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Effect of Chinnodbhavadi Arka in Amlapitha an Analytical Evaluation

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ABSTRACT

Ayurvedic concepts was one of the primordial aspects to be properly evaluated for better understanding scientifically. Detailed analytical study of any classical combination increase its acceptance in scientific manufacturing and therapeutic industry. Very few studies are conducted on Arka kalpana mentioned in Ayurvedic classics. Since the procedure extracted maximum active principles the drug process surely a better choice than many of the conventional Ayurvedic formulations. *Amlapitha* is one of the most common disease in today's fast paced world. Normally stomach produces acids for the digestive process. Improper food (*virudhahara*) untimely diet, alcoholism and smoking leads to excess production of acids and it results in the condition hyperacidity. Excess of *Pittaja ahara* and *vihara* also leads to *Amlapitha.* In this context *Chinnodbhavadi Arka in Arkaprakasha* got immense importance. It is a simple and economic compound. The results obtained in the study showed that the drug is safe and effective for internal use in the condition of Amlapitha.

INTRODUCTION

According to researches 70% of the world population was affected with *Amlapitha*. In the normal state our body is slightly alkaline in nature. Intake of *virudhahara, vihara*, emotional problems, untimely food, alcoholism and smoking will increase the production of acids and it leads to the condition hyperacidity[1]. Hyperacidity is commonly known as heart burn characterized with the symptoms like burning pain in the chest. Another common cause for the disease is the excess use of gastric irritating drugs. It has been estimated that 40% of adverse drug reactions affect the gastrointestinal tract, which highlights the contribution of drug in the manifestation of this painful condition[2]. Drugs such as antacids, pro kinetics and gastric anti-secretary agents, etc. are available in modern science, which can effectively treat this condition. But at the same time they are capable of producing many complications too.

Ayurveda the well-developed eternal medical system which contains time tested combinations of herbal and herbo-mineral drugs are potent enough to manage all types of major and minor ailments affecting body and mind. The maintenance of Agni or digestive fire in its normal state is the sole reason for our health. Peptic ulcer disorders hence need prime attention in its early stage.

In this context *Chinnodbhavadi Arka* mentioned in the *Panchama sathaka* of *Arkaprakasha*[3] got more importance because it is a simple and economical compound. Most of the Ayurveda medicines are time tested still analytical study will helpful for the safety administration. This is an attempt to standardize *Chinnodbhavadi Arka* analytically. The study mainly includes organoleptic characters, microbial contamination, HPTLC, GCMS, Test for heavy metals and test for pesticides. The combination after careful evaluation using various analytical studies revealed that it is safe enough to use internally for peptic ulcer condition.

METHODOLOGY

Ingredients:

	Sanskrit Name	Botanical name	Part used	Proportion
1	Chinnodbhava	*Tinospora cordifolia*	Stem	Equal
2	Nimba	*Azadiracta indica*	leaf	Equal
3	Patola	*Trichosanthes cucumerina*	leaf	Equal

Method of preparation:

All the ingredients mentioned in the combination was collected and properly identified from the reputed institution. It was weighed properly for its required quantity washed, dried and are made in to coarse powder (pass through sieve number 44). To the coarse powder add 10 times of water, soaked well and kept it for one day. Next day the well soaked drug is transferred to the distillation apparatus[4]. The mixture is continuously heated till 60% of the distillate is collected. First and last portion of distillate will be discarded and remaining portion is used after proper mixing and cooling, and is stored in air tight bottle.

Analytical study:

1. Organoleptic characters:

Organoleptic characters of arka were identified by using sensory characters like eye, nose and tongue and were documented[5].

2. Clarity test:

Visual inspection: Performed under a good light, baffled against reflection into the eye and viewed against black and white background with twisting action.

3. Volatile matter:

10 ml of arka was extracted 2 times with 20 ml *n*-hexane. Hexane soluble portion was taken in a pre-weighed china dish and evaporated to room temperature. The weight difference was noted and calculate the volatile matter.

4. Specific gravity:

Specific gravity bottle was cleaned by shaking with acetone and then with ether. Dried the bottle and noted the weight. The arka was cooled to room temperature. The specific gravity bottle was filled with the test liquid carefully, the stopper was inserted and removed the surplus liquid. Weight difference was noted. Repeated the procedure using distilled water in place of sample solution.

