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PHYSICO-CHEMICAL ANALYSIS OF INGREDIENTS OF KETAKI MOOLAADI TAILA & VACHAADI UPANAHA

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ABSTRACT:

Introduction – *Sandhigatavata* is a *Vardhakyajanya Vyadhi* and *Dhatukshayajanya Vyadhi* in which vitiated *Vayu* gets localized in *Sandhi*. Osteoarthritis is a degenerative disease affecting weight bearing joints, often described as wear and tear arthritis. Osteoarthritis is a major cause of morbidity and disability, limiting activity and impaired quality of life especially among the elderly. **Objectives:** 1. To study the Physico-chemical and Phyto-chemical analysis of *Vachadi Lepa 2.* To study the Physico-chemical and Phyto-chemical analysis of *Ketakimoola Taila* 3. To compare the obtained values of analysis of both drugs with *Ayurvedic P*harmacopia of India. **Materials & Methods–** Experimental Study involving the Physico-Chemical Study of following parameters- Loss on drying (moisture content), Refractive Index, Specific Gravity, Acid Value, Iodine Value, Saponification Value, TLC, Test for Carbohydrates, Test for proteins, Test for steroid, Test for Saponins, Test for Alkaloids, Test for Tannins, Test for cardiac Glycosides, Test for anthroquinone Glycosides, Test for Saponin Glycosides. **Discussion:** The article enlightens about the Physico-Chemical analysis of the drug. The isolated secondary metabolite otherwise known as the phyto-constituents are therapeutically significant and hence studied here. Since it is multi-herbal combination has water and alcohol soluble contents like Carbohydrates, Flavonoids, Anthraquinone glycosides. Ingredients like Monosaccharides, Proteins, Amino acids, Tannins are water soluble. Steroids and Cardiac Glycosides are alcohol soluble. **Conclusion –** Physico-chemical and Phyto-chemical analysis both drugs were had standard composition as per the reference values of Ayurvedic Pharmacopia of India.

Keywords: *Vachadi Upanaha* (Poultice), Ketakimooladi tail (Oil), physic chemical analysis.

INTRODUCTION

Sandhigatavata is a *Vardhakyajanya Vyadhi* and *Dhatukshayajanya Vyadhi* in which vitiated *Vayu* gets localized in *Sandhi*. Osteoarthritis is a degenerative disease affecting weight bearing joints, often described as wear and tear arthritis. Osteoarthritis is a major cause of morbidity and disability, limiting activity and impaired quality of life especially among the elderly. *Janu Sandhigatavata* is considered as Osteoarthritis in modern parlance due to the resemblance in the aetio-pathological factors and clinical features. Osteoarthritis is the second most common rheumatologic problem.

The reported prevalence of Osteoarthritis from a study is 28.7% in the overall [1]. The prevalence is higher in villages (31.1%) and big cities (33.1%) as compared to towns (17.1%) and small cities (17.2%). There is a steady rise in prevalence from age 30 such that by age 65. 80% of people will have radiographic evidence of Osteoarthritis. Ayurveda describes treatment principle of *Sandhigata Vata* as *Sneha Upanaham Agnikarma Bandhana.*[2,3]

Charaka has advised *Punaha Punaha Snehana and Swedan* should be done in *Vatavyadhi*.[4] Prime importance to *Snehana Chikitsa* and *Upanaha* (poultice) in the management of *Sandhigatavata*. *Snehana* can be performed both *Bahya* (External) and *Abhyantara* (Internal). *Matrabasti* and *Upanaha*(poultice) both are well established and having more efficacious results in *Janu Sandhigata Vata, Matra Basti* is advised as best in the treatment for *Vata* disorders & Acts as *Bramhana*. [5,6] *Upanaha* (poultice) is a type of *Sweda and Swedana Karma* plays a major role in alleviating the pain and stiffness of joints.[7] *Ketakimooladi taila*(oil) is *Tridoshahara, Asthigatavata Shaman, Asthiposhana,balya, bhrumhan and Shoolahara.* It is *Madhura Rasa Pradhan Samashitoshna Veerya Oushada*. It has *Asthi Sandhi Visheshatvam* [8], *Vachadi Upanaha churna* (powder) is *Vatashamana, Srotovivarana, Sweda Janana, Shoolaprashamana* [9].

