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ANTI-OXIDANT STUDY OF AN HERBOMINERAL HEPATO-PROTECTIVE COMPOUND YAKRITSHULA VINASHINI VATIKA

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ABSTRACT

Yakritshula Vinashini Vatika (YSV), mentioned in Bhaishajya Ratnavali, is a herbo-mineral compound used in the management of Liver Diseases. It is a combination of Navasadara and Saindhava lavana and 6 herbal ingredients with Putikambu (Chirabilva swarasa) levigation. Liver diseases are considered as fatal and life threatening. Natural functions of the liver include secretion of bile salts and pigments and also digestion of fats. Not only this, the liver actually fights toxins naturally. The toxins immediately get spread in the body through blood. According to Ayurvedic point of view antioxidants can be used for Rasayana treatment for longevity of life, delaying of ageing and improving the health status of an individual. Antioxidants are molecules that slow or prevent the oxidation of other chemicals by neutralizing free radicals by accepting or donating an electron to eliminate the unpaired condition. Antioxidant activity of methanolic extract of Yakritashula vinashini vatika was evaluated by calculating the free radical scavenging activity of that drug using DPPH scavenging, Nitric oxide radical scavenging and reducing power. The results were compared with standard at different concentration of Ascorbic Acid (20, 40, 60, 80, 100 µg). The strength of YSV methanolic extract in DPPH, Nitric oxide radical scavenging and reducing power assay methods of 100 µg concentration were 75.54%±0.875(Ascorbic Acid-94.23%±0.532), 66.97%±0.236 (Ascorbic Acid-84.45%±0.328) and 0.28±0.0096 (Ascorbic acid 0.30±0.0001) respectively. Extract of Yakritashula vinashini vatika showed significant antioxidant activity in all the concentrations.

KEYWORDS: Yakritshula vinashini vatika, Herbomineral compound, Antioxidant property.

INTRODUCTION

Rasayana

Rasayana is one of the eight major clinical disciplines of Ashtanga Ayurveda.[1] Sushruta describes Rasayana as the branch that deals with 'Vayah Sthapana' (delay ageing process), prolongs longevity, develops positive physical and mental health. It develops resistance and immunity in the body to counteract the diseases. [2]

Acharya Dalhana, in his commentary 'Nibandha Sangraha' on Sushruta, while commenting on 'Vayasthapana' has explained in two ways.

a. That promotes the age of hundred years or more.
b. This helps in alleviating the senility and maintains youth fullness for a long time.

Dalhana while explaining on the pharmacokinetics of Rasayana Dravya that explains the specific action through Rasa, Guna, Virya, Vipaka and Prabhava.[3] Dalhana defines that natural tendency to hunger; thirst, old age, death, sleep etc are inhibited by the use of Rasayana.[4]

Acharya Charaka defines Rasayana as the procedure by which a healthy person obtains the best qualities of Rasadi Dhatus, i.e. all the Dhatus are nourished by the process of Rasayana.[5] While commenting on this verse Chakrapani has advised to include Smriti, Buddhi and other mental faculties along with Rasadi Dhatus.[6] Acharya Chakrapani has mentioned Rasayana as a particular measure by which alleviates Jara-vyadhi.

In Bhava Prakash, it has been mentioned that Rasayana prevents ageing and disease, maintains young age, maintains eyesight and also develops growth etc.

From the above description we can say that antioxidants can be used for Rasayana treatment for longevity of life, delaying of ageing and improving the health status of an individual. Though not by the name, antioxidant therapy is being used for the Rasayana. Thus we can conclude that Antioxidant constitute a part of Rasayana.

Garavisha [7], a special type of toxin described in Ayurved, affect liver & hepatomegaly may take place. The reason for hepatomegaly is that blood had special affinity towards toxins. The toxins immediately get spread in the body through blood.

The Pittashaya can be considered as Liver including gallbladder. Ayurveda has clearly stated that whatever the food etc. have been ingested through mouth, have to reach the Pittashaya and there that will be metabolized, assimilated and excreted.

