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Preliminary Phytochemical Investigation, Fluorescence analysis and Determination of Ash Content of leaf extracts

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Abstract
The demand for herbal medicines is increasing because of fewer toxicity and side effects of the medicines. The conventional medicine system engages the exercise of various plant extracts or active principles. Ethno medical study intensely signifies that one of the greatest opportunity in searching novel cost-effective plants for medication. This investigation generally provides the health claim at reasonable price. The present study is aims to assess the preliminary qualitative and quantitative phytochemical constituents in hexane, Ethyl acetate, Ethanol and Hydro alcohol of *Plectranthus mollis, Elaeagnus conferta* and *Grewia tilaefolia* leaf extracts. These leaf extracts were subjected to Physicochemical analysis investigations like Fluorescence report for the leaf extracts and Determining Ash value of leaf extracts for the identification of different Parameters.

Keywords
Plant extracts, Phytochemical, Fluorescence, qualitative investigation, Ash content.

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1. INTRODUCTION
The Phytochemicals are naturally occurring substances in medicinal plants to treat various diseases. The Phytochemical screening as qualitative analysis to explore the Phytochemicals present in various parts of plants Dalbergia sissoo and to examine the therapeutic uses. The medical plants that had been investigated are the rich source of natural medicinal agents [1-2]. Dalbergia sissoo, the hard woody plants of hilly area and river side area had been reported for its medicinal importance from original age [3]. The plants leaves, pod and bark of various extracts with ethanol, aqueous and ethyl acetate has been recognized through Phytochemical screening test includes a series of Phytochemicals as flavonoids, tannins, polyphenols, reducing sugars, carbohydrates, proteins, saponins, glycosides, steroids and terpenoids, free amino acids, anthraquinones, alkaloids fats and oils [4-5]. Various observational studies indicate the regular consumption of foods including flavonoids may reduce the risk of several diseases including fever,

pain reliever, neurodegenerative diseases and certain forms of cancer [6].

The herbal formulation also possesses significant unpleasant activity if used without standardization. The present work concentrate on some of the standardizing parameters used to assess the quality parameters of polyherbal formulation. The formulation contains rhizome of Curcuma longa, leaf 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 comprehensive fluorescence character, 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, aqueous extract showed highest yield followed by ethanol and methanol correspondingly [7].

Phytochemical investigation of the methanolic and aqueous extracts of Faidherbia albida legumes indicated the presence of terpenes, cardiac glycosides, monosaccharides and carbohydrates type of compounds in both extracts. While alkaloids and saponins were found in aqueous extract only, flavonoids were found to be missing in both extracts. The aqueous and methanolic extracts exhibited a potent growth inspiration effect. Inhibition of both the rootlet and shoot showed a dose dependent response. Aqueous extract has a greater inhibitory effect on rootlet growth than shoot growth. The methanolic extract has a greater inhibitory effect than the aqueous extract. Both extracts and some fractions were tested against three pathogenic bacterial species; Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, pathogenic fungal species; Fusarium oxysporum, Alternaria alternate, and Aspergillus niger. Most of the plant extracts inspire the studied fungal growth specially the aqueous extract. Meanwhile, it shows interesting results by inhibiting the growth of the studied pathogenic bacterial species with most extracts and fractions [8].

Medicinal plants are playing a key part in traditional systems for the remedy of various maladies. However, the primary obstacle of using conformist medicine in the developed nations is absence of documentary proof and stringent quality control measures. There is need of documentation for the research work carried out on traditional medicines and in this context, with the present increase in the phytotherapeutics, the availability of genuine plant material becoming food shortage. With this difficulty, there is need to standardize the plants and its parts to be utilized as a medicine. The process of standardization can be acquired by step wise pharmacognostic and phytochemical studies. Proper identification and quality assurance of beginning material is a significant stride to guarantee reproducible quality of herbal medicine which will helps us to confirm its safety and effectiveness [9].

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 necessary 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 construction. Standardization of medicinal plants and its extracts have great magnitude since the cosmetics and nutraceuticals production are important and emerging segments in the global market [10]. Standardization of drugs mean, confirming its identity and determination of its quality and purity. The phytomedicines on hand in market are standardized herbal preparation consisting of mixture of one or more plants which are used in most countries for the management of different 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 several factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken from the proper classification of plant, season area of collection their extraction and purification processes [11]. The present studies are preliminary phytochemical investigation, fluorescence analysis and determination of ash content of leaf extracts for the identification of special parameters.

