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Quality standardization and toxicity study of ayurvedic formulation

Nhawkar Smita V.*, Mullani Ashish K., Magdum Chandrakant S. and D'Souza John I.
Tatyasaheb Kore College of Pharmacy, Warananagar, Tal- Panhala, Dist- Kolhapur-416 113, Maharashtra, India.

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Abstract: Memorin is an ayurvedic remedy for improving mental function. Standardization of Ayurvedic formulations is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs. WHO specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization are of importance. The aim of present study is Quality standardization and toxicity study of Memorin. Standardization of drug was done on the basis of evaluating the drug for its Physico-chemical parameters and presence of phytochemical constituents. It includes total ash value, extractive values, loss on drying, bulk density, tapped density etc. Drug was evaluated for presence of alkaloids, proteins, steroids, tannins, glycosides etc. Qualitative evaluation of the drug was performed by means of FT-IR, GC-MS and HPLC. In HPLC, C18 column was used. Flow rate was 1 ml/min. Mobile phase for HPLC analysis was methanol: ammonium acetate and it was run at 350nm for detection of compounds. Toxicity study of drug was done by using animals (Wistar albino rats). It can be concluding that the drug was safe and there was no sign of toxicity and mortality.

Key Words: Standardization; Quality; Physico-Chemical Parameters; Phytochemical Screening.

Introduction

Ayurveda or Ayurvedic medicine is an ancient system of health care that is native to the Indian subcontinent. It is presently in daily use by millions of people in India, Nepal, Sri Lanka, China, Tibet, and Pakistan. It is now in practice for health care in Europian countries. The word "Ayurveda" is a tatpurusha compound of the word ayus meaning "life" or "life principle" and the word veda, which refers to a system of "knowledge". According to Charaka Samhita, "life" itself is defined as the "combination of the body, sense organs, mind and soul, the factor responsible for preventing decay and death." According to this perspective, Ayurveda is concerned with measures to protect "ayus", which includes healthy living along with therapeutic measures that relate to physical, mental, social and spiritual harmony. According to tradition, Ayurveda was first described in text form by Agnivesha, named - Agniveshtantra. The book was later redacted by Charaka, and became known as the Charaka Samhita. Another early text of Ayurveda is the Sushruta Samhita, which was compiled by Sushrut, the primary pupil of Dhanvantri, sometime around 1000 BC. Dhanvantri is known as the Father of Surgery. Ayurveda is considered by many scholars to be the oldest haling science. In Sanskrit, Ayurveda means 'The science of life' Ayurvedic knowledge originated in India more than 5,000 years ago and is often called the "Mother of All Heaing". It stems from the ancient Vedic culture and was taught for many thousands of years in an oral tradition from accomplished master to their disciples. Ayurveda places great emphasis on prevention and encourages the maintenance of health through close attention to balance in one's life, right thinking diet lifestyle and the use of herbs. Knowledge of Ayurveda enables one to understand hope to create this balance of body, mind and consciousness accordingly to one's own individual constitution and to make lifestyle changes to bring about and maintain this balance. In Ayurveda body, mind and consciousness work together in maintaining balance. They are simply viewed as different facets of one's being learn how to balance the body, mind and consciousness requires an understanding of how vata, pitta, and kapha work together. According to Ayurvedic philosophy the entire cosmos is an interplay of the energies of the five great elements- Space, Air, Fire, Water and Earth. Vata, Pitta and Kapha are combinations and permutations of these five elements that manifest as patterns presents in all creation.