5. Refractive index:

A drop of water was placed on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre. Refractive index of the test sample was measured at 29˚C.

6. Determination of pH:

One ml of Arka was taken and make up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

7. Viscosity:

The arka was filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the formula:

$$\eta_1 = \frac{\rho_1 t_1}{\rho_2 t_2} \times \eta_2$$

η_1 – Viscosity of sample

η_2 - Viscosity of water

t_1 and t_2- Time taken for the sample and water to pass the meniscus

ρ_1 and ρ_2 – Density of sample and water

8. Total Acidity: .

Take 1 gm of the sample in a suitable titration flask & Dissolved in 75ml of CO_2 free water.Mix throughly,titrate against std NaOH solution using 4-6 drops of phenolphthalein indicator till the pink colour persists for 10 sec.

$$\% \text{ Acidity} = \frac{0.23 \times V}{M}$$

V=Corrected volume of 0.05 NaOH used

M=Weight in grams of the sample taken for the test.

9. HPTLC:

10ml of Sample of arka was extracted with 20.0ml of *n-Hexane*. 4, 8 and 12µl of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (8.0: 2.0). The developed plates were visualized short UV, long UV and then derivatised with vanillin sulphuric acid, observed under white light and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded.

10. Microbial Contamination:

Methodology: Preparation of casein Soya bean Digest Agar medium (CSDAM), Casein peptone (15g), soya peptone (5g) and sodium chloride (5g) were taken and dissolved in 990 ml distilled water and pH was adjusted to 7.3±0.2 and make up the volume to 1000ml. Finally add 15 g agar to the media and autoclaved at 121^{o}c for 20 minutes.

11. Test for Heavy Metals:

Test method adopted was STH/SOP/ICPMS/01 and STH/SOP/GCMSMS/01.

12. Test for pesticides:

Test method adopted was STH/SOP/GCMSMS/01.

13. GC-MS Study:

Preparation of Sample**:** 100µL sample was reconstituted in 0.5mL of ethanol, filtered through a syringe filter (Nylon 13 mm 0.2um) into vials and injected to GCMS.

GC-MS Analysis: Analysis was performed by injecting 2 μL of the sample with a split ratio of 5:1. Helium gas (99.9995%) was used as the carrier gas at a flow rate of 1 mL/min. The analysis was performed in the EI (electron impact) mode with 70 eV of ionization energy.

The injector temperature was maintained at 280°C (constant). The column oven temperature program is

Oven	Rate °C/min	Value °C/min	Hold Time
Initial		50	10
Ramp 1	10	100	0
Ramp 2	7	150	0
Ramp 3	5	280	15

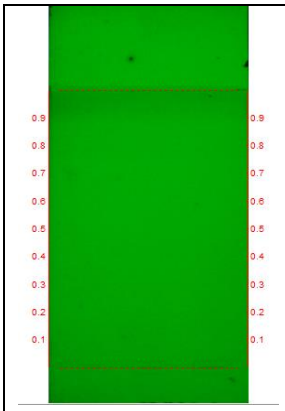
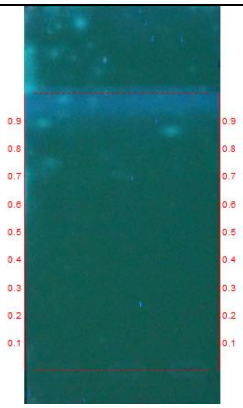
The compounds were identified after comparing the spectral configurations obtained with that of available mass spectral database (NIST -08 SPECTRAL DATA)

RESULTS:

Sl no.	**Parameter**	***Results n = 3% w/w*** ***Chinnodbhavadi arka***
1.	Colour	Colourless
	Odour	Strong, Characteristic
	Taste	Bland
2.	Clarity test	Clear solution (Devoid of particles)
3.	Volatile matter (%)	0.017
4.	Specific gravity	0.9964
5.	Refractive index	1.33167
6.	pH	7.0
7.	Viscosity	0.9660
8.	Total acidity	0.018

HPTLC Study:

