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ORI- EXPERIMENTAL STUDY

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PHYTOCHEMICAL AND CHROMATOGRAPHICAL EVALUATION OF MADANPHALA (RANDIA DUMETORUM LAMK.) FROM DIFFERENT DESHA BASED ON AKASHADI PANCHAMAHABHOOTA MADHUSHREE C S¹, MANJUNATH H. DUNDI^{2*}

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ABSTRACT:

Background: Madanaphala is the best vamana dravya (emetic drug). As per classics the best place to collect these vamana dravya is the land which is predominant of the Agni (fire), Vayu (air) and Akash (ether) mahabhoota. In this study we are going to evaluate the phytochemical and chromatographical qualities of the Madanaphala collected from Jangaladesha (dry land) that is Kappatagudda which is the ideal collection area mentioned by Acharya's for vamana karma and the Madanaphala collected from the Anupadesha (marshy land) that is Jamboti. Objectives: Evaluation of phytochemical and chromatographical constitution of Madanaphala, collected from different desha (land) based on Akashadi mahabhoota (ether etc., elements). Methodology: 2 samples of Madanaphala, collected from the two different desha's, one sample collected from Kappatagudda region and the other sample collected from Jamboti region. The collected fruits are dried, cleaned, powdered and were analyzed for phytochemical and chromatographical evaluation. Conclusion: The phytochemical analysis of Madanaphala collected from two different region Kappatagudda and Jamboti didn't show any changes. TLC of both the samples showed the presence of phytochemicals at different Rf values but the sample collected from Kappatagudda has more number of Rf values which indicate presence of more number of phytochemicals than in Madanaphala collected from Jamboti. The standard Rf value of Oleanolic acid is 0.57. In the HTPLC result the nearest Rf value observed was 0.51 which is found in the Madanaphala collected from Kappatagudda, but no nearest Rf value was observed in Madanaphala collected from Jamboti. So we can justify the reference which is explained by our Acharya Sushruta in the context of Bhumipravibhaqiyamadhyaya that vamana dravya should be collected from desha predominant of Akash, Vayu and Agni mahabhoota. As our study also showed that Madanaphala collected from Kappatagudda region which is predominant of Agni, Vayu and Akash mahabhoota predominant is of superior quality than the Madanaphala collected from Jamboti region having Parthiva(earth) and Aap(water) mahabhoota.

Key Words: Madanaphala, Phytochemical, Chromatographical, Desha

INTRODUCTION:

In Indian system of medicine *Madanaphala* (*Randia dumetorum* Lamk) family Rubiaceae is an important medicinal plant popularly known as emetic nut. It is found in waste places & jungles all over India, extending northwest to the Bias River & ascending to outer Himalaya to 4000 ft. ^[1].

Fruit globose or broadly ovoid, 1.8-4.5cm long, crowned with persistent calyx-limb, longitudinally ribbed or smooth yellowishbrown, fruit contains numerous seeds, 0.4-0.6 cm long, very hard, brown, smooth and compressed. Fruit is bitter & sweet, carminative, purgative, aphrodisiac, emetic, antipyretic. lt cures abscess, ulcers, inflammation, wounds, tumors, skin diseases and have anti-bacterial activity. The pulp of fruit is believed by many practitioners to also have anthelmintic properties, and also used as an abortifacient as folklore remedy [2].

The bark is astringent and is given in cases of diarrhea and dysentery. It is applied externally in the form of paste in rheumatism and to relieve pain of bruises and bone aches during fevers and to disperse abscesses and used internally. The aqueous extract of the root bark of the tree is used as an active insecticide

Madanaphala fruits are considered as the agrya (superior) dravya for vamana karma [4]. They are considered as agrya as they are free

from complications and adverse side effect^[5]. This Vamana karma is the process by which Apakva Kapha (unripen kapha) and Pitta are expelled out forcefully through Urdhwa bhaga^[6] (upper clavicular region). Vamana dravya should be collected from soil which possesses the qualities of Agni, Vayu and Akasha mahabhoota [7]. So Madanaphala should be collected from the soil predominant of Agni, Vayu and Akasha mahabhoota. The qualities of these Agni, Vayu and Akasha mahabhoota is mostly found in Jangala desha [8,9,10]

In this study we are going to evaluate the phytochemical and chromatographical qualities of the *Madanaphala* collected from *Jangala desha* which is the ideal collection area mentioned by Acharya's for *vamana karma* and the *Madanaphala* collected from the *Anupa desha*. Here we have selected Kappatagudda for *Jangaladesha* and Jamboti (near Belagavi) which belongs to *Anupa desha*.

