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To Perform Phytochemical Analysis of Extracted Materials

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ABSTRACT

Pharmacovigilance is defined as 'the study of the safety of marketed drugs under the practical conditions of clinical usage in large communities'. The objective is to extend safety monitoring and detect drug adverse events that have previously been unrecognised despite evaluation in clinical trials. Although these methods were developed for monitoring pharmaceutical medicines they are also used for additional evaluation of the safety of other medicinal products including herbals, blood products, vaccines and even medical devices. As the use of herbal medicines has increased, so too have the reports of suspected toxicity and adverse events. Such unwanted reactions can be due to (i) side effects (usually detectable by pharmacodynamics and often predictable); (ii) reactions occurring as a result of overdose, overduration, tolerance, dependence-addiction (detectable either by pharmacodynamics or pharmacovigilance), (iii) hypersensitivity, allergic and idiosyncratic reactions (detectable by pharmacovigilance), (iv) mid-term and long-term toxic effects including liver, renal, cardiac and neurotoxicity also genotoxicity and teratogenicity (detectable by in vitro and in vivo toxicological studies or by pharmacovigilance). As many herbal products on the market have not been thoroughly tested for their pharmacology and toxicology, pharmacovigilance has paramount importance in detecting unwanted reactions.

Keyword: Herbals, Blood Products, Vaccines, Medical Devices, Liver, Renal, Cardiac

INTRODUCTION

Pharmacovigilance of herbal medicines refers to the science and activities involved in detecting, assessing, understanding, and preventing adverse effects or any other problems related to the use of herbal products. With the growing global use of herbal medicines—often perceived as natural and safe—there is an increasing need to monitor their safety and efficacy. Herbal preparations can cause adverse reactions due to factors such as poor quality control, contamination, adulteration with synthetic drugs or heavy metals, misidentification of plants, and herb–drug interactions. Unlike conventional drugs, many herbal products lack standardization, clinical evidence, and proper labelling. leading to unpredictable therapeutic outcomes. The World Health Organization (WHO) has recognized the importance of integrating herbal pharmacovigilance into national drug monitoring systems to ensure patient safety. In India, the Ministry of AYUSH has established the Pharmacovigilance Programme for Ayurveda, Siddha, Unani, and Homeopathy (ASU&H) to monitor and document adverse drug reactions associated with traditional medicines. The main objectives of herbal pharmacovigilance include ensuring safe use, identifying risks, maintaining product quality, and promoting rational therapy. However, challenges such as underreporting, lack of awareness among healthcare professionals, and limited scientific data still exist. Therefore, strengthening herbal pharmacovigilance systems is essential to enhance the credibility, safety, and effectiveness of herbal medicines in global healthcare.

Pharmacovigilance (PV) is the science and activities related to the detection, assessment, understanding, and prevention of adverse effects or any other drug-related problems. While traditionally focused on synthetic drugs, the principles of PV are increasingly being applied to herbal medicines, traditional remedies, and dietary supplements. This has become crucial due to the global increase in the use of herbal products, driven by factors such as a perception of them being "natural" and therefore safe, and a growing interest in alternative therapies. However, this perception is often flawed, as herbal medicines are not without risks and can cause significant adverse reactions (ADRs) and drug-herb interactions.

1.1 Need for pharmacovigilance of herbal medicine-

The need for robust PV systems for herbal medicines stems from several key factors:

- **Safety Concerns**: Herbal products can cause serious adverse effects, including hepatotoxicity (liver damage), nephrotoxicity (kidney damage), and allergic reactions. Case studies have documented instances of liver injury from ingredients like Tinospora cordifolia and severe skin reactions from metal-based Siddha medicines.

- **Herb-Drug Interactions**: Herbal medicines are frequently used alongside conventional drugs, leading to potential interactions. These interactions can alter the efficacy or increase the toxicity of either the herb or the drug. For example, St. John's Wort is known to interact with various conventional drugs, including antidepressants and oral contraceptives.

- **Adulteration and Contamination**: The lack of standardized manufacturing and quality control can lead to herbal products being adulterated with undeclared potent substances (e.g., steroids, heavy metals) or contaminated with pesticides, microbial toxins, or other hazardous materials.

- **Misidentification of Plants**: Improper identification of plant species used in preparations can lead to unintended toxic effects.

- **Dosage and Formulation Issues**: Unlike conventional medicines, the active ingredients, dosage, and standardization of herbal products can vary significantly. Traditional herbal preparations often contain multiple ingredients, making it difficult to pinpoint the causative agent of an ADR.

