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International Journal of Applied Ayurved Research ISSN: 2347- 6362

PHARMACEUTICAL ANALYSIS OF *DENIBADIYA* DECOCTION IN SURVEY ON TREATMENT LINE FOR HEMIPLEAGIA (*PAKSHAGHATA)*

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ABSTRACT

Complete paralysis of one half of the body called hemiplegia . Stroke is the major consequence of cerebrovascular disease, known as second most common cause of death. Annually, 15 million people worldwide suffer from strokes. Hemiplegia is a condition similarto *Pakshaghata*. It is one of the *Vata Vyadhis*. In Ayurveda *Nidana Parivarjana*, *Shodana* and *Shamana Chikitsa* used for treatment line of *Pakshaghata*.*Denibadiya* decoction commonly used in traditional system of medicine in Sri Lanka for *Pakshaghata*.Objectives were, to find out most commonly used decoction in treatment line for *Pakshaghata*, and to study pharmaceutical analysis of selected decoction.*Denibadiya* decoction is identified as most commonly used decoctionin survey in Ayurveda teaching Hospital, Borella. According to survey, total 21 types of preparation of drugs used as treatment line of *Pakshaghata* within 8 weeks. In those preparations; 4 types of *Kashaya* (19.04%), 3 types of *Vati* (14.28%), 2 types of *Kalka*(09.52%), 5 types of *Taila* (23.80%), 1 type of *Leha* (04.76%), 1 type of *Pattu*/*Mallum*/Plaster (04.76%) , and 5 types of*Choorna* (23.80%) were identified as treatment line. Among the used 4 types of *Kashaya* the most importantly*Denibadiya* used during 6 weeks. Pharmaceutical analysiscompleted for 8 types of ingredients of *Denibadiya* decoction in *DravyagunaVignana* Laboratory of Institute of Indigenous Medicine, University of Colombo. According to physical and chemical analysis of 8 types of ingredients of *Denibadiya* decoction, it can be concluded as observed physical analysis, Foreign matter value (1.58%), Color (brownish red), Odor (pungent), Taste (*Thiktha* and *Kashaya*), P^H value (6.19), Moisture content (15.44%), Total ash value (9.025%),Water soluble ash content (6.45%), Acid insoluble ash content (17.75%). According to the observed chemical analysis, Flavonoids, Saponins, Terpenoids, Alkaloids, Glycosides, Carbohydrates, Tannins, Proteins were present except Steroids. Results of Thin Layer Chromatography was done (R_{f1}- 9.6, R_{f2}-1.4,R_{f3} 1.04). It could be recommended that statistical analysis of revealed data and efficacy of *Denibadiya* decoction will be evaluated in further clinical study for treatment according to progress of the patients of *Pakshaghata*as continuation of this study.

Key Words: *Pakshaghata* ,*Denibadiya* decoction, Pharmaceutical analysis

Pharmaceutical analysis of *Denibadiya* decoction

INTRODUCTION: Pharmaceutical analysis is a chemical process for

identification, determination, qualification and purification of a substance separation of the components of a solution , mixture or determination of structure of chemical compounds. The substance may be single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are from animals, plants, microorganisms and various synthetic products.

Nature has been given a large source of medicinal agents from plants. An impressive number of modern drugs have been isolated from natural plants sources.Many of these isolations were based on the use of the plants used in traditional medicine.The indigenous medicine system continues to play an essential role in health care using these medicinal plants.*Denibadiya* decoction is predominantly used in years in Sri Lankan traditional system of medicine for *Pakshaghata*

Medicinal plants are containing inherent active ingredients used to cure diseases.The use of traditional medicines and medical plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO,1996).Healing with medicinal plants is as old as mankind itself.

