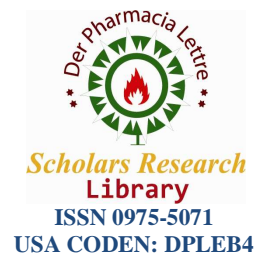




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## The study of antioxidant activities of an Ayurvedic medicine Ayaskriti

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### ABSTRACT

Ayaskriti is an Ayurvedic preparation for the treatment of anemia, weight loss and skin related diseases. This is made of a number of plants and plant parts. The present study deals with antioxidant activities of Ayaskriti, such as DDPH assay, FRAP assay and Hydrogen peroxide scavenging activity assay. It was found that all the three assays indicated that Ayaskriti has antioxidant activity. This is one step towards understanding the medicinal efficacy of this medicine.

**Key words** Ayaskriti, Ayurvedic, Anemia, DDPH, FRAP, Hydrogen Peroxide Scavenging Assay, Antioxidant.

### INTRODUCTION

Ayaskriti is an Ayurvedic medicine in liquid form mainly used in anaemia, weight loss therapy, skin diseases etc. It contains iron as one of the ingredient. The word Ayas in Sanskrit means Iron. This medicine is made of a number of medicinal plants and plant parts along with metallic iron. 12- 24 ml of this medicine is taken twice a day after food or as advised by the medical practitioner. Ayaskriti is made of the following ingredients which are divided into three sections: One part is known as Kwatha Dravyas, the second is Sandana Dravyas and the third is Prakshepaka dravyas. All the ingredients of Kwatha dravyas are taken at 960 grams each and mixed with 98 litres of water. The ingredients of Sandana dravyas are 9.5 kg of Jaggery and 1.6 litres of Honey. The Prakshepaka dravyas are taken at 48 grams each including pure iron foil.

The Kwatha dravyas are made into Kashayam by boiling in water till the quantity is reduced to one fourth of the original volume and cooled. The Kashayam is strained and then jaggery, honey and Prakshepa dravyas are mixed. Red hot iron foil is immersed in the resultant liquid till it melts. The prepared fluid is closed tightly in a vessel and kept for fermentation for a month. The resultant mixture is Ayaskriti which is filtered and stored in glass bottles for use as medicine.

The list of ingredients is mentioned below:

The Kwatha Dravyas: Asana (*Pterocarpus marsupium*), Tinisha (*Anogeissus lactifolia*), Bhurja (*Betula utilis*), Swethavaha (*Calotropis procera*), Prakeerya (*Sapindus trifoliatus*), Khadira (*Acacia catechu*), Kadara (*Acacia polyantha*), Bhandi (*Rubia cordifolia*), Shimshapa (*Dalbergia sisoo*), Meshashrunji (*Prosopis spicegera*), Chandana (*Santalum album*), Raktachandana (*Pterocarpus santalinus*), Daruharidra (*Berberis aristata*), Tala (*Borassus flabellifer*), Palasha (*Butea monosperma*), Jonkaka (*Aquilaris agallocha*), Shaka (*Grewia populifolia*), Shala (*Shorea robusta*), Kramuka (*Phyllanthus reticulatus*), Kalinga (*Holarrhena antidysenterica*), Ajakarna (*Acacia leucophloea*), Aswakarna (*Cassia fistula*) and water 98 litres.

The Prakshepa dravyas are Kalinga (*Holarrhena antidysenterica*), Murva (*Marsdenia tenacissima*), Bharangi (*Clerodendron serratum*), Katuki (*Picrorrhiza kurroa*), Maricha (*Piper nigrum*), Ativisha (*Aconitum heterophyllum*), Gandira (*Coleus forskohlii*), Ela (*Elettaria cardamomum*), Pata (*Cyclea peltata*), Ajaji (*Carum carvi*), Kadvanga phalam (*Oroxylum indicum*), Ajamoda (*Trichyspermum roxburghianum*), Sarshapa (*Brassica alba*), Vacha (*Acorus calamus*), Jeeraka (*Cuminum cyminum*), Hingu (*Ferula foetida*), Vidanga (*Emblia ribes*), Pasugandha (*Cleome gynandra*), Pippali (*Piper longa*), Pippalimoola (Root of *Piper longa*), Chavya (*Piper retrofractum*), Chitraka (*Plumbago zeylanica*), Shunti (*Zingiber officinale*), Shudha loha patra (Pure iron foil).

