

Pharmacognosy and phytochemical study of *Amalakyas Rasayana*; an anti ageing Ayurvedic formulation

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ABSTRACT

Pharmacognostical study revealed that the ingredients of *Amalakyas Rasayana* are authentic. Physicochemical parameters of AR are within the accepted range. The 30.6% w/w of total ash and acid insoluble ash 3.45% w/w may be due to its iron (Fe_2O_3) content. AR contains 50% w/w water soluble extractives, which indicates that AR is easily soluble in water. AR gives 2 and 6 spots on short and long UV light respectively, which indicate that its chemical compounds are easily separable. AR contains 27.5% w/w iron (Fe_2O_3) indicating it's a good source of iron. The total phenolic content was given satisfactory linearity against Gallic acid equivalents ($R^2 = 0.998$). The DPPH ($R^2=1$ in polynomial plotting) assay showed good linearity against ascorbic acid indicating its free radical scavenging activity. EDTA & NBT assays showed sufficient linearity ($R^2 = 0.976$) against ascorbic acid indicating potent superoxide radical scavenging activity, whereas ferrous reducing power was also showed satisfactory linearity ($R^2 = 0.986$). Total tannin was estimated 2.82 % w/w. The extract was also showed ROS scavenging activities ($R^2=0.976$; $\text{EC}_{50}=77.5\mu\text{g/ml}$). These pharmacognostical, physico-chemical and analytical parameters will help to establish further standards of Ayurvedic drugs to assure their safety, purity and efficacy.

Key Words: Pharmacognosy, standardization, antioxidant, *Amalakyas Rasayana*, *in vitro*

INTRODUCTION

Ayurveda is globalizing and demand for herbal based drugs is also evolving to greater extent. When Ayurveda is commercialized, the basic fundamentals of Ayurvedic drug manufacturing modifies to an extent which could possibly affect safety, quality and efficacy of drugs. Standardization and quality control strategies are more required to provide standard and quality drugs with safety and efficacy to achieve goal of therapeutics of Ayurveda. Concept of standardization is not

new to Ayurveda and the seed of standardization conceived in Ayurveda itself. The basic facts that scattered in ancient Ayurvedic treaties mainly lay the foundation to today's standardization aspect. Charaka has given the accepted qualities of drug as; *bahuta* (to be sufficient in quantity), *yogyatva* (to be efficacious-quality), *anekavidha kalpana* (To be in various forms) and *sampat* (to be rich in pharmacodynamic properties such as *rasa*, *guna*, *vipaka*, *virya* and *prabhava*)⁽¹⁾. *Amalakyas Rasayana* (AR), poly-herbal formulation added with *lauha bashama* is said to have *vayasthapana* (anti ageing), *medhya* (enhancing intellectual power) and *balakara* (body strengthening) properties.

STANDARDIZATION ASPECTS

There are three specific stages where Standardization should be considered which are areas related to the raw material (*dravya*),

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processing procedure (*bhaisajya kalpana*) and finished product (*aushadha*). Only when the former two areas are properly monitored, the latter and essentially the most important area of finished product will be of good quality. Standardization and quality control of Ayurvedic formulations is complex as herbal drug preparation involves multi steps and incorporates many plant and mineral materials. It is therefore essential to identify specific characters of raw plant materials and their authentication. In this context, mere standardization of final product is not sufficient and pharmacognocny of individual ingredients and analytical aspect of raw plant materials and finished drug should be given priority too. This paper discusses importance of some aspects of standardization of crude drug and finished product.

Standardization of crude drug includes organoleptic, microscopic chemical and biological parameters further more *in vitro* anti-oxidant activity also observed using prescribed methods.

PLAN OF STUDY

Aims and objectives

The present study was designed to authenticate the individual crude drugs of AR, analyze finished drug using different physico-chemical parameters, find out UV absorption pattern of the drug which can be utilized for its analysis, develop the TLC finger print profile for the drug and to assess anti-oxidant & free-radical scavenging activity (*in vitro*) of AR. The pharmacognocny was included identification of crude plant materials, collection & sampling, processing, organoleptic parameters of crude drugs & powders and powder microscopy. The analytical study of finished product was carried out in which organoleptic parameters, weight variation test, loss on drying, water soluble extractives, methanol soluble extractives, total ash, acid insoluble ash, estimation of iron content (Fe_2O_3), qualitative study of different biologically active groups and thin layer chromatography were covered.

