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Physico-chemical properties and chromatographic findings of different natural solvent extracts of *Vitex negundo Linn.*

Research Article

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Abstract

Introduction: Ayurveda, uses various herbs that are converted into different dosage forms mostly in presence of *Jala* (water) as solvent. In addition to water; seers also advocated certain other solvents for extraction of active principles from the herbs. Works on using traditional solvents other than water in extracting principles from different herbs are not reported till date. **Aims:** Considering lack of such evidences, an attempt has been made to prepare *Nirgundi Ghana* (solid extract of *Vitex negundo* Linn.) in three different solvents i.e. *Jala* (water), *Kanji* (sour gruel) and *Gomutra* (cow's urine) and analyze respective physico-chemical profiles. **Materials and Methods:** *Ghana* was prepared by classical methods described in Ayurveda. The samples were analyzed through relevant physico-chemical parameters. **Results:** In pharmaceutical study; yield was found more in presence of *Kanji* and *Gomutra*. Physico-chemical profiles showed few differences, but in most of the cases, they were insignificant. **Conclusion:** Based on preliminary physico-chemical profiles, it cannot be ascertained the usefulness of the finished products in therapeutics. Relevant experimental studies to identify and characterize the active phyto-constituents and evaluate therapeutic utilities of the principles extracted into the respective solvents are essential in further studies.

Keywords: Extraction, *Ghana, Gomutra, Kanji, Nirgundi, Vitex negundo*

Introduction:

Ayurveda emphasizes on maintenance, promotion of health and curing diseases through natural resources in a comprehensive way.(1) As these resources cannot be used in their natural forms, are converted into different dosage forms mostly in presence of *Jala* (water) as the *drava dravya* (extracting media / solvent).

It is evident from preliminary screening through the classics that, seers used other *dravas* (liquid medias) like *Ksira* (milk),(2) *Go-mutra* (cow's urine), (3) *Takra* (butter milk), (4) *Kanji* (sour gruel),(5) and *Ksarodaka* (alkaline water)(6) etc. in addition to *Jala* in different pharmaceutical procedures based on necessity. This infers that, seers have knowledge about rate and transfer of medicinal properties of any *dravya* (drug) on the virtue of *drava dravya.* As some of these solvents are alkaline and some are acidic, it is to be investigated and determined the ideal extracting media for various herbs on scientific basis. Published information on such kind of studies are not found till date and is expected to revolutionize the methods of preparations to bring out more effective preparations than those of prepared by conventional methods.

Considering this, it is planned to prepare *Nirgundi Ghana* (solid extract of *Vitex negundo* Linn.) in presence of three different medias (*Jala*, *Kanji* and *Gomutra*) and analyze preliminary physico-chemical profiles. *Nirgundi* (*Vitex negundo* Linn.) is selected in the present study, as the plant is abundantly, easily available and frequently used in different ailments by Ayurvedic physicians and folklore healers as well.

Materials and Methods:

Procurement of raw material:

Fresh leaves of *Nirgundi* were collected from botanical garden of Gujarat Ayurved University, Jamnagar and authenticated at Pharmacognosy laboratory. *Kanji* was prepared in the laboratory of *Rasashastra & Bhaishajya kalpana*, IPGT & RA, GAU, Jamnagar by following standard methods of preparation. (7) *Gomutra* was procured from local *Goshala* (cow-shed), Jamnagar.

Preparation of test formulations:

Ghana was prepared from fresh leaves of *Nirgundi* that were crushed thoroughly and mixed with required amounts (8 parts) of potable water.(8) The contents were subjected to mild heat maintaining temperature in between 95-100^{0}C. The contents were

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stirred constantly, in order to avoid possibilities of settling down of the contents and their charring. When the volume was reduced to one quarter; the contents were filtered through a clean cotton cloth into a stainless steel container to obtain *Kwatha* (decoction). The decoction was further subjected to the process of *punah paka* (re-boiling) with continuous stirring till the contents become semi-solid, were shifted into stainless steel tray and subjected to drying maintaining temperature around 50^0C. The dried aqueous extract (W) was collected carefully with the help of scraper and used in the study. Similar procedure was repeated in presence of *Kanji* and *Gomutra* as solvents to obtain solid extracts that were labeled as K and G respectively. In addition to these practicals; attempts were also made to extract *Ghana* of *Gomutra* and *Kanji* individually to calculate the possible extract of solvents that enter into the finished product (W, K and G).

