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Research Article

# Pharmacognostic Evaluation of Polyherbal Marketed Formulation

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**ABSTRACT**
Marketed polyherbal tablet contain Shankhpushpi and Brahmi main ingredient. The polyherbal formulation widely used in Ayurvedic clinical practice to improve the memory and intellect by their action .There is no modern methods of standardization was not reported for analysis of this formulation. Hence the study highlights the physiochemical characterization, dissolution, stability and HPLC profiling which can be applied for authentification of this polyherbal formulation. The polyherbal tablet contain a Shankhpushpi (*Evovoulous alsinoide*), Brahmi (*Centela asiatica*),Gulab (*Rosa centiofolia*),Vaj (*Acorous calamous )* .The main ingredients authenticated botanically .Ayurvedic polyherbal tablet formulation subjected to standardization according to official guideline. The standardization of the formulation was carried out as per official guidelines in which the polyherbal tablet formulation was subjected physiochemical characterization Standardization parameter would serve as the identity of this polyherbal formulation.HPLC fingerprinting profile was developed serve as fingerprint for the identification of the formulation. The proposed method for standardization of polyherbal formulation is simple, rapid and effective method .The developed quality standard can be used as quality check for polyherbal formulation.

**Key word**: Ayurvedic polyherbal formulation, Standardization, HPLC

## INTRODUCTION

Ayurveda derives from the Sanskrit words Ayus (life) and Veda (knowledge) is the most Ancient system of traditional medicine of the world .It has been practiced in India since 5000BC .Ayurveda is the holistic approach towards the life ,health, disease management through medicinal herbs, minarals,diet and lifestyle[1] .As per WHO, the 70 -95% of global population particularly in developing countries mostly use traditional medicines for their health care.[2]Wolrd wide acceptance of this holistic science and increasing demand at global level create a great need for standardization of herbal medicine to maintain its safety and efficacy. According WHO the use of herbal medicine thought the world exceeds that of the conventional drugs by two to three times.

The herbal medicine is natural product containing one or more herb in formulation. Effectiveness phytoconstituent depends on time, region and processing and storage. Variation in the collection, processing or storage of herb was produced impact on its efficacy profile. Hence the proper knowledge for collection and usage of most medicinal plants exist in traditional medicine system, it can be used as guide to quality standards.[3]

Standardization of herbal formulation is nothing but confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observation.[4,5,6] The purpose of this work is to evaluate the polyherbal Ayurvedic tablet formulation popularly used containing herbs namely Shankhpushpi (*Evovoulous alsinoide*),Brahmi (*Centela asiatica*),Gulab (*Rosa centiofolia*),Vaj (*Acorous calamous* ).These are the group of drugs widely used in Ayurveda specially to improve the memory and intellect. The increased quality standards at global market for this drug create need for proper standardization methodology for safety and efficacy.In traditional medicinal system testing parameters for quality of material (Dravya), consist of rasa (taste),guna(properties, potency),vipaka (post digestion effect) and karma (action)are very different from the modern method. There is no direct written protocol available for traditional medicine for testing action. Hence necessity to develops modern and objective standards for evaluation of the safety, quality and efficacy of this Ayurvedic polyherbal nootropic tablet is aim of the current study.

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Table 1: Physiochemical standards for polyherbal formulation

| Parameters | Result |
|---|---|
| $P^H$ | 6.6 |
| Ethanol soluble extractive value (%) | 12 |
| Water soluble extractive value (%) | 9.6 |
| Total ash(%w/w) | 4.5 |
| Acid insoluble ash (%w/w) | 1.25 |
| Water soluble ash(%w/w) | 1.5 |
| Hardness (kg/cm$^2$ ) | 3.5 |
| Friability (%) | 0.94 |
| Disintegration time (min) | 5.5 |
| Weight variation (gm) | 0.475-0.24 |

