IAMJ

INTERNATIONAL AYURVEDIC MEDICAL JOURNAL

Review Article ISSN: 2320-5091 Impact Factor: 6.719

ANTIOXIDANT ACTIVITY OF DHATRYADI RASAYANA- LITERARY REVIEW

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https://doi.org/10.46607/iamj3710102022

(Published Online: October 2022)

Article Received: 29/09/2022 - **Peer Reviewed:** 10/10/2022 - **Accepted for Publication:** 12/10/2022

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ABSTRACT

The current scenario is witnessing a number of lifestyle disorders and many of them are related to an increased level of various oxidative stress which leads to excessive production of free radicals such as OH^-, O_2^-, NO^- etc in the body. Excessive production of free radicals is associated with various oxidative changes like cellular damage including DNA, proteins, and lipids, leading to declining in mitochondrial integrity and exacerbating cellular damage, contributing to cellular senescence which results in premature ageing. In Ayurveda, *Rasayana Karma* was advocated by *Acharyas* to tackle these changes. *Rasayana* suppresses the activity of oxidative stressors and reduces the production of free radicals, thus *Rasayana* prevents various oxidative changes which lead to premature ageing. One such formulation is *Dhatryadi Rasayana* mentioned in different textbooks of Ayurveda. This formulation helps to attain long, strong, and black hair, maintain proper functioning of sense organs, and long life devoid of diseases.

Keywords: *Rasayana*, Antioxidant, Ayurveda, Oxidative stress

INTRODUCTION

Oxidants and antioxidants in humans are maintained in balance in a normal physiological state and overproduction of oxidants in certain conditions such as smoking, hazardous environmental exposures or infectious diseases can cause oxidative stress leading to oxidative damage to biomolecules and cells [1]. Overproduction of free radicals and inadequate antioxidants have been implicated in the pathogenesis and complications of some disease conditions like diabetes, cancer, Alzheimer's disease, arthritis, neurodegenerative disease, and the ageing process [2]. A cardinal property of an antioxidant is the ability to scavenge free radicals and inhibit further damage. In Ayurveda, *Rasayana* is possessed to have antioxidant properties. *Rasayana* revitalizes the nervous system, optimizes the functioning of enzymes in the cells, stimulates the functioning of the brain, and boosts concentration. All these factors slow down ageing and boost overall health. *Dhatryadi Rasayana* is one such valuable formulation mentioned in *Chakradutta* [3], *Bhaishajya Ratnavali* [4], *Rasa Kamadhenu* [5], *Vrindamadhava or Siddha Yoga* [6], and Yoga Ratnakara [7] consisting of three ingredients: *Dhatri* (*Emblica officinalis* Gaertn.), *Bhringraja* (*Eclipta alba* Hassk.) and *Tila* (*Sesamum indicum* Linn.). This *Rasayana* revitalizes the human body, helps in maintaining health, and eliminates the ill effects of various diseases.

Drug Review:

Dhatryadi Rasayana is an esteemed formulation consisting of three ingredients: *Dhatri* (*Emblica officinalis* Gaertn.), *Bhringraja* (*Eclipta alba* Hassk.) and *Tila* (*Sesamum indicum* Linn.). This *Rasayana* provides long life devoid of diseases, maintains proper activities of sense organs as well as provides long, strong and black hair.

***Emblica officinalis* Gaertn.**

Synonyms [8,9,10]: *Amalaki, Dhatriphala, Amritaphala, Shiv, Vrishya, Shriphala, Jatiphalarasa, Vayastha, Panchrasa.* A small or medium-sized, deciduous tree. Leaves subsessile, closely set along the branchlets, distichous, narrowly linear, obtuse, having the appearance of pinnate leaves. Flowers greenish yellow, in axillary fascicles on the leaf-bearing branchlets, often on the naked portion below the leaves. Fruits are fleshy, globose, with obscure vertical furrows, pale yellow, Seeds 6, trigonous, and the parts used are fruit, root bark, stem bark, leaf, and seed [11].

