



EVALUATION OF PHYSICOCHEMICAL AND PHYTOCHEMICAL STANDARDIZATION PARAMETERS OF SIDDHA FORMULATION *OMA LEGIUM* BY MODERN ANALYTICAL TECHNIQUES

M. Sumathi ^{*1}, C. Shanmugapriya², R. Meenakumari³

^{*1} P.G Scholar, Post Graduate Department of Kuzhanthai Maruthuvam, Government Siddha Medical College, Chennai 600 106, Tamil Nadu, India

² Post Graduate Department of Kuzhanthai Maruthuvam, Government Siddha Medical College, Chennai 600 106, Tamil Nadu, India

³ Head, Department of Kuzhanthai Maruthuvam, Government Siddha Medical College, Chennai 600 106, Tamil Nadu, India

ABSTRACT

*Siddha system of medicine pioneering in emphasizes the biological activity of the various phytochemicals with respect to the etiology and pathophysiology of various dreadful diseases emerging in humans and animals. It is evident that there are some medicinal plants used in siddha has potency of acting as an anesthetics, analgesics, anti-microbial, immune modulators, Hepato, neuro and nephron protectant etc. But the most critical scenario is that information about most of the novel preparations is now extinct due to lack of proper documentation and standardization. Hence the main aim of the present investigation is to standardize the formulation *Oma Legium* (OL) as per AYUSH guideline and to reveal the property of the formulation to the scientific community for better understanding about the standards of the formulation. The results obtained from standardization and physicochemical analysis clearly reveals that total ash value of OL is about 5.66%, Loss on drying 39.43%, Total reducing sugar 22.76% and Total fat content 6.75%. The result of the phytochemical analysis indicates that the formulation OL shows the presence of alkaloids, flavonoids, glycosides, coumarins, triterpenoids, phenols, tannins, saponins, betacyanins, proteins and carbohydrates. HPTLC finger printing analysis of OL indicates the presence of 14 significant peaks with R_f value ranging from 0.01 to 0.86. From the results of the present investigation it was concluded that the formulation OL is highly sterile, safe and also possess biologically significant phytochemicals which may be used as an ailment for treating various disease.*

Keywords: Siddha system, *Oma Legium*, Standardization, Physicochemical, AYUSH, HPTLC,

INTRODUCTION

Siddha formulations offers tremendous advantage in clinical practice against metabolic and lifestyle disorders including neuro degenerative diseases. Often investigation on siddha preparations attempted on reverse pharmacology basis. Hence nearly 80% of the formulation already have proven track record clinically

and now several investigation are being made on its preclinical aspect. Siddha pharmacopoeia established in recent times has imposed more on standardization aspect of the formulation. Starting from preparatory phase to storage each and individual step involved in formulating siddha preparation has its own quality check evaluations [1].

The chances of occurrence of adverse event is very minimal in siddha when compare to any other therapies in the world this is mainly because the 90 % of ingredients used in the preparing formulations are compatible with the biological system of the humans and animals. Hence event of adverse events are less. Anti-dotes and counter therapy modifications are even available in siddha system in case of rare occurrence of some unexpected interactions [2].

Bioactive phytocomponents present in preparations like legium, chooranam and other oils have unique advantage of multiple mode of action. Synergy of using combined phytomedicine has well established in traditional medicine like siddha Before emergence of chromatographic and analytical technique siddhars traditionally established the evaluation and standardization methods made manually to ascertain the quality and stability of the formulation before offering the same to the patients. Development of monograph for indigenous and novel preparations has been considered a right platform for future researcher to select their drug of choice for their research work. As a measure of focusing upon the need of drug standardization the present investigation work undertaken to standardize the traditional polyherbal siddha formulation *Oma Legium* (OL) which has been used for the treatment of various ailment. Still now there is no proper documentary evidence available on standardization and phytochemical investigation aspect of this formulation this prompted us to peruse the systematic standardization of OL by phytochemical and physicochemical evaluation by AYUSH guidelines.

MATERIALS AND METHODS

Source of raw drugs:

The herbs is collected from southern zone of Tamil Nadu ,and other required raw drug is procured from a well reputed indigenous drug shop from Parrys corner ,Chennai, Tamil Nadu, India .All the herbs were authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India

Ingredients

The siddha formulation *Oma Legium* (OL) comprises of the following herbs as phyto ingredients

1. Omam (<i>Tachyspermum ammi</i>)	-3500gms
2. Amuukurak-kizhangu (<i>Withania Somnifera</i>)	-35gms
3. Kukil (<i>Shorea Robusta</i>)	-35gms
4. Parangi-pattai(<i>Smilax china</i>)	-35gms
5. Karpokaarisi-(<i>PsoraleaCorylifolia</i>)	-35gms
6. Sugar	-350grm
7. Nei	-2lit

Preparation [3]

3500grams of omam (*Tachyspermum ammi*) was added to 21.5 lit of water and it was allowed to boil and then reduced to 8 parts followed by filtration. Further to this about 340grms of sugar was added to make it as syrup form and add powdered form of amukura, kukil, parangipattai, karpogaarisi, each 35grms and ghee 2 lits were added to bring it to semisolid consistency.

