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# Evaluation of Antioxidant Potential and Total Phenol Content Of Amruth Jeevan Rasayan: A Herbomineral Ayurvedic Formulation

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#### ABSTRACT

Amruth Jeevan Rasayan is comprising of the purely herbal 'avaleha' / herbal jam named Amruth Jeevan Rasayan Avaleha along with the mineral-rich Amruth Jeevan Rasayan Tablets, formulated following the principles of treating aging and age-associated diseases. Being an anti aging drug, Amruth Jeevan Rasayan must have antioxidants and free radical scavenging activity to minimize free radical-induced damage, which is a key cause of aging. The methanolic extract of Amruth Jeevan Rasayan was evaluated in vitro for total phenolic and free radical scavenging activity. The total phenolic content was measured using Folin-ciocalteu reagent against gallic acid. Free radical scavenging activity was measured by 2,2-diphenyl-1-picryl hydroxyl (DPPH) and 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) di ammonium salt (ABTS) assay. The studies showed that Amruth Jeevan Rasayan possesses antioxidant activity. The results of this study suggest that the antioxidant and free radical scavenging activity of Amruth Jeevan Rasayan may explain its Rasayan effect and justify its use as a medicine for age associated diseases.

Keywords: Antioxidant, Phytochemicals, Amruth Jeevan Rasayan, DPPH, and ABTS

#### 1. INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidizes, through xanthine oxidize activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics.<sup>[1]</sup> Reactive oxygen species (ROS), besides of their useful role in signal transudations, are also involved in the dispersion of several degenerative diseases like malignant tumors, rheumatic joint inflammation, cataracts, Parkinson's and Alzheimer's disease, hypertension, diabetes, oxidative stress, tissue damages and atherosclerosis.<sup>[2]</sup> Oxidative damage to cellular bimolecular such as lipids, proteins and DNA is thought to play a crucial role in the incidence of several chronic diseases.<sup>[3–7]</sup>



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In addition to antioxidant enzymatic system, there are non-enzymatic bimolecular and proteins in living organisms, which act as antioxidant and free radical scavengers to protect the body from such effects,. Food supplementation containing ascorbate, carotenoids, tocopherols, flavonoids and phenols play a significant role in this matter. <sup>[8,9]</sup> Flavonoids are a group of polyphenolic compounds found abundantly in the plant kingdom. Interest in the possible health benefits of flavonoids and other polyphenolic compounds has increased in recent years owing to their potent antioxidant and free-radical scavenging activities. <sup>[10–16]</sup>

These bioactive natural compounds scavenge the reactive oxygen species and prevent free radicals to cause deterioration. They have the aptitude to scavenge oxygen-nitrogen derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes and inhibiting oxidizes.<sup>[17-19]</sup> Based on such a type of incredible results, interest in exploration of bioactive compounds extracted from medicinal plants was increased in recent years to replace the use of synthetic drugs, which were restricted due to side effects. On the other hand, polyphenol, used as natural antioxidants, are gaining importance, due to their health benefits for humans, decreasing the risk of cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation.<sup>[20,21]</sup>

Ayurveda arguably is the oldest medical system in the world, providing innumerable leads to find active and therapeutically useful compounds from plants. The health promoting, disease preventing and rejuvenating approach available in the Indian systems of medicine like 'Ayurveda' is gaining greater attention and popularity in many regions of the world. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products, including medications for ethno veterinary medicine. Rasayan tantra is a unique branch of Ayurveda and the drugs mentioned in this chapter have been described to both cure disease and also promote health.<sup>[22]</sup> Rasayan medicines improve the quality of 'Rasa' (plasma) and thus strengthen or promote the health of all tissues of the body.<sup>[23]</sup>

