

## Phytochemical Screening and Physicochemical Parameters of Crude Drugs: A Brief Review

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### ABSTRACT

The plant has been used as a medicine in ancient. Now day's pharmaceutical companies start processing of medicinal and aromatic plants in their formulation by using extraction of active components. Now days there are several processes like distillation, effleurage, maceration, expression, solvent extraction and fluid extraction are available for extraction of plant component. This review also summarizes the characters of phytoconstituents, procedures for extraction of herbal drugs and treatment of drug residue after extraction. Standardisation of crude drug plays a very important role in identifying the purity and quality of crude drugs. The present investigation reveals standardization which includes Moisture content, total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, Phytochemical screening, and further isolation and identification of phytoconstituents. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydro fluorocarbon solvents). For aromatic plants, hydro distillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be employed. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, micro distillation, thermomicrodistillation and molecular distillation.

**Keywords:** Extraction, phytochemicals, standardization, thermomicrodistillation.

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### INTRODUCTION

The World health organization (WHO) estimates that 4 billion people (80%) of the world's population presently use herbal medicine for one form of primary health care or another. Its history is inextricably intertwined with that of modern medicine, but pharmacologists, rather than use a whole plant identify, isolate, extract and synthesize individual components, thus capturing the active properties as against the herbalist who considers that the power of a plant lies in the interaction of all its ingredients [1].

Herbal medicines are promising choice over modern synthetic drugs. They show minimum or no side effects and are considered to be safe. Generally herbal formulations involve the use of fresh or

dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained [2]. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Many of these indigenous medicinal plants are used as spices and food. Medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, locations and oils (essential

and fixed) [3]. Higher plants produce both primary and secondary chemical metabolites, the former being vitally important in normal development and reproduction of plants. On the other hand, secondary metabolites are known to play important roles in plant survival as defense mechanisms against adverse biotic and abiotic conditions [4]. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portion of plant (and animal) tissue using selective solvents through standard procedures. The products so obtained from plants or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use, these include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pillar (semi solid) extracts or powdered extracts [5].

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstruum. The extract thus obtained, after standardization, may be used as a medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain a complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, Flavonoids and lignans.

**The basic parameters influencing the quality of an extract are:**

1. Plant part used as starting material
2. A solvent used for extraction
3. Extraction procedure

**Effect of extracting plant phytochemicals depends on:**

1. Nature of the plant material
2. Origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect the quantity and secondary metabolite composition of an extract depend upon [6].

1. Type of extraction
2. Time of extraction

3. Temperature
4. Nature of solvent
5. Solvent concentration
6. Polarity

#### **Extraction of medicinal plants**

Extraction, as the term is used in pharmaceuticals, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pillular (semisolid) extracts and powdered extracts.

#### **Methods of extraction of medicinal plants**

##### **Maceration**

- In this process, the whole or coarsely powdered crude drug is placed in a stopper container with the solvent.
- And allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved.
- The mixture then is strained; the Marc (the damp solid material) is pressed.
- And the combined liquids are clarified by filtration or decantation after standing.

##### **Infusion**

- Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water.
- These are dilute solutions of the readily soluble constituents of crude drugs.

##### **Digestion**

- This is a form of maceration in which gentle heat is used during the process of extraction.
- It is used when moderately elevated temperature is not objectionable.
- The solvent efficiency of the menstruum is thereby increased.

##### **Decoction**

- In this process, the crude drug is boiled in a specified volume of water for a defined time.
- It is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat stable constituents.

- This process is typically used in preparation of Ayurvedic extracts called “quath” or “kawath”. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16.
- The volume is then brought down to one-fourth its original volume by boiling during the extraction procedure.
- Then the concentrated extract is filtered and used as such or processed further.

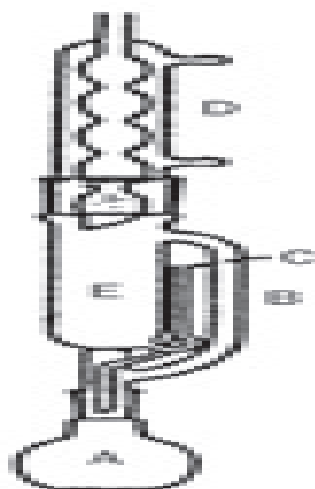
#### **Percolation**

- This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts.
- A percolator (a narrow, cone-shaped vessel open at both ends) is generally used.
- The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed.
- Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h.
- The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly.
- Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product.