Herbal Medicine

Herbal medicines make up an important component of the trend toward alternative medicine. A Harvard study recently found that one in three respondents acknowledged use of at least one alternative therapy within the past year. Extrapolated, these findings suggest that up to $13.7 billion were spent in 1990 alone for these treatments. Tyler defines herbal medicines as "crude drugs of vegetable origin utilized for the treatment of disease

Corresponding Author:
Dr. Nhawkar Smita V.,
Tatyasaheb Kore College of Pharmacy,
Warananagar, Tal- Panhala, Dist- Kolhapur-416 113,
Maharashtra, India.

states, often of a chronic nature, or to attain or maintain a condition of improved health. Current demands for herbal medicines have resulted in an annual market of $1.5 billion and increasingly widespread availability. Standardization of herbal formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, phytochemical, standardization, and In-vitro, In-vivo parameters. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. In India, the department of Ayush, Government of India, launched a central scheme to develop a standard operating procedure for the manufacturing process to develop pharmacopeia standards for Ayurveda preparations.

Standardization of Herbal Drugs

Standardization of drugs is a confirmation of its identity and determination of its quality and purity. Phyto-therapeutic agents are standardized herbal formulation which consisting of plants which are used in most countries for the management of various diseases. WHO define herbal drugs as a drug containing as active ingredients plant materials in the crude or processed state plus certain excipients, solvents, diluents or preservatives. Standardized herbal products of consistent quality and containing well-defined constituents which are required for reliable clinical trials and to provide beneficial therapeutic effects. Pharmacological properties of an herbal formulation depend on phytochemical constituents present therein. Standardization can be of two types as follows:

True standardization: Represents a definite phytochemical or group of constituents known to have activity.

Pseudo standardization: This is based on manufacturers guaranteeing the presence of a certain percentage of marker compounds; these are not indicators of therapeutic activity or quality of the herb.

In standardization, the triple 'P' based protocols are

1. Pharmacogenetic
2. Physico-Chemical
3. Phytochemical

Standardization of herbal formulations is important for assessing the quality drugs, based on the concentration of their active principles, physical, chemical, phytochemical, standardization, and In-vitro, In-vivo parameters. Standardization of herbal formulations is important for assessing the quality drugs, based on the concentration of their active principles, physical, chemical, phytochemical, standardization, and In-vitro, In-vivo parameters. One of the major problems faced for herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations.

Toxicity

Toxicity is the degree to which a substance can damage an organism.

Why study toxicology?

Benefit –risk ratio can be calculated. Prediction of therapeutic index can be calculated with smaller ratio, better safety of the drug. Therefore, toxicity study is important.

$$\text{Therapeutic index} = \frac{\text{Maximum tolerated dose}}{\text{Minimum curative dose}}$$

Why do we require non-clinical studies in animals before administered to man??

Pharmacological effects are same in man as in animals. Toxic effect in species will predict adverse effects in man. High doses in animals improves predictability to man. Risk assessment which can be made by comparison of toxic doses in test species with predicted therapeutic dose in man. Therefore, non-clinical studies are necessary in animal. Type of toxicity can be divided into following:

Systemic toxicology studies

1. Single dose studies
2. Repeated dose studies

Reproductive toxicology studies

1. Male fertility
2. Female reproduction & Developmental studies
3. Local toxicity studies
4. Hypersensitivity studies
5. Genotoxicity studies
6. Carcinogenicity studies

From these, we are going to study single dose (acute toxicity) and multiple dose study (sub-acute toxicity).

Acute toxicity (Single dose studies)

Acute toxicity is defined as "the adverse effect occurring within a short time of administration of single dose of a substance or multiple doses given within 24hrs". Acute toxicity studies in animal are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies is useful in choosing dose for repeat dose studies, providing preliminary identification of target organs of toxicity and occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting dose for phase 1 human studies and provide information relevant to acute over dosing in humans.

Sub-acute toxicity (Repeated dose studies)

The Globally Harmonized System (GHS) defines sub-acute as it "specific target organ/systemic toxicity arising from a repeated exposure" Repeated dose toxicity testing using oral administration of a test substance in rodents for 28 and 90 days is used to evaluate chronic toxic effects, primarily effects on various organ systems, and to establish a no observed effect level. Sub-acute toxicity testing consists of oral, dermal, and inhalation sub-chroic repeated dose studies (28-day) and sub-acute repeated dose studies (90-day) in rodents. The endpoints for repeat dose testing consist of an evaluation of clinical observations, blood analysis, whole body gross necropsy, and microscopic examination of all organs and tissues.

