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Research Article

PRELIMINARY PHYTO CHEMICAL ANALYSIS AND ACUTE ORAL TOXICITY STUDY OF NADI HINGU NIRYASA

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Abstract

Nadi hingu botanically known as *Gardenia gummifera* is used for its medorogahara, Krimihara, Rakshogna etc medicinal properties. Kaiyadeva Nighantu and other Vedic literature told that Nadi hingu possess potent biological activities like Medhohara; Yakrututtejaka and Hridhya properties. The present research has been under taken to evaluate the preliminary phyto chemical analysis and acute oral toxicity study of Nadi hingu niryasa (*Gardenia gummifera*). Acute oral toxicity study was done preliminary to fix the particular dose for further studies. Photochemical analysis of Nadi hingu shows the presence of Alkaloids, Carbohydrates, Flavanoids, Phenols, and Resin. The study showed Loss on Drying- 5.05%, Total ash is 4.03%, Water soluble ash value is .59%, Acid insoluble ash value is 0.30%, Water soluble extractive value is 8.43%, Alcohol soluble extractive value is 59.97%. The LD50 is greater than 2000 mg/kg.

Keywords: *Gardenia gummifera*; Nadi hingu; Toxicity study.

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INTRODUCTION

Nadi hingu (*Gardenia gummifera* Linn.) belonging to the family Rubiaceae is a large medicinal shrub with resinous buds. It is one of the endangered plant species of India and is traditionally used in conditions of cardiac debility, obesity and lipolytic disorders. Resin from the leaf buds of *Gardenia gummifera* is used in cardiac debility. There are many references available in Ayurvedic classics about this drug. Nadi hingu is katurasa pradhanadravya, laghu, ruksha and tikshna guna, ushna veerya and katuvipaka. Main karma is kapha vata samaka. Nadi hingu is a strong deepana pachana and a vatanulomana drug. Its medicinal uses are Kaphanissaraka, sthoulya hara, agnimandhya, atisaragna, yakrututhejaka, pleehanashana, jwara, shoola, udavartha. In all this conditions Nadi hingu niryasa choorna is an effective remedy.

Gardenia gummifera belonging to the family Rubiaceae is a small tree growing up to 8 m high with bright yellow resinous exudation coming from leaf buds and from bark of the plant. Commonly seen on degraded slopes, rocky strata in deciduous forests, usually found in dry laterite forests, hills above 1000m, peninsular Indian, northern circars, Deccan and carnaticwestwards at the foot of the Ghats on the Malabar Coast. The plant remains fairly common but is sparsely distributed; it is classified as 'Least concern'in the IUCN Red List of Threatened Species (2011).

Chemical constituents like Gardenin, Nevadensin, Desmethyl tangeret in, Wogonins, Isocutell are in, Pigenin, Demethylsudachit in and Volatile oil are present; Garden in act as a coloring matter. From stem bark Oleanonic aldehide, Sitosterol, D- mannitol, Erythrodiol and a new compound 19a-hydroxyerythrodiol were isolated. The present study was carried out to analyze the sample by using different phytochemical test, physicochemical parameters and to develop HPTLC.

MATERIALS AND METHODS

The work has been carried out in SDM centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India.

1) Organoleptic character assessment

Perception of taste is done by Rasanendriya. Rasa recognized first is Pradhana rasa and perceived subsequently is Anurasa. Process for Analysis: 20 volunteers were selected and served with five grams of powder of the drug. Assessment of odour is by the perception of smell i.e. Nasendriya. Assessment of colour is by the perception of vision i.e. Chakshurendriya. Assessment of texture is done by the perception of touch i.e. Sparshanendriya.

2) Phyto-chemical parameters [1][2][3][4][5][6][7]

a)Loss on drying at 105°C - Determination of moisture content value (loss on drying)

The loss on drying test is designed to measure the amount of water and volatile matters in a sample, when a sample is dried under specific conditions. This test method is useful for design purposes, service evaluation, regulatory statutes, manufacturing control, quality control, specification acceptance, development and research. This test method determines only the mass of material lost, not its identity. Present experiment is determined by Hot air oven method.

Procedure

Accurately weighed 5 g of coarsely powdered drugs are taken in dried, weighed Petri dish. Petri dish is kept in hot air oven at 105ºC for 5 hrs. Petri dish is taken out, cooled in desiccators and weighed. Again the Petri dish is kept in the hot air oven at 105ºC for 30 min.