Analytical Study

All the samples of *Nirgundi Ghana* (W, K, and G) were analysed to evaluate organoleptic characters, preliminary physico-chemical analysis (including estimation of pH,(9) Total ash,(10) Acid insoluble ash, (11) Water Soluble Extractive,(12) Alcohol Soluble Extractive, Loss on Drying at 105^0 C), qualitative estimation of functional groups.(13)

HPTLC Profile

Ethyl Acetate: Methanol: Water: Glacial Acetic Acid (7.8:1.2:0.7:0.3) was selected as solvent system and Vaniline Sulphuric Acid as spray reagent. All tracks of *Ghana* (W, K, and G) were scanned under 254 nm and 366 nm and Rf values are recorded accordingly.

HPTLC condition

Application mode	:	CAMAG Linomat V Hamilton Syringe
Development chamber	:	CAMAG Twin trough chamber (20 x 10 cm)
Plates	:	Precoated silica gel GF_{254} plates
Chamber saturation	:	30 min
Development time	:	30 min
Scanner	:	CAMAG Scanner III
Scanning mode	:	Linear at 254 nm and 366 nm
Detection	:	Deutarium lamp, Mercury lamp
Photo documentation	:	CAMAG reprostar
Data system	:	WINCATS software (Ver. 3.17)
Drying device	:	Oven
U.V. Spectrum	:	200 nm to 700 nm

Results

Brief details of *Nirgundi Ghana* obtained in presence of different solvents and details of *Ghana* obtained from individual solvents (*Gomutra* and *Kanji*) are placed at Table 1. Organoleptic characters (Table - 2), comparative preliminary physico-chemical profiles (Table - 2), qualitative estimation of different functional groups (Table - 3), percentage of alkaloids (Table - 3), observations of HPTLC study along with Rf values (Table - 4) of all the three samples are placed at respective tables. (Fig.-1)

Discussion:

Apart from certain organoleptic changes; no significant differences were observed during pharmaceuticals stages of *Ghana* preparation. It is evident from table-1 that, the quantity of extract of *Nirgundi* is more in *Kanji* and *Gomutra* than *Jala*. At the end of the preparation, it is found that *Kanji* yielded more percentage of *Ghana* (40.4%) followed by *Gomutra* (34.5%), and least in *Jala* (6.8%). *Kanji,* being a media of *amla pradhana* is expected to liquefy (*kledayati*), disintegrate (*jarayati*), and possibly facilitate more extraction of principles (*apakarshayati*) from the raw material.(14) This may be the probable reason behind extraction of more percentage of *Ghana* in presence of *Kanji* as solvent. Similarly, with the other solvent *Gomutra* that is alkaline in nature.(15) Subtracting *Ghana* of respective solvents from the total yield infers that, the solvents other than water facilitate extraction of more principles into the end product.

Total Ash was high in case of sample G, followed by W, and K. Alcohol Soluble Extractive was very low in sample K and was almost equal in other two samples. Variations in other physico-chemical profiles are insignificant. Alkaloids, Tannins, Saponins, Phenols, and Flavanoids were found in all the three samples, while Glycosides, Protein, Quinines were absent in all the three samples. Sterols, and Triterpenoids were present in W, and G amples (and absent in K), and Carbohydrate was absent in G. Quantitatively, the percentage of alkaloids present in sample K was found to be lesser (0.19), in comparison to sample W (0.44), and sample G (1.09).

During the HPTLC study, spots in three samples were observed at different frequencies, few of them were appeared to be in the same frequencies. The three samples (W, K, and G) revealed 14, 13, and 10 spots at 254 nm; while 7, 8, and 4 spots at 366 nm respectively. After spray with Vanillin - Sulphuric acid, twelve spots in sample W, and nine spots each in sample K and G were observed. These differences indicate the presence of variable functional groups in both the trial groups.

Conclusion:

References available in the classics reveal that, the seers of ancient India identified importance of different solvents other than *jalam* (water) in specific cases. *Sushruta* opines that, activity of substances increases by *Arishta Dravya* (solvents of alcoholic base) *Samyoga.*(16) Though, the yield is more in case of *Kanji* or *Gomutra*, it cannot be ascertained that, the extracted priciples are useful in therapeutics. Hence, relevant experimental studies are needed to justify comparative efficacies. In addition, identification and characterization of the phyto-constituents extracted through respective solvents is also essential.