Amruth Jeevan Rasayan is a herbomineral product, which composed of the purely herbal jam named Avaleha, along with the mineral-rich Tablets. Amruth Jeevan Rasayan Avaleha contains an ideal group of 26 unique herbs, in a base of Ghee, Honey and Sugar, whereas Amruth Jeevan Rasayan Tablets contain 6 rich herbs and 6 nutritious minerals. Both Avaleha & Tablets belong to the wonderful group of herbal formulations called 'Rasayana'. This combination capable of re-inventing, rejuvenating and strengthening the human body, resisting aliments, preserving comprehensive health, supporting convalescence (regaining of complete health after getting cured from disease) and promoting long-life by countering the debilitating effects of ageing. On this ground, because of being a combination of herbomineral known to have health promoting effects, this study was designed to provide scientific basis to the efficacy of Amruth Jeevan Rasayan mentioned in the texts. The objective of this study was to screen the phytochemicals of its ingredients and to evaluate the total phenol content and antioxidant potential / free radical scavenging activity of methanol extract of Amruth Jeevan Rasayan.

# 2. MATERIAL AND METHODS

## **2.1** Plant materials

All ingredients in Amruth Jeevan Rasayan were collected from Thodupuzha, Kerala, India in the month of May and June. The specimens were identified and voucher specimens of plants were deposited in the Herbarium in the Department of Pharmacognosy, Nagarjuna Herbal Concentrates Ltd, Kerala, and India. The different plant materials (Table 1) were cut into small pieces, dried at 40-50°C shade for one week and powdered.

#### 2.2 Preparation of extract

Extraction was performed by 10g of each plant powder/ Amruth Jeevan Rasayan Avaleha was soaked with methanol for 24 hours at room temperature. After filtration the residue was extracted twice in the same conditions. The methanol was completely evaporated at 40°C using a rotary vacuum evaporator. The yield of extract was given in the Table 1.

## 2.3 Preparation of bhasma/ Amruth Jeevan Rasayan tablet for the test

Bhasma (Calcined form of metals)/ Amruth Jeevan Rasayan tablet were collected and used for the study. Very fine powder of Bhasma were taken and suspended in methanol for the analysis.

## 2.4 Preliminary Phytochemical Analysis

The various extracts of Amruth Jeevan Rasayan and its ingredients were tested for different phytoconsituents like alkaloids, Flavonoids, Saponins, carbohydrates, Protein, Steroids, Glycosides and Tannins using standard procedures.<sup>[24]</sup>

### 2.5 Determination of Total Polyphenols

The amount of total phenolic contents in extracts was determined by using Folin-Ciocalteu procedure. In this method, the samples ( $100\mu g/ml$ ) were introduced into test tubes; 1ml of Folin-Ciocalteu reagent, 2 ml of sodium carbonate (35%) and 2ml of de-ionized water were added. The tubes were mixed and allowed to stand for 30 min. Absorbance at 765 nm was measured using Chemito 2600 UV/visible Spectrophotometer (Nasik, India). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry weight (DW). The values were obtained from three different experiments performed in duplication.

#### 2.6 In-vitro Anti-oxidant activity

# 2.6.1 DPPH radical scavenging activity

The ability of the plant extract to scavenge 1,1-diphenyl-2-picryhydrazyl (DPPH) free radicals was assessed by the standard method.<sup>[25]</sup> The stock solution of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 20, 40, 60, 80 and 100µg/ml. Diluted solutions (1ml each) were mixed with 3ml of methanolic solution of DPPH (DPPH, 0.004%). After 30 min of incubation at room temperature, the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as control. Ascorbic acid was used as standard. The experiment was carried out in triplicate. Percentage inhibition was calculated using equation (1), whilst IC50 values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values  $\pm$  standard deviation (n = 3).

(I (%) = [(A blank - A sample) / A blank] x 100

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. The extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition (%) against extract concentration. Test was performed in three different experiments with duplication. Ascorbic acid standard was used for comparison.