Assessment of antioxidant and free-radical scavenging activity (*In vitro*) was done in which estimation of total phenolic content, free-radical scavenging activity by DPPH, EDTA & NBT assays, hydroxyl & superoxide radical scavenging activity, estimation of total tannin and iron reducing power were covered.

MATERIALS AND METHODS

The authentic individual ingredients of AR were collected from the pharmacy of I.P.G.T. & R.A., G.A.U. Jamnagar. Pharmacognostical study was done in the department of pharmacognocny followed by preparation of the test drug was carried out in the pharmacy as per textual reference ⁽²⁾.

Chemicals: 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH, Lancaster-UK), Riboflavin (Loba-India), N.B.T (Nitro Blue Tetrazolium Chloride-Loba-India), Methanol GR (99.8%) (Loba-India), Gallic Acid (Loba-India), and EDTA (Ethylene diamine tetra acetate (Loba-India) and Pre coated plates Merck (India),

RESULTS

Pharmacognocny: The ingredients of AR were selected for detailed pharmacognostic investigation. They are *Tinospora cordifolia* Willd. Miers. ex Hook. F. & Thomas. (*amruta*), *Terminalia chebula* Retz. (*abhaya*), *Emblica officinalis* Gaertn. (*dhatri*), *Pluchea lanceolata* Oliver & Hiern. (*mukta*), *Alpenia galangal* Willd. (*sweta*), *Leptadenia reticulate* Retz. Wt. & Arn. (*jeevanti*), *Asparagus racemosus* Willd. (*atirasa*), *Centella asiatica* Linn. Urban. (*mandukaparni*), *Desmodium gangiticum* Linn. D.C. (*stira*) and *Boerhavia diffusa* Linn. (*punarnava*). Sample collection, processing and prevention were done according to the API standards. Pharmacognostical evaluation of individual drugs is mentioned as follows;

Tinospora cordifolia Willd ⁽³⁾, family: Menispermaceae

Macroscopic Characteristics: Drug consists of matured and dried pieces of stem of climber. Thickness varies from 0.6 - 5 cm in diameter,

light brown surface marked with warty protuberances, transversely smooth surface shows a radical structure with conspicuous medullary rays. Taste is bitter. Organoleptic characters: The fine powder is whitish yellow in colour, bitter and astringent in taste. Microscopic characters are starch grains, simple fibres, phloem parenchyma (polygonal elongated cells), epidermal cells, calcium oxalate crystals and vascular fragments are seen in photo-microscopy in x10.

Terminalia chebula Retz⁽⁴⁾, family: Combretaceae

Macroscopic Characteristics: Fruits are ovoid, yellowish brown in colour, 20-35 cm long and 13-25 cm wide wrinkled and ribbed longitudinally, pericarp of mature fruits is fibrous, 3-4 cm thick, non-adherent to the seed and astringent in taste. Organoleptic Characters: The fine powder is dull yellowish and bitter and astringent in taste. Microscopic characters are clerids, starch grains, and pitted vessels are seen in photo-microscopy in x10.

Emblica officinalis Gaertn⁽⁵⁾, family: Euphorbiaceae

Macroscopic Characteristics: Fresh fruits are globose, 2.5-3.5 cm in diameter, fleshy, smooth with six prominent lines; light yellowish coloured, sour and astringent taste followed by delicately sweet taste. Dried fruit is of curled pieces of pericarp of single segment of 1-2 cm long, black to gray in colour, rough cartilaginous texture and sour and astringent in taste. Organoleptic Characters: The fine powder is light brown in colour and Sour, bitter, Astringent in taste. Microscopic characters are tracheids, pitted vessels, fibres, epidermal cells and stone cells of different shapes are seen in photo-microscopy in x10.

Pluchea lanceolata Oliver & Hiern⁽⁶⁾, family: Asteraceae

Macroscopic Characteristics: The whole plant is taken for medicinal purpose. Dried leaves are annual, simple, board lanceolate, pubescent, and ash in colour. Simple small hairs are prominent near veins. Roots are slender and brown in colour. Odour is very characteristic and astringent and slightly bitter in taste. Organoleptic Characters: The

fine powder is dark brown in colour and bitter in taste. Microscopic characters are trichome with spiral vessels, fragments of parenchyma, oil globule with starch cells and rosette crystals are seen in photo-microscopy in x10.