Material and Methods

Plant Materials

All Raw materials and drug were procured from Local Market of Kolhapur, Maharastra.

Determination of Foreign Organic Matter

Weigh 100 to 500 g of the sample. Spread the sample on a white tile or a glass plate uniformly without overlapping. Inspect the sample with necked eyes or by means of lens. Separate the foreign organic matter. After complete separation, weigh the matter and determine % w/w present in sample.[12]

Determination of Ash Values:

Determination of total Ash values: Weigh and ignite flat, thin, porcelain dish or a tared silica crucible. Weigh about 2g of the powered drug into the dish/crucible. Supports the dish on a pipe-clay triangle placed on a ring of retort stand. Heat with a burner, using a flame about 2 cm. high and supporting the dish about 7 cm. above the flame, heat till vapors almost lower the dish and heat more strongly until all the carbon is burnt off. Cool in a desiccator. Weigh the ash and calculate the percentage of total ash with reference to the air-dried sample of the crude drug.[12]It can be calculated by using formula

Total ash value of the sample =

$$\frac{100\times(Z-X)}{Y}\times 100$$

Determination of Acid- Insoluble Ash Values: Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Using 25 ml of dilute hydrochloric acid, wash the ash from the dish used for the dish used for total ash into a 100-ml beaker. Place wire gauze over a Bunsen burner and boil for five minutes. Filter through an 'ashless' filter paper, wash the residue twice with hot water. Ignite a crucible in the flam, cool and weigh. Put the filter- paper and residue together into the crucible; heat gently until vapors cease to be evolved and then more strongly until all carbon has been removed. Cool in desiccator. Weigh the residue and calculate acid-insoluble ash of the crude with reference to the air- dried sample of the crude drug8. It can be calculated by using formula.[12]

Acid-insoluble ash values of the sample=

$$\frac{100\times a}{Y}\%$$

Determination of water soluble ash values: Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Using 25 ml of water, wash the ash from the dish used for total ash into a 100ml beaker. Place a wire gauze over a Bunsen burner and boil for five minutes. Filter through an 'ash less' filter paper, wash the residue twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter-paper and residue together into the crucible; heat gently until vapors cease to be evolved and then more strongly until all carbon has been removed. Cool in desiccator. Weigh the residue and calculate acid-insoluble ash of the crude with reference to the air-dried sample of the crude drug.[12]

Determination of Extractive values

Determination of Alcohol-Soluble Extractive: Weigh about 5 g of the powdered drug in a weighing bottle and transfer it to a dry 250 ml

conical flask. Fill a 100-ml graduated flask to the delivery mark with the solvent (90% alc). Wash out the weighing bottle and pour the washings, together with the reminder of the solvent into the conical flask. Cork the flask and set aside for 24 hours, shaking frequently. Filter into a 50-ml cylinder. When sufficient filter has collected, transfer 25 of the filtrate to a weighed, thin porcelain dish, as used for the ash value determinations. Evaporate to dryness on a water bath and complete the drying in an oven. Cool in a desiccator and weigh. Calculate the percentage w/w of extractive with reference to the air-dried drug.[12]

Determination of water-soluble extractives: about 5 g of the powdered drug in a weighing bottle and transfer it to a dry 250 ml conical flask. Fill a 100-ml graduated flask to the delivery mark with the solvent (chloroform). Wash out the weighing bottle and pour the washings, together with the reminder of the solvent into the conical flask. Cork the flask and set aside for 24 hours, shaking frequently. Filter into a50 ml cylinder. When sufficient filter has collected, transfer 25 of the filtrate to a weighed, thin porcelain dish, as used for the ash values determinations. Evaporate to dryness on a water bath and complete the drying in an oven. Cool in a desiccator and weigh. Calculate the percentage w/w of extractive with reference to the air-dried drug.[12]

