининг амалий аҳамияти ва татбиқи. Ишлаб чиқилган суртма ва шамча дори турлари гемостатик таъсирли бошқа препаратларга нисбатан самарали ва иқтисодий жиҳатдан арзонлиги сабаб Республикамиз ишлаб чиқариш корхоналарига тавсия этиш мумкин.

Иш тузилиши ва таркиби. Кириш, адабиётлар шарҳи, тадқиқот давомида фойдаланилган объектлар ва қўлланилган усуллар тавсифи боби ҳамда олиб борилган изланишлар, уларнинг муҳокамаси, натижалари бобларидан иборат. Шунингдек, умумий хулосалар, адабиётлар рўйхати ва иловалар келтирилган.

Бажарилган ишнинг асосий натижалари. Хомашёни тайёрлаш, майдалаш, экстракциялаш, экстрактни тозалаш, қуритиш, сифат кўрсаткичларини аниқлаш, турғунлигини, яроқлилик муддатини аниқлаш, суртма дори тури таркиби ва технологиясини ишлаб чиқиш, уларнинг сифатини баҳолаш.

Хулоса ва таклифларнинг қисқача умумлаштирилган ифодаси. Икки уйли газанда, куш тарон, аччик таррон ўсимликларидан гестомин таъсирли суртма ва шамча дори воситаси технологиясини ишлаб чиқилади ҳамда меъёрий хужжатлар мажмуаси тузилади.

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Topic: Standardization of medicinal plants with hemostatic effect.

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Annotation of Master's Dissertation

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REPUBLIC OF UZBEKISTAN
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ANNOTATION OF MASTER'S DISSERTATION

The relevance of the topic. Subtracted extracts from the plants and the medicines based on their derivatives are giving wide range of treatment options in various diseases. Reserves are sufficient local raw materials like like Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper, are used in folk medicine to treat various diseases from ancient times. In scientific medicine medications in this content used for as an agent to stop bleeding. It is known that majority of the hemostatic medicines are prepared from synthetic substances, therefore along with the therapeutic effect they have side effects. Taking into account that pharmaceutical medicine which is taken from the herbal plants does not have any side effects on humans body, producing scientific based technology of hemostatic ointment and suppository medicine from the herbal plants like Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper, is one of the actual tasks.

Purpose and tasks of work. Selection of the method of extracting bioactive substances from the like Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper, based develop the composition and the technology of the ointment and suppository medicine based on the subtracted extracts.

Scope and object of research. Dry extracts of the plants like Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper the method of subtraction extracts from the plant, the composition and technology of the ointment and suppository medicine, determination of end indicators, standardization, stability.

Research Methods and Techniques. Subtraction of dry extracts from the plants of like Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper. Development of the composition and the technology of ointment and suppository medicine. Physical and chemical methods used to determine quality parameters.

Scientific degree of innovation in research results. For the first time, from the dry extracts of like Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper will be produced ointment and suppository medicine with hemostatic effect.

The practical significance and application of research results. Due to its effective and economical in comparison with other hemostatic drugs, developed ointment and suppository medicine can be recommended to the industrial enterprises of our Republic.

Structure of work and composition. Introduction consists of chapters, description of the literature, description of the objects used and the description used in the research, as well as their researches, their discussion and results. There are also general conclusion, a list of publications, and apps.

The fundamental results of the completed work. Preparation, crushing, extraction, cleaning of extracts, drying, determination of quality parameters, determination of durability, expiration date, development of the composition and the technology of ointment remedies, estimation of their quality.

A brief overview of conclusions and recommendations. From the plants of like Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper, gestomin affected ointment and suppository medicines will be produced and a set of normative documents will be created.

CONTENT

INTRODUCTION

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history (Barnes et al., 2007). Early humans recognized their dependence on nature for a healthy life and since that, time humanity has depended on the diversity of plant resources for food, clothing, shelter, and medicine to cure myriads of ailments. Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or inactive, and also which combinations or processing methods had to be used to gain consistent and optimal results. Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeias. Physical evidence of the use of herbal remedies some sixty thousand years ago has been found in a burial site of a Neanderthal man uncovered in 1960 in a cave in northern Iraq (Solecki, 1975). Indeed, well into the twentieth century, much of the pharmacopeia of scientific medicine was derived from the herbal lore of native people. The knowledge of plant-based drugs developed gradually and was passed on, thus, laying the foundation for many systems of traditional medicine all over the world. In some communities, herbal medicine is still a central part of their medical system. Plants are widely distributed throughout the world but most abundantly in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (WHO, 2005, 2002a,b, 1999a,b, 1998a,b, 1990, 1981, 1979; De Smet, 1995; Duke and Martinez, 1994; Majno, 1975; Ackerknecht, 1973). Thus, herbal medicine has led to the discovery of a number of new drugs, and non-drug substances.

The world is witnessing an unprecedented growth in the usage of herbal products. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. For global harmonization WHO specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines are of utmost importance. Standardization of drug means confirmation of its identity, quality and purity throughout all phases of its cycle.

The medicinal plants are important source for pharmaceutical manufacturing. Medicinal plants & herbal medicines account for a significant percentage of the pharmaceutical market. As the side effects of Synthetic medicine have started getting more apparent, majority of formulation are prepared from herbs. The herbal medicines however, suffer from lack of standardization parameters. The main limitation is the lack of standardization of raw materials, of processing methods and of the final products, dosage formulation, and the non-existence of criteria for quality control. It is necessary to introduce measures on the regulation of herbal

medicines to ensure quality, safety, efficacy of herbal medicines by using modern techniques, applying suitable standards & GMP.

The basic resources of medicines come from nature and they are used as medicaments from ancient time to present day. People around the world possess unique knowledge of the natural resources on which they depend, including tremendous botanical expertise. The traditional medicines cater about 85% of the world population for their health needs. It is essential to maintain safety, quality and efficacy of the plant and their products to avoid and serious health problems. [1] WHO defines traditional medicine as including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well being, as well as to treat, diagnose or prevent illness. WHO has provided some terms related to herbal drugs, according to their definitions. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products. In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials). Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir baking with honey, alcoholic beverages or other materials. Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials. Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term “mixture herbal product” can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal. Herbal medicines are used very commonly in various health practices or therapies of Traditional Medicines like Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy and Homeopathy. [3]

Traditional system of medicines has become significantly more popular all over the globe because of the effective and curative nature for chronic disease with less toxicity. Herbal medicines are not a simple task since many factors influence the biological efficacy and reproducible therapeutic effect [1, 2]. Standardization

of herbal formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, phytochemical, standardization, and In-vitro, In-vivo parameters. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine [3]. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations [4]. The task of lying down standard for quality control of herbal crude drug and their formulation involves biological e particular disease area, chemical profiling of the material and lying down specification for the finished product. Therefore, in case of herbal drugs and product, the word "standardization" should encompass entire field of study from cultivation of medicinal plant to its clinical application. Plant material and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality control are necessary. WHO has emphasized the need to ensure quality control of medicinal plant products by using modern technique and by applying suitable parameters and standards. In order to overcome certain inevitable shortcoming of the Pharmacopoeia monograph other quality control measures must be explored.

CHAPTER 1. REVIEW OF LITERATURE.

1.1. Modern order standardizes herbal liquid dosage forms. Standardization.

In recent years, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics [4]. In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of the herbal drugs [5]. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular medicines. Hence standardization is a tool in the quality control process [6]. American Herbal Product association defines: “Standardization refers to the body of information and control necessary to product material of reasonable consistency. This achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes [7]. “Standardization” expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a respectively by adding excipients or by mixing herbal drugs or herbal drug preparations [8]. “Evaluation” of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration [9]. Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs. The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compound/s would serve as an additional parameter in assessing the quality of the sample.

In olden days vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection of its Rishis, Vaidyas and Hakims. Unlike in olden times where traditional practitioners prepared and tested the qualities of herbal medicines, the

problem faced today are these of economics of industrial scale production, shelf life and distribution to long distances. These have necessitated development of modern and objective standards for evaluating the safety, quality and efficacy of these medicines. People are also becoming aware of the potency and side effect. To gain public trust and to bring herbal product into mainstream of today health care system, the researchers, the manufacturers and the regulatory agencies must apply rigorous scientific methodologies to ensure the quality and lot to lot consistency of the traditional herbal products [11]. Need of Quality control and standardization of herbal products can be summarized as follows-

1. When traditional medicines were developed technology and concept of standardization was quite different.

2. During past thousand years dynamic process of evolution may have changed the identity of plant material.

3. Due to commercialization, supply of genuine raw material has become a challenge.

4. Properties of botanicals may have undergone change due to time and environmental factors [12]. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic [13]. Environmental conditions such as sunlight, rainfall, altitude, temperature, soil, storage conditions as well as different harvesting procedures, time and method of collection, manufacturing processes such as selecting, drying, purifying, extracting, and genetic variability can create substantial variability in product quality and in the concentration of plant chemicals within different products. Ecological conditions like insect feeding, microbial infections may affect secondary metabolites and in turn chemical composition of the plant. Also different parts of same plant (example roots, stem and leaves) contain different concentration of chemical constituents. At the same time diurnal variations (for example paclitaxel, opium alkaloids) and seasonal changes also account for variability in herbal medicines. The therapeutic or toxic components of plant vary depending on the part of the plant used as well as stages of ripeness14. Products from different manufacture vary considerably and it is not possible to control all the factors that affect the plants chemical composition [15;16].Due to complex nature and inherent variability of the constituents of plant based drugs, it is difficult to establish quality control parameter and modern analytical technique are expected to help in circumventing this problem. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. Most of the herbal formulations, especially the classical formulations of traditional medicine, are polyherbal. Many preparations are either liquid or semisolid. For such formulations it is very difficult to establish parameters for quality control. Even official standards are not available. The unique processing methods followed for the manufacture of these drugs turn the single drugs into very complex mixture, from which separation,

identification and analysis of the components is very difficult. Standardization of herbal products can be divided into two categories, first , an active constituents extract, where biochemical principles are known and have therapeutic values, and second, a marker extract, where the active principle is not known and a characteristic compound is used as marker to assess the presence of other therapeutic biochemical compounds [17]. Standardization has limitations because only isolated compounds are considered, ignoring the whole constituents of the herb, which may have synergistic or buffering activities to reduce the side effects. Standardization of traditional medicine starts right from the collection of raw materials to the extreme clinical application. In case of traditional medicine, the therapeutic efficacy is a total effect of its chemical constituents. So, the quality and purity refers to the total profile of the drug rather than any of its character. Therefore, a multidimensional approach is essential for standardization of traditional medicine. This multidimensional approach should cover every minute aspect of drug specifically the name, botanical source, geographical source, organoleptic, morphological, anatomical, physical, chemical and biological activities. World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose [13]. The authenticity, quality and purity of herbal drugs are established by references given in pharmacopoeia. These documents publish traditional and standardized therapeutic uses of herbs and provide a foundation for clinical practice. Monographs consist of a description of the herb, including botanical information, laboratory analysis, therapeutic indications and drug interactions. The pharmacopoeia prescribes (numerical value) like structural, analytical, physical standards for the drugs [18].

Conventional methods for standardization of crude drug. Standardization of herbal raw drug includes passport data of raw plant drugs. It includes medico-botanical survey, identification, botanical authentification, macroscopic, examination. Testing of drugs as per approved Pharmacopoeial testing protocol-Fully pharmacognostical profile, Identification by various chromatographic techniques, Assessment of purity by physico-chemical profile, Assessment of strength by active marker or assay estimation and Safety by heavy metal profiling, microbiological limit test analysis, aflatoxins analysis, pesticides residue and biological activity [19]. Macroscopic identity of medicinal plant materials is based on sensory evaluation parameters like shape, size, colour, texture, odour and taste while microscopy involves comparative microscopic inspection of powdered herbal drug. Further, advances in microscope technology have increased the accuracy and capabilities of microscopy as a mean of herbal crude material identification due to the implication of light and scanning electron microscopes

(SEM) in herbal drug standardization [5; 20]. The phytochemical evaluation for standardization purpose includes the following- Preliminary testing for the presence of different chemical groups, quantification of chemical groups of interest (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins), establishment of fingerprint profiles, multiple marker-based ingerprint profiles and quantification of important chemical constituents [21].

Standardization of herbal/ polyherbal formulation. The herbal formulation in general can be standardized as to formulate the medicament using raw material collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. The preparations with better clinical efficacy are to be selected. All the routine physical, chemical and pharmacological parameters are checked for all the batches in order to select the final finished product and to validate the whole manufacturing process. Standardization is an important aspect for maintaining and assessing the quality and safety of the polyherbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect [22]. Standardization minimizes batch to batch variation; assure safety, efficacy, quality and acceptability of the polyherbal formulations [23].

Standardization of herbal formulation requires implementation of Good Manufacturing Practices (GMP) [24]. In addition, study of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, self-life, toxicity evaluation, chemical profiling of the herbal formulations is considered essential. Heavy metals contaminations, Good Agricultural Practices (GAP) in herbal drug standardization are equally important [25].

In indigenous/traditional system of medicine, the drugs are primarily dispensed as water decoction or ethanol extract. Thus the medicinal plant parts should be authentic and free from harmful material like pesticides, heavy metals, microbial, radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts. The extract should then be checked for indicated biological activity in an experimental animal model. The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints. The next important step is stabilization of the bioactive extract with a minimum shelf life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies. Determination of the probable mode of action will explain the therapeutic profile. The safe and stable herbal extract may be marketed if its therapeutic use is well documented in indigenous systems of medicine, as also viewed by WHO. A limited clinical trial to establish its therapeutic potential would promote clinical use. The herbal medicines developed in this mode should be dispensed as prescription drugs or even OTC products depending upon disease consideration [26; 27].

Who guidelines for quality standardized herbal formulations.

1) Quality control of crude drugs material, plant preparations and finished products.

2) Stability assessment and shelf life

3) Safety assessment; documentation of safety based on experience or toxicological studies.

4) Assessment of efficacy by ethno- medical information and biological activity evaluations.

The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC, and GC).

Generally, all medicines , whether they are synthetic or of plant origin, should fulfil the basic requirement of being safe and effective [28; 29]. The term 'herbal drugs' denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying and storage [30].

1. Quality Control Of Herbal Drugs - Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. In general, quality control is based on three important pharmacopeia aspects:

a. Identity or authenticity- it should have one herb;

b. Purity – it should not have any contaminant other than herb;

c. Assay or Content -the active constituents should be within the defined limits.

Identity can be achieved by macro and microscopical examinations. In addition to this identity tests, which include simple chemical tests, eg.colour or precipitation and chromatographic tests are also necessary. These chemical and chromatographic tests help to provide batch to batch comparability and the chromatogram may be used as a 'fingerprint' for the herbal ingredient by demonstrating the profile of some common plant constituents such as flavonoids, alkaloids and terpenes. To prove identity and purity, criteria such as type of preparation, sensory properties, physical constants, adulteration, contaminants, moisture, ash content, and solvent residues have to be checked. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification [31; 32]. Purity is closely linked with safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues.

Analytical methods such as photometric analysis, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), and Gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Depending on whether the active principles of the preparation are known or unknown, different concepts such as "normalization versus standardization" have to be applied in order to establish relevant criteria for uniformity.

Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used. In all other cases, where no active constituents or marker can be defined

for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeia [33; 34]. A special form of assay is the determination of essential oils by steam distillation. When active constituents (e.g. sennosides in senna) or markers (e.g. alkydamides in Echinacea) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy(UV/VIS), TLC, HPLC, HPTLC, GC, mass spectrometry, or a combination of GC and MS(GC/MS), can be employed [35].

2. Stability Assessment And Shelf Life. Prolonged and apparently uneventful use of a substance usually offers testimony of its safety. In a few instances, however, investigation of the potential toxicity of naturally occurring substances widely used as ingredients in these preparations has revealed previously unsuspected potential for systematic toxicity, carcinogenicity and teratogenicity. Regulatory authorities need to be quickly and reliably informed of these findings. They should also have the authority to respond promptly to such alerts, either by withdrawing or varying the licenses of registered products containing suspect substances, or by rescheduling the substances to limit their use to medical prescription.

Assesement Of Quality. All procedures should be in accordance with good manufacturing practices.

Crude Plant Material. The botanical definition, including genus, species and authority, description, part of the plant, active and characteristics constituents should be specified and, if possible content limits should be defined. Foreign matter, impurities and microbial content should be defined or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period. A lot number should be assigned and this should appear on the product label.

Plant Preparations. The manufacturing procedure should be described in detail. If other substances are added during manufacture in order to adjust the plant preparation to a certain level of active or characteristics constituents or for any other purpose, the added substances should be mentioned in the manufacturing procedures. A method for identification and, where possible, assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances to ensure consistent quality of the preparation.

Finished Product. The manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined to ensure consistent quality of the product. The finished product should comply with general requirements for particular dosage forms.

Stability. The physical and chemical stability of the product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf-life should be established.

3. Safety Assessment: Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of

serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration. However, some of the plants used in herbal medicines can also be highly toxic. As a whole, herbal medicines can have a risk of adverse effects and drug-drug and drug-food interactions if not properly assessed.

Assessment of the safety of herbal products, therefore, is the first priority in herbal research.

These are various approaches to the evaluation of safety of herbal medicines. The toxic effects of herbal preparation may be attributed mainly to the following: Inherent toxicity of plant constituents and ingredients and Manufacturing malpractice and contamination.

Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phyto-chemical and pharmacological studies. It is, however, safe to assume that, based on human experiences in various cultures, the use of toxic plant ingredients has already been largely eliminated and recent reports of toxicity could largely ne due to misidentification and overdosing of certain constituents 36. Adulteration of botanical preparations is another important issue. Due to over exploitation of certain plants, habitat loss and fragmentation of the forest, many medicinal plants have reached to the level of the endangered or rare species. These and many other factors (like cost of raw material) cause problem for availability of genuine drug, which encourages the adulteration of plant by substitution with inferior commercial varieties, artificially manufactured substances, exhausted drugs or cheaper plant or by another vegetative part [37]. Several reports suggest that many herbal products contain undisclosed pharmaceuticals and heavy metals [38]. The intentional use of pharmaceutical adulterant is possible.

Agrochemicals are used to protect the plant from the crude plant material. More over mechanism of action, pharmacokinetics and drug-drug interactions of many herbs are still in infancy. At the same time growing number of reports about fatal or adverse effects of herbal preparations intensifies need for national regulation and registration of herbal medicines and establishment of safety monitoring. Clinicians should not prescribe or recommend herbal remedies without well-established efficacy as if they were medications that had been proved effective by rigorous study [39].

Assessment Of Toxicity. Toxicity investigation will also be required because the analysis alone is unlikely to reveal the contributions to toxicity itself. In assessing toxicity of an herbal medicine, the dose chosen is very important [40].

Toxicity assessment involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern technique, standardization and techniques to adequately model toxicity.

4. Assessment Of Efficacy. Herbal medicines are inherently different from conventional pharmacological treatments, but presently there is no way to assess their efficacy other than by currently used conventional clinical trial methodologies, in which efficacy is conventionally assessed by clinical, laboratory, or diagnostic outcomes: Clinical outcomes include parameters such as improved morbidity, reduced pain or discomfort, improved appetite and weight gain,

reduction of blood pressure, reduction of tumor size or extent, and improved quality of life. Laboratory /other diagnostic outcomes include parameters such as reduction of blood glucose, improvement of hemoglobin status, reduction of opacity as measured by radiological or imaging techniques, and improvement in electrocardiogram (ECG) findings.

Implementation of a standardized approach for the herbal practitioners and collection of the prospective data necessarily creates an interventional design which, if planned properly, may closely resemble single-blind randomized trials. Even if it differs from double-blind randomized trials in the degree of rigor, the design may be the optimum, both biologically and economically, for rapid evaluation of herbal products. Standardization, however, may sometimes be incompatible with the existing legislative framework and caution is needed regarding the ethical implications of such studies. Although randomized clinical trials (with double blind trials as the gold standard) are relatively difficult to be implemented in the case of herbal medicine, they are not ruled out per se in assessing the efficacy of these products. Data from case series studies may provide sufficient scientific and ethical validity to conduct such trials, but acceptance of this protocol needs a paradigm change in the methodoly of drug evaluation as understood in conventional medicine. Standardization and Quality control of herbal drugs involve wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical method and tools.

Physical Evaluation- Each monograph contains detailed botanical, macroscopic and microscopic descriptions with detailed illustrations and photographic images which provide visual documentation of accurately identified material. A microscopic analysis assures the identity of the material and as an initial screening test for impurities.

Chemical Evaluation- Chemical analysis of the drug is done to assess the potency of vegetable material in terms of its active principles. It covers screening, isolation, identification, and purification of the chemical components. It help to determine the identity of the drug substance and possible adulteration.

Biological Evaluation- Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animals and on their intact or isolated organs can indicate the strength of the drug or their preparations.

Analytical Methods- It helps in determining identity, quality and relative potency. The most important step in the development of analytical methods for botanical and herbal preparations is sample preparation. The basic operation includes steps such as pre- washing, drying of plant materials or freeze drying and grinding, to obtain a homogenous sample and often improving the kinetics of extraction of the constituents. In the pharmacopoeia monographs, method such as sonication, heating under reflux, Soxhlet extraction, and others are commonly used [41; 42] However, such methods can be time-consuming, require the use of a large amount of organic solvent, and may have lower extraction efficiencies. New methods are continuously being sought to address this issue. As target compounds may be polar or nonpolar and even thermally labile, the suitability of the methods

of extraction must be considered. To reduce or eliminate the use of organic solvents and improve the extraction processes, newer sample preparation methods, such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE) have been introduced for the extraction of targeted constituents present in plant materials.

Chromatography - Separation of individual components from the herbal mixture is the key step to enable identification and bioactivity evaluation. Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds, even from a complex matrix. These include paper chromatography (PC), thin-layer chromatography (TLC), gas chromatography (GC), HPLC, and capillary electrophoresis (CE). TLC is used extensively in the phytochemical evaluation of herbal drugs because It enables rapid analysis of herbal extracts with minimum sample clean-up requirement, It provides qualitative and semi quantitative information of the resolved compounds. In TLC fingerprinting, the data that can be recorded using a high performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, λ max and shoulder inflection/s of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and absorption spectra (recorded with a photodiode array detector) with different mobile phases. Similarly, GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal drugs [43; 44]. There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar) [45]. HPTLC has been investigated for simultaneous assay of several components in a multicomponent formulation [46]. It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC [47]. HPTLC technique is widely employed in pharmaceutical industry in process development, identification and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health foods [48]. LC-MS has become method of choice in many stages of drug development [49]. The chemical standardization of an aqueous extract of the mixture of the herbs provided chemical compounds serving as reference markers using LC-MS [50].