Alpenia galanga Willd⁽⁷⁾, family: Zingiberaceae

Macroscopic Characteristics: The leaves are ovate, ovate lanceolate or oblong ovate, brown in color and sometimes exceed 50cms in length. The margin is entire, the apex is acute, the surface glandular and hairy, and the texture brittle. The venation is pinnate, the lateral veins anastomosing near the margin, and the base is decurrent. The odor is characteristic and the taste nauseous, bitter and acid. Organoleptic Characters: The fine powder is whitish yellow in colour and pungent in taste. Microscopic characters are Starch grains, epidermal cells with starch grains and pitted vessels are seen in photo-microscopy in x10.

Leptadenia reticulata Retz. Wt. & Arn⁽⁸⁾, family: Asclepiadaceae

Macroscopic Characteristics: The roots are used, yellowish in colour. Organoleptic Characters: The fine powder is whitish yellow bitter in taste. Microscopic characters are starch grains (in group), trichome (unicellular), stone cells(x10) and stone cells (x20) are seen in photo-microscopy in x10.

Asparagus recemosus Willd⁽⁹⁾, family: Liliaceae

Macroscopic Characteristics: The roots are tuberous, 10-30 cm in length and 0.1-0.5 cm thick, tapering at both ends with longitudinal wrinkles, cream coloured and sweetish in taste. Organoleptic Characters: The fine powder is whitish cream in colour and sweet in taste. Microscopic characters are stone cells (corn shaped), pitted vessels, spiral vessels with epidermal cells and stone cells (elongated) are seen in photo-microscopy in x10.

Centella asiatica Linn. Urban⁽¹⁰⁾, family: Apiaceae

Macroscopic Characteristics: The dried whole plant is a prostrate, faintly aromatic, stoloniferous perennial herb. Roots are thin,

leaves orbicular, reniform and base cordate. Organoleptic Characters: The fine powder is brown in colour and astringent and pungent in taste. Microscopic characters are spiral vessels, simple fibres, trichome (granulated), epidermal cells (elongated), crystals and starch grains (oval) are seen in photo-microscopy in x10.

Desmodium gangiticum Linn. DC⁽¹¹⁾, family: Fabaceae

Macroscopic Characteristics: Salaparni consist of dried root of plant which is a nearly erect under shrub. Lateral roots 0.1-0.8 cm thick, uniformly cylindrical with a number of branches. Roots are light yellow in colour, sweet in taste and slight mucilaginous while odour is not characteristics. **Organoleptic Characters:** The fine powder is brown in colour and sweet and bitter in taste.

Microscopic characters are trichome, cork cells, pitted vessels; rosette crystals and surface cells and vessels and starch grains and; spiral vessels and starch grains are seen in photo-microscopy in x10.

Boerhavia diffusa Linn⁽¹²⁾, family: Nyctaginaceae

Macroscopic Characteristics: Drug consists of matured dried whole plant of punarnava. Stem is greenish purple, cylindrical and minutely pubescent where as roots are fairly long, cylindrical, brown colored, no distinct odour and slightly bitter in taste. **Organoleptic Characters:** The fine powder is brownish in color and bitter and astringent in taste. **Microscopic characters** are starch grains, Vessels (pitted, elongated, pointed), cork cells with pitted vessels (brownish color) and trichome are seen in photo-microscopy in x10.

ANALYTICAL STUDY

Organoleptic characters: The fine powder of AR is smooth in texture, black in color, sour in taste and non-irritant in odour.

Physico-chemical Parameters: Table.-1 shows the data of physic chemical values

Table 1: Physicochemical parameters of Amalakayas Rasayana

Name of the Test	Values obtained
Weight Variation Test	549±0.055 mg
Loss on Drying	3.45 % w/w
Water Soluble Extractives	50 % w/w
Methanol Soluble Extractives	37.6 % w/w
Total Ash	30.6 % w/w
Acid Insoluble Ash	3.45 % w/w
Iron Content (Fe ₂ O ₃)	27.50 % w/w

observed in experiment as per method explained in textual reference^{(13), (14)}

Thin Layer Chromatography (TLC):⁽¹⁵⁾ TLC is widely used for separation of an individual compound from a mixture. Observing the

intensity one can identify the compounds and R_f value of separated spots. The principle of separation is adsorption.

The TLC study of Amalakayas Rasayana was carried out by using different conditions to evolve suitable TLC pattern.