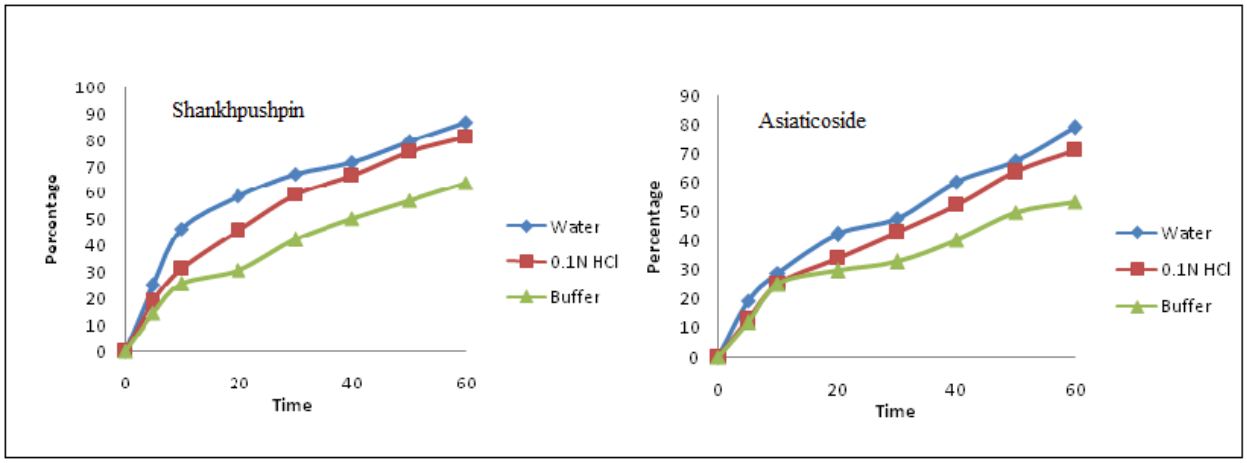


*Fig1.Percentage drug release from tablet*

**MATERIAL AND METHOD**

Instrumentation: Dissolution test apparatus, Electro lab – USP TDT-08L. Disintegration test apparatus, Electro lab ED-2SAPO, Friabilator, Electro lab –USP EF-1W, Monsanto hardness tester, Dolphin, Incubator, Lab Hosp Corporation, Digital pH meter (pico, Lab India) were used, HPLC, Shimadzu (Isocratic)- 11355/17904, UV-visible spectrophotometer (Double beam), Shimadzu 1650 PC were used.

Material: All chemical used were of analytical grade. The marketed polyherbal formulation was used for the standardization. Required pant medicine such as Shankhpushpi and Brahmi were collected form authorized raw drug supplier. The raw drug were identified and authenticate in lab.

Methodology: Standardization parameters include-

Phytochemical analysis[7:] The polyherbal tablet was evaluated for the presence of alkaloid, tannins and phenols, flavonoids, glycosides, saponins.

Physicochemical standardization

pH- 1 tablet was dissolved in distilled water and pH measured by digital pH meter. The calibration of pH meter was done by using solution of pH 4, 7, 9.2 respectively.[12]

Ethanol soluble extractive: 25 gm of sample were powdered and macerated with 50 ml of ethanol in an enclosed flask for 24 hours. Flask were shaken frequently during first 6 hours and allowed to stand for next 18 hours. After 24 hours filtration was done rapidly. Solvent evaporated in vacuumed evaporator under reduced pressure and temperature. From the weight of dried residue ethanol soluble extractive were calculated.[12]

Water Soluble extractive*:* 25 gm of sample were powdered and macerated with 50 ml of water in an enclosed flask for 24 hours. Flask were shaken frequently during first 6 hours and allowed to stand for next 18 hours. After 24 hours filtration was done rapidly. Solvent evaporated in vacuumed evaporator under reduced pressure and temperature. From the weight of dried residue water soluble extractives were calculated.[12]

Ash value

Determination of total ash: For Total ash determination, 2 gm of powdered material was placed in tare crucible of silica previously ignited and weighed. The powdered drug was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450ºC until free from organic content, cooled in desiccators, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible and that of crucible with total ash.