Determination of Flow Properties:

Determination of bulk density and tapped density: Weigh accurately 25 g of powder (w1). Place it in dried graduated measuring cylinder and note volume as V1 ml. Place the cylinder containing sample in bulk density apparatus. Adjust apparatus for 100 tapping and operate it. Record the volume occupied by the power as V_2ml. Formula for calculation of bulk density and tapped density as follows:

$$\text{Bulk Density} = \frac{\text{Mass}}{\text{Bulk volume}}$$

$$\text{Tapped Density} = \frac{\text{Mass}}{\text{Tapped Volume}}$$

Determination of angle of repose: Take a clean and dry funnel with a round steam of 20 to 30mm diameter with flat tip and attach it to burette stand. Place a graph paper sheet below the funnel on clean and dry platform. Adjust the distance between lower tip of the funnel and sheet to some specified height. (1cm to 2cm). Gently pour sample in funnel from top till a heap of powder forms and touches the lower tip of the funnel. Using a pencil draw a circle around the heap covering approximately 90% of total powder. Repeat the procedure four times to obtain average reading. Find out average diameter and radius of each drawn circle 8. It can be calculated by using formula,[12]

$$\textbf{Angle of Repose}(\theta) = \tan^{-1}(h/r)$$

Where h= height of pile; r= radius

Determination of Loss on Drying

Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100 0C or 105 0C Cool in a desiccator and watch the loss in weight is usually recorded as moisture.

Phytochemical screening[12]

Following tests were performed for different phyto-constituents:

Test for Protein

i. **Birute Test**- In solution of protein, there is addition of dilute NaOH and few drops of $CuSO_4$ solution. Violet or pink colored solution observed.
ii. **Million's test**- Mix 3 ml of protein solution with 5 ml of Million's reagent. White ppt. Warm ppt turns brick red or the ppt dissolve giving red colour solution.

Test for carbohydrate

i. **Molish Test**-In dilute solution of carbohydrate, molish reagent added and few drops of conc. Hcl. Purple color observed.
ii. **Fehling's test**-On addition of Fehling's solution, color of solution changed from blue to red/ brown.

Test for alkaloids

Evaporate the aqueous, alcoholic and chloroform extract separately. To residue, add dil. HCl shake well and filter. With filtrate, perform following tests:

1. **Dragendroff's test:** 2 to 3 ml filtrate with few drops of dragendroff's reagent. Orange brown ppt is formed.
2. **Mayer's test:** 2 to 3 filtrate with few drops Mayer's reagent gives ppt.
3. **Hager's test:** 2 to 3 ml. filtrate with Hager's reagent gives yellow ppt.
4. **Wager's test:** 2 to 3 ml. filtrate with few drops Wagner's reagent gives reddish brown ppt.

Test for tannins
Small quantity of extract mixed with water and heated on water bath then filtered it and add ferric chloride solution which gives dark green color.

Test for steroid
In 0.5 g of extract add 2 ml acetic anhydride with each 2 ml of sulfuric acid, color changes from violet to blue.

Test for flavonoids
0.2gm of extract dissolved in diluted hydrochloric acid and add sodium hydroxide, yellow solution turns to colorless.

