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Identification and Quantitative Estimation of Claimed Components in Herbal Products

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ABSTRACT- In this study 7 Natural Samples have been taken namely S1 (Neem), S2 (Pudina), S3 (Alovera), S4 (Tulsi), S5 (Turmeric), S6 (Pepper), S7 (Sauf). All the samples were extracted with standard extraction procedure using Buffer, Ethanol, Methanol, Chloroform reagent. The present work is concerned with the identification and quantities estimation of herbal products based on the application of Paper Chromatography, SDS PAGE and Quantitative Determination. Examination of the entire Natural and suspected Herbal product (drug) sample were based on the Rf Value, Band formation and O.D Value. The accurate percentage was analysed using these methods and then compare with that claimed on the product. In many of the samples scenario was totally different i.e the claimed percentage on the product was not at all matching.

INTRODUCTION-

Herbal drugs have been used since ancient times as medicines for the treatments of range of diseases. [2].Medicinal plants have played a key role in world health. There is a growing focus on the important of medicinal plants in the traditional health care system (Ayurveda, unani homeopathy, yoga) in solving health care problems. Systematic approach and well design methodologies for the standardization of herbal raw material and herbal formulation are developed. In view of the growing interest in herbal medicines, methods for standardization of herbal drugs are developed and used different formulation. Standardization of drugs means confirmation of its identity and determination of its quality and purity phototherapeutic agents or phytomedicines are standardized herbal preparations consisting of complex mixture one or more plants which are used in most countries for the management of various diseases.[3] The importance of medicinal plants in the traditional health care system (Ayurveda, unani Homeopathy, yoga) is solving health care problems. [4] Medicines are designed to prevent or treat illnesses or relive symptoms. Any effective medicine can cause side effects. Side effects may not be discovered until many people have used the medicines over a period. These allopathic drugs are although popular in the country. Some patient avoid to take allopathic medicine for different diseases such as diabetes , blood pressure , weight loss, weight gain etc. [6]because they do not rely on allopathic drugs as they have their side effects. Therefore, they move to herbal products, the aim of this study is to identify the ingredients in the Herbal products as the claimed quantity of Natural product in such Herbal product are not in sufficient amount to affect the body in a positive manner, where as in some cases the quantity exceeds the prescribed limit which may also affect adversely.

METHODOLOGY

Table:-1 Different type of Natural Herbal and Herbal product samples.

S.N	Sample No	Name of Herbal Product	Name of Claimed component
1	(S1)	Neem Alovera	Neem
2	(S2)	Pudin Hara	Pudina
3	(S3)	Neem Alovera	Alovera
4	(S4)	Sardi ja	Tulsi
5	(S5)	Sardi ja	Turmeric
6	(S6)	Sardi ja	Pepper

7	(S7)	Divya udar kalp	Sauf

Extraction Method:-.Taken 1g of the required plant sample (leaf, stem) and wash or rinse the samples with distilled water to minimize any contamination then grinded the samples in mortar and pestle by adding 1ml of extraction buffer and Incubated the samples at 55°C for 15 minutes in water bath then Cooled and centrifuged the sample at 8000 rpm for 2-3 minutes and Collected the sample after centrifugation and pipette out the supernatant or upper aqueous layer**,** Preserved the supernatant and marked as sample - 1.To the pellet, added 1ml of Ethanol (100%) and vortex the sample and centrifuged the sample at 8000 rpm for 2–3 minutes**.** Collected the supernatant and preserved it as **Ethanol Extract, Methanol, and Chloroform.**

(1)-Paper chromatography:-

Take samples on paper, run sample at 1 hour, and then incubate sample at 37°C for 30-40 minute.
Noted the value of distance travelled by solvent and solute in different mobile phase and calculated the Rf value by using following formula-Rf value=Distance travel by Solute/ Distance travel by solvent.

(2)-Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE)

A-Resolving gel:-Separation according to their molecular weight and mobility of the molecule are free in resolving gel.

B-Stacking gel:-Stacking gel help in segregation of molecule forming path at in stacking.

Sample processing for SDS GEL:-Pipette out 50µl of sample and 50µl of 10x sample buffer into new appandrofe tube then incubate the sample at 50°C for 2 minute and taken out the sample, cooled the sample and add into the well of stacking gel ,After loading the samples into the wells, run the sample at a voltage of 50-100 for 45-1hours **,** After 1-hour turn of the power, take out a gel slap carefully and place into the staining tray or rack and 2times staining and de-staining the sample one by one with the help of staining and destining solution then Keep on the sample over night for better staining and Observe the gel plate and consider the different band of the compound. Which molecule or compound get segregated depending on their molecular weight and forming band at regular interval.