Ayurvedic Properties of *Emblica officinalis* Gaertn: It is *Amla* (sour) predominant *Panchrasa* (except *Lavana Rasa*) in nature, *Guru* (heavy), *Ruksha* (dry) and *Sheeta* (cold) in *Guna* (qualities), *Sheeta* (cold) in *Virya* (potency) and *Madhura* in *Vipaka* (taste developed through digestion). It is *Tridoshahara* in nature as it pacifies *Vata Dosha* due to *Amla Rasa*, *Pitta Dosha* due to its *Madhura Rasa* and *Sheeta Virya* and pacifies *Kapha Dosha* due to *Kshaya Rasa* and *Ruksha Guna* [12].

Chemical Constituents of *Emblica officinalis* Gaertn: It primarily contains tannins, alkaloids, phenolic compounds, amino acids, and carbohydrates [13]. Fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B [14], one on hydrolysis gives gallic acid, ellagic acid, and glucose wherein the other gives ellagic acid and glucose respectively. The fruit also contains Phyllemblin [15]. It contains Hydrolysable Tannins-Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corilagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin; Alkaloids- Phyllantine, Phyllembein, Phyllantidine; Phenolic compounds-Gallic acid, Methyl gallate, Ellagic acid, Trigallayl glucose; Amino acids- Glutamic acid, Proline, Aspartic acid, Alanine, Cystine, Lysine; Carbohydrates-Pectin; Vitamins-Ascorbic acid; Flavonoids- Quercetin, Kaempferol; Organic acids- Citric acid. Compounds isolated from EO were gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O - galloyl beta D glucose, 3 Ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid) and isostrictiniin [16].

Pharmacological action of *Emblica officinalis* Gaertn:

Antioxidant and free radical scavenging activity [17-25]: Gallic acid equivalent to total phenolic content from fruit and seed of *E. officinalis* has excellent antioxidant properties and plays an important role as free radical scavengers required in the maintenance of 'redox homeostasis' responsible for diverse degenerative diseases [17]. The methanolic seed extract of *Emblica officinalis* has promising free radical scavenging activity of 1,1, Diphenyl-2-picryl-hydrazyl (DPPH) in a concentration-dependent manner [18]. Methanolic extract of fruit pulp also has antioxidant and free radical scavenging activity [19,20,21,22,23]. Methanolic extracts of dried leaves of *Phyllanthus emblica* were used for the comparative study of antibacterial and antioxidant activity and the research work ended positively showing the extract has both of these activities[24]. In separate research work, it is seen that the water extract of *E. officinalis* fruit prepared according to Thai Herbal Pharmacopoeia has a strong potential for free radical scavenging, ferric reducing as well as inhibiting ROS (reactive oxygen species) production[25].

***Eclipta alba* Hassk.**

Synonyms [26,27,28]: *Bhringraja, Markava, Kesharanjnana, Kesharaja, Bhring, Bhringrenu, Angaraka*. Erect, slender, roughly pubescent herbs, 30-60cm high. Leaves opposite, very variable, sessile, narrowly lanceolate, toothed, or nearly entire. Heads radiate, 6-8mm in diameter, terminal on erect stalks. Involucral bracts leaf-like, outer large, Flowers white, pappus of 2–5-minute teeth. Achenes are narrowly oblong, ribbed, and tipped with pappus teeth and the parts used are whole plants and seeds [29].

Ayurvedic Properties of *Eclipta alba* Hassk: This drug is *Katu* and *Tikta* in *Rasa, Ruksha* and *Laghu* in *Guna, Ushna* in *Virya, Katu* in *Vipaka*. It pacifies *Kapha Dosha* due to its *Ruksha, Laghu Guna, Ushna Virya*, and *Katu, Tikta Rasa,* and also pacifies its *Vata Dosha* due to *Ushna Virya* [30].

Chemical Constituents of *Eclipta alba* Hassk: *Eclipta alba* (L.) contains a wide range of active principles which includes coumestans, alkaloids, flavonoids, glycosides, polyacetylenes, triterpenoids. The leaves contain stigmasterol, β-terthienylmethanol, wedelolactone, demethylwedelolactone, and demethylwedelolactone-7-glucoside [31]. The roots give hentriacontanol and heptacosanol. The roots contain polyacetylene-substituted thiophenes. The aerial part is reported to contain a phytosterol, β-amyrin in the n-hexane extract, and luteolin-7-glucoside, βglucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone in the polar solvent extract. The polypeptides isolated from the plant yield cystine, glutamic acid, phenylalanine, tyrosine, and methionine on hydrolysis. Nicotine and nicotinic acid are reported to occur in this plant [32].