These solvents might have been used by seers in selected cases to extract desired principles that may be useful in specific conditions. On the other hand, use of

such solvents may extract specific active principles leaving behind many other principles from the drug, which possibly contribute a lot in the treatment. Whether using such solvents in Ayurvedic practice is useful or not, is questionable and there is a need to explore this issue.

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Table 1: Average details of *Ghana* obtained in different batches.

Sl. No.	Group	Quantity of *Nirgundi*	Solvent added	Quantity of Solvent	Decoction obtained	*Ghana* obtained (g)	% of *Ghana* obtained
1	W	200 g	*Jala*	1600 ml	400 ml	13.7	06.8
2	K	200 g	*Kanji*	1600 ml	400 ml	80.9	40.4
3	G	200 g	*Gomutra*	1600 ml	400 ml	68.1	34.5
Sl. No.		**Quantity of Media**	**Media**			***Ghana* obtained (g)**	**% of *Ghana* obtained**
1		100 ml	*Gomutra*			4.9	4.9
2		100 ml	*Kanji*			6.8	6.8

Table 2: Organoleptic and Physico-chemical characters of *Nirgundi Ghana* of three samples

	Characters	Batch		
		W	K	G
1	**Color**	Brown	Brown	Brown
2	**Odor**	Characteristic	Characteristic	Characteristic odor of urine
3	**Appearance**	Dark	Dark	Dark
4	**Taste**	Bitter	Bitter with mild acidic	Bitter with characteristic odor of urine
5	**P^H (5% Aqu. solution)**	6.69	6.73	6.75
6	**Loss on drying at 105^0C**	4.96	4.22	4.71
7	**Total ash**	10.22	5.58	44.21
8	**Acid insoluble ash**	0.82	0.71	1.21
9	**Water Soluble extractive**	83.68	90.02	84.74
10	**Alcohol Soluble Extractive**	52.106	12.21	50.85

Table 3: Qualitative estimation of different functional groups in *Nirgundi Ghana*

	Functional Group	W	K	G
1	Alkaloid	+ve	+ve	+ve
2	Carbohydrate	+ ve	+ ve	-ve
3	Tannin	+ve	+ve	+ve
4	Saponin	+ ve	+ ve	+ ve
5	Glycosides	-ve	- ve	- ve
6	Flavanoids	+ve	+ve	+ve
7	Phenols	+ve	+ve	+ve
8	Protein	- ve	-ve	-ve
9	Sterols	+ve	-ve	+ve
10	Quinines	-ve	-ve	-ve
11	Triterpenoids	+ve	-ve	+ve
Quantitative estimation of Alkaloids in *Nirgundi Ghana*				
12	Total Alkaloids (%)	0.44	0.19	1.09

+ve = Present, -ve = Absent

Table 4: Rf values in *Nirgundi Ghana* (Before Spray)

Track	Visualization	No. of spots	Rf Values
Track 1 (W)	λ Max 254 nm	14	0.02, 0.07, 0.10, 0.14, 0.21, 0.27, 0.33, 0.39, 0.52, 0.63, 0.69, 0.74, 0.86, 0.99
	λ Max 366 nm	7	0.02, 0.09, 0.24, 0.36, 0.45, 0.48, 0.64
Track 2 (K)	λ Max 254 nm	13	0.02, 0.09, 0.21, 0.26, 0.37, 0.44, 0.53, 0.60, 0.67, 0.72, 0.80, 0.84, 0.99
	λ Max 366 nm	8	0.02, 0.09, 0.25, 0.34, 0.44, 0.52, 0.62, 0.87
Track 3 (G)	λ Max 254 nm	10	0.03, 0.11, 0.27, 0.37, 0.38, 0.50, 0.61, 0.72, 0.81, 0.99
	λ Max 366 nm	4	0.03, 0.14, 0.39, 0.98
Rf values in *Nirgundi Ghana* (After Spray)			
Track 1 (W)		12	0.02, 0.06, 0.15, 0.20, 0.31, 0.37, 0.43, 0.59, 0.65, 0.71, 0.82, 0.95
Track 2 (K)		9	0.02, 0.06, 0.14, 0.23, 0.35, 0.50, 0.57, 0.63, 0.69
Track 3 (G)		9	0.04, 0.11, 0.22, 0.34, 0.40, 0.51, 0.59, 0.70, 0.95