LC-NMR improves speed and sensitivity of detection and found useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process. The online LC-NMR technique allows the continuous registration of time changes as they appear in the chromatographic run automated data acquisition and processing in LC-NMR improves speed and sensitivity of detection [51]. Gas chromatographic equipment can easily interfaced with rapid scan mass spectrometer of various types. The flow rate of the capillary column is generally low but enough that the column. Output can easily fed directly into ionization chamber of MS. In this the simplest mass detector in GC is the Ion Trap Detector

[46; 52]. GC equipment can be directly interfaced with rapid scan mass spectrometer of various types. GC and GC-MS are unanimously accepted methods for the analysis of volatile constituents of herbal medicines, due to their sensitivity, stability and high efficiency. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the complex constituents [53].

Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. SFC permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been applied to a wide variety of materials including natural products, drugs, food and pesticide [54].

UV absorption has been the most commonly used detection method for the preliminary identification of the separated components. However, various other detectors, such as fluorescence (FD), flame ionization (FID), electron capture (ECD), refractive index (RI), and most recently, evaporative light scattering (ELSD), are also available for specific cases. Most of these detection methods allow the quantification of chemical compounds present in plant material or herbal product. The availability of high-speed computing and the appropriate software allows detection by using mass spectrometry (MS). This method not only allows the detection of component peaks of a mixture separated by chromatography but also in combination with UV (using a photodiode array detector), multistage MS and nuclear magnetic resonance spectrometry (LC–UV–MS–NMR), allows its molecular characterization [55; 56]. More recently, NMR metabonomics, in combination with chemometrics, especially principal component analysis (PCA) and simulated independent modeling of class analogy (SIMCA) algorithms, has been recognized as a very powerful tool to classify samples according to their total chemical composition. The resolution of high-field NMR can provide information in the orders of magnitude higher than of other fingerprinting technologies such as usual NMR spectrometry or HPLC. This is a nonreductuive fingerprinting method of the total chemical composition of samples [57; 58; 59; 60].

The presence of toxic metals is also one of the parameters included in pharmacopoeias. The tool primarily used to detect and quantify the elements in most analyses is based on atomic absorption spectrometry (AAS).

Currently, there have been a number of instruments developed based on the same principle, such as inductively coupled plasma–optical emission spectrometry (ICP–OES). Detection and quantification based on mass spectrometry has also been available using inductively coupled plasma–mass spectrometry (ICP–MS) [61].

Dna fingerprinting technique. DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remain the same irrespective of the plant part used while the phytochemical content will vary with the plant part used, physiology and environment [67]. This concept of fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants, animals and other microorganisms. Genotypic

characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains. Additional motivation for using DNA fingerprinting on commercial herbal drugs is the availability of intact genomic DNA from plant samples after they are processed. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug [68]. The other useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs which helps in distinguishing adulterants even in processed samples [69]. DNA markers are helpful to identity cells, individuals or species as they can be used to produce normal, functioning proteins to replace defective ones. Moreover, these markers help in treatment of various diseases and help in distinguishing the genuine herb from adulterated drug. Cannabis sativa and Arabidopsis thaliana L. Heyne have been differentiated from their adulterated species by using ISSR markers [70].

Role of genetic markers in the standardization of herbal drugs. A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism SNP), or a long one, like minisatellites. Some commonly used types of genetic markers are RFLP (or Restriction fragment length polymorphism), AFLP (or Amplified fragment length polymorphism), RAPD (or Random amplification of polymorphic DNA), VNTR (or Variable number tandem repeat), Micro satellite polymorphism- SNP (or Single nucleotide polymorphism), STR (or Short tandem repeat), SFP (or Single feature polymorphism). They can be further categorized [71]. RAPD based molecular markers have been found to be useful in differentiating different accessions of neem collected from different geographical regions [72]. Germplasm analysis to study genetic diversity is another important area in which a lot of efforts have been put in. Fingerprinting of crops like rice wheat, chickpea, pigeon pea, pearlmilletetc is being carried out extensively [73]. Sequence characterized amplified region (SCAR), AP– PCR,RAPD and RFLP have been successfully applied for differentiation of these plants and to detect substitution by other closely related species**.** e.g. P. ginseng is often substituted by P. quinquefolius (American ginseng) [74]. RAPD markers have been successively used for selection of micropropogated plants of Piper longum for conservation [75; 76].

Quality control and standardization of herbal medicines – concept and scope. Generally, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective (EMEA, 2005; WHO, 2002c, 1998c, 996, 1991a, b, 1990, 1988). The term “herbal drugs” denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage (EMEA, 1998). Hence they are capable of variation. This variability is also caused by differences in growth, geographical location, and time of harvesting. Standardization of herbal medicines is the process of prescribing a set of standards or inherent

characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process. Several problems not applicable to synthetic drugs often influence the quality of herbal drugs. For instance:

1. Herbal drugs are usually mixtures of many constituents.
2. The active principle(s) is (are), in most cases unknown.
3. Selective analytical methods or reference compounds may not be available commercially.
4. Plant materials are chemically and naturally variable.
5. Chemo-varieties and chemo cultivars exist.
6. The source and quality of the raw material are variable.

The methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) also affect herbal quality. At present no official standards are available for herbal preparations. Those manufacturers, who are currently doing some testing for their formulations, have their own parameters, many of which are very preliminary in nature. Presently it is very difficult to identify the presences of all the ingredients as claimed in a formulation. Hence the first important task is to evolve such parameter by which he presence of the entire ingredient can be identified, various chromatographic and spectrophotometric methods and evaluation of physicochemical properties can be tried to evolve pattern for identifying the presence of different ingredient. Wherever possible these methods can be applied for quantitative estimation of bioactive group of compounds like alkaloids, flavonoids, polyphenolic components or estimation of particular compound (Wani, 2007).

The need for standardization – Producers' and consumers' perspective.

In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent. It is the cardinal responsibility of the regulatory authorities to ensure that consumers get the medication, which guarantees purity, safety, potency and efficacy. The regulatory authorities rigidly follow various standards of quality prescribed for raw materials and finished products in pharmacopoeias, formularies and manufacturing operation through statutory imposed good manufacturing practices. These procedures logically would apply to all types of medication whether included in modern system of medicine or one of the traditional systems. Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety. However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is

difficult to establish quality control parameter though modern analytical technique are expected to help in circumventing this problem. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. This is further complicated by the use of combination of herbal ingredients as being used in traditional practice. It is common to have as many as five different herbal ingredients in one product. Thus batch to batch variation starts from the collection of raw material itself in the absence of any reference standard for identification. These variations multiply during storage and further processing. Hence for herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application. Plant materials and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary.

Standardization and quality control of herbal crude drugs – Processes and procedures.

According to WHO (1996a and b, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Attention is normally aid to such quality indices such as:

1. Macro and microscopic examination: For Identification of right variety and search of adulterants.

2. Foreign organic matter: This involves removal of matter other than source plant to get the drug in pure form.

3. Ash values: These are criteria to judge the identity and purity of crude drug – Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.

4. Moisture content: Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.

5. Extractive values: These are indicative weights of the extractable chemical constituents of crude drug under different solvents environment.

6. Crude fibre: This helps to determine the woody material component, and it is a criterion for judging purity.

7. Qualitative chemical evaluation: This covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance.

8. Chromatographic examination: Include identification of crude drug based on the use of major chemical constituents as markers.

9. Quantitative chemical evaluation: To estimate the amount of the major classes of constituents.

10. Toxicological studies: This helps to determine the pesticide residues, potentially toxic elements, safety studies in animals like LD50 and Microbial assay to establish the absence or presence of potentially harmful microorganisms.

The processes mentioned above involves wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical methods and tools. The specific aims of such investigation in assuring herbal quality are as varied as the processes employed.

Physical evaluation.

Each monograph contains detailed botanical, macroscopic and microscopic descriptions of the physical characteristics of each plant that can be used to ensure both identity and purity. Each description is accompanied by detailed illustrations and photographic images which provide visual documentation of accurately identified material.

Microscopic evaluation.

Full and accurate characterization of plant material requires a thorough physical examination. Microscopic analyses of plants are invaluable for assuring the identity of the material and as an initial screening test for impurities.

Chemical evaluation.

This covers screening, isolation, identification and purification of the chemical components. Chemical analysis of the drug is done to assess the potency of vegetable material in terms of its active principles. The chemical screening or tests may include color reaction test, which help to determine the identity of the drug substance and possible adulteration.

Biological evaluation.

Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animal and on their intact or isolated organs can indicate the strength of the drug or their preparations. These assays are known as Biological assays or Bioassay.

Purity determination.

Each monograph includes standards for purity and other qualitative indices already mentioned above.

Analytical methods.

Critical to compliance with any monograph standard is the need for appropriate analytical methods for determining identity, quality, and relative potency. There are a plethora of analytical methods available. However, it is often difficult to know which is the most appropriate to use, but critical among know analytical tools in monograph standardization is chromatography

Chromatography.

Chromatography is the science which studies the separation of molecules based on differences in their structure and/or composition. In general, chromatography involves moving a preparation of the materials to be separated, “the "test preparation”, over a stationary support. The molecules in the test preparation will have different interactions with the stationary support leading to separation of

similar molecules. Test molecules which display tighter interactions with the support will tend to move more slowly through the support than those molecules with weaker interactions. In this way, different types of molecules can be separated from each other as they move over the support material. Chromatographic separations can be carried out using a variety of supports, including immobilized silica on glass plates (thin layer chromatography), very sensitive High Performance Thin Layer Chromatography (HPTLC), volatile gases (gas chromatography), paper (paper chromatography), and liquids which may incorporate hydrophilic, insoluble molecules (liquid chromatography). High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed collectively in different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific type of analysis.

Quantitative analysis.

The most appropriate quantitative analytical method with accompanying chromatograms is desirable. The primary goal of the methods is to provide validated methods to be used to quantify the compounds most correlated with pharmacological activity or qualitative markers (Wani, 2007).

Control of starting material.

Control of the starting materials is essential in order to ensure reproducible quality of herbal medicinal products (De Smet, 2004; Gaedcke and Steinhoff, 2003; WHO, 2002b; Phillipson, 1993). The following points are to be considered in the control of starting materials:

Authentication and reproducibility of herbal ingredients.

The problems associated with unregulated herbal products highlight the major public health issues that can arise when their herbal ingredients have not been authenticated correctly. Herbal ingredients must be accurately identified by macroscopic and microscopic comparison with authentic material or accurate descriptions of authentic herbs (Houghton, 1998). It is essential that herbal ingredients are referred to by their binomial Latin names of genus and species; only permitted synonyms should be used. Even when correctly authenticated, it is important to realise that different batches of the same herbal ingredient may differ in quality due to a number of factors such as:

1. Inter- or intra-species variation: The variation in constituents is mostly genetically controlled and may be related to the country of origin.

2. Environmental factors: The quality of a herbal ingredient can be affected by environmental factor like climate, altitude and other conditions under which it was cultivated.

3. Time of harvesting: For some herbs the optimum time of harvesting should be specified as it is known that the concentrations of constituents in a plant can vary during the growing cycle or even during the course of a day.

4. Plant part used: Active constituents usually vary between plant parts and it is not uncommon for a herbal ingredient to be adulterated with parts of the plant not normally utilized. In addition, plant material that has been previously subjected to extraction and is therefore 'exhausted' is sometimes used as adulterants to increase the weight of a batch of herbal ingredient.

5. Post-harvesting factors: Storage conditions and processing treatments can greatly affect the quality of a herbal ingredient. Inappropriate storage after harvesting can result in microbial contamination, and processes such as drying may result in a loss of thermo-labile active constituents.

Adulteration/substitution.

There are instances when herbal remedies have been adulterated with other plant material and conventional medicines. Reports of herbal products devoid of known active constituents have reinforced the need for adequate quality control of herbal remedies.

Identity and purity.

In order to try to ensure the quality of licensed herbal medicines, it is essential not only to establish the botanical identity of a herbal ingredient but also to ensure batch-to-batch reproducibility. Thus, in addition to macroscopic and microscopic evaluation, identity tests are necessary. Such tests include simple chemical tests, e.g. colour or precipitation and chromatographic tests. Thin-layer chromatography is commonly used for identification purposes but for herbal ingredients containing volatile oils, a gas–liquid chromatographic test may be used. Although the aim of such tests may be to confirm the presence of active principles, it is frequently he case that the nature of the active principle has not been established. In such instances chemical and chromatographic tests help to provide batch-to-batch comparability and the chromatogram may be used as a 'fingerprint' for the herbal ingredient by demonstrating the profile of some common plant constituents such as flavonoids, alkaloids and terpenes. Identity and purity ask the most important question “is the herb the one it should be?” In answering this, a lot of quality determinants are critically examined. Such determinant as purity and chemical constituents are very important. To prove identity and purity, criteria such as type of preparation, sensory properties, physical constants, adulteration, contaminants, moisture, ash content and solvent residues have to be checked. Identity can be achieved by macro- and microscopical examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification (De Smet, 1999). At times an incorrect botanical quality with respect to the labelling can be a problem. For example, in the 1990’s, a South American product labelled as “Paraguay Tea” was associated with an outbreak of anticholinergic poisoning in New York. Subsequent chemical analysis revealed the presence of a class of constituents that was different from the metabolites normally found in the plant from which Paraguay tea is made. Assaying for those herbal ingredients with known active principles is another method of ensuring product’s identity and purity. An assay should be established in order to set the criterion for the minimum

accepted percentage of active substances. Such assays should, wherever possible, be specific for individual chemical substances, and high-pressure liquid chromatography and gas–liquid chromatography are the methods of choice. Where such assays have not been established, then non-specific classical methods such as titration or colorimetric assays may be used to determine the total content of a group of closely related compounds. Purity is closely linked with the safe use of drugs and deals with factors such as values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Depending on whether the active principles of the preparation are known or unknown, different concepts such as “normalization versus standardization” have to be applied in order to establish relevant criteria for uniformity. Content assay is the most difficult area to perform in quality control since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeias. The choice of the extracting solvent depends on the nature of the compounds involved, and might be deduced from the traditional uses. For example, when an herbal drug is used to make a tea, the hot water extractable matter, expressed as milligrams per gram of air-dried material, may serve this purpose (WHO, 1998b, 1996b). A special form of assay is the determination of essential oils by steam distillation. When the active constituents (for example, sennosides in Senna) or markers (for example, alky amides in Echinacea) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, GC, mass spectrometry (MS), or a combination of GC and MS (GCMS), can be employed (Watson, 1999).

Standardization of herbal medicines.

This involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. botanical extracts made directly from crude plant material show substantial variation in composition, quality, and therapeutic effects. Standardized extracts are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes. No regulatory definition exists for standardization of dietary supplements. As a result, the term “standardization” may mean many different things. Some manufacturers use the term standardization incorrectly to refer to uniform manufacturing practices, but following a recipe is not sufficient for a product to be called standardized. Therefore, the presence of the word “standardized” on a supplement label does not necessarily indicate product quality. When the active principles are unknown, marker substances should be established

for analytical purposes and standardization. Marker substances are chemically defined constituents of a herbal drug that are important for the quality of the finished product. Ideally, the chemical markers chosen would also be the compounds that are responsible for the pharmacological effects in the body. There are two types of standardization. In the first category, “true” standardization, a definite phytochemical or group of constituents is known to have activity. Ginkgo with its 26% ginkgo flavones and 6% terpenes is a classic example. These products are highly concentrated and no longer represent the whole herb, and are now considered as phytopharmaceuticals. In many cases they are vastly more effective than the whole herb. However the process may result in the loss of efficacy and the potential for adverse effects and herb–drug interactions may increase. The other type of standardization is based on the guarantee of the manufacturers for the presence of a certain percentage of marker compounds which are not indicators of therapeutic activity or quality of the herb. In the case of herbal drug preparations, the production and primary processing of the medicinal plant or herbal.

1.2. Information of the composition of the Persicaria hydropiper and Polygonum aviculare, Urtica leaves and Calendula flowers.

Calendula (Calendula officinalis)

Botanical Family: Asteraceae syn. Compositae - Aster Family (Picture 1). Calendula is an annual or biennial herbaceous aromatic plant native to central, eastern and southern Europe and North Africa. There are approximately 15 different species of calendula. The plant grows about 1 to 2 feet high, has medium-green leaves and a much branching fragrant (resinous) stem with daisy-like flowers.

Picture 1.

The flowers are a delightful variety of light orange, yellow to golden and dark yellow flowers. Calendula will flower all summer long and well into autumn depending on climate. The term ‘calends‘ refers to the plant’s tendency to bloom in accordance with the calendar— every month in some regions, or during the new moon. It is one of the easiest plants to grow and one that provides great joy, bringing sunshine to the heart and mind. Calendula grows in the full sun although here in North Carolina and other southern states, it is best grown in part sun/part shade.

Traditional use: Calendula was used in German folk medicine as a remedy for wounds and glandular problems. (Wood). Used historically as ‘poor man’s saffron,’ calendula adds both color and flavor to some foods, typically rice and

chowders. It was prevalent in European marketplaces during the Middle Ages and was a common soup-starter.

Chemistry: Over thirty chemical components have been identified in calendula. These constituents include the flavonol glycosides isoquercitrin, narcissin, neohesperidoside, and rutin, terpenoids a- and b-amyrin, lupeol, longispinogenin, and sterols, volatile oils, arvoside A, carotenoid pigments, calendulin, and polysaccharides. (HerbalGram, Expanded Commission E) The plant contains a number of pentacylic alcohols including faradol, brein, arnidiol, and caldenduladiol. Rutin, quercitin, and isorhamnetin are among the flavonoids in the plant. (Foster).

Therapeutic benefits: Calendula has been shown to be an effective bacteriostatic, anti-inflammatory, antipyretic, antifungal, and vulnerary herb. Clinical trials have shown that calendula increases cell proliferation and encourages the granulation process of wound healing. According to Matthew Wood (*The Earthwise Herbal*) calendula possesses at least 7 main properties:

Externally: 1. Applied externally to wounds as an antiseptic, bacteriostatic (inhibits growth or multiplication of bacteria), and hemostatic. Calendula can be used to treat minor scratches and serious lacerations, it prevents the appearance of pus and inflammation, encouraging the body to heal the tissues at its own pace. It has a specific affinity for swollen, hot, painful, pus-filled tissue, especially where there is no vent. *Calendula has been shown to help activate the body's own cells, which gobble-up foreign debris or invaders at the sight of infection then help to activate other defense mechanisms.

Internally: 2. it is therapeutic for swollen glands and lingering, unresolved infections, cleansing the lymphatic glands and ducts. It helps resolve stagnation in the lymphatics from wounds, gland removal, or sickness.

3. it lowers high enzyme counts from damaged liver

4. it soothes the digestive mucosa and other mucus membranes

5. it warms the stomach, drives heat to the periphery, thins fluids, and causes sweating in fever (antipyretic). Considered one of the best herbs for deep fever particularly when the bones hurt.

6. it promotes the period (menstruation). According to Julia Graves in communication with Wood: Calendula stimulates *upana vayu,* the downward bearing wind, or in other words, really increases the life force in the pelvic region. It is therefore indicated for amenorrhea and dysmenorrhea.

7. it is a traditional European peasant tonic taken to prevent sickness in winter (immune tonic). It prepares for the stress of winter by removing old lymphatic congestion and lingering infections.

The herb Calendula is approved by the German commission E for internal and topical use: Inflammation of the oral and pharyngeal mucosa and for external use on poorly healing wounds and Ulcus cruris**.

Persicaria hydropiper.

Persicaria hydropiper (L.) Delarbre belongs to the family of Polygonaceae. The synonyms for this species include *Persicaria hydropiper* (L.) Spach, *P. hydropiper* (L.) Opiz, *P. hydropiper* (L.) H. Gross, *Polygonum hydropiper* L., and *P. hydropiper* var. *projectum* Stanford [1–4]. The species is commonly known as marsh-pepper smartweed, marsh-pepper knotweed, smartweed, or water pepper [5–7] and is also called la liao in China [8], bishkatali or pakarmul in Bangladesh [9], and daun senahun in Malaysia [10].

Picture 2.

Based on the information stated in the Flora of North America and Flora of China [4, 8], *P. hydropiper* is an annual plant of 40–70 cm tall. Briefly, it has a decumbent to ascending or erect branched and glabrous stem. The leaves are lanceolate or elliptic-lanceolate (4–10 × 0.4–2.5 cm) and glabrous with petiole (0.1–0.8 cm), cuneate base, acute to acuminate apex, ciliated margin, and sessile attachment with stipule. The terminal and axillary inflorescences (0.3–18.0) × 0.5–0.9 cm) are either erect or nodding with glabrous peduncle (0.1–0.5 cm), ascending pedicels, and 3–5 flowers. The flowers have greenish proximal and white or pink distal perianth, obovate tepals, 6–8 stamens, and 2-3 styles. *P. hydropiper* is distributed worldwide and found native in temperate and tropical Asia including Western Asia, Caucasus, Siberia, Middle Asia, Russian Far East, China, Eastern Asia, Indian Subcontinent, Indo-China, and Malesia; Europe region such as Northern Europe, Middle Europe, East Europe, Southeastern Europe, and Southwestern Europe; Northern Africa and Australia [11]. The plant generally grows in wet areas at watersides and in marshes [12] and is usually predominant in agricultural fields [13]. It is also commonly distributed to highland sites with highly organic, moist, or silty areas [14].

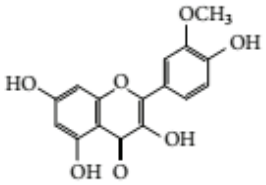
2. Traditional Uses.

