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Quality Standardization and Acute Oral Toxicity Evaluation of *Vishavilwadi Gutika* - A Potent Antitoxic Formulation

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ABSTRACT

Vishavilwadi Gutika is a traditional *Agada* (anti-poisonous) formulation recommended for the treatment of *Sarpa Visha* (snake venom), as mentioned in the text *Kriya Kaumudi* by Kuttikrishna Menon, a renowned *Visha Vaidya* from Kerala. This polyherbal formulation consist of 16 herbal drugs were triturated in *Bastha Mootra* (goat's urine) which possess properties like *Vishagna* (antitoxic), *Shophagna (*antiinflammatory) and *Krimigna* (anthelminthic), while also aiding metabolism. This study endeavours to standardize and analyse acute oral toxic response of *Vishavilwadi Gutika*. Various parameters, including uniformity of weight, hardness, disintegration time, pH, loss on drying, total ash, acid-insoluble residue, alcohol and water extractive esteem and HPTLC profiling, were surveyed agreeing to the measures set by the Indian Pharmacopeia and Ayurvedic pharmacopeia of India to guarantee quality control of the herbal ingredients. Acute oral toxic response was assessed in conformity with OECD 425 protocols, evaluating the formulation's safety at maximum lethal dosage. The findings indicated that the reference standard profile for *Vishavilwadi Gutika* is non-toxic and safe for internal use. Consequently, this study has established a standardised, affordable and effective anti-toxic herbal formulation for future researches.

Key words: *Vishavilwadi Gutika, Visha, Agada, Standardization, Acute oral toxicity.*

INTRODUCTION

Traditional polyherbal formulations have been employed worldwide for centuries in both the management and prevention of a wide range of acute and chronic illness. Recently, research has placed increasing attention on herbal medicines because of their wide range of biological effects, accessibility, affordability and generally safe usage.[1] In contrast, the safety and efficacy of herbal formulations have become a significant concern in recent times due to excessive over the counter usage of drugs, limited regulatory oversight, adulteration, contamination and increased global demand. As a result, standardization and acute oral toxicity studies have gained importance in order to establish and assess the safety profile of the herbal formulations. The process of formulating and applying standards that are results of industry, user, interest group and governmental consensus is known as standardization.[2] The standardization process guarantees that results are linked to established reference systems, providing increased confidence in their accuracy and proximity to the true value. A proper standardization involves several key steps. First, it requires the development and characterisation of appropriate reference materials, with their values assigned in meaningful units using validated reference

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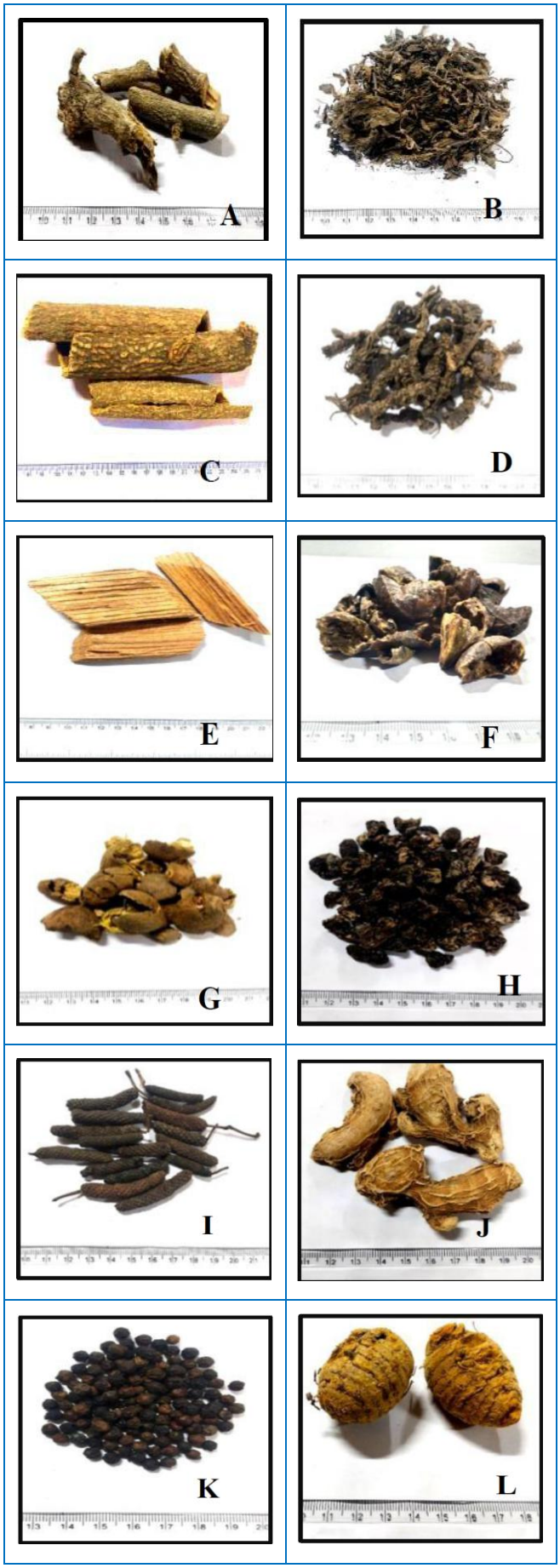
measurement procedures. Next, commercial routine assays or field assays, must be established to provide results that are traceable to higher-order reference materials and methods. Finally, suitable reference intervals and decision limits must be available to ensure proper interpretation and decision- making based on the results.[3] Acute oral toxicity responses are checked to evaluate the harmful effects that arise within a short period after administering a single, often high dose of chemical, physical, or biological agents. The purpose of these studies is to detect any adverse health effects associated with the test drug, covering direct and deferred biochemical, physiological or morphological alterations, as well as long term effects that may suggest secondary damage to organs or tissues.[4]