Liver is the largest gland in the human body and one of the five major organs vital to life. Natural functions of the liver include secretion of bile salts and pigments and also digestion of fats. Not only this, the liver actually fights toxins naturally.

In case of liver disease, the liver which is a very significant organ of our body becomes inflamed and intoxicated and it consequently loses its work efficiency. The ailing and intoxicated liver results into formation of a number of ailments like anorexia, impairment of digestion, anemia, jaundice, hepatitis etc.

Antioxidant[8],[9], [10]

Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically this means that, the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non-free radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized.

Action of Antioxidants - Antioxidant attacks in different phases of LPO (lipid peroxidation) they are:-

a. Inhibit the initiation process (abstracting the allylic hydrogen from α - methylene carbon (e.g. Vitamin E)
b. Inhibit the formation of hydroperoxides.
c. Degrade the hydroperoxides formed without producing radicals
d. Remove the free radical (Scavenger activity e.g. – Vitamin E & A)

Sources of Antioxidant - In biological systems, physiologically two types of antioxidants are having proved useful against free radical pathogenesis.

a. Endogenous antioxidants -

i. Enzymatic and ii. Non enzymatic

b. Exogenous antioxidants -

i. Natural and ii. Synthetic

A. Endogenous antioxidants

Endogenous antioxidants are natural antioxidants occurring in biological systems under physiological conditions. Enzymatic antioxidants includes- Cytochrome oxidase, system Super oxide, dismutase Catalase, Glutathione peroxidase and non-enzymatic includes - α-tocopherol (vit-E), β-carotene, Glutathione, Ascorbic acid, Vit–A, Ceruloplasmin, Transferrin and Ferritin.

B. Exogenous antioxidants

Exogenous antioxidants are occurring in biological systems under patho-physiological conditions. These includes natural (Citrus fruits and juices, Spinach, Tomato, Spinach, carrot etc.) and synthetic antioxidants.

MATERIAL AND METHODS

a) Selection of the Formulation

Reviewing thoroughly the classical literature and the official AFI 'Yakrita shula vinashini vatika' mentioned under vati kalpana of AFI [11] was selected**.**

Table No. 1 Ingredients of Yakritashula vinashini vatika

S. No	Name	Origin	Latin Name	Part used	Proportion
1.	Navasadara	Mineral	Ammonium chloride	-	1 Kasha
2.	Saindhava lavana	Mineral	Rock salt halite / Chloride of sodium	-	2 Kasha
3.	Chitraka	Herbal	*Plumbago zeylanica*	Root bark	10 Kasha
4.	Kokilaksha	Herbal	*Asteracantha longifolia*	Seed	10 Kasha
5.	Rohitak	Herbal	*Tacoma undulate*	Bark	10 Kasha
6.	Yavani	Herbal	*Tachyspermum ammi*	Fruits	10 Kasha
7.	Putikambu (Swarasa)	Liquid for levigation	*Holoptelia integrifolia*	Leaves.	Q.S.

b) Procedure

Antioxidant activity of particular drug extract can be evaluated by calculating the free radical scavenging activity of that drug using DPPH and spectrophotometer (Institute of Biomedical and Industrial Research-Registered under Rajasthan societies act 1958). This laboratory method is easy, convenient and reliable.

Preparation of drug extracts: Sample (Yakritashula vinashini vatika) was subjected to solvent extraction using methanol as solvent.