# 2.6.2 Determination of Antioxidant Potential (ABTS assay)

The total radical scavenging capacity based on the ability of a compound to scavenge the stable 2,2'azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical in 6 min. For the total antioxidant assay, ABTS was dissolved in de-ionized water to a 7 M concentration. The ABTS radical cation (ABTS+) was produced by reacting ABTS stock solution with a 2.45mM potassium persulfate (final concentration) and incubating the solution in the dark at room temperature for 12–16 h before use. The radical stock solution was diluted with a 5mM solution of phosphate-buffered saline (PBS; pH 7.4) to obtain a spectrophotometric absorbance value at 734nm.

Ascorbic acid standard was prepared in de-ionised water over the range 2-10µg/ml. Extracts were prepared in different concentrations (20-100µg/ml) in methanol. To 40µl of extract/standard solution, 1.96 ml of ABTS+ solution was added and the tubes were kept in dark for 6 min and read at 734 nm using Chemito 2600 UV/visible Spectrophotometer (Nasik, India). This was compared to a control where 40µl of the solvent was added to 1.96 ml of ABTS+ solution. The assay was performed in three different experiments with duplication. Antioxidant activity was expressed as the % of ABTS radical reduction. Radical scavenging activity was expressed as the inhibition percentage and was calculated as % radical scavenging activity = [(control OD - sample OD)/control OD]  $\mu$  100. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition (%) against extract concentration.

#### **3.RESULT AND DISCUSSION**

#### 3.1 Photochemical screening of ingredients of Amruth Jeevan Rasayan

Thousands of diverse natural products are synthesized by plants and many of these are involved in plant defense. The phytochemical diversity of antioxidant compounds include terpenoids, saponins, phenolics and phenyl propanoids, alkaloids, glucosinolates and also elemental sulphur, the sole inorganic compound [26]. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals ('phyto-' from Greek - *phyto* meaning 'plant') or phytoconstituents. <sup>[27, 28]</sup>

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Many of phytochemicals such as alkaloids, saponins, cabohydrats, glycosides etc were present in Withania somnifera, Zingiber officinala, Terminalia chebula, Terminalia bellirica and Eclipta alba. Almost all plants contain alkaloids. Important medicinal phytochemicals such as alkaloids, carbohydrates, flavonoids, Tannins, Glycosides, Protein, Saponins and Steroids were present in many samples. The result of the phytochemical analysis shows that the medicinal plants were rich in at least one of alkaloids, flavonoids, terpenoids, and sugars. Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources.<sup>[29]</sup> The results of preliminary phytochemical analysis are shown in Table 111.

#### 3.2 Total phenol content of Amruth Jeevan Rasayan and its ingredients

The total phenolic contents of the extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: y = 7.026x - 0.0191, r2 = 0.999). The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract (Table IV). The amount of total polyphenolics varied widely in medicinal plant materials and ranged from 84.77 to 695.4mg GAE per gram of dry weight (Table IV). A very high amount of total polyphenolic contents (695.4 mg GAE/g extract) was found in *Terminalia chebula* fruit pulp. The fruit pulp extract of *Terminalia bellerica* and *Emblica officinalis* had next highest amount of polyphenolic contents (677.01 and 592.81 mg GAE/g extract). *Holostemma adakodien, Piper nigrum, Withania somnifera, Curculigo orchioides, Glycyrrhiza glabra, Mucuna pruriens* contain more than 200 mg GAE/g extract. Earlier, a wide variation was observed on total phenolic content in different aromatic and medicinal plants were 6.80-32.10 mg gallic acid equivalents per g dry weight basis <sup>[30]</sup>. The other medicinal plants had the polyphenolic contents (84.77 mg GAE/g extract). Amruth Jeevan Rasayan Avaleha and Amruth Jeevan Rasayan Tablets contain the total phenol 93.10, 31.35 mg

GAE/g extract respectively. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores.<sup>[31]</sup>