Vishavilwadi Gutika is a polyherbal antitoxic formulation mentioned in in the context of *Uragavisha Samanya Chikitsa Prakarana* (Snake bite treatment protocol) of *Kriya Kaumudi*, a Keraliya *Visha Chikitsa Grantha*.[5] It consists of a remarkable blend of 16 herbal ingredients that are economically feasible and readily available such as *Vilwa, Tulasi, Karanja, Natam, Devadaru, Amalaki, Vibheethaki, Harithaki, Pippali, Maricha, Shunti, Haridra, Daruharidra, Patha, Ishwaramooli* and *Neeli* grounded in freshly collected *Basthamootra* and made into *Gutika* (tablet) form. This study focuses on the pharmaceutico-analytical standardization and acute oral toxicity assessment of *Vishavilwadi Gutika*, performed in accordance with the OECD 425 protocol, to determine its safety and efficacy for internal use.

Materials and Methods

Plant Materials and Preparation

All sixteen ingredients were gathered in equal quantities, authenticated and grounded into a *Sookshma Choorna* (fine powder) that passes through sieve no 85.[6] This powder was then triturated with freshly collected goat's urine and formed into 500 mg pills at the G.M.P. certified S.D.M. Ayurveda Pharmacy, Kuthpady, Udupi, Karnataka, India. Ingredients of *Vishavilwadi Gutika* are tabulated in Table no.1 and pictures are depicted from Fig.1.



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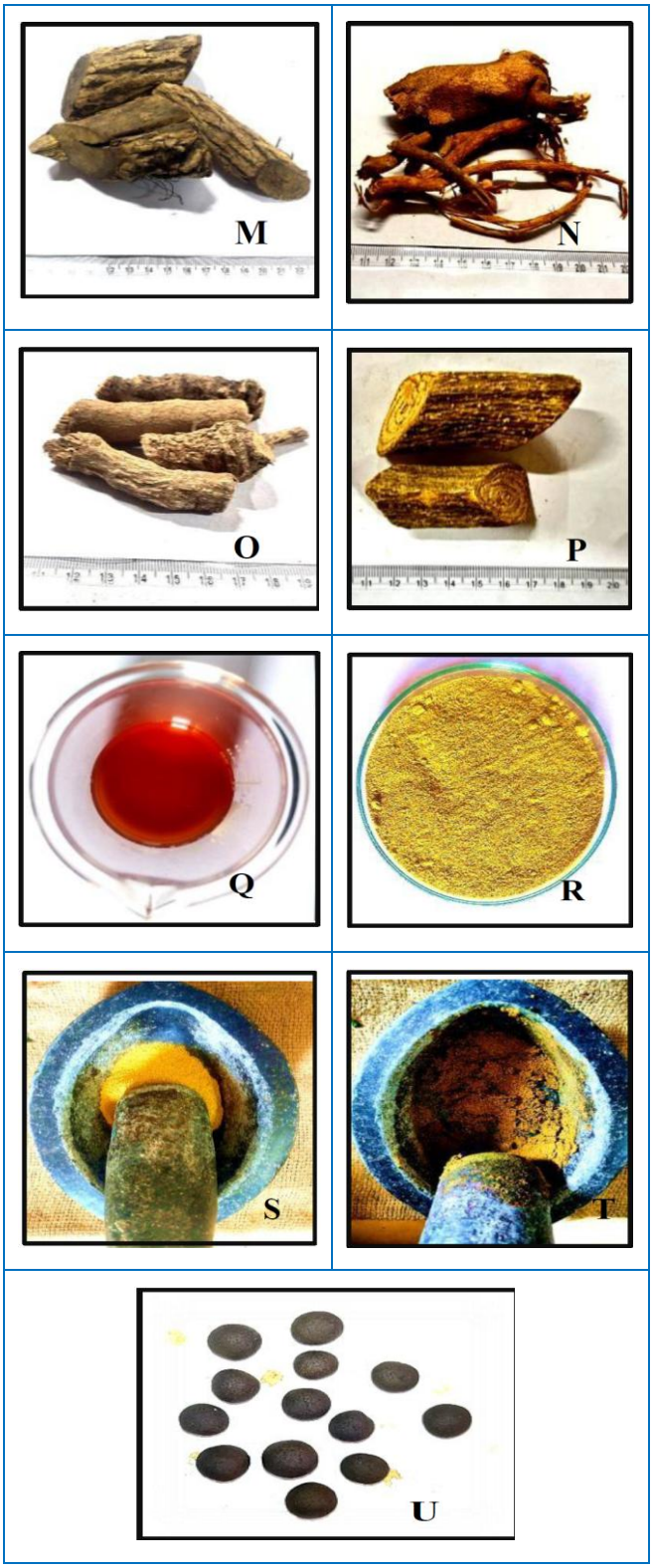