Chromatographic Conditions

Sample preparation :	Methanol soluble extractive of Amalakayas Rasayana prepared earlier was used for TLC study.
Mobile phase :	Methanol + Chloroform +Toluene (2:4:3)
Stationary phase :	Silica gel G
Detection :	Under long U.V. (366nm) and short U.V. (254nm).
	Spraying with Anisaldehyde sulphuric acid and subsequent heating at 110°C for about 10 minutes.

By visualization under short U.V. there were 2 two spots at 0.18 and 0.67, while under long U.V. exposure 6 spots has been found at 0.083, 0.18, 0.3, 0.5, 0.66 and 0.88. Components represented by the R_f 0.18 and 0.67 were common in both light exposures. There were four additional long U.V. sensitive components which were represented by the R_f 0.083, 0.3, 0.5, & 0.88. On derivatization by spraying Anisaldehyde sulphuric acid which is indicative for the presence of the phenols, steroids, sugars and terpenes has shown 6 spots at 0.083, 0.18, 0.74, 0.3, 0.66

and 0.77 after subsequent heating at 110°C for about 10 minutes.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phyto chemical screening of AR shows the presences of Flavonoid, Alkaloides, glycosides, Tannins, Steroids, Carbohydrate and Proteins. General method was used in this qualitative test as per textual references⁽¹⁶⁾. The results are tabulated in table - 2.

Table No 2: Results of the qualitative test of Amalakayas Rasayana

Qualitative Test	Result
Alkaloids	Positive
Flavonoide	Positive
Steroid	Positive
Glycoside	Positive
Carbohydrate	Positive
Protein	Negative
Tannin	Positive
saponin	Negative

SCREENING OF ANTIOXIDANT AND FREE-RADICAL SCAVENGING ACTIVITY

Preparation of methanol extract: Five (5gm) grams of Sample is extracted with 100 ml of methanol (99.8%) in conical flask by Maceration followed by soaking it for about 12 hours. Then, the extract was filtered and methanol was evaporated on water bath and solid extract was collected. This extract used for further Antioxidant study.

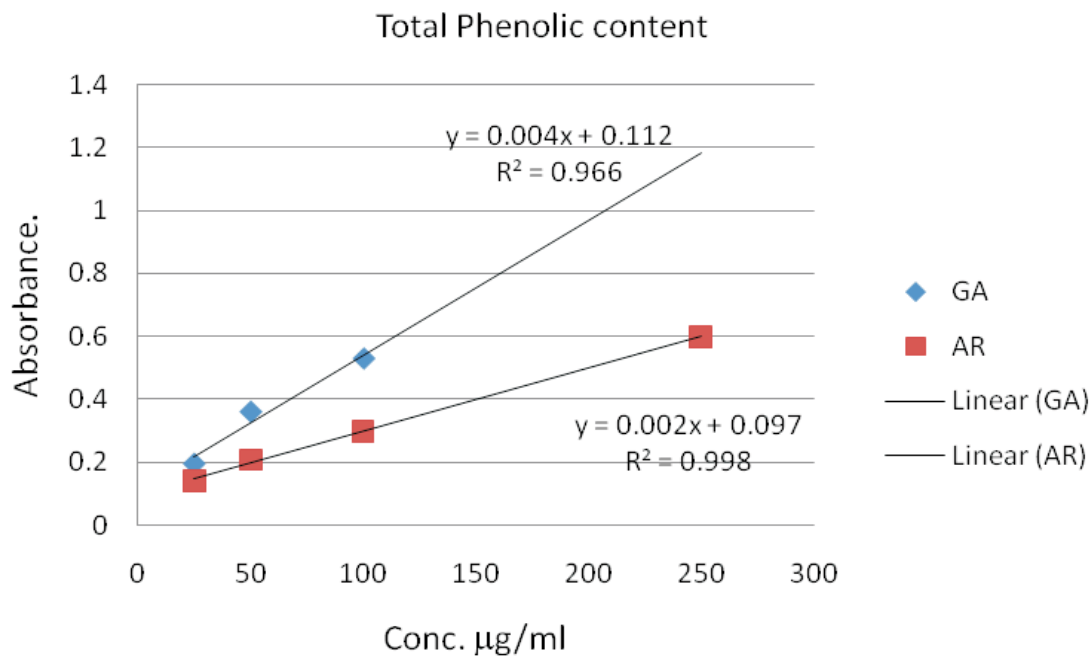
Estimation of total phenolic content⁽¹⁷⁾: The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi⁽¹⁸⁾. Briefly the method is as follows; Take a AR Extract and Prepared 1mg/1ml stock solution. From the above stock solution 0.5 to 2.5 ml of aliquots were pipetted out into 25 ml volumetric flasks. And 10 ml of water and 1.5 ml of *Folin Ciocalteu reagent* were added and kept for 5 minutes, and then 4 ml of 20% sodium carbonate solution was added and made up to 25 ml with double distilled