Dose	:	5 gms, twice a day
Duration	:	2-3 weeks
Age	:	3 - 7 yrs.

Physicochemical Evaluation [4, 5]

Percentage Loss on Drying

10gm of OL was accurately weighed in evaporating dish and was air dried at 105°C for 5 hours and then weighed.

Determination of Total Ash

3 g of OL was accurately weighed in silica dish and incinerated at the furnace a temperature 400 °C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of pH

About 5 g of OL will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation using pH meter.

Determination of Total Reducing Sugar Content

20gm wt. equivalent to 20ml of the sample OL was taken and to which 10ml of conc HCl were added and kept it aside for overnight. Neutralize this solution with approximately 1M NaOH add 10 ml of 21.9 g zinc acetate, 3ml glacial acetic acid followed by 10.6 g potassium ferrocyanide and distilled water was added to make a volume of 100ml. 10ml of Fehling solution was taken and burette solution was added drop wise and heat to boiling over hot plate till blue color appeared. At this time, two drops of methylene blue was added and the titration was carried on till brick red color was obtained

Mg of sugar in 100 ml = Total reducing sugar x 100 / Titer value

Determination of total fat content

Weigh 4gms of sample in the thimble and place it in soxhlet fitted condenser. Take 100 ml of petroleum ether in round bottom flask and boil for 4 hours. Take the extract in pre weighed conical flask and evaporate the petroleum ether on water bath. Remove trace of pet ether with vacuum pump. Take the weight of the residue to constant fat weight.

$$\text{Percentage of fat content} = \frac{\text{weight of petroleum ether extract} \times 100}{\text{weight of the sample taken}}$$

Sterility Test [6,7]

About 1ml of the test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37° C for 24-48 hours. Grown colonies of organism was then counted and calculated for CFU.

Preliminary phytochemical Evaluation [8]

Test sample OL was extracted with ethanol and the extract was subjected to class of preliminary phytochemical screening of the following components

Test for Alkaloid- Mayer's reagent

To the test drug about 2ml of Mayer's reagent was added and was observed for the presence of alkaloids. Appearance of dull white precipitate indicates the presence of alkaloids.

Test for flavonoid

To 0.1ml of the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for Glycosides -Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for Triterepnoids

To the test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) at the side of the test tube. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Test for Steroids - Salkowski test

To the test solution 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Test for Carbohydrates - Benedict's test

To 0.5 ml of test drug about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Test - Phenol- Lead acetate test

The test sample is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for tannins

About 0.5ml of test sample is boiled in 20 mL of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. The 0.1% FeCl₃ is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins.

Test for Saponins

The test drugs were shaken with water vigorously for 10 mins, copious lather formation indicates the presence of saponins.

Test for Proteins (Biuret Test)

Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids.

Test of Coumarins

1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for Quinones

The test samples were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

Test for Anthocyanin

About 0.2 ml of the extract was weighed in separate test tube, 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at 100 ± 2°C. Observed for the formation of bluish green color which indicates the presence of anthocyanin.

Test for Betacyanin

To 2 ml of the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of yellow colour indicates the presence of betacyanin.

Heavy Metal analysis by ICPMS [9, 10]

Digestion of sample is carried out by transforming 2.5 ml of the sample into a closed beaker and 5 ml of concentrated HNO₃ was added and digested to near dryness. 16 M nitric acid was further added each time to the sample and digested until the clear solution was obtained. 5ml of 12 M Hydrochloric acid was added to ensure complete digestion .The digested solution was cooled to room temperature and made to the final volume of 100 ml with deionized water. Sample solutions were then filtered through membrane (0.45micron) filter. Finally, the digested samples were used for metal analysis using inductively coupled plasma Mass Spectrometry (Perkin Elmer DRC-e Model) .Each sample was digested in triplicate. A blank solution was also prepared in a similar manner.