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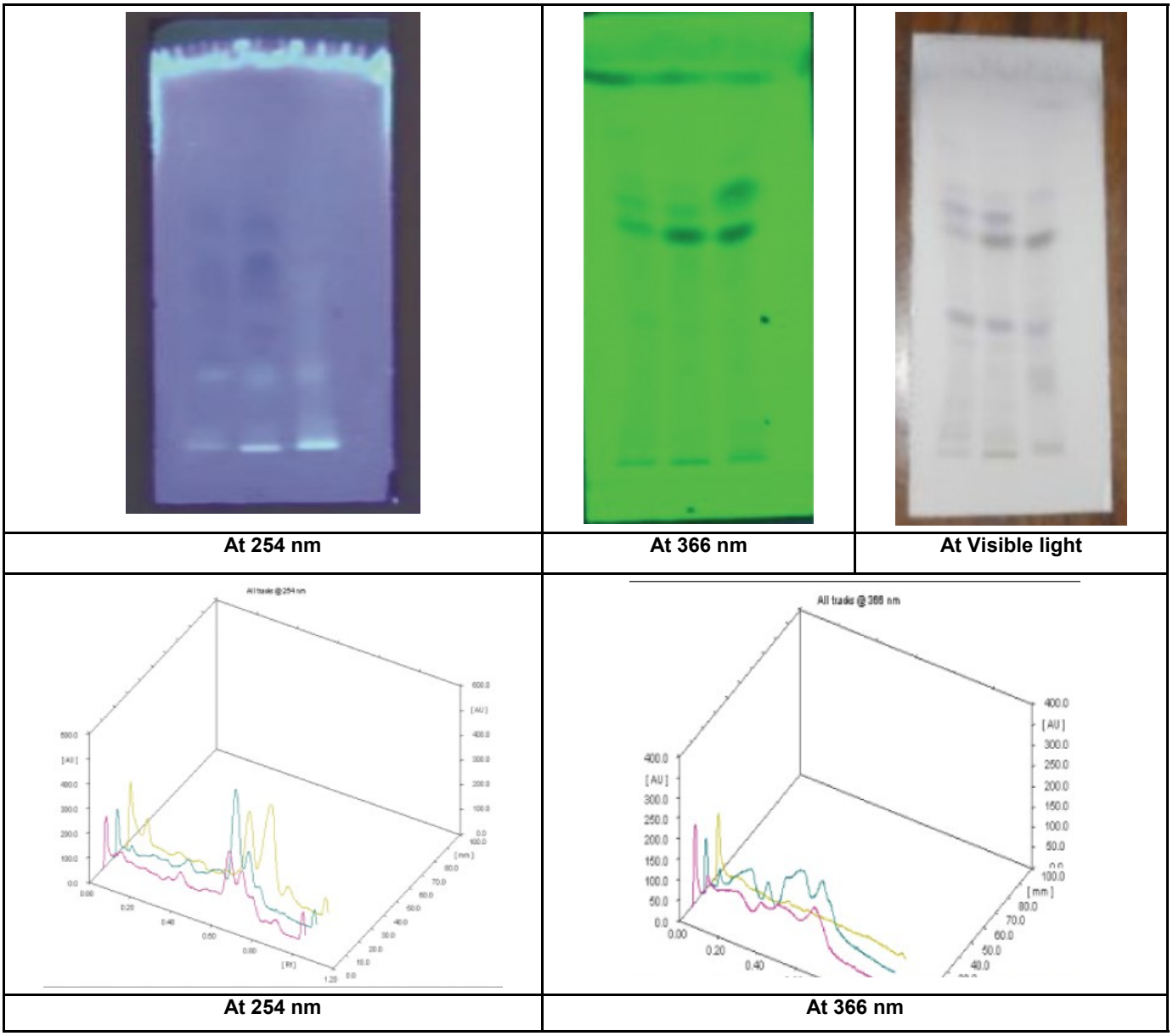

Fig.1: HPTLC of WKG

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