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CONGLOMERATION OF POLY HERBAL DRUGS AS LEHYA FORMULATION HAVING ANTIOXIDANT ACTIVITY WITH EXTRACTS OF ANNONA SQUAMOSA, CASSIA FISTULA AND ILLICIUM VERUM

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ABSTRACT

Herbal medicine also called Botanical medicine or phyto medicine refers to using a plant parts such as seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Recently, World Health Organization estimated that 80% of people worldwide rely on herbal medicines. Now a day, Digoxin is a purified cardiac glycoside that is extracted from the foxglove plant, *Digitalis lanata*. Digoxin is widely used in the treatment of various heart conditions, namely atrial fibrillation and atrial flutter. Elements that are more reluctant to lose electrons are not easily oxidized. Nonmetals like nitrogen, oxygen, and chlorine are not easily oxidized. Antioxidants block the process of oxidation by neutralizing free radicals. The intensity of the yellow colour formed was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards. The percentage of inhibition was determined by comparing test with standard. The polyherbal formulation in the form of Lehya meant for antioxidant activity is prepared by using suitable plants reported to possess antioxidant properties such as leaves of Annona squamosa, leaves of Cassia fistula and fruits of Illicium verum. The preparation for standardization included loss on drying, total ash, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value and PH was estimated. Formulated poly herbal lehya was proved to show pronounced in-vitro antioxidant activity and its percentage inhibition was at 200µg concentration was found to be 95.42 of DPPH inhibition, and 69.49 of Nitric oxide.

KEYWORDS: Lehya formulation, in-vitro antioxidant activity, Annona squamosa, Cassia fistula, Illicium verum, Glutathione peroxidase, Arginine, Grie's reagent.

INTRODUCTION

***Herbal medicine*:** Herbal medicine also called Botanical medicine or phyto medicine refers to using a plant parts such as seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control in the treating and preventing disease. A large amount of archaeological evidence exists which indicates that humans were using medicinal plants during the Paleolithic, approximately 60,000 years ago. Furthermore, other non-human primates are also known to ingest medicinal plants to treat illness. In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Almost one fourth of pharmaceutical drugs are derived from botanicals. Recently, World Health Organization estimated that 80% of people worldwide rely on herbal medicines. In Germany, about 600 - 700 plant based medicines are available and are prescribed by some 70% of German physicians. Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. The *Sushruta* Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates (father of medicines) e.g. *De herbis et curis* and - especially - Galen (e.g. *Therapeutics*), provided the pattern for later western medicine. Now a days, Digoxin is a purified cardiac glycoside that is extracted from the

foxglove plant, *Digitalis lanata*. Digoxin is widely used in the treatment of various heart conditions, namely atrial fibrillation and atrial flutter.

***Oxidation*:** oxidation is the loss of electrons. It occurs when an atom or compound loses one or more electrons. Metals such ad sodium, magnesium, and iron lose electrons more easily and are said to be *easily oxidized*. Elements that are more reluctant to lose electrons are not easily oxidized. Nonmetals like nitrogen, oxygen, and chlorine are not easily oxidized. When a compound is oxidized, its properties get changed. For example, when an iron object undergoes oxidation, it is transformed such that unoxidized iron is a strong, structurally sound metal, while oxidized iron will be brittle, reddish powder.

Oxidation-Reduction (redox) reactions: Mostly, oxidation occurs in tandem with a process called reduction. Reduction is the process of gaining one or more electrons. In an oxidation-reduction or redox reaction, one atom or compound will steal electrons from another atom or compound. A classic example of a redox reaction is rusting. When rusting happens, oxygen steals electrons from iron. Oxygen gets reduced while iron gets oxidized. The result is a compound called iron oxide, or rust. There is a very handy pneumonic device for remembering the differences between oxidation and reduction -- *LEO the lion says GER*. The letters in LEO and GER stand for Loss of Electrons is Oxidation, Gain of Electrons is Reduction.

Antioxidant process: Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized. Hence, there is a constant need to replenish our antioxidant resources.

Chain-breaking - When a free radical releases or steals an electron, a second radical is formed. This molecule turns around and does the same to a third molecule, continuing to generate more unstable products. The process continues until termination occurs -- either the radical is stabilized by a chain-breaking antioxidant such as beta-carotene and vitamins C and E.

Preventive - Antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase prevent oxidation by reducing the rate of chain initiation. They also prevent oxidation by stabilizing transition metal radicals such as copper and iron.

