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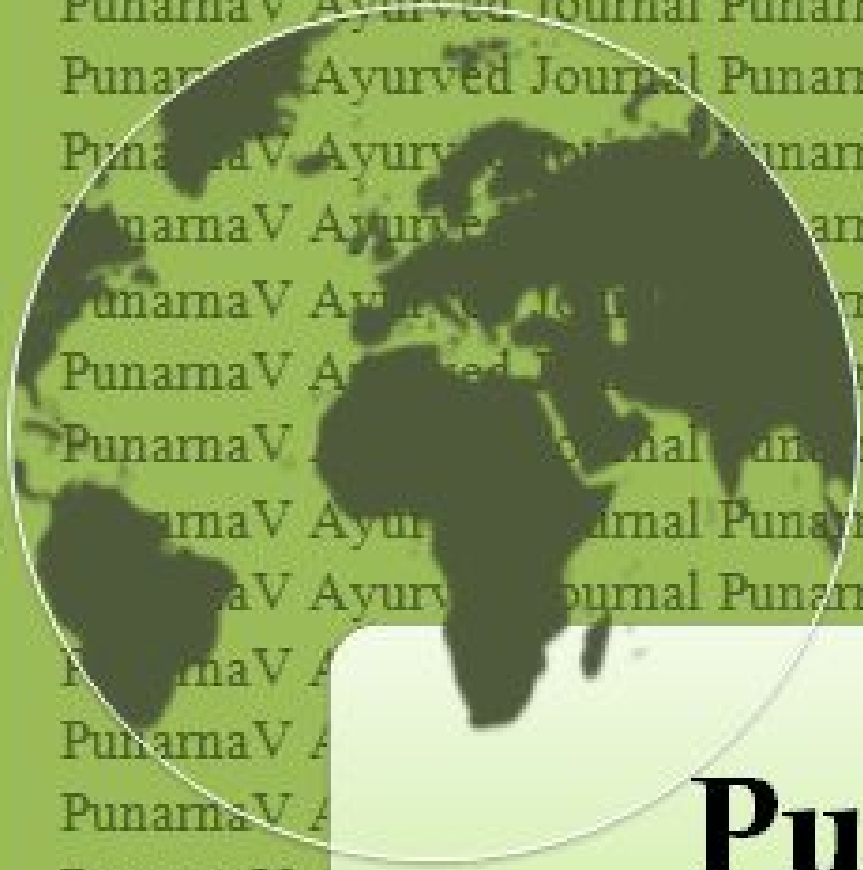

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DETERMINATION OF TERMINALIA ARJUNA CONTENT IN NAGARJUNABHRA RASA

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ABSTRACT:

Nagarjunabhra Rasa is an important formulation in Rasashastra widely used by Ayurvedic physicians for the treatment of hridshula particularly stable angina. Many renowned Ayurvedic Pharmacies and even physicians themselves manufacture it. It has two ingredients: 100 puti Abhraka Bhasma smeared and dried by triturating for seven times in Terminalia Arjuna decoction. Arjuna is a prominent and known cardio-active herb in Ayurveda proved by modern research studies. Abhraka Bhasma potentiates action of Arjuna by synergy in addition to its own therapeutic effects. In an initial trial conducted in chronic stable angina patients clinical efficacy of few market samples of Nagarjunabhra Rasa was found unsatisfactory. While these samples were analyzed in laboratory; Arjuna content in them was found missing. Availability of substandard drugs and adulteration and substitution of raw materials is a common practice in the market. This necessitated quality control tests on Ayurvedic drugs before clinical administration. Therefore to ascertain the quantity and quality of ingredients of Nagarjunabhra Rasa this unique study was carried out. In this attempt organoleptic, physico-chemical tests and preliminary phyto-chemical screening for tannins, saponins, steroids and terpenoids, flavonoids, cardiac glycosides, combined anthraquinones, reducing compounds and alkaloids on T. Arjuna bark, Nagarjunabhra Rasa and its Arjuna extractive were performed. Nagarjunabhra Rasa manufactured by a highly reputed company was taken for examination. All the samples qualified API standards. Quantity of TA content per 300 mg tablet was found to be 70.23 mg and that of Abhraka Bhasma 205.17 mg. The outcome could be used for standardisation of Nagarjunabhra Rasa in future.