1.2 Challenges in Herbal Medicine Pharmacovigilance-
Applying the conventional PV model to herbal medicines presents unique and significant challenges:

1. **Underreporting**: This is a major issue. Both healthcare professionals and the public often fail to report ADRs associated with herbal products, believing them to be safe. Patients may not even disclose their use of herbal remedies to their doctors.

2. **Lack of Standardization and Quality Control**: The quality of herbal products can be highly variable. Factors such as the plant's origin, the season of collection, processing methods, and the presence of adulterants can all influence the safety and efficacy of the final product.

3. **Complex Chemical Composition**: Herbal products are typically a mixture of many chemical compounds, not a single active molecule. This makes it difficult to establish a clear dose-response relationship or identify the specific component responsible for an adverse effect.

4. **Misinformation and Lack of Awareness**: The public's belief in the inherent safety of "natural" products, coupled with a lack of awareness among healthcare professionals about potential risks, contributes to the problem. Many practitioners are not formally trained in traditional medicine, making it difficult to assess and interpret causality reports.

5. **Difficulty in Causality Assessment**: Establishing a causal link between an herbal product and an ADR can be challenging. It requires a detailed understanding of the herb's pharmacology, potential interactions, and a thorough patient history, including the use of all concomitant medications.

6. **Regulatory Hurdles**: In many countries, herbal products are classified as dietary supplements or foods, not medicines. This classification often means they are not subject to the same stringent regulations for safety and efficacy as conventional drugs.

SIGNIFICANCE OF THE STUDY

Traditional medicine is becoming increasingly popular as a result of its lack of or reduced side effects and withdrawal symptoms. Ayurveda, siddha, homoeopathy, Unani, Tibetan, and Chinese medicine are among the traditional medical systems mentioned in the ancient script. The majority of rural populations rely on the Ayurveda system of medicine, which has a 3000 year tradition. The tridoshas ideas, which include Sanskrit terminology like vata, pitta, and kapha, are the foundation of functional ayurveda models. The Sanskrit word 'vata' refers to a mixture of air and water that involves movement and can cause discomfort in various parts of the body if it is out of balance. The Sanskrit word 'pitta' refers to the mix of fire and water that regulates the body's temperature and digestion, while 'kapha' refers to the combination of earth and water that provides strength and nourishment. Any shift in the equilibrium of one of the three doshas can lead to sickness or shifts in the other two doshas.

The primary notion for disease treatment is to remove the causal elements and bring the body's processes back into appropriate balance. Prior to medication, the patient must follow a healthy diet and avoid certain habits. Food can affect gastrointestinal tract, dermatological, neurological, cardiovascular, gynaecological, and orthopaedic illnesses both directly and indirectly. In the therapy of any disease, herb–food interactions are extremely important. In terms of treatment, ayurvedic physicians' advise should always be followed. The treatments in the ayurveda system are given on an empty stomach because they are plant-based and therefore slow-acting. Plants provide approximately 90% of

ayurvedic remedies. Jayasundar, 2014; Kumar et al., 2017). Anemia, asthma, bronchitis, arthritis, cough, cancer, constipation, diarrhoea, diabetes, epilepsy, gastritis, obesity, paralysis, migraine, and other disorders and diseases are treated with Ayurvedic botanicals. (Samy and colleagues, 2008)

REVIEW OF LITERATURE

Begum et al. (2011) investigated the antioxidant and hepatoprotective properties of Andrographis paniculata in Albino rats with Acetaminophen-induced hepatotoxicity. According to the findings of this study, A.Paniculata caused a considerable drop in serum liver marker enzymes as SGOT, SGPT, and ALP. As a result, the ethanolic extract of A.Paniculata was found to have antioxidant and hepatoprotective properties.

Begum et al. (2011) compared the hepatoprotective effect of three Phyllanthus species, P. urinaria, P. niruri, and P. simplex, in CCl4-induced liver injury in rats. According to the findings, treatment of alcoholic events of P. niruri, P. urinaria, and P. simplex orally at three different doses resulted in considerable reversal of increased serum levels of transaminases (GOT, GPT). As a result, only the extracts of P. niruri and P. urinaria were found to protect the liver in a chronic condition. In compared to P. niruri, P. urinaria has a 60 percent hepatoprotective action. As a result, in actual practice, P. urinaria may be an acceptable substitute for P. niruri in cases with liver diseases.

Rasool et al. (2007) investigated the therapeutic effects of Triphala, an Indian ayurvedic herbal preparation, on acetaminophen-induced hepatotoxicity in mice. Triphala extracts (100mg kg 1, bwt-1, i.p.) prevented acetaminophen (900mg kg-1, bwt-1, i.p.) produced hepatotoxicity in mice, as evidenced by decreased serum amino transferases, alkaline phosphatases, inflammatory mediator, and hepatic lipid per oxidation, according to the findings. Acetaminophen-induced hepatic enzyme antioxidant and glutathione depletion were likewise rescued by triphala extract. Triphala therapy appears to reduce acetaminophen-induced hepatotoxicity in rats, according to these findings.