Determination of acid insoluble ash: 25 ml of dil. HCl is added to the crucible containing the total ash and covered with a watch glass. It is boiled gently for 5 min. Watch glass was rinsed with 5 ml of hot water and the liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until

Table2: Accelerated stability study for tablet

| Quality Control parameter | Standard limit | Observation | | |
|---|---|---|---|---|
| | | 0 month | 3 month | 6month |
| Hardness (kg/cm$^2$)(n=5) | 2-5 kg/cm$^2$ | 3.5 ±0.1 | 3.4±0.07 | 3.24±0.083 |
| Friability (%) (n=5) | NMT 1 | 0.94±0.1 | 0.93±0.01 | 0.91±0.014 |
| Disintegration time (n=6) | NMT 15 | 7.5±0.70 | 7.3±0.1 | 7±0.1 |
| Weight variation (n=20) | ±5% | 0.474-0.524 | 0.478-0.529 | 0.499-0.529 |
| pH (n=5) | 5-7 | 6.6±0.014 | 6.96±0.015 | 7.24±0.1 |
| Microbial load | No growth | No growth | No growth | No growth |

*(*NMT-not more than)*

filtrate was neutral. The filter paper containing the insoluble matter was transferred to crucible, dried on hot plate and ignited to a constant weight. The content of acid insoluble ash was calculated as a percentage acid insoluble ash.

Water soluble ash: The total ash obtained, boiled for 5 min. with 25 ml of water. The insoluble matter was collected on Gooch crucible or an ash-less filter paper. It was then washed with hot water, and ignited for 15 min. at temp. Not exceeding 450ºC. The total weight of insoluble matter should be subtracted from the weight of the ash. The difference in weights represents the water-soluble ash. The percentage of water soluble ash was calculated.

Quality control test of tablet as per IP (2010)

Hardness*:* 5 tablet of sample were taken to measure hardness. The hardness calculated and compared to IP standard.

Friability: 20 tablet of sample were used for test. Friabilator was run for 4 min at 25 rpm and percentage friability was calculated and compared with IP standard.

Disintegration Test: Disintegration test was performed using 6 tablet of sample. The media used for this test was distilled water and time required to disintegrate the tablet were recorded.

Weight Variation Test: 20 tablets were weighed individually. Average weight and % weight variation was calculated and compared with IP limit.

Estimation of pesticide residue: 20 Tablet of sample were powdered and kept in RBF, 100ml sodium sulphide was added with 100ml n-hexane and refluxed for 1hr. Filtrate from RBF was taken in separating funnel and extraction was done with 50 ml n-hexane and 25 ml of acetonitrile. The acetonitrile layer was mixed with 500 ml of demineralized water with 2.5 ml saturated sodium sulphide and again shaken in separating funnel with n-hexane layer and evaporated on water bath. The residue was used for analysis of organochlorine, organophosphate, carbamates.

Organochlorine colour test: Residue + Iso propyl alcohol (IPA)

Organophosphate: 1 ml of residue in 5ml ethanol + KOH

Carbamates: 1 ml of residue in 5 ml ethanol + 1 drop furfural + 1 drop HCl

Colour of test solution was observed and compare with std. solution of each organochlorine, organophosphate, carbamates marketed pesticide[11].

Determination of microbial load[12]

Preparation of media

- Nutrient agar: 2.8 gm in 100 ml sterile distilled water
- MacConkey agar: 1.5 gm in 100 ml sterile distilled water.

Preparation of sample: Sample was prepared by dissolving tablet powder equivalent to one tablet, in a 50 ml of sterilized distilled water.

Methods: Microbial load was calculated by two different methods as:

Spread plate method*:* Three plate of nutrient agar and three plate of MacConkey agar were prepared. 1 ml sample was added using sterile pipette in both Petri plate containing nutrient agar and MacConkey agar. Sample was spread uniformly over the media and kept for incubation for 24-48 hr at 37$^0$C and growth was observed.

Pour plate method: Nutrient agar medium and MacConkeyt agar medium was prepared and transfer 15 to 20 ml medium in three tubes. Sterilized all test tubes in autoclave at 121$^0$C, 15 lbs for 15 min., simultaneously sterilized three Petri plate. One loop of sample was taken in first test tube, then from first tube to second test tube and one loop of sample from second test tube to third test tube and mixed well during each step. Then poured in Petri plate and kept for incubation for 24-48 hr at 37$^0$C and growth was observed.