#### 3.3 Antioxidant property

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 mm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment <sup>[32].</sup> The methanolic extracts of Amruth Jeevan Rasavan and its ingredients were found to possess concentration dependent scavenging activity on DPPH and ABTS radicals and the results were given in table IV. The mean IC50 values for DPPH can show that fruit pulp of Terminalia chebula, Terminalia bellirica and Emblica officinalis have good scavenging activity than vitamin C ie 3.82, 3.69 and 4.26 µg/ml instead of this the ascorbic acid have 8.69µg/ml. Zingiber officinale also shows a good free radical scavenging activity of 19.41µg/ml. Curculigo orchioides, Mucuna pruriens, Withania somnifera, Piper nigrum and Glycyrrhiza glabra were shows more than 100 µg/ml. Asparagus racemosus (rhizhome) having very low free radical scavenging activity as indicated by 579.29µg/ml of IC 50 value in DPPH assay. AJRA and AJRT also showed 43.46±2.54 and 42.46±2.54 µg/ml. Calcined forms of bhasmam also showed a very good radical scavenging activity, ie Sankhu bhasmam, Swarna bhasmam, Abhra bhasmam, Annabhedi sindhooram and Kanmada bhasmam showed 44.61±0.12, 44.76±0.12, 45.89±1.331, 48.49±0.85 and 45.84±0.48 µg/ml respectively. These results by ABTS assay evidence that Terminalia chebula, Terminalia bellirica and Emblica officinalis were also found to have a very good scavenging activity.

All bhasmams also shows very good IC50 values i.e below 50µg/ml for DPPH and below 100µg/ml in ATS. Other ingredients like ghee, sugar and honey were also showed god IC 50 values. Amruth Jeevan Rasayan Tablets and Amruth Jeevan Rasayan Avaleha also had the IC 50 values of 40.03 and 38.85µg/ml.

Many synthetic drugs are said to protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines. Recently, many natural antioxidants have been isolated from different plant materials. Amruth Jeevan Rasayan is a formulation developed by R&D of NHCL, which is said to have properties to work against age-related deterioration in the body. Amruth Jeevan Rasayan is a herbomineral preparation composed of *A. racemosus*,<sup>[33]</sup> *B. diffusa*,<sup>[34]</sup> *T. chebula*,<sup>[35]</sup> *T. cordifolia*, <sup>[36]</sup> *E. officinalis*, <sup>[37]</sup>, *W. somniferra* <sup>[38]</sup> and Swarna bhasma,<sup>[39]</sup> ie, are all well known to exert immunomodulator, antioxidant, and free radical scavenging activities, as individually identified plants. When all the ingredients are made into a single formulation, their pharmacodynamic actions may differ from their individual effects. The hypothesis of this study was that all the ingredients may act synergistically to have potent phytochemical combination by which more antioxidant activity could be shown than the individual ingredients had.

# 4. Conclusion

From the results obtained in this study, It can be concluded that methanol extract of Amruth Jeevan Rasayan and its ingredients, which composed of large amounts of phenol compounds, exhibits high antioxidant and free radical scavenging activities. *In vitro* assays indicate that both Amruth Jeevan Rasayan Avaleha & Tablets belong to the wonderful group of herbal formulations called 'Rasayana', which are capable of completely re-inventing, rejuvenating and strengthening the human body, resisting aliments, preserving comprehensive health, supporting convalescence (regaining of complete health after getting cured from disease) and promoting long-life by countering the debilitating effects of ageing. They help the body cells to shell out unnecessary chemicals accumulated as a byproduct of natural ageing and oxidation which might be helpful in preventing and preventing premature aging. However, the components responsible for the antioxidant activity are currently unclear.

Therefore, further investigation is needed to isolate and identify the antioxidant compounds present in the extract of Amruth Jeevan Rasayan.