A. DPPH radical scavenging activity

The stable 2, 2- diphenyl-1-picrylhydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the extract. 1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3 ml of various concentrations of methanolic extract of Sample (5, 10, 25, 50 and 100 µg) and the reference compound. After 30 min, absorbance was measured at 517 nm. Ascorbic acid (5, 10, 25, 50 and 100 µg) was used as the reference material because it is a known anti-oxidant positive control. The DPPH free radical is a stable free radical, which has been widely used to determine free radical scavenging activities of free radical scavenger or anti-oxidant. The measured scavenging effect increased with increasing concentration of extract .All the tests were performed in triplicate and the percentage of inhibition was calculated.

$$\text{DPPH Scavenging (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

B. Nitric oxide radical scavenging activity

This method is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric ions that can be estimated using Griess reagent. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and methanolic extract of Sample in different concentrations (5, 10, 25, 50 and 100 µg) and the reference compound were incubated at 25°C for 150 min. Each 30 min, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546nm. All the tests were performed in triplicate and the results averaged. Ascorbic acid used as the reference compound. The percentage decrease in absorbance was calculated.

$$\text{\% increase in reducing power} = \frac{A_{test} - 1 \text{ X } 100}{A_{blank}}$$

C. Reducing power method of Free radical scavenging determination

Fe^{+3} reductions are often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. Different concentration of methanolic extract of Sample in different concentration (5, 10, 25, 50 and 100 µg) extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50º C for 20 min. A

portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the results averaged. Increased absorbance of the reaction mixture indicates increase in reducing power.

RESULT AND DISCUSSION

The methanolic extract of Yakritashula vinashini vatika was used for the DPPH Scavenging, Nitric oxide radical scavenging and reducing power. It possess significant antioxidant activity in all the concentrations and the results were comparable with standard at different concentration of Ascorbic Acid (20, 40, 60, 80, 100 µg).

In DPPH method of 100 µg concentration show the strength of Yakritashula vinashini vatika methanolic extract 75.54%±0.875 and Ascorbic Acid have 94.23%±0.532. Sample showed good DPPH Scavenging.

Nitric oxide radical scavenging methods, it was found that the radical scavenging activity of methanolic extract of Yakritashula vinashini vatika increase in concentration in dose dependent manner and exhibited significant activity at 100 µg i.e. 66.97%±0.236 and Ascorbic acid 84.45%±0.328. Sample showed good Nitric oxide radical scavenging.

In reducing power assay method, sample show dose dependent response of reducing power where the activity of methanolic extract of Yakritashula vinashini vatika increased steadily with increase in concentrations. Sample Absorbance at 100 µg was 0.28±0.0096 and Ascorbic acid 0.30±0.0001. Extract of Yakritashula vinashini vatika showed good Reducing power.

Table No. 2 Showing DPPH Scavenging in percentage (%)

S. No	Concentration (µg)	DPPH Scavenging %	Ascorbic Acid ±SD%
1.	20	19.67±0.788	34.23±1.282
2.	40	31.56±0.356	42.43±1.304
3.	60	42.46±0.896	53.76±0.330
4.	80	53.28±0.369	72.84±0.599
5.	100	75.54±0.875	94.23±0.532

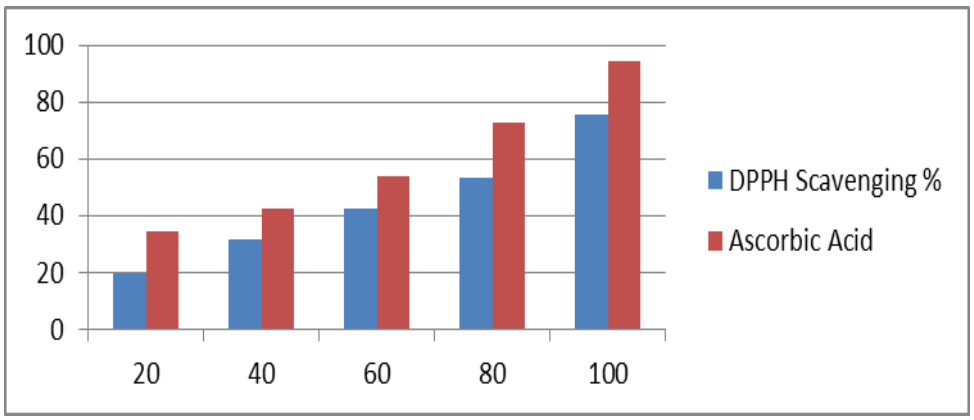
Table No. 3 Showing Nitric Oxide Scavenging percentage in (%)