Figure 1: *Vishavilwadi Gutika* ingredients and preparation image (A)*Vilwa* Root (B) *Tulasi* Flower (C) *Karanja* Root (D) *Tagara* Root (E) *Devadaru* heartwood (F) *Harithaki* fruit (G) *Vibhithaki* fruit (H) *Amalaki* fruit (I) *Nagara* rhizome (J)*Maricha* fruit (K) *Pippali* fruit (L) *Haridra* rhizome (M) *Daruharidra* rhizome (N) *Neeli* root (O) *Ishwara mooli* root (P) *Patha* rhizome (Q) *Bastha mootra* (R) *Sookshma Choorna* of all drugs (S) Before trituration (T) After trituration (U) Final form of *Vishavilwadi Gutika*

Table 1: Ingredients of *Vishavilwadi Gutika* along with its latin name, parts used and Quantity.

SN	Drugs	Botanical name	Used part	Quantity
1.	*Vilwa*	*Aegle marmelos Corr.*	Root	100g
2.	*Surasa*	*Ocimum Sanctum Linn.*	Flower	100g
3.	*Karanja*	*Pongamia glabra Vent.*	Root	100g
4.	*Tagara*	*Valeriana wallichii DC*	Root	100g
5.	*Devadaru*	*Cedrus deodara Roxb*	Heartwood	100g
6.	*Haritaki*	*Terminalia chebula Retz*	Fruit	100g
7.	*Amalaki*	*Emblica officinalis Gaertn*	Fruit	100g
8.	*Vibhitaki*	*Terminalia bellerica Roxb*	Fruit	100g
9.	*Nagara*	*Zingiber officinale Roxb.*	Rhizome	100g
10.	*Maricha*	*Piper nigrum Linn*	Fruit	100g
11.	*Pippali*	*Piper longum Linn*	Fruit	100g
12.	*Haridra*	*Curcuma longa Linn.*	Rhizome	100g
13.	*Daruharidra*	*Berberis aristata DC*	Stem	100g
14.	*Nili*	*Indigofera tinctoria Linn*	*Root*	100g

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15.	*Ishwari mula*	*Aristolochia indica Linn*	*Root*	100g
16.	*Patha*	*Cissampelos pareira Linn*	Rhizome	100g
17.	*Bastha mootra*	Goats' urine	----------	Q. S

Instrumentation and techniques of standardization

The analytical studies were carried out at the S.D.M. Centre for Research in Ayurveda and Allied Sciences, located in Kuthpady, Udupi, Karnataka, India.

Assessment of key parameters for standardization

1. Organoleptic evaluation

It includes assessment of color, taste, odour and appearance as the sensory attributes significantly influence consumer preference.

2. Physicochemical parameters

a) Uniformity of weight

Instruments used: Weighing balance and petridish.