AIM & OBJECTIVE:-

 Evaluation of phytochemical and chromatographical constitution of Madanaphala, collected from different desha based on Akashadi mahabhoota.

MATERIALS AND METHODS:-

Plant materials:- 2 Samples of *Madanaphala* fruits are collected. One sample is collected from the Kappatagudda region which belongs to *Jangaladesha* that is predominant of *Agni*,

Vayu and Akash Mahabhoota and the another sample is collected from the Jamboti region which belongs to Anupadesha that is predominant of Pruthvi and Aap Mahabhoota. The Madanaphala fruits were got identified and authenticated at AYUSH approved Central Research Facility at Shri B.M.K Ayurveda Mahavidyalaya and PG centre, Shahapur, Belagavi and voucher number (CRF/Auth/74/2023) and (CRF/Auth/75/2023) of the drugs was given in Central Research Facility.

Churna preparation:-For testing purpose *Madanaphala* fruits are made into *churna* (powder). Here *Madanaphala* fruits *churna* is prepared by using Pulveriser and sieved through fine clean sieve in our college KAMCH RSBK Pharmacy.

Physico-chemical Phyto-chemical Screening and TLC test: Physico-chemical, Preliminary Phyto-chemical screening and TLC test was done at Central research facility, KLEU'S Shri BMK Ayurveda Mahavidyalaya, Belgaum, Karnataka.

HPTLC test:-Ethanolic extract of Madanaphala from Kappatagudda and Jamboti has been sent to SDM centre for research in Ayurveda and allied sciences, Udupi, Karnataka.

Method:-

100mg of each Ethanolic extract of Madanaphala from Kappatagudda and Ethanolic extract of Madanaphala from Jamboti was dissolved in 100µl of ethanol. On a pre-coated silica gel F2544 and 8µl of each of the above extract was applied on aluminum plates to a band width of 7mm using Linomat 5 TLC applicator. The plate was developed under Toluene : Ethyl acetate : Formic acid (7.0:3.0:0.3). The developed plates were visualised in short UV, long UV, White light after derivatization with Anisaldehyde sulphuric acid reagent. Developed plate was scanned at 254nm and 530nm (After derivatization with ASA reagent). Rf, colour of the spots, densitometry scan was recorded.

RESULTS

Results described under following headings

- 1. Pharmacognostic results
- 2. Analytical results
- 3. Experimental result
- 1) Pharmacognostic results:

A) Macroscopic evaluation of Madanaphala:-

Madanaphala {fruit} of Kappatagudda region:-

More or less globose, dark brown in colour, 2.5-3cm length, 1.4 – 1.8 cm width with one end slightly depressed and the other end slightly raised, a depressed end scar for the pedicle and at raised end there remains scar for calyx.

• Madanaphala (fruit) of Jamboti region :-

More or less globose, dark brown in colour, 3.8-4cm length, 2-2.2cm width with

one end slightly depressed and the other end slightly raised, a depressed end scar for the pedicle and at raised end there remains scar for calyx. Results described under following headings.



Figure -1: Pharmacognostic Study of Madanaphala

B) Powder microscopy:-

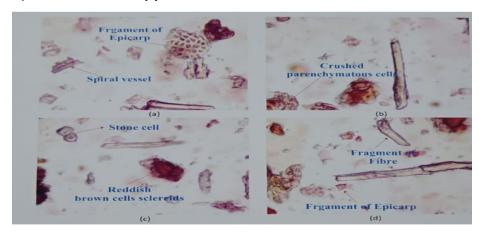


Figure-2: Powder Microscopy of Kappatagudda

Powder microscopy shows: Fragment of Epicarp, spiral vessels (a) crushed parenchymatous cells (b) stone cells, reddish

brown sclerides (c) Fragment of fibre and Fragment of epicarp (d)