(3)-Quantitative determination of drug (conc.):-

Concentration of the sample is different in different amount and noted the value of concentration after that calculate the O.D value by using following formula-

Mean concentration of the sample = O.D value of Unknown 1 + O.D Value of Unknown 2 /2.

Graph:- Graph is plotted between O.D value and Concentration value.

RESULT AND DISSCUTION

Table 2:- R*f* value of Natural and Suspected Herbal Product in different mobile phase (S1) to (S7).

S. NO	NATURAL SAMPLE PRODUCT				CLAIMEDE HERBAL PRODUCT		
	Mobile phase	Distance travel by solvent	Distance travel by solute	Rf Value	Distance travel by solvent	Distance travel by solute	Rf Value
S1	Ethanol	30	29.3	1.02	31	29.0	1.06
S2	Ethanol	30	29.8	1.00	30	29.9	1.00
S3	Ethanol	30	28.7	1.04	31	29.0	1.06
S4	Ethanol	30	30	1	29.5	29.0	1.01
S5	Ethanol	29.5	28.7	1.02	30	29.5	1.01
S6	Ethanol	29.5	29.5	1	30	27.1	1.10
S7	Ethanol	29.5	29.5	1	31	29.8	1.04

Table 2 Shows that the difference in Rf value of Natural compound and suspected Herbal Product indicate that their might be difference in their composition or the quantity of claimed in Natural product is either increased or decreased in the suspected Herbal product.

SDS PAGE

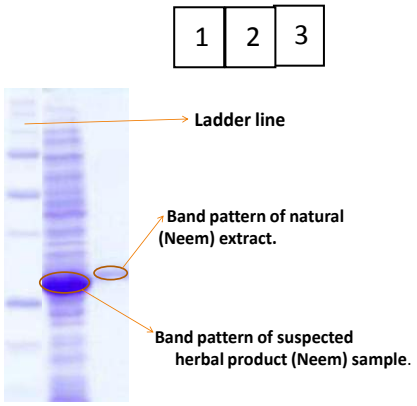

Fig-1:Line 1 Ladder line, Line 2 Suspected Herbal product line and Line 3 Neem extract line.

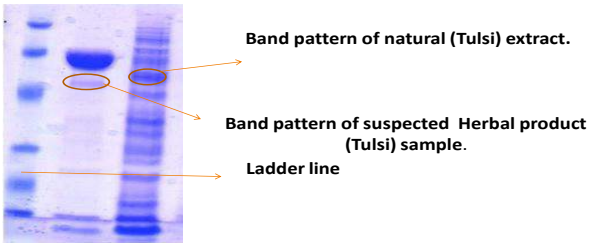

fig-4Line 1-Ladder line,Line 2-Tulsi Herbal product line and Line 3-Tulsi extract line.

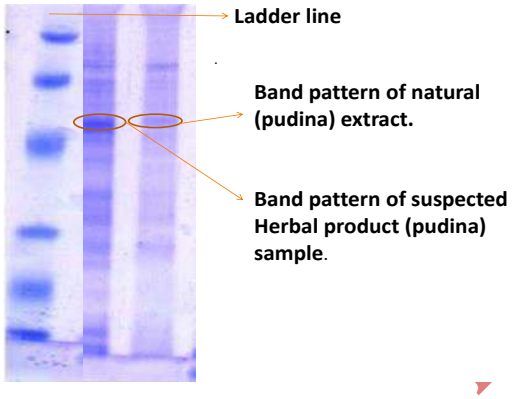

Fig-2:Line 1-Ladder line, Line 2- Suspected Herbal product line, and Line 3-Puddina extract line.

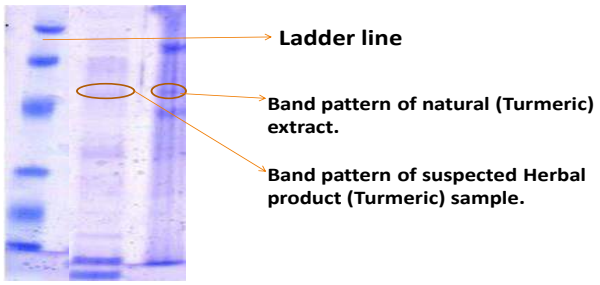

fig-5: Line 1- Ladder line, Line 2-Turmeric Herbal product line and Line 3-Turmeric extract line.

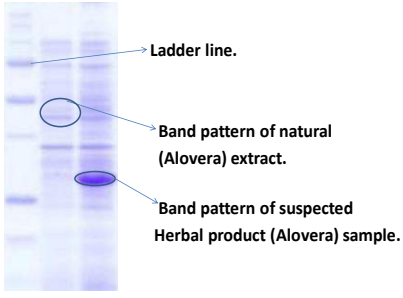

fig-3:Line 1-Ladder line, Line 2-Alovera extract line and Line -3 Alovera Herbal product line.