TLC Analysis [11]

Test sample OL was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Ethyl acetate: Methanol: Water (100:13.5:10). After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm Sample Spotting

HPTLC Analysis [12]**Chromatogram Development**

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

HPTLC Chromatographic condition

Sample	:	OL
Der Solvent	:	Anisaldehyde
Stationary phase:		Silica gel GF ₂₅₄
Mobile phase	:	Chloroform: n-butanol: methanol: water: Acetic acid (4:1:1:0.5:0.5)
Scanning wavelength	:	366 nm
Sample concentration	:	10mg/ml
Applied volume	:	5 µl
Application mode	:	CAMAG HPTLC

RESULTS**Physico-chemical Evaluation and standardization of OL**

The results obtained from the physicochemical evaluation reveals that the total ash value of OL was found to 5.66 %. Similarly loss on drying value at 105°C was found to be 39.43% respectively. Total Reducing sugar level of OL was 22.76 % and the total fat content was found to be 6.75 %, pH of the OL was found to be 6. The results obtained from qualitative elemental analysis of OL by ICPMS showed the below detective level of Cadmium (Cd), The results were tabulated in Table 01.

Table 1: Physicochemical Evaluation and Heavy Metal Analysis of *Oma Legium*

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	39.43 ± 1.98
2.	Total Ash (%)	5.66 ± 1.69
3.	Total Reducing sugar (%w/v)	22.76 ± 1.09
4.	Total fat content (%w/v)	6.75 ± 1.14
5.	pH	6
6.	Cadmium (mg/L)	BDL

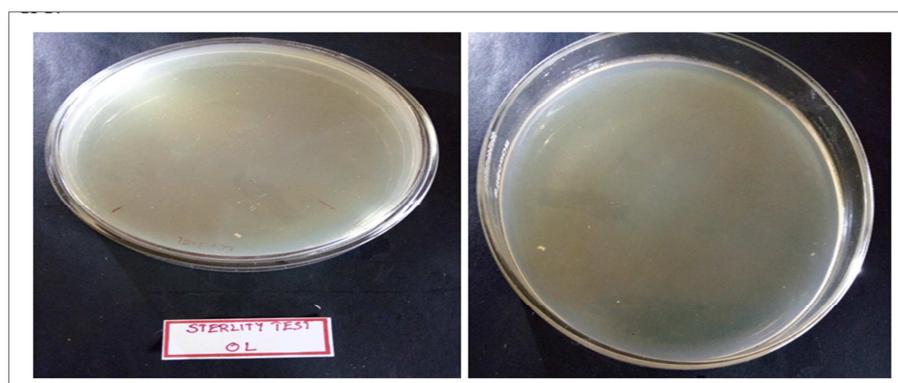
Evaluation of OL formulation

The results of the sterility test of OL by pour plate technique reveals that the formulation OL is of high sterile and the culture after incubation shown the absence of pathogens such as *E-coli*, *Salmonella Staphylococcus Aureus*, *Pseudomonas Aeruginosa*. The results were tabulated in Table 02 and illustrated in Figure 1.

BDL- Below Detective Level

Table 2: Sterility Evaluation of *Oma Legium*

Test	Specification	Result	Method
<i>E-coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus Aureus</i>	Absent	Absent	
<i>Pseudomonas Aeruginosa</i>	Absent	Absent	

Figure 1: Sterility Evaluation of *Oma Legium***Qualitative Phytochemical evaluation of OL**

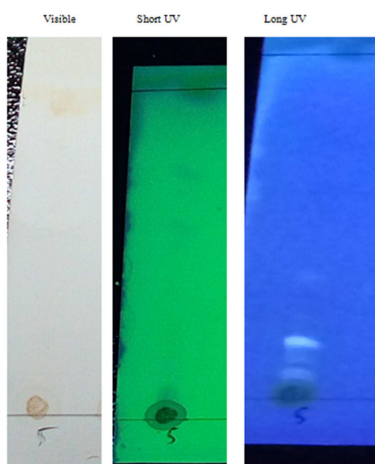
The result of the qualitative phytochemical analysis indicates that the formulation OL shows the presence of biologically significant phytochemicals such as alkaloids, flavonoids, glycosides, coumarins, triterpenoids, phenols, tannins, saponins, betacyanins, proteins and carbohydrates. The results were tabulated in Table 03.

