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COMPARATIVE ANTIOXIDANT STUDYOF HERBAL FORMULATIONS WITH REFERENCE OF ASHWAGANDHA

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Abstract: Antioxidants are compounds that inhibit oxidation chemical reaction that can produce free radicals and chain reactions that may damage the cells of organism. This research was conducted in order to compare the antioxidant activity in ayurveda, Siddha & Homeopathic systems of medicine with reference to Ashwagandha herbal formulations and to investigate which formulation have the more antioxidant property among the three system of medicines. The antioxidant properties of the formulation was identified by using : Ferric reducing power, hydrogen peroxide radical scavenging activity, DPPH assay, nitric oxide radical scavenging activity. This study concluded that Ayurveda system shows better antioxidant property than the other two systems. So it is preferable to use ayurvedic system than other two system of medicine in cases to get the effect of antioxidant properties.

INTRODUCTION:

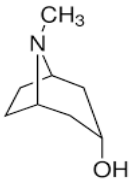
Pharmacognosy is the study of plants and other natural substances as possible sources of drugs. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical, and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources".[1]

The system of medicines which are considered to be Indian in origin or the systems of medicine, which have come to India from outside and got assimilated in to Indian culture are acknowledged as Indian Systems of Medicine. India has unique distinction of having six systems of medicine in this category. They are:- Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homeopathy.

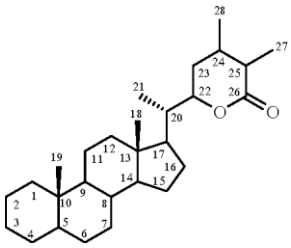
Ayurveda literally means the Science of life. It is presumed that the fundamental and applied principles of Ayurveda got organized and enunciated around 1500 BC. Siddha system of medicine is practiced in some parts of South India especially in the state of Tamilnadu. Homeopathy, also known as homeopathic medicine, is a medical system that was developed in Germany more than 200 years ago. It's based on two unconventional theories: "Like cures like" & "Law of minimumdose".[2]

Ashwagandha is a short, tender perennial shrub growing 35–75 cm (14–30 in) tall. Tomentose branches extend radially from a central stem. Leaves are dull green, elliptic, usually up to 10–12cm (3.9–4.7 in) long. The flowers are small, green and bell-shaped. The ripe fruit is orange-red.The main phytochemical constituents are withanolides, which are triterpene lactones - withaferin A, alkaloids, steroidal lactones, tropine, and cuscohygrine. Some 40 withanolides, 12 alkaloids, and many sitoindosides have been isolated. Withanolides are structurally similar to the ginsenosides of Panax ginseng ,leading to a

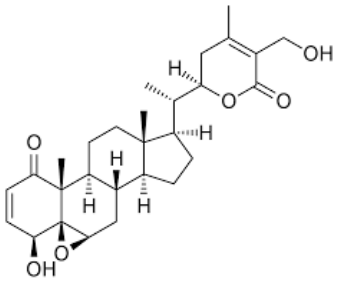
common name for *W. somnifera*, "Indian ginseng".[3]



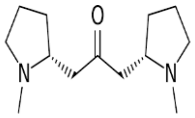
Tropine



Steroidal Lactone



Withaferin A



Cuscohygrine

Uses include: Helps Fight Depression, Increases Muscle Mass, Increase Fertility in Men, Controls Diabetes, Enhances Memory, Helps reduce Stress & Anxiety, Boosts Immunity, Antibacterial Properties, Lowers Cholesterol, boosts Thyroid Function. [4]

Antioxidants are compounds that hinder oxidation, a chemical reaction that can create free radicals and chain reactions that may damage the cells of organisms. To balance oxidative stress, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione.[5]
Antioxidant properties can be estimated by various methods like:-

- Ferric reducing power
- Hydrogen peroxide radical scavenging activity
- DPPH assay
- Nitric oxide radical scavenging activity.