According to indigenous texts (*Vatikaprakaranaya*), *Denibadiya* is an *Anupana* of S*eetharamavati* and it is a decoction used for various types of *Vata – Kaphajaroga*. In indigenous medical system, it is called as *Sanni roga*. It has ability to clear the *Srotas*(channels), remove the abnormalities in *Srotas*,widely used in *Shodhana treatment* in *Pakshaghata*condition. In *Vatikaprakaranaya* it has mentioned;

Denibathdebatuthtotilathdiymiti
Warunathiguruthkasayakaradethi
Guli dethhinguthsulununupodilathi
Emahathsannithkaphavabiduwathi”
(*Vatikaprakaranaya)*

The ingredients of *Denibadiya*decoction are,

1. Binkohomba whole plant - *Munronia pinnata*
2. Kohomba root bark - *Azadirachta indica*
3.Ela Batu root bark - *Solanum melongena*
4. Katuwelbatu whole plant - *Solanum virginianum*
5. Thotila bark - *Oroxylum indicum*
6. Diyamitta - *Cissempelus pareira*
7. Lunuwarana root bark – *Crateva adansoniissp.Odora*
8. Viyaliiguru - *Zingiber officinale*

1. Binkohomba - *Munronia pinnata*

Figure 01 - *Munronia pinnata* plant

- Scientific name - *Munronia pinnata*
- Family name - Meliaceae

Figure 02- Dried whole plant

- Description - Shrublets 10-50 cm tall, stems usually not branched. Leaves odd pinnate, seeds yellowish gray
- Status - Native

- Ayurvedic usage - Fever, Dysentery, Asthma, Blood disorders
- Parts used in treatments -Whole plant
- Related medical properties- Purifies the blood**,** Anti pyretic

2. Kohomba - *Azadirachta indica*

Figure 03-*Azadirachta indica* plant

Figure 04 - Dried leaves and roots

- Scientific name - *Azadirachta indica*
- Family name - Meliaceae
- Description - A tall tree with spreading branches, stem and young parts glabrous. Leaves imparipinnate compound, one seeded.
- Status - Native
- Ayurvedic usage - Fever, Skin aliments, Asthma, Cough
- Parts used in treatment - Leaves, Seeds, Roots, Bark
- Related medical properties- Reduces aggravation of *Kapha*and *Pitta dosha*

3. Ela Batu -*Solanum melongena*

Figure 05 - *Solanum melongena* plant

Figure 06 - Dried root

- Scientific name - *Solanum melongena*
- Family name - Solanaceae
- Description - Stout, armed, densely pubescent herb or shrub up to 1m tail, seeds discoid.
- Status - Only under cultivation
- Ayurvedic usage - Fever, Asthma, Cough, Facial paralysis
- Parts used in treatment - Whole plant, Fruits, Seeds, Flowers
- Related medicinal properties - Reduce aggravation of *Vata,Pitta* and *Kapha dosha*, Appetizer, Detoxification

4. Katuwelbatu - *Solanum virginianum*

Figure 07 - *Solanum virginianum* plant

Figure 08 - Dried whole plant

- Scientific name -*Solanum virginianum*
- Family name - Solanaceae

- Description - Small shrub about 20-40 cm high, prickles numerous, bright yellow; leaves deeply pinnately.
- Status - Native
- Ayurvedic usage - Fever, cough, Asthma, chest pains
- Parts used in treatment - Seeds, Root
- Related medicinal properties - Pacifies vitiated *Kapha* and *Vata* disorders

5. Thotila - *Oroxylum indicum*

Figure 09 - *Oroxylum indicum* plant

Figure 10 - Dried root bark

- Scientific name - *Oroxylum indicum*
- Family - Bignoniaceae
- Description - Small tree, 5-8 cm high, sometimes up to 13m, bark thick. Leaves deltoid ovate in outline.
- Status - Native
- Ayurvedic usage - Rheumatism, Diarrhoea, Piles, Dysentery
- Parts used in treatment - Stem bark, Root
- Related medicinal properties - Astringent

6. Diyamitta - *Cissempelus pareira*

Figure 11 - *Cissempelus pareira* plant

Figure 12 - Dried whole plant

- Scientific name -*Cissempelus pareira*
- Family name - Menispermaceae
- Description - Slender woody climber occurs throughout the island up to 1200 m
- Ayurvedic usage - Fever, Asthma, Diarrhea, Pain
- Parts used in treatment - Roots, Stem, Leaves
- Related medicinal properties - Alleviates *Tridosha* mainly *Vata* and *Kapha*.

