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Fluorescence analysis and extractive values of herbal formulations used for wound healing activity in animals

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Abstract
The demand for herbal medicines is increasing because of fewer toxicity and side effects of the medicines. But, the herbal formulation also possess significant untoward activity if used without standardization. The present work focused on some of the standardizing parameters used to assess the quality parameters of polyherbal formulation. The formulation contains rhizome of *Curcuma longa*, leaves of *Murraya koenigii* and *Psidium guajava* were analyzed for fluorescence activity, extraction yield and presence of heavy metals respectively. The results of the study revealed multifaceted fluorescence character has been displayed by herbal formulation and a limit test for heavy metals proved the absence of all heavy metals in the formulation. Among the extractive yield with various solvents, acqueous extract showed highest yield followed by ethanol and methanol respectively. So, the present study concludes, all the ingredients of the herbal combination are within the set limits of WHO Guidelines for Herbal drugs.

Keywords: Herbal formulation, standardizing, fluorescence, extractive yield, heavy metal

Introduction
Plants have been used for medicinal purposes long before pre historic period, food is the major source for serving the nutritional needs, but with growing modernization some traditional methods are being given up. Hence, the modern food habits are affecting the balanced nutrition [1]. Our countries herbal wealth constitutes more than 8000 species and account for about 50% of all higher plant varieties. The emerging field of herbal products industry holds a great potential to the economic development of the Indian region. According to World Health organization (WHO) nearly 80 per cent of the world population depends on traditional medicines. Recent surveys have revealed that almost 50 per cent of the prescription drugs are based on natural products and raw materials. India and China are the largest users of herbal medicines [2]. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. However, some can cause health problems, some are not effective and some may interact with other drugs. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. Standardization starts at the initial stages from the production of quality materials. Quality control plays a major role in the drug production. Standardization of medicinal plants and its extracts have great importance since the cosmetics and nutraceuticals production are important and emerging segments in the global market [3]. Standardization of drugs mean, confirming its identity and determination of its quality and purity. The phytomedicines available in market are standardized herbal preparation consisting of mixture of one or more plants which are used in most countries for the management of multifarious diseases. World health organization has also set specific guidelines for the assessment of safety, efficacy and quality of herbal medicines. Standardization of herbal drugs is not an easy task as numerous factors influence the bioefficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plant, season and area of collection and their extraction and purification processes [4].
Turmeric (*Curcuma Longa*), a widely used traditional medicinal and dietary tuber has been scientifically studied for its anti-inflammatory and choleretic effects and topically used in surgery, wounds, ulcers and burns healing [5, 6]. *Murraya Koenigii* is a highly valued plant for its characteristic aroma and medicinal value.

It is an important export commodity from India as it fetches good foreign revenue. Green leaves are eaten raw for cure of dysentery, diarrhea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders [7, 8, 9]. *Psidium guajava* L. is a small medicinal tree that is native to South America. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments [10]. Leaves, pulp and seeds are used to treat respiratory and gastrointestinal disorders, and as an antispasmodic, anti-inflammatory, as a cough sedative, anti-diarrheic, in the management of hypertension, obesity and in the control of diabetes mellitus. It also possesses anticancer properties [11]. The seeds are used as antimicrobial, gastrointestinal, anti-allergic and anticarcinogenic activity [12-15]. The present study was aimed to standardize herbal formulation containing rhizome of *Curcuma longa*, leaves of *Murraya koenigii* and *Psidium guajava* for wound healing activity in animals.

Material and Methods

Collection, Authentication of plant samples

The rhizome of *Curcuma longa*, leaf parts of *Murraya koenigii* and *Psidium guajava* were collected during the month of Aug - Nov 2017. The identification and authentication of plants and their parts was done by M S Swaminathan Research Foundation and a voucher specimen has been maintained in the Department. The rhizomes and the leaves of plants were after collection, cleaned in running tap water, shade dried. The test samples were then grinded to coarse powder in an iron mortar and pestle. The powdered materials were passed through sieve no 25. All the three powders were of equal quantity (100 g) mixed thoroughly and used for further analysis including extraction.