KEY WORDS: *Nagarjunabhra Rasa, Physico-chemical tests, Preliminary phyto-chemical screening, Terminalia Arjuna extractive content, T. Arjuna Bark,*

INTRODUCTION

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Availability of substandard products, adulteration and substitution of raw material and non compliance of standard operative procedures in manufacturing of Ayurvedic medicines are few challenging problems faced by clinicians. WHO and Government of India have prescribed GMP guidelines for Ayurvedic drug manufacturer which are now mandatory for analysis of raw materials as well as finished products before releasing medicine into the market.[1,2] In this backdrop *Nagarjunabhra Rasa* was examined for quantitative and qualitative standards to ensure the quality, efficacy and safety of the drug.

Nagarjunabhra Rasa is a famous formulation generally prescribed by ayurvedic physicians for number of disease conditions and particularly *hridshula*[23] i.e. stable angina. This formulation is mentioned almost in every classical *Rasa Shastra* compendium under '*hridroga chikitsa*'.[4,5,6,] It has two ingredients; *Abhraka Bhasma* and Arjuna bark. It is prepared from 100 *puti Abhraka Bhasma* by triturating it with seven bhavnas of Arjuna bark's decoction for seven days.[7] This is indicated for treatment of various heart diseases, all types of pain, piles, nausea, vomiting, dyspepsia, diarrohea, loss of appetite, bleeding disorders, post-traumatic cachexia, generalized body swelling, abdominal diseases, hyperacidity, recurrent fever and many more. It improves strength, stamina and fertility.[4] It cures aneamia and jaundice and is a nervine and brain tonic.[3] It is recommended in the dose of 1-2 tablets twice daily with honey or Arjuna bark's decoction .[3,7] This is manufactured by many renowned Ayurvedic Pharmacies and even by Ayurvedic physicians themselves.

Arjuna, one of the ingredients of *Nagarjunabhra Rasa* is said to be efficacious in various heart diseases. In Ayurvedic Dravya Guna (Pharmacology) it is specified as *'hridya'* by special effect (*prabhava*).[8] Various modern research studies found Arjuna bark extract as an inotropic agent, effective in angina, hypertension and dyslipidemia.[9] It has antioxidant, anti platelet aggregation, anti-inflammatory properties and has salutary effects on coronary endothelium.[10] It is proved to be effective in chronic stable angina.[11] Arjuna's potency is further increased by *Abhraka Bhasma* through its synergistic effect according to classical references besides its own therapeutic value.[5] Previous randomised clinical assessment of poor efficacy of few samples of *Nagarjunabhra Rasa* in the treatment of hridshula led to need of determination of its contents. In this unique study organoleptic,

physico-chemical and phyto-chemical analysis were carried out on Terminalia Arjuna Bark along with *Nagarjunabhra Rasa* and its Arjuna extractive isolate. Both ingredients of *Nagarjunabhra Rasa* were quantified. For this study *Nagarjunabhra Rasa* manufactured by a reputed pharmacy was selected. Quantity of *Abhraka Bhasma* was calculated by deducting weight of Arjuna content from total weight of *Nagarjunabhra Rasa* tablet. No test of identification for *Abhraka Bhasma* was to carry out in this study. It was taken for granted as the compound was manufactured by a highly reputed company; hence relied.

AIMS AND OBJECTIVES

Aim of the study was to determine the quantity and quality of ingredient *Arjuna* content in *Nagarjunabhra Rasa* with an objective to develop quality control standards for the formulation.