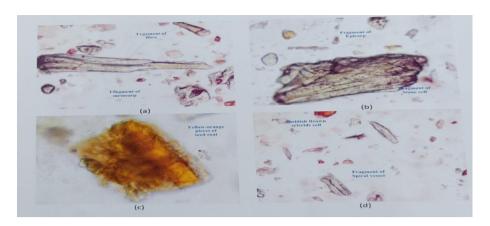


Figure- 3: Powder microscopy of Jamboti

Powder microscopy shows; Fragment of Fibre and Mesocarp (a) Fragment of stone cells and Epicarp (b) Yellow-orange pieces of seed coat

(c) Reddish brown sclerids cells and Fragment of spiral vessels(d).

2) Analytical result:

A. Organoleptic Characters of Madanaphala fruit

Table-1:- Organoleptic Characters of Madanaphala fruit

SL no.	TESTS	FRUIT OF KAPPATAGUDDA REGION	FRUIT OF JAMBOTI REGION	
1.	Part	Fruit	Fruit	
2.	Colour	Yellowish brown	Yellowish brown	
3.	Taste	Characteristic	Characteristic	
4.	Odour	Characteristic	Characteristic	

B. Physico-chemical Properties of *Madanaphala* fruit *churna*:-

The available standard protocols for various procedures were adopted. The obtained results are tabulated below in Table no 2.

Table no-2: Represent Physico-chemical properties of Madanaphala fruit powder

SL NO	TESTS	LIMITS	FRUIT OF KAPPATAGUDDA REGION	FRUIT OF JAMBOTI REGION
1.	Foreign matter	Not more than 2%	Nil	Nil
2.	Ash value	Not more than6%	3.523%	3.698%
3.	Acid insoluble ash	Not more than 0.25%	0.198%	0.243%

4.	Water	soluble	Not less than 16%	17.133%	18.325%
	extractive				
5.	Alcohol	soluble	Not less than 19%	20.239%	21.065%
	extractive				

C. Preliminary phytochemical screening for organic components: Aqueous and Alcoholic extracts of *Madanaphala churna* was prepared with cold maceration technique. That was

further subjected for qualitative phytochemical screening. The results are mentioned below in Table no. 3

Table no 3: Illustrates the results of phytochemicals in *Madanaphala churna* aqueous and alcoholic extract

		SAMPLE OF KAP	PATAGUDDA REGION	SAMPLE OF JA	SAMPLE OF JAMBOTI REGION	
SL	ORGANIC	WATER	ALCOHOL	WATER	ALCOHOL	
NO	ELEMENT	EXTRACT	EXTRACT	EXTRACT	EXTRACT	
1.	Carbohydrates	Positive	Positive	Positive	Positive	
2.	Reducing sugar	Positive	Positive	Positive	Positive	
3.	Monosaccharaides	Positive	Positive	Positive	Positive	
4.	Pentose sugar	Negative	Negative	Negative	Negative	
5.	Non reducing sugar	Negative	Negative	Negative	Negative	
6.	Hexose sugar	Negative	Positive	Negative	Positive	
7.	Proteins	Negative	Negative	Negative	Negative	
8.	Amino acids	Positive	Positive	Positive	Negative	
9.	Steroids	Negative	Positive	Negative	Positive	
10.	Flavonoids	Positive	Positive	Positive	Positive	
11.	Alkaloids	Negative	Negative	Negative	Negative	
12.	Tannins	Negative	Negative	Negative	Negative	
13.	Cardiac glycosides	Positive	Positive	Positive	Positive	
14.	Anthraquinone	Negative	Negative	Negative	Negative	
	glycosides					
15.	Saponin glycosides	Positive	Negative	Positive	Negative	

D. TLC - Profile of MADANAPHALA Churna

Table no. 4: Illustrates Rf values of phytochemicals separated during TLC from alcoholic ext. of Madanaphala churna with solvent system Chloroform : Ethanol (9.5 : 0.5)