Table 3: Qualitative chemical and preliminary phytochemical analysis of *Oma Legium*

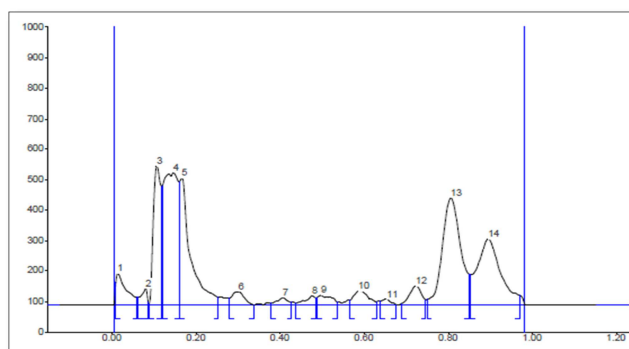
S.No	Name of Chemical/ Phyto Components	Presence or Absence
1.	ALKALOIDS	Presence
2.	FLAVONOIDS	Presence
3.	GLYCOSIDES	Presence
4.	STEROIDS	Absence
5.	CARBOHYDRATES	Presence
6.	TRITERPENIDS	Presence
7.	COUMARINS	Presence
8.	PHENOLS	Presence
9.	CARDIAC GLYCOSIDES	Absence
10.	TANNINS	Presence
11.	SAPONINS	Presence
12.	PROTEINS	Presence
13.	ANTHOCYANIN	Absence
14.	BETACYANIN	Presence
15.	QUINONES	Absence

TLC and HPTLC analysis of OL

Preliminary TLC analysis of the sample OL emits fluorescence indicates the presence of fluorescent emitting compound as illustrated in Figure 2. The results of HPTLC analysis of the sample OL reveals the presence of 14 prominent peaks corresponds to 14 different compound's with Rf value ranging from 0.01 to 0.86 with percentage area of 3.41 to 21.41%. The results were tabulated in Figure 3 and illustrated in Figure 2 and 4.

Figure 2: TLC Chromatogram of *Oma Legium*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	72.0	0.01	100.9	4.46	0.06	22.9	1821.5	3.41
2	0.06	23.4	0.08	52.0	2.30	0.09	9.0	560.9	1.05
3	0.09	2.1	0.11	453.7	20.04	0.12	388.3	6218.2	11.63
4	0.12	390.1	0.15	432.7	19.11	0.16	402.0	11281.8	21.11
5	0.16	402.9	0.17	415.6	18.35	0.25	23.0	7986.5	14.94
6	0.28	21.9	0.30	42.2	1.86	0.34	0.2	937.9	1.75
7	0.38	4.5	0.41	22.1	0.98	0.43	7.7	413.9	0.77
8	0.44	4.4	0.48	29.6	1.31	0.49	22.0	509.4	0.95
9	0.49	22.3	0.50	30.1	1.33	0.54	8.5	745.7	1.40
10	0.57	14.7	0.59	43.9	1.94	0.63	9.5	1173.1	2.19
11	0.64	11.9	0.66	17.4	0.77	0.68	0.3	282.9	0.53
12	0.69	3.8	0.72	59.8	2.64	0.75	15.9	1270.0	2.38
13	0.75	16.9	0.81	349.7	15.44	0.85	98.5	11443.5	21.41
14	0.86	96.3	0.90	214.8	9.49	0.97	30.6	8804.3	16.47

Figure 3: HPTLC analysis of *Oma Legium*Figure 4: HPTLC Chromatogram of *Oma Legium*

DISCUSSION

As per the World Health Organization medicinal plant have been identified as the best source for bioactive components from which new drug entities has been developed which acts by multiple mechanism. According to the global survey it has been identified that nearly 80% of individuals from developed and developing countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to understand their properties, safety and efficacy [14].

In ASU systems plants, minerals, and animal products are used as main drugs to cure various ailments [15,16]. Herbal medicine also called botanical medicine or phytomedicine refers to the use of plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine [17]. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease [18]. The results obtained from the physicochemical evaluation reveals that the total ash value of OL was found to 5.66 %. Similarly loss on drying value at 105°C was found to be 39.43% respectively. Total Reducing sugar level of OL was 22.76 % and the total fat content was found to be 6.75 %, pH of the OL was found to be 6. The results obtained from qualitative elemental analysis of OL by ICPMS showed the below detectable level of Cadmium (Cd).

Advancement of sophisticated instrument method of analysis offers varying degree of improving the usage and exploration of siddha preparations among the people of lower economic zone. Medicinal plants and other herbs collected from the cultivators often have chance of heavy metal traces like arsenic, mercury, lead and chromium in the preparation if not be properly purified. Upon continuous administration of such heavy metal containing preparations leads to lethal effects. Hence recent technology like AAS, ICPMS has advantage of detecting heavy metals even with PPQ level. The use of modern analytical techniques is a must to characterize the drug, its interaction with specific body tissues/organs and to provide a molecular basis for the curative aspects. In the task of bringing into the mainstream, the unutilized potential of the ancient science of healing, a close interaction of practitioners of traditional Indian Systems of Medicine (ISM). Preliminary TLC analysis of the sample OL emits fluorescence indicates the presence of fluorescent emitting compound as illustrated in Figure 2. The results of HPTLC analysis of the sample OL reveals the presence of 14 prominent peaks corresponds to 14 different compound's with Rf value ranging from 0.01 to 0.86 with percentage area of 3.41 to 21.41%.