Reference

- 1. Sreedhar V, Ravindra Nath LK, Madana Gopal N, Sanjith Nath M. *In-vitro* antioxidant activity and free radical scavenging potential of roots of *Vitex trifoliate* Res J Pharm, Biol and Che Sci 2010; 1(4):1036-1044.
- 2. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. J Biochem 1984; 219:1–14.
- 3. Simonian NA, Coyle JT. Oxidative stress in neurodegenerative diseases. Annu Rev Pharmacol Toxicol 1996; 36: 83–106.
- 4. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. J Hypertens 2000;18:655–673.
- 5. Vant Veer P, Jansen MC, Klerk M, Kok FJ. Fruits and vegetables in the prevention of cancer and cardiovascular disease. Public Health Nutr 2000;3:103–107.
- 6. Bokov A, Chaudhuri A, Richardson A. The role of oxidative damage and stress in aging. Mech Ageing Dev 2004;125:811–826.
- 7. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. *Arterioscler*. Thromb Vasc Biol 2005; 25:29–38.
- 8. Fritz KL, Seppanen CM, Kurzer MS, Csallany AS: The in vivo antioxidant activity of soybean isoflavone in human subjects. Nut Res 2003;23:479–487.
- 9. Patricia I, Oteiza AG, Erlejman S, Verstraeten V, Keen CL, Fraga CS. Flavonoid membrane interactions: A protective role of flavonoids at the membrane surface. Clin Develop Immunol 2005;12:23–25.
- 10. Bravo L. Polyphenol: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998;56:317–333.
- 11. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002;13:572–584.
- 12. Pier-Giorgio P. Flavonoids as antioxidants. J Nat Prod 2000;63:1035–1042.
- 13. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenoilc compounds. Trends Plant Sci 1997;2:152–159.
- 14. Sealbert A, Johnson J, Saltmarsh M. Polyphenols: Antioxidants and beyond. Am. J Clin Nutr 2005;81:2155–2175.
- 15. Ross JA, Kasum CM. Dietary flavonoids: Bioavailability, metabolic effects, and safety. Ann Rev Nutr 2002;22:19–34.
- 16. Rice-Evans CA, Miller N. Maxwell SJ. Prospects for the use of antioxidant therapies. Drugs1995;49:345.
- 17. Ames SN, Shigrenaga MK, Hagen TM. Oxidant, antioxidant and degradative disease of aging. Proc Nat Acad Sci USA 1993;90:7915–7922.
- 18. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxides anions. Biochem Pharmacol 1988;37:837-841.
- 19. Ardestani A, Yazdanparast R. Antioxidant and free radical scavenging potential of Achillea santolina extracts. Food Chem 2007;104:21–29.
- 20. Bingham M, Gibson G, Gottstein N, Pascual-Teresa SD, Minihane AM, Rimbach G: Gut metabolism and cardio protective effects of dietary isoflavones. Current Top Nut Res 2003;1:31–48.
- 21. Silva BM, Andrade PB, Valentãoo P, Ferreres F, Seabra RM, Ferreira MA: Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. J Agric Food Chem 2004;52:4705–4712.