2. MATERIALS AND METHODS

2.1 Phytochemical analysis of leaf extracts

2.1.1 Preparation of extracts

Crude Sample extract was prepared by Soxhlet extraction method. About 20g of powdered sample material was uniformly packed into a thimble and extracted with 250ml of different solvents (Hexane, Ethyl acetate, Ethanol and Hydroalcohol). The process of extraction was continued for 24 hours or till the solvent in siphon tube of extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40^{0}C till all the solvent gets evaporated. Dried extract was kept in refrigerator at 4°C till future use.

Plectranthus *mollis* **Elaeagnus *conferta*** **Grewia *tilaefolia***

Image:1 Experimental plants images

2.1.2 Phytochemical screening

Preliminary phytochemical analysis was carried out for all the extracts of the sample as per standard methods described by Brain and Turner 1975 and Evans 1996.

2.1.2.1 Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

a) **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Mayer's reagent: Mercuric chloride (1.358g) was dissolved in 60ml of water and potassium iodide (5g) is dissolved in 10ml of water. The two solutions are mixed and made up to 100ml with water.

b) **Wagner's test**: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Wagner's reagent: Iodine (1.2g) and potassium iodide (2g) is dissolved in 5ml of water and made up to 100ml with distilled water.

2.1.2.2 Detection of Flavonoids

a) **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) **H_2SO_4 test**: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

2.1.2.3 Detection of Steroids

Liebermann- Burchard test: 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

2.1.2.4 Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inner face indicates the presence of terpenoids.

2.1.2.5 Detection of Anthroquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

2.1.2.6 Detection of Phenols

a) **Ferric chloride test**: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

b) **Lead acetate test**: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

2.1.2.7 Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

2.1.2.8 Detection of Tannins

2.1.2.9 Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

2.1.2.10 Detection of Carbohydrates

a) **Fehling's test:** 0.2g filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

b) Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

c) Fehling's solution B: Potassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

2.1.2.11 Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

2.2 Physico chemical analysis

2.2.1 Physicochemical evaluation

The following physicochemical parameters were carried out (WHO, 2002; The Ayurvedic Pharmacopoeia of India 2008; Vaghasiya*et al*., 2008) in dried powder sample of *G.tilifolia*.

2.2.2 Fluorescence analysis

The powdered drug was examined under UV and ordinary light with different reagents. About 10gms of the powdered drug was taken in a petridish and treated with different reagents viz., Aqueous, aqueous sodium hydroxide, 50% sulphuric acid, 1N hydrochloric acid and 1N methanolic sodium hydroxide. These were observed under different wavelengths *i.e*., visible rays and ultraviolet rays (254 nm and 365 nm). Various colour radiations emitted were observed and noted (Pradhan and Dayal, 1981).

2.2.3 Total ash (Mukherjee, 2008**)**

About 3 gm of the powdered drug was accurately weighed and taken in a silica crucible, which was previously ignited and weighed. The powdered drug was spread in a fine even layer at the bottom of the tarred crucible. The crucible was kept inside the muffle furnace and the temperature increased to make it dull red hot not exceeding 450°C until free from carbon. The crucible was cooled, kept in desiccators and weighed. The procedure was repeated to obtain the constant weight. The percentage of total ash was calculated with reference to the air dried drug. The total ash value of the sample was noted.

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis of leaf extracts

The presence of Phyto constituents make the plant useful for treating different diseases and have a potential of providing useful drugs of human use. The present studies have found that most of the biologically active Phytochemicals were in Hexane, Ethyl acetate, Ethanol and Hydro alcohol extracts of Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia after further investigations.