Persicaria hydropiper has a strong peppery taste and is commonly used as a hot-tasting spice, food flavor, and garnish for a variety of traditional dishes [15–17].The Japanese people use the young shoot as spice and garnish with raw fish such as “sashimi” for its pungent taste [18], while the water or ethanol leaf extract served as a food additive to preserve pickles, dressing, and cooked foods [19]. In Southeast Asia, the Chinese and Malays use the leaves in traditional laksa dishes [16]. Most importantly, *P. hydropiper* also has a wide range of traditional uses for medicinal purposes. In Europe, the plant has been used as diuretic and emmenagogue [20] and to regulate menstrual irregularities [21]. In addition, decoction of the whole plant, either alone or mixed with other medicinal plants, is also given for diarrhea, dyspepsia, itching skin, excessive menstrual bleeding, and hemorrhoids [22]. The leaves and seeds are used in a folk medicine against cancer

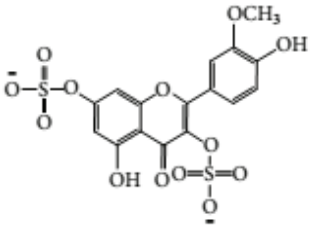
[23]. The Romanian people in Oltenia utilized infusion of the aerial part as astringent and cicatrising, as well as for gastric, pulmonary problems, and uterine hemorrhages [24]. The use of bruised leaves and seeds as vesicantsh as also been reported [25]. In India, the Mishingwomen in Assamtake the dried root powder of *P. hydropiper* for termination of pregnancy and it may lead to permanent sterility if taken continuously for more than a year [26, 27]. Leaf's juice is consumed for uterine disorders [28]. In Arunachal Pradesh, the whole plant extract and ground plant paste are used as fish poisons [28, 29], whereas the leaf infusion is used to relieve colic pain [30].The plant has also been utilized as natural dyes [31]. In Bangladesh, the Garo tribe uses the leaf juice for menstrual pain, the leaf paste to stop bleeding, and the whole plant as pesticide for stored grains [32]. Another tribe of Tripura uses the mixture of crushed *P. hydropiper* leaf with black pepper for headache [33]. In a district of Sylhet, the crushed plant helps to arrest hemorrhage and in Rema- Kalenga, the leaves are used for stomach pain [34]. The leaf juice has been given for treating many health problems like headache, pain, toothache, liver enlargement, gastric ulcer, dysentery, loss of appetite, and dysmenorrhea, while the roots are used as stimulant and their juice is applied to wounds, skin diseases, and painful carbuncles [35]. In Vietnam, the stems and leaves are taken for snake-bite and as diuretic and anthelmintic [12]. In China, the plant is consumed to prevent ovulation and cease pregnancy [36], while the root is used as stimulant, diuretic, carminative, tonic, and anthelmintic [37]. This plant has been found to be toxic to pigs and sheep [38].

3. Phytochemical Constituents.

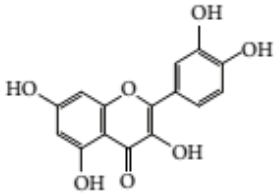
P. hydropiper has been reported to contain mainly flavonoids, sesquiterpenes, sesquiterpenoids, and phenylpropanoids (Table 1). Various extracts andfractions of *P. hydropiper* whole plant and herbs were found to contain flavonoids, such as (+)-catechin, (−)-epicatechin, hyperin, isoquercitrin (Figure 1), isorhamnetin (Figure 1), kaempferol, quercetin (Figure 1), quercitrin, rhamnazin and rutin [39–41]; drimane-typed sesquiterpenes, such as 3-



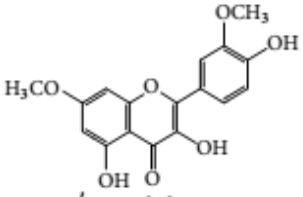
Isorhamnetin



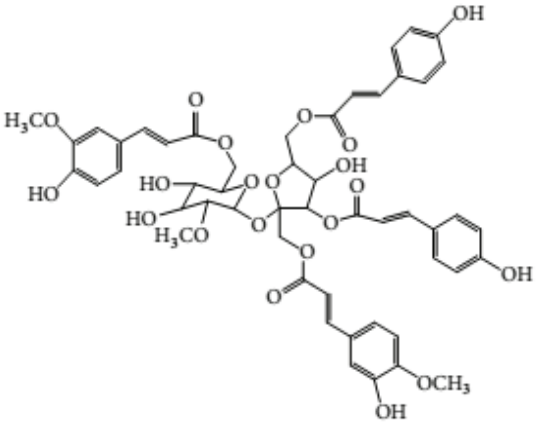
Isorhamnetin-3,7-disulphate



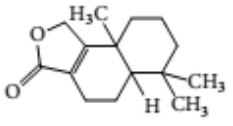
Quercetin



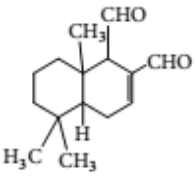
7,4′-Dimethylquercetin



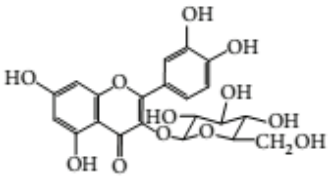
Hydropiperoside B



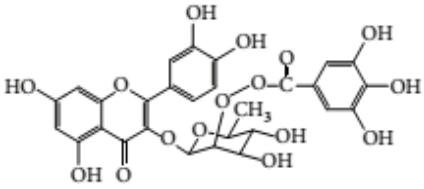
Confertifolin



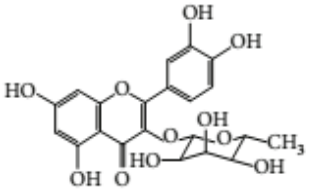
Polygodial



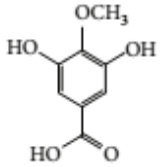
Isoquercitrin



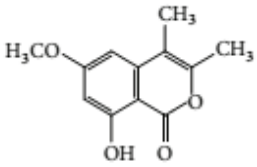
Galloyl quercitrin



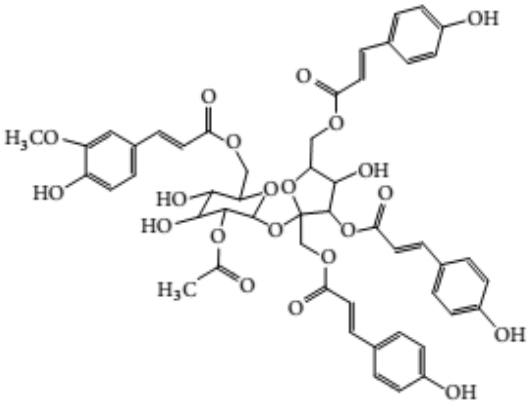
Quercetin-3-*O*-rhamnoside



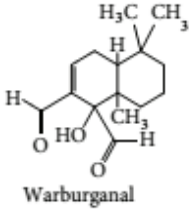
3,5-Dihydroxy-4-methoxybenzoic acid



Polygonolide



Vanicoside A



Warburganal

Polygonum aviculare L.

The *Polygonaceae* family consists of about 800 species and the Polygonum genus comprises about 300 species distributed throughout the world - in Europe (31 species), Asia, North Africa, North America and is also introduced into South America. *Polygonum aviculare* L. is native to Europe and Asia (China, Korea etc).

Picture 3.

In European Union (EU), it is mainly collected in Eastern Europe. It is a sturdy annual herbaceous plant and widely distributed mainly in fields or roadsides (Światek *et al.* 1986; Karlsson 2000; Sawicka *et al.* 2002; Costea and Tardif 2005, Wyk and Wink 2005 Narasimhulu *et al.* 2014; Styles 1962).

Knotgrass is procumbent and spreads along the ground. The stem is slender (diameter 0.5 – 2 mm), branched, with nodes, cylindrical and longitudinally striated. Average length of the stem is 30 cm. Leaves are small, narrow, sessile and glabrous entire. The characteristic of the plant is the sheath-like stipules (ochrea) which have form of a lacerated membrane in silver and brown colour at the base. Very small flowers have 5 greenish-white perianth segments, the tips of which are often coloured red, generally flowered from June-September. The fruits (2 – 4 mm) are brown to black ovate, triangular nuts, often punctate or striate (European Pharmacopoeia 8th ed., 07/2013:1885; Österreichisches Arzneibuch 1981; Wichtl 2004; Wyk and Wink 2005).

Polygoni avicularis herba is known under the following other names: knotgrass (English); Vogelknöterichkraut (German); maura sūrene (Latvian); takažolių žolė (Lithuanian); Ziele rdestu ptasiego (Polish); nať rdesna ptačího (Czech); madárkeserűfű virágos hajtás (Hungarian); Stavikrvová vňať (Slovak language); Vejpileurt (Danish); Vogelknöterichkraut (German).

Constituents

The following constituents of knotgrass (Polygoni avicularis herba) according to existing references are reported in the literature:

Flavonoids (0.1-1%; rarely 2.5-3%): derivatives of kaempferol, quercetin and myricetin, particularly avicularin (quercetin-3-O-arabinoside approximately 0.2%), juglanin (kaempferol-3-O-arabinoside), hyperoside, quercitrin, quercetin-3-galactoside, as well as vitexin, isovitexin, rhamnetin-3-Ogalactoside, rhamnazin hydrogen sulphate, myricetin-3-O-fhamnoside, rutin, astragalin, isoquercitrin, miquelianin, spiraeoside, orientin, myricitrin, desmanthin-1, luteolin, betmidin, taxifolin, isorhamnetin, apigenin. Recently some new flavonoids were isolated: liquiritin and cinaroside, 5,3'- dihydroxy-4'-O-angeloxyflavone-7-O-β-D-glucopyranoside (Sun et al. 2002). Five new flavones were isolated: 5,7-

dihydroxy-6-methoxyflavane, 5,7- dihydroxy flavane, 5,7- dimethoxy-4'-hydroxyflavane, morin-7-O-β-D-glucoside and 5- hydroxyl-3'-methoxyflavanone-7-O-rutinoside (Zheng et al. 1999). New dimeric procyanidin glucoside: catechin 3-O-acetate-(4α→8)-catechin 3-O-acetate-3'-O-β-Dglucopyranoside was isolated in 2012 (Cong et al. 2012). Tannins (3.5- 4%): rhatannin, gallo- and catechol tannins. Phenolic carboxylic acids: caffeic, chlorogenic, gallic and protocatechuic acids (Sawicka et al. 2002; Światek et al. 1986; Wichtl 2004; WHO monographs NIS 2010). Naphthoquinone: 6-methoxyplumbagin (Al-Hazimi and Haque 2002).

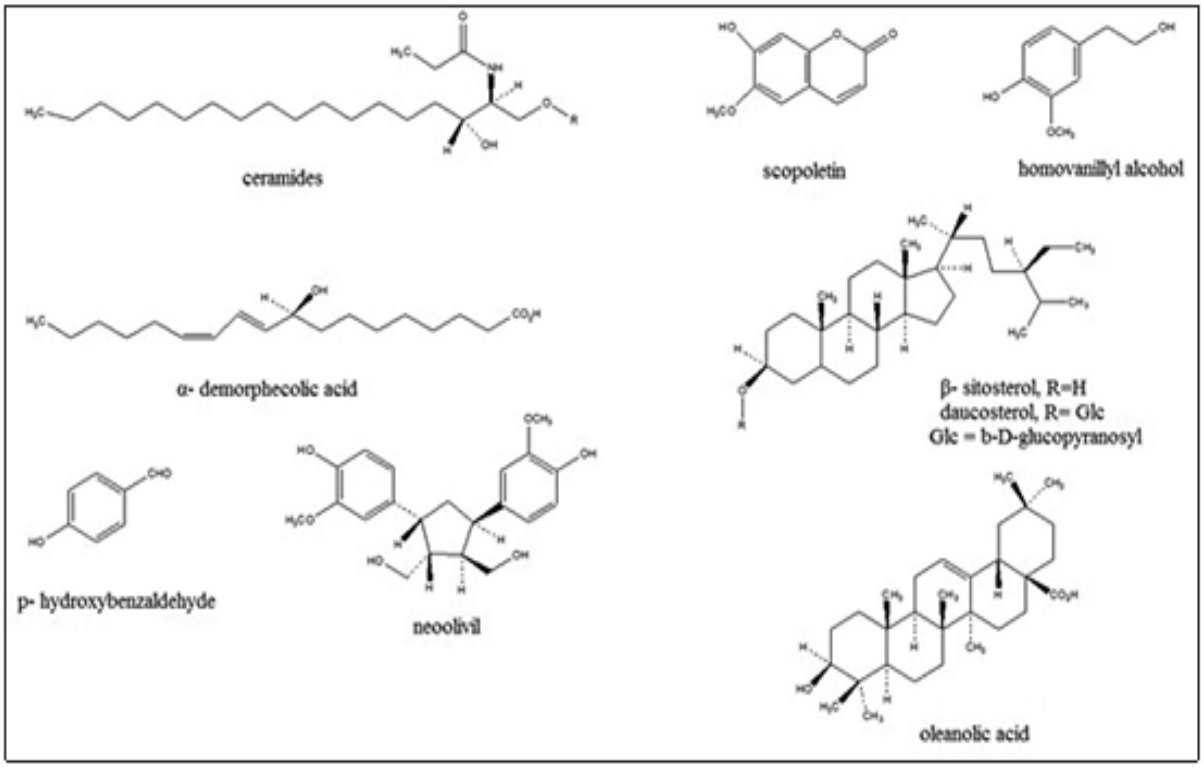
Urtica dioica L.

Urtica dioica L. of family Urticaceae, is a perennial plant which is commonly known as stinging nettle. It is widely distributed throughout the temperate and tropical areas around the world.[1] It is found in the Himalayas from Kashmir to Kumaon at altitudes of 2,100-3,200 m.[2,5] Since olden eras, public have taken advantage of this sting by flailing arthritic or paralytic limbs with fresh plant to stimulate circulation and bring warmth to joints and extremities in a management known as urtication.[6]

Picture 4.

Traditionally, the leaves and roots of plant are used internally as a blood purifier, emmenagogue, diuretic, nasal and menstrual haemorrhage, rheumatism, eczema, anaemia, nephritis, haematuria, jaundice, menorrhagia and diarrhea. [4,7,8] The plant elaborates different classes of organic compounds of medicinal importance including phytosterols, saponins, flavanoids, tannins, sterols, fatty acids, carotenoids, chlorophylls, proteins, amino acids and vitamins [1,9,10] The compounds which are reported from the plant are beta-sitosterol, trans-ferulic acid, dotriacotane, erucic acid, ursolic acid, scopoletin, rutin, quercetin and p-hydroxylbenzalcohol. [11] The plant has been reported to have various pharmacological activities [9,12] such as antioxidant [13] anti-inflammatory, ntiulcer [14] anti-colitis, antiviral [1] anticancer [15] antibacterial, antimicrobial, antifungal [14,16-18] antiandrogenic [19] insecticide [20] immunomodulatory [21] hypocholesterolemic [22] hypoglycemic [23] cardiovascular effects [22] analgesic [14] natriuretic, hypotensive [24] hepatoprotective [25] and rheumatoid arthritis. [26] This review intent to summarize diverse studies on this plant and critically evaluates the issues associated to ethnomedicinal uses, phytochemistry, pharmacology and toxicology of U.dioica. Phytochemical Studies The main chemical constituents of Urtica dioica are flavonoids, tannins, volatile compounds and fatty acids, polysaccharides, isolectins, sterols, terpenes, protein, vitamins and minerals. [1,8,40-44] The compounds responsible for the burning sensation

properties of leaves trichomes are acetylcholine, histamine, 5-hydroxytryptamine (serotonin), leukotrienes and formic acid. [45-47] The main components of essential oil of U. dioica are carvacrol (38.2%), carvone (9.0%), naphthalene (8.9%), (E)-anethol (4.7%), hexahydrofarnesyl acetone (3.0%), (E)-geranyl acetone (2.9%), (E)-β-ionone (2.8%) and phytol (2.7%).[40] The flavonoids are mainly kaempferol, isorhamnetin, quercetin, isoquercitrin, astragalin, rutin and their 3-rutinosides and 3-glycosides. [11,42,48] The shikimic acid derivatives like phenylpropanes, caffeic acid and various esters of this acid such as chlorogenic acid and caffeoyl malic acid have been identified. [49-52] The carotenoid such as β-carotene, hydroxy-β-carotene, lutoxanthin, lutein epoxide and violaxanthin are reported. [42,53-55] The leaves are rich in vitamins B, C, K and minerals such as calcium, iron, magnesium, phosphorus, potassium and sodium. [45,56] Other chief constituents present are essential amino acids, glucokinnins and a very high content of chlorophyll. [41,57-59] The chemical structures of various isolated chemical compounds from Urtica dioica Linn. are shown in Figure 2.



1.3. Pharmacological properties of these medicinal plants.

Calendula. ***Antiinflammatory activity*** - The acute inflammatory response during the early stages of injury generates factors that are essential for tissue growth and repair [22]. When prolonged, however, chronic inflammation can be detrimental, preventing wound remodelling and matrix synthesis, leading to delays in wound closure and an increase in wound pain [23]. Thus, it is plausible that an antiinflammatory effect could facilitate wound healing and improve patient comfort. Even though traditional texts and animal studies indicate that Calendula extract exerts an antiinflammatory effect [11,24-25], there is a paucity of clinical evidence to support this claim. In one single-blind, randomised controlled trial of

254 patients with breast cancer, the topical administration of *Calendula officinalis* ointment twice daily to irradiated skin resulted in a significantly lower incidence of acute radiation induced dermatitis ($p<0.001$) as well as lower maximal pain scores ($p=0.03$) when compared to Trolamine, a topical analgesic [16]. Whilst a reduction in the occurrence of dermatitis could be explained by an antiinflammatory effect, it is also possible that the dermatitis was prevented by way of an antioxidant effect. Thus, the efficacy of Calendula as an antiinflammatory agent in humans remains inconclusive.

Antioxidant effect The production of free radicals at or around the wound bed may contribute to delays in wound healing through the destruction of lipids, proteins, collagen, proteoglycan and hyaluronic acid [26]. Agents that demonstrate significant antioxidant activity may therefore preserve viable tissue and facilitate wound healing. Given that the butanolic extract of Calendula demonstrates free radical scavenging activity against superoxide radicals and hydroxyl radicals *in vitro* in a dose dependent manner; that the same extract inhibits iron ascorbate-induced lipid peroxidation in rat liver microsomes [27]; and that several organic solvent extracts of Calendula inhibit lipid peroxidation of liposomes *in vitro* [12], it is argued that Calendula may facilitate wound healing via an important antioxidant effect. Even so, clinical research is needed to validate these findings.

Antimicrobial activity Wound healing can also be delayed when microorganisms are present in large enough numbers [22, 28]. Therefore, reducing the bacterial load of a wound may be necessary to facilitate wound healing, as well as reduce local inflammation and tissue destruction [29]. An ideal agent for the prevention and control of wound infection would therefore be one that directly destroys pathogens, whilst also stimulating immune activity. Calendula is one agent that possesses both of these properties. For instance, studies have shown that the polysaccharide fraction of *Calendula officinalis* stimulates phagocytic activity of human granulocytes *in vivo* [30] and phagocytic activity in mice [31-32], whilst the ethanolic extract of Calendula stimulates mixed human lymphocyte proliferation *in vitro* [33]. Adding to this, when applied to rats with ellipsoid cutaneous excisions, the daily application of four percent Calendula and one percent allantoin ointment for 21 days generated greater phagocytic activity, macrophage differentiation, granulation and epithelialization than the use of either allantoin or plain ointment alone [34]. The clinical significance of this immunomodulatory effect is further highlighted in the following studies where wound infection was used as the main outcome measure. An open, randomised, controlled, multicentre trial involving 156 patients compared the effectiveness of daily applications of three different ointments in the management of second and third degree burns over a mean period of seventeen days [20]. Although the prevention of eschar formation and local infection was similar between the Calendula (ointment containing 20% fresh plant in a Vaseline base) and Elase (proteolytic ointment) groups, a marginally significant difference in these

outcomes favoured Calendula over Vaseline (p=0.05). The open design of this study, however, suggests that these findings be considered with caution. In another randomised, controlled trial, eighteen patients with trophic ulceration were randomly allocated to one of three blinded treatments: ten percent Calendula ointment, topical neomycin or placebo paraffin ointment. The topical application of Calendula ointment prevented secondary infection and demonstrated a 30-40 percent reduction in wound diameter and depth within four weeks [18]. The comparative effects of neomycin and placebo ointment on wound healing, however, were not detailed. The ability of Calendula to prevent wound infection may not only be attributed to the immunomodulating effect of the plant, but also to an antimicrobial effect. Experimental studies have demonstrated that extracts of Calendula flower have a high degree of activity against eighteen different strains of anaerobic and facultative aerobic periodontal bacteria *in vitro* [35], and against four different types of fungi, with the inhibitory effect being comparable to that exerted by the antifungal agents Amphotericin B and Nystatinb[36]. Whilst this antibacterial effect has been demonstrated clinically in 65 patients with chronic suppurative otitis using a twenty percent tincture of Calendula flowers [37], details of this study are limited, and therefore, these findings should be interpreted with caution. It should also be noted that in order for the abovementioned laboratory findings to be clinically relevant, further investigation is needed to identify whether the antimicrobial effect of Calendula can be altered by the presence of body fluids or by the carrier agent used in the formulation, alike that reported in studies on essential oils [38].