- The marc is then pressed and the expressed liquid is added to the percolate.
- Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting [26].

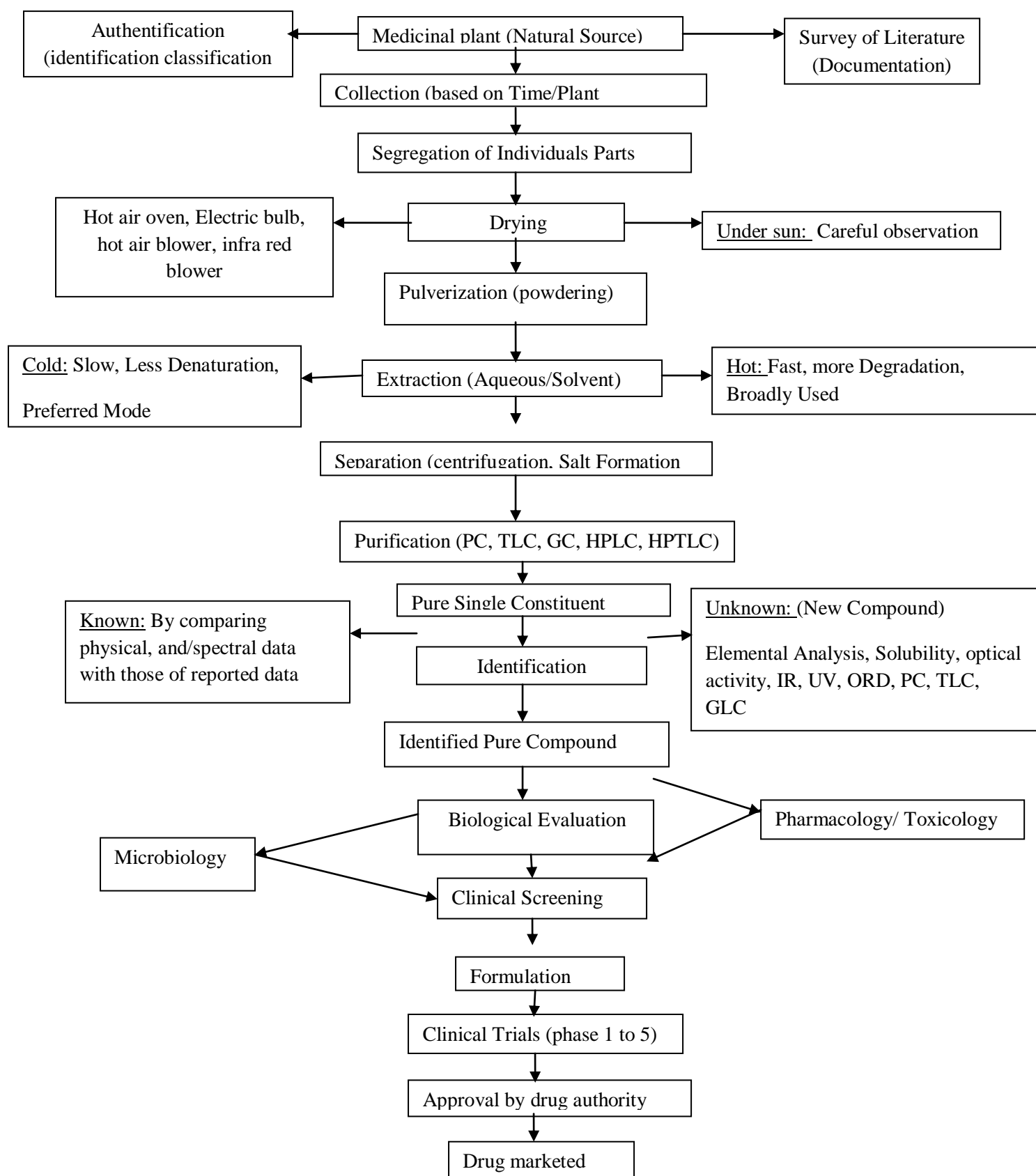
#### **Hot continuous extraction (Soxhlet)**

- In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus (Figure).
- The extracting solvent in flask A is heated, and its vapors condense in condenser D.
- The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact.
- When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A.
- This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.
- The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent.
- This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale [27].