water. The mixture was incubated at room temperature for 30 minutes and the absorbance was recorded at 765 nm in a spectrophotometer. The same method was used for standard preparation too. Percentage of total phenolics was calculated from calibration curve of Gallic acid (50"250 ìg) plotted using the above procedure and total phenolics were expressed as % Gallic acid. The standard Gallic acid indicated 0.196, 0.36 and 0.528 absorbance (765 nm) at 25,50,100 (ìg/ml) concentration respectively, whereas methanol extract of AR showed 0.14,0.208,0.298 and 0.601 absorbance (765 nm) at 25, 50, 100 and 250 (ìg/ml) concentration respectively (figure-1).

Free-radical scavenging activity (DPPH assay)⁽¹⁹⁾:

Antiradical activity was measured by a decrease in absorbance at 516 nm of a solution of colored DPPH in methanol brought about by the sample. A stock solution of DPPH (1.3

Figure 1: Absorbance (at 765nm) of Gallic acid and methanol extract of AR in different concentrations ($\mu\text{g/ml}$)



mg/ml in methanol) was prepared such that 75 μl of it in 3 ml methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of sample extract at different concentrations was noted after 15 minutes. EC_{50} was calculated from % inhibition. A blank reading was obtained using methanol instead of the extract. Ascorbic acid was used as positive control. Decrease in absorbance is the presence of sample extract and standard at different concentrations was noted after 30 minutes. A blank reading was taken using methanol instead of extract. Ascorbic acid is used as positive standard. Absorbance was read out at 517 nm using Double Beam U.V. Spectrophotometer: 2201. Percentage Inhibition: $= (A_{\text{blank}} - A_{\text{test}}) / A_{\text{blank}} \times 100$. Absorbance for Blank = 0.946

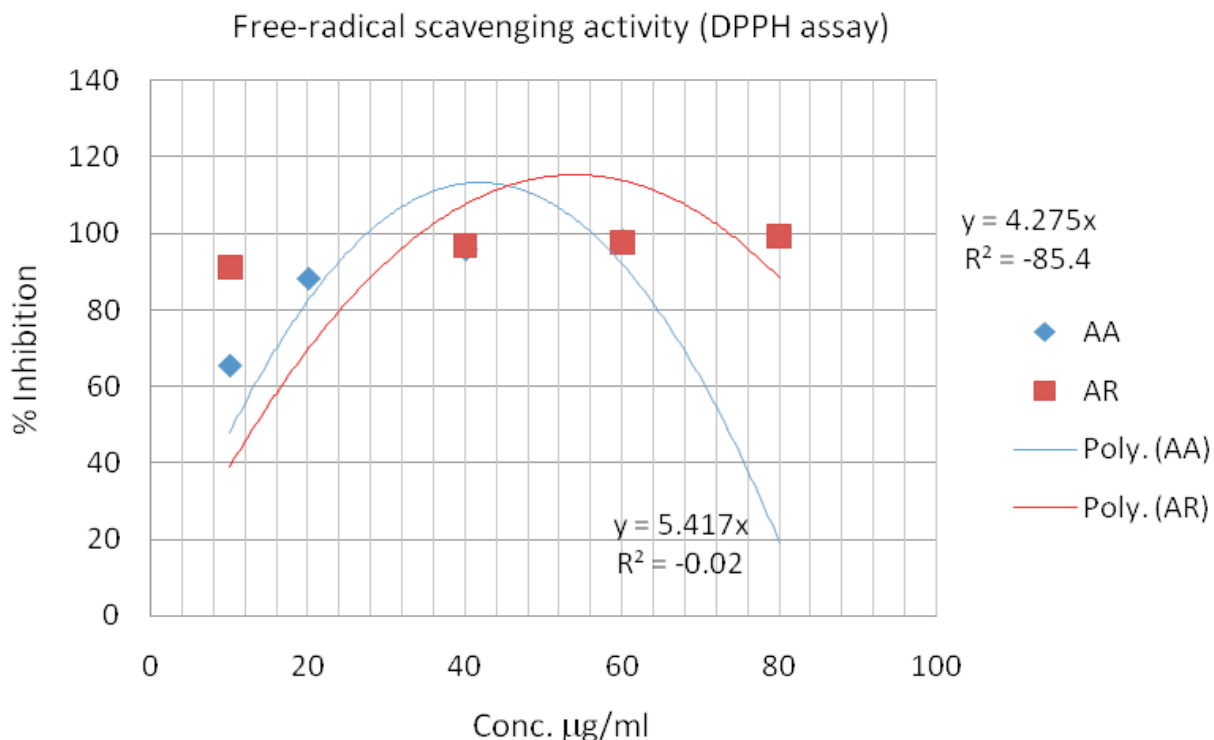
EC_{50} for Test and Standard are $\text{EC}_{50}@10-12 \mu\text{g/ml}$ with polynomial trend line extrapolated on graph. The percentage inhibition of ascorbic acid was 65.54, 88.37, 95.98 and 98.30 at 10, 20, 40 and 60 $\mu\text{g/ml}$ concentration, whereas % Inhibition of methanol extract of AR was 91.12, 96.83, 97.57