UV Fluorescence analysis

Take about 0.5gms of plant powder into clean and dried test tubes. To each tube 5ml of different organic solvents like distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, glacial acetic acid, sulphuric acid, nitric acid, hydrochloric acid, 5% FeCl3, 5% I_2, picric acid, 1N NaOH and 1N NaOH + methanol were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible day light and UV light of short wavelength (254 nm) and UV light of long wavelength (365 nm) for their characteristic colour [16].

Determination of extractive values

The dry powdered plant materials of *Curcuma longa*, *Murraya koenigii* and *Psidium guajava* were extracted with petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water using maceration process. Weigh about 5 gm of coarsely powdered plant material and transfer to a dry 250 ml conical flask. Then the flask was filled with 100 ml of the solvent separately. The flasks were corked and kept aside for 24 hrs with frequent agitation for first 6 hours at room temperature than allowed to stand for eighteen hours. The mixtures were filtered through Whatman No. 1 filter paper into a measuring cylinder. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish at 105^0 C to a constant weight and weighed. The extractive value in percentage was calculated by using following formula and recorded [17, 18].

$$\text{Extractive value} = \frac{\text{Weight of dried extract}}{\text{Weight of Plant materials}} \text{X } 100$$

Heavy metal test

The herbal formulation was tested for the presence of cadmium, bismuth and lead respectively by standard protocol [19]

Results and Discussion

Table 1: Components of Polyherbal formulation.

Sl no	Plants	Parts used	Quantity required
1	*Curcuma longa*	Rhizome	50 gms
2	*Murraya koenigii*	Leaves	50 gms
3	*Psidium guajava*	Leaves	50 gms

Table 2: UV fluorescence analysis of Herbal formulation

S. No	Experiments	Visible light	UV Fluorescence	
			254 nm	365 nm
1	Powder as such	Darkish green	Light green	Dark green
2	Powder + 1N Acqueous NaOH	Greenish yellow	Light blue	Dark blue
3	Powder +1N Alcoholic NaOH	Yellowish green	Dark green	blue
4	Powder + 1N HCl	Dark green	Dark blue	Blue
5	Powder + conc. H_2SO_4	Brown	Dark green	Dark brown
6	Powder + 50% H_2SO_4	Light brown	Green	Dark green
7	Powder +conc. HCl	Light green	Green	Dark green
8	Powder +conc. HNO_3	Brown	Fluorescent green	Dark green
9	Powder + 50% HNO_3	Yellowish green	Fluorescent green	Violet
10	Powder +Acetic acid	Yellowish green	Light green	Darkish green
11	Powder +Ferric chloride	Brown	Yellow	Darkish yellow
12	Powder + NH_3	Green	Fluorescent green	Darkish green
13	Powder +Benzene	Green	Fluorescent green	Blue
14	Powder +Petroleum ether	Light green	Fluorescent green	Blue
15	Powder + Chloroform	Green	Dark green	Blue
16	Powder +Acetone	Yellowish green	Light green	Blue
17	Powder +Ethyl acetate	Yellowish green	Greenish blue	Dark brown
18	Powder +Acetonitrile	Dark brown	Dark green	brown
19	Powder + Di ethyl ether	Greenish yellow	Fluorescent green	Dark brown
20	Powder + Picric acid	Yellow	Pale yellow	Pale yellow
21	Powder +2 propanol	Brown	Fluorescent yellow	brown

22	Powder +Methanol	Light yellow	Light blue	Blue
23	Powder +Ethanol	Yellowish	Fluorescent yellow	Blue
24	Powder +Water	Brown	Yellowish	Yellowish