Table: 1 Phytochemicals screening of leaf extracts in different solvents

Plant Name	*Plectranthus mollis*				*Eleagnus conferta*				*Grewia tilaefolia*			
Phytochemicals	**Hexane**	**Ethyl acetate**	**Ethanol**	**Hydro alcohol**	**Hexane**	**Ethyl acetate**	**Ethanol**	**Hydro alcohol**	**Hexane**	**Ethyl acetate**	**Ethanol**	**Hydro alcohol**
Alkaloids												
Mayer's test	-	-	+	+	-	-	+	+	-	-	+	+
Wagner's test	-	-	+	+	-	-	+	+	-	-	+	+
Flavonoids												
Lead acetate test	+	+	++	++	+	+	++	++	-	-	+-	+
H2SO4 test	+	+	+	++	+	+	++	++	-	-	+-	
Steroids												
Liebermann-Burchard test	+	+	++	+	+	+	++	++	+	+	+	+
Terpenoids												
Salkowski test	-	+	+	+	-	+	++	++	-	+	+	++
Arthroquinone												
Borntrager's test	-	-	-	-	-	-	+	+	-	-	-	+
Phenols												
Ferric chloride test	-	+-	+	++	-	+-	++	++	+-	+	++	++
Lead acetate test	-	+-	+	++	-	+-	++	++	+-	+	++	++
Saponin	-	-	-	+	+	-	++	++	-	-	+-	+
Tannin	-	-	-	-	-	-	+	+	-	-	-	+
Carbohydrates	+	+	+	++	+	+	+	++	+	+	+	++
Oils & Resins	+	+	-	-	+	+	-	-	+	+	-	-

Note: – Negative = Absent. + Positive = Present.

The medicinal properties are explored due to the presence of above-mentioned Phytochemicals. These Phytochemicals are essential for our body to keep it healthy. They have healing properties for various diseases. It is necessary to explore their therapeutic uses in different diseases and to explore the exact Phytochemical for peculiar disease. The presence of various Phytochemicals in *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* extracts of Hexane, Ethyl acetate, Ethanol and Hydro alcohol in leaf were found to be therapeutic in various diseases. From phytochemical screening, we observed that the Ethanol and Hydro alcohol extracts gave a positive result with maximum test, which indicated the presence of Alkaloids, Flavonoids, Steroids, Terpenoids, Arthroquinone, Phenols, Saponin, Tannin, Carbohydrates, Oils and Resins in both extracts. Based on the general test for the ferric chloride test and Lead acetate test for phytochemicals gave positive results in both extracts. Test for tannins and phenolic compounds gave positive results in maximum extract.

3.2 Physicochemical analysis

3.2.1 Fluorescence analysis

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material under UV light. This can be used to characterize the crude drugs [13]. It is also one of the pharmacognostic procedures useful in the identification of authentic samples and recognizing adulterants [12]. Fluorescence studies helps in the identification of drugs that are more or less difficult to distinguish. In a mixture of drugs of two or more species, fluorescence studies help to identify a particular drug by the use of estimates of intensity of fluorescence. The comparison of the unknown should be made with a sample of known identity. The fluorescence analysis of extracts of leaf of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* were observed under visible light and under UV light (365 nm) and the results were recorded in the Table 2.

S.No	Test	Plant	Observation under different wave lengths		
			White light	Long wavelength	Short wavelength
		Plectranthus mollis	green	Light Red	Light green
1	Sample + H_2SO_4	*Eleagnus conferta*	Pale green	Pale Red	Light green
		Grewia tilaefolia	Dark green	Red	Dark green
		Plectranthus mollis	Green	Dark red	Red
2	Sample + NaoH in water	*Eleagnus conferta*	Light brown	Red	Straw
		Grewia tilaefolia	Brown	Brick red	Brick red
		Plectranthus mollis	Pale green	Straw	Light green
3	Sample + NaoH in Methanol	*Eleagnus conferta*	straw	Pale green	Pale green
		Grewia tilaefolia	Light straw	Light green	Straw
		Plectranthus mollis	Light green	Green	Green
4	Sample + HCL	*Eleagnus conferta*	Green	Brown	Light green
		Grewia tilaefolia	Light green	Fluorescent orange	Light green
		Plectranthus mollis	Light yellow	Pale Green	Fluorescent orange
5	Sample + Water	*Eleagnus conferta*	Light Green	Pale green	Pale green
		Grewia tilaefolia	Light straw	Light green	Straw

Table: 2 Fluorescence analysis of leaf extracts of plant

A correlation exists between a compound present in the drugs and their fluorescent behaviour under different conditions. Fluorescent study of extracts of leaf of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* showed characteristic colouration under visible light and UV light (365 nm). Florescence analysis of different extracts of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* leaves gives a clue whether these extracts are in adulteration, thus can be used as a diagnostic tool for testing the adulteration. Such studies were done previously in *Morinda tinctoria* [14] and *Abutilon indicum* [15]. Colour variation was observed in visible light and UV light and it can be used as a standard parameter for quality control of the drug. The quality control is necessary if plant products are to fill the needs for cheap and reliable medicines or when natural products are to be used as template for new drug molecules.