Dissolution study: Dissolution study of tablet was carried out using 0.1 N HCL, Distilled Water and phosphate buffer pH 6.8 as a dissolution media. The samples were withdrawn for 60 min at the interval of 10 min. The absorbance of sample measured on UV spectrophotometer and percentage release was calculated.[12]

Accelerated stability study: According to ICH guidelines Q1A (R2) the accelerated stability study was performed at 40$^0$ c ± 2$^0$ c and humidity condition were maintained at 75% ±5% RH for 6 month. Tablets were placed in marketed container as it is. Sampling were done at 0, 3 and 6 month time interval and evaluated. Parameters evaluated are %drug content, microbial load, quality control parameter for tablets. Quality control parameters for tablet evaluated are pH, hardness, friability, and weight variation and disintegration time[12].

HPLC fingerprinting

HPLC condition

Column: C18 (25 cm×4.6 mm, i.d.), 5µm

Mobile phase: Methanol: Acetonitrile: Water (40:30:30%v/v)

Detection: at 280 nm

Flow rate: 1 ml/min

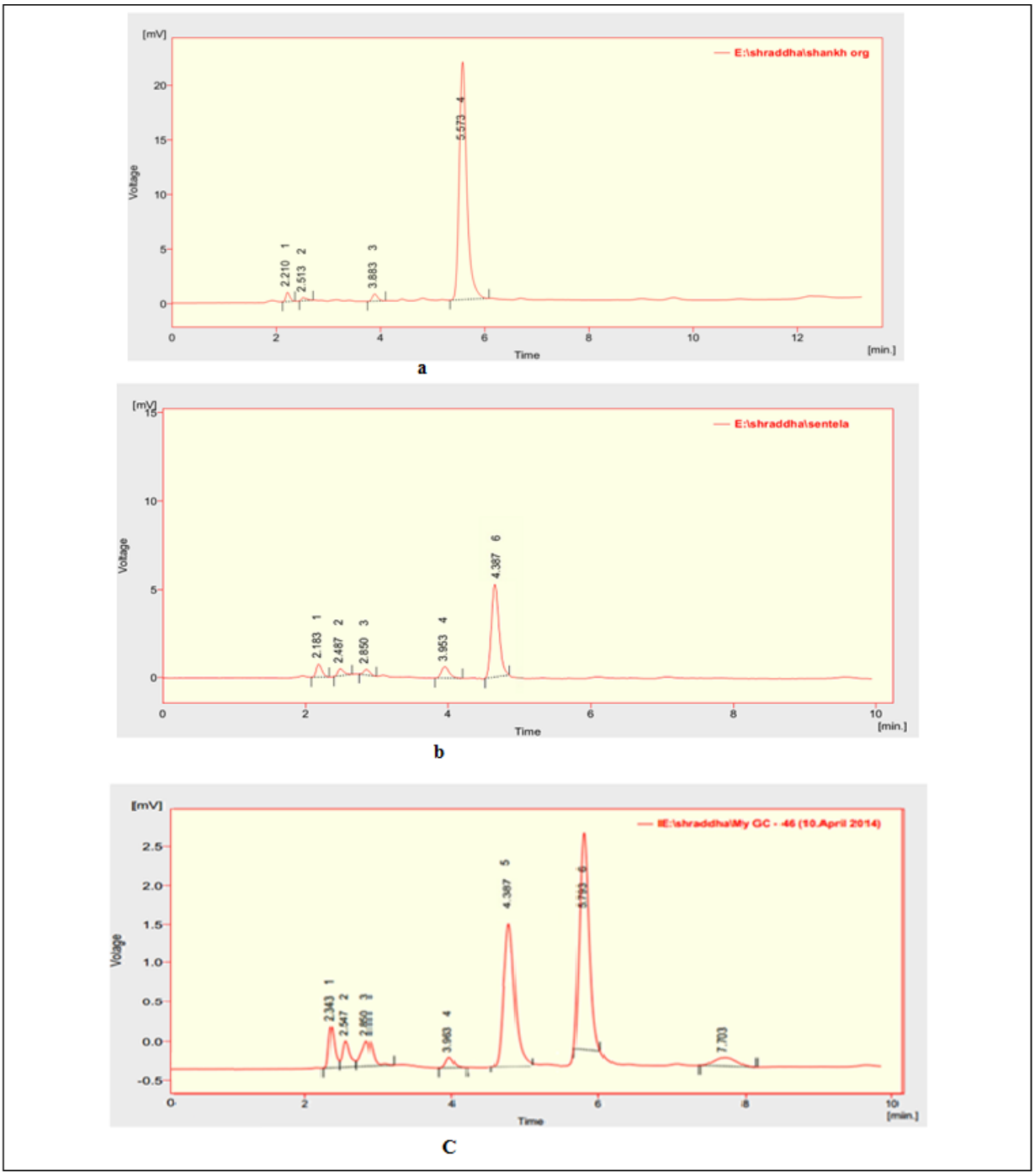


Fig2: HPLC chromatogram for tablet and standard marker (a) Shankhpushpi, (b) Brahmi, (c) Tablet

HPLC fingerprinting of tablet and its ingredient were obtained by extracting of sample. For tablet extraction 3gm of tablet powder was extracted in 10 ml methanol by cold maceration for 24 hours. Extraction and isolation of Shankhpushpi was done by using general method for extraction of alkaloid. Extraction and isolation of Brahmi was done by methanol by cold maceration method and the mark residue was extracted with pet ether. The resultant 1µ/ml of each solution was injected to record the chromatogram.

**RESULTS AND DISCUSSION**

Phytochemical constituent

The phytochemical analysis showed the presence of alkaloid, glycoside, saponin, tannin and flavonoid.Naturaly occurring secondary plant metabolite are mainly responsible for therapeutic activity. The presence of phenolic and flavonoids are responsible for antioxidant activity of the formulation.

Physiochemical Analysis

Standardization of marketed polyherbal formulation as per pharmacopeia was carried out based on the physiochemical parameters. The marketed sample passed all the pharmacopeial test.

Estimation of pesticides residue: Chemical color test method was used for detection of pesticide having

sensitivity up to 0.6ppm. Standard for each category was used for comparison. Pesticide residue was harmful to the individual consuming the formulation. The major harmful groups like organochlorine, organophosphate, carbamates were absent in formulation indicating safety of formulation.