Wound healing activity The most important clinical endpoint in wound management is wound closure, or one hundred percent epithelialization [39]. Given that wound closure is so important, it is argued that any agent demonstrating significant woundhealing activity should be seriously considered in conventional practice. Calendula, for example, may facilitate wound healing by increasing both wound angiogenesis [40] and collagen, nucleoprotein and glycoprotein metabolism [34,41], leading to improvements in both local circulation and in the formation of granulation tissue [42]. Several experimental studies lend support to these claims, demonstrating that the daily application of calendula cream [44] or a 1:10 alcoholic extract of Calendula [43] to paravertebral incisions in rats facilitates collagen maturation and epithelialization within 1043 to 2544 days. In a poorly defined trial of fifty patients with slowly healing wounds and amputation stumps, treatment with a topical Calendula preparation for an unknown period of time was examined [45]. The trial found wound granulation appeared within several days of initiating the Calendula treatment, and secondary skin development had occurred around 10-14 days. Researchers also found that the Calendula treatment reduced discomfort during dressing changes, and that the treatment was more cost-effective than other medicines used. However, given the lack of details on the study design and interventions, these results must be considered with caution. The wound healing

effect of two different Calendula preparations were compared in another poorly defined trial involving 38 patients with venous ulceration, burns or skin lesions [21]. Patients from each wound class were equally divided into two groups. In the first group (n=19), wounds were cleaned daily with a solution containing 90% distilled water and 10% *Calendula officinalis* tincture until the wound healed. In the second group (n=19), the Calendula solution was also used to clean the wound, but in addition, wounds were dressed with a thin film of Carbopol 900 gel containing two percent Calendula tincture. The study found that cleaning venous ulcers, burns and skin lesions with 10% Calendula solution, followed by the daily application of 2% Calendula gel, resulted in a greater number of healed wounds, as well as a reduction in the median time to heal when compared to using Calendula solution alone. The outcome of this study is further supported by a more recent controlled trial, in which the effect of twice-daily applications of 7.5% Calendula ointment were compared to daily applications or saline dressings in 34 patients with venous leg ulceration [17]. After three weeks of treatment, the total surface area of wounds in the Calendula group decreased by 42 percent, compared to 15 percent in the control group. The difference between the two groups was statistically significant ($p<0.05$). Whilst these results are promising, there is a lack of information from both trials about the controls used, the selection of participants, and the secondary dressings applied, which casts doubt over the generalisability of these findings. A number of other studies have examined the healing effects of Calendula within combination preparations [46-48], but given the nature of these formulations, it is impossible to isolate the effects of Calendula from the other extracts. Therefore, these studies were omitted from this review.

Analgesic activity. Given that open wounds can generate pain and subsequent disability, it is important that the dressing applied does not increase pain, and if possible, lessens pain. Whilst no studies to date have specifically investigated 12 the analgesic effects of Calendula, there is some suggestion that Calendula may decrease wound pain. For example, in one open-label study, thirty patients with grade 1 and 2a burns were treated topically with a hydrogel preparation containing 10% Calendula officinalis tincture, three times a day for 13-14 days [49]. While details of this trial are limited, researchers indicate that improvement was noted for pain, erythema, swelling, soreness, blistering and heat sensitivity, and tolerance to the topical preparation was good. Even so, no firm conclusions can be made from this small, uncontrolled pilot study until more rigorous research is conducted.

Persicaria hydropiper.

Several reports on pharmacological properties of *P. hydropiper* are available to support the ethnomedicinal uses of the plant including antioxidant, antibacterial, antifungal, antihelminth, antifeedant, cytotoxicity, anti-inflammatory, antinociceptive, oestrogenicity, anti-fertility, anti-adipogenicity, anticholinesterase, and neuroprotection.

Antibacterial Activity. Confertifolin isolated from the leaf essential oil of *P. hydropiper* showed strong/good antibacterial activity against *Enterococcus faecalis* (MIC 31.25

Depending on the amount of persicarin in *P. hydropiper*, its extract could potentially possess neuroprotective activity.

Polygonum aviculare (Polygonaceae) is an herb commonly distributed in Mediterranean coastal regions in Egypt and used in folkloric medicine. Organic and aqueous solvent extracts and fractions of Polygonum aviculare were investigated for antimicrobial activities on several microorganisms including bacteria and fungi. The phytochemical constituents of the air dried powered plant parts were extracted using aqueous and organic solvents (acetone, ethanol, chloroform and water). The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of inhibition zone against both Gram-negative and Gram-positive bacteria and fungi using paper disc diffusion method. Results of the phytochemical studies revealed the presence of tannins, saponins, flavonoids, alkaloids and sesquiterpenes and the extracts were active against both Gram - negative and Gram - positive bacteria. Chloroform extract gave very good and excellent antimicrobial activity against all tested bacteria and good activity against all tested fungi except Candida albicans. Structural spectroscopic analysis that was carried out on the active substances in the chloroform led to the identification of panicudine (6- hydroxy-11- deoxy- 13 dehydrohetisane). Evaluation of the antimicrobial activity of panicudine indicated significant activity against all Gram-negative and Grampositive organisms tested. Panicudine displayed considerable activity against the tested fungi with the exception of Candida albicans. The antimicrobial activity of the extracts were unaffected after exposure to different heat treatments, but was reduced at alkaline pH. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of panicudine on the test organisms showed that the lowest MIC and the MBC were demonstrated against Salmonella paratyphi, Bacillus subtilis and Salmonella typhi and the highest MIC and MBC was exhibited against Staphylococcus aureus.

Urtica dioica. ***Antioxidant Activity*** **-** Antioxidants are emerging as prophylactic and therapeutic agents which scavenge free radicals or reactive oxygen species and prevent their damaging effect. Free radicals have been associated with pathogenesis of disorders like cancer, diabetes, cardiovascular diseases, autoimmune diseases, neurodegenerative disorders and are implicated in aging.[64] The hydro-alcoholic extract of *Urtica dioica* plant has shown significant results for antioxidant activity with half inhibitory concentration (IC50) value of 88.33 ± 2.88 µg/ml.[34] The aqueous (5% decoction) and methanolic extracts at the concentration 50-500 mg/ ml have shown significant antioxidant potential.[13] The aqueous extract in a dose-dependent manner 12.5- 800 mg/ml inhibit lipopolysaccharide-stimulated nitric oxide productions.[58] The aqueous extract has significant reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities. The 50, 100 and 250 µg/ml dose of aqueous extract has shown 39, 66 and 98% inhibition

on peroxidation of linoleic acid emulsion, respectively while 60 µg/ml of alpha-tocopherol, exhibited only 30% inhibition.[14]

Antidiabetic Activity - The aqueous extract of plant 250 mg/kg has shown a significant glucose lowering effect against alloxan induced diabetes in rats.[59] The fructose induced insulin resistance in male rats has been shown to decrease serum glucose level on administration of hydro-alcoholic leaf extract.[65] The leaf extract was administered in perfused islets of langerhans both in normal and streptozotocin induced diabetic rats which showed a significant enhancement of insulin secretion thereby decreasing the blood sugar level.[66] The cold methanolic extract of leaves (250 mg/kg) has also shown significant antihyperglycemic effect in alloxan induced diabetes.[67]

Hepatoprotective Activity - Hepatoprotection is the ability to prevent damage to the liver, prevent the liver affections prophilactically and maintains balance in liver enzymes. The leaves extract of plant has shown maximum hepatoprotective activity at dose 400 mg/kg as suggested by decreased level of serum alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin levels and significant decrease in malonyldehyde (MDA) level as well as a significant increase in superoxide dismutase (SOD) level.[10,68] In CCl 4 induced hepatotoxicity plant extract has shown significant hepatoprotective effect in isolated rat hepatocytes (*in-vitro*) and same in rabbits (*in-vivo*) with protective effect against hepatocellular degeneration and necrotic changes.[25,69,70] The *Urtica dioica* seed extract has also shown protective effect on hepatic damage created with ischemia-reperfusion and it exhibited liver protection effect by increasing the activity of paraoxonase, arylesterase and liver tissue catalase activity.[71]

Anti-hyperlipidemic Activity - The plant has very potent antihyperlipidemic activity as it lowers the levels of lipids and lipoproteins in blood. The aqueous extract 150 mg/kg given for 30 days to rats fed on normal or high-fat diet, improved the blood lipid profile. The significant decrease in total cholesterol, low density/high density cholesterol (LDL/HDL) ratios via lower concentrations of LDL and plasma total apo-protein B has been observed.[72] The ethanolic extract of the plant at dose 100 and 300 mg/kg has shown significant reduction in the level of total cholesterol and LDL level in hypercholesterolemic rats.[73,74]

Diuretic Activity - The aqueous extract of whole plant has been reported to produce diuretic and natriuretic effects in rabbits.[75] The aqueous extract of aerial part of the plant was administered at low dose (4 mg/kg/h) and high dose (24 mg/kg/h) which shows diuresis effect by increase diuresis (11 and 84% respectively) and natriuresis (28 and 143% respectively). Hence, the plant has shown to have potential diuretic effect.[24] The ethanolic extract of *Urtica dioica* at dose 1 g/kg (p.o) has no effect on diuretic activity but the urine output increased significantly at dose 500 mg/kg (i.p).[76] Carceres *et al*., 1987,[76] reported an increase in urine production by 20% after 1g/kg oral dose in 10% decoction in rats.

The diuretic effect of stinging nettle was approximately 25% of that achieved with hydrochlorothiazine (25 mg/kg).[77]

Antiviral Activity - The N-acetyl glucosamine-specific lectin from *Urtica dioica* is a strong inhibitor of syncytium formation between HUT-78 cells and CD4 + Molt/4 cells permanently infected by HIV-1 and HIV-2.[78] The mannose binding site of HIV virus is highly susceptible after due to mutation in HIV. However the N-acetyl glucosamine region is the conserved site. Therefore *U. dioica* extract posses affinity for N-acetyl glucosamine region exhibit better anti-HIV activity. Further plant extract exhibit specificity for N-glycosylation of GP-120 may serve as an better alternative to prevent the development of drug resistance.[79] The aqueous extract of the plant indicates a significant inhibition on the development of syncytia with low doses (0.5-1 µg/ml) and increased when the concentration rose until it reached an inhibition level of 84% which, however began to show cytotoxic effects.[80].

Antimicrobial Activity - The plant has been tested for antimicrobial activity against various Gram positive and Gram-negative bacteria: *Bacillus subtilis* IP 5832, *Lactobacillus plantarum* 299v (Lp299v), *Pseudomonas aeruginosa* and *Escherichia coli*. The result has shown minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract ranged from 9.05 to more than 149.93 mg/ml-1.[17] Screening of antibacterial activity of plant methanolic extract has been done on six bacteria strain such as *E. coli, P. aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, S. aureus* and *S. epidermidis*. It showed significant inhibitory activity against *S. pyogenes, S. aureus* and *S. epidermidis.*[81] The antimicrobial activity of stinging nettle extract has been reported for *S. areus, Enterococcus faecalis, B. subtilis* and *E. coli.*[82,83] The flavonoids patuletin isolated from plant extract has also been tested for antimicrobial properties and the compound showed significant activity against *S. aureus, S. faecalis, E. coli* and *C. albicans* with MIC of 0.02, 0.02, 0.002 and 0.001 g/ml, respectively.[84].

Cardiovascular Effect - On intravenous (i.v) administration of *Urtica dioica* fraction 0.1 mg/kg cause a decrease of MAP mean arterial pressure (79.59/0.5 mmHg) in comparisons to basal value (96.59/0.5 mmHg) which show that plant have antihypertensive property.[85] The aqueous extract (1 and 2 g/l) has been studied on the isolated, spontaneously beating, Langendorff rat heart and the isolated rat thoracic aorta in order to characterize the cardiac and vascular effects.[86] The increase in the concentration of KCl (40-60 mM) that is by the raise of the levels of membrane depolarization due to decrease in the vaso-relaxation action of plant. These effects proposed the involvement of hyper-polarization factors, probably bound to potassium channels opening.[87]

Immunomodulatory Activity - In mouse splenocytes there was stimulation of lymphocyte proliferation and an increase in the proportion of T-lymphocytes due to immunomodulatory action of aqueous extract (400 g/ml).[58] The ethanolic

extract of plant at dose 50 and 100 mg/kg body weight (b.w) given orally for 14 days showed significantly lower activity of cytochrome P450, lactate dehydrogenase (LDH), NADPH-cytochrome P450 reductase (cyt P450 R), total sulfhydryl groups (T-SH), non-protein sulfhydryl groups (NP-SH) and protein bound sulfhydryl groups (PB-SH).[92] The compounds quercetin-3-O-rutinoside, kaempherol-3-O-rutinoside and isorhamnetin-3-O-glucoside present in the methanolic extract of the aerial parts of the plant contribute to the immunomodulatory activity of the plant.[21]

Hypotensive Effect - The aqueous extract of *Urtica dioica* reported to have positive inotropic effect associated with a marked decrease in heart rate without effecting heart pressure.[86] However, the continuous intravenous perfusion of the aqueous extract at a dose of (4 and 24 mg/kg/h) has shown decreased in blood pressure by 15% and 38% respectively [24].

Conclusions on the review of the literature

Based on the analyzed data, it can be concluded that Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper as a source of medicinal plant raw materials have been studied in detail and variously studied: detailed phytochemical studies have been carried out, and its pharmacological properties have been studied.

According to the literature data used medicinal plants contain such biologically active substances as tannins, flavonoids, alkaloids, essential oils, protein substances, vitamins C, K1, B1, B2, macro and microelements, etc. Preliminary experiments have shown that The drug has capillary-strengthening, hemostatic and anti-inflammatory activity. To optimize the method for producing the above liquid extract, standardizing the preparation obtained, it is very important to develop a methodology for the qualitative and quantitative determination of bioflavonoids. Which is simple, uncomplicated and has good accuracy and reproducibility.

It can be noted that for the time being there are practically no official medications of this composition, despite the fact that the composition of raw materials has been sufficiently studied and the raw material base is significant. Thus, it is obvious that the development and standardization of liquid dosage forms based on phytopreparations in the flowers of Calendula, Urtica dioica, Polygonum hydropiper, Persicaria hydropiper is quite a modern task. Particularly interesting for external use are such pharmacological aspects of the action as anti-inflammatory, wound-healing, capillary-strengthening. Accumulated material in the domestic literature testifies.

CHAPTER 2. OBJECTS AND METHODS OF RESEARCH

2.1. Purpose of research

The main goal of the present work is the development of medicinal forms from calendula collection, nettle, polygonum hydropiper and persicaria hydropiper, their pharmacological research and determination of quality standards. To achieve this goal, it is necessary to resolve the following tasks:

- to carry out the experimental and theoretical justification of the optimal technology for extract and tincture;
- to develop methods for qualitative and quantitative analysis, quality standards for extract and tincture;
- perform pharmacological studies: toxicity and hemostatic effect of the resulting medicinal forms.
-

2.2. Objects of research

The present work was carried out at the departments of technology of medicines, pharmacognosy, pharmaceutical chemistry of the Tashkent Pharmaceutical Institute in Tashkent in 2016-2018. Also in LLC “Scientific Center For Standardization Of Drugs”, “Uzbek Scientific Research Institute Of Chemistry And Pharmaceutical” N. A. Sultanova and “Med Standart”.

In experimental studies, substances, auxiliary substances, reagents and raw materials were used, which corresponded to normative documentation (GF, GOST, FS, TU). Samples of raw materials "Calendula flowers" were obtained from the scientific institute of “Uzbek Scientific Research Institute Of Chemistry And Pharmaceutical” N. A. Sultanova, Tashkent.

The leaves of the nettle, the mountain pepper and the bird were obtained from the Pharmacognosy Department of Chemistry of the Tashkent Pharmaceutical Institute, Tashkent.

For the execution of this work, the following instruments and equipment were used:

Laboratory balance VLTE-150, II class of accuracy according to GOST 24104-2001.

Hand scales VR-20.

Thermostat TS-80M-2

Drying cabinet 31

Extractor

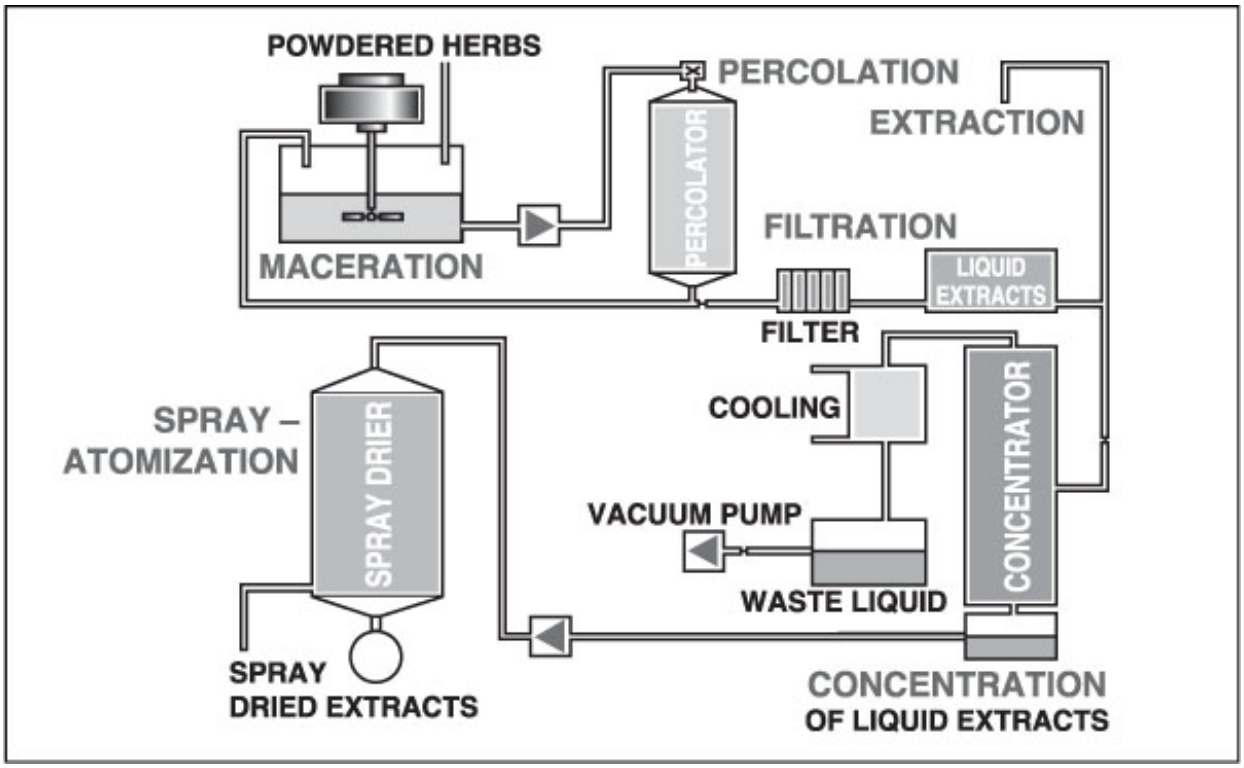
CHAPTER 3. DEVELOPMENT OF THE TECHNOLOGY FOR OBTAINING EXTRACT AND TINCTURE

3.1. The choice of extractant to obtain tinctures and extracts.

We received the original medicinal product in the form of a liquid extract. The extract was obtained by maceration. The raw material for obtaining the liquid extract "Hemostav" was medicinal plants such as nettle leaves, the herb urtica dioica and persicaria hydropiper and the flowers of marigold. According to the literature data used medicinal plants contain such biologically active substances (BAS) as tannins, flavonoids, alkaloids, essential oils, protein substances, vitamins C, K1, B1, B2, macro and microelements, etc. [1,2,3]. Preliminary experiments have shown that medicinal product has capillary-strengthening, hemostatic and anti-inflammatory activity [4].

Picture 5

Scheme 1.



CHAPTER 4. STUDY OF BIOLOGICAL ACTIVE SUBSTANCES IN RECEIVED MEDICINAL FORMS

4.1. Studies of flavonoids in liquid extract and tincture

Table 1.

Comparative table of pharmacopoeia on standardization of extract and tincture

EXTRACTS / TINCTURES	State Pharmacopoeia of the Russian Federation XIII	British Pharmacopoeia 2013	United States Pharmacopeia 2015
Definition	**Extracts** are concentrated extracts from medicinal raw materials, less often from raw materials of animal origin. **Tinctures** are a liquid dosage form, which is usually colored alcoholic or hydroalcoholic extracts obtained from medicinal plant raw materials (dried or fresh), as well as from raw materials of animal origin without heating and removing the extractant.	**Extracts** are preparations of liquid (liquid extracts and tinctures), semi-solid (soft extracts and oleoresins) or solid (dry extracts) consistency, obtained from herbal drugs or animal matter, which are usually in a dry state. **Tinctures** are liquid preparations that are usually obtained using either 1 part of herbal drug or animal matter and 10 parts of extraction solvent, or 1 part of herbal drug or animal matter and 5 parts of extraction solvent.	Extracts may be defined as preparations with liquid, solid, or semisolid consistency. The products obtained by extraction are fluidextracts, powdered extracts, semisolid extracts, and tinctures.

Types	**EXTRACTS** Consistency: - Extracts dry (extracta sicca) - powdery masses, having the property of flowability, with a moisture content of not more than 5%; - Extracts thick (extracta spissa) - viscous mass with a moisture content of not more than 25%; - Extracts liquid (extracta fluida) - thick, mobile, sometimes oily liquids. Extracts-concentrates are extracts of different consistency, standardized with respect to medicinal plant raw materials in certain proportions. They are used mainly to obtain infusions and decoctions, replacing in specified ratios of medicinal plant raw materials. **The extractant used is distinguished:** - water extracts obtained using purified water as an extractant; - alcohol extracts, obtained with the	Different types of extract may be distinguished. Standardised extracts are adjusted within an acceptable tolerance to a given content of constituents with known therapeutic activity; standardisation is achieved by adjustment of the extract with inert material or by blending batches of extracts. Quantified extracts are adjusted to a defined range of constituents; adjustments are made by blending batches of extracts. Other extracts are essentially defined by their production process (state of the herbal drug or animal matter to be extracted, solvent, extraction conditions) and their specifications.	**FLUIDEXTRACTS,** also known as liquid extracts, are preparations of plant matter, containing alcohol as a solvent or as a preservative, or both, and are so made that each mL contains the extracted constituents of 1 g of the crude material that it represents, unless otherwise specified in the individual monograph. They may be prepared from suitable extracts and may contain suitable antimicrobial or other preservatives. Pharmacopeial fluidextracts are made by percolation, often following a period of maceration. The required solvent is specified in the individual monograph. The common manufacturing procedure includes concentration of the more diluted portion of percolate by evaporation or distillation under vacuum at temperatures below 60°. The time of

	use of ethyl alcohol of different concentrations as an extractant; - oil extracts obtained with the use of vegetable oil as an extractant; - Extracts obtained using various organic solvents (carbon tetrachloride, dichloroethane, etc.); - Extracts obtained by sequential extraction of medicinal plant raw material by extractants, including different polarities. **TINCTURES** Tinctures are divided into simple, based on one type of medicinal plant material, and complex (complex) - from a mixture of several types of medicinal raw materials.		maceration and the rate of flow during percolation may be varied to adjust for the quantity and nature of the crude material under extraction, provided that the composition of the extracted constituents of interest is not adversely affected. The rate of flow of the percolate can be slow, moderate, or rapid. With reference to the extraction of 1000 g of the starting material, at a slow rate, not more than 1 mL of percolate is produced per minute; at a moderate rate, between 1 and 3 mL per minute is produced; and at a rapid rate, between 3 and 5 mL per minute is produced. A fluidextract that tends to deposit sediment may be aged and filtered, or the clear portion may be decanted, provided that the resulting clarified liquid conforms to the Pharmacopeial standards.