OBJECTIVES OF THE STUDY

1. To extract various phytochemicals from the selected plants.
2. To perform phytochemical analysis of extracted materials.

RESEARCH METHODOLOGY

The chosen plant powder (dried leafs) weighed around 600 gms, and each plant powder (dried leaf) weighed 200 gms. The mixture was mixed with 4000 mL of sterile water and left for 30 minutes. Later, the mixture was cooked until the total volume was one-fourth of its original size (i.e. 1000 mL). The mixture was filtered after cooling. Polyherbal extract-I (Gynandropsis gynandra (Cleome gynandra), Luffa cylindrica (Luffa aegyptiaca), and Artocarpus heterophyllus) and polyherbal extract-II (Gynandropsis gynandra (Cleome gynandra), Luffa cylindrica (Luffa a (Lawsonia Inermis, Euphorbia Nerifolia and Pongamia Pinnata). Previous studies have revealed that plant Artocarpus heterophyllus leaves and Euphorbia Nerifolia leaves have anti-diabetic effect, but there is no evidence for their hepatoprotective potential. It is possible to obtain a synergistic impact of these plants by retaining this repotted plant in each.

Phytochemical screening was used to identify distinct phytoconstituents in the produced polyherbal extraxcts.

PHYTOCHEMICAL SCREENING OF POLYHERBAL PREPARATION

Identification of phytoconstituents in the plant material was carried out using various qualitative and quantitative chemical tests.

FOR NTICANCER ACTIVITY

Preparation of *Memecylon Edule* extracts:

In November, dried leaves of Memecylon Edule were collected in the Tirumala Tirupathi hills. Assistant Professor.K.Madhava chetty Department of Botany, S.V.University, Tirupathi, Andhra Pradesh, India validated the plant material. Shade dried Memecylon edule leaves were crushed to a coarse powder using a high-speed mechanical blender. n-hexane, chloroform, ethyl acetate, ethanol, and water were used to extract the powdered material (500g). Hexane, chloroform, ethyl acetate, ethanol, and water were filtered and concentrated at reduced pressure below 400C in rota vapour (Rotavac, Heidolf, Germany). In addition, preliminary phytochemical screening was carried out.

ASSAY FOR CYTOTOXICITY

In the last few years, scientific methodologies for evaluating natural compounds with biological activity in vivo have evolved. The highly automated bioassay screening based on colorimetric methods to quantify the proliferation of cell cultures is a new invention (Mosmann, 1983; Denizot & Lang, 1986). The assessment of surviving and/or growing mammalian cells is required in many biological tests. This can be accomplished using the MTT assay, which is a non-

radioactive, quick, and cost-effective method for determining cell viability and proliferation. As a result, the MTT assay is used to assess cytotoxicity.

RESULT AND DATA INTERPRETATION

Organoleptic qualities are difficult to assess because there are no standard lab tests and the process necessitates experts with extensive experience. For the Gynandropsis gynandra, Luffa cylindrical, Artocarpus heterophyllus, Lawsonia inermis Linn, Euphorbia nerifolia, and Pongamia pinnata, the following organoleptic qualities of the plant components were surveyed: physical appearance, aroma, and taste.

Table No. 6.1: The organoleptic properties of the plant extracts

Properties	*Gynandropsis gynandra*	*Luffa cylindrica*	*Artocarpus heterophyllus*	*Lawsonia inermis Linn*	*Euphorbia nerifolia*	*Pongamiapinnata.*
Color	Green	Brown	Greenishbrown	Green	Green	Green
Odour	Characteristic	Charact eristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	High Bitter	Bitter	Bitter	High Bitter	Bitter	Bitter

Determination of foreign matter:
Molds, insects, and other pollutants, including animal excreta, should be absent from therapeutic plant materials. Before cutting or grinding therapeutic plant materials for testing, any dirt, stones, sand, dust, or other natural matter had to be removed. For the detection of foreign particles in whole or cut plant materials, microscopic inspection was used. The percentage of foreign matter present in Gynandropsis gynandra, Luffa cylindrical, Artocarpus heterophyllus, Lawsonia inermis Linn, Euphorbia nerifolia, and Pongamia pinnata was determined, and the results are presented in Table.