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and cooled in desiccators and weighed. This step is repeated till constant weight is obtained by 2 consecutive weighing percentage of moisture content with reference to the air dried drugs were calculated using the formula,

The weight loss i.e. loss on drying was calculated and expressed as %w/w.
W1 = Wt. of the Petri dish
W2= Wt. of sample
W3 = Wt. of the dried sample with dish

Loss on Drying (LOD) = (W1+W2)-W3x 100% (w/w) W

Determination of ash values

Total ash

Incinerate about 2 to 3 g. accurately weighed, of the ground drug in a tarred platinum or silica dish at a temperature not exceeding 450°C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate evaporate to dryness, and ignite at a temperature not exceeding 450°C. Calculate the percentage of ash with reference to the air dried drug.

Total ash % = W3-W1x100` W2 W1= weight of empty dish.

W2= weight drug taken.
W3= weight of ash with dish.

Acid insoluble Ash

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ash less filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hotplate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

Water soluble ash

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ash less filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample.
Calculate the percentage of water-soluble ash with reference to the air dried drug.

Water soluble ash = Total ash – Water insoluble ash.

Determination of Extractive values in Water, Alcohol and Chloroform

This method determines the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of single solvent can be the means of providing preliminary information of the quality of a particular drug sample, for example in a drug where the extraction procedure for the constituents commences with water as the solvent, any subsequent aqueous extraction on the re dried residue will give a very low yield of soluble matter

Alcohol soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any

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solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in a hot air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

3) Preliminary phytochemical tests

a) Tests for alkaloids

Dragendroff's test

To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Wagner's test

To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

Mayer's test

To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's test

To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

b) Tests for carbohydrates

Molisch's test

To the extract, 1 ml of α-naphthol solution and conc. Sulphuric acid were addedalong the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's test

A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's test

To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

c) Test for steroids

Libermann-Burchard test

To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

Salkowski test

The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added.

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Table 1: Preliminary Phytochemical screening of *Gardenia gummifera* (resin) extract

Tests	Colour if positive	Alcoholic extract
	Alkaloids	
Dragendroff's test	Orange red precipitate	Orange red precipitate
Wagners test	Reddish brown precipitate	Reddish brown precipitate
Mayers test	Dull white precipitate	Dull white precipitate
Hagers test	Yellow precipitate	Yellow precipitate
	Steroids	
Liebermann- buchard test	Bluish green colour	No Bluish green colour
Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	No bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
	Carbohydrate	
Molish test	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate
	Tannin	
With $FeCl_3$	Dark blue or green or brown	Black color
	Flavanoids	
Shinoda's test	Red or pink	Red color
	Saponins	
With $NaHCO_3$	Stable froth	Stable froth
	Triterpenoids	
Tin and thionyl chloride test	Pink	Brown color
	Coumarins	
With 2 N NaOH	Yellow	Red color
	Phenols	
With alcoholic ferric chloride	Blue to blue black	Black color
	Carboxylic acid	
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
	Amino acid	
With ninhydrine reagent	Purple colour	Brown color
	Resin	
With aqueous acetone	Turbidity	Turbidity
	Quinone	
Conc. sulphuric acid	Pink/purple/red	Brown color

Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

d) Test for saponins

To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponins.

e) Test for tannins

To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

f) Test for flavonoids
Shinoda's test

To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath.

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Formation of red to pink colour indicates the presence of flavonoids.

g) Test for phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

h) Test for coumarins

To the extract in alcohol, a few drops of 2 N sodium hydroxide solutions were added. Dark yellow colour formation indicates the presence of coumarins.

i) Test for triterpenoids

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

j) Test for carboxylic acid

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

k) Test for resin

Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of turbidity.

l) Test for quinine

A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinine. (Table 1)

4) HPTLC (High performance thin layer chromatography)

1g of *Gardenia gummifera* powder was extracted with 10 ml of *alcohol*. 4, 8 and 12µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Chloroform: Methanol (9.6: 0.4). The developed plates were visualized in Short UV, Long UV and under white light and then derivatised with vanillin sulphuric acid, scanned under UV 254nm, 366nm and 620nm. R_f, colour of the spots and densitometric scan were recorded. (Figure 1 to Figure 9)

Acute toxicity study

Acute toxicity study was conducted at SDM Center for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India.

Wistar albino rats, rat cage, feeding bottles, rat feeding catheters, weighing machine, graduated conical flask, permanent marker, beaker, distilled water, gloves, 5ml syringe, fine powders of the drugs etc. were the materials used for the study. Mortality, Weight change, Food consumption, Water consumption, Changes in skin/Fur/Eyes, Diarrhea, Behavioral patterns like tremors, salivations, lethargy and sleep/comma of rats were determined as parameters to evaluate the toxicity.