Pharmacological action of *Eclipta alba* Hassk:

Antioxidant Activity: The methanol and hydrolyzed extract of *E. alba* has been assessed for its antioxidant potential in both *in vitro* and *ex vivo* models. The *in vitro* antioxidant activity was evaluated through 2,2-diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging and nitric oxide radical inhibition activity. The *ex vivo* antioxidant activity was determined through lipid peroxidation inhibitory activity on mice liver homogenate by thiobarbituric acid reactive substances (TBARS) method. The methanolic extract and hydrolyzed extract both showed potent antioxidant activity in both models proving to be powerful scavengers of DPPH free radicals and nitric oxide radicals, as well as inhibitors of lipid peroxidation [33]. Antioxidant activity as assessed by DPPH free radical scavenging method has also been described for ethanol extract of the plant [34]. Methanolic and aqueous extracts of *E. alba* demonstrated antioxidant activity in hydrogen peroxide scavenging assays, total antioxidant capacity, and reducing ability assay [35]. The antioxidant potential of the plant methanolic extract has been shown through DPPH free radical scavenging and 2,2-azinobis-(3-ethylbenzthiazoline6-sulfonic acid) (ABTS) assays [36]. The ethanolic extract of the plant also demonstrated antioxidant potential in DPPH and ABTS assays [37]. Ethanol and ethyl acetate extracts of leaves of the plant showed antioxidant activity in the ferric thiocyanate method; aqueous and hexane extracts also showed antioxidant effects but less than ethanol and ethyl acetate extracts [38]. The possible cerebroprotective and antioxidant effects of the

hydroalcoholic extract of E. alba have been evaluated in global cerebral ischemia in rats. The global cerebral ischemia-reperfusion injury was induced by occluding bilateral common carotid arteries (BCCA) for 30 min, followed by 4 h reperfusion. BCCA caused significant depletion in superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), catalase (CAT), glutathione-S-transferase (GST), and glutathione reductase (GR) and a significant increase in malondialdehyde (MDA) in the brain. Pre-treatment with hydroalcoholic extract significantly reversed the levels of biochemical parameters and significantly reduced the edema and cerebral infarct size as compared to the ischemic control group [39].

***Sesamum indicum* Linn.**

Synonyms [40,41]: *Homdhanya, Pavitra, Papghana, Putdhanya, Pitartarpana*. An erect, glandular-pubescent, annual herb up to 95cm tall, branching from the base. Leaves alternate or lower opposite and often deeply 3 lobed; lobes lanceolate, 3-15×1.5-6 cm, serrate, puberulous beneath; upper leaves entire, lanceolate much smaller passing into bracts. Flowers ill-smelling, white or pink with yellow marks, axillary, solitary, forming a false raceme at the end of branches. Fruits quadrangular, oblong, compressed capsules, deeply 4-grooved, dehiscent to halfway down. Seeds are many, obovoid, compressed, black or white and the parts used are root, leaf, seed, and oil [42].

Ayurvedic Properties of *Sesamum indicum* Linn: It is *Madhura* (sweet) in *Rasa, Kshaya* (astringent), and *Tikta* (bitter) in *Anurasa, Guru* (heaviness) and *Snigdh* (smooth) in *Guna, Ushna* in *Virya* (potency), *Madhura* in *Vipaka*. It pacifies *Vata Dosha* due to its *Guru, Snigdh Guna* and *Ushna Virya* as well as *Madhura Vipaka*, and enhances *Kapha* and *Pitta Dosha* [43].

Chemical Constituents of *Sesamum indicum* Linn: Sesamin and sesamolin are the most abundant lignans of sesame seeds and the major fat-soluble lignans [44]. Sesame seeds are a rich source of copper, calcium, phosphorous, iron, magnesium, vitamin B1, and tryptophan. A chlorinated red naphthoquinone pigment possessing antifungal activity, named chlorosesamone (2-chloro5, 8-dihydroxy-3-3methyl-2-butenyl)-1, 4- naphthoquinone) was reported from the roots of *Sesamum indicum* [45]. From the water extract of the whole plant, two new phenylethanoid glycosides, and three new triglycosides which had the same sugar sequence were isolated [46]. Two anthraquinone derivatives, named anthrax sesamone D and E, were isolated from the roots of *Sesamum indicum*. Their respective structures were determined to be 1, 2, 4-tetrahydroxy-3-(4-methylpent-3-enyl) anthrax quinone and 1, 2-dihydroxy-3- (4-methylpent-3-enyl) anthrax quinone [47]. 2-Geranyl-1, 4-naphthoquinone was isolated from the hairy root culture of *Sesamum indicum*. The structure was determined to be 2-[(e)-3, 7-dimethylocta-2, 6-dienyl]-1, 4- naphthoquinone [48].