Table No. 6.2: Determination of foreign matter in the plant extract

S.No	**Name of the plant samples**	**Values obtained (%w/w)**
1.	*Gynandropsis gynandra*	7.7 %
2.	*Luffa cylindrica*	5.3 %
3.	*Artocarpus heterophyllus*	6.4 %
4.	*Lawsonia inermis Linn,*	4.9 %
5.	*Euphorbia nerifolia*	5.5 %
6.	*Pongamia pinnata*	3.9 %

Density, Bulk Density, Tapping density
The densities of Gynandropsis gynandra, Luffa cylindrical, Artocarpus heterophyllus, Lawsonia inermis Linn, Euphorbia nerifolia, and Pongamia pinnata extracts were 5.11-6.98 g/ml, 0.25-0.89 g/ml, and 0.29-0.69 g/ml, respectively. The flow qualities of the plant extract powders may all be classified as excellent or good.

Table No. 6.3: Determination of density, bulk density and tapping density of the plant extract.

S.No	**Name of the plantsamples**	**Density(%w/w) g/ml**	**bulk density g/ml**	**Tapping density g/ml**
1.	*Gynandropsis gynandra*	5.12	0.46	0.69
2.	*Luffa cylindrica*	6.33	0.42	0.57
3.	*Artocarpus heterophyllus*	5.99	0.53	0.50
4.	*Lawsonia inermis Linn,*	6.12	0.26	0.70
5.	*Euphorbia nerifolia*	5.33	0.59	0.46
6.	*Pongamia pinnata*	6.99	0.88	0.30

Ash content of the plants:

Table No. 6.4: Determination of Total ash, Acid soluble ash and Acidinsoluble ash

S.No	Name of theplant	Total ash (%W/W*)	Acid soluble ash (%W/W*)	Acid insolubleash (%W/W*)	Loss on Drying %
1.	*Gynandropsisgynandra*	4.13	0.59	0.69	5.9
2.	*Luffa cylindrical*	3.83	0.25	0.74	5.4
3.	*Artocarpus heterophyllus*	2.89	0.13	0.46	6.4
4.	*Lawsonia inermis Linn,*	3.69	0.59	0.22	2.4
5.	*Euphorbianerifolia*	4.03	0.46	0.52	6.5
6.	*Pongamiapinnata*	5.22	0.99	0.37	4.9

The ash level of the studied plant extracts ranges from 4.13% to 25%. The acid soluble ash concentration ranged from 0.13 percent (Artocarpus heterophyllus) to 0.98 percent (Pongamia pinnata), whereas the acid insoluble ash content was 0.22 percent (Lawsonia inermis Linn) to 0.74 percent (Pongamia pinnata) (Luffa cylindrical). The Luffa cylindrical had the greatest ash content (0.74 percent w/w), indicating the amount of organic and inorganic components contained in plant extract.

CONCLUSION

Plants from Gynandropsis gynandra, Luffa cylindrica, and Artocarpus heterophyllus, and Lawsonia inermis Linn, Euphorbia nerifolia, and Pongamia pinnata. The anti-diabetic and hepatoprotective properties of these formulations were investigated. Plant samples were examined for organoleptic features, such as appearance, color, taste, and odor, as well as physicochemical properties, such as pH, total ash, sulphated ash, acid-insoluble ash, total solids, and specific gravity, as well as phytochemical screening for secondary metabolites (glycosides, alkaloids, tannins, saponins, flavonoids, anthraquinones, anthracene).

The size of the capsule shell that could be employed was largely determined by the bulk density of the contents (Cole, 1987). The bulk densities of Lawsonia inermis and Luffa cylindrica were higher than the bulk densities of the other plant extracts, according to the results of the pre-preparation investigation. The bulking qualities of a powder are determined by how the sample was prepared, treated, and stored, in other words, how it was handled. The particles can be packed to have a variety of bulk densities, and even little disturbances in the powder bed can cause a change in bulk density. The flowability of bulk solids is determined by the range of bulk density and tapping density of the samples (Fitzpatrick, 2003).

In this study, bioactive compounds were extracted from the plants of Gynandropsis gynandra, Luffa cylindrica, and Artocarpus heterophyllus in poly herbal preparation-I, and Lawsonia inermis Linn, Euphorbia nerifolia, and Pongamia pinnata in poly herbal preparation-II using a sequential extraction involving solvents of decreasing polarity (Petroleum ether, eth Because the nature and polarity of the selected plants were unknown, a sequential extraction approach was employed. Petroleum ether is known to extract low-polarity molecules such fatty acids, waxy fatty acids, alkaloids, and terpernoid aglycones (Ayaffor et al., 1994; Cowan, 1999). Flavonoids, tannins, and certain terpenoids are known to be extracted using ethyl acetate, which has a medium polarity (Eloff, 1998; Scalbert et al., 2005). Alcohol, on the other hand, is known to extract highly polar molecules such glycosides, polysaccharides, amino acids, and derivatives (Bruneton, 1999; Scalbert, et al., 2005).

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