Housing and feeding conditions of rats

The animals were selected from animal house attached to department of Pharmacology, SDM Center for Research in Ayurveda and Allied Sciences, Udupi. The animals were housed in standard laboratory condition of light and dark cycle of 7am to 7pm, temperature of 25^0 C and 30-60% relative humidity in well ventilated polypropylene cages. Animals were provided with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water ad libitum.The animals were randomly selected, marked with the help of picric acid to permit individual identification, and kept in their cages for 7 days prior to the start of dosing. The experiment was conducted after obtaining the permission from the institutional ethics

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committee in accordance with the guide line formulated by CPCSEA.

Preparation of Nadi hingu powder

Nadi hingu resin was dried properly and ground to fine powder using mortar and pestle and thoroughly sieved. After which stored in tight container and used for the experimentation. This Choorna was thoroughly mixed with 50mg of gum acacia powder and distilled water and filtered well by a nice cloth to make a stock solution. This was administered orally with a Gastric catheter .The powder obtained from single batch was used throughout the experimental study.

Rat dose:

The rat dose would be derived from LD 50 determined by acute oral toxicity study carried out according to the OECD guidelines 425 (guidelines-staircase).

Single animals are dosed in sequence usually at 48 hours intervals. However, the time intervals between dosages are determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose would be delayed until one is confident of survival of the previously dosed animal. For selected the starting dose, all available information, including information on structurally related substances and results of any other toxicity tests on the test material, would be used to approximate the LD50 as well as the slope of the dose response curve.

The first animal is dosed a step below the best preliminary estimate of the LD50. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears morbid, the second animal receives a lower dose. If no estimate of the substance's lethality is available, dosing would be initiated at 175mg/kg.

Dosing continues depending on the fixed – time interval (e.g. 48-hour) outcomes of all the animals up to that time. The testing stops when following stopping criteria first is met i e; 3 consecutive animals survive at the upper bound; when the stopping criteria have been attained, the estimated LD50 should be calculated from the maximum likely-hood calculation.

Dose fixation was done on the basis of AOT (acute oral toxicity study) study. It was done by (OECD Test Guideline 425). Human dose× surface area ratio convertibility factor for rat or mouse as required, conversion of the dose obtained above to dose in mg or g/kg by multiplying with suitable factor based on the average weight of the animal. AOT 425statical program (Version: 1.0) was followed.

OBSERVATION AND RESULTS

The organoleptic characters of the drug was Katu Tikta rasa is the Pradhana rasa, Kashaya rasa is the Anurasa, the powdered Nadi hingu was having the smell of strong Pungent particular smell, The powder of Nadi hingu is having the colour of Yellowish brown, The powdered Nadi hingu is having the touch of sticky and rough texture.

The results of Physico-chemical, phytochemical are depicted in Table 2 and Table 3 respectively.

Acute oral toxicity study

Test Results and Recommendations

Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program
Date/Time: Wednesday, November 16, 2016, 9:34:17 AM
Data file name: Nadi hingu.
Last modified: 16-Nov-16 9:34:15 AM
Test/Substance: Nadi hingu
Test type: Main Test
Limit dose (mg/kg): 2000

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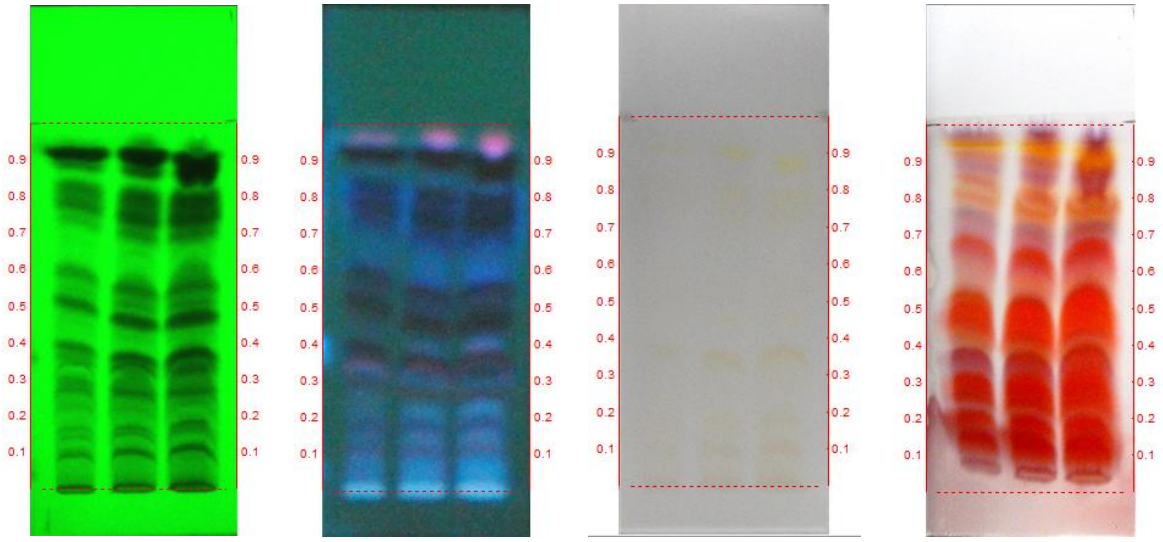
Figure 1: HPTLC photo documentation of Ethanol extract of *Gardenia gummifera*