Procedure: Twenty tablets of *Vishavilwadi Gutika* were randomly selected and weighed. The pills' individual weights were noted and the average weight was computed. It must remain aligned with the mean weight without exceeding the specified proportions of 10 ,7.5 and 5 percentage deviation with respect to average weight of tablet as 80mg or less, more than 80mg but less than 250mg and 250mg or more, and no weight should deviate by more than twice that limit.[7]

b) Hardness test

Instruments used: Monsanto hardness tester

Procedure: Five tablets of *Vishavilwadi Gutika* were selected and tested for hardness. A threaded bolt was used to force the higher plunger against a spring until the tablet broke, while the bottom actuator in proximity to the tablet. The amount of force needed to break the tablet was noted.[7]

c) Disintegration time

Instruments used: Disintegration apparatus

Procedure: The disintegration unit tank was filled with distilled water up to the specified level. A total of 750 ml of distilled water was poured into each of the 1000 ml beakers. The instrument's timer was set for 60 minutes. The water in the beakers was maintained at a temperature of 37°C, while the water in the main tank was kept at 37.5°C. One tablet was placed in each tube, with a disk added to each. The assembly was then suspended in the beakers containing the water, and the apparatus was activated. The disintegration time of each tablet was recorded.[7]

d) Determination of pH

Instruments used: pH meter, Electrodes, Tablets of 4,7 and 9.2

Procedure: Preparation of Buffer Solutions - One tablet each of pH 4, 7, and 9.2 was dissolved in 100 milliliters of distilled water to prepare standard buffer solutions. To measure the pH, one milliliter of the sample was taken, diluted with 10 milliliters of distilled water, thoroughly mixed, and then filtered. The experiment was performed using the filtrate. After turning on the device, the pH meter was allowed to warm up for 30 minutes. The pH was first adjusted to 4.02 at room temperature (30°C) using the knob after adding the pH 4 solution. The pH meter was then set to 7 by adjusting the knob after the pH 7 solution was added. After adding the pH 9.2 solution, the pH reading was taken without further adjustments. Finally, a 10% solution of *Vishavilwadi Gutika* was added, and the pH reading was recorded. The procedure was repeated four times, and the average of the readings was used as the final result.[8]

e) Moisture content (Loss on drying at 105^0C)

Instruments used: Hot air oven and Petridish

Procedure: Ten grams of the sample (*Vishavilwadi Gutika*) were placed in a tared evaporating dish and dried at 105°C for 5 hours in a hot air oven. Afterward, the sample was weighed. Drying continued until the difference between two consecutive weights did not exceed 0.01 g, after cooling in a desiccator. The percentage of moisture was then calculated based on the initial weight of the sample.[9]

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f) Total ash

Instruments used: Crucible, Ashless filter paper, Muffle furnace.

Procedure: Two grams of the sample (*Vishavilwadi Gutika*) were burned in a tared platinum crucible at a temperature not exceeding 450°C until all carbon had been eliminated, leaving behind ash. The percentage of ash was then calculated based on the initial weight of the sample.[10]

g) Acid insoluble ash

Instruments used: Crucible, Ashless filter paper, Muffle furnace.

Procedure: Dilute HCl (25 ml) was added to the crucible containing the total ash and brought to a boil. The insoluble material was collected on ashless filter paper (Whatman 41), and the filtrate was washed with hot water until it reached a neutral pH. The filter paper with the insoluble material was then transferred back to the original crucible, dried on a hot plate, and ignited to a constant weight. After cooling for 30 minutes in an appropriate desiccator, the residue was promptly weighed. The acid-insoluble ash content was calculated, considering the air-dried weight of the drug.[10]

h) Water soluble ash

Instruments used: Crucible, Ashless filter paper, Muffle furnace.

Procedure: The ash was boiled with 25 ml of water for 5 minutes. The insoluble material was collected on ashless filter paper, washed with hot water, and incinerated at a temperature not exceeding 450˚C for 15 minutes. The water-soluble ash was calculated by subtracting the weight of the insoluble material from the total ash weight, using the air-dried sample as a reference.[10]

i) Alcohol soluble extractive

Instruments used: Weighing balance, Alcohol, Graduated cylinder, Filter paper, Stoppered conical flask.

Procedure: Four grams of the sample (*Vishavilwadi Gutika*) were accurately weighed and placed in a glass-stoppered flask. To this, 100 ml of approximately 95% alcohol was added, and the mixture was periodically shaken for six hours, then allowed to stand for 18 hours. The solution was carefully filtered to prevent solvent loss. A 25 ml portion of the filtrate was pipetted into a pre-weighed 100 ml beaker, and the solvent was evaporated on a water bath. The beaker was then heated in an air oven at 105°C for six hours, cooled in a desiccator for 30 minutes, and weighed. The proportion of alcohol-extractable materials was calculated, and the experiment was repeated twice to record the average value.[9]

j) Water soluble extractive

Instruments used: Weighing balance, Water, Graduated cylinder, Filter paper, Stoppered conical flask.