7. Lunuwarana - *Cratevaadansoniissp.Odora*

Figure 13 - *Cratevaadansoniissp.Odora*plant

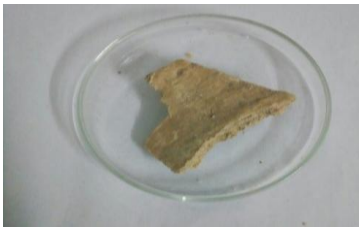


Figure 14 - Dried root bark

- Scientific name - *Cratevaadansoniissp.Odora*
- Family name - Capparaceae

- Description - Tree 3-12 m tall, leaves long petiolate, fruit globose
- Status - Native
- Ayurvedic usage - Urine calculi, Dysuria, Catarrh
- Parts used in treatment - Root bark, Leaves, Bark
- Related medicinal properties - Pacifies *Vata dosha*

8. Viyaliiguru - *Zingiber officinale*

Figure 15 - *Zingiber officinale* plants

Figure 16 - Rhizome and dried rhizome

- Scientific name - *Zingiber officinale*
- Family - Zingiberaceae
- Description - Leafy stem 2m, leaves sessile, flowers are dull yellowish, have rhizomes.
- Status - Native
- Ayurvedic usage - Fever, Asthma, Cough, Abdominal pains
- Parts used in treatments - Rhizome
- Related medicinal properties - Alleviate *Kapha* and *Vata*

Methodology: Literature on *Ayurveda* and traditional medicine were studied and modern pharmaceutical standardization were reviewed. Pharmaceutical Analysis of this research work was carried out in the *DravyagunaVignana* Laboratory, Institute of Indigenous Medicine.

- **Physical studies included,**
- Foreign matter examination
- Color
- Odor
- Taste
- p^H Value
- Moisture Content
- Total Ash Value
- Determination of water soluble ash content & acid insoluble ash
- **Chemical studies included,**
- Detection of Alkaloids
- Detection of Saponins
- Detection of Flavonoids
- Detection of Glycosides
- Detection of Carbohydrates
- Detection of Proteins
- Thin Layer Chromatography (TLC)
- **Analysis of physical properties**
- **Determination of foreign matter**

Measured 40g of the sample and spreaded as a thin layer on a suitable platform. Examined in day light and separated the foreign matter. Weighed the sorted foreign matter and calculated the foreign matter content in percentage with reference to the drug sample.

- **Colour analysis**

Sample colour analysis was done according to the eye perception

- **Odour analysis**

Odour analysis was done according to the perception and considered whether odour existed for 10 minutes time duration.

- **Taste analysis**

Taste analysis was done according to the gustatory perception

- **Determination of pH Value**

To standardize the pH meter, two solutions were selected whose difference in pH does not exceed four units such that the expected pH of the material under test falls

between them. The pH value was measured by using pH meter in *DravyagunaVignana* Laboratory, Institute of Indigenous Medicine.

- **Determination of Total Ash**

4g of power was added in to a crucible and heated in the muffle furnace at 600^0C for 2 hours. Then it was cooled in the desiccator and measured the ash weight and other calculations.

- **Determination of Moisture Content**

Took 4g power from the sample and put into moisture analyzer.

- **Determination of acid soluble ash content**

Two crucibles were taken and put ash (2g) to it, then measured the weight. Then 25 ml of HCl and 25 ml of distilled water were added into the two crucibles separately. Then heated them using bunsen burner. Crucibles were heated in the muffle furnace and after cooling in the desiccator weight was measured.

- **Analysis of chemical properties**
- **Detection of Flavonoids**

Lead acetate test – 0.1 g extract was mixed with 1 ml of 10% Lead acetate and observed for yellow precipitate.