- 22. Shasthri K, Chaturvedi G. Charaka Samhita with Vidyotini Commentoty. Varanasi: Chaukambha Bharati Academy 2007;pp.35–6.
- 23. Govindarajan R, Vijayakumar M, Pushpangadan P. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. Journal of Ethnopharmacology 2005;99:165–178.
- 24. Baxi AJ, Shukla VJ, Bhatt UB. Guideli Monograph on Testing of Some Ayurvedic Formulations. Jamnagar: Gujarat Ayuurveda University; 2002.p.5-15.
- 25. Cooper RM, Resende MLV, Flood J, Rowan MJ, Beale MH, Potter U. Detection and cellular localization of elemental sulphur in disease-resistant genotypes of theobroma calcao. *Nature* 1996;379:159-162.
- 26. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of Action. Journal of Nutrition 2004;134: (12 Suppl):3479S-3485S.
- 27. Abo KA, Ogunley, VO & Ashidi JS. Antimicrobial poteintial of *Spondias mombin, Croton zambesicus* and *Zygotritonia crocea. Journal of Pharmacological Research*. 1991;5(13):494-497.
- 28. Nweze EL, Okafor JL & Njoku O. Antimicrobial Activityies of Methanolic extracts of *Trume* guineesis (Scchumn and Thorn) and *Morinda lucinda* used in Nigerian Herbal Medicinal practice. *Journal of Biological Research and Biotechnology* 2004;2(1): 34-46.
- 29. Doughari JH & Obidah JS. Antibacterial potentials of stem bark extracts of *Leptadenia lancifoli*.against some pathogenic bacteria. *Pharmacologyonline* 2008;3: 172-180.
- 30. Bajpai M, Pande A, Tewari SK and Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. In J Food Scnces and Nutrition 2005;56(4): 287-291.
- 31. Vaya J, Belinky PA, Aviram M. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity toward LDL oxidation. Free Radical Biol. Med 1997;23(2):302-313.
- 32. Eyob S, Martinsen BK, Tsegaye A. Antioxidant and antimicrobial activities of extract and essential oil of korarima (Aframomum corrorima (Braun) P.C.M. Jansen). African Journal of Biotechnology 2008;7(15):2585-2592.
- 33. Bhatnagar M, Sisodia SS, Bhatnagar R. Antiulcer and antioxidant activity of Asparagus racemosus Willd.and Withania somnifera Dunal. in rats. Ann N Y Acad Sci 2005;1056:261–78.
- 34. Sumanth M, Mustafa SS. Anti-stress, adoptogenic and immunopotentiating activity roots of Boerhaavia diffusa in mice. Int J Pharmacol 2007;3:416–20.
- 35. Raju D, Illango K, Chitra V, Ashish K. Evaluation of anti-ulcer activity of methanolic extract of Terminalia chebula in experimental rats. J Pharm Sci Res 2009;1:101–7.
- 36. Sheth MD, Rege NN, Dahanukar SA. Effect of Tinospora cordifolia on gastrointestinal dysmotility induced by chronic, unpredictable wrap-restraint. Indian J Pharmacol 2001;33:135.
- 37. Mehrotra V, Mehrotra S, Kirar V, Radhey S, Misra K, Srivastava AK, Nandi SP. Antioxidant and antimicrobial activities of aqueous extract of Withania somnifera against methicillin-resistant Staphylococcus aureus, Journal of Microbiology and Biotechnology Research 2011;1(1), 40-45.
- 38. Karimi E, Oskoueian E, Hendra R, Jaafar HZ. Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity. 2010;15(9):6244-56.
- 39. Mahapatra Arun Kumar, Nisha Kumari Ojha\$, Abhimanyu Kumar. Rationality of Swarna Prashan in Pediatric Practice. In J Ayurvedic And Herbal Medicine 2013;3:3, 1191:12003

Table I:- Malayalam name bota	nical name, parts used an	l of yield of extract	of ingredients of Amrut
Jeevan Rasayan Avaleha			