Short UV **Long UV** **White light** **After Derivatisation (White light)**

Track 1- Gardenia gummifera – 4 µl
Track 2– Gardenia gummifera – 8 µl
Track 3- Gardenia gummifera – 12 µl
Solvent system-Chloroform: Methanol (9.6: 0.4)

Figure 2: Densitometric data of *Gardenia gummifera* at 254nm

Track 3, ID: Gardenia gummifera

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.04 Rf	547.7 AU	14.93 %	0.09 Rf	05.6 AU	19863.1 AU	13.73 %
2	0.10 Rf	303.0 AU	0.15 Rf	463.6 AU	12.64 %	0.18 Rf	58.4 AU	16601.3 AU	11.48 %
3	0.18 Rf	260.3 AU	0.22 Rf	382.3 AU	10.42 %	0.26 Rf	40.2 AU	16575.1 AU	11.46 %
4	0.26 Rf	240.6 AU	0.32 Rf	391.0 AU	10.66 %	0.35 Rf	08.8 AU	17695.4 AU	12.23 %
5	0.35 Rf	305.7 AU	0.38 Rf	384.7 AU	10.49 %	0.41 Rf	63.9 AU	11836.5 AU	8.18 %
6	0.41 Rf	364.4 AU	0.43 Rf	415.8 AU	11.34 %	0.47 Rf	69.4 AU	13421.3 AU	9.28 %
7	0.48 Rf	163.9 AU	0.54 Rf	378.2 AU	10.31 %	0.58 Rf	52.6 AU	17673.5 AU	12.22 %
8	0.60 Rf	222.7 AU	0.63 Rf	263.4 AU	7.18 %	0.73 Rf	3.8 AU	12614.5 AU	8.72 %
9	0.74 Rf	0.2 AU	0.79 Rf	168.4 AU	4.59 %	0.81 Rf	65.1 AU	3988.2 AU	2.76 %
10	0.81 Rf	166.0 AU	0.84 Rf	219.7 AU	5.99 %	0.93 Rf	0.7 AU	13299.7 AU	9.20 %
11	0.94 Rf	0.2 AU	0.97 Rf	53.2 AU	1.45 %	0.99 Rf	20.4 AU	1070.1 AU	0.74 %

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Figure 3: Densitometric scan of *Gardenia gummifera* at 254nm