The medicinal efficacy of Ayaskriti through pharmacological and other medical standards is not reported and this is the first attempt in this direction. It is important to have a correlation of the activities of each constituent plant along with that of Ayaskriti in understanding the logic behind choosing these plant constituents for making this medicine. The medicinal roles of each major constituent of Ayaskriti are mentioned below:

**Asana (*Pterocarpus marsupium*)**

The medicinal properties of *P. marsupium* are reviewed by Badkhane *et al*, 2010 in which they have mentioned its role as anti diabetic, anti inflammatory, cardio tonic, and antibacterial. [1]

**Tinisha (*Anogeissus latifolia*)**

This plant has been reported to have pharmacological potentials such as wound healing, anti ulcer, hypo lipidemic, antibacterial, hepato protective and antioxidant. [2]

**Bhurja (*Betula utilis*)**

This medicinal plant possesses anticancer, anti-HIV, antimicrobial and antioxidant activities. [3]

**Swethavaha (*Calotropis procera*)**

Among the many scientific reports available on medicinal values of *Calotropis* a few are being sighted here. Sharma *et al*, 2012, have reviewed the therapeutic potential of *Calotropis*. This plant is used for leprosy, elephantiasis, fever, menorrhagia, malaria and snake bite by local people. It is reported to be schizonticidal, nematocidal, anti microbial, anti inflammatory, anticancer, antipyretic, antioxidant, hepato-protective and anti-nociceptive. It contains important bioactive and medicinal compounds like organic carbonate, cystine protease, procerain, alkaloids, flavonoids, sterols etc. [4]

**Prakeerya (*Sapindus trifoliatus*)**

The pericarp of this fruit is reported for its various medicinal properties. [5] It is regarded as a tonic, stomachic, spermicidal, and a thick aqueous solution is used in the treatment of hemicranias, hysteria and epilepsy. [6]

**Khadira (*Acacia catechu*)**

The medicinal values of this plant were reviewed by Stohs and Bagchi, 2015, such as antioxidant, anti-inflammatory and chemo protective properties. [7]

**Kadara (*Acacia polyantha Willd*)**

The decoction of pods of *Acacia polyantha* is used to treat urino-genital diseases, the leaves are used to treat diarrhea and dysentery, the bark decoction for sore throat and the dry powder is used to treat external ulcers. [8, 9]

**Bhandi (*Rubia cordifolia*)**

This plant is reported to have medicinal properties like anti-acne, anti-inflammatory, antibacterial and antioxidant. [10]

**Shinshapa (*Dalbergia sisoo*)**

This is a medicinal plant with many therapeutic roles like anti diabetic, anti inflammatory, analgesic, anti nociceptive and anthelmintic.[11]

**Meshasrungi (*Prosopis specigera*)**

Gupta *et al*, 2014 have described the various medicinal properties of the bark extract of this plant such as anti diabetic, anti hyperglycemic, antioxidant, antibacterial, antidepressant, skeletal muscle relaxant, analgesic and antipyretic, hypo lipidemic and anti atherosclerotic, nootropic, and anticonvulsant effects. [12]

**Chandana (*Santalum album*)**

Sandal is an age old medicinal plant and it is used for many diseases. It has curative roles such as anti hyperglycemic and anti hyper lipidemic, cardio protective, as a brain tonic and anti ulcerogenic. [13, 14]

**Rakta chandana (*Pterocarpus santalinus*)**

This plant is one of the oldest medicinals having properties like hepato protective, gastro protective, anticancer, antioxidant, anti diabetic and apoptotic. [15]

**Daruharidra (*Berberis aristata*)**

*Berberis aristata* is ethno botanically important herb that is used from time immemorial by mankind for the treatment of various ailments. Sharma *et al*, 2011 has reviewed this plant's therapeutic role such as hepato-protective, hypoglycemic, anticancer, antimicrobial, anti-inflammatory, antioxidant etc. among many other medicinal values. [16]

**Tala (*Borassus flabellifer*)**

The plant mainly contains gums, albuminoids, fats, steroidal glycosides, and carbohydrate like sucrose which possess medicinal activities like antimicrobial, anti-inflammatory, anthelmintic, diuretic, antioxidant, immuno modulatory and anti malarial activities. [17]

**Palasha (*Butea monosperma*)**

*Butea* has antifungal, antimicrobial, anti inflammatory, anticonvulsive, anti esterogenic, anti fertility, anti diabetic, anti diarrheal, thyroid inhibitory, anti peroxidative and hypoglycemic effects. [18]

**Jonaka (*Aquilaria agallocha*)**

This plant has pharmacological potentials such as antioxidant, anti diabetic, hepato protective, anti inflammatory, analgesic and antipyretic, antihistaminic, laxative, anti microbial, Central Nervous system (CNS) activity, sedative, anxiolytic and anti-convulsant activities. [19]