EXPERIMENTAL METHODS :

SELECTION OF FORMULATIONS:

For this study we purchased the following two tablets and a tincture:

- Himalaya ashwagandha general wellness tablet (Ayurveda)
  Indications: Stress, Improve immunity, Low sexual vitality, Depression[6]
- Ashwagandha tincture (homeopathy)
  Indications: Nervous disorder, Insomnia, Aphrodiasiac, Enhance immunity, Reduces stress, Increase the quantity & quality of the sperm[7]
- Ashwagandha siddha tablet (siddha)
  Indications: Protects the immune system, relieve stress ,Improves learning skills and memory Reduces anxiety and depression , Helps reduce brain-cell degeneration Stabilizes blood sugar, Helps lower cholesterol, anti-inflammatory , Natural diuretic, Enhance vitality and vigor[8]

1. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) Assay:

The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity.The method is commonly used due to requirement of short time for the analysis. The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical is very stable, reacts with compounds that can donate hydrogen atoms, and has a UV–vis absorption maximum at 515 nm. The method is based on the scavenging of DPPH by antioxidants, which upon a reduction reaction decolorizes the DPPH methanol solution. The assay measures the reducing ability of antioxidants toward the DPPH radical.[6]

PRINCIPLE:

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which convet to yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as, (DPPH) + (H-A) ◊ DPPH-H + (A) Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration shows the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION:

Dissolve 4mg of DPPH in 100ml of ethanol to prepare 0.1mM of DPPH solution.

PROCEDURE:

Different volumes of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark conditions at room temperature for 20 min. After 20 min, the absorbance of the mixture was measured at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated by the following formula,

$$\%RSA=(Abs\ Control - Abs\ sample) / Abs\ control \times 100$$

Where,

RSA is the Radical Scavenging Activity
Abs control is the absorbance of DPPH radical + ethanol
Abs sample is the absorbance of DPPH radical + plant extract. [7]

2.Ferric Reducing Power:

The ferric reducing antioxidant power (FRAP) assay is a typical ET-based method that measures the reduction of ferric ion (Fe^{3+})-ligand complex to the intensely blue-colored ferrous (Fe^{2+}) complex by antioxidants in an acidic medium.[8]

PRINCIPLE:

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to compose ferric–ferrous complex that has an absorption maximum at 700 nm.[9]

PROCEDURE:

Methanolic extract (1.0 ml) of varying concentrations (20-100 µg/ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 %). The mixture was incubated at 50 °C for 20 minutes with TCA (10 %: 2.5 ml). The mixture is then centrifuged at 3000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (1%). The absorbance of the reaction mixture was measured at 700 nm by using the UV-Visible spectrophotometer. The higher absorbance of the reaction mixture indicated greater reducing power. The reducing power of methanolic extracts of *Withania somnifera* was compared with that of standard antioxidant L-ascorbic acid (1000 µg/ml).[10]

3.Hydrogen Peroxide Radical Scavenging Activity:-

PRINCIPLE

Hydrogen peroxide (H_2O_2) scavenging activity of natural antioxidants present in plant extracts has been determined widely by measuring decrement of H_2O_2 in an incubation system containing H_2O_2 and the scavenger using the classical UV-method at 230 nm.[11]

PROCEDURE

Methanolic extract of *Withania somnifera* aliquots of concentrations ranging 20-100 µg/ml were added to 0.6 ml hydrogen peroxide (40 mM) solution in an already prepared phosphate buffer (pH 7.4). Then the reaction mixtures were then incubated for 15 minutes at room temperature and absorbance reading at 230 nm against phosphate buffer was taken as blank. The percentage of inhibition calculated based on the formula as:

$$Chelating\ Activity\ (\%) = (A1 - A2) /A1 \times 100$$

Where

A1 :- absorbance of the reaction mixture without extract
A2 :- absorbance of the reaction mixture with extract [10]

4.Nitric Oxide Radical Scavenging Activity:-

PRINCIPLE

To determine the scavenging activity of the plant extracts against nitric oxide radical. Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. The extracts reduces the amount of nitrite formed between oxygen and nitric oxide generated from sodium nitroprusside. The absorbance was measured at 546nm and the percentage inhibition is calculated using the below formula.[26]

PROCEDURE

Nitric oxide radicals are produced using sodium nitroxide solution. 1ml of 10 mM sodium nitroprusside solution was mixed with 1 ml of methanolic extract of *Withania somnifera* formulations of different concentrations (20-100 µg/ml) in phosphate buffer (0.2 M pH 7.4). The mixture is then incubated at 30 °C for 160 minute. After incubation the reaction mixture was mixed with 1.0 ml of pre-prepared Griessreagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dichloride, and 2% phosphoric acid). The absorbance was calculatef at 546 nm and the percentage of inhibition was calculated using the formula

$$\text{Percentage of inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where