Analytical Study
Thin Layer Chromatography: The stationary phase was applied onto the plate uniformly and then allowed to dry and stabilize. A thin mark is made at the bottom of the plate with a pencil to apply the sample spots. Then samples solution were apply on the spots marked on the line at equal distances. The mobile phase is poured into the TLC chamber to a level few centimeters above the chamber bottom. Then the plate prepared with sample spotting was place in TLC chamber such that side of the plate with sample line was towards the mobile phase. The chamber was closed. The plate was immerse such that sample spots were well above the level of mobile phase but not immersed in the solvent. Allow sufficient time for development of spots. Then the plates were removed and allowed to dry. The sample spots visualized by using iodine chamber or UV light.[13]

Atomic Absorption Spectroscopy: Heavy metal detection: Accurately weigh 2 g of the sample in a Kjeldahl flask. An acid mixture of HNO3: HClO4 (4:1) was added in the flask and heated continuously till it becomes colourless. The sample was then transferred to a 20-ml volumetric flask and volume was made up with distilled H2O. A reagent blank was prepared accordingly to the procedure. The standard of lead (Pb), cadmium (Cd), arsenic (Ar).

Gas Chromatography- Mass Spectroscopy: GC-MS spectra of the Memorin was recorded by using GC-MS (Schimadzu GC- 2010). The GC is usually coupled to a Mass Spectrometer (detector) which has the function of recording the mass spectrum of the chemical compounds as they come out of the GC and after fragmentation processes by a stream of electrons in the mass spec.

Helium gas is often used a carrier gas, although other gases like nitrogen may also be used. It is recommended that the GC oven should be initially held at 80°C for 1 min., and then warmed at 4-6 min-1 about 300°C, where it is to be held for 20 min., and the total length of time for running the analysis determined and programmed by the analytical geochemist (GC-MS analyst). Peaks in the chromatograms produced by these analyses were identified by a combination of references to their mass spectra and by compare retention time and elution orders with those of known standards previously analyzed on the same gc-ms.[13]

FT-IR
FT-IR spectra of Memorin recorded using FT-IR (Cary 63, Agilent Technology). The crystal was cleaned properly. A sample quantity of Memorin was placed on the sample accessary of the instrument. Knob of the instrument was pressed down the spectrum was recorded over the range of wave number 4000-650 cm-1 with Micro Lab software. FT-IR spectra of other excipient were also recorded in similar manner. For liquid sample like olive oil and glycerin, there was no need to press the knob.[13]

HPLC: High Performance and Liquid Chromatography
It is a chromatographic technique that is used to separate a mixture of compounds. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector that provides a characteristic retention time for the analyte. The pump provides the higher pressure required to propel the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography.[13]

Chromatographic Conditions:
Mobile Phase: methanol: ammonium acetate
Detection: 350 nm
Pressure: 121kgf/cm2
Temperature: Room Temperature
Flow Rate: 1 ml/min
Column: luna C 18 250 x 4.6 mm (5 µ)
Detector: UV Visible

Toxicity

For Acute Toxicity: The acute toxicity study for drug was performed using rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. To find the LD50 of drug three groups of rats such, having one rat in each group, were given PM in the doses of 500, 1000, and 2000 mg/kg orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 hours after treatment for any behavioral changes/mortality. They will further observe daily for 7 days for mortality and their behavioral changes/mortality will determine whether drug is safe for use or not.[9-10]

For Sub-acute Toxicity: Repeated dose oral toxicity study was carried out according to OECD Guideline 407. The animals were divided into four groups and each group having one animal. Group 1 received 10 ml/kg body weight of normal saline and served as control. Groups 2, 3 and 4 received drug doses of 125, 250 and 500 mg/kg body wt, respectively. These rats will observe for their Mortality, body weights, food and water consumption as well as general toxicity signs of the animals will evaluated daily for 28 days. [9-10]

Results and Discussion

Organoleptic Characters:

Color, odour, taste etc. of the powders were recorded.

Name	Mandukparni	Shankhpushpi	Jatamansi	Yashtimadhu
Color	Grayish green	Brownish- ash	Dark brown	Yellowish
Odor	Slightly aromatic	Aromatic	Strongly aromatic	Characteristic
Taste	Slight bitter	Bitter Astringent	Acrid, slightly bitter	Sweet
Touch		Smooth	Fibrous	Hard

Forgein matter

All the raw materials were checked for presence of Forgein matter.