**Shaka (*Grewia tenax*)**

This plant is used ethno medically for tonsillitis, anemia, bone injury etc. The plant is reported to have antitumor, anti carcinogenic and help in iron absorption in the gut. [20]

**Shala (*Shorea robusta*)**

*Shorea* is a tree with valued timber. Apart from its commercial value it has medicinal properties like anti diabetic, anti hyper lipidemic, antimicrobial, analgesic, anti-inflammatory, anti-obesity and immuno modulatory effects. [21]

**Kramuka (*Phyllanthus reticulatus*)**

This is another medicinal plant with pharmacological activities like anti diabetic, anti plasmodial, hypo cholesterolemic, antimicrobial, cytotoxic activity, hepato protective, anti bacterial, anti nociceptive, anti-hyperglycemic activity, analgesic, anti-inflammatory, antioxidant and anti-hepatitis B viral. [22]

**Kalinga (*Holarrhena antidysenterica*)**

The name of the plant itself indicates that it is a good anti dysentric medicine. This plant has other activities like brocho pneumonia, anticancer, appetizer, diuretic, tonic, immune modulating etc. [23]

***Ajakarna (Acacia leucophloea)***

Traditionally the bark is used as astringent, demulcent, constipating, expectorant and antipyretic, vulnerary, demulcent, bitter, thermogenic, styptic, alexeteric, anthelmintic, vulnerary, constipating, bronchitis, cough, diarrhea, dysentery, vomiting, wounds, ulcers, internal and external haemorrhages, oral ulcers, proctoptosis, stomatitis and intermittent fevers. [24]

***Aswakarna (Cassia fistula)***

*C. fistula* is known as rich source of tannins, flavonoids and glycosides. Pharmacological activities of *Cassia* include antibacterial, anti diabetic, anti fertility, anti-inflammatory, antioxidant, hypato protective, antitumor and as antifungal. [25]

***Murva (Marsdenia tenacissima)***

This plant has many medicinal roles of which its anti-angiogenic, apoptotic, anticancer, enzyme inhibitory activities are important. [26-29]

***Bharangi (Cleodendron serratum)***

It is traditionally valued and reported for treating pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever in India with a long history. [30]

***Katuki (Pycorrhiza kurroa)***

*Pycorrhiza kurroa* also known as Kutki has two active bitter compounds, Picroside I and Picroside II. These molecules are known for their hepato protective activity and against toxins. [31-32] The antioxidant role of this plant was studied by Tiwari *et al*, 2012 and Kant *et al*, 2013. [33-34]

***Maricha (Piper nigrum)***

Pepper plays a great role in digestions, useful for low appetite, sluggish digestion, abdominal pain, toxins and borborygmus. It has other medicinal qualities like anthelmintic, digestive, antimicrobial, antioxidant, anticonvulsant, sedative, muscle relaxant, antipyretic, anti-inflammatory, antifungal, hepato protective, antimicrobial, antiulcer and lipolytic. [35]

***Ativisha (Aconitum hetrophyllum)***

This plant has many pharmaceutical characteristics like cardio tonic, analgesic, anaesthetic, anti-inflammatory and blood pressure elevation effects. Another species of *Aconitum* has antibacterial, antidysentric, antirhematic and CNS stimulant activities. [36]

***Sunthi (Zingiber officinale)***

Ginger is also one of the household medicines used against common cold, cough and indigestion. Its medicinal values are well documented. Adel and Prakash, 2014, have reported its antioxidant properties. Ginger controls vomiting and nausea during pregnancy. It controls blood pressure by blocking calcium channels. [37]

***Gandira (Coleus forskohlii)***

This plant has medicinal properties like antihypertensive, anti obesity, anti glaucoma, anti asthma, anti metastatic, anti depression, ant psoriasis and antithrombotic. [38]

***Ela (Elettaria cardamomum)***

Cardamom is another important culinary ingredient used for its characteristic aroma. Apart from the aroma it has medicinal value. Verma *et al*, 2009, have reported blood pressure lowering, fibrinolysis enhancing and antioxidant activities of Cardamom. [39] Khan *et al*, 2011 have shown the pharmacological basis of cardamom as medicine for asthma. [40]

***Pata (Cyclea peltata)***

*C. peltata* has pharmacological activities such as diuretic, anticancer, anti oxidative, anti toxic, anti-inflammatory etc. [41]