MATERIALS

Nagarjunabhra Rasa tablets manufactured by a highly standard company were procured from local grocer. Crude Terminalia Arjuna Bark purchased from *Khari Bawali* market Delhi. The material was identified and authenticated by senior chemist working in Naresh Hospital Pharmacy Lab situated at Jhajjar (Haryana)-Delhi NCR; where all the tests were conducted.

METHODS

Terminalia Arjuna Dry Extract from *Nagarjunabhra Rasa*:[12]

Sample of *Nagarjunabhra Rasa* 20 grams dissolved in 200 ml of demineralised water; stirred for 6 hours and then kept still for 18 hours. Thereafter supernatant water decanted and centrifuged at 2000 rpm for 20 minutes. Thus obtained clean water extractive was dried in a porcelain china dish in hot air oven at 105^0 temperature. Kept in desiccator and weighed.

Terminalia Arjuna Dry Extract from Bark

1 Kg of T. Arjuna Bark cleaned and dried in sun; coarsely powdered and soaked in 16 liters of DM water for 24 hours; thereafter boiled on gentle heat till reduced to ¼ i.e. 4 liters.[13] Filtered through fine muslin cloth and kept still for next day. Supernatant clean water decanted and separated in a steel cauldron. It was dried further on a steam bath until brown, lustrous, crispy, soft dry extract is obtained.[14]

Physico-chemical Parameters

Physico-chemical parameters were determined according to methods described in Ayurvedic Pharmacopoeia of India and WHO guidelines for herbal medicines.[15, 16]

Moisture Content (Loss on Drying)

10 gm powdered material from each sample placed in a tarred evaporating dish and dried to a constant weight in an oven at 105°C. Loss of weight of air dried drug was calculated in percentage.

Total Ash

2 gm each of all samples incinerated in a silica dish at a temperature not exceeding 450° until free from carbon; cooled and

weighed till a constant weight obtained. Percentage of ash was calculated with reference to air dried drug.

Acid- Insoluble Ash

The obtained total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; filtered and the insoluble matter was collected in an ash-less filter paper; washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water Soluble Ash

The total ash was boiled for 5 minutes with 25 ml of water; insoluble matter was collected on a ash-less filter paper; washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°; subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the air dried drug.

Alcohol Soluble Extractive

5 grams of accurately weighed each sample was taken in a closed conical flask with 100 ml of 90% alcohol for 24 hours; shaking frequently during 6 hours and allowed to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent; evaporated 25 ml of the filtrate to dryness in a china dish at 105° to constant weight and weighed. Percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Water Soluble Extractive

5 gram of accurately weighed each sample was taken in a closed conical flask with 100 ml of chloroform water shaking constantly for 6 hours and kept to stand for 18 hours and then filtered carefully; 25 ml of filtrate evaporated to dryness at 105° to constant weight. Weight of the extractive was taken and percentage was calculated.

Preliminary Phyto-chemical screening

Tests were conducted on dry powders of specimens using standard procedures to identify the chemical constituents as described by Sengupta et al[17]; Ajayi I.A. et a1[18]; Venkata SSN Kantamreddy[19], Amol A Dambal[20] and Vidita V. Bhargawa et al.[24]

Test for Tannins

500 mg of each sample powder was boiled with 10 ml DW for 5 minutes in water bath and filtered. 1 ml of filtrate was diluted with 5 ml DW and 2-3 drops of 10% ferric chloride were added. A bluish-black colour change and precipitates were observed indicating the presence of tannins.

Test for Saponins

1 gram of powdered sample was boiled in 10 ml of DW in a bottle bath and filtered. Filtrate was diluted with 5 ml of DW and shaken vigorously for stable persistent froth. 2 drops of olive oil were added to the solution and further shaken for few minutes to form emulsion with no froths indicating presence of saponins.

Test for Terpenoids and Steroids

500 mg of each extract dissolved in 5 ml of chloroform separately by shaking for five minutes and filtered through filter paper. To the filtrate 1 ml of acetic acid was added and then ½ ml of concentrated sulfuric acid was run down the sides of the test tube. Appearance of pink colour ring at the

interface indicated the presence of terpenoids. Appearance of violet to blue colour in combination later indicated the presence of steroids with terpenoids.