The *Vachadi Upanaha Sweda* has *Snighda*, *Guru* and *Ushnaguna* which counteracts with *Ruksha, Laghu and Sheetaguna* of *Vata*. Thus, acts as *Vatashamaka*, *Shoola* and *Sthambha. Upanaha* (poultice) has to be tied for 12 hours of duration with restricting the movement of knee joint.[10]

Ketaki Mooladi Taila (oil) is having *Ketaki Moola, Bala and Atibala* indicated in *Astigata vata* and acts as *Vatahara* and *Brumhana*. The pharmacological analysis, HPTLC etc of the drug was done to analyse the

active components for better understanding of mode of action.

OBJECTIVES

- To study the Physico-chemical and Phyto-chemical analysis of *Vachadi Lepa*
- To study the Physico-chemical and Phyto-chemical analysis of *Ketakimoola Taila*
- To compare the obtained values of analysis of both drugs with *Ayurvedic P*harmacopia of India

MATERIALS AND METHODS

Experimental Study involving the Physico-Chemical Study of following parameters- Loss on drying (moisture content), Refractive Index, Specific Gravity, Acid Value, Iodine Value, Saponification Value, TLC, Test for Carbohydrates, Test for proteins, Test for steroid, Test for Saponins, Test for Alkaloids, Test for Tannins, Test for cardiac Glycosides, Test for anthroquinone Glycosides, Test for Saponin Glycosides.

Trial drug details:

Table. No.01 *Ketakimooladi taila* [11]

Sl.no	Ingredient	Latin Name	Part Used
1.	*Ketakimool (1part)*	Pandanus Odorifer	*Moola*
2.	*Bala (1part)*	SidaCordifolia	*Moola*
3.	*Atibala (1part)*	Abutilon Indicum	*Moola*
4.	*Kanji (* Equal quantity of kashaya)	-	
5.	*Tila Taila (4part)*	Sesamum indicum	*Beeja*

Table.No.02 *Vachadi Upanaha Churna* [12]

Sl.no	Ingredient	Latin Name	Part Used
1.	*Vach (1part)*	Acorus Calamus	*Rhizome*
2.	*Shatapushpa (1part)*	Anethum Graveolens	*Beeja*
3.	*Devadaru (1part)*	Cedrus Deodara	*Stem bark*
4.	*Rasna (1part)*	Pluchea Lanceolata	*Moola*
5.	*Kushta (1part)*	Saussurea Lappa	*Moola*
6.	*Tila (1part)*	Secamum Indicum	*Beeja*
7.	*Masha (1part)*	Vigna Mungo	*Beeja*
8.	*Kulathi*	Macrotyloma Uniflorum	*Beeja*

9.	*Eranda (1part)*	Ricinus Communis	*Moola*
10	*Amla dravya(Kanji)50ml*	Fermented sedimentation	
11.	*Tila Tail-for abyanga- 30 ml* *For upahanaha- 50ml*	Sesame oil	*Beeja*
12.	*Lavana-20-30gm*	Rock Salt	
13.	*Takra -30 ml*	Butter Milk	
14.	*Go Ksheera-30ml*	Cow's Milk	

Morphological/ Organoleptic evaluation:

Qualitative evaluation based on sensory profile refers to observation by colour, odour, taste and touch, respectively.

Physico-chemical standards

Loss on drying (moisture content)

1.0gm of powdered test sample was weighed & placed in China/glass dish and dried in oven at 100- 500ºC. The sample was taken out and cooled in desiccators and loss in weight was recorded. This procedure was repeated till constant weight was obtained.

Loss on drying (%) = Loss in weight x 100 /w**,** where 'W' is = Weight of the drug powder in gram.

Refractive Index

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line inter- sects the separatrix exactly at the center. Noted the reading. Distilled water has a refractive index of 1.3315 at 29°C. The difference between the reading and 1.33206 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+), and the correction is minus (-). The Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get an accurate refractive index. The Refractive index of the test samples was measured at 28°C.

Specific Gravity

Cleaned a specific gravity bottle by shaking it with acetone and then with ether. Dried the bottle and not- ed the weight. Cooled the sample solution to room temperature. Carefully filled the specific gravity bot- tle with the test liquid, inserted the stopper, and re- moved the surplus liquid. Noted the weight. Repeat- ed the procedure using distilled water in place of the sample solution.

Acid Value

Weighed 2- 10g of oil in a conical flask. Added 50 ml of the acid-free alcohol-ether

mixture (25 +25ml) previously neutralized with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. The endpoint is the appearance of pale pink colour. Repeated the experiment twice to get concordant values.