Malayalam Name	Botanical Name	Parts used	Yield of extract in %		
Adapathiyan	Holostemma adakodien	Rhizhome	8.81		
Amukkuram	Withania somnifera	Root	7.4		
Chukku	Zingiber officinala	Rhizhome	10.47		
Iarttimadhuram	Glycyrrhiza glabra	Root	17.23		
Idavakam	Microstylis muscifera	Root tuber	5.22		
Jeevakam	Maxalis acuminata	Root tuber	38.73		
Kadukka	Terminalia chebula	Fruit pulp	57.06		
Kakoli	Lilium polyphyllum	Root	5.32		
Kattupayar	Atylosia goinsis	Whole plant	3.62		
Kattuzhunnu	Phaseolus trilobus	Whole plant	4.7		
Kayyunnyam	Eclipta alba	Whole plant	4.22		
Koval	Coccinia grandis	Stem	2.07		
Ksheerakakoli	Fritillaria roylei	Root	37.23		
Kumkumapoovu	Crocus sativus	stamen	1.07		
Kurumulaku	Piper nigrum	Seed	13.96		
Mahaameda	Polygonatum verticillatum	Root tuber	7.2		
Meda	Polygonatum cirrhifolium	Root tuber	5.12		
Munthiri	Vitis vinifera	Fruit	79.6		
Naykurana	Mucuna pruriens	Seed	7.15		
Nellikka	Emblica officinalis	Fruit pulp	32.73		
Nliapanna	Curculigo orchioides	Rhizhome	5.32		
Palmuthakku	Lpomoea paniculata	Rhizhome	5.12		
Sathavary	Asparagus racemosus	Rhizhome	39.73		
Thaannikka	Terminalia bellirica	Fruit pulp	28.04		
Thazhuthama	Boerhavia diffusa	Root	3.91		
Thippali	Piper longum	Seed	26.06		
Amruth jeevan rsayan	-	-	64.7		

Table II:- Malayalam name botanical name, parts used and of yield of extract of ingredients of Amruth Jeevan Rasayan Tablets

Malayalam Name	Botanical	Name/	Chemical	Used as
	name			

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Amukkuram	Withania somnifera	Root
Kadukka	Terminalia chebula	Fruit pulp
Naykurana	Mucuna pruriens	Seed
Nellikka	Emblica officinalis	Fruit pulp
Thaannikka	Terminalia bellirica	Fruit pulp
Abhra bhasmam	Calcined Mica (ferrus silicate	Bhasmam
	and aluminium)	
Annabhedi	Calcined Ferri Sulphas (ferric	Bhasmam
sindhooram	oxide)	
Kanmada bhasmam	Calcined Asphaltum	Bhasmam
	(potassium nitrate)	
Sankhu bhasmam	Calcined Conch (calcium	Bhasmam
	carbonate calx)	
Swarna bhasmam	Calcined Aurum (gold)	Bhasmam

Table III:- Preliminary phytochemical screening of ingredients in Amruth Jeevan Rasayan medicinal plant species

Botanical Name	Alkaloi	Flavano	Saponi	carbohy	Protei	Steroi	Glycos	Tann
	ds	ids	ns	drates	n	ds	ides	ins
Asparagus	+	+	-	-	-	-	-	-
racemosus								
Atylosia goinsis	+	-	-	+	+	+	+	-
Boerhavia diffusa	+	+	-	-	-	+	-	-
Coccinia grandis	+	+	-	-	-	+	-	-
Crocus sativus	+	+	-	+	+	+	-	-
Curculigo orchioides	+	+	-	+	-	-	+	-
Eclipta alba	+	+	-	+	+	+	+	-
Emblica officinalis	+	-	+	+	-	-	+	+
Fritillaria roylei	+	+	-	-	-	-	-	-
Glycyrrhiza glabra	+	+	-	+	-	+	+	+
Holostemma adakodien	+	-	-	+	-	+	+	-
Lilium polyphyllum	+	-	-	-	-	-	-	-
Lpomoea paniculata	+	-	-	-	-	-	-	-
Maxalis acuminata	+	+	-	-	-	-	-	-
Microstylis muscifera	+	-	-	-	-	-	-	-
Mucuna pruriens	+	-	+	+	-	+	-	-
Phaseolus trilobus	+	_	-	+	+	+	+	+
Piper longum	+	+	-	+	+	-	-	-
Polygonatum cirrhifolium	+	+	-	+	-	-	+	-
Polygonatum verticillatum	+	-	+	+	+	+	+	-
Terminalia bellirica	+	-	+	+	-	+	+	+
Terminalia chebula	+	-	+	+	-	+	+	+
Vitis vinifera	+	-	-	+	-	+	-	-
Withania somnifera	+	-	+	+	+	+	+	-
Zingiber officinala	+	+	-	+	+	+	+	-