S. No.	Concentration (µg)	Nitric Oxide Scavenging %	Ascorbic Acid ±SD%
1.	20	17.47.±0.479	27.32±0.455
2.	40	23.97±0.579	43.68±0.481
3.	60	40.79±0.986	58.34±0.598
4.	80	54.97±0.470	70.32±0.266
5.	100	66.97±0.236	84.45±0.328

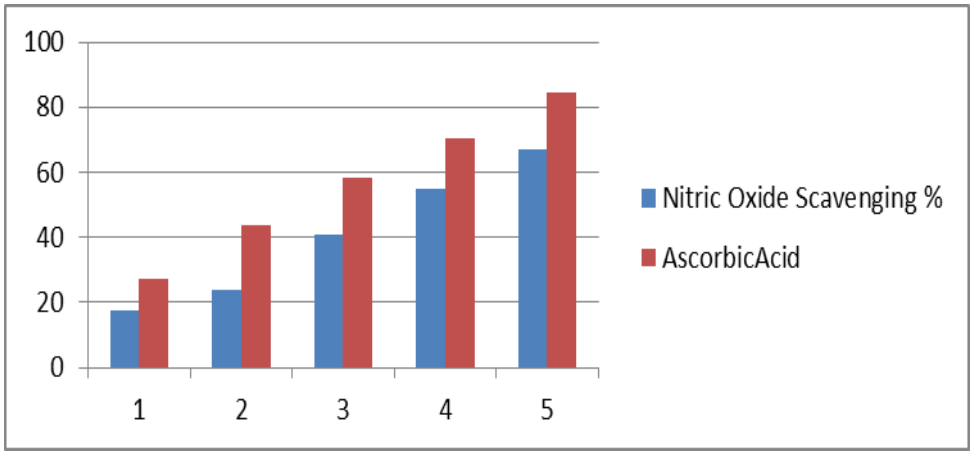
Table No. 4 Showing Reducing power (Absorbance)

S.No.	Concentration (µg)	Reducing power (Absorbance)	Ascorbic Acid ±SD
1	20	0.16±0.0065	0.20±0.0006
2	40	0.21±0.0045	0.22±0.001
3	60	0.23±0.0096	0.24±0.0003
4	80	0.25±0.0057	0.28±0.0001
5	100	0.28±0.0096	0.30±0.0001

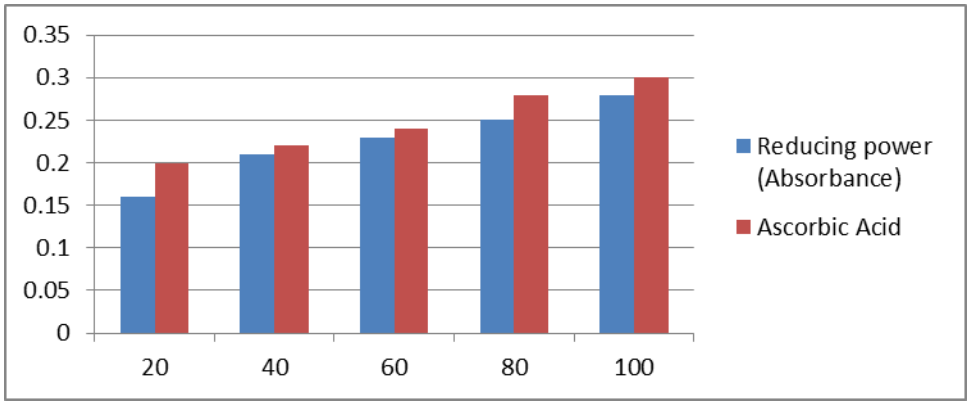
Graph 1



Graph 2



Graph 3



CONCLUSION

It can be concluded that extract of Yakritashula vinashini vatika showed good antioxidant property. Therefore it will be beneficial in liver disorders for both prophylactic and therapeutic purposes.

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