Acontrol:- absorbance of reaction mixture
Asample:- absorbance of reaction mixture (with Sample)

A decrease in the absorbance indicates a high nitric oxide scavenging activity[10]

RESULT

1- DPPH ASSAY

Table 1

S.NO	sample	concentration (µg/ml)	Absorbance (at 517nm)	Percentage Inhibition (%)
1	control	_	0.095	-
2	standard	20	0.017	82
		40	0.011	88
		60	0.008	91
		80	0.005	94
		100	0.002	97
3	Ayurvedha	20	0.027	71.57
		40	0.025	73.68
		60	0.023	75.78
		80	0.017	82.10
		100	0.015	84.21
4	Siddha	20	0.032	87.36
		40	0.028	91.57
		60	0.027	92.63
		80	0.025	94.73

		100	0.022	97.89
5	Homeopathy	20	0.091	4.21
		40	0.079	16.84
		60	0.073	23.15
		80	0.067	29.47
		100	0.059	37.89

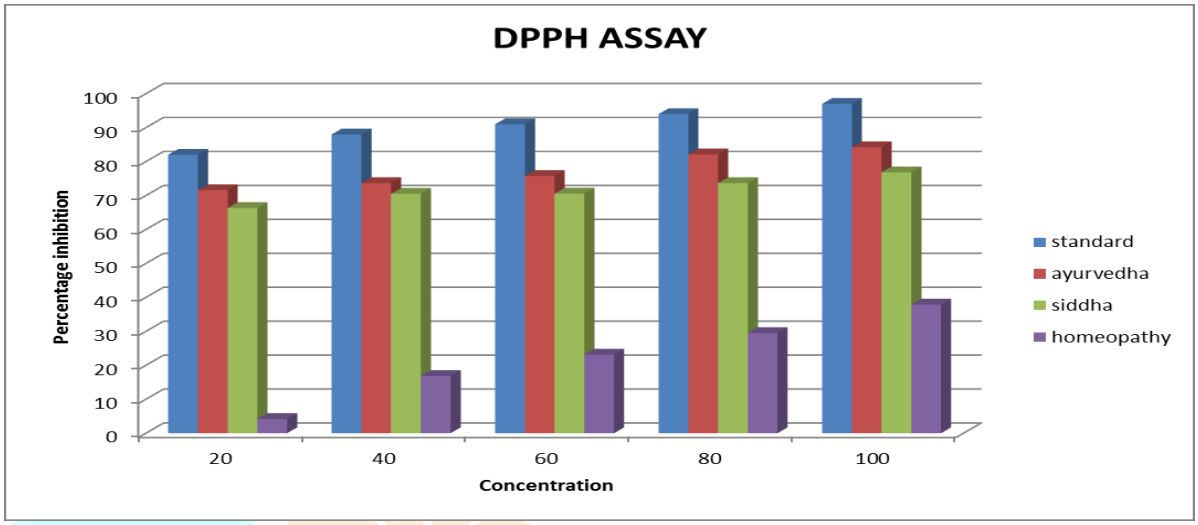


Figure 1

2- FERRIC REDUCING POWER

Table 2

SL.NO	SAMPLE	CONCENTRATION(µg/ml)	ABSORBANCE (at 700nm)
1	Standard	20	0.61
		40	0.82
		60	0.89
		80	1.08
		100	1.24
2	Ayurveda	20	0.151
		40	0.170
		60	0.173
		80	0.190
		100	0.237
3	siddha	20	0.042
		40	0.065
		60	0.125
		80	0.178
		100	0.193
4	homeopathy	20	0.010
		40	0.012
		60	0.020
		80	0.023
		100	0.026