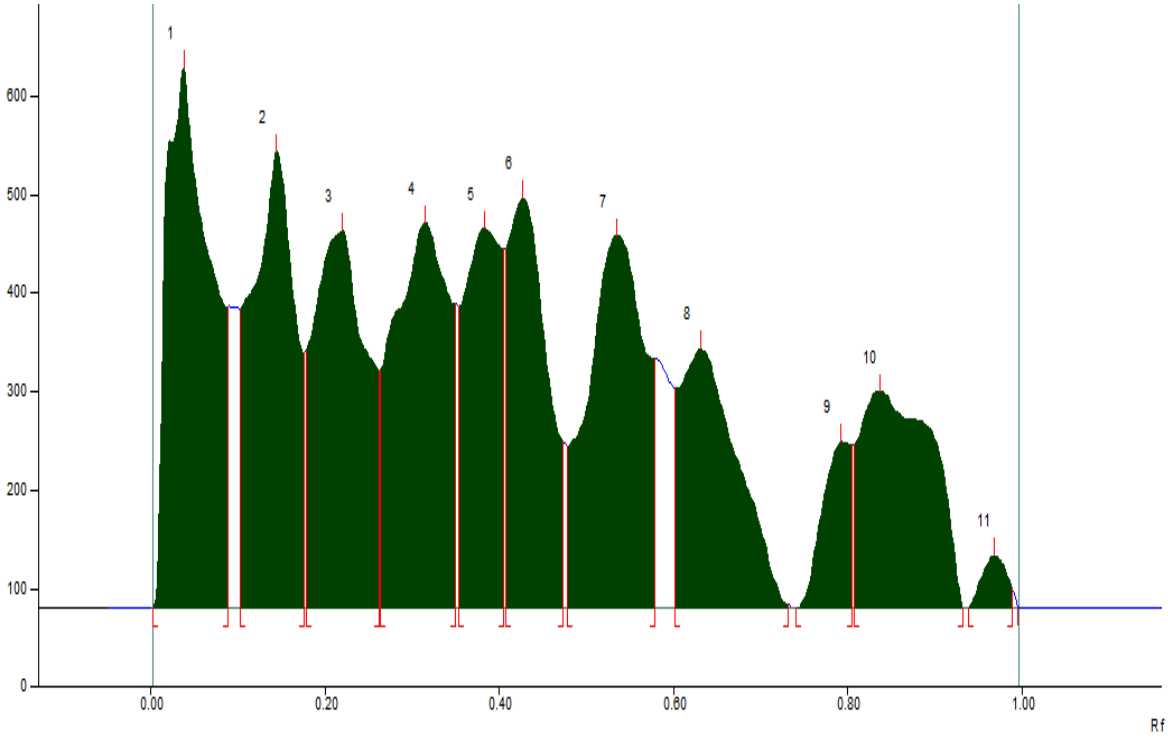

Figure 4: Densitometric data of *Gardenia gummifera* at 366nm

Track 3, ID: Gardenia gummifera

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	2.5 AU	0.03 Rf	15.1 AU	1.11 %	0.04 Rf	0.0 AU	90.7 AU	0.18 %
2	0.04 Rf	0.8 AU	0.09 Rf	83.8 AU	6.18 %	0.14 Rf	2.4 AU	3590.4 AU	7.06 %
3	0.15 Rf	3.0 AU	0.18 Rf	91.1 AU	6.72 %	0.21 Rf	51.2 AU	2469.0 AU	4.86 %
4	0.22 Rf	53.5 AU	0.27 Rf	139.3 AU	10.28 %	0.32 Rf	0.7 AU	5237.2 AU	10.30 %
5	0.32 Rf	0.8 AU	0.36 Rf	121.1 AU	8.94 %	0.38 Rf	32.3 AU	2924.8 AU	5.75 %
6	0.39 Rf	32.4 AU	0.41 Rf	71.3 AU	5.26 %	0.43 Rf	51.1 AU	1458.0 AU	2.87 %
7	0.43 Rf	51.1 AU	0.47 Rf	281.7 AU	20.79 %	0.55 Rf	35.0 AU	11872.4 AU	23.35 %
8	0.55 Rf	35.2 AU	0.57 Rf	62.7 AU	4.62 %	0.59 Rf	42.4 AU	1246.6 AU	2.45 %
9	0.59 Rf	42.5 AU	0.60 Rf	48.3 AU	3.56 %	0.63 Rf	0.2 AU	820.7 AU	1.61 %
10	0.64 Rf	0.3 AU	0.71 Rf	190.7 AU	14.07 %	0.80 Rf	21.6 AU	12422.3 AU	24.43 %
11	0.83 Rf	10.6 AU	0.94 Rf	250.0 AU	18.45 %	1.00 Rf	3.0 AU	8708.3 AU	17.13 %

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Figure 5: Densitometric scan of *Gardenia gummifera* at 366nm