Alkaline reagent test – To 2ml of extract few drops of 2% NaOH was added and observed for intense yellow colour and disappearance of the yellow colour adding few drops of dilute HCL.

Shinoda test – To 2ml of the extract few drops concentrated HCl and then few pieces of Magnesium were added and observed for pinkred colour.

- **Detection of Saponins**

Foam test – From the extract 0.1g was taken and mixed with 5 ml of distilled water. Then shaken vigorously and observed for stable foam of honey comb appearance.

- **Detection of Terpenoids /Terpenes**

Salkowski test – 0.1g of the extract was mixed with 2ml Chloroform and then was followed by the addition of 30ml of con.H_2SO_4 along the side of the test tube. This was kept for some time without shaking. Then observed the solution for reddish brown ring in the interface.

- **Detection of Steroids / Phytosterols –**

Lieberman – Burchard test – 0.5g of the extract was shaken with Chloroform in a test tube and few drops of Acetic anhydride was added to the test tube. This was boiled in a water bath and rapidly cooled in iced water. Then 2ml of con. H_2SO_4 was added alongside of the tube. Formation of a brown ring at the junction of two layers and turning the upper layer in to green colour was observed.

- **Detection of Glycosides**

Keller kiliani test – The extract was mixed with 2ml of Glacial acetic acid, one drop of 5% $Fecl_2$ and 2ml of con. H_2SO_4 and kept for some time without shaking. Then observed the solution for reddish brown ring in the interface.

- **Detection of Carbohydrates**

Benedict's test – To the extract 3ml of benedict's qualitative reagent was added and boiled the solution for about 2 minutes. Then observed whether the solution progress in the colours of blue, green, yellow, orange and finally to brick red precipitate.

Fehling's test – 1ml from each fehling's A and B solutions was added, heated using a water bath and observed brick red precipitate.

- **Detection of Alkaloids**

0.1g of the extract was dissolved in 1% dil. HCl and 2ml of the solution was taken.

Mayer's test – Few drops of Mayer's reagent was added and observed for cream precipitate.

Wagner's test – Few drops of Wagner's reagent was added and observed for reddish brown precipitate.

Hager's test –Few drops of Hager's reagent was added and observed for yellowish precipitate.

- **Detection of Tannins**

Ferric Chloride test – To 2ml of the extract 2-3 drops of 5% $FeCl_3$ were added and observed for dark green (condensed tannins) or dark blue (hydrolysable tannins) solutions.

- **Detection of Proteins**

Biuret test – To the extract 2ml of 1% NaOH and few drops of $CuSO_4$ were added and observed for purple colour.

Ninhydrin test – To 2ml of the extract few drops of Ninhydrin reagent was added, boiled and observed for blue colour solution.

- **Thin Layer Chromatography (TLC)**
- TLC is a chromatography technique, works on the principle that different compounds will have different solubility's and absorption to the two phases between which they are to be partitioned. TLC is a solid- liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase).
- Materials and Equipment – Toluene, Ethyl acetate, Methanol, *Denibadiya Kashaya*Water extract powder,100ml beaker, Watch glass, Filter paper ,TLC plate, Capillary Tube, UV lamp.
- Methodology -
- Preparation of solvent system – Chloroform, Dichloromethane and methanol were mixed in the ratio of 2:2:2
- Preparation of sample solution – A sample of *Denibadiya*decoction water extract powder was re dissolved 0.1-0.2ml methanol.
- Preparation of TLC container – To aid in the saturation of the TLC chamber with the solvent vapors, a half of the inside of the beaker was lined with a filter paper. Then it was filled with solvent system (mobile phase) to a depth of 5 mm. The beaker was covered with the watch glass.
- Preparation of the TLC plate – A line was drawn about 1cm from the bottom of the plate and 1cm from the upper end of the plate. Then a small dot was marked on the middle of the bottom line for the sample spot. Make sure not to touch the middle of the plate and not to press hard on the plate with the pencil.
- Spotting the TLC plate – A drop from the sample solution was taken into the capillary tube and a sample spot was placed on the TLC plate without pressing hard. The plate was allowed to dry.
- Developing the plate –The plate was placed nearly vertical as possible inside the chamber ensuring that the sample spot is above the surface of the solvent system. Then chamber was closed with the watch glass and allowed the solvent to ascend. Before the solvent reached to the upper line, the plate was removed from the chamber and marked the position of the solvent front (a). Plate was dried, observed under the UV lamp and the center of the spots visible were marked. The distance to each spot was measure from the point of the application (b).