			POWDERED EXTRACTS are solid preparations having a powdery consistency obtained by evaporation of the solvent used for extraction. They may contain suitable added substances such as excipients, stabilizers, and preservatives. Standardized powdered extracts are adjusted to the defined content of constituents, using suitable inert materials or a powdered extract of the plant matter used for preparation. Where applicable, a limit for the solvent used for extraction is specified in the individual monograph. **SEMISOLID EXTRACTS,** also known as soft extracts or pillular extracts, are preparations having consistencies between those of fluidextracts and those of powdered extracts, and are obtained by partial evaporation of the solvent,

			water, alcohol, or hydroalcoholic mixtures being used as extracting solvents. They may contain suitable antimicrobial or other preservatives. A semisolid extract and a powdered extract obtained from the same material are interchangeable as drugs or as supplements, but each has its own advantages.
Methods of extraction/Features of technology/Production	**EXTRACTS** Extracts can be obtained by percolation, repercolation, maceration, circulation extraction, and other suitable validated methods. Liquid extracts after completion of the extraction process should be surely maintained at a temperature of 8 - 10 ° C for at least 2 days to precipitate the ballast substances	**EXTRACTS** Extracts are prepared by suitable methods using ethanol or other suitable solvents. Different batches of the herbal drug or animal matter may be blended prior to extraction. The herbal drug or animal matter to be extracted may undergo a preliminary treatment, for example, inactivation of	**EXTRACTS** In the extraction practice for articles of botanical origin, the constituents of interest are completely or partially separated from other components with the aid of water, alcohol, alcohol-water mixtures, or other suitable solvents. This extraction process involves the removal

	that are separated by filtration and to obtain a clear liquid. In the preparation of dry extracts and thick they are released from dietary fiber by adding alcohol to the resulting extract ethyl, adsorbents boiling hoods and other conventional methods, followed by filtration. The purified extracts are concentrated by evaporation under vacuum to the desired consistency (thick extracts). Dry extracts are obtained by drying thick extracts or directly from purified extracts using methods that ensure maximum preservation of active substances: spraying, lyophilization, sublimation, etc. When extracting concentrate extracts, they are diluted to the desired active ingredient content using dextrin and other excipients.	enzymes, grinding or defatting. In addition, unwanted matter may be removed after extraction. Herbal drugs, animal matter and organic solvents used for the preparation of extracts comply with any relevant monograph of the Pharmacopoeia. For soft and dry extracts where the organic solvent is removed by evaporation, recovered or recycled solvent may be used, provided that the recovery procedures are controlled and monitored to ensure that solvents meet appropriate standards before re-use or admixture with other approved materials. Water used for the preparation of extracts is of a suitable quality. Potable water	of the desired constituents from the plant matter with suitable menstrua, the evaporation of all or nearly all of the solvent, and the adjustment of the residual fluids, masses, or powders to the prescribed standards. Suitable inert substances may be added as carriers or diluents to improve physical characteristics. Suitable antimicrobials and other preservatives may be added to preserve the integrity. Extracts may be subjected to processes that increase the content of characterized constituents, decrease the content of unwanted constituents, or both. Extracts with no added inert substances and no processing beyond the

	Hygroscopicity of dry extracts is reduced by adding lactose, aerosil and other auxiliary substances to them. **TINCTURES** Tinctures are obtained by maceration, percolation or other validated method, using ethyl alcohol as an extractant in the required concentration. From one mass part of the medicinal plant raw material, 5 parts of the tincture are obtained. From one mass portion of the medicinal plant raw material containing alkaloids and cardiac glycosides, 10 parts by volume of the tincture, unless otherwise indicated in the pharmacopeial article or regulatory documentation. After completion of the extraction process, tinctures are set at a temperature of no higher than 8-10 ° C for at least 2 days until a clear liquid is obtained and filtered. In the process of storing a number of tinctures, mainly complex ones, the formation of an insignificant	may be suitable if it complies with a defined specification that allows the consistent production of a suitable extract. Where applicable, concentration to the intended consistency is carried out using suitable methods, usually under reduced pressure and at a temperature at which deterioration of the constituents is reduced to a minimum. Essential oils that have been separated during processing may be restored to the extracts at an appropriate stage in the manufacturing process. Suitable excipients may be added at various stages of the manufacturing process, for example to improve technological qualities such as homogeneity or consistency. Suitable stabilisers and antimicrobial preservatives may also be added. Liquid extracts are prepared by using ethanol of a suitable	extraction are called native extracts. In some preparations, the plant matter may be pretreated by inactivation of enzymes and microbial contaminants, grinding, defatting, or a similar procedure. **METHODS OF EXTRACTION** **Percolation -** In the manufacture of extracts, percolation is a commonly used method. The crude material being extracted is reduced to pieces of suitable size, if necessary, then mixed thoroughly with a portion of the specified solvent, and allowed to stand for about 15 minutes. The mixture is transferred to a percolator, sufficient amount of the specified solvent is added to cover the entire solid mass, and the mixture is allowed to percolate slowly (at a rate of not more than 1 mL per

	sediment of ballast substances is allowed, provided that there are no biologically active substances in which standardization is carried out. Tinctures can be used as medicinal herbal preparations for internal or external use or be part of other medications, for example, elixirs, drops for ingestion, etc.	concentration or water to extract the herbal drug or animal matter, or by dissolving a soft or dry extract (which has been produced using the same strength of extraction solvent as is used in preparing the liquid extract by direct extraction) of the herbal drug or animal matter in either ethanol of a suitable concentration or water. Liquid extracts may be filtered, if necessary. **TINCTURES** Tinctures are prepared by maceration or percolation (outline methodology is given below) using only ethanol of a suitable concentration for extraction of the herbal drug or animal matter, or by dissolving a soft or dry extract (which has been produced using the same strength of extraction solvent as is used in preparing the tincture by direct extraction) of the herbal drug or animal matter in ethanol of a suitable	minute for 1000 g of material), the matter to be extracted being always covered with a layer of solvent. The residue may be pressed, and the obtained fluid is combined with the percolate. The entire percolates are concentrated, generally by distillation under reduced pressure, so as to subject the constituents of interest in the article under extraction to as little heat as possible. **Maceration** - Unless otherwise specified, the crude material being extracted is reduced to pieces of suitable size, mixed thoroughly with the specified extracting solvent, and allowed to stand at room temperature in a closed container for an appropriate time, with frequent agitation until soluble matter is dissolved. The mixture is filtered, the insoluble material is washed

		concentration. Tinctures are filtered, if necessary. Tinctures are usually clear. A slight sediment may form on standing, which is acceptable as long as the composition of the tincture is not changed significantly. **Production by maceration** Unless otherwise prescribed, reduce the herbal drug or animal matter to be extracted to pieces of suitable size, mix thoroughly with the prescribed extraction solvent and allow to stand in a closed container for an appropriate time. The residue is separated from the extraction solvent and, if necessary, pressed out. In the latter case, the 2 liquids obtained are combined. **Production by percolation** If necessary, reduce the herbal drug or animal matter to be extracted to pieces of suitable size. Mix thoroughly with a portion of the prescribed	with the same solvent used for maceration, and the filtrates are combined and concentrated, usually under reduced pressure, to the desired consistency. **TINCTURES** TINCTURES are liquid preparations usually prepared by extracting plant materials with alcohol or hydroalcoholic mixtures. Traditionally, tinctures of potent articles of botanical origin represent the activity of 10 g of the drug in each 100 mL of tincture, the strength being adjusted following the test for content of active principles or marker compounds. Most other plant tinctures represent 20 g of the respective plant material in each 100 mL of tincture. Different tinctures are not always diluted to obtain the same ratio of starting plant material to final tincture. This ratio will depend on the

		extraction solvent and allow to stand for an appropriate time. Transfer to a percolator and allow the percolate to flow at room temperature slowly making sure that the herbal drug or animal matter to be extracted is always covered with the remaining extraction solvent. The residue may be pressed out and the expressed liquid combined with the percolate.	requirements prescribed in the specific tests for content of active principles or marker compounds included in the individual monographs. As tinctures are being prepared, they are assayed in accordance with these content tests. Using the values obtained from such assays, the final concentration of a tincture is adjusted by adding more solvent or by evaporating part of the solvent. Unless otherwise specified, tinctures are usually prepared from coarse powder or fine cuttings of plant materials either by a percolation process or a maceration process.

General Pharmacopeial Requirements	**Appearance**	**EXTRACTS** Indicate the color and odor of the extract, if any. If necessary, for liquid extracts, the presence of opalescence, the possibility of precipitate formation during storage, etc. **TINCTURES** Tinctures must correspond in appearance and smell to the requirements of a pharmacopeial article or regulatory documentation.		
	Solubility	**EXTRACTS** If provided by a pharmacopeial article or regulatory documentation, for oil extracts, solubility is determined in accordance with the requirements of the FSF "Solubility".		

	Relative density	**EXTRACTS** For oil extracts, the density is determined in accordance with the requirements of the FPD "Density". **TINCTURES** The determination is made if it is stipulated by a pharmacopeia article or normative documentation in accordance with the requirements of the DFS "Density". The density should correspond to the limits specified in the pharmacopeia article or regulatory documentation.	Where applicable, the liquid extract/tinctures complies with the limits prescribed in the monograph.	
	Water		The oleoresin (semi-solid extracts)/ dry extract complies with the limits prescribed in the monograph.	
	Refractive index	**EXTRACTS** If provided by the pharmacopoeial article or normative documentation, the oil extracts are determined by the refractive index in accordance with the requirements of the FF Refractometry.		

	Ethanol	**EXTRACTS** For alcohol-containing extracts, the definition of ethyl alcohol is carried out in accordance with the requirements of the CFA "Determination of ethyl alcohol in liquid pharmaceutical preparations". **TINCTURES** The test is carried out in accordance with the CFC "Determination of ethyl alcohol in liquid pharmaceutical preparations", unless otherwise specified in the pharmacopeia article or regulatory documentation. The value of the ethyl alcohol content should be indicated in percentages and correspond to the limits established in the pharmacopeia article or normative documentation.	For alcoholic liquid extracts//tinctures, carry out the determination of ethanol content. The ethanol content complies with that prescribed.	Between 90% and 110% of the labeled amount of C_2H_5OH is found in Fluidextract and Semisolid Extract.

	Methanol and 2-propanol	**TINCTURES** In tinctures, the content is not more than 0.05% methanol and not more than 0.05% 2-propanol, unless otherwise specified in the pharmacopeia article or regulatory documentation. The determination is carried out by gas chromatography in accordance with the OPS "Residual organic solvents".	Maximum 0,05 per cent V/V of methanol and maximum 0,05 per cent V/V of 2-propanol for alcoholic liquid extracts, unless otherwise prescribed.	
	Particle-size distribution	**EXTRACTS** Dry extracts are controlled according to the "Granulometric composition" parameter in accordance with the requirements of the FBS "Sieve analysis". Norms are given in the relevant pharmacopoeial articles or regulatory documents.		

	Heavy metals	**EXTRACTS** All extracts must withstand the requirements for the content of heavy metals - not more than 0.01%, unless otherwise provided for by a pharmacopeia article or regulatory documentation. The determination is made in accordance with the requirements of the FEA "Heavy Metals". **TINCTURES** 10 ml of the tincture is evaporated in a porcelain dish to dryness in a water bath, 1 ml of sulfuric acid is concentrated, carefully burned and calcined at a temperature of 600 ° C. To the obtained residue, 5 ml of a saturated solution of ammonium acetate are added under heating, filtered through an ashless filter, washed with 5 ml of water and the filtrate is brought to 100 ml with water; 10 ml of the obtained solution must withstand the tests for heavy metals (FEA "Heavy metals", method 1). The permissible content of heavy metals should not exceed 0.001%.		20 mg per g (Method II)

	Acid, peroxide, iodine, saponification value	**EXTRACTS** If provided by the pharmacopoeial article or regulatory documentation, for the oil extracts, the acid number, peroxide number, iodine number, saponification number is determined in accordance with the requirements of the corresponding FBS.		
	Pesticide Residues			Proceed as directed under Articles of Botanical Origin: meets the requirements.
	Residual organic solvents	**EXTRACTS** In the case of using organic solvents in the production of extracts, their residual content is monitored in accordance with the requirements of the CFA "Residual Organic Solvents".	Where applicable, the soft extract/oleoresins/dry extract complies with the limits prescribed in the monograph.	If prepared with solvents other than alcohol, water, or alcohol-water mixtures, it meets the requirements for Residual Solvents.

	Residue on Evaporation			Transfer promptly about 2 mL, accurately measured, of Fluidextract, about 0.5 g of Powdered Extract, or about 2 g of Semisolid Extract to a suitable tared, round-bottom flask. Evaporate to dryness on a water bath, and dry the residue at 100° to 105° for 3 hours. Allow to cool in a desiccator over phosphorus pentoxide, and determine the weight of the residue obtained: not less than 95% of Powdered Extract specimen remains as residue; or not less than 70% of Semisolid Extract specimen remains as residue.
	Loss on drying	**EXTRACTS** For extracts of dry and thick, the loss in weight during drying is determined in accordance with the requirements of the FSC "Loss in mass during drying".	Where applicable, the dry extract complies with the limits prescribed in the monograph.	

	Dry residue	**EXTRACTS** For liquid extracts, the dry residue is determined by the following procedure: 5.0 ml of the liquid extract is placed in a suspended pump, evaporated in a water bath and dried for 3 hours at (102.5 ± 2.5) ° C, then cooled in a desiccator for 30 min and weighed. The dry residue content must meet the requirements given in the pharmacopeia article or regulatory documentation. **TINCTURES** 5.0 ml of the tincture is placed in a pre-dried at a temperature of 100 - 105 ° C to constant weight and a precisely weighed porcelain cup with a diameter of 5 cm or a bug, weighed to the nearest 0.0001 g, evaporated in a water bath to dryness, dried in an oven in for 2 hours at a temperature of (102.5 ± 2.5) ° C, cool in a desiccator (over anhydrous silica gel, calcium chloride anhydrous or other suitable desiccant) for 30 minutes and weigh. The result is expressed	Where applicable, the liquid/soft extract/tinctures complies with the limits prescribed in the monograph, corrected if necessary, taking into account any excipient used.	

		as a percentage. The content of the dry residue must correspond to the limits specified in the pharmacopoeial article or normative documentation.		
	Assay	**TINCTURES** The content of active compounds or biological activity is determined using validated procedures and expressed as a percentage or U / mL.		
	Packaging, labeling and storage	Packaging. In accordance with the requirements of the FSC "Medicinal forms". In a package providing protection from light, unless otherwise provided by a pharmacopeia article or regulatory documentation (Extracts). In accordance with the requirements of the CFS "Medicinal forms", in bottles of orange glass (Tinctures). Marking. In accordance with the requirements of the FSC "Medicinal forms". For liquid extracts, if there is the possibility of formation (during storage) of the sediment, the label indicates	**Labelling.** The label states: — the herbal drug or animal matter used; — whether the extract is liquid, soft or dry, or whether it is a tincture; — for standardised extracts, the content of constituents with known therapeutic activity; — for quantified extracts, the content of constituents (markers) used for quantification; — the ratio of the starting material to the genuine extract (extract without excipients)	**Labeling.** Label it to indicate the name of the plant part used; the names of solvents, other than the hydroalcoholic solvents, used in preparation; the content, in percentage, of active principles or marker compounds identified in the individual monograph; and the name and concentration of any added antimicrobial or other preservative. Where active principles are unknown, the ratio of starting material to final product is stated. For

		"Possible formation of sediment", "Shake before use" (Extracts). In accordance with the requirements of the FSC "Medicinal forms". On the packaging indicate the amount of feedstock in grams and the amount of ethyl alcohol of the specified concentration, sufficient to obtain 1 liter of tincture (Tincture). Storage. In accordance with the requirements of the CFS "Storage of medicines". In the dark place at a temperature of 15 to 25 ° C, unless otherwise indicated in the pharmacopeia or regulatory documents (Extracts). In accordance with the requirements of the CFS "Storage of medicines". Store in a dark place at a temperature of 15 to 25 ° C, unless otherwise indicated in the pharmacopeial article or regulatory documentation (Tinctures).	(DER); — the solvent or solvents used for extraction; — where applicable, that a fresh herbal drug or fresh animal matter has been used; — where applicable, that the extract is 'refined'; — the name and amount of any excipient used including stabilisers and antimicrobial preservatives; — where applicable, the percentage of dry residue. **Storage.** Protected from light (all extracts). In an airtight container (oleoresins, dry extracts)	semisolid extracts and powdered extracts, the identity and quantity of any added excipient is also indicated. In such cases the percentage of native extract may also be stated. **Storage.** Store in tight, light-resistant containers.

Table 2.

The results obtained extract and tinctures

№	Parameters	Extract	Tincture
1	Description	The liquid is green-brown with a fragrant smell, bitter-astringent taste	The liquid is green-brown with a fragrant smell, bitter-astringent taste
2	Heavy metals	Not more than 0,01%	Not more than 0,01%
3	Density	0,9067	0,9053
4	Dry residue	0,1302	0,1406
5	Quantitative determination of ethyl alcohol	69,2%.	72,6%.
6	pH	6,5	6,7

4.2. Studies of macro-microelement composition .

ICP mass spectral analysis.

Mass spectrometric analysis - using an inductively coupled plasma mass spectrometer ICP MS (inductively coupled plasma mass-spectrometer) allows qualitative and quantitative elemental analysis of substances, determining the concentration of elements.

The sample is fed to the mass spectrometer at a rate of ~ 1 ml / min using a peristaltic pump in a nebulizer. The particles of the sputtered sample enter the central channel of the inductively coupled plasma, where they evaporate and decay into atoms. Ions from the plasma through a series of cones fall into the mass detector, where they are separated based on the mass-to-charge ratio. The mass detector receives a signal proportional to the particle concentration with such a ratio. Concentration can be determined by calibration using multielement standards.

ISP-MS analysis allows to determine elements with atomic mass from 7 to 250, that is, from Li to U.

Sample preparation: to determine trace amounts of heavy metals, 1 ml was taken from the object to a 10 ml volumetric flask, brought to the mark with bidistilled water, and then used for direct injection into the spray chamber of the device (inductively coupled plasma mass spectrometer) Agilent Technology 7500.

Instrument parameters: plasma power 1200 W, integration time 0.1 s, rotation speed of peristaltic pump 0.1 r / s. The remaining parameters of the instrument are set during the adjustment and remain unchanged during the period between maintenance periods. As a standard, a multielement (27 component) standard solution of Agilent Technology was used with the content of the target components 10.0 mg / l.

EKSTRAKT

File Name : 1.D
Acq Time : Jun 30 2017 02:31 pm

	Mass	Conc.	Counts(CPS)	Bkg count	Time(sec)
Li	7	2.500 mg/l	454,720.6	---	0.1
Be	9	0.01800 mg/l	473.3333	---	0.1
B	11	7.500 mg/l	167,461.5	---	0.1
Na	23	360.0 mg/l	23,743,550	---	0.1
Mg	24	4.800 mg/l	178,815.6	---	0.1
HYDRIDE					
Al	27	0.1900 mg/l	14,213.33	---	0.1
Si	29	68.00 mg/l	457,126.5	---	0.1
P	31	5.700 mg/l	18,750.00	---	0.1
S	34	3.600 mg/l	11,590.00	---	0.1
K	39	340.0 mg/l	22,594,020	---	0.1
Ca	43	6.000 mg/l	1,733.333	---	0.1
Sc	45	0.1500 mg/l	11,223.33	---	0.1
Ti	47	0.4700 mg/l	2,356.667	---	0.1
V	51	0.03000 mg/l	1,553.333	---	0.1
Cr	53	0.7700 mg/l	4,286.667	---	0.1
Mn	55	0.1500 mg/l	7,106.667	---	0.1
Fe	57	2.800 mg/l	3,626.667	---	0.1
Co	59	0.005200 mg/l	220.0000	---	0.1
Ni	60	0.08500 mg/l	696.6666	---	0.1
Cu	63	1.600 mg/l	34,826.67	---	0.1
Zn	66	0.5700 mg/l	2,420.000	---	0.1
As	75	0.07700 mg/l	396.6667	---	0.1
Se	82	<0.1100 mg/l	23.33333	---	0.1
Br	79	1.600 mg/l	1,276.667	---	0.1
ARGIDE					
Rb	85	2.000 mg/l	105,050.0	---	0.1
Sr	88	0.02900 mg/l	1,756.667	---	0.1
Y	89	<6.500E-4 mg/l	36.66667	---	0.1
Zr	90	<1.300E-3 mg/l	23.33333	---	0.1

Nb	93	<7.500E-4 mg/l	3.333333	---	0.1
Mo	95	0.007200 mg/l	83.33333	---	0.1
Rh	103	<7.200E-4 mg/l	26.66667	---	0.1
Pd	105	<4.100E-3 mg/l	40.00000	---	0.1
Ag	107	0.009800 mg/l	273.3333	---	0.1
Cd	111	<0.01300 mg/l	23.33333	---	0.1
In	115	<1.100E-3 mg/l	3.333333	---	0.1
Sn	118	<3.800E-3 mg/l	33.33333	---	0.1
Sb	121	<2.900E-3 mg/l	16.66667	---	0.1
I	127	0.07200 mg/l	1,060.000	---	0.1
Cs	133	0.6100 mg/l	51,530.00	---	0.1
Ba	137	0.01300 mg/l	140.0000	---	0.1
W	182	<1.700E-3 mg/l	6.666667	---	0.1
Ir	193	<9.200E-4 mg/l	0.0000000	---	0.1
Pt	195	<2.100E-3 mg/l	16.66667	---	0.1
Au	197	<1.500E-3 mg/l	46.66666	---	0.1
Hg	202	0.00100 mg/l	283.333	---	0.1
Tl	205	<8.300E-4 mg/l	13.33333	---	0.1
Pb	208	0.01500 mg/l	666.6666	---	0.1
Bi	209	<7.500E-4 mg/l	16.66667	---	0.1
U	238	<4.400E-4 mg/l	46.66666	---	0.1

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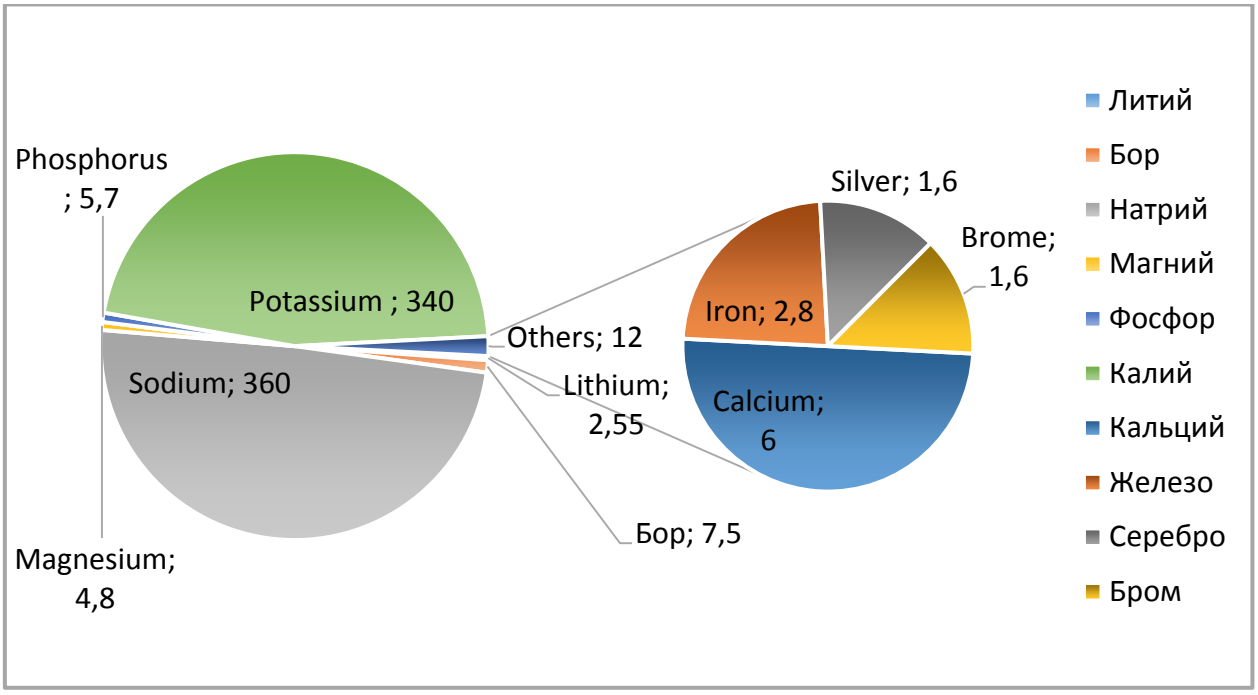
Diagram 1.