Test for Flavonoids

500 mg of crude sample was dissolved in 5 ml of ethanol by shaking vigorously for 5 minutes the filtered through filter paper. 1 ml of this ethanolic extract was treated with 4 pieces of magnesium turnings and ½ ml of concentrated hydrochloric acid. Development of pink or magenta-red colour indicated the presence of flavonoids.

Test for Cardiac-glycosides

5 ml of distilled water extract from each sample was treated with 2 ml of glacial acetic acid. Two drops of ferric chloride solution added in it. 1 ml of concentrated sulfuric acid was mixed in it carefully through the test tube walls. Appearance of reddish brown ring at the interface of liquids indicated the presence of de-oxysugars.

Tests for Combined Anthraquinones

500 mg of powdered sample of each specimen was boiled with 5 ml of 10% dilute hydrochloric acid for 5 minutes in bottle water bath and filtered. The cooled filtrate was partitioned with equal volume of chloroform. The chloroform layer was separated with a clean pipette carefully and transferred into a separate test tube. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken kept to rest. The separated aqueous layer showed delicate rose-pink colour indicating the presence of an anthraquinones.

Testing for free Anthraquinones

500 mg of dry powder of samples separately dissolved in 5 ml of chloroform by shaking vigorously and then filtered. The filtrate was then mixed with equal volume of 10% ammonia solution. No presence of pink colour in aqueous layer suggestive of absence of free anthraquinones.

Test for alkaloids

Powdered sample in 500 mg of each was separately boiled with 5 ml of 10% HCL on a water bottle bath and filtered. 10% ammonia solution added to adjust the pH of the filtrate to 6-7. Small quantity of Mayer's reagent added and observed for turbidity or precipitates which were taken as evidence of presence of alkaloids in the extracts.

Tests for Reducing Sugars

500 mg powder of each sample was boiled separately with 5 ml distilled water for 5 minutes then filtered. On cooling filtrates were made alkaline with 10% sodium hydroxide. The resulting solution was boiled with an equal volume of Benedict's reagent solution on a water bath. Formation of brick red precipitates represented the presence of reducing sugars.

RESULTS AND DISCUSSION

Guidelines for quantitative and qualitative standards were followed during extract preparation and various tests. Physico-chemical analysis and preliminary phyto-

chemical screening were performed using standard laboratory techniques for determinations.[12, 15, 16, 22]

The study showed from table no. I that TAE quantity obtained from NAR was 23.41% and that of Abhraka Bhasma was 68.39 %. Thus, one approximate 300 mg tablet of NAR was containing 70.23 mg of TAE which seems though very less in quantity with comparison to modern effective therapeutic dose of the extract which is 250-500 mg per dose.[21] Notwithstanding, it is mentioned in Rasa Shastra texts that Abhraka Bhasma augments efficacy of a drug when added with that. This could be justified through a separate study. Quantity of Abhraka Bhasma was found 205.17 mg. Organoleptic characters such as odour, colour, luster, crispy consistency and touch of TAE were the same which are seen in pharmaceutically prepared dry extract of T. Arjuna bark and/or obtained by another experiment through method as explained in Rasatrangini.[14]

Table no. 2 shows the physico-chemical determinants. To identify the purity of samples, these tests were undertaken. Total ash value of TA Bark was 24% (≤ 25%). Though it was within normal limits yet on higher side in comparison with other studies available (15-16%).[17, 20, 25] Ash value of NAR was 53% with acid insoluble ash = 47.5%; which may be due to presence of Abhraka Bhasma. Total ash value of TAE-NAR was 12% showing least earthy, inorganic matter and impurities. Acid insoluble ash value of TA Bark measures 15.5% (≤ 1%); NAR = 56%; TAE-NAR = 4.5%; which were high and may be due to presence of Abhraka Bhasma silica and siliceous earth and starch, gum acacia and talcum powder excipients used for tablet manufacturing. Extractive values of TA Bark were: Alcohol soluble extractive of TA Bark was 20.8% (≥20%) and water soluble extractive 22.4% (≥20%) suggesting standards compliance. Whereas in NAR and its TAE content; alcohol and water soluble extractive values were nil & 20%; 0.2% & 85.6% respectively. This shows that the NAR compound and its TAE content have least alcohol soluble constituents but water soluble phyto-chemicals were within standard limit.