SL	SAMPLE OF KAPPATAG	DDA REGION	SAMPLE OF JAMBO	OTI REGION
NO	SHORT WAVE	LONG WAVE	SHORT WAVE	LONG WAVE
1.	0.13	0.13	0.11	0.15
2.	0.23	0.66	0.21	0.25
3.	0.37	0.76	0.33	0.63
4.	0.61	0.87	0.50	0.75
5.	0.75	0.97	0.62	
6.	0.93		0.68	
7.	0.96		0.95	

E. HPTLC OF MADANAPHALA :-

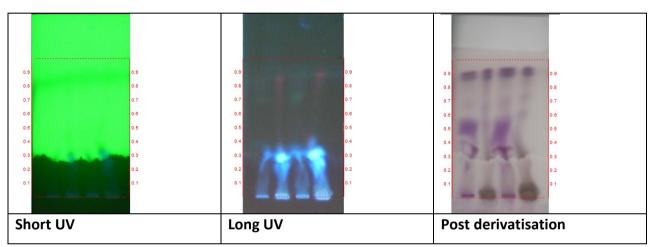


Figure 4. HPTLC photo documentation of Ethanolic extract of *Madanaphala* from Kappatagudda and Ethanolic extract of *Madanaphala* from jamboti

Track 1 - Ethanolic extract of *Madanaphala* from Kappatagudda–4µl

Track 2 - Ethanolic extract of *Madanaphala* from jamboti– 4µl

Track 3 - Ethanolic extract of *Madanaphala* from jamboti– 8µl

Solvent system – Toluene: Ethyl acetate: Formic acid (7.0: 3.0: 0.3)

Track 3 - Ethanolic extract of *Madanaphala* from Kappatagudda– 8µl

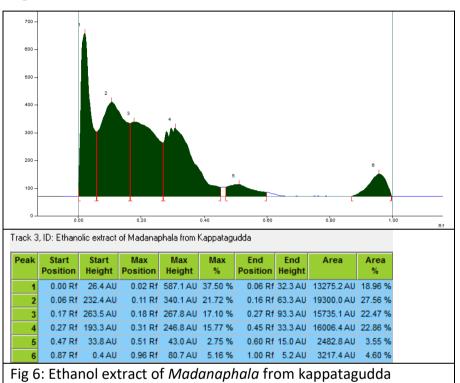
Table 5:R_f values of sample of Ethanol extract of Madanaphala

Short UV		Long UV		Post derivatisation	
Kappatagudda Jamboti		Kappatagudda	Kappatagudda Jamboti		Jamboti
-	-	0.31(F. blue)	0.31(F. blue)	-	-
-	-	-	-	0.49 (Purple)	-
-	-	-	-	0.53 (Purple)	-

-	-	-	-	0.60 (Purple)	0.61 (Purple)
-	-	-	-	0.68 (Purple)	0.67 (Purple)
-	-	0.73 (F. green)	-	-	-
-	-	-	0.85(F. red)	0.87 (Purple)	-
-	-	-	-	0.90 (Purple)	-

^{*}F - Fluorescent; L -Light; D - Dark

Figure 5:Densitometric scan of at 254nm



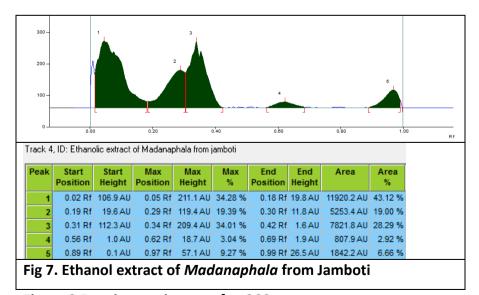
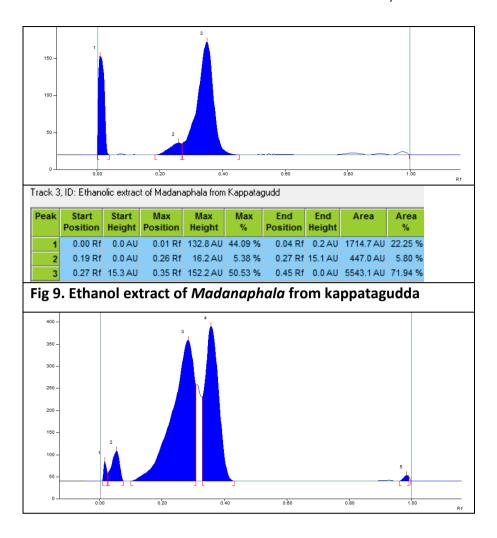
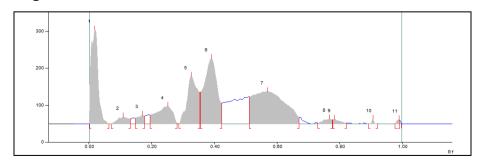