Microbeal lod: The nutrient agar, MacConkey agar and Macconkey broth were used for the evaluation of microbial load. The Pour plate method, spread plate method were used for evaluation of microbial growth. Both the methods showed absence of microbial growth. The absent of microbial growth in marketed formulation indicates its safety and quality.

Dissolution study: The dissolution study was performed using shankhpushpin for Shankhpushpi and asiaticoside from Brahmi as marker compound in order to measure release pattern of formulation by UVmethod.The release in the 0.1N HCl media was found to be more than the phosphate buffer pH 6.8. The shankhpushpin being alkaloid in nature forms water insoluble free bases in the basic pH media limiting its solubility and the release. The percentage relese of asiaticoside was release more in the distilled water than in 0.1N HCl and phosphate buffer. May be related to water solubility of asiaticoside glycoside.The dissolution study has been carried out in the three different media to find out the best media. For both the drugs, distilled water and HCL is better media for drug release.

The percentage of drug release of tablet showed in graphs fig.no.1

From the stability study data the formulation was found to be stable for 6 months. There is little variation in the physicochemical properties. The microbial growth was not found in any formulation. Accelerated stability testing on herbal formulation provide a evidence on the quality of a drug substance or drug product varies with time under the influence of variety of environmental factors such as temp, humidity, and light .

HPLC fingerprinting: The chromatogram of shankhpushpin showed the retention time (RT) 2.210, 2.513, 3.883, 5.573min.The asiaticoside extract showed the retention time (RT) at2.183,2.487,2.850,3.953,4.387min.The tablet extract showed the retention time (RT) at 2.343,2.547,2.860,3.963,4.387,5.793,7.703min.(fig2)The unique RT value of the tablet and standard extract gives idea about fingerprinting for active constituent. Chromatogram shows in figure2.

## CONCLUSION

The current investigation can be used as simplified standardization protocol for polyherbal formulation. This standardization protocol is help to maintain quality standards of the polyherbal formulations. Further validated HPLC method can be developed for quantitative estimation of major phytoconstituent in polyherbal tablet.

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