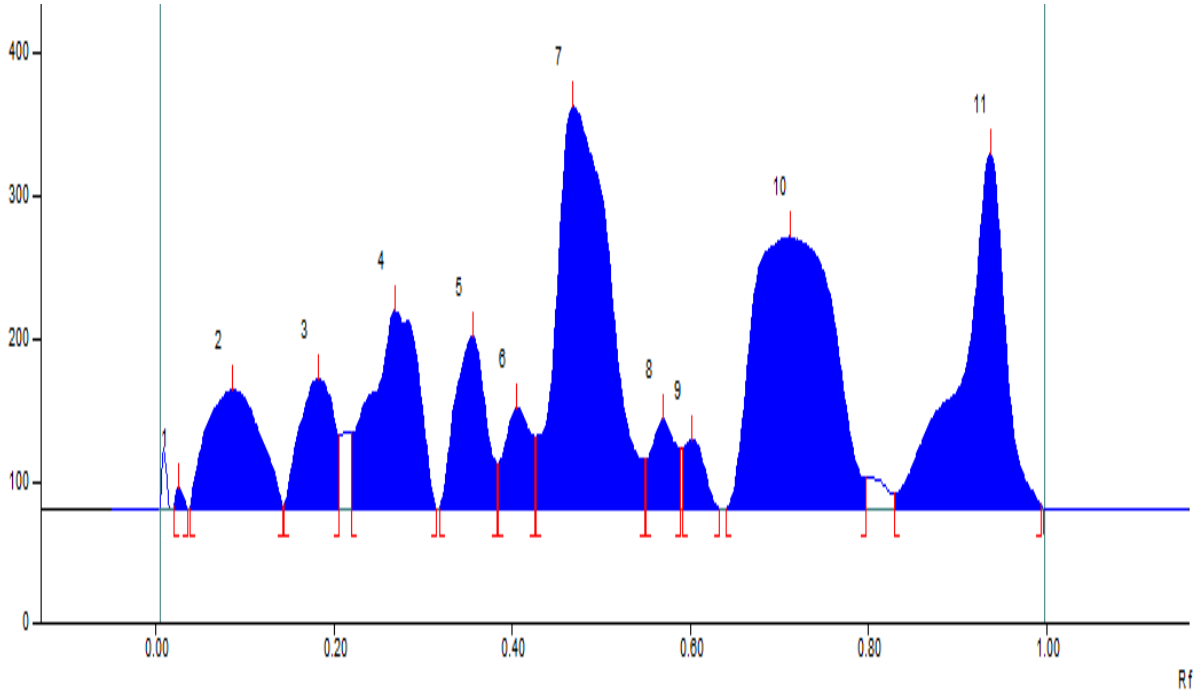

Figure 6: Densitometric data of *Gardenia gummifera* at 540nm

Track 3, ID: Gardenia gummifera

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.6 AU	0.02 Rf	62.5 AU	21.80 %	0.03 Rf	55.1 AU	584.5 AU	8.57 %
2	0.03 Rf	55.4 AU	0.04 Rf	62.7 AU	21.86 %	0.08 Rf	14.5 AU	1237.8 AU	18.14 %
3	0.09 Rf	14.5 AU	0.14 Rf	41.7 AU	14.53 %	0.18 Rf	10.1 AU	1310.9 AU	19.21 %
4	0.18 Rf	10.0 AU	0.22 Rf	18.3 AU	6.39 %	0.27 Rf	4.7 AU	677.8 AU	9.93 %
5	0.36 Rf	6.7 AU	0.43 Rf	49.6 AU	17.28 %	0.47 Rf	3.0 AU	1469.7 AU	21.54 %
6	0.51 Rf	2.0 AU	0.54 Rf	12.2 AU	4.25 %	0.56 Rf	6.5 AU	296.3 AU	4.34 %
7	0.78 Rf	3.5 AU	0.84 Rf	16.1 AU	5.62 %	0.86 Rf	14.5 AU	514.2 AU	7.54 %
8	0.86 Rf	14.3 AU	0.90 Rf	23.7 AU	8.27 %	0.94 Rf	0.1 AU	731.5 AU	10.72 %

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Figure 7: Densitometric scan of *Gardenia gummifera* at 540nm