4.3. Determination of alcohol by the method of gas liquid chromatography

Chromatography was performed under the following conditions: DB-624 Capillary 30.0m x 250µm x 1.40µm nominal; the temperature of the stove from 40 ° C to 120 ° C, the duration of the analysis is 8.0 min, injection 1µl, split ratio 50: 1, the injector temperature is 120 ° C; temperature of the detector is 260 ° C;

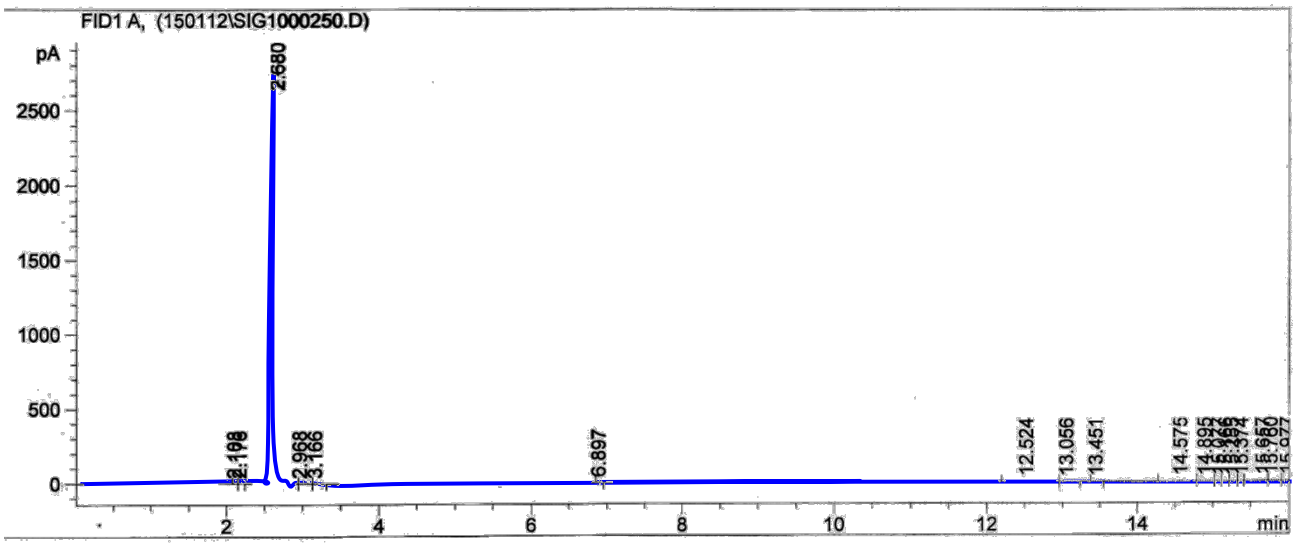
mobile phase-0.8 ml / min helium (He); detector-flame ionization (FID); air and hydrogen velocity of 450 ml / min and 40.0 ml / min, respectively.

Preparation of the test solution: 10 ml of the test extract is pipetted into a 100 ml volumetric flask and the volume is adjusted to the mark with purified water and mixed. The solution is filtered through a membrane filter with a size of 0.45 mkm..

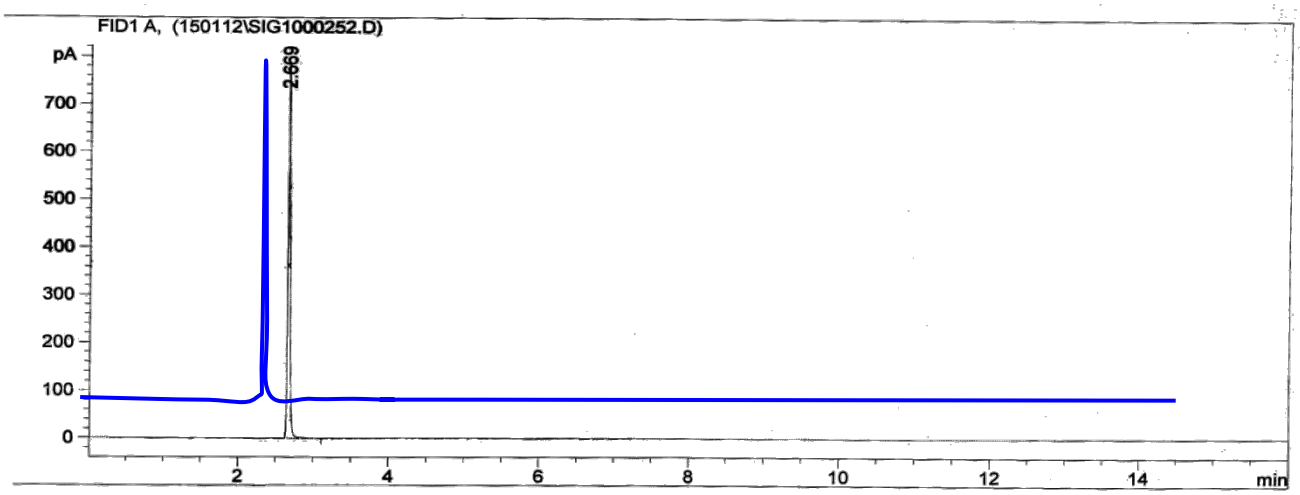
Preparation of a 70% solution of a standard sample (PCO) of ethanol. To do this, 66.5 g (exact sample) of 99% ethyl alcohol is placed in a 100 ml volumetric flask, the volume is adjusted to the mark with purified distilled water and the solution No. 1 is mixed. 10 ml solution No. 1 is placed in a 100 ml volumetric flask, the volume is adjusted to the mark with purified water and mixed.

The identification of ethyl alcohol on the chromatograms of the test sample was carried out by comparing the retention times of the PCO. Alternately, 1.0 mkm of the test solution and the ethanol solution of PCO was chromatographed, obtaining not less than 3 chromatograms for each of the solutions.

The reliability of the results of the analysis was checked by determining the suitability of the chromatographic system for: -resolution of (R) alcohol spirits of ethyl (not less than 2.0); -the asymmetry coefficient (T) of the peak of ethyl alcohol (does not exceed 2.0); -relative standard deviation (RSD) (does not exceed 2.0%). The identification of ethyl alcohol on the chromatograms of the test samples was carried out by comparing the retention times of a standard sample. The results of the studies are presented in Pic. 6-7.



Pic.6. Chromatogram of standard sample of ethyl alcohol



Pic.7. Chromatogram of the extract "Gemostav"

On the chromatogram peak of retention time 2,68 min with PCO of ethyl alcohol with retention time 3,66. Quantity of ethyl alcohol in the extract «Hemostat» calculated by the formula

$$X = \frac{H_1 \bullet m_0 \bullet 10 \bullet 100 \bullet P}{H_0 \bullet 100 \bullet 100 \bullet V}$$

where H_1-height peak of ethyl alcohol from the chromatogram of the test solution; H_0-height peak of ethyl alcohol from the chromatogram of PCO solution of ethanol; m_0-mass 96% ethyl alcohol in grams; V- volume of test solution used for the analysis , in milliliters; P- volume 70% ethyl alcohol, for the preparation of PCO solution of ethanol, in percentage.

As a results of researches revealed, that quantitative content of ethyl alcohol in the extract «Hemostat» is not less than 69,2%.

Conclusion by chapter:

Phytoextract "Gemostav" contains all the necessary macro and microelements, among which the greatest amount are: sodium, potassium, magnesium, calcium and iron. It should be noted that the above elements refer to essential, that is, vital - essential elements.

High content of mineral substances allows to expand the spectrum of application of alcohol phytoextract and testifies to the prospects of its use in medicine.

As a result of the studies, GLC conditions were found for the analysis of ethyl alcohol in the liquid extract "Hemostav" which makes it possible not only to identify the latest, but also to determine the quantitative content, and also to evaluate its purity. The data obtained in the future will serve as the basis for the drafting of the VFS.

CHAPTER 5. STANDARDIZING LIQUID EXTRACTS AND TINCTURES

5.1. Development of a technique for the qualitative analysis of flavonoids by the TLC method.

The technique of qualitative and quantitative determination of flavonoids in liquid extract "Hemostav" is developed. For the identification of flavonoids, the TLC method and for quantitative determination the spectrophotometry method.

Till the date, the technology of manufacturing phytopreparations is an interesting direction of modern pharmacy. Particularly significant is the component associated with the technology of the appropriate dosage forms. Medicinal forms based on medicinal plant as a raw materials are less toxic in comparison with their synthetic analogs, together they have a wide pharmacotherapeutic spectrum of action. Native substances of medicinal plants are more gently mixes in the natural processes of the human body, which leads to a reduction in possible allergic reactions and side effects.

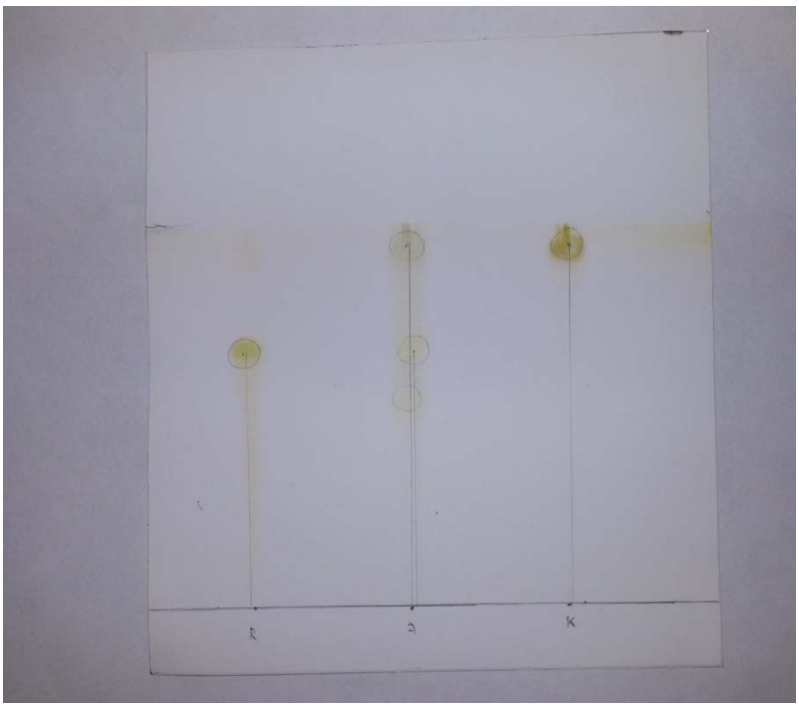
Expanding the range of domestic medicines, especially with a guaranteed raw material base, is one of the promising areas of the pharmaceutical science of the Republic of Uzbekistan.

To optimize the method for producing the above mentioned liquid extract, standardizing the preparation of obtaining, it is very important to develop methods for the qualitative and quantitative determination of bioflavonoids. Which is simple, uncomplicated and has good accuracy and reproducibility.

The purpose of this work was to develop a methodology for the qualitative and quantitative determination of flavonoids, macro- and microelements in a new product.

Experimental part.

Authenticity was determined by chemical reactions to flavonoids and also by TLC. 0.02 ml of the drug is applied to a plate of "Silufol" (15x15 cm). The plate with the applied sample is air-dried, placed in a chamber with a butanol-acetic acid-water solvent mixture (4: 1: 1) and chromatographed in an ascending manner.



Picture 8. Result TLC chromatography

When developing with ammonia vapors or spraying a solution of vanillin in concentrated sulfuric acid, two spots should appear (Rf about 0.48, and 0.68). These spots were identified as quercetin and rutin. We used a lot of a solvent mixture for TLC chromatography.

Table 3.

List of used systems for TLC chromatography

#	Name solvent mixture	Ratio	Result
1	Butanol-acetic acid-water solvent mixture	4: 1: 1	Rf rutin = 0.48 Rf quercetin =0.68 Rf x = 0.37
2	Butanol-acetic acid-water solvent mixture	4: 1: 5	Rf rutin = 0.03 Rf quercetin =0.2
3	Butanol-acetic acid-water solvent mixture	4: 5: 5	Rf rutin = 0.02 Rf quercetin =0.04
4	Xroform – ethanol	2:5	Rf rutin = 0.07 Rf quercetin =0.1

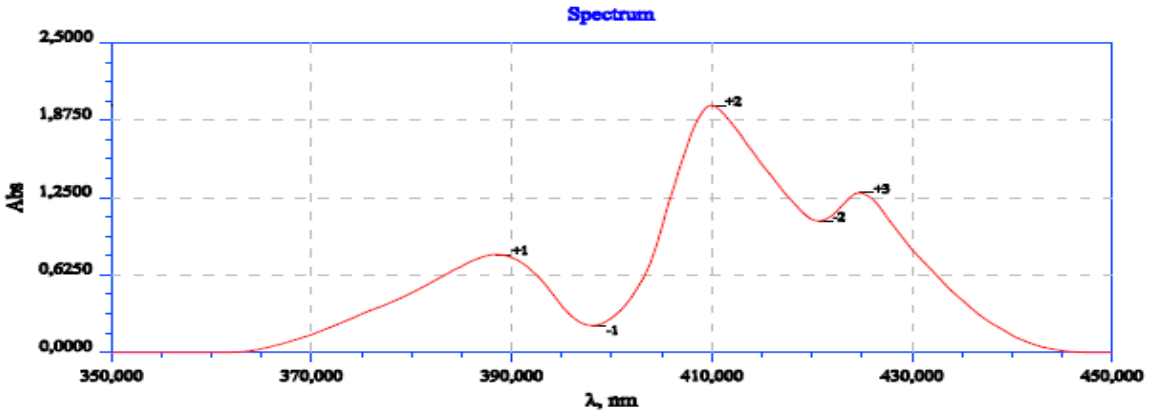
5.2. Development of a methodology for the quantitative determination of flavonoids by the SF method

The quantitative content of the sum of flavonoids in the liquid extract was determined by the spectrophotometric method in terms of the routine, based on the reaction of complexation with aluminum chloride.

The following techniques were used to determine the amount of flavonoids:

1) 1.0 ml of the drug is placed in a 25 ml volumetric flask, than 5 ml 96% ethanol, 5 ml of a 2% solution of aluminum chloride is added, one drop, acetic diluted acids and adjusted to 70% with a solution of ethanol. The solution is stirred and stirred in a dark place. After 30 minutes immediately measured and optical density of the resulting solution on a spectrophotometer at a wavelength of 408 nm in a cuvette with a layer thickness of 10 mm. As a reference solution, a solution prepared in the same manner is used, but without adding an aluminum chloride solution. In parallel, the optical density of the routine solution of a standard sample prepared similarly to the test is measured.

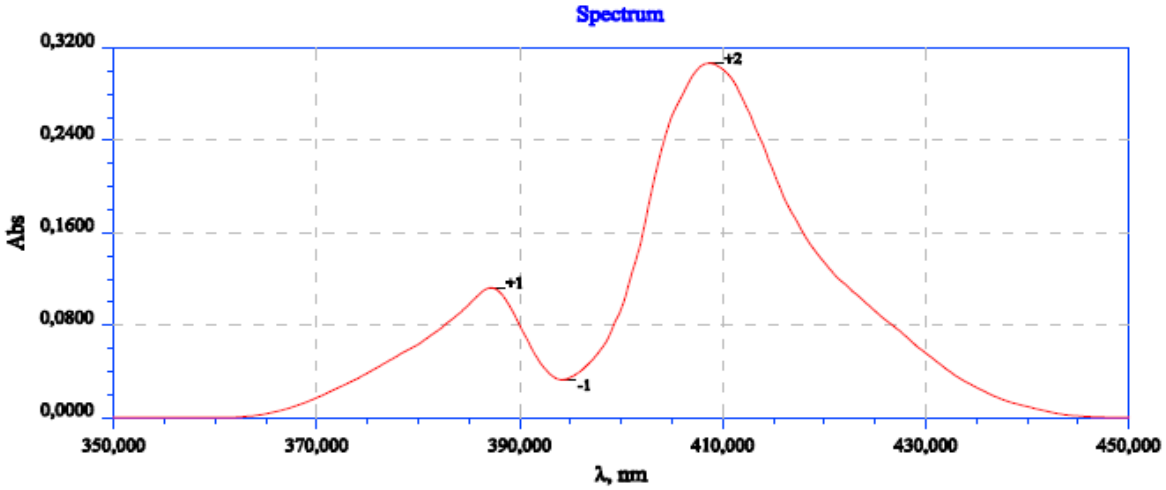
Operator: Саида Умурзакова
Sample : Испытуемый раствор
Date/time: 15.01.2017 17:24:39



peaks	λ, nm	Abs
+1	388,412	0,7911
-1	398,171	0,2191
+2	409,941	1,9958
-2	420,712	1,0634
+3	424,789	1,2884

Picture 9. UV spectrum of liquid extract "Gemostav"

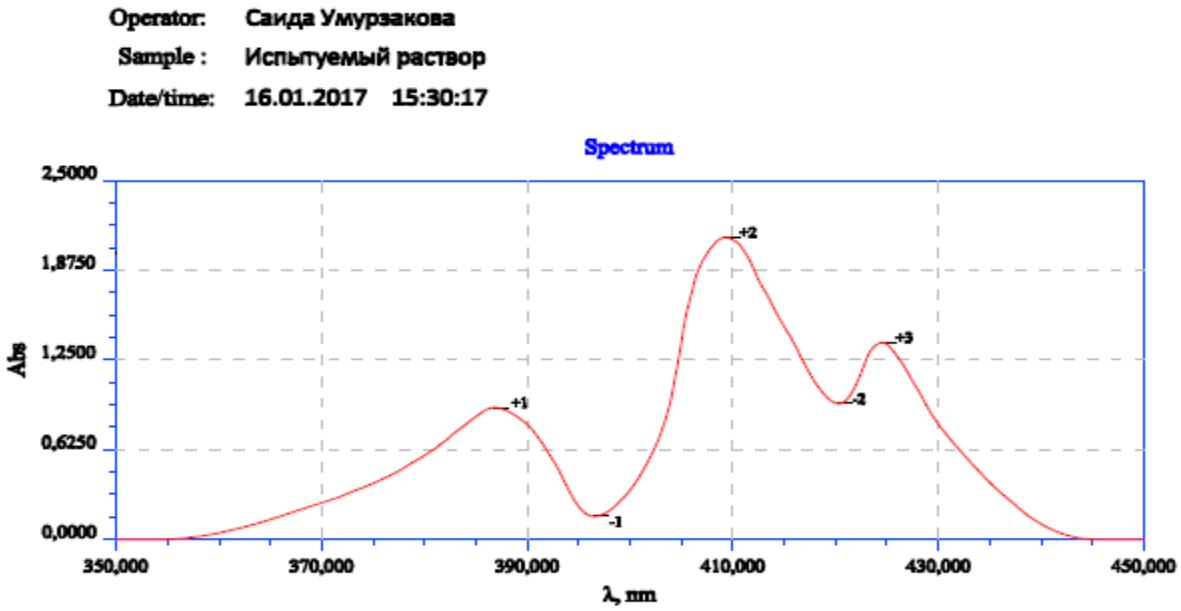
Operator: Саида Умурзакова
Sample: PCO
Date/time: 15.01.2017 17:22:46



peaks	λ, nm	Abs
+1	387,171	0,1124
-1	394,124	0,0327
+2	408,688	0,3068

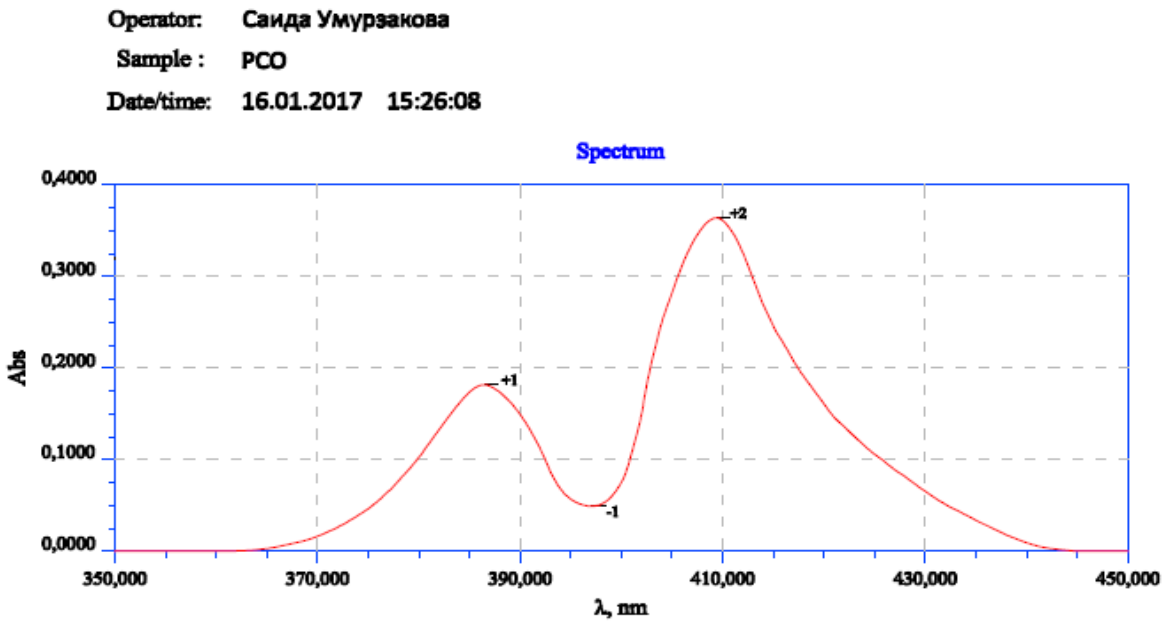
Picture 10. UV spectrum of RSO.