**Soxhlet apparatus**

**Schematic development of a 'Drug' from a 'Medicinal Plant' [28].**



**Phytochemical screening:**

Phytochemical examinations were carried out for all the extracts as per the standard methods.

**1. Test for Alkaloids****a) Dragendorff's Test:**

About 0.2 g of the extracts was warmed with 2% H<sub>2</sub>SO<sub>4</sub> for two minutes. It was filtered and few drops of Dragendorff's reagent were added. Orange red precipitate indicates the presence of alkaloids [7].

**b) Mayer's test:**

To a few ml of filtrate, a few drops of Mayer's reagent were added by the side of the tube. A creamy white precipitate indicates the presence of alkaloids [8].

**2. Test for Flavonoid****a) Alkaline reagent test:**

Extract was treated with 10 % NaOH solution; formation of intense yellow color indicates presence of Flavonoid.

**b) NH<sub>4</sub>OH test:**

3 ml of extract was 10 % NH<sub>4</sub>OH solution development of yellow fluorescence indicates a positive test.

**c) Mg turning test:**

Extract were treated with Mg turning and add conc. HCl to this solution add 5ml of 95 % ethanol, formation of crimson red colour indicates Flavonoid.

**d) Zn test:**

2 ml extract were treated with Zn dust and conc. HCl development of red colour indicates presence of Flavonoid [9].

**3. Test for Phenolic compounds**

The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of

neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds [10].

**4. Test for gum and mucilage**

About 10ml of the extract was slowly added to 25ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gum and mucilage.

**5. Test for fixed oils and fats**

A drop of concentrated extract was pressed in between two filter papers and kept undisturbed. Oil stain on the paper indicates the presence of oils and fats [11].

**6. Test for glycoside**

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genuine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer [12].

**7. Test for steroid**

20mg of the extract was treated with 2.5 ml of acetic anhydride and 2.5 ml of chloroform. Then concentrated solution of sulfuric acid was added slowly and red violet color was observed for terpenoids and green bluish color for steroids [13].

**8. Test for tannins**

To 0.5 ml of extract solution 1ml of water and 1- 2 drops of ferric chloride solution were added. Blue color was observed for Gallic tannins and green black for catecholic tannins [14].

**Table 1: Phytochemical examinations of some therapeutic plants [15, 16, 17, 18].**

Plant extracts	Alkaloids	Glycosides	Flavonoid	Tannin
<i>Ocimum sanctum</i>	+	+	+	+
<i>Zanthoxylum aromaticum</i>	+	-	-	+
<i>Rhododendron</i>	-	-	-	+
<i>Ocimum santrum</i>	+	+	-	+
<i>Azadiracta indica</i>	-	-	+	+
<i>Aloe barbandesis</i>	+	+	+	+
<i>Polyalthia longifolia</i>	+	+	-	+
<i>Curcuma longa</i>	-	+	+	-
<i>Hyptis suaveolens</i>	+	+	+	-

**9. Test for amino acids****a) Ninhydrin test**

To 1ml of sample, add 5drops of Ninhydrin Reagent. Heated in a boiling water bath for

2 min. A purple color indicates the presence of amino acids.

#### b) Xanthoproteic test

To 3ml of the sample, add 1ml of concentrated nitric acid and heated for 3min. Then cooled and added 0.5 ml of NaOH. Reddish orange color indicates the presence of aromatic amino acids.

#### c) Folin's test

To 1ml of sample, add 1ml of Folin's phenol reagent followed by the addition of 1N sodium carbonate. The blue color indicates presence of tyrosine and tryptophan.

#### d) Million's test

To 1ml of sample, add 1ml of Million's reagent and heated for 3 minutes. Then 1% sodium nitrate is added. Red color formed indicates the presence of Tyrosine.

#### e) Pauly's test

To 1ml of sample, add 1ml of 1% sulphanilic acid and cooled in ice. Then 1ml of sodium nitrite added. After 5 min, 2ml sodium carbonate added. Presence of cherry red color indicates the presence of Histidine [7].