Raw Material	Foreign matter	Standard (NMT)
Mandukparni	Absent	2%
Shankhpushpi	Absent	2%
Jatamansi	Absent	5%
Yashtimadhu	Absent	3%

Physico-chemical parameters: Drug were evaluated for various Physico-chemical parameters like total ash, water soluble extractive and alcohol, soluble extractive values, loss on drying, bulk density, tapped density, angle of repose.

Ash Values of raw materials and drug:

Total Ash Value: Total Ash Values of raw material and drug shown in table.

Raw Materials	Total Ash Values (% W/W)	Standards(NMT) (%)
Mandukparni	2	17
Shankhpushpi	2	17
Jatamansi	8.5	9
Yashtimadhu	7.9	10
Memorin(Drug)	3.8	-

Acid Insoluble Ash Values: Acid Insoluble Ash Values of raw material and drug shown in table.

Raw Materials	Acid Insoluble Ash Values (% W/W)	Standards (NMT) (%)
Mandukparni	1.9	5
Shankhpushpi	1.5	7
Jatamansi	3.8	5
Yashtimadhu	0.6	2.5
Memorin(Drug)	1.6	-

Extractive Values of Raw Materials and Drug:

Alcohol Soluble Extractive Values: Alcohol soluble extractive Values of raw material and drug is shown in table.

Raw Materials	Alcohol Soluble Extractive Values (% W/W)	Standards (NLT) (%)
Mandukparni	0.11	9
Shankhpushpi	0.1	6
Jatamansi	0.10	2
Yashtimadhu	0.26	10
Memorin(Drug)	0.4	-

Water Soluble Extractive Values: Water soluble extractive values of raw material and drug is shown in table.

Raw Materials	Water Soluble Extractive Value (% w/w)	Standards (NLT) (%)
Mandukparni	0.20	20
Shankhpushpi	0.3	10
Jatamansi	0.13	5
Yashtimadhu	0.31	20
Memorin(Drug)	0.36	-

Loss on Drying of raw materials and drug (LOD): LOD of raw materials and drug is shown in table.

Ingredients	Mandauk-parni	Shankh-pushpi	Jata-Mansi	Yashti-madhu	Memorin (Drug)
LOD (%)	5.4	6.3	3.8	3.8	6.5

Bulk and Tapped Density: Data of bulk and tapped densities are shown in table.

Ingredients	Bulk Density (g/ml)	Tapped Density (g/ml)
Mandukparni	0.6	0.8
Shankhpushpi	0.22	0.40
Jatamansi	0.37	0.52
Yashtimadhu	0.4	0.5
Memorin(Drug)	0.25	0.5

Phytochemical screening

Drugs and raw materials were tested for presence of different phyto-constituents.

Drug Ingredients	Shankhpushpi	Mandookparni	Jatamansi	Yashtimadhu
Protein	+	+	+	+
Steroid	-	-	-	-
Tannins	+	-	+	+
Flavonoids	+	-	+	+
Alkaloids	+	-	+	+
Glycosides	+	+	-	+

Screening of Phytoconstituents (Present: +, Absent: -)

Solubility, pH and Melting Point of ingredient

Test	Mandukparni	Shankhpushpi	Jatamansi	Yashtimadhu
Solubility	Water	Water	90% alcohol	Water
PH	7	5.6	7.4	7
Melting point	82-83 ^{0C}	162-164 ^{0C}	133-34 ^{0C}	190 ^{0C}

Angle of Repose: Angle of Repose of raw materials and drug is shown in table.

Raw Materials	Angle of Repose (Θ)	Criteria
Mandukparni	28.03	Excellent
Shankhpushpi	32.6	Good
Jatamansi	26.74	Excellent
Yashtimadhu	28.74	Excellent
Memorin(Drug)	25.60	Excellent

Thin Layer Chromatography

TLC was run for raw material. Different mobile phase were used for this purpose. Result of thin layer chromatography are shown in table.