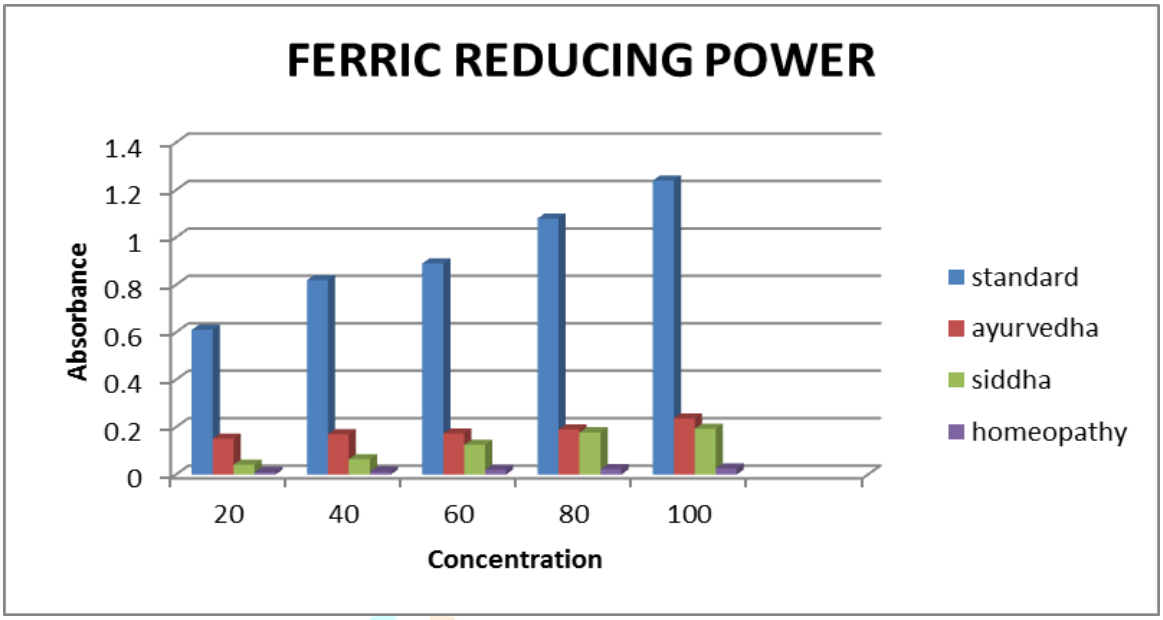


Figure 2

3. HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY:

Table 3

SL.NO	SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE (at 230nm)	PERCENTAGE INHIBITION (%)
1	CONTROL		0.830	
2	STANDARD	20	0.473	43
		40	0.348	58
		60	0.332	60
		80	0.315	62
		100	0.240	71
3	AYURVEDHA	20	0.668	19.51
		40	0.653	21.32
		60	0.649	21.80
		80	0.643	22.53
		100	0.632	23.85
4	SIDDHA	20	0.733	11.68
		40	0.731	11.92
		60	0.728	12.28
		80	0.711	14.33
		100	0.700	15.66
5	HOMEOPATHY	20	0.745	10.24
		40	0.736	11.32
		60	0.734	11.56
		80	0.724	12.77
		100	0.719	13.37