and 99.26 at 10, 40, 60 80 $\mu\text{g/ml}$ concentration respectively (figure-2).

Assay for superoxide radical scavenging activity was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. 0.5 ml of each phosphate buffer, riboflavin, EDTA, NBT and Sodium cyanide solutions were taken in 'Blank', 'Standard' & 'Test' Test-tubes. 0.5 ml of each of homogenate solution & distilled water was added to T & B tubes respectively & mixed well. After notice the initial reading all the tubes were kept under incandescent lamp for 15 min. Then absorbance was taken at 530 nm in UV Spectrophotometer and percentage of inhibition was calculated by comparing the results of control and test samples (equation). Superoxide radical scavenging activity is expressed in terms of EC_{50} . Percentage Inhibition $= (A_{\text{Blank}} - A_{\text{Test}}) / A_{\text{Blank}} \times 100$ Ascorbic acid showed 13.25, 38.95, 52.49 and 59.11 percentage inhibition at 40, 60, 80 and 100 concentration ($\mu\text{g/ml}$) at 530nm, whereas methanol extract of AR showed 34.80, 45.02, 49.45 and 60.50

Figure 2: Free radical scavenging activity, % inhibition versus concentration for Standard Ascorbic acid (AA) and AR (polynomial plotting of second degree)

Superoxide anion radical scavenging activity:



at same concentrations respectively of ascorbic acid (figure-3).

the reaction mixture indicated greater reducing power (figure-4).

DETERMINATION OF REDUCING POWER ⁽²⁰⁾

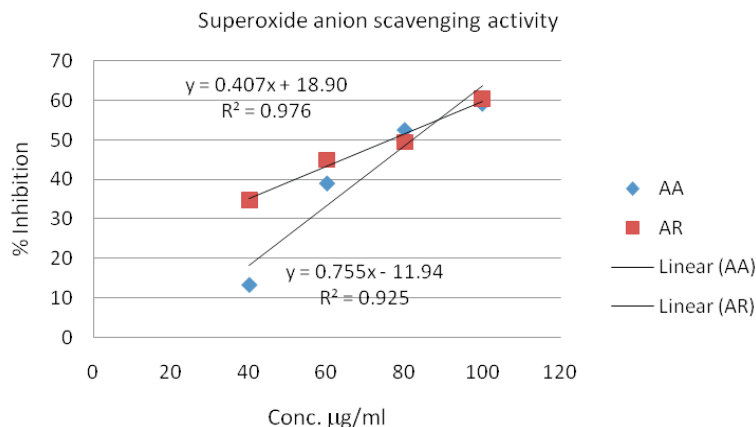
The Fe^{3+} -reducing power of the extract was determined by the method of Oyaizu with a slight modification. Different concentrations (0.0–0.4 mg/ml) of the extract (0.5 ml) were mixed with 5 ml phosphate buffer (0.2 M, pH 6.6) and 5 ml potassium hexacyanoferrate (1%), followed by incubation at 50°C in a water bath for 20 min. After incubation, 5 ml of TCA (10%) was added to terminate the reaction and centrifuged at 3000 rpm for 10 min. The upper portion of the solution (5 ml) was mixed with 5 ml distilled water, and 1 ml FeCl_3 solution (0.1%) was added and the absorbance was measured at 700 nm against an appropriate blank solution. All tests were performed six times. A higher absorbance of

As illustrated in figure 4, Fe^{3+} was transformed to Fe^{2+} in the presence of *Amalakyas Rasayana* extract. This result indicates that increase in abs of the reaction mixture indicated reducing power.