Pharmacological action of *Sesamum indicum* Linn:

Antioxidant Activity: The total phenolic content, total antioxidant status, free radical scavenging capacity, inhibition of low-density lipoprotein cholesterol, and metal chelating capacity of extracts of the whole black and whole white sesame seeds and their hull fractions in 80% aqueous ethanol were investigated. Results were found that Sesame products displayed good ferrous ion chelating capacities. besides, it was demonstrated that there was considerable antioxidant activity in sesame products especially black sesame hulls [49]. The antioxidant activity of ethanolic extracts of sesame coat (EESC) was investigated. The antioxidant activity of 1.0 mg EESC was equal to 1.0 mg tocopherol but was weaker than 1.0 mg butylated hydroxyanisole on peroxidation of linoleic acid. EESC showed an inhibitory effect against the formation of thiobarbituric acid reactive substances in a liposome model system. Besides, phenolic compounds and tetranortriterpenoids indicating antioxidant activity, are present in EESC. The result showed that the antioxidant activity of this plant is due to its free radical reaction, metal-binding ability, and quenching of reactive oxygen [50]. Sesaminol is extracted from the acetone extract of a sesame seed. it was shown that sesamolin in unprocessed sesame oil is the source of sesaminol. Sesaminol was not so greatly removed by the

deodorization process. The antioxidative activity of Sesaminol was relatively equal to those of sesamol and γ-tocopherol by the thiocyanate method. Therefore, it seems that the antioxidative activity of refined unroasted seed oil is mainly attributed to Sesaminol [51]. The effective components of a mashed sesame seed with acetone were investigated. The acetone extract showed strong antioxidative activity with the thiocyanate method and offer 4 active antioxidative substances as P1, P2, P3, and P4. The antioxidative activities were in the order of P3 >P2>P1 >P4. The same components were also obtained from the 80% ethanol extractable polar fraction of the sesame oil cake treated with β-glucosidase, which suggested the presence of the active substances also as their glycosides in sesame seed [52]. Chemical constituents of sesame oil from two different colored seed varieties were examined. These antioxidative components are effective via synergistic action. Results indicated that both USM (unroasted sesamum) and RSM (roasted sesamum) had antioxidant activity in a dose-dependent manner. Compared to USM, the RSM was a better antioxidant in most cases. this could be used as an alternative natural antioxidant for food applications [53]. Differences between two different varieties [cv. Orhangazi and cv. Cumhuriyet] of Sesamum indicum in growth parameters, lipid peroxidation, antioxidative enzyme activities, and proline accumulation were tested. Results indicated that both parameters differ according to the ability of the variety in coping with oxidative stress caused by salinity. The antioxidative activity of both varieties was almost the same. however, the antioxidant enzyme activity of cv. Cumhuriyet was more when subjected to salt stress [54]. In a study antioxidant agent of sesame was introduced to be related to the Presence of various new antioxidative lignan phenol compounds in sesame seed and oil. Sesaminol as a new antioxidative principle in raw sesame salad oil was introduced. The mechanism of the superior antioxidative activity of roasted sesame oil was associated with its synergistic effect of the browning products with tocopherol, sesamol, and sesamin [55]. Antioxidative constituents of unroasted and roasted sesame seed oil were investigated. The main active constituent in fresh unroasted seed oil was γ-tocopherol, and that of roasted seed oil was sesamol, which was produced by hydrolysis of sesamolin that is present to a large degree in roasted sesame seed oil. This conversion of sesamolin to sesamol is catalyzed by acids [56]. In an animal study, the effects of sesamin on hepatic fatty acid oxidation were examined in rats that were fed experimental diets containing various amounts of sesamin for 15 days. Dietary sesamin increased oxidation rates in a dose-dependent manner. Mitochondrial activity almost increase 2-fold in rats on the 0.5% sesamin diet. Peroxisomal activity became10 times more in rats fed a 0.5% sesamin diet in relation to rats on the sesamin-free diet [57]. Sesame cake was extracted with methanol to obtain a crude antioxidant extract. Its antioxidant activity was evaluated using the β-carotene bleaching method, linoleic acid peroxidation method, and free radical scavenging assay, using 2,2-diphenyl-1-picryl hydrazyl radical. Results showed that crude extract was effective at 100 and 200 ppm levels and comparable with butylated hydroxy toluene at 200 ppm, whereas purified extract showed comparable or better activities at 5, 10, 50, 100, and 200 ppm levels [58]. In a vitro study, the antioxidant properties of Sesaminol were investigated. Sesaminol inhibited lipid peroxidation in LDL in a concentration-dependent manner. Besides, findings suggest that sesaminol is a potentially effective antioxidant that can protect LDL against oxidation [59]. The protective and antioxidant potential of sesame oil [SO] and [or] alpha-lipoic acid [ALA] against DZN toxicity in male Wistar albino rats was investigated. DZN-treated animals exhibited macrocytic hypochromic anemia. SO, and [or] ALA supplementation ameliorated the deleterious effects of DZN intoxication. Results showed that Seasame oil can reduce the side effects of DZN through its antioxidant and free radical-scavenging activities [60].