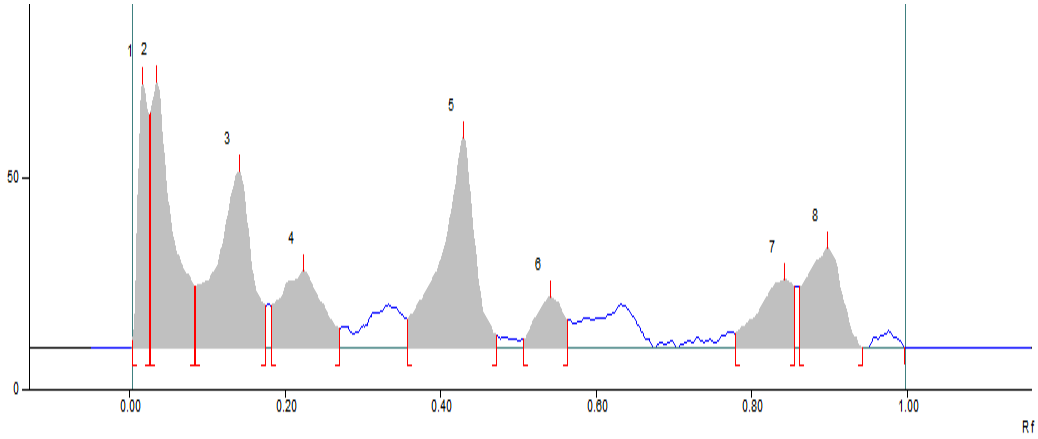

Figure 8: Densitometric data of *Gardenia gummifera* at 620nm

Track 3, ID: Gardenia gummifera

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.6 AU	0.02 Rf	62.5 AU	21.80 %	0.03 Rf	55.1 AU	584.5 AU	8.57 %
2	0.03 Rf	55.4 AU	0.04 Rf	62.7 AU	21.86 %	0.08 Rf	14.5 AU	1237.8 AU	18.14 %
3	0.09 Rf	14.5 AU	0.14 Rf	41.7 AU	14.53 %	0.18 Rf	10.1 AU	1310.9 AU	19.21 %
4	0.18 Rf	10.0 AU	0.22 Rf	18.3 AU	6.39 %	0.27 Rf	4.7 AU	677.8 AU	9.93 %
5	0.36 Rf	6.7 AU	0.43 Rf	49.6 AU	17.28 %	0.47 Rf	3.0 AU	1469.7 AU	21.54 %
6	0.51 Rf	2.0 AU	0.54 Rf	12.2 AU	4.25 %	0.56 Rf	6.5 AU	296.3 AU	4.34 %
7	0.78 Rf	3.5 AU	0.84 Rf	16.1 AU	5.62 %	0.86 Rf	14.5 AU	514.2 AU	7.54 %
8	0.86 Rf	14.3 AU	0.90 Rf	23.7 AU	8.27 %	0.94 Rf	0.1 AU	731.5 AU	10.72 %

Figure 9: Densitometric scan of *Gardenia gummifera* at 620nm

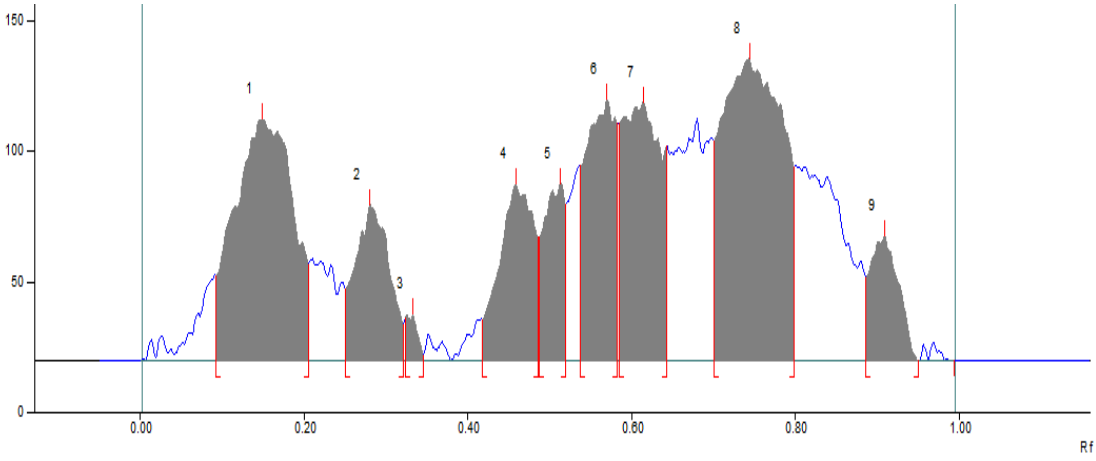

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Table 2: Results of standardization parameters of *Gardenia gummifera* (resin)

Parameter	Results n = 3 %w/w
Loss on drying	5.05
Total Ash	4.03
Acid Insoluble Ash	0.30
Water soluble Ash	0.59
Alcohol soluble extractive value	59.97
Water soluble extractive value	8.43

Table 3: Preliminary Phytochemical screening of *Gardenia gummifera* (resin) extract

Test	Inference	Test	Inference
Alkaloid	+	Coumarins	-
Steroid	-	Phenols	+
Carbohydrate	+	Carboxylic acid	-
Tannin	-	Amino acids	-
Flavanoids	+	Resin	+
Saponins	+	Quinone	-
Terpenoid	-		