Ingredients	Name of mobile phase	Rf values
Mandukparni	Tolune: Methanol (5:5)	0.7
Shankhpushpi	Tolune: Ether (1: 1)	0.8
Jatamansi	Hexane: Ethyl Acetate: Acetic Acid (8:2)	0.7
Yashtimadhu	Tolune: Ethyl Acetate: Acetic acid (5: 4 : 0.8)	0.8
Memorin	Methanol: Hexane	0.6

Atomic Absorption Spectroscopy

Memorin was detected for the presence of heavy metals by using atomic absorption spectroscopy. Result are shown in table.

Data of Heavy Metal Present In Memorin

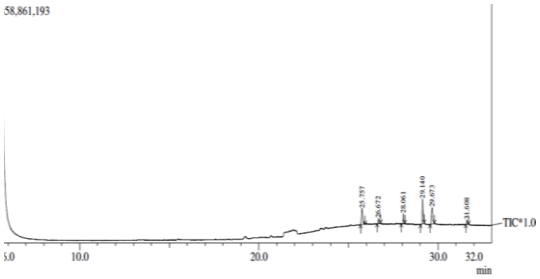
Sr. No.	Name of metals	Percentage	Standard (ppm)
1.	Lead (Pb)	0.049 mg/L	10
2.	Cadmium (Cd)	Absent	0.3
3.	Arsenic (Ar)	Absent	3

GC-MS

Qualitative determination of the Memorin was done by means of GC-MS. It is a GC-MS spectrum of drug. There are different constituents present in drug. Out of these, six fragments of the constituents are observed. Retention time and peak area can be calculated. Result are shown in table.

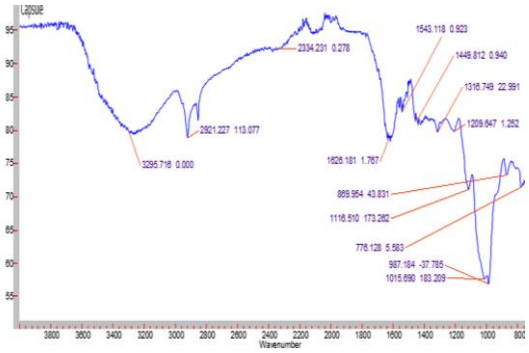
List of compounds present drug

Name of compound	Molecular formula	Molecular weight
Decyl oleate	$C_{28}H_{54}O_2$	422
Tetracosane	$C_{24}H_{50}$	338
Petacosane	$C_{25}H_{52}$	352
Di-actyl phthalate	$C_{24}H_{38}O_4$	390
Butylaldehyde,4-benzyloxy-4-[2,2-dimethyl-4-dioxolany-1]	$C_{16}H_{22}O_4$	278
Tricosane	$C_{23}H_{48}$	324



GC-MS Data of Memorin capsule

FT-IR: IR of Memorin: IR spectra of Memorin. Result shown in table.



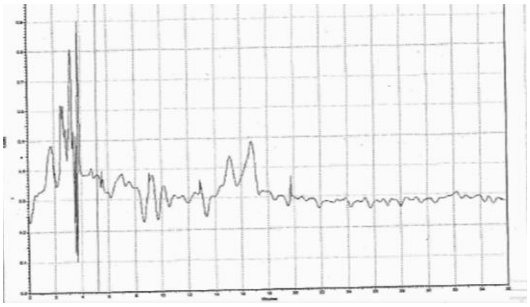
IR spectra of Memorin capsule

IR data of Memorin capsule

Observed wavenumber	Functional group	Reported wavenumber
2921.227	O-H	2500-3300
1626.181	C6H5	1600-1700
1116.510	C-O	675-1000

HPLC (For Memorin capsule only)

HPLC chromatogram of Memorin is shown in fig. 15,16,17,18. Memorin extract was prepared and analyzed using HPLC at different wavelength such as 320, 350, 480, 620nm. The HPLC chromatogram shows about 6-7 peaks corresponding to different constituents of Memorin extract.