QUANTITATIVE ESTIMATION OF TANNIN ⁽²¹⁾

Accurately weighed sample (2.5g) was taken in a 250ml beaker with about 150ml of distill water and boiled on water bath for 30 min, cooled and filtered in a 250ml volumetric flask, washed and diluted up to the mark. From this stock solution 5ml was taken in a 250ml conical flask, 100ml of water and 12.5ml of indigocarmine solution was added to it. And then titrated against 0.1N KMnO_4 solution with constant stirring, color changes

Figure 3: Superoxide radical scavenging activity, % inhibition versus concentration for Standard (AA) and test drug (*Amalakyas Rasayana*)

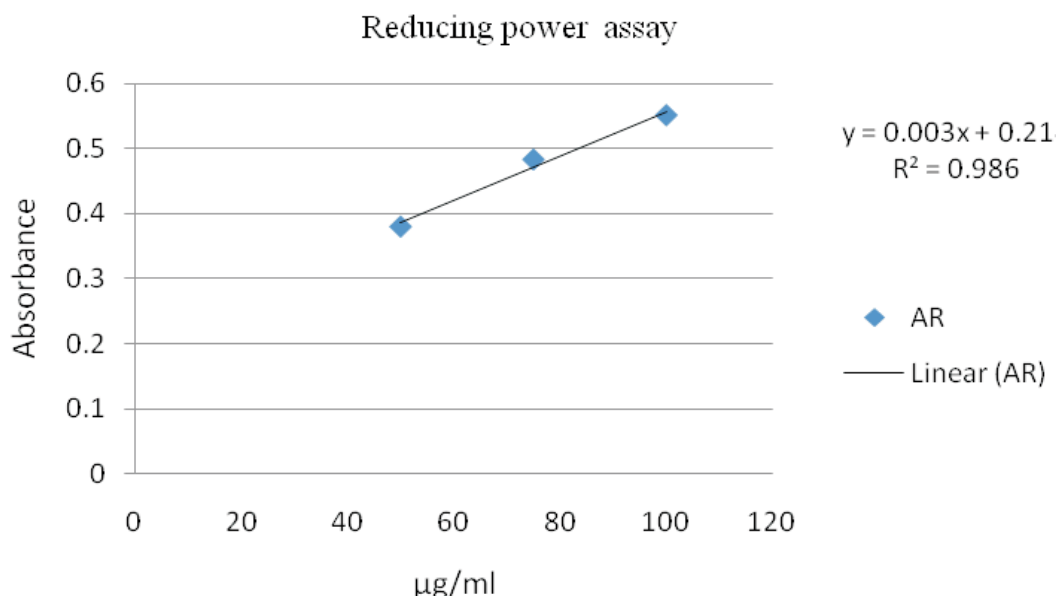


from blue to green to bright yellow (Reading A). In a beaker 50ml of stock solution, 25ml gelatin solution and 50ml of acidified sodium chloride solution was taken and 5gm of kaolin powder was added to it and shaken well for few minutes. The mixture was allowed to settle and filtered through filter paper. From this filtered solution 12.5ml was taken into a 250ml conical flask, 12.5ml of indigo carmine solution and 100ml of water was added to it and finally titrated against 0.1N KMnO_4 solution (Reading B).