Procedure: Four grams of *Vishavilwadi Gutika* were precisely weighed and placed in a glass-stoppered flask. For six hours, the mixture was shaken periodically after adding 100 milliliters of distilled water. After then, it was left for eighteen hours to stand. Quick filtering was done on the solution to prevent solvent loss. A 25 ml portion of the filtrate was transferred to a pre-weighed 100 ml beaker and evaporated on a water bath. The beaker was then heated at 105°C in an air oven for six hours, cooled in a desiccator, and weighed. The experiment was repeated twice, and the average value was recorded.[9]

3. Analytical method: HPTLC (High Performance Thin-Layer Chromatography)

Instruments used: Linomat 5 TLC applicator

Procedure: 1 gram of *Vishavilwadi Gutika* was dissolved in 10 ml of ethanol. Samples measuring 3, 6, and 9 µl of the solution were applied to pre-coated silica gel F254 aluminum plates with a 7 mm band width using a Linomat 5 TLC applicator. The plates were developed using a solvent system of toluene and ethyl acetate (9.0:1.0). After development, the plates were examined under short-wave and long-wave UV light and post-derivatized with sulfuric acid and vanillin. They were then scanned under UV light at wavelengths of 254 nm, 366 nm, and 620 nm (post-derivatization). The Rf values, spot colors, and densitometric scans were recorded.[11,12]

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Acute Oral Toxicity Study

Approval from Animal ethics committee: The study was performed after getting approval from IAEC of S.D.M Ayurveda college in its meeting held on 16/04/2024 - Ref. No. SDMCRA/IAEC/S-A-04.

Selection of Animal species:

a) Animal species - Wistar strain albino rats

b) Source - Animal house attached to SDM Research centre, Kuthpady, Udupi.

c) Selection - Five healthy rats of both sexes weighing between 160 and 200 grams were chosen using AOT software.

d) Acclimatization period - Prior to dosage, all of the chosen animals were housed in an acclimation period of seven days.

e) Numbering and identification - The animals were marked with saturated picric acid solution in water and numbered with respect to its body parts for proper identification as shown in (Figure 2 A and B).

Husbandry condition: [13]

a) Housing: Polypropylene cages with stainless steel top covers were used to house the rats. The bedding was made of dry husk, which was replaced every morning.

b) Environment: The animals were kept in an environment with a temperature of 22 ± 3°C, a 12-hour light and 12-hour dark cycle, and a relative humidity between 50% and 70%.

c) Diet: Throughout the trial, rats were fed pellet feed from Sai Durga Feed in Bangalore, with the exception of the night before dosing, when the animals fasted. Unlimited access to drinking water was provided in polypropylene bottles with stainless steel sipper tubes.

Dose Preparation and Schedule: [13]

a) Test Drug: *Vishavilwadi Gutika*

b) Dose fixation: According to the AOT Software

c) Dose: 175mg/kg, 550mg/kg, 2000mg/kg test substance as shown in (Figure 2 C)

d) Schedule: Single dose per animal

e) Administration: Oral route via an oral feeding needle at varying dosage amounts to the individual animal, as demonstrated in (Figure 2 D)

Figure 2 (A): Identification mark with picric acid

Figure 2 (B): Marked Group of Rats

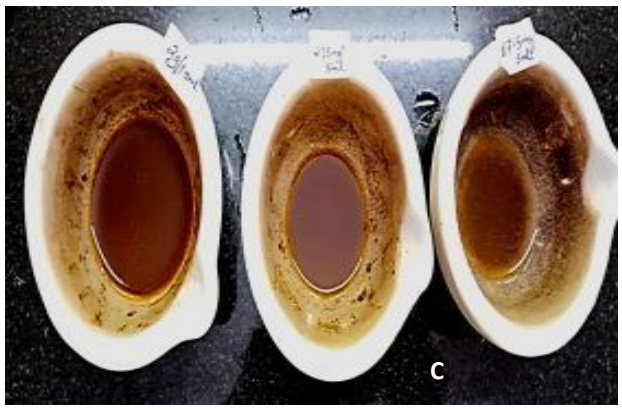

Figure 2 (C): 175mg/kg, 550mg/kg, 2000mg/kg dose preparation of test substance

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Figure 2 (D): Oral feeding of test sample

Procedure of Acute oral toxicity assay[14]