(+) Present; (-) Negative

Table 4: R_f values of all the samples

Short UV	Long UV	White light	After derivatisation (White light)
-	0.07 (F. blue)	-	0.07 (Red)
0.11 (D. green)	0.11 (F. blue)	0.11 (Yellow)	-
0.15 (D. green)	0.15 (F. blue)	-	-
0.16 (D. green)	-	-	0.16 (Red)
-	0.19 (FD. blue)	0.19 (Yellow)	0.19 (Red)
0.23 (D. green)	0.23 (F. blue)	-	0.23 (Red)
-	-	-	0.24 (Red)
0.28 (D. green)	0.28 (F. blue)	-	-
-	0.31 (FD. blue)	-	0.31 (Red)
0.34 (D. green)	0.34 (FD. pink)	-	-
0.36 (D. green)	0.36 (FD. pink)	0.36 (Yellow)	0.36 (Red)
-	0.42 (F. blue)	-	-
0.47 (D. green)	0.47 (FD. blue)	-	-
0.52 (D. green)	-	-	0.52 (Red)
0.56 (D. green)	0.56 (FD. blue)	-	-
-	0.60 (F. blue)	-	0.60 (Red)
0.65 (D. green)	-	-	-
0.70 (D. green)	-	-	0.70 (Red)
0.75 (D. green)	0.75 (FD. blue)	-	0.75 (Purple)
0.79 (D. green)	0.79 (FD. blue)	0.79 (Yellow)	-
-	-	-	0.82 (Orange)
0.88 (D. green)	-	-	0.88 (Purple)
0.92 (D. green)	0.92 (FD. blue)	0.92 (Yellow)	0.92 (Purple)
-	-	-	0.95 (Orange)

***L-Light, D-Dark, F-Fluorescence**

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Assumed LD50 (mg/kg): Default
Assumed sigma (mg/kg): 0.5
Recommended dose progression: 2000, 550, 175, 55, 17.5, 5.5, 1.75

Data

Seq.	Test Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	175	O	O
2	2	550	O	O
3	3	2000	O	O
4	4	2000	O	O
5	5	2000	O	O

(X = Died, O = Survived)

Dose Recommendation: The main test is complete. Stopping criteria met: 3 at Limit Dose.

Summary of long-term results

Dose	O	X	Total
175	1	0	1
550	1	0	1
2000	3	0	3
All Doses	5	0	5

Statistical Estimate based on long term outcomes: The LD50 is greater than 2000 mg/kg.

Rat dosage = 2000mg/kg
Test group -1st =1/10th of Highest dose tested without evidence of toxicity =400mg/kg =0.4g
Test group-2nd =1/5th of LD 50 =0.8g

HPTLC

1g of *Gardenia gummifera* powder was extracted with 10 ml of *alcohol*. 4, 8 and 12µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Chloroform: Methanol (9.6: 0.4). The developed plates were visualized in Short UV, Long UV and under white light and then derivatised with vanillin sulphuric acid, scanned under UV 254nm, 366nm and 620nm. R_f, colour of the spots and densitometric scan were recorded. (Table 4)

DISCUSSION

The organoleptic study of Nadi hingu has proved that it is having Katu Tikta Rasa, Strong pungent odour and Yellowish brown colour. The drug Nadi hingu has shown 10% of loss when powdered sample kept in hot air oven for 105^{0}C. which means less moisture content. Total ash value of Nadi hingu is 4.03%, in which acid insoluble ash is .30% and water soluble ash is .59%. Alchohol has got 59.97 extractive values while water has got 8.43. Dragendroff's test, Mayer's test etc indicates presence of alkaloids, Liebermann's test shows presents of steroids, Molish test indicates presents of carbohydrates. $FeCl_3$test confirms presence of Tannins. Test for saponins indicates t the presents of saponins. HPTLC finger printing *Gardenia gummifera* the sensitivity was found to be 0.07 µg and the linearity was observed in the range of 0.07µg to .95µg. The gardenin content of 1.76% was observed in test sample. (Figure 1 to Figure 9)

The proposed method being precise and sensitive can be used for detection, monitoring and quantification of gardenin in *G. gummifera*.

Mortality is the main criteria in assessing the acute toxicity of any drug. No animals were died at dosage of 500mg /kg body weight. From the above findings it can be inferred that the LD50 value is higher than 2000mg /kg body weight.

CONCLUSION

Phytochemical analysis of Nadi hingu has revealed some of the significant phytochemical constituents in it. The study showed Loss on Drying - 5.05%, Total ash is

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4.03%, Water soluble ash value is .59%, Acid insoluble ash value is 0.30%, Water soluble extractive value is 8.43%, Alcohol soluble extractive value is 59.97%. Qualitative test of the drug shows presence of Alkaloids, Carbohydrates, Flavanoids, Phenols, and Resin. Acute toxicity study - Statistical Estimate based on long term outcomes: The LD50 is greater than 2000 mg/kg.

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