The fluorescent colour is specific for each compound. Plant materials give different colouration when treated with various chemicals. Some plant

constituents showed characteristic fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, addition of different reagents results in the conversion into fluorescent derivatives or decomposition products. Crude drugs are often assessed qualitatively in this way and fluorescence analysis was an important parameter for pharmacognostic evaluation of crude drugs [16]. The colour formation with respect to the particular reagents was noted and was aid in the determination of quality and purity of the leaf powder. For fluorescence analysis, the powdered samples of leaves of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* were treated with various chemical agents and were observed under visible light as well as under UV light (356 nm). The results obtained are reported in Table 2.

The crude drug when viewed under UV light showed different fluorescence at different wavelengths. This is due to the presence of different phytochemical constituents in the drug [17]. Flavones which are light yellow in aqueous condition, under UV light, turns to bright yellow under alkaline conditions. Phytosterols, when treated with 50% H_2SO_4 shows green fluorescence under UV light. Coumaric acid appears yellowish green in alkaline condition under UV radiation. Terpenoids, exhibits yellow green fluorescence under short UV light [18]. Berberin showed light yellow colour of fluorescence [19]. According to World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant was the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [20].

The results of the fluorescence analysis of various extracts and powder of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* leaves showed characteristic colouration on treatment with various chemical reagents. The major bioactive compounds present in the crude drugs of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* were found to be flavones, sterols, terpenoids and berberin. Proper control of starting material was most essential one for ensuring the reproducible quality of herbal drugs. In recent years there has been a great emphasis in the standardization of medicinal plants because of therapeutic benefits. Pharmacognostical studies are more reliable, accurate and inexpensive means for the identification and evaluation of plant drugs. Pharmacognosy is a simple and reliable base, by which complete information of the crude drug can be obtained.

3.2.2 Ash value

Ash value is a validity parameter to assess the degree of purity and in evaluating the quality of crude drugs [21]. Ash constitutes the inorganic residues obtained after complete combustion of a drug. It indicated the presence of various impurities like carbonate, oxalate and silicate. Total ash represents the total content of physiological ash and non-physiological ash. Water soluble ash was the content of total ash soluble in hot water and acid insoluble ash was represented by the non-physiological ash especially sand and soil [22].

S.No	Parameters analysed	Plant	(%W/W)
1	Total Ash	Plectranthus mollis	9.25
		Eleagnus conferta	7.15
		Grewia tilaefolia	10.26
2	Water Soluble Ash	Plectranthus mollis	1.2
		Eleagnus conferta	1.06
		Grewia tilaefolia	2.15
3	Water Insoluble Ash	Plectranthus mollis	7.13
		Eleagnus conferta	5.23
		Grewia tilaefolia	8.06
4	Acid Soluble Ash	Plectranthus mollis	5.2
		Eleagnus conferta	5.18
		Grewia tilaefolia	8.23
5	Acid Insoluble Ash	Plectranthus mollis	0.65
		Eleagnus conferta	0.02
		Grewia tilaefolia	1.58
6	Sulphated Ash Value	Plectranthus mollis	0.74
		Eleagnus conferta	0.09
		Grewia tilaefolia	0.96