Calculation of Retention factor (R_f)-

Retention factor =$\frac{\text{Distance travelled by the compound (b)}}{\text{Distance to the solvent front (a)}}$

RESULTS AND DISCUSSION

- **Analysis of physical properties**
- **Foreign matter analysis**

Foreign matter value = 1.58 %

- **Colour analysis**

Sample colour was brownish red.

- **Odour analysis**

Sample had its own specific odour to perception and it similar to smelling of pungent type sharp odour.

- **Taste analysis:**Sample had its own specific taste to perception and it similar to *Thiktha* and *Kasaya rasa.*
- **Determination of pH Value**

According to the analysis, sample gives pH as acidic (6.19).

- **Determination of ash content**

Total ash content = 9.025 %

- **Determination of moisture content**

According to the moisture analyzer,
Moisture content is = 15.44 %

- **Determination of acid insoluble and watersoluble ash content**

Acid insoluble ash content = 17.75%
Water soluble ash content = 6.45%

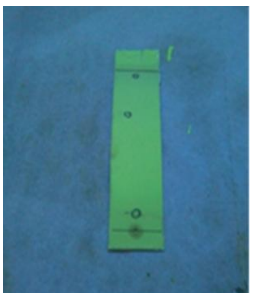
- **Analysis of chemical properties**

Table 01 – Results of the detection of phytochemicals

Phytochemicals	Result
Flavonoids	+
Saponins	+
Terpenoids	+
Alkaloids	+
Steroids	-
Glycosides	+
Carbohydrates	+
Tannins	+
Proteins	+

- Thin Layer Chromatography

Retention factor = Distance travelled by the compound (b)
Distance travelled by the compound (a)



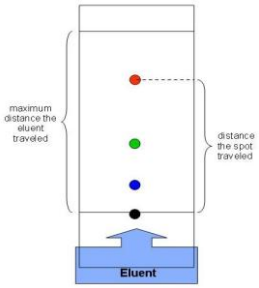


Figure 17- Observation of TLC plate (Laminar Flow), Figure 18-Calculation of R_f value

OBSERVATIONS&CONCLUSION:

According to the analysis of physical properties, foreign matter content (1.58%) was relatively low, pH (6.19) was slightly acidic, ash values were relatively low (Total ash = 9.025%, Water soluble ash=6.45% and Acid insoluble ash=17.75%) and moisture content was moderately low (15.44%).

According to phytochemical analysis, Flavanoids, Saponins, Terpenoids, Alkaloids, Glycosides, Carbohydrates, Tannins, Proteins were present except Steroids.

TLC was done for the separation of compounds present in the extract and three spots were separated from the TLC of this study. (R_{f1}= 9.6, R_{f2} =1.4,R_{f3} =1.04) It can be concluded that, according to the foreign matter content, ash values and moisture content the raw material sample was less contaminated. The decoction was slightly acidic which was a favorable value for the gut.

According to the TLC, a spot with high Rf value indicated that the compound is more polar while a spot with low R_f value indicated that the compound is less polar.

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Source of support: Nil Conflict of interest: None Declared

Cite this Article as : [pharmaceutical Analysis Of Denibadiya Decoction in Survey on Treatment Line for Hemipleagia (Pakshaghata)] *www.ijaar.in : IJAAR VOL V ISSUE VIII MAY-JUNE 2022Pg No:646-654*