2) 1.0 ml of the drug is placed in a 25 ml volumetric flask, than 5 ml 96% ethanol, 5 ml of a 2% solution of aluminum chloride is added, 2 ml acetic diluted acids and adjusted to 70% with a solution of ethanol. The solution is stirred and stirred in a dark place. After 30 minutes immediately measured and optical density of the resulting solution on a spectrophotometer at a wavelength of 408 nm in a cuvette with a layer thickness of 10 mm. As a reference solution, a solution prepared in the same manner is used, but without adding an aluminum chloride solution. In parallel, the optical density of the routine solution of a standard sample prepared similarly to the test is measured.



peaks	λ, nm	Abs
+1	386,811	0,9178
-1	396,514	0,1606
+2	409,306	2,1032
-2	420,296	0,9446
+3	424,465	1,3699

Picture 11. UV spectrum of liquid extract "Gemostav"



peaks	λ, nm	Abs
+1	386,533	0,1818
-1	397,075	0,0492
+2	409,389	0,3642

Picture 12. UV spectrum of RSO.

In the end result, this method was chosen. To do this, 1.0 ml of the drug is placed in a 25 ml volumetric flask, 3 ml of a 2% solution of aluminum chloride is added, one drop, acetic diluted acids and adjusted to 96% with a solution of ethanol. The solution is stirred and stirred in a dark place. After 40 minutes, the solution is filtered through a paper filter "white tape" (TU-6-09-1678-77), 5.0 ml of the preparation is placed in a 25 ml volumetric flask, the volume of the solution is adjusted with 96% ethyl alcohol to the label and immediately measured and optical density of the resulting solution on a spectrophotometer at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm. As a reference solution, a solution prepared in the same manner is used, but without adding an aluminum chloride solution. In parallel, the optical density of the routine solution of a standard sample prepared similarly to the test is measured.

The content of sum of flavonoids (X) in percent in recalculation on routine is calculated by the formula:

$$X = \frac{A \times m_0 \times 1 \times P}{A_0 \times m \times 5}$$

Where,

A is the optical density of the researched solution;

- the optical density of the routine standard sample solution;

- Mass routine standard solution

Note: preparation of standard sample solution

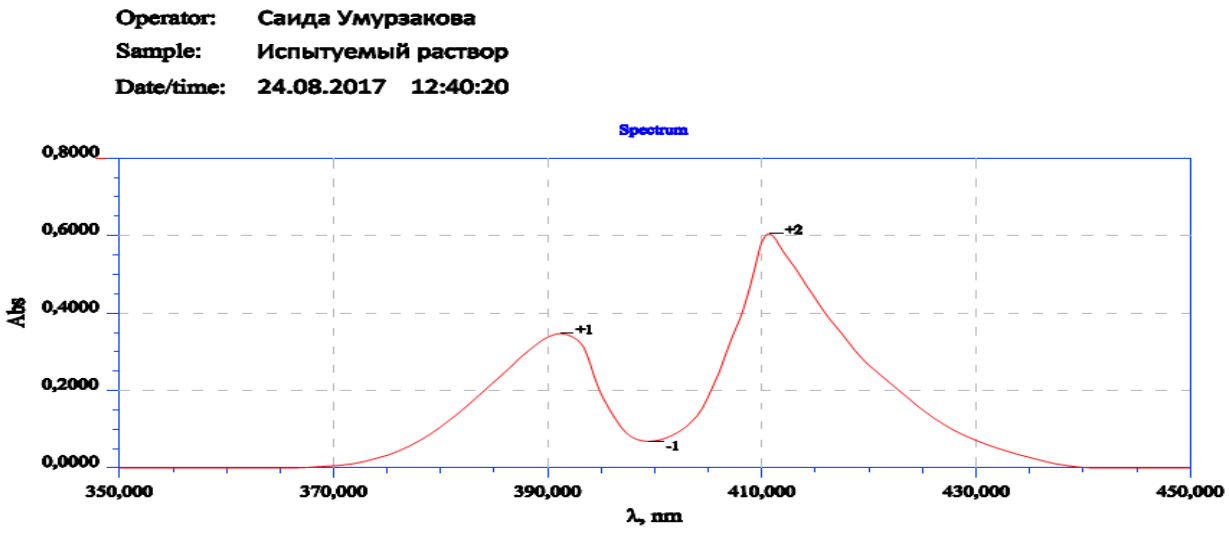
About 0.05 g (exact sample) routine of routine sample (TU-64-4-1297-96), previously dried at a temperature of 130-135 ° C for 3 hours, is added to a 100 ml volumetric flask and dissolved in 85 ml of 96 % of ethyl alcohol when heated in a water bath. After cooling to room temperature, the volume of the solution is adjusted to the mark with the same solvent and mixed. 1.0 ml of the resulting solution is placed in a measuring flask with a capacity of 25 ml and then goes the same way as the preparation of the test solution beginning with the words "... add 3 ml of a 2% solution of aluminum chloride ..." to "... without adjusting the aluminum chloride solution."

Shelf life of the solution is 1 month.

Preparation of 2% aluminum chloride solution 2.00 grams of aluminum chloride is placed in a 100 ml volumetric flask, dissolved in 96% ethyl alcohol, and the volume of the solution is adjusted to the mark with the same solvent.

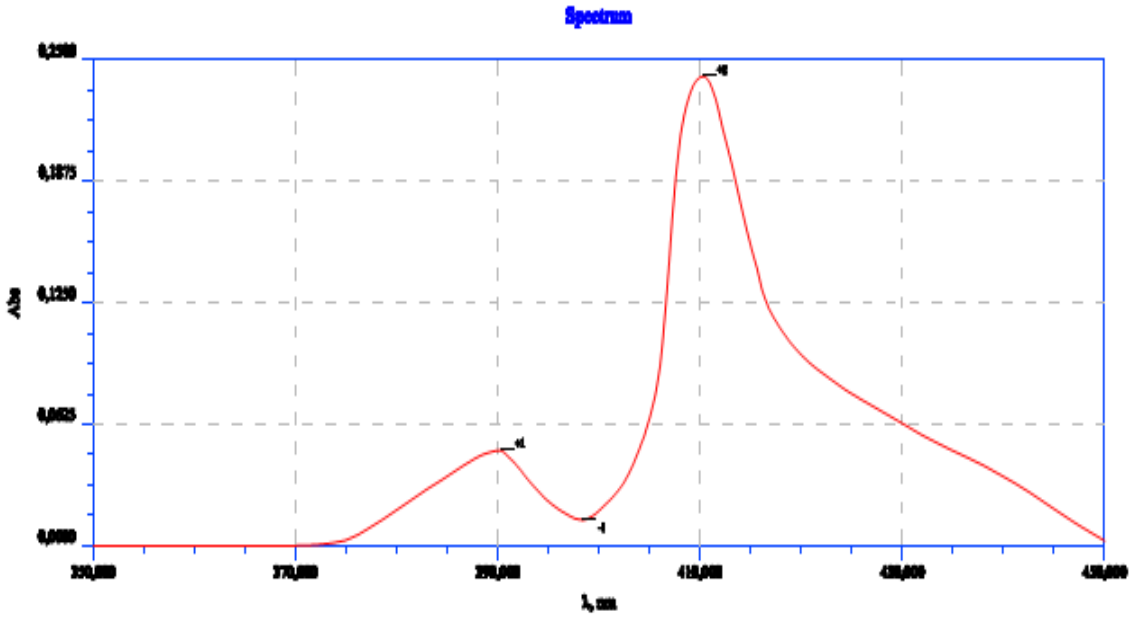
The shelf life of the reagent is 3 months.

The results of the study are presented in picture 13 and 14 for extract and picture 15 and 16 for tincture.



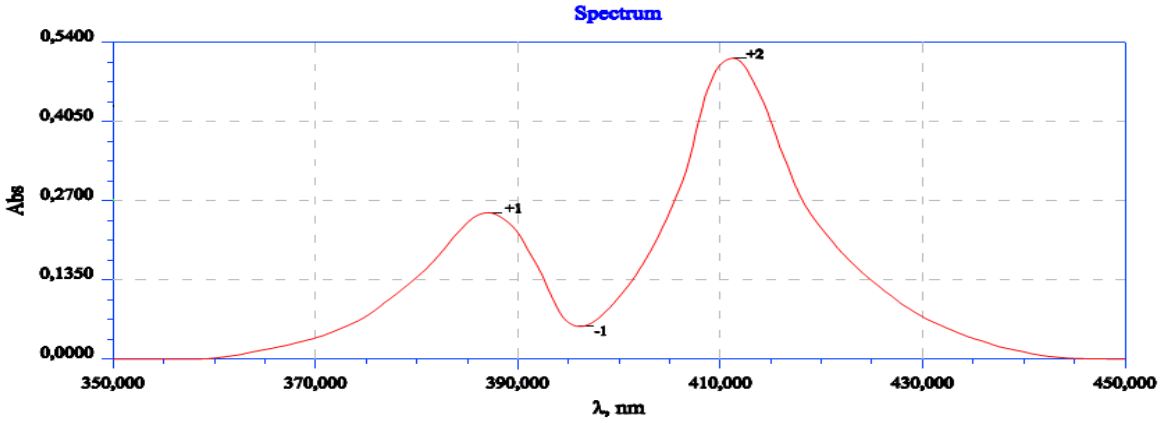
peaks	λ, nm	Abs
+1	391,156	0,3470
-1	399,492	0,0693
+2	410,681	0,6046

Picture 13. UV spectrum of liquid extract "Gemostav"



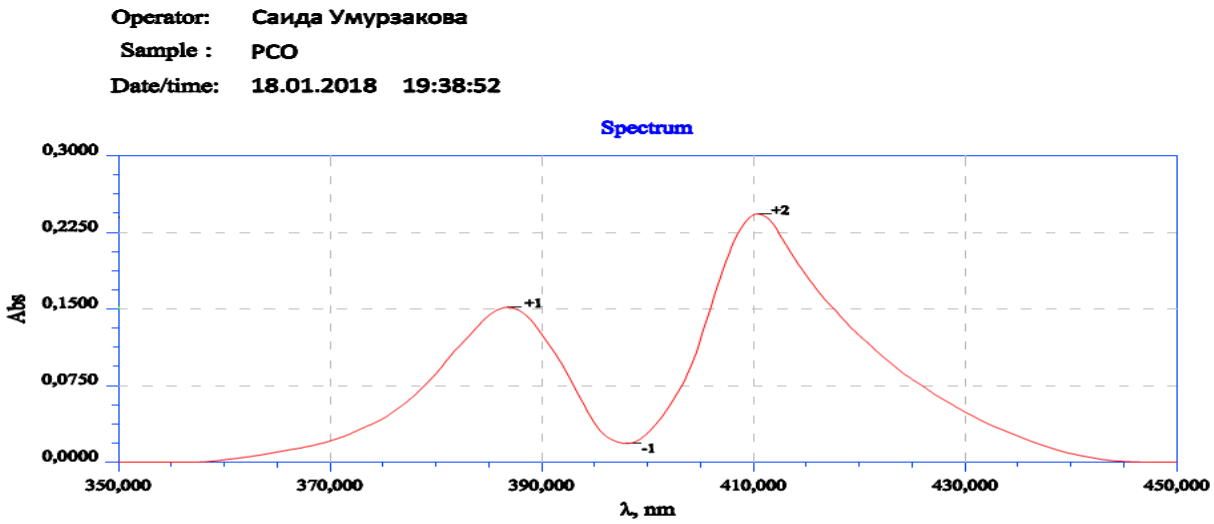
Picture 14. UV spectrum of RSO.

Operator: Саида Умурзакова
Sample : Испытуемый раствор
Date/time: 18.01.2018 19:43:48



peaks	λ, nm	Abs
+1	387,091	0,2496
-1	396,121	0,0557
+2	411,248	0,5131

Picture 15. UV spectrum of tincture "Gemostav"



peaks	λ, nm	Abs
+1	386,821	0,1518
-1	397,992	0,0183
+2	410,350	0,2430

Picture 16. UV spectrum of tincture "Gemostav"

Table 4.

Metrological characteristics of the results of quantitative determination of the sum of flavonoids of the liquid extract "Gemostav" (n = 5; P = 95%; t (p, f) = 2.78)

X_i, %	$\overline{X}$, mg/ml	f	S^2	S	Sx	$\overline{\varepsilon}$,%
X_1=0,62 X_2=0,62 X_3=0,64 X_4=0,64 X_5=0,64	0,632	4	0,00012	0,0109	0,0048	2,15

5.3. Definitions of microbiological purity of liquid extract and tincture.

Introduction: Recently, interest in medicinal plants and developing on its basis of effective, safe and affordable medicines has significantly increased worldwide. Herbal remedies are close to the human body and are widely used both for treatment and for the prevention of various diseases in medical practice. This is due to such advantages as soft effect on the human body and minimization of side effects, which makes it possible to recommend over-the-counter release of developed phytopreparations (FP).

At the present stage of development of the domestic pharmaceutical industry and in connection with the introduction of the rules of proper manufacturing

(GMP) and laboratory (GLP) practices, approaches to quality control of medicines (LP) are changing. The need to control the quality of medicines is justified by the importance of ensuring their safety and effectiveness. Therefore, starting with the development of new pharmaceuticals, at all stages from production to the consumer, it is necessary to assess the likelihood of the risk of producing substandard products and to improve control systems and ensure their quality. A special place in this case is the control of microbiological purity of drugs.

Objectives of the study: The study of the microbiological purity of the new phytopreparation in the form of a liquid extract obtained on the basis of domestic herbal raw materials of marigold medicinal, mountain pepper, and nettle, recommended for medical practice as a hemostatic and anti-inflammatory agent.

Materials and methods: Determination of microbiological purity was carried out in accordance with the requirements of GF XI "Methods of microbiological control of medicinal products".

The tests were carried out under aseptic conditions, using methods and nutrient media to control all types of non-sterile drugs. The analysis was carried out in Petri dishes with sterile nutrient media: meat - peptone agar - for quantitative determination of grown colonies, blood agar - for determination of hemolytic bacterial strains, yolk-salt agar - for detection of pathogenic staphylococci, Endo - for detection of intestinal bacterial groups, Saburo - for excreting fungi. To the nutrient media, the test preparation was added in various concentrations (1: 2: 4), not less than in three Petri dishes. All plates with crops were incubated in a thermostat at 37 ° C, except for Saburo 20-25 ° C. The initial recording of the results was carried out after 24 h and 48 h, the final recording after 5 days. After incubation, the counting of the grown up colonies and their identification were carried out.

The obtained results: According to the results obtained, in the liquid extract the total number of aerobic bacteria in 1 g was 2 colony forming unit (CFU) (no further microbiological analysis was found), the total number of yeast and mold fungi was not found. E.Coli, Salmonella, Ps. aeruginosa, St. aureus and other gram-negative bacteria were not detected at all time.

The proposed liquid drug meets the requirement for this indicator, according to which such preparations should contain no more than 5 103 aerobic bacteria, 102 yeast molds in the absence of Pseudomonas aureginosa, Staphylococcus aureus and not more than 102 other intestinal bacteria. Assessment of the quantitative and qualitative composition of the contaminating flora did not reveal any deviations from the requirements of GF XI.

Picture 17. The results of analysis A

Picture 18. The results of analysis B

5.4. Pharmacological studies of the liquid extract and tincture

Acute toxicity and homeostatic activity of the liquid extract named "Gemostav" had been conducted as various study, as consequences the liquid extract "Gemostav" was found absolutely harmless. To be precise, the “Gemostav” with high doses were orally tested and as a result this experiment was positively accepted as well as there were not shown animal death. Findings between the homeostatic activity of the liquid extract “Gemostav” and infusion of nettle leaves with dioecious indicated that used medicine obtained a reliable homeostatic activity superior to the nettle leaves infusion.

Key words: acute toxicity, homeostatic activity, liquid extract "Gemostav".

In different medical fields, such as in hematology, surgery, traumatology, oncology, obstetrics as well as other fields, preventing and stopping the bleeding is extremely important . Regularly, medicines which are being used by traditional medical activities to stop the bleeding are not effective enough and cannot highly result an effective reduction in blood loss, which is expected to cause dangerous result named hemorrhagic shock, actually DIC syndrome, ischemia and necrosis of vital organs. The only reason could be wide diversity of causes which contribute to the emergence of hematologic and/or microcirculatory bleeding type. Clinical experience shows that in elective surgery on the liver, kidneys, spleen, when the integrity of the main arteries and veins of the capillary-parenchymal bleeding are

the main sources of intraoperative blood loss. Alternatively, to stop their local hypothermia, using laser irradiation bleeding tissue applique liquid nitrogen, heat and ultrasonic coagulation administered parenteral fresh frozen plasma and its products - blood coagulation factors (in isolation or in various combinations), ambenom, epsilon-aminocaproic acid, inhibitors of serine proteases (trasilol, gordoks) adrokson, etamthialat, serotonin, oxytocin, pituitrin and other various medicines with different mechanisms of stopping bleeding. Obtaining local hemostatic effect among different tools such as thrombin, fibrinogen, Factor XIII, aprotinin, fibrin, and polymeric film and the composition are being commonly used [1].

All of abovementioned factors make it relevant to create and study the pharmacological properties of new phytocompositions with a pronounced hemostatic effect.

The main aim of the research was to study the acute toxicity and haemostatic activity of the liquid extract "Gemostav".

Materials and methods of the research. All researches were tested on healthy animals which have been isolated no more than 10-14 days [2, 3, 4].

The object of our study was (pharmacological preparation) liquid extract named "Gemostav", which includes following compositions as: calendula officinalis (flowers) 200.0; nettle (leaves) 100,0; mountaineer bird (grass) 100,0; mountaineer pepper (grass) 100,0; ethanol 70% (in the ratio 1: 1).

Since the pharmacological effect of medicinal plants extracts can be determined by the content of extractive substances (dry residue) [3], in order to conduct the research as well as possess accurate calculation of the selected doses, initially, we determined the dry residue of the liquid extract "Gemostav" (according to GF XI USSR) , which was resulted 6.3 percent.

There were conducted numerous researches about acute toxicity according to the generally existed and accepted methods [2, 3, 4]. The experiments were carried out with 36 white mongrel mice (of both sexes) weighing 18-22 g, additionally, on

the next steps by separating animals in groups which consisted of 6 animals in each one (five test groups and one intact).

As the liquid extract "Phytoallergoderm" contains of 70% ethanol in its composition, we performed de-alkalization of the preparation by evaporation under mild conditions (the extract was poured into an evaporation cup and dried under a hood, at room temperature) to an almost dry residue (after such a procedure , ethanol was almost completely absent in the extract), followed by bringing the water purified to the original volume, and as a result 6.34% de-alkalized extract was obtained (for the study of specific activity). Moreover, in order to obtain 12.68% of the de-alkalized extract, the dried extract was adjusted with water purified to half the original volume (to analyze acute toxicity). This procedure was assessed to eliminate the nonspecific activity of ethanol [3].

12.68% de-alkalized extract was directly tested on animals once (by means of a special probe) at following doses: 2000 mg / kg (0.32 ml / 20 g), 3000 mg / kg (0.47 ml / 20 g), 4000 mg / kg (0.63 ml / 20 g), 5000 mg / kg (0.79 ml / 20 g) and 6000 mg / kg (0.95 ml / 20 g). However, according to the literature, the maximum volume for a single oral administration is 0.5 ml / 20 g [2, 3], so we complied fractional administration method for it. Before 4000 mg / kg dose was utilized, initially, 0.13 ml / 20 g was added, passing half an hour there was used by 0.5 ml / 20 g. Additionally, as a same way we resulted 5000 mg / kg dose, 0.29 ml / 20 g was a result at the first stage, then after 30 minutes, 0.5 ml / 20 g was administered. When a dose of 6000 mg / kg was administered, 0.45 ml / 20 g was first administered, then 0.5 ml / 20 g was given after 30 minutes.

Furthermore, the animals were divided into particular cells by grouping, and controlled continuous monitoring during the first hour, on the first steps the monitoring was verified during the first day, and it continued once a day, as well it controlled during the subsequent 13 days of the experiment (a total observation period was 14 days).

The general condition of the animals, their features behavior, intensity and nature of the motor activity, presence and nature of the convulsions, coordination

of movements, tone of the skeletal muscles, response against external stimuli, frequency and depth of the respiratory movements, the rhythm of the heartbeats, the condition of the wool and skin, coloring of the mucous membranes, position of the tail, amount and consistency of fecal matter, feed and water intake, as well as other indicators those illustrating the toxic medicine effect. Additionally, the timing of the intoxication development and animals' death were recorded relatively.