Animals are dosed individually in sequence, typically with a 48-hour gap between each dose. However, the time intervals may be adjusted based on the timing, severity, and duration of any toxic effects that appear. The next dose is administered only when it is reasonably certain that the previous animal will survive. To determine the starting dose, all relevant data are considered, including information from structurally similar compounds and results from any previous toxicity tests on the substance, to estimate the LD50 and the pharmacodynamic slope. The initial animal's dosage is one step below the expected LD50. The subsequent animal receives a larger dosage if it lives. In the event that the first animal passes away or exhibits toxicity, the second animal is given a lower dose. Dosing starts at 175 mg/kg in the absence of any previous fatality evidence. Based on the observed outcomes, dosing is continued for each animal at predefined intervals (e.g., 48 hours). When the initial stopping condition is satisfied which can be when three consecutive animals survive at the maximum dosage or when another predetermined criterion is reached, the testing comes to an end. After that, maximum likelihood estimation is used to get the LD50. The animals were under constant observation for four hours after dosing. In order to document behavioural changes, each animal was separately placed in an open arena and at the end of each hour careful cage-side observations were recorded without disturbing them. These symptoms include convulsions, Straub's reaction, muscle spasms, opisthotonus, changes in motor activity (increased or decreased), convulsions, arching and rolling, lacrimation, salivation, diarrhoea, writhing, respiratory changes and signs of CNS depression such as hypoactivity and other pertinent symptoms. Mortality Monitoring: Throughout the 14-day trial period, all animals were examined once daily for any indications of morbidity or mortality after being dosed at ½, 1, 2, 3, 4, 24 and 48hrs.

Results and Discussion

Table 2: Results of Organoleptic evaluation

Parameters	Result
Colour	Brownish black (Fig 1 U)
Odour	Characteristic strong pungent smell of Goat's urine
Taste	Astringent and bitter
Apperance	Smooth globular

Table 3: Results and Implications of the standardization values.

SN	Parameters	Result n = 3 (% w/w)	Implications
1.	Uniformity of wt	0.566±0.01	All the tablets in the batch were consistent in both quantity and weight, ensuring its uniformity, which is a crucial criterion for passing quality control tests.
2.	Hardness (kg/cm2)	1.5	It measures the force needed to crush or break a tablet, which is crucial for ensuring tablets remain intact during packaging, shipping, and handling. [15] It is permissible to use tablets that cause weight loss of less than 1.0%. Maintaining hardness within specified limits supports proper dissolution and

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			disintegration in the gastrointestinal tract.
3.	Disintegration time (min:sec)	2:00	Tablets are formed by compressing multiple granules, so it's crucial for them to break down from granular form to release their bioactive components for ensuring a rapid onset of action and quick drug release. [16]
4.	pH	6.76	Weakly acidic, partially neutral because of which it enhances the partition coefficient. This optimal balance between ionized and unionized forms enhances drug permeability, promoting better absorption and bioavailability, especially in lipid-rich environments like cell membranes.
5.	Loss on drying	3.79±0.01	An optimal moisture was maintained, indicating a safe shelf life for the product and ensuring it remained free from any fungal or bacterial contamination.
6.	Total Ash	14.59±0.74	Indicates the optimal amount of organic and inorganic compounds in the sample.
7.	Acid Insoluble Ash	5.29±0.67	Indicates the optimum number of inorganic materials in the sample like presence of oxalates, carbonates, phosphates, oxides and silicates.
8.	Alcohol soluble extractive value	9.31±0.01	The value indicates a mid polar and non polar phytoconstituents level in the sample.
9.	Water soluble extractive value	17.85±0.01	The value reflects higher polarity, suggesting greater water solubility, which supports effective drug metabolism, rapid action, efficient absorption and excretion giving a higher therapeutic value.

Table 4: Rf values of *Vishavilwadi Gutika*

Short UV	Long UV	After derivatisation
-	0.09 (F. blue)	-
-	0.12 (F. blue)	-
0.16 (Green)	-	0.16 (Purple)
-	0.23 (F. blue)	0.22 (Purple)
0.24 (Green)	-	-
0.29 (Green)	0.28 (F. blue)	0.29 (Yellow)
-	0.33 (F. blue)	0.34 (Purple)
0.40 (Green)	-	0.40 (D. Purple)
-	0.37 (F. blue)	-
-	-	0.47 (Purple)
-	0.50 (F. red)	-

To understand the separation of phytoconstituents and to determine their bioactivity within the formulation, HPTLC was done (Figure 4). Given that this polyherbal formulation comprises of 16 different ingredients from various plant parts, standardization poses significant challenges. With this in mind, we selected a solvent system likely to elute multiple constituents present in the formulation. In the future, we are planning to further standardize the formulation using HPTLC with four or more phytopharmaceutical markers to achieve marker-based standardization profile.