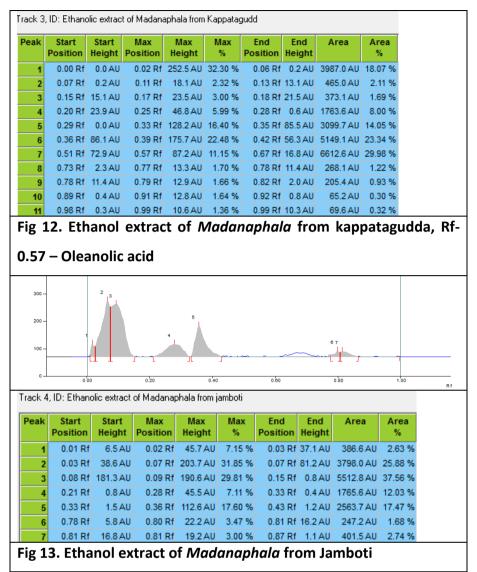
Figure 8.Densitometric scan of at 366nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.4 AU	0.02 Rf	44.5 AU	5.61 %	0.03 Rf	17.4 AU	285.3 AU	1.00 %
2	0.03 Rf	17.7 AU	0.05 Rf	68.4 AU	8.63 %	0.08 Rf	0.2 AU	1194.4 AU	4.19 %
3	0.10 Rf	0.0 AU	0.28 Rf	318.1 AU	40.10 %	0.31 Rf	16.8 AU	15911.8 AU	55.83 %
4	0.33 Rf	190.1 AU	0.36 Rf	349.3 AU	44.04 %	0.43 Rf	0.0 AU	10964.6 AU	38.47 %
5	0.96 Rf	0.0 AU	0.99 Rf	12.9 AU	1.62 %	0.99 Rf	9.7 AU	143.2 AU	0.50 %

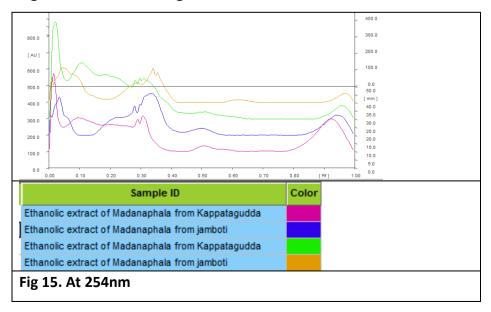
Figure 11. Densitometric scan after derivatisation with ASA at 530nm

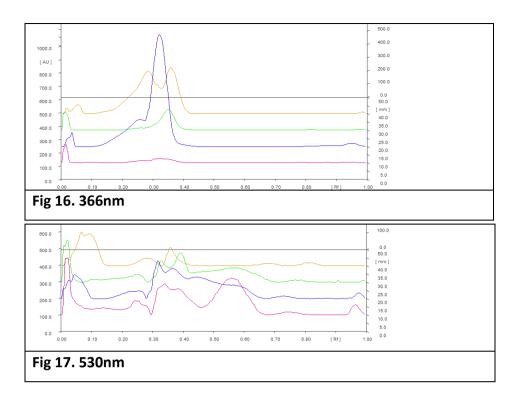




Rf-0.57 - Oleanolic acid

Figure 14. 3D Chromatogram





DISCUSSION

- The macroscopic evaluation of both the sample of *Madanaphala* did not showed significant changes. The sample of *Madanaphala* collected from Kappatagudda is 2.5-3cm in length and 1.4-1.8 cm in width and the *Madanaphala* collected from Jamboti is 3.8-4cm in length and 2-2.2cm in width.
- ➤ Both the sample are free from foreign matter. Ash value of sample collected from the Kappataguda and Jamboti is 3.523% and 3.698% in both the sample it is under API limit of not more than 6% which is mentioned for the dried fruits of Randia dumentorm Lamk.
- Acid insoluble ash values of both samples are; Sample collected from Kappatagudda
 0.198% and Sample collected from jamboti