Table 3: Heavy metal testing for Herbal formulation

S. No	Experiment	Observation	Results
Test for Cadmium			
1	NH_4OH added in to the sample solution	White precipitate is absent	Absent of cadmium
2	Potassium Ferrocyanide added to the sample solution	White precipitate is absent	Absent of cadmium
Test for Bismuth			
1	H_2S gas added in the sample solution	Dark brown precipitate is absent.	Absent of bismuth
2	NH4OH added in to the sample solution	White precipitate is absent	Absent of bismuth
Test for Lead			
1	Dilute Hcl added in sample solution	White precipitate of CaCl2 is absent.	Absent of lead
2	KI is added in sample solution.	Yellow precipitate is absent.	Absent of lead

Table 4: Extractive value of Herbal formulation in different solvents and their yield

S. No	Type of extract	Amount of extract (gms)	Yield in % w/w
1	Petroleum Ether	3.72	1.86
2	Chloroform	9.94	4.97
3	Ethyl Acetate	12.46	6.23
4	Ethanol	15.78	7.89
5	Methanol	16.42	8.21
6	Water	26.46	8.23

According to the World Health Organization (WHO, 1998), before testing any herbs for the corresponding pharmacological activity, it needs to be standardize by a set of guidelines for establishing identity and purity of the drug materials [20]. In the present work an attempt has been made to assess the polyherbal formulation intended for wound healing activity by standardizing some parameters according to the guidelines put forth by WHO. Fluorescence is an important phenomenon displayed by various phytoconstituents present in plant materials. Some show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products, which do not visibly fluoresce in daylight. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents and chemicals though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation [21-23]. The results of fluorescent analysis of leaf powder of polyherbal formulation was depicted in Table.02, the herbal formulation showed characteristic coloration upon treatment with multifarious chemical reagents.

The presence of heavy metals in herbs is an indicative of its purity and contamination, the heavy metals like Arsenic, Lead and Cadmium have been shown to be contaminants of herbal ingredients [24, 25]. A simple limit test for the detection of heavy metals found in many pharmacopeias, the protocol and its procedure and results has been shown in Table 03. A chemical test performed for cadmium, bismuth and lead detection in the herbal formulation, as the result shows, the sample is negative for heavy metals.

The extraction yield (mass of extract / mass of dry matter) used as an indicator of effects of the extraction conditions [26]. In the present experiment the extraction yield has increased with respect to the polarity of solvents. The maximum yield was noticed in acqueous extract. Possibly, this might be due to the increased polarity of water and at elevated extraction temperature, water has similar dielectric constants as that of organic solvents like methanol. Direct heat generation within the volume with a significant impact on heating kinetics and also on the pressure effects on the cell wall membrane structure resulting into the higher and faster diffusion or partition rate of the solute from the solid matrix into solvent may be the probable reason for the highest yield in acqueous extract and also may be due to cavitational effects caused by high intensity ultrasound [27, 28].

Estimation of extractive value determines the amount of the active constituents in a given amount of plant material when extracted with solvent. The extractive value of extracts of herbal formulation containing *curcuma longa* rhizome, *murraya koenigii* leaf and *psidium guajava* leaf was investigated and presented in Table No. 04. From the present study it was found that, the extractive value of herbal formulation in acqueous extract was maximum (26.46%) as compare to other extracts. The methanolic extract showed slightly lower extractive value (16.42%) than ethanolic extract.

Conclusion

In developing countries more than 80 per cent of the population relies on traditional medicines, mostly plant drugs, for their primary healthcare. The present study was conducted to evaluate the standardizing parameter for herbal preparations used for wound healing activity with respect to fluorescence activity, heavy metal and extractive activity. The herbal extract showed varied fluorescence character which is an essential parameter for standardization of herbs and the formulation was free from any heavy metals. Furthermore, the extractive value of the formulation was assessed to find the effective solvent for extraction process and to get an idea about the nature of chemical constituents present and expressed in combination. In the present study, acqueous extract showed highest yield as compared to ethanol and methanol solvents. From the present study it can be concluded that the herbal formulation was in accordance with the guidelines set forth by World health organization (WHO). The formulation can further carried to understand the pharmacological activity against wound healing models either invitro or *in vivo* studies by characterizing and isolation of active ingredients.

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Conflict of Interest: none declared

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