DISCUSSION

At present no official standards are available for quality control of the herbal preparation. Combined, well coordinated efforts from scientific workers of different disciplines are required for this purpose. Ash value is such a parameter by which purity of drug can be measured. Amount of materials remain after ignition is the total ash, where as acid insoluble ash is of inorganic substances such as silica. Water soluble ash is ash of

Figure-4: Reducing power of methanol extract of AR



physiological material of plant itself. The sample of AR contained 30.6% w/w total ash and acid insoluble ash 3.45% w/w that may be due to its iron content. When moisture content is high, the drug is at risk of getting degraded soon and infected with microorganisms. Loss on drying of AR is 3.45% w/w, which is quite normal. Extractive values indicate amount of chemical constituents of the drug. Water soluble extractives indicate its soluble materials such as sugar, glycosides, mucilage and tannin. AR contains 50% w/w water soluble extractives, which indicates that AR is easily soluble in water, whereas methanol soluble extractive is 37.6% w/w.

$$\text{Percentage of total tannin} = \frac{A - B \times 0.0042 \times 250}{\text{Wt. of sample} \times \text{vol. taken}} \times 100 \quad (\% \text{ of tannin} = 2.82\% \text{ W/W})$$

much valued to have good effect against wear and tear associated with ageing. AR is a preparation composed of well known plants material to have rich antioxidant and immunomodulatory properties and free radical scavenging activities. The free radical scavenging activity of AR has been studied by the DPPH, EDTA and NBT assay where as total phenolic content was assessed by using Gallic acid standard and Folin Ciocalteu's reagent. Reducing power was measured by Oyaizu method and the total tannin content by the method described by Sims Stephen. All the studies acquired promising results which suggests that AR possesses good antioxidant activities. The superoxide radical scavenging activity of extract of AR is increased markedly with increasing concentrations with RSD 0.976 in comparison to RSD Ascorbic acid 0.925 shows better correlation. EC_{50} @ 77.5 $\mu\text{g/ml}$ observed from graph for AR. The results suggest that the AR extract is a more potent scavenger of superoxide radicals. Variation in DPPH assay lead focus on reducing capacity of AR, The reducing capacity of AR shows RSD = 0.986 indicating good correlation ship between readings against ferrous ion but when the drug tested against DPPH molecule linear correlation is not possible, polynomial equation of third

Weight variation is the measure of uniformity of the tablets or capsules. The capsules of AR have $549 \pm 0.005\text{mg}$ which certifies that powder within the capsules are uniform, particle sizes are equal and mixing is satisfactory. TLC uses to separation of natural compound in a mixture on TLC plate with the help of a solvent. AR gives 2 and 6 spots on short and long UV light respectively, which indicate that its chemical compounds are easily separable.

Free radicals are constantly generated and they cause extensive damage to tissues and bio-molecules in living organisms leading to various disease conditions, especially degenerative diseases, and also accelerated ageing. Ayurveda rasayana drugs are very

degree show RSD=1 for test as well as standard, from polynomial equation of second degree trend line EC_{50} was extrapolated. Thus the experiment requires further validation and robustness study for DPPH reduction assay. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. The reducing power of the extract of AR found to be superior. The results indicate that AR contains significant amounts of tannins and phenolic compounds. Both these classes of compounds have good antioxidant potential and their effect on human nutrition and health is considerable. The total phenolic content shows 0.998 R^2 (RSD) hence considered good correlation between readings but the slope values is exactly half of the standard tested (Gallic acid). It shows that the change in absorption is low with respect to concentration change that may be due to matrix effect and that may be responsible for variation in DPPH assay. Phenolic compounds are also very important plant constituents because their hydroxyl groups confer scavenging ability.

CONCLUSION

On the basis of the results obtained in the present study, the ingredients of AR are pure without adulterant and authentic plant materials. The pharmacognostical characteristics are in accordance with those are given in Ayurvedic pharmacopeia of India. AR contains alkaloids, flavonoids, steroids, tannins and phenolic compounds, cyanogenic glycosides and carbohydrates and most of them are known to be antioxidants. Methanol extract of AR is rich in phenol compounds, which may be a possible cause to possess high antioxidant and free radical scavenging activities. The observed values of physicochemical parameters such as loss on drying, water soluble extractives, methanol soluble extractives, total ash and acid insoluble ash are within acceptable normal ranges. AR also contains 27.5% w/w iron (Fe_2O_3) indicating it's a good source of iron and could be used in iron deficiency anemia. It also has reducing power, which is assumed that AR has anti oxidant properties. The *in vitro* assays indicate that this combination of plant is a source of natural antioxidant, which might be helpful in preventing the progress of numerous oxidative stresses which trigger at various age related disease and in retarding ageing as well as preventing pre-mature ageing. Though, it is evident that AR has potent antioxidant activity, further investigations are needed to isolate and identify the other compounds present in *Amalakyas Rasayana* and to authenticate therapeutic value noticed in classics..

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