Iodine Value

The sample was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCL 20ml of iodine monochloride solution was added. The stopper was inserted, which was previously moistened with a solution of potassium iodide, and the flask was kept in a dark place at a temperature of about 17° C for 30 min. 15ml of potassium iodide and 100ml of water were added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as an indicator. The number of ml of 0.IN sodium thiosulphate required was noted. The experiment was repeated with the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required was noted. The experiment was repeated to get concordant values.

Saponification Value

Weighed 2g of the Oil/ Fat into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash. Refluxed in a water bath for 30 minutes. Cooled and added 1 ml of Phenolphthalein solution and titrated immediately with 0.5 M Hydro- chloric acid (an ml). Repeated the operation omitting the substance being examined (blank) (b ml). Repeat- ed the experiment twice to get concordant values

TLC

The TLC profiles of Alcoholic extract of were applied on track 1 and 2 whereas 10 µl of Merck TLC aluminium plates silica gel 60F 254 100×100 mm The plate was developed in solvent system Toluene: Ethyl acetate (3:7 v: v:). TLC photos were captured at UV 254 and UV 366nm.

Details of Various Phytochemical Tests

Test for Carbohydrates

Molisch's Test (General Test): In 2 – 3 ml aq. Extract, few drops of alpha naphthol solution and alcohol was added and shaken well. Then concentrated $H_2SO_4$was added from sides of test tube to observe violet ring formed at the junction of 2 liquids.

Tests for reducing sugars

a) **Fehling's test –** Mix 1ml of Fehling's A and 1ml of Fehling's B solutions and boil for 1 minute. Add equal quantity of test solution. Heat in boiling water bath for 5-10 mins to observe yellow or brick red precipitation.
b) **Benedict's Test –** Mix equal volume of Benedict's reagent and test solution in a test tube. Heat in boiling water bath for

5 min. Observe the change in colour of solution which appears green, yellow or red depending upon on amount of reducing sugar present.

Test for non-reducing polysaccharides (Starch)

Iodine test: Mix 3ml test solution and few drops of dilute iodine solution. Blue colour appears and it disappears on boiling and reappears on cooling.

Test for proteins

Biuret test-To 3ml of test solution add 4% NaOH and few drops of 1% $CuSO_4$ solution to observe violet or pink colour.

Test for Amino acids

Ninhydrin test- Heat 3ml of test solution and 3 drops of 5% Ninhydrin solution in boiling water bath 10 min to observe purple or bluish colour.

Test for steroid

Salkowski reaction – To 2ml. of extract, add 2ml chloroform and 2ml of conc.H_2SO_4 shake well. Observe red colour in chloroform layer and for acid layer greenish yellow florescence.

Test for Saponins

Foam test – Shake the drug extract vigorously with water to observe persistent foam.

Test for Flavonoids – To small quantity of residue, lead acetate is added to observe yellow coloured precipitation.

Test for Alkaloids

Dragendorff's test – To 2-3ml filtrate, few drops of Dragendorff"s reagent is added to observe orange coloured precipitation.

Test for Tannins

Lead acetate test – To 2-3 ml extract few drops of lead acetate solution is added to observe to observe white ppt.

Test for cardiac Glycosides

A solution of 0.5 mL, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 mL of extract. Later, 1 mL of concentrated H_2SO_4, was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycosides

Test for anthroquinone Glycosides

Borntrager's Test: Add a 1ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of dilute ammonia. The formation of rose pink to red colour of the ammoniacal layer shows the presence of anthraquinone glycosides.

Test for Saponin Glycosides

5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was

mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

RESULTS

Table No. 03. Showing Organoleptic Characters of *Ketaki Mooladi Taila* .

TESTS	**RESULTS**
Colour	Light greenish
Odour	Characteristic
Form	Oil

Table No. 04. Showing Physicochemical Standards of *Ketaki Mooladi Taila*.

TESTS	**RESULTS**
Loss on Drying at 110 C	0.601%
Refractive Index at 40 C	1.468
Acid value	6.705
Iodine value	118.01
Saponification Value	240.46
Specific gravity	0.918

Note: **API** Standards are not available for above preparation. Given results are of the submitted obtained through this sample only.