**Ajaji (*Carum carvi*)**

*Carum carvi* is reported to have hypoglycaemic, antioxidant, antibacterial, nootropic, antifungal, anti colitic, anti cholinesterase, anti-inflammatory, anti carcinogenic, immune modulatory, anticancer, anti mutagenic and diuretic activities. [42]

**Kadavanga Phalam (*Oroxylum indicum*)**

The therapeutic potential of this plant is reviewed by Ahad *et al*, 2012. It has antibacterial, anti-inflammatory, anti hyperlipidemic, analgesic, hepato protective, anti diabetic, nephro protective, immune modulatory, anticancer, anti mutagenic and gastro protective. [43]

**Ajamoda (*Trachyspermum roxburghianum*)**

The pharmacological evidences shows, its use in traditional system of medicine to treat diarrhea, abdominal spasm (colic), asthma, bronchitis cough, common cold, dyspepsia, lethargy, loss of consciousness, palpitation, vomiting, pain in bladder and kidneys as well as considered useful as anthelmintic, anti gout, antimicrobial, cardio tonic, carminative, condiment, digestive, emmenagogue, stimulant and stomachic. [44]

**Sarshapa (*Brassica alba* Syn. *Sinapsis alba*)**

This species is known to be of great medicinal importance due to its antineoplastic, antimicrobial, and insecticidal activities. [45-47]

**Vacha (*Acorus calamus*)**

The medicinal properties of *A. calamus* were reported by Kumar and Vandana, 2012. [48] This plant has activities like antiulcer and cyto protective, analgesic, antispasmodic, anti-inflammatory, anticonvulsant and antibacterial.

**Jeeraka (*Cuminum cymium*)**

Sahoo *et al* 2014 have described the medicinal properties of *C. cymium* such as antidiabetic, antioxidant, anti bacterial, antifungal, broncho dialatory, hepato protective, renal protective, chemo preventive, antiepileptic, memory enhancer and hypo lipidemic. [49]

**Hingu (*Ferula foetida*)**

*Ferula* is medicinal herb used for the treatment of different diseases, in ayurveda and traditional medicinal practice particularly in gastrointestinal disorders, nervous disorders and respiratory problems. It has several pharmacological activities such as anti ulcerogenic, anti flatulent, digestive, antibacterial, antiviral, anti fungal, cancer chemo preventive, anti-diabetic, antispasmodic, anti haemolytic, antihelmintic, anti parasitic, anti infertility, anti-hepatotoxic and chemo protective and nephro protective. [50, 51] There is also some evidence of the beneficial cardiovascular properties of *F. foetida* including anti cholesterolemic, anticoagulant, anti-inflammatory and antioxidant activities. [52]

**Vidanga (*Embelia ribes*)**

*Embelia ribes* is reported to have analgesic. anthelmintic, anti anxiety, anti bacterial, anti cancer, anti convulsant, antidepressant, anti fertility, antifungal, anti mitotic, anti genotoxic, antioxidant, neuro protective, antihistaminic, cardio protective, nephro protective and anti diabetic activities. [53]

**Pasugandha (*Cleome gynandra* Syn. *Gynandropsis pentaphylla*)**

*Gynandropsis pentaphylla* plant has been traditionally used as anthelmentic, rubefacient and used internally for expulsion of round worms & externally as counter-irritant. [54, 55] This plant is also used in cough and as ant scorbutic, diaphoretic; emollient. It also finds application on wounds and cobra bites. [56]

**Magadhi (*Piper longum*)**

Kumar *et al*, 2011 have reviewed the various health benefits of *Piper longum*. with many important medicinal values such as anticancer, antioxidant, hepato protective, anti-inflammatory, immune modulatory, antimicrobial, anti hyperlipidemic, analgesic, antidepressant, anti amoebic, vasodialtory, bioavailability enhancer due the presence of piperine in it, anti obesity activity, radio protective, cardioprotective and antifungal activities. [57]

**Chavya (*Piper retrofractum*)**

Traditionally it has been used as stimulant, carminative, tonic, anti hypertensive, muscle relaxant, antifungal and antibacterial. [58]

**Chitraka (*Plumbago zeylanica*)**

This plant has medicinal roles such as antimicrobial, anti ulcer, anti obesity, anti-inflammatory, hypo cholestrolemic, hepato protective, wound healing, cytotoxic, anticancer and antiproliferative. [59]

**Sunthi (*Zingiber officinale*)**

Ginger is also one of the household medicines used against common cold, cough and indigestion. Its medicinal values are well documented. Abel and Prakash, 2014, have reported its antioxidant properties. Ginger controls vomiting and nausea during pregnancy .It controls blood pressure by blocking calcium channels. [60]

**Materials and methods**

Ayaskriti medicine was procured from standard Ayurvedic shop from Chennai. Antioxidant studies, namely, DPPH assay, FRAP assay, Hydrogen Peroxide scavenging activity assay and were conducted by standard methods.