HPTLC Photo documentation of Hexane fraction of *Chinnodbhavadi arka*

		
Short UV	**Long UV**	**After derivatisation**

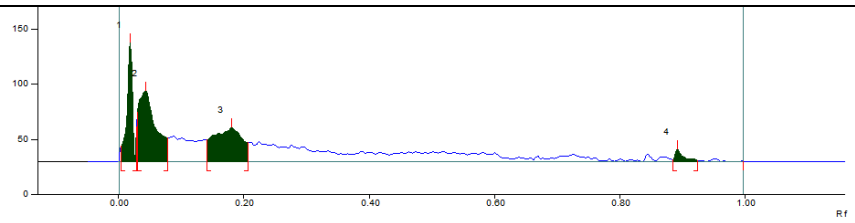
Track 1: ***Chinnodbhavadi arka***- 4µl

Track 2: ***Chinnodbhavadi arka***- 8µl

Track 3: ***Chinnodbhavadi arka***- 12µl

Solvent system-Toluene: Ethyl acetate (8.0: 2.0)

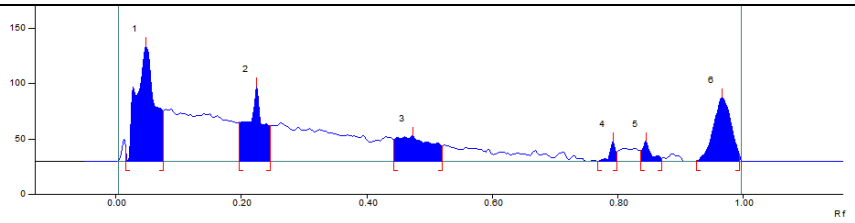
Densitometric scan of the sample of *Chinnodbhavadi arka*



Track 3, ID: Chinnodhbhavadi arka

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	13.9 AU	0.02 Rf	108.0 AU	50.09 %	0.03 Rf	31.7 AU	645.7 AU	21.33 %
2	0.03 Rf	44.8 AU	0.04 Rf	64.4 AU	29.87 %	0.08 Rf	21.2 AU	1260.7 AU	41.65 %
3	0.14 Rf	19.6 AU	0.18 Rf	31.3 AU	14.54 %	0.21 Rf	17.5 AU	1005.1 AU	33.21 %
4	0.89 Rf	1.7 AU	0.89 Rf	11.9 AU	5.50 %	0.93 Rf	1.0 AU	115.3 AU	3.81 %

Fig 2a. At 254nm



Track 3, ID: Chinnodhbhavadi arka

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	12.4 AU	0.05 Rf	103.9 AU	36.07 %	0.08 Rf	45.8 AU	2217.1 AU	37.95 %
2	0.20 Rf	35.2 AU	0.22 Rf	67.4 AU	23.38 %	0.25 Rf	32.0 AU	1246.1 AU	21.33 %
3	0.44 Rf	19.6 AU	0.47 Rf	23.0 AU	7.97 %	0.52 Rf	13.7 AU	916.1 AU	15.68 %
4	0.77 Rf	0.0 AU	0.79 Rf	17.8 AU	6.19 %	0.80 Rf	8.2 AU	115.5 AU	1.98 %
5	0.84 Rf	10.0 AU	0.85 Rf	18.4 AU	6.38 %	0.87 Rf	2.7 AU	185.4 AU	3.17 %
6	0.93 Rf	0.6 AU	0.97 Rf	57.7 AU	20.02 %	1.00 Rf	5.7 AU	1161.2 AU	19.88 %

Fig 2b. At 366nm

Test Microbial contamination:

Sl no.	Dilution	Number of colonies (NOC)			CFU/ml
1	Direct	02	02	02	02

The study shows 2 CFU (colony forming units), is within the permissible limit and will not produce any ill effect by the conception.