A model which describes the parenchymal hemorrhage of the liver of intact animals includes the hemostatic activity of the liquid extract [1, 2, 3, 4]. The results of experiments illustrated white mongrel rats (in both sexes) weighing 160-195 g, in a group of 6 animals, overall 54 animals.

As a reference (reference preparation) for hemostatic medical plant can be shown followings, "Foliage of dioecious leaves" (Folia Urtica diotica), JSC "QASHQADARYO DORI-DARMON" Uzbekistan, registration certificate number in RU: DV / M 00950/08/16 05/08 / 16 (Medicines register of Uzbekistan in 2017).

In order to study the hemostatic activity from the leaves of nettle, an infusion was prepared according to GF XI, in a ratio of 1: 5, 1:10 and 1:15, (taking into account the water absorption coefficient of 1.8 ml / g, the degree of crushing of the leaves was no more than 5.0 mm).

While choosing effective doses for studying the specific activity of the liquid extract of "Gemostav", were guided by data of acute toxicity with oral administration (LD50> 6000 mg / kg) according to which the estimated effective dose was searched in the range of tolerated doses.

Animals with ethereal anesthesia (ether injected inhalation) resulted an autopsy of the abdominal cavity as well as the liver was removed outside and as a result cut wounds were made got its surface (5 notches, 2.5 cm long and 2-3 mm deep) by means of a scalpel, as a result, a capillary- parenchymal hemorrhage. Further on, the entire surface of the bleeding liver was superimposed with a four-layer gauze (3.5x3.5 cm2) moistened with preparations:

1. Control group (control) - gauze moistened with physiological saline was applied to animals in a volume of 2.0 ml / 180 g;

2. Test group number 1 - gauze moistened with 3.17% de-akalogized extract of "Gemostava", at a dose of 352 mg / kg, in a volume of 2.0 ml / 180 g was applied to the animals;

3. Test group number 2 - gauze moistened with 2.11% de-akalogized extract of "Gemostava" was applied to animals, at a dose of 234 mg / kg, in a volume of 2.0 ml / 180 g;

4. Test group №3 - gauze moistened with 1,585% de-akalogized extract of "Gemostava", at a dose of 176 mg / kg, in the volume of 2.0 ml / 180 g was applied to the animals;

5. Test group number 4 - gauze moistened with 1.268% de-akalogenated extract of "Gemostava", at a dose of 141 mg / kg, in a volume of 2.0 ml / 180 g was applied to the animals;

6. Test group №5 - gauze moistened with 1,057% de-akalogized extract of "Gemostava", in a dose of 117 mg / kg, in the volume of 2.0 ml / 180 g was applied to the animals;

7. Comparison group (reference group) No. 1 - gauze moistened with 3.22% aqueous infusion of nettle leaves, at a dose of 358 mg / kg, in a volume of 2.0 ml / 180 g was applied to the animals;

8. Comparison group (reference group) No. 2 - gauze moistened with 2.09% aqueous infusion of nettle leaves, at a dose of 232 mg / kg, in a volume of 2.0 ml / 180 g was applied to the animals;

9. Comparison group (reference group) No. 3 - gauze moistened with 1.47% aqueous infusion of nettle leaves at a dose of 163 mg / kg in a volume of 2.0 ml / 180 g was applied to the animals.

Time when the bleeding stopped evaluated by stopwatch (in seconds). The criterion for evaluating the moment of bleeding stop was the complete absence of blood penetration through the surface and the edges of the superimposed gauze tampon.

The criterion for assessing hemostatic activity was resulted decrease in the required time to stop bleeding, compared with control.

During the experiment, all animals were kept in standard vivarium conditions, and were on a full-fledged diet and water diet. During the experiments, the room temperature was in the range between 18-25 ° C, relatively to humidity in the range between 40-70%.

The results were processed by the method of variation statistics by the Student's test for p = 0.05 [2, 3]. The tables illustrate the average arithmetic meanings (M), the corresponding standard errors of the mean value (m), the Student's criterion (t), the number of samples (n), the confidence limits (the lower confidence limit ÷ the upper confidence limit).

Results of the research

After oral administration of the drug at doses of 2000 mg / kg, 3000 mg / kg, 4000 mg / kg, 5000 mg / kg and 6000 mg / kg, symptoms of intoxication, changes in general condition, and death of animals were not observed.

The calculation of LD50 due to the absence of dead animals after oral administration of liquid extract was impossible, which indicates that there is no toxicity in the dose range of 2000-6000 mg / kg, so LD50> 6000 mg / kg is assumed (see Table 1).

Table 5

The results of the study of acute toxicity, after oral administration of the liquid extract "Gemostav"

Doses mg / kg	Number of animals dead / total
2000 mg / kg	0/6
3000 mg / kg	0/6
4000 mg / kg	0/6
5000 mg / kg	0/6
6000 mg / kg	0/6
LD50> 6000 mg / kg	

During the research hemostatitic activity of drugs, it was established (Table 2) that the drug "Gemostav" in doses of 350 mg / kg (51.07%), 234 mg / kg (51.40%), 176 mg / kg (56.91%) and 141 mg / kg (62.06%) have a significant hemostatic effect. At a dose of 117 mg / kg (42.28%), the hemostatic effect is also observed, but it is not statistically significant. It should be noted that when the dose is 141 mg / kg (62.06%), the highest hemostatic effect is observed.

It should also be said that if you compare the experimental data of all five doses of the liquid extract "Gemostav", then it turns out that the difference between them is not statistically reliable.

The results of the study of the hemostatic activity of the leaves of the nettle nettle showed (see Table 2) that the infusion at doses of 358 mg / kg (49.88%) and 232 mg / kg (54.16%) had a statistically significant hemostatic effect. At a dose of 163 mg / kg (42.04%), hemostatic effects are also observed, but not statistically significant. It should be noted that exposure to the dose of 232 mg / kg (54.16%) has the highest hemostatic effect.

During comparison of the most effective doses of liquid extract "Gemostav" and infusion of nettle leaves it was found that the liquid extract "Gemostav" has a significantly greater hemostatic effect than the infusion of nettle.

Table 6

The results of the study of hemostatic activity (M±tm; p = 0,05; n = 6)

Group	**Time when the bleeding stopped (in seconds)**	**Percentage of Effectiveness**
Control	350,83 (217,14÷484,53)	-
«Gemostav» (Dose of 352 mg / kg)	171,67 (137,71÷205,62)	51,07%
«Gemostav» (Dose of 234 mg / kg)	170,50 (129,44÷211,56)	51,40%
«Gemostav»	151,17 (130,81÷171,52)	56,91%

(Dose of 176 mg / kg)		
«Gemostav» (Dose of 141 mg / kg)	132,50 (118,92÷146,08)	62,06%
«Gemostav» (Dose of 117 mg / kg)	202,50 (161,21÷243,79)	42,28%
Infusion of nettle leaves with dioecious (Dose of 358 mg/kg)	175,83 (147,98÷203,69)	49,88%
Infusion of nettle leaves with dioecious (Dose of 232 mg/kg)	160,83 (150,65÷171,02)	54,16%
Infusion of nettle leaves with dioecious (Dose of 163 mg/kg)	203,33 (176,64÷230,02)	42,04%

Discussion of the results

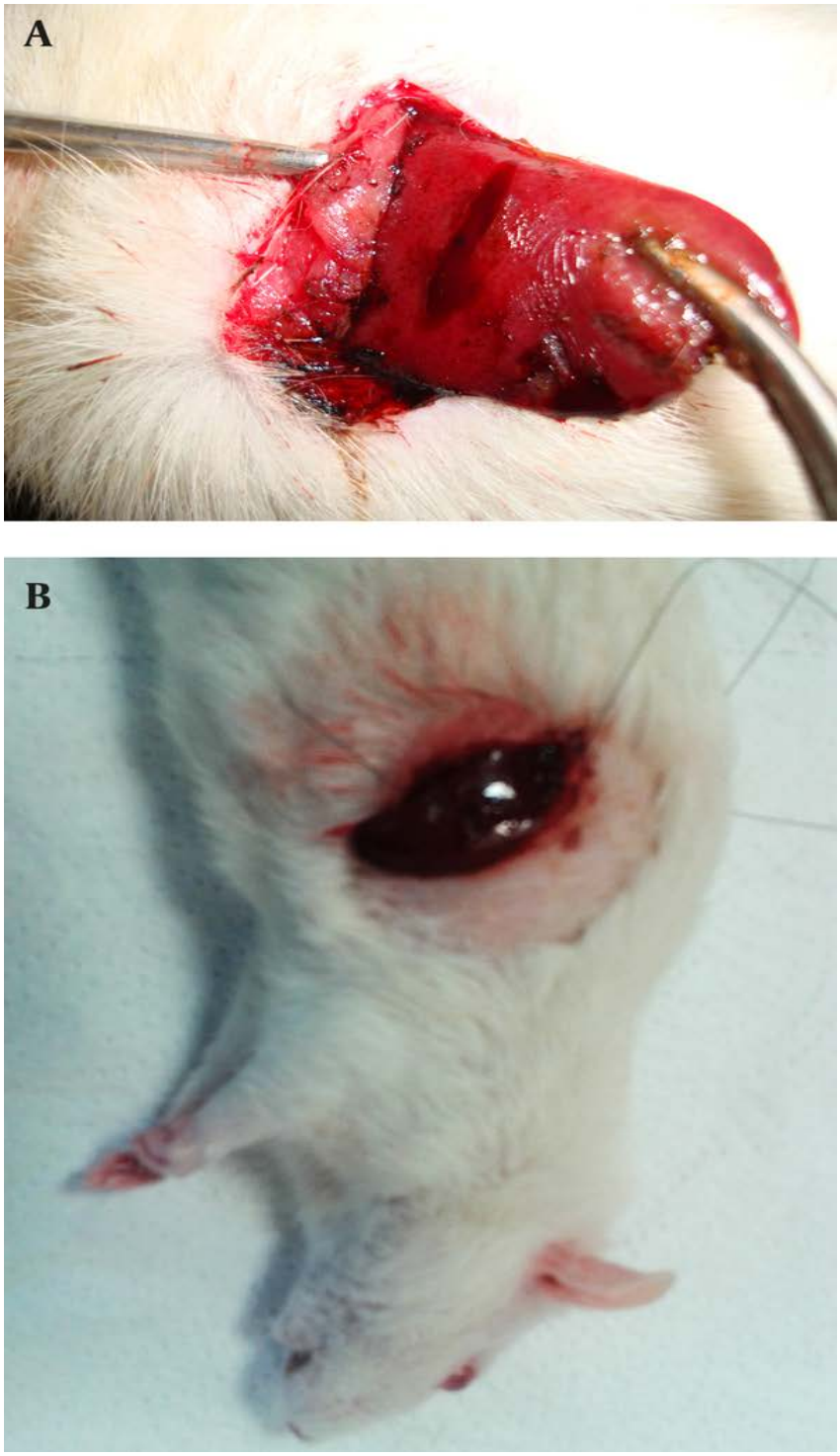
On the basis of the data obtained, we can conclude that the liquid extract of "Gemostav" has a good tolerance, because with oral administration of high doses, there is no death of animals, and no symptoms of intoxication.

Based on the received data on the indicator of the average dose, we determined the hazard class in accordance with GOST 12.1.007-76 (the classifier contains four levels of classification, for the safety of substances) [5], for a liquid extract that corresponds to the fourth hazard class (low-hazard substances). The results showed that it is not feasible to further investigate acute toxicity, for oral administration of the drug, since the maximum dose administered corresponds to the last hazard class.

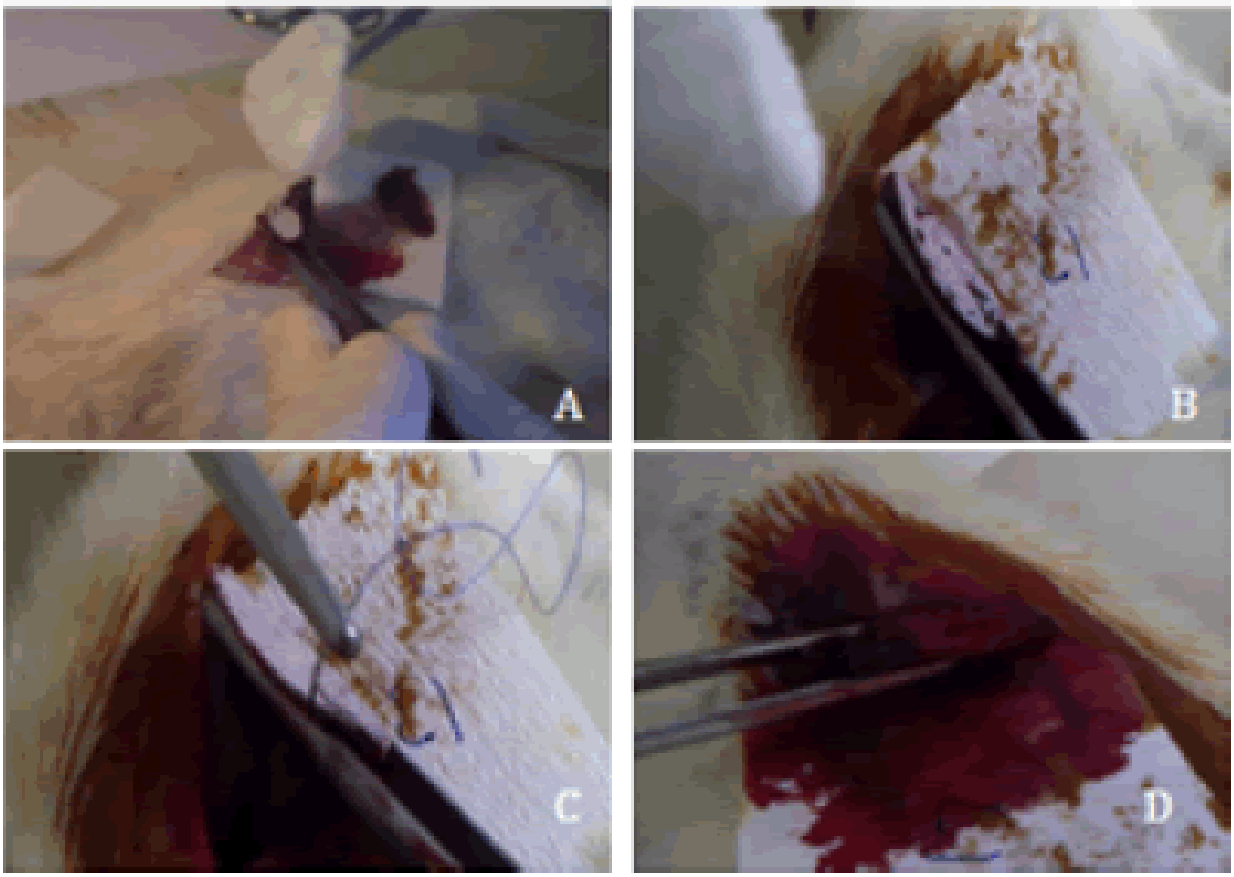
It should be noted that according to the instructions for studying the parameters of acute toxicity, described in the methodological guide to preclinical drug research, edited by Stefanova A.V. [4], it is said that any substance injected in large quantities can cause toxic effects, so the limiting indicator is the maximum

dose of the fourth toxicity class (low-toxic substances), taking into account the route of administration.

We also evaluated the toxicity data, described in the methodical guide to preclinical drug research, edited by A. Stefanova. [4]. According to this researcher's view, it may be concluded that according to the guidelines for studying acute toxicity parameters, described in the methodical guide to preclinical drug research, edited by Stefanova A.V. [4], it is said that any substance injected in large quantities can cause toxic effects, so the limiting indicator is the maximum dose of the fourth toxicity class (low-toxic substances), taking into account the route of administration. And if a dose of the drug corresponding to the maximum dose of the fourth toxicity class is not observed, death is not observed, then a larger dose is usually impractical. In case of death of animals due to the effect of the test substance, it is necessary to conduct the tests in full.



Picture 19



Picture 20

Conclusion by chapter:

1. Thus, on the basis of the studies carried out, the TLC method for the identification of flavonoids was developed, and for the quantitative determination of the spectrophotometry method in the sample under study. The maintenance routine in the liquid extract was 0.63%, respectively. The results of studying the metrological characteristic [6] of the developed methodology showed that the relative error of the mean result was ± 2.15%, which meets the requirements for instrumental analysis methods. Phytoextract "Hemostav" contains all the necessary macro and microelements, among which the greatest amount are: sodium, potassium, magnesium, calcium and iron. It should be noted that the above elements refer to essential, that is, vital - essential elements.

High content of mineral substances allows to expand the spectrum of application of alcohol phytoextract and testifies to the prospects of its use in medicine.

2. The proposed dosage form in the form of a liquid extract obtained from medicinal plants: Calendula officinalis, Polygonum aviculare, Polygonum hydropiper, Urtica dioicae meets the requirements of GF XI, Issue 2, c 193, change 2, from 12.10.2005 category 3 B, Microbiological purity". The results obtained are included in the draft VFS for this dosage form.

3. From the data on the qualitative and quantitative content of active substances, we can conclude that the contribution of a group to the manifestation of an extract of a specific pharmacological action.

Summarizing the all abovementioned, it can be concluded that the further study of acute toxicity by oral administration of the liquid extract of "Hemostava" is completed.

The obtained data of the research of haemostatic activity testify to the reliable haemostatic activity of the liquid extract of "Gemostava" in doses of 350 mg / kg, 234 mg / kg, 176 mg / kg and 141 mg / kg. However, it should be emphasized that the dose of 141 mg / kg being the most effective dose, has a significant advantage in hemostatic activity before the infusion of nettle leaves.

To conclude, acute toxicity and hemostatic activity of the liquid extract "Gemostav" was studied. As a result, it was found that the drug is highly harmless, since when administered orally at high doses it is well tolerated and does not cause death of animals. Based on the data obtained, a hazard class was determined according to GOST 12.1.007-76, for a liquid extract that corresponds to the fourth hazard class (low-hazard substances). Additionally, on the basis of the data obtained, the toxicity class described in the methodical manual for preclinical drug research, edited by Stefanova AV, was assigned, which corresponds to the fifth toxicity class (practically non-toxic). The results of the assessment of the safety class showed the completeness of studies of acute toxicity parameters with oral administration.

The study of the haemostatic activity of the liquid extract "Gemostav" in comparison with the infusion of nettle leaves with dioecious, showed that the test preparation possesses a reliable haemostatic activity superior to the infusion of nettle leaves.

CONCLUSION

1. Phytoextract "Gemostav" contains all the necessary macro and microelements, among which the greatest amount are: sodium, potassium, magnesium, calcium and iron. It should be noted that the above elements refer to essential, that is, vital - essential elements.
2. High content of mineral substances allows to expand the spectrum of application of alcohol phytoextract and testifies to the prospects of its use in medicine.
3. As a result of the studies, GLC conditions were found for the analysis of ethyl alcohol in the liquid extract "Hemostat" which makes it possible not only to identify the latest, but also to determine the quantitative content, and also to evaluate its purity. The data obtained in the future will serve as the basis for the drafting of the VFS.
4. TLC method was developed for the identification of flavonoids, and for the quantitative determination of the spectrophotometry method in the test sample
5. Maintenance routine in the liquid extract was 0.63%, respectively
6. Metrological characteristic [6] of the developed methodology result showed relative error of the mean result was ± 2.15%
7. Phytocoagulant "Hemostav" contains all the necessary macro and microelements which are considered as vital-essential elements
8. High content of mineral substances allows to expand the spectrum of alcohol phytoextract application and testifies to prospects of its use in medicine
9. The contribution of a group to the manifestation of an extract of a specific pharmacological action can be identified by data on the qualitative and quantitative content of active substances
10. Thus, on the basis of the studies carried out, the TLC method for the identification of flavonoids was developed, and for the quantitative determination of the spectrophotometry method in the sample under study. The maintenance routine in the liquid extract was 0.63%, respectively. The results of studying the metrological characteristic [6] of the developed methodology showed that the relative error of the mean result was ± 2.15%, which meets the requirements for instrumental analysis methods. Phytoextract "Hemostav" contains all the necessary macro and microelements, among which the greatest amount are: sodium, potassium, magnesium, calcium and iron. It should be noted that the above elements refer to essential, that is, vital - essential elements.

High content of mineral substances allows to expand the spectrum of application of alcohol phytoextract and testifies to the prospects of its use in medicine.

11. The proposed dosage form in the form of a liquid extract obtained from medicinal plants: Calendula officinalis, Polygonum aviculare, Polygonum hydropiper, Urtica dioicae meets the requirements of GF XI, Issue 2, c 193, change 2, from 12.10.2005 category 3 B, Microbiological purity". The results obtained are included in the draft VFS for this dosage form.

12. From the data on the qualitative and quantitative content of active substances, we can conclude that the contribution of a group to the manifestation of an extract of a specific pharmacological action.

Summarizing the all abovementioned, it can be concluded that the further study of acute toxicity by oral administration of the liquid extract of "Hemostava" is completed.

13. The obtained data of the research of haemostatic activity testify to the reliable haemostatic activity of the liquid extract of "Gemostava" in doses of 350 mg / kg, 234 mg / kg, 176 mg / kg and 141 mg / kg. However, it should be emphasized that the dose of 141 mg / kg being the most effective dose, has a significant advantage in hemostatic activity before the infusion of nettle leaves.

To conclude, acute toxicity and hemostatic activity of the liquid extract "Gemostav" was studied. As a result, it was found that the drug is highly harmless, since when administered orally at high doses it is well tolerated and does not cause death of animals. Based on the data obtained, a hazard class was determined according to GOST 12.1.007-76, for a liquid extract that corresponds to the fourth hazard class (low-hazard substances). Additionally, on the basis of the data obtained, the toxicity class described in the methodical manual for preclinical drug research, edited by Stefanova AV, was assigned, which corresponds to the fifth toxicity class (practically non-toxic). The results of the assessment of the safety class showed the completeness of studies of acute toxicity parameters with oral administration.

The study of the haemostatic activity of the liquid extract "Gemostav" in comparison with the infusion of nettle leaves with dioecious, showed that the test preparation possesses a reliable haemostatic activity superior to the infusion of nettle leaves.

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APPENDIX