- 0.243%. Then water soluble extraction of Kappatagudda and Jamboti sample is 17.133% and 18.325% respectively and the Alcohol soluble extract value of both Kappatagudda and Jamboti sample is 20.239% and 21.065% respectively. In these Acid insoluble ash value and extractive values of water and alcohol soluble, the extractive value is higher in the sample collected from Jamboti region.
- Preliminary phytochemical results of water extract of both samples of Madanaphala collected from Kappatagudda and Jamboti showed presence of Carbohydrate ,Reducing sugar, Monosaccharaides, Amino acid, Flavonoids, Cardiac glycosides, Saponin glycosides. Then the alcohol extract of both the sample showed the presence of Carbohydrates, Reducing

sugar, Môn saccharides, Hexose sugar, Steroids, Flavonoids, Cardiac glycosides in common but the same extract of the sample collected from Kappatagudda showed the presence of Amino acids in addition to the above phytochemicals.

- TLC profiling of both extract given an impressive result that directing towards presence of number of phytochemicals. Sample of *Madanaphala* collected from Kappatagudda showed 5 spots of Rf values i.e., 0.13, 0.66, 0.76, 0.87, 0.97 in long wave whereas sample of *Madanaphala* collected from Jamboti region showed 4 spots of Rf value i.e., 0.15, 0.25, 0.63, 0.75 in long wave.
- As of more number of Rf values present in the sample of Kappatagudda, it shows that presence of phytochemicals are more in that sample than the sample collected from Jamboti.
- In HPTLC graph, the maximum height travelled and area covered in the graph of *Madanaphala* sample collected from Kappatagudda than that of the *Madanaphala* collected from Jamboti. So we can consider the *Madanaphala* collected from the Kappatagudda as more pure than that of the sample collected from the Jamboti.
- ➤ The standard Rf value of oleonolic acid is 0.57. In the graph of ethanol extract of

Madanaphala collected from the Kappatagudda, the position of graph showed 0.51 whereas graph of Ethanol extract of Madanaphala collected from Jamboti does not showed any nearby Rf value to 0.57. This shows the presence of oleonolic acid in the sample collected from Kappatagudda but it is absent in the sample collected from Jamboti.

SCOPE FOR FURTHER STUDIES

- Different areas and places can be taken in to the consideration for the further study which is predominant of Agneya, Vayuvya and Akashiya mahabhoota, excluding the area which we have been considered in the study.
- Qualitative HTPLC analysis can be carried out to know the exact phytochemical present in the sample.

CONCLUSION

- On observation of morphology of Madanaphala collected from Kappatagudda and Jamboti, they showed variation in size but there was no marked variation in colour, taste and other organoleptic characters.
- The phytochemical analysis of Madanaphala collected from two different region i.e., Kappatagudda and Jamboti didn't show any significant changes.

- > TLC of both the samples showed the of phytochemicals presence at different Rf values but the sample collected from Kappatagudda has more number of Rf values which indicate number presence of more of phytochemicals in it than Madanaphala collected from Jamboti.
- The standard Rf value of Oleanolic acid is 0.57. In the HTPLC result the nearest Rf value observed was 0.51 which is found in the *Madanaphala* collected from Kappatagudda, but no nearest Rf value was observed in *Madanaphala* collected from Jamboti.
- As our study also showed that Madanaphala collected from Kappatagudda region which comes under Jangala desha having Agni, Vayu and Akash mahabhoota predominant is of superior quality than Madanaphala collected from Jamboti region which comes under Anupa desha having *Parthiva* and mahabhoota. So by this study we can justify the reference which is explained by our Acharya Sushruta in the context of Bhumipravibhaqiyamadhyaya that vamana dravya should be collected from desha predominant of Akash, Vayu and Agni mahabhoota.

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