Test for Heavy Metals:

TEST PARAMETER	UNITS	RESULTS	TEST METHOD
Arsenic	ug/kg	<0.01	STH/SOP/ICPMS/01
Lead	ug/kg	<0.01	
Cadmium	ug/kg	<0.01	
Mercury	ug/kg	<0.01	
2,4-DichlorophenoxyAcetic Acid	ug/kg	<0.01	STH/SOP/GCMSMS/01
Aldicarb	ug/kg	<0.01	
Aldrin and Dieldrin(Aldrin and Dieldrin combined expressed as Dieldrin)	ug/kg	<0.01	
Ametyrn	ug/kg	<0.01	
Atrazine	ug/kg	<0.01	
Bifenthrin	ug/kg	<0.01	
Captafol	ug/kg	<0.01	
Carbofuran	ug/kg	<0.01	
Carbofuran(Sum of carbofuran and3 hydroxy carbofuran expressed as Carbofuran)	ug/kg	<0.01	
Chlorantraniliprole	ug/kg	<0.01	
Chlordane(sum of cix and trans Chlordane)	ug/kg	<0.01	
Chlothianidin	ug/kg	<0.01	
Thiazolymethylguanidine(TMG)	ug/kg	<0.01	
Thiazolymethylurea(TZMU)	ug/kg	<0.01	
Methylnitroguanidin (MNG/TMG)	ug/kg	<0.01	

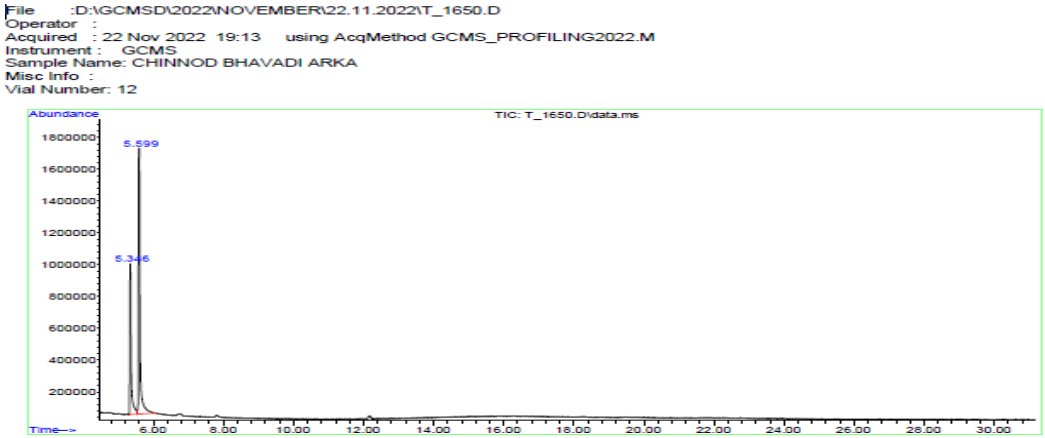
Test for the heavy metals shows that the heavy metals like arsenic, lead, cadmium etc. are less than 0.01% that means the trial drug is safe to use.

Test for Pesticides:

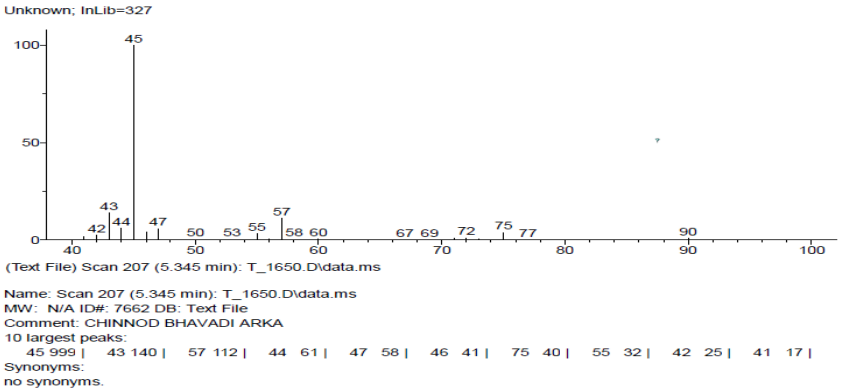
TEST PARAMETER	UNITS	RESULTS	TEST METHOD
DDT(sum ofp.p-DDT,pp-DDE andp.p-TDE(DDD)expressed as DDT	ug/kg	<0.01	
Diazinon	ug/kg	<0.01	STH/SOP/GCMSMS/01
Diuron	ug/kg	<0.01	
Endosulfan (all isomers)	ug/kg	<0.01	
Fenitrothion	ug/kg	<0.01	
Fenthion	ug/kg	<0.01	
Ferbam	ug/kg	<0.01	
Fipronil	ug/kg	<0.01	
Formothion	ug/kg	<0.01	
Halosulfuron methyl	ug/kg	<0.01	
Heptachlor	ug/kg	<0.01	
Hexazinine	ug/kg	<0.01	
Hydrogen cyanamide	ug/kg	<0.01	
Imidacloprid, Lindane (BHC gamma isomer)	ug/kg	<0.01	
Methomyl	ug/kg	<0.01	
Metribuzin	ug/kg	<0.01	
Metsulfuron Methyl	ug/kg	<0.01	
Parathion-ethyl	ug/kg	<0.01	
Phorate(sum of Phorate, its oxygen analogue and their sulphoxides and sulphones, expressed as phorate)	ug/kg	<0.01	
Phosphamidon	ug/kg	<0.01	
Simazine	ug/kg	<0.01	
Thiamethoxam	ug/kg	<0.01	