HPLC chromatogram of Memorin at 350nm

The chromatogram of 350nm shows more intense peaks as compare to wave no. 220, 480, 620nm. From the HPLC analysis, we can predict the presence of six major probable constituents in the extract. This result is in accordance with GC-MS analysis that show 6 different probable constituents present in Memorin extract.

Toxicity Studies

Acute toxicity data of Memorin capsule

Extract Used	No.of animals	Limit of Dose	Duration of period	Sign of toxicity	Mortality
Aqueous extract (Memorin capsule)	6 rats (female)	2000 mg/kg	24 hours	No	No

Sub-acute toxicity:

Sub-acute toxicity data of Memorin capsule

Extract Used	No.of animals	Limit of dose	Duration of period	Sign of toxicity	Mortality
Aqueous extract (Memorin capsule)	6 rats (female)	2000 mg/kg	28 days	No	No

Effect of aqueous extract of Memorin capsule on the body weight of rats

Groups	Dose	Body weight		Result
		Before Test	After Test	
I	500 mg/kg	150 gm	130 gm	No mortality
II	1000 mg/kg	175 gm	140 gm	No mortality

For acute toxicity

No mortality and morbidity or any signs of behavioral changes or toxicity were observed throughout the 24 hours. Morphological characteristic (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors and forth were observed, posture, reactivity of handling or sensory stimuli, grip strength were all normal. There are slightly changes in body weight of animals.

Effect of aqueous extract of Memorin capsule on body weight of rats.

Groups	Dose	Body weight		Result
		Before Test	After Test	
Control	Water for injection	200	190	No mortality
I	100 mg/kg	180	175	No mortality
II	500 mg/kg	170	165	No mortality
III	2000 mg/kg	175	172	No mortality

For sub-acute toxicity

The animals were healthy with no differences being noted with respect to the control group. There was slightly change in body weight of treated groups as compare to control groups. No mortality was observed during the experiment.

Discussion

The present study was undertaken to evaluate the ayurvedic formulation which was Memorin capsule by quality standardization and toxicity study. Quality standardization of Memorin capsule was performed by physical parameters analysis, and phytochemical analysis. The physical parameters such as total ash value, extractive value, bulk density, tapped density, LOD, were calculated. All calculated values were passing the Ayurvedic Pharmacopial Standard. The water-soluble ash values are not mentioned in Ayurvedic Pharmacopeia. The phytochemical screening showed presence of alkaloids, glycosides, tannins, saponins, steroids, and carbohydrates in extract of drug. Different tests were performed for the screening. There was no any heavy metal present. Pb was found in drug but it does not have any side effect.

Our in-vivo study indicates that there was no toxic effect of aqueous extract of Memorin capsule on Wister albino rats. Only difference in body weight of animals was observed. There was normal behavior of animals after administration of dose. There was no any sign of toxicity. Mortality was not found during experiment. So it was concluding that drug was safe. Safe dose of drug was found to be 2000 mg/kg.

Conclusion

Standardization of a drug was performed by evaluating its physical parameters and phytochemical analysis. Result for quantitative analysis for water soluble ash, acid insoluble ash values, water soluble extractive, and alcohol soluble extractive values, loss on drying, foreign organic matter were calculated and found to be within range and these all values pass the Ayurvedic Pharmacopial limits. Phytochemical analysis revealed the presence of glycoside, carbohydrates, saponins, tannins, and steroid.

From the experiment performed as per the OECD guidelines 423, the result shows that aqueous extract of Memorin capsule have been found nontoxic at dose 2000 mg/kg. No changes were observed for skin, far, eyes, behavioral pattern, salivation, sleep of treated as well as control animal. Tremors, lethargy, diarrhea and coma did not occur in any of the animal. There is only change in body weight of the animal.

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