#### Evaluation of crude drugs

##### Determination of Moisture (Loss on drying)

###### Procedure:

- Weigh about 1.5g of the powdered drug into a weighed flat and thin Porcelain dish.
- Dry it in the oven at 100 °C or 105 °C.
- Cool in desiccators and watch the loss in weight is usually recorded as moisture.

**Note:** A very useful form of dish for the determination of moisture and of ash is a thin flat porcelain dish. If a platinum dish available it may be used.

The burning of the powder should proceed slowly and the material must not be allowed to catch fire or to give off smoke as dense fumes [19].

#### Ash values

The total ash, acid insoluble ash and water-soluble ash values were determined from air-dried samples using the procedure described in the IP.

##### Procedure

###### Total ash value

- About 2gm of powdered drug was weighed accurately into a tarred silica crucible.
- Incinerated at 450°C in a muffle furnace until free from carbon.
- The crucible was cooled and weighed.
- Percentage of total ash was calculated with reference to air-dried substance.

###### Acid insoluble ash

- Ash obtained from the total ash was boiled with 25ml of 2N HCl for a few minutes.
- Filtered through an ash less filter paper.
- The filter paper was transferred into a tarred silica crucible.
- Incinerated at 450°C in a muffle furnace until free from carbon.
- The crucible was cooled and weighed.
- Percentage of acid insoluble ash was calculated with reference to air-dried substance.

###### Water soluble ash

- Ash obtained from the total ash was boiled with 25 ml of distilled water for a few minutes
- And filtered through an ash less filter paper.
- The filter paper was transferred into a tarred silica crucible.
- Incinerated at 450°C in a muffle furnace until free from carbon.
- The crucible was cooled and weighed.
- Percentage of water-soluble ash was calculated with reference to air-dried substance [20].

**Table 2: Total ash and acid in soluble ash of some medicinal plants [21, 22, 23, 24].**

S.N.	Drugs	Total ash (%w/w)	Acid insoluble ash (w/w)
1.	<i>Oscimum sanctum</i> (leaves)	15.21	17.6
2.	<i>Azadirachta indica</i> (leaves)	12.81	9.66
3.	<i>Sesbania rostrata</i> (leaves)	8.70	1.97
4.	<i>L.aestuans</i> (leaves)	14.44	1.52
5.	<i>Pimenta dioica</i>	90	13

### Determination of total ash value formula:

$$\text{Total ash value of the sample} = \frac{100(z-x)}{y}\%$$

X= weight of empty dish

Y= weight of the drug taken

Z= weight of the dish + ash (after complete incineration) [19].

### Determination of extractive values

Extracting values are useful for determining of crude drugs & it gives an idea about the nature of the chemical constituents present. The solvent used for the extraction should be in position to dissolve appropriate quantities of desired substances.

### Determination of alcohol soluble extractive value

- About 5gms of air dried coarse powdered drug was weighed
- And macerated with 100ml of 90% alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hrs & these allowed standing for 18 hrs.
- Thereafter it was filtered rapidly taking precautions against loss of the solvent.
- 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed swallowed dish, dried at 105°C & weighed.
- The %of the alcohol soluble extractive values was calculated with reference to the air-dried drug.

### Determination of water soluble extractive value

- About 5gm of air-dried powdered drug was taken & macerated with 100 ml of chloroform water in a closed flask for 24 hrs shaking frequently during the first 6 hrs.
- And then allowed to stand for 18 hrs.
- Thereafter, it was filtered rapidly taking precautions against loss of the solvent.
- 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105 & weighed.
- The percentage of the water soluble extractive value was calculated with reference to the air-dried drug [25].

### Other parameters

- Foreign matter
- Moisture content
- Tannin content
- Bitterness value

- Swelling index
- RF values
- Microbial contamination
- Toxic residues
- Heavy metal accumulation

### CONCLUSION

Natural product drug invention program a time-consuming capital-intensive program. Several extraction, fractionation, taking apart and isolation methods are developed after which isolation of the active moiety and their compound examination is performed. The phytochemical examination of a plant involves the selection, collection, identification and authentication, extraction of the plant material. Examination of the biosynthetic pathways of particular compound, quantitative evaluations and pharmacological activities.

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