DISCUSSION

Antioxidants act as a defence mechanism that protects against the deleterious effects of oxidative stress leading to oxidative changes which result in cell membrane disintegration, membrane protein damage, and

DNA mutations which further result in ageing and initiate or propagate the development of many diseases such as cancer, liver injury, skin damages, coronary artery diseases, etc. Vitamin C is regarded as the first line of natural antioxidant defence in plasma and a powerful inhibitor of lipid peroxidation. It also regenerates the major antioxidant tocopherol in lipoproteins and cell membranes. *Emblica officinalis* Gaertn. is a rich source of vitamin C. A recent study has demonstrated that Vitamin C is not present in fresh juice extractives of *Emblica Officinalis* Gaertn. It was suggested that vitamin C-like activity of *Emblica officinalis* Gaertn. is due to the presence of tannoids, mainly emblicanin A and B.

Eclipta alba Hassk. showed the presence of phenolics, flavonoids, tannins, and ascorbic acid contents. The presence of alkaloids, phenolic compounds, flavonoids, and tannins has been associated with various degrees of antioxidant activities. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Antioxidant properties of sesame fractions are related to lignans, an innate non-enzymatic antioxidant defense mechanism against reactive oxygen species which play a vital role in health promotion. The main antioxidant agent of sesame is Tocopherols. They are a class of plant phenolics that have important antioxidant and nutritional properties. They are natural antioxidants that inhibit oil oxidation. They have free radicals scavenging properties. The main function of α-tocopherol is that of a radical-chain-breaking antioxidant in membranes and lipoproteins, as well as in foods. Γ-tocopherol is the major tocopherol in sesame seeds, whereas α-and δ-tocopherols are present in smaller amounts. It is more potent than α-tocopherol in decreasing platelet aggregation, low-density lipid [LDL] oxidation, and delaying intra-arterial thrombus formation. Various other phytoconstituents such as sesamin, sesamolin, sesamol, their glucosylated forms sesaminol glucosides, etc are responsible for the antioxidant activity of *sesamum indicum* Linn.

The antioxidant property of different ingredients is due to their secondary metabolites present in them. Secondary metabolites such as tannin, flavonoids, alkaloids, phenolics, etc. are responsible for the antioxidant properties of different ingredients of *Dhatryadi Rasayana*.

CONCLUSION

To the best of our knowledge, this review is the first attempt to compile the antioxidant activity of ingredients of *Dhatryadi Rasayana*. The antioxidant activity of the ingredients of *Dhatryadi Rasayana* is significantly effective in preventing the deleterious effects of oxidative stress, therefore revitalizing the human body and helping in maintaining health.

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Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL:Sheenam Rani et al: Antioxidant Activity of Dhatryadi Rasayana- Literary Review. International Ayurvedic Medical Journal {online} 2022 {cited October 2022} Available from: http://www.iamj.in/posts/images/upload/2905_2913.pdf