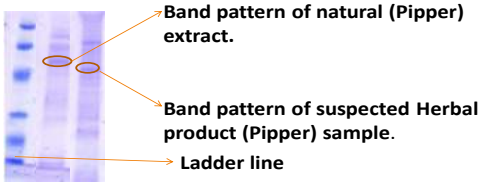

fig-6:Line 1- Ladder line, Line 2-Pepper extract line and Line 3-Pepper Herbal product line.

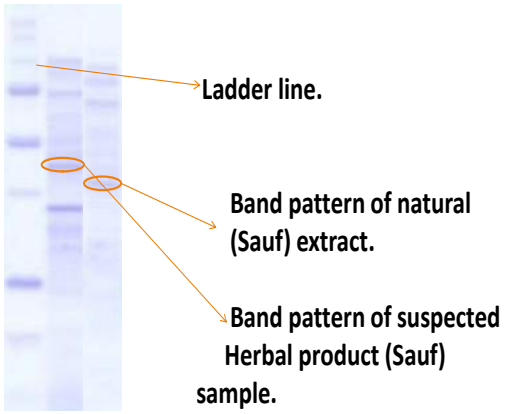

fig-7:Line 1- Ladder line, Line 2-Sauf Herbal product line and Line 3-Sauf extract line.

The electrophoretogram is shows in fig 1, 3, 4, 5,6and7 clearly indicate a difference in band pattern of Natural compound and Herbal product suggesting a variation in components of the Herbal product or a difference in the quantity of the claimed Natural compound in the suspected Herbal product.

Table 3:-For the determination of quantity, following parameters were taken.

S. NO	Unknown Sample 1	Unknown Sample 2	Quantity of Natural sample	Quantity of Claimed Herbal Product
S1	2.69 mg/ml	3.08 mg/ml	2.88 mg/ml	2.5 mg/ml
S2	2.82 mg/ml	4.2 mg/ml	3.51 mg/ml	3.50 mg/ml
S3	1.28 mg/ml	1.8 mg/ml	1.54 mg/ml	2.0 mg/ml
S4	2.6 mg/ml	4.0 mg/ml	3.3 mg /ml	20 mg/ml
S5	26.7 mg/ml	3.7 mg/ml	15.2 mg/ml	2.0 mg/ml
S6	1.41 mg/ml	2.29 mg/ml	1.85 mg/ml	1.2 mg/ml
S7	3.0 mg/ml	4.8 mg/ml	3.9 mg/ml	400mg/ml

Mean concentration of the sample = O.D value of Unknown 1 + O.D Value of Unknown 2 /2.

Graph:- Graph is plotted between O.D value and Concentration value.

Table:3 shows that the sample of Herbal product showing variation in Rf and band pattern was analysed quantitatively to estimate the quantity of Natural product in the suspected Herbal product, on testing the sample by pH conductometer, it was observed that the quantity of Neem in Herbal product sample Neem Alovera was more than it is claimed on the product.

SUMMARY AND CONCLUSION

In present study 7 Samples were taken such as S1(Neem), S2(Pudina), S3(Alovera), S4(Tulsi), S5(Turmeric), S6(Pepper),S7(Sauf), S8(Elaechi), S9(Clove), S10(NeemBark). All the sample was extracted with standard extraction procedure using Buffer, Ethanol, Methanol, Chloroform reagent. The present work is concerned with the application of Paper Chromatography, SDS PAGE and Quantitative Determination. Examination of all the Natural and suspected Herbal product (drug) sample were based on the Rf Value, Band formation and O.D Value. After analysis it was found that Sample S2 (Pudina), Showed same Rf Value, SDS PAGE and O.D Value as compare to control Sample , but S1 (Neem), S3(Alovera), S4(Tulsi), S5(Turmeric), S6(Pepper), S7(Sauf), showed different Rf Value, SDS PAGE and O.D Value as compare to the control sample.

From this study it was concluded that by the use of pH conductometer through O.D value can be used successfully for Qualitative determination of claimed component in Herbal product. This method is very reliable for the analysis of quantity in various herbal products. This method can be applied in forensic science laboratory for the determination of quantity in Herbal product. From the result, it has been clearly and conclusively demonstrated that sample S2, all value are approximately same as claimed in Herbal product and sample S1(Neem), S3(Alovera), S4(Tulsi), S5(Turmeric), S6(Pepper), S7(Sauf), all value are some different in their composition and quantity as claimed in Herbal product.

It clearly shows that in Herbal product in place of Natural compound some value is different as. This kind of formulation faults are the important concern of medico-

legal issues because the people consuming it may suffer severe fatalities.

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