**Antioxidant Study**

Antioxidant study was performed by DPPH Assay, FRAP Assay and Hydrogen Peroxide Scavenging Activity assay.

**DPPH Assay (1,1-diphenyl-2-picrylhydrazyl) (Bliss, 1958) [61]**

The sample was dissolved in Ethanol in 1mg/ml concentration and used as stock. From the stock, various concentrations (100, 200, 300, 400mg) were taken for further analysis.

Respective solvents were taken as negative control.

Conc. = Concentration of the sample

OD = OD of the sample

Neg. Control = The Solvent

Activity =  $\text{Neg. Control} - \text{OD} / \text{Neg. Control}$

% of Activity =  $\text{Activity} / 100$

IC<sub>50</sub> =  $50 - c \text{ value} / m \text{ value}$

IC<sub>50</sub>/ml =  $\text{IC}_{50} / 3$  (3 ml of DPPH for the assay. To find the activity in 1 ml, the value had been divided by 3).

**FRAP Assay (Ferric Reducing/Oxidant Power) (Pulido *et al.*, 2000) [62]**

Ayaskriti was dissolved in Ethanol. Triplicates had been put for all the Processes.

Conc. = Concentration of the sample

OD = OD of the sample

Linearity (y) =  $mx + c$

M = Slope

C = The point x crosses y axis

X =  $\text{OD} - c \text{ value} / m \text{ value}$

mM Fe/mg =  $X \text{ value} / \text{concentration} \times 1000$

Mean = Average of mM Fe/mg

STDEV = Standard Deviation for mM Fe/mg.

**Hydrogen Peroxide Scavenging Activity (Ruch *et al.* 1989) [63]**

Ayaskriti was dissolved in Ethanol.

Triplicates had been put for all the Processes.

Conc. = Concentration of the sample

OD = OD of the sample

Neg. control = The solvent

Activity =  $\text{Negative control} - \text{OD} / \text{Negative control}$

% of activity =  $\text{Activity} / 100$

Mean = Average of % of Activity

STDEV = Standard Deviation of % of Activity

Graph = (For Mean of % of Inhibition vs samples) Drawn using 2D clustered column.

## RESULTS AND DISCUSSION

**No. 1 Indicates the results of DDPH assay with Ethanol Ayaskriti.**

Sl.No	Solution	Conc.	OD	Neg.Control	% Activity	m value	C value	IC50	IC50/ml
1	Ethanol	100	0.493	0.989	53.18504	0.1814	17.533	178.982	59.66005
2		200	0.378		61.77958				
3		300	0.261		73.60971				
4		400	0.193		80.48534				

From the results it shows that IC50/ml was lowest value (59.66005) indicating highest activity.

### FRAP test Results are mentioned in Table No.2

**Table No .2 Indicates the FRAP assay patterns of Ayaskriti in Ethanol solution**

Solvent	Conc.	OD	m Value	c Value	X	mM Fe(II)/mg	Mean	STDEV
Ethanol	10	0.347	0.0274	0.1086	8.70073	87.00729927		
	10	0.317	0.0274	0.1086	7.60583	76.05839416		
	10	0.325	0.0274	0.1086	7.89781	78.97810219	80.68	5.67

From the Table no. It is clear that ethanol solution Ayaskriti indicated antioxidant activity (80.68).

**Table 3 Indicates the Hydrogen Peroxide scavenging activity of Ayaskriti**

Solvent	Conc	OD	Neg. Control	% Activity	Mean	STDEV
Ethanol	100	0.448	0.748	40.10695		
	100	0.414	0.748	44.05241		
	100	0.498	0.748	33.42246	39.39	5.65

From table no. 3 it is clear that Ayaskriti has antioxidant activity as averaged to 39.39 % with regard to Hydrogen peroxide scavenging activity.

The above results clearly indicate the antioxidant activity of Ayaskriti. Antioxidants play a vital role in the homeostasis of the body. The antioxidant property of Ayaskriti could be one of the several other reasons to use it as a medicine to treat anemia. The present work is one step in the direction to understand the medicinal efficacy of Ayaskriti by using various other parameters and the work is in progress.

## CONCLUSION

From the positive antioxidant results of Ayaskriti it is understood that this medicine reflects similar properties of most of its constituent ingredients. This indicates that the Ayurvedic proponents have intelligently used the ingredients to make Ayaskriti towards reaching a particular medicinal goal.

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