Table No. 05. Showing TLC of *Vachadi Upanaha Choorna*

TESTS	**RESULTS**
TLC: (Alcohol extract)	Rf Values
Mobile phase – Toluene: Ethyl acetate Ratio: 7: 3	Short Wave (254 nm): 0.67, 0.77 Long Wave (366 nm): 0.25, 0.30,0.42, 0.49, 0.67, 0.73

Table No. 06. Showing Preliminary Phytochemical Screening of *Vachadi Upanaha Choorna*

TESTS	**WATER**	**ALCOHOL**
Carbohydrates	+	+
Reducing sugar	+	+
Monosaccharides	+	–
Pentose sugar	–	–
Non reducing sugar	–	–
Hexose sugar	–	–
Proteins	+	–
Amino acids	+	–
Steroids	–	+
Flavonoids	+	+
Alkaloids	–	–
Tannins	+	–

Table No. 07. Showing Test for Glycosides of *Vachadi Upanaha Choorna*

Cardiac Glycosides	–	+
Anthraquinone Glycosides	+	+
Saponin glycosides	–	–

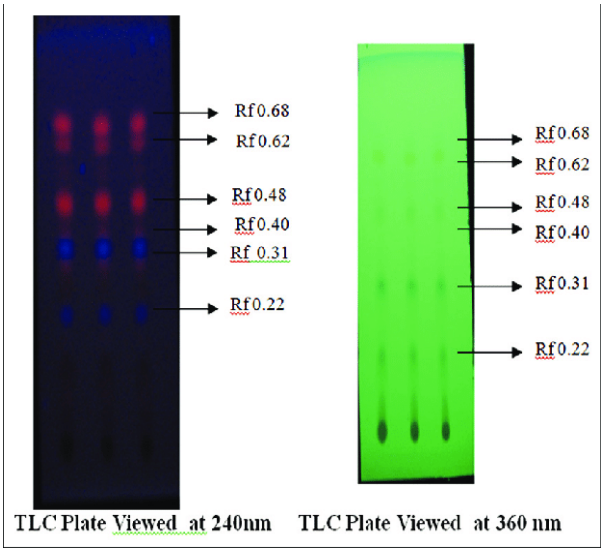

Fig. 1: TLC Image of *Ketaki Moola Taila*(oil)

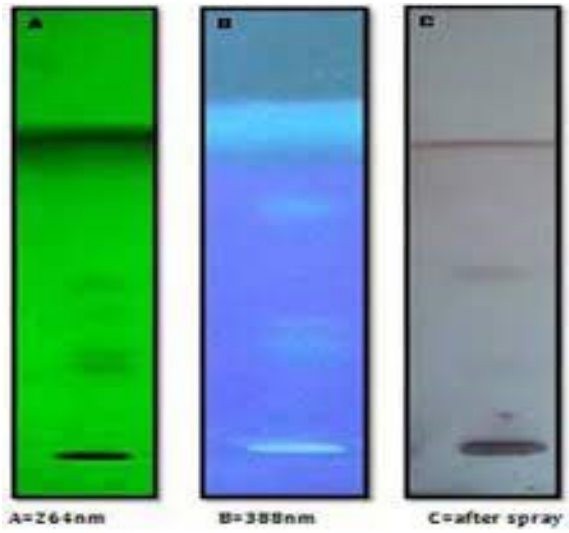

Fig. 2: TLC Image of *Vachadi Upanaha Churna*(powder)

DISCUSSION

The isolated secondary metabolite otherwise known as the phytoconstituents are therapeutically significant and hence studied here. Among the lot Carbohydrates, reducing sugar, Flavonoids, Anthraquinone glycosides are both Water and Alcohol soluble. Monosaccharides, Proteins, Amino acids, Tannins are water soluble. Steroids and Cardiac Glycosides are alcohol soluble.

The Qualitative Phytochemical Analysis of *Vachadi upanaha churna*(powder):

TLC results showed the presence of 2 spots at 254 nm and 6 spots at 366 nm. The therapeutically active components present in the final product are Carbohydrate, Reducing sugar, flavonoids and anthraquinone glycosides.

The preliminary phytochemical analysis of *Vachadi upanaha churna*(powder):

The therapeutically active components present in Vachadi Upanaha Choorna are Carbohydrate, Reducing sugar, flavonoids and anthraquinone glycosides.

Discussion on *Ketakimooladi Taila* (oil):

The organoleptic characteristics of Ketakimooladi taila(oil) is light greenish in colour having characteristic odour having moisture content 0.601% Refractive index at 40c is 1.468, Acidic value of 6.705, Iodine value of 118.01, Saponification value of 240.46 and specific gravity of 0.918 is found in physico-chemical study of *Ketaki mooladi Taila*(oil).

Conclusion

All the ingredients of *Ketakimoolaadi Taila*(oil) and *Vachaadi Upanaha*(poultice) were subjected for physic-chemical analysis and were found as per the API standards.

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