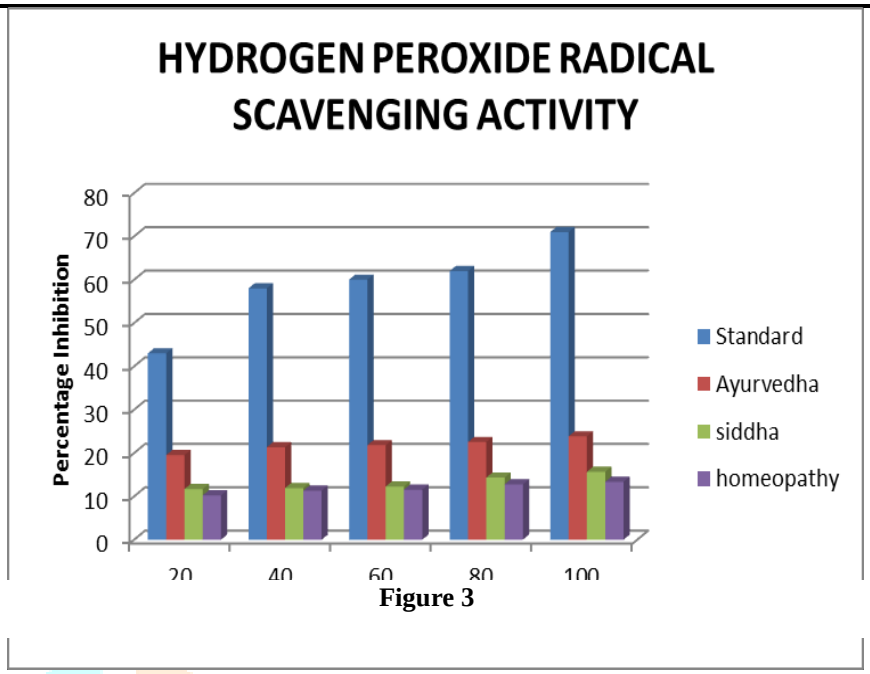


Figure 3

4. NITRIC OXIDE RADICAL SCAVENGING ACTIVITY:

Table 4

SL.NO	SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE (at 546nm)	PERCENTAGE INHIBTION (%)
1	CONTROL		0.359	
2	STANDARD	20	0.104	71
		40	0.071	80
		60	0.039	89
		80	0.021	94
		100	0.007	98
3	AYURVEDHA	20	0.131	63.5
		40	0.127	64.62
		60	0.095	73.53
		80	0.077	78.53
		100	0.038	89.41
4	SIDDHA	20	0.136	62.1
		40	0.131	63.5
		60	0.118	67.1
		80	0.115	67.9
		100	0.113	68.52
5	HOMEOPATHY	20	0.198	44.84
		40	0.190	47.07
		60	0.160	55.43
		80	0.141	60.72
		100	0.117	67.40

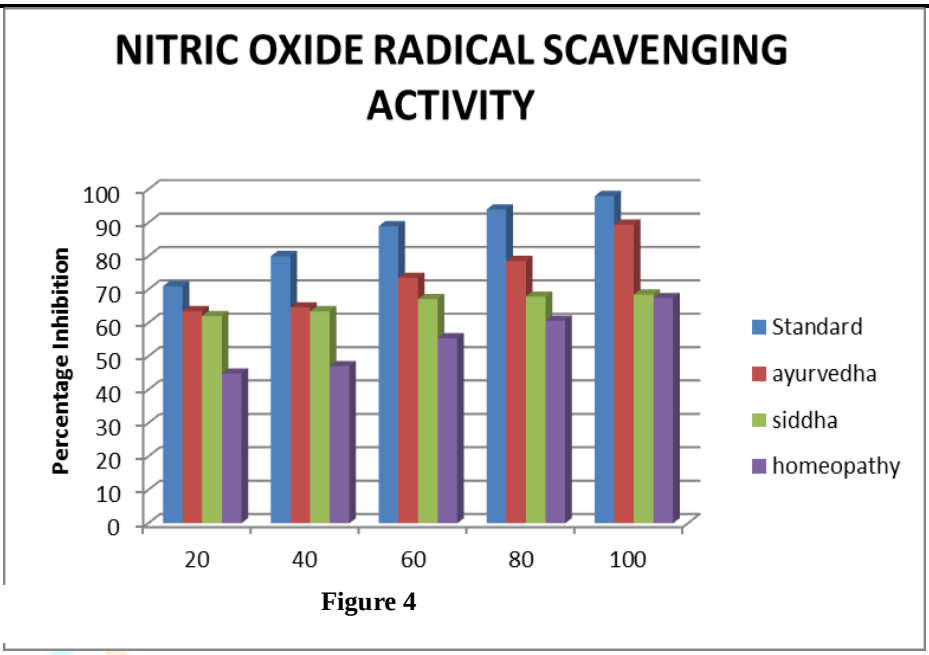


Figure 4

CONCLUSION:

Antioxidants are substances, which can inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals which may damage the cells of organisms. Comparative antioxidant study of herbal formulations with reference of Ashwagandha in Ayurveda, Siddha, Homeopathy system of medicine was carried out using different methods: DPPH Assay, ferric reducing power, hydrogen peroxide radical scavenging activity, nitric oxide radical scavenging activity. On evaluating the results, DPPH Assay illustrated that Ayurveda had greater percentage inhibition value, ferric reducing power showed more absorbance for Ayurveda formulation, Ayurveda system produced better percentage inhibition for hydrogen peroxide radical scavenging activity & nitric oxide radical scavenging activity. Hence it was concluded that, Ayurveda system showed a stronger antioxidant property than other two systems, followed by siddha & then homeopathy. Hence, formulations in ayurveda system can be used for the indications which can be cured by aswagandha using their antioxidant properties.

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