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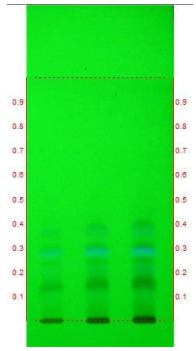
Figure 3: HPTLC photo documentation of alcoholic extract of *Vishavilwadi Gutika*



Short UV	Long UV	After derivatization

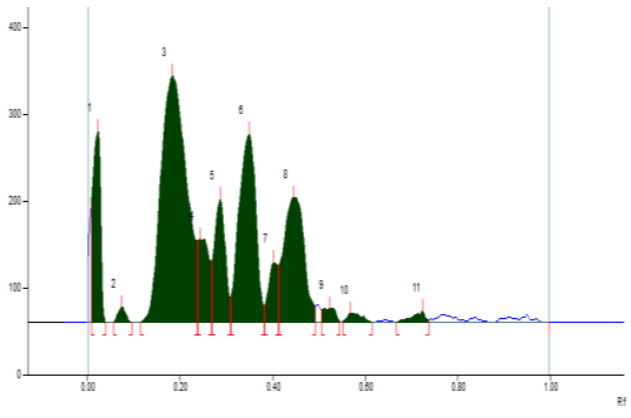
Track 1- *Vishavilwadi Gutika* – 3µl

Track 2- *Vishavilwadi Gutika* – 6µl

Track 3- *Vishavilwadi Gutika* – 9µl

Solvent system - Toluene: Ethyl acetate (9.0:1.0)

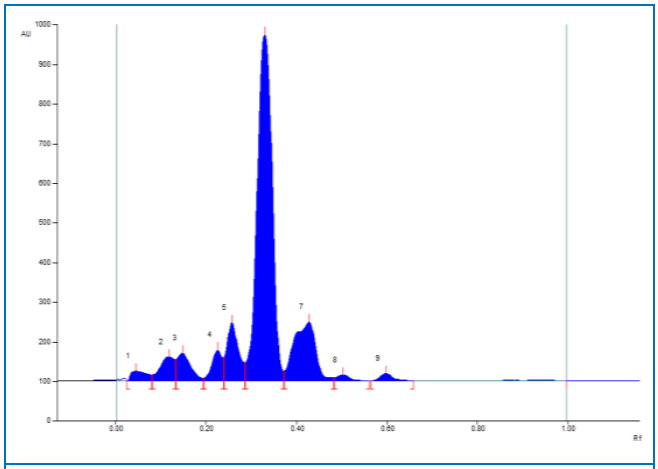
Figure 4. Densitometric scan of *Vishavilwadi Gutika*



Track 7, ID: Vishavilwadi gutika

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	137.1 AU	0.02 Rf	220.4 AU	17.95 %	0.04 Rf	0.7 AU	2766.4 AU	9.10 %
2	0.06 Rf	0.4 AU	0.07 Rf	17.6 AU	1.44 %	0.10 Rf	0.0 AU	215.4 AU	0.71 %
3	0.11 Rf	0.1 AU	0.18 Rf	283.3 AU	23.08 %	0.24 Rf	93.6 AU	11252.9 AU	37.03 %
4	0.24 Rf	93.9 AU	0.24 Rf	95.6 AU	7.79 %	0.27 Rf	70.0 AU	1673.1 AU	5.51 %
5	0.27 Rf	71.3 AU	0.29 Rf	141.6 AU	11.53 %	0.31 Rf	27.6 AU	2419.0 AU	7.96 %
6	0.31 Rf	29.7 AU	0.35 Rf	216.5 AU	17.64 %	0.38 Rf	19.0 AU	5663.0 AU	18.63 %
7	0.38 Rf	19.7 AU	0.40 Rf	68.8 AU	5.61 %	0.41 Rf	65.6 AU	1004.4 AU	3.30 %
8	0.41 Rf	66.2 AU	0.45 Rf	143.8 AU	11.72 %	0.49 Rf	18.9 AU	4587.7 AU	15.10 %
9	0.51 Rf	13.9 AU	0.52 Rf	16.2 AU	1.32 %	0.55 Rf	0.7 AU	325.5 AU	1.07 %
10	0.55 Rf	1.5 AU	0.57 Rf	10.2 AU	0.83 %	0.62 Rf	0.0 AU	236.2 AU	0.78 %
11	0.67 Rf	0.1 AU	0.73 Rf	13.5 AU	1.10 %	0.74 Rf	3.3 AU	248.4 AU	0.82 %