Table IV:- Free radical scavenging activity (DPPH and ABTS) and total phenol cont of Amrujeevan rasayan and its ingredients

Botanical Name	DPPH radical	ABTS radical		
	scavenging assay (IC <sub>50</sub> )(µg/ml)	scavenging assay (IC 50) (µg/ml)	Total phenol(mg of gallic acid equivalents/g	
Assorbia asid	9 60 1 219	<u> </u>	of dry weight extract)	
Ascorbic acid	8.09±1.318	8.85± 3.32	-	
Asparagus racemosus	279.29±6.62	92.42±4.45	$197.70\pm2.68$	
Atylosia goinsis	207.26±2.29	79.11±3.52	$189.94 \pm 1.78$	
Boerhavia diffusa	239.74±2.20	43.21±3.32	$128.73 \pm 2.43$	
Coccinia grandis	229.17±3.69	46.90±2.38	$109.77 \pm 6.50$	
Crocus sativus	519.15±5.29	57.20±3.98	$162.06 \pm 138$	
Curculigo orchioides	43.40±2.15	61.27±3.41	271.55 ±3.98	
Eclipta alba	148.64±2.12	49.45±3.98	$190.51 \pm 1.78$	
Emblica officinalis	4.26±1.06	5.20± 3.69	592.81 ±6.86	
Fritillaria roylei	239.29±3.62	90.42±3.45	$175.70 \pm 3.68$	
Glycyrrhiza glabra	100.68±1.61	37.96±1.25	276.72 ± 10.15	
Holostemma adakodien	173.98±1.91	121.95±4.95	216.37±2.31	
Lilium polyphyllum	205.70±3.21	55.67±4.35	135.20± 4.68	
Lpomoea paniculata	216.07±4.41	45.87±3.15	$125.28 \pm 5.68$	
Maxalis acuminata	179.29±6.62	91.42±4.45	$187.70 \pm 2.68$	
Microstylis muscifera	200.27±2.21	48.77±3.22	$115.38 \pm 4.78$	
Mucuna pruriens	45.1±2.15	40.65±2.15	414.94 ±3.87	
Phaseolus trilobus	227.68±2.18	50.96±3.16	$197.98 \pm 2.23$	
Piper longum	229.32±6.52	78.24±3.91	$110.05 \pm 10.97$	
Polygonatum	43.40±2.15	61.27±3.41	271.55 ±3.98	
cirrhifolium				
Piper nigrum	99.43±2.15	49.70±2.91	$267.24 \pm 3.98$	
Polygonatum verticillatum	70.20±3.59	17.50± 2.91	209.25 ±5.90	
Terminalia bellirica	3.82±0.20	5.911±2.18	677.01 ±6.81	

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Terminalia chebula	3.69±0.43	$4.591 \pm 2.49$	695.40 ± 5.93
Vitis vinifera	202.02 ±5.50	79.77±3.45	84.77 ± 2.39
Withania somnifera	78.20±4.59	7.53± 3.91	269.25 ±6.90
Zingiber officinala	19.41±7.42	5.85±2.75	123.56 ±2.438
Abhra bhasmam	45.89±1.331	193.05±2.29	-
Annabhedi sindhooram	48.49±0.85	69.73±4.51	-
Kanmada bhasmam	45.84±0.48	39.71±1.28	-
Sankhu bhasmam	44.61±0.12	189.39±2.29	-
Swarna bhasmam	44.76±0.12	39.68±1.24	-
Sugar	107.69±1.62	73.31±4.82	-
Ghee	201.95±2.65	72.25±4.23	-
Honey	254.58±2.74	41.32±3.81	-
Amruth jeevan Tablet	42.46±2.54	40.03±2.18	-
Amruth jeevan rasayan	43.46±2.54	38.85±1.34	31.35 ± 1.58