Tests for pesticides also shows that the pesticides like DDT, Diazion, Endosulfan etc. are less than 0.01% only.

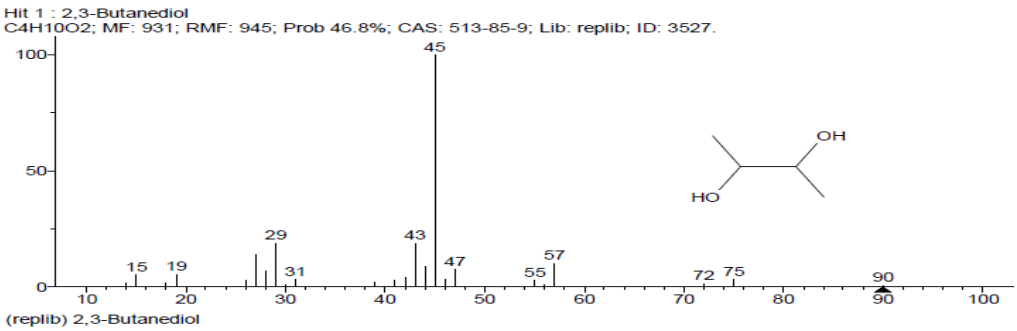
GCMS Study: Acq method GCMS Profiling 2022.M



GCMS Finding of Chinnodbhavadi Arka:



Identified content:



The gas chromatography shows 10 peaks, among them only one was identified which is similar to Butanediol present in the spectral data base. Remaining nine peaks are not

identified because the data base does not contains similar compounds. Which shows that the Arka contains not only the volatile principles but other active compounds also present in it.

DISCUSSION:

For the standardization of Chinnodbhavadi Arka, have conducted analytical study in detail. The findings are as follows.

The organoleptic characters like colour, odour and tastes are according to the standard of arka preparation and the clarity is clear. It contains 0.017% of volatile matter, its specific gravity is 0.9964 and its refractive index is 1.33167. Its pH is 7.0 only ie. the arka is neutral in nature. Its viscosity is 0.9660 and its total acidity is 0.018.

The microbial contamination study shows 2 CFU s, this is also within the permissible limit. As it is mentioned for individuals suffering from GIT disorders, it can be administered very safely.

HPTLC study was conducted to standardize the combination, but can't identify the chemical constituents as one of the limitation noticed.

Test for the heavy metals shows that the heavy metals like arsenic, lead, cadmium etc. are less than 0.01% that means the trial drug is safe to use according to standards.

The pesticides like DDT, Diazion, Endosulfan etc. are less than 0.01%only in tests for pesticides. This also shows that Chinnodbhavadi arka is safe for internal use.

The GCMS study shows 10 peaks, among them only one is identified by comparing with the spectral data base. The remaining nine contents showing peaks are not identified because it was not include in the GCMS spectral data base.

CONCLUSION:

Chinnodbhavadi Arka is a unique combination for Amlapitha which is made out of Guduchi, Nimba patra and Patola patra by distillation method. The main highlight of the analytical study is it doesn't contains any heavy metals or pesticides. The GCMS study shows ten peaks, among them one compound was identified from the combination. All the possible standardization parameters are performed in this combination for presenting it to the scientific world and popularizing it among the common people.

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