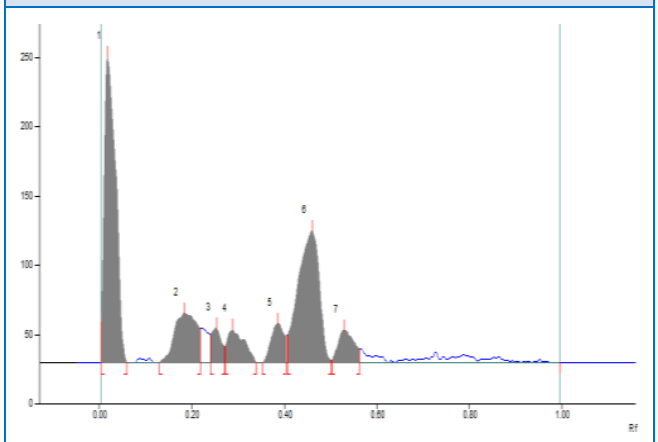
Fig 4 (A). At 254nm



Track 7, ID: Vishavilwadi gutika

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	2.5 AU	0.05 Rf	24.1 AU	1.68 %	0.08 Rf	15.3 AU	631.9 AU	1.85 %
2	0.08 Rf	15.4 AU	0.12 Rf	60.8 AU	4.24 %	0.13 Rf	53.3 AU	1383.7 AU	4.04 %
3	0.13 Rf	53.8 AU	0.15 Rf	69.6 AU	4.85 %	0.19 Rf	6.7 AU	1516.2 AU	4.43 %
4	0.20 Rf	6.9 AU	0.23 Rf	77.6 AU	5.41 %	0.24 Rf	56.9 AU	1285.9 AU	3.76 %
5	0.24 Rf	59.2 AU	0.26 Rf	147.2 AU	10.26 %	0.29 Rf	45.9 AU	2721.7 AU	7.96 %
6	0.29 Rf	46.3 AU	0.33 Rf	873.1 AU	60.83 %	0.37 Rf	24.9 AU	20835.2 AU	60.91 %
7	0.37 Rf	25.3 AU	0.43 Rf	148.7 AU	10.36 %	0.48 Rf	8.0 AU	5148.1 AU	15.05 %
8	0.48 Rf	8.1 AU	0.50 Rf	15.3 AU	1.07 %	0.56 Rf	0.0 AU	309.6 AU	0.91 %
9	0.56 Rf	0.0 AU	0.60 Rf	18.7 AU	1.30 %	0.66 Rf	0.0 AU	375.1 AU	1.10 %

Fig 4 (B). At 366nm



Track 7, ID: Vishavilwadi gutika

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	29.0 AU	0.02 Rf	220.6 AU	48.92 %	0.06 Rf	0.4 AU	3919.2 AU	37.36 %
2	0.13 Rf	0.0 AU	0.18 Rf	35.5 AU	7.88 %	0.22 Rf	24.9 AU	1257.0 AU	11.98 %
3	0.24 Rf	20.7 AU	0.25 Rf	24.8 AU	5.50 %	0.27 Rf	11.5 AU	397.4 AU	3.79 %
4	0.27 Rf	11.7 AU	0.29 Rf	23.6 AU	5.23 %	0.34 Rf	0.2 AU	627.3 AU	5.98 %
5	0.35 Rf	0.1 AU	0.39 Rf	28.1 AU	6.24 %	0.40 Rf	19.3 AU	571.7 AU	5.45 %
6	0.41 Rf	19.7 AU	0.46 Rf	94.8 AU	21.03 %	0.50 Rf	1.6 AU	3139.1 AU	29.93 %
7	0.50 Rf	1.7 AU	0.53 Rf	23.4 AU	5.19 %	0.56 Rf	9.9 AU	577.5 AU	5.51 %

Fig. 4 (C). At 620nm

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Results of Acute oral toxicity profile

An LD50 of more than 2000 mg/kg is shown by statistical estimates from both short- and long-term results, indicating that the formulation is safe for internal use and non-toxic.

CONCLUSION

As the WHO promotes the proper application of ethnomedicinal methods and highlights the significance of safety studies for herbal medications, phytotherapy is becoming more and more popular. The FDA and WHO both emphasize that scientific studies are necessary to confirm the safety and effectiveness of herbal remedies. Therefore, it is crucial to carry out first toxicological evaluations in order to guarantee the safety of herbal medicines.[17] The current research was conducted to standardize and evaluate the safety profile of *Vishavilwadi Gutika* for internal administration in different medical emergencies. The pharmaceutico-analytical parameters of *Vishavilwadi Gutika* were determined to be optimal, setting a standard for future research. Even at an oral dosage of 2000 mg/kg, or 22.4 g for a 70 kg adult, the test medication demonstrated no fatality in compliance with OECD recommendations 425.[18] Neither the treated nor control animals' skin, ears, eyes, behaviour, salivation, or sleep were found to have changed. Additionally, none of the animals showed signs of unconsciousness, diarrhoea, lethargy or tremors. Over the course of 14-day observation period following treatment, every animal in the treated group survived. These findings imply that *Vishavilwadi Gutika* is non-toxic and safe to take internally. Furthermore, this study offers preliminary data that can guide future investigations to identify safe and efficient dosages for preclinical testing.

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