Table: 3 Ash value of leaves of leaf extracts

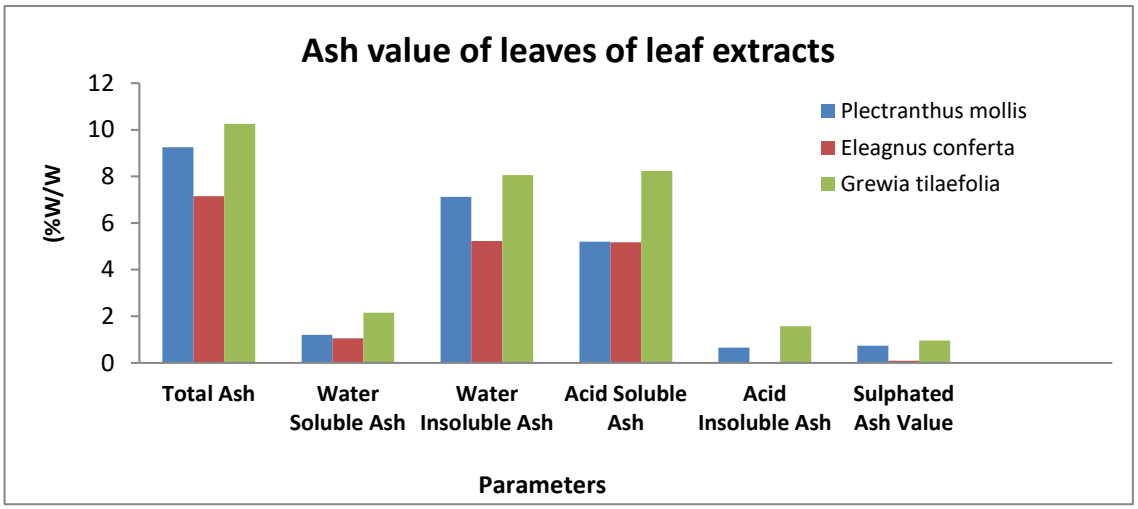

Image: 2 Graphical representation of Ash value of leaves of leaf extracts of Hydro alcoholic leaf extracts

The purpose of ashing plant material was to remove all traces of organic matter that might otherwise interfere in analytical determination. The total ash value represents both physiological and non-physiological ash. Physiological ash is the ash inherent in the plant due to biochemical processes and the non-physiological ash is the contaminants from the environment. These may be carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals which were taken up from the soil [23]. For the evaluation of purity of drugs, total ash value was particularly important. A high percentage of total ash value revealed the presence of inorganic constituents and very low value of acid insoluble ash indicated the presence of negligible amount of siliceous matter. The acid insoluble ash was a part of total ash that was insoluble. Water soluble portion of the total ash constitutes the water soluble ash. Water soluble ash can be used as an important indicator for the presence of exhausted material.

In this evaluation, in leaf extracts, total ash value was higher followed by acid soluble ash and water insoluble ash. The total ash, acid soluble ash and water insoluble ash were found to be higher in *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* leaf extracts respectively. Appreciable amount of ash values obtained for leaf powder of *Plectranthus mollis and Grewia tilaefolia* implied that the leaf powder of the two plants had higher organic content and fairly low inorganic content.

4. CONCLUSIONS

The Phytochemical screening provides the qualitative analysis to explore the presence of flavonoids, tannin carbohydrate, reducing sugars, antroquinones, steroids and phenoids, saponins, glycosides, alkaloids, proteins, free amino acids, oils and fats. Flavnoids have been possess potent antipyretic, analgesic and antihistaminic properties. These enhanced to cure many diseases. To therapeutice effect the plant leaf extracts of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* reveals to be significant.

Pharmacochemical analyses of medicinal plants are a parameter of quality control. So it becomes a necessary step in the study of pharmacognostic characteristic of the plant before its use in the field of research and also in pharmaceutical formulation. From this study, it may be concluded that the analysis showed the purity of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* leaf extracts. Moreover, extracts yielded good results. The results of phytochemical analysis of *Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia* plants leaf extracts of Hexane, Ethyl acetate, Ethanol and Hydro alcohol in leaf were found to be therapeutic in various diseases. From phytochemical screening, we observed that the Ethanol and Hydro alcohol extracts gave a positive result with maximum test, which indicated the presence of Alkaloids, Flavonoids, Steroids, Terpenoids, Arthroquinone, Phenols, Saponin, Tannin, Carbohydrates, Oils and Resins in both extracts. Based on the general test for the ferric chloride test and Lead acetate test for phytochemicals gave positive results in both extracts. Test for tannins and phenolic compounds gave positive results in maximum extract.

The total ash, acid soluble ash and water insoluble ash were found to be higher in Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia leaf extracts respectively. Appreciable amount of ash values obtained for leaf powder of Plectranthus mollis and Grewia tilaefolia implied that the leaf powder of the two plants had higher organic content and fairly low inorganic content.

The results of the fluorescence analysis of various extracts and powder of Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia leaves showed characteristic colouration on treatment with various chemical reagents. The major bioactive compounds present in the crude drugs of Plectranthus mollis, Eleagnus conferta and Grewia tilaefolia were found to be flavones, sterols, terpenoids and berberin. Proper control of starting material was most essential one for ensuring the reproducible quality of herbal drugs. In recent years there has been a great emphasis standardization of medicinal plants because of therapeutic benefits. Pharmacognostical studies are more reliable, accurate and inexpensive means for the identification and evaluation of plant drugs. Pharmacognosy is a simple and reliable base, by which complete information of the crude drug can be obtained. All these plants may be a good source of minerals to treat number of diseases that are mainly caused due to the deficiency of those minerals and can be utilized in Ayurvedic system to cure disease. So these extracts can be used for further studies of this plant material as potential source in pharmaceutical preparations.

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