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. The result of the qualitative phytochemical analysis indicates that the formulation OL shows the presence of biologically significant phytochemicals such as alkaloids, flavonoids, glycosides, coumarins, triterpenoids, phenols, tannins, saponins, betacyanins, proteins and carbohydrates.

CONCLUSION

From the data's of the present investigation it was concluded that the siddha formulation *Oma Legium* prepared and analyzed according to the standard procedures. Results of the study generated an evidence based data with respect to the phytochemical and physiochemical parameters of the trial drug OL. The result of the qualitative phytochemical analysis indicates that the formulation OL shown the presence of biologically significant phytochemicals further the physiochemical standard value of the OL proves its genuinity and also sterility and ICPMS test result revealed the sterility and purity of the drug. TLC study result projects the presence of fluorescent emitting compound. HPTLC analysis reveals the presence of 14 significant compound's. Further studies have to be carried with special emphasis on molecular biology aspect of the drug and its target receptor in the biological system in near future.

Acknowledgement

I wish to acknowledge my thanks to The Noble research solutions, Chennai for their technical support for this research work.

REFERENCES

- 1) Arvind Chopra. Ayurveda-modern medicine interface: A critical appraisal of studies of Ayurvedic medicines to treat osteoarthritis and rheumatoid arthritis. J Ayurveda Integr Med. **2010**; 1(3): 190-198.

- 2) Mohd Fauzi F. Chemogenomics approaches to rationalizing the mode-of-action of traditional Chinese and Ayurvedic medicines. *J Chem Inf Model.* **2013**; 53(3):661-73.
- 3) Athmarakshaamirtham Ennum VaithiyaSarasangiragam. **1972**; 07:446-447.
- 4) The Ayurvedic Pharmacopoeia of India. Part II. Volume II, Fifth Edition, Department of AYUSH, New Delhi, **2008**.
- 5) Department of AYUSH.The Ayurvedha Pharmacopoeia of India. Vol I, Ministry of Family health and welfare .**2008**; 59: 83.
- 6) Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems. Department AYUSH .Ministry of Health & Family Welfare, Govt. of India
- 7) Indian standard methods of sampling and test for oils and fats Indian standard institution New Delhi.**1964**; 47-50.
- 8) Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol:Wright- Scientecnica; **1975**; 36-45
- 9) Smille TJ and Khan IA, A Comprehensive approach to Identifying and authenticating Botanical Products, Clinical Pharmacology and therapeutics. **2010**; 87: 175-186.
- 10) Limmatvapirat CJ, Charoenteeraboon, Phaechamud T. Simultaneous analysis of eleven heavy metals in extracts of *Sonneratiacaseolaris* (L.) Engl. By ICP-MS. Res. J. Pharm., Biol. Chem.Sci. **2012**; 3:744-50.
- 11) Lukasz Komsta, Monika Waksmundzka-Hajnos, Joseph Sherma. Thin Layer Chromatography in Drug Analysis .CRC Press, Taylor and Francis.
- 12) Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium; **2002**; 305: 227.
- 13) Meena Devi VN, Nagendra Prasad P, Kalirajan K, Infrared spectral studies on Siddha drug- Pavala parpam, International journal of pharma and biosciences, **2010**; 4: 474-483
- 14) Subrat N, Ayurvedic and herbal products industry an overview. In: Paper presented at workshop on wise practices and experimental learning in the conservation and management of Himalayan medicinal plants, Katmandu, Nepal: **2002**; 15-20.
- 15) Binu S, Uses of pteridophytes among the tribals in pathanamthitta district, Kerala, India. Journal of non timber forest products, **2008**; 5: 129-131.
- 16) Mensah J.K. Phytochemical Analysis of Medicinal Plants Used for the Management of Hypertension: Ethnobotanical Leaflets .**2009**; 13: 1273-87.
- 17) Bose DM, Sen SN and Subbarayappa BV(Ed) Subbarayappa BV (Au), Chemical practices and alchemy: In a Concise History of Science in India, Indian National Science Academy, New Delhi, **1971**; 315-335.
- 18) Grover J.K., Yadav S., Vats V. Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol.* **2002**; 81:81- 100.
- 19) S Sasidharan. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *Afr J Tradit Complement Altern Med.* **2011**; 8: 1-10