TA bark, NAR and its TA extractive material were examined for preliminary phyto-chemical screening to find out quality. Table no. 3 showed that phyto-chemical present in samples were identified as tannins, saponins, terpenoids, steroids, flavonoids, cardiac glycosides, combined anthraquinones, alkaloids and reducing sugars. It was found that TA Bark contains maximum numbers of active chemical constituents as highly positive. NAR and TAE-NAR showed weak presence of tannins, saponins and reducing sugars and no presence of steroids and flavonoids thus limiting their pharmacological activity; but presence of terpenoids, cardiac glycosides, alkaloids and combined anthraquinones shows their cardio selective property.

Table No. 1 showing weights of TAE obtained from NAR and TABark:*

Sample	TA Dry Ext. obtained from 20 gm of NAR	Wt. of Abhraka Bhasma from 20 gm of NAR	TA Dry Ext. obtained from 1 kg TA bark
I	4.32 gm	13.08 gm	70.10 gm
II	4.95 gm	13.73 gm	70.84 gm
III	4.78 gm	14.28 gm	70.41 gm
Average	4.68 gm	13.69 gm	70.41 gm
%	23.41 %	68.39 %	7.4 %

***TAE** = Terminalia Arjuna dry Extract; **NAR** = Nagarjunabhra Rasa; **TA** = Terminalia Arjuna.

Table No. 2 showing Determination of Physico-chemical Analysis of different samples:

Sample	Moisture-content	Total – Ash	Acid-insoluble Ash	Water soluble Ash	Alcohol soluble extractive	Water soluble extractive
TABark	4.2% (<8.0%)	24% (≤25%)	15.5% (≤1%)	0.5%	20.8% (≥20%)	22.4% (≥20%)
NAR	8.0% (<8.0%)	53%	47.5%	2%	Nil	20%
TAE-NAR	5.6% (<8.0%)	16%	4.5%	11%	0.2%	85.6%

***TABark**=Terminalia Arjuna Bark; **NAR**= Nagarjunabhra Rasa; **TAE-NAR**= Terminalia Arjuna dry Extract obtained from Nagarjunabhra Rasa.

*** API reference values are given in brackets with sample values.**

Table No. 3 showing results of phyto-chemical screening of different samples:

Constituent	TABark	NAR	TAE-NAR
Tannins	+	+/-	+/-
Saponins	+	+/-	+/-
Terpenoids and Steroids	+ -	+ -	+ -
Flavonoids	+	-	-
Cardiac –glycosides	+	+	+
Combined Anthraquinones	+	+/-	+
Free Anthraquinones	-	-	-
Alkaloids	+	+	+
Reducing sugars	+	+/-	+/-

+ ve = present; - ve = absent; +/- = weakly present

CONCLUSION

This study concluded that TA bark sample was contaminated in terms of acid insoluble ash values while other quantitative values were within standard limit whereas qualitative parameters showed presence of all phyto-chemicals. T.A. bark contains maximum number of chemical constituents highly positive. *Nagarjunabhra Rasa* and its TA extract content showed presence of cardio selective constituents only. Phyto-chemical concentrations in *Nagarjunabhra Rasa* and its T. Arjuna extractive content isolated appeared low in comparison to T. Arjuna bark. Quantity of T. Arjuna extract in one tablet was 70.23 mg and that of *Abhraka Bhasma* 205.17 mg. The present study could also be useful for developing standards for *Nagarjunabhra Rasa* and verification of quality before administration. Further scope of research and measurements of TA extractive through TLC and HPTLC methods remains high.

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