The effectiveness of any given antioxidant in the body depends on involvement of free radical. Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radicals intermediates and inhibit other oxidation agents such as thiols, ascorbic acid or polyphenols.[1] As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis etc.

Classification of anti-oxidants

1. Based on solubility

(a) Hydrophilic antioxidants:- They are soluble in water. Water soluble antioxidants react with oxidants react with oxidants in the cell cytoplasm and blood plasma.

(b) Hydrophobic antioxidants:- They are soluble in lipids. Lipid soluble antioxidants protect cell membranes from lipid peroxidation.

2. Based on line of defense

(a) First line defense (preventive antioxidant):-These are enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GTX), glutathione reductase and some minerals like Se, Mn, Cu etc. SOD mainly acts by quenching of superoxide (O2), catalase by catalyzing the decomposition of hydrogen peroxide (H2O2) to water and oxygen. GTX catalyzes the reduction of H2O2 and lipid peroxide generated during lipid peroxidation to water using reduced glutathione as substrate.

(b) Second line defense (Radical scavenging antioxidant):- These are glutathione, Vitamin C, uric acid, albumin, bilirubin, vitamin E, carotenoids; flavonoid etc. β-carotene is an excellent scavenger of singlet oxygen. Vitamin C interacts directly with radicals like O2, OH. GSH is a good scavenger of many free radicals like O2, OH and various lipid hydro peroxides and may help to detoxify many inhaled oxidizing air pollutants like ozone.

(c). Third line defense (Repair and de-novo enzymes):- These are a complex group of enzymes for repair of damaged DNA, protein, oxidized lipids and peroxides and also to stop chain propagation of peroxyl lipid radical. These enzymes repair the damage to biomolecules and reconstitute the damaged cell membrane.

Anti-oxidant nutrients

Antioxidant action is also part of the role of vitamins C, E, folate and beta carotene and also the minerals selenium, manganese, copper and zinc. Much of the marketing of antioxidants concentrates on these nutrients. However, studies of antioxidant minerals and vitamins taken as supplements have been disappointing and it appears that the complex array of antioxidants present naturally in plants as well as those the body produces in reaction to stress may be more important.

Antioxidants & Cardiovascular disease: There is widespread agreement from many studies that a diet high in fruits and vegetables reduces the risk of cardiovascular disease and some types of cancer. Growing evidence also shows that whole grains offer protective effects against both types of disease, although this may not be related specifically to their antioxidant capacity. Nuts are also protective against cardiovascular disease, but current evidence suggests this is due to their essential fats, minerals and an amino acid called arginine that is plentiful in nut protein. The jury is still out on whether antioxidants in green and black tea help prevent cardiovascular disease.

With red wine, there is good evidence for protection against cardiovascular disease, but benefits are wiped out if intake is high. It is still unclear how much of the benefit comes from alcohol's effect of raising HDL (so-called 'good') cholesterol and how much the antioxidant compound, resveratrol, is responsible. The evidence is insufficient to suggest that non-drinkers should start drinking. Much research is attempting to elucidate the potential benefits of dark chocolate, although researchers agree that fruit and vegetables are a wiser dietary choice, since their antioxidants come without chocolate's high load of fat and kilojoules.

Antioxidants & Cancer: Research continues to examine possible protective roles against cancer due to various foods, including spices, herbs and tea, fruit, vegetables and antioxidants in extra virgin olive oil. Results of recent studies do not support antioxidant supplements, but health authorities continue to find benefits of a high intake of fruits and vegetables. There is concern about possible interactions between high doses of some antioxidant supplements and chemotherapy drugs that work by using free radicals to kill cancerous cells.

Antioxidants & Macular degeneration: Some positive messages were expected from studies of particular antioxidants in macular degeneration, the major cause of blindness in elderly people. Some (but not all) studies initially suggested that specific antioxidant supplements helped protect against further degeneration, while others backed greater benefits from vegetables and fruits rich in the antioxidants lutein and zeaxanthin. Egg yolk is also a good source of these compounds. A recent and extensive review reports no benefits of vitamin E, beta carotene or any antioxidant supplements for preventing age-related macular degeneration.

Antioxidants & Immune system: Frequent claims suggest that antioxidants benefit the immune system. In theory, that sounds valid, but specific evidence continues to elude scientists.

Antioxidants & Anti-ageing: For many people, the greatest interest is in antioxidants' anti-ageing potential. Since the body's production of its own antioxidants decreases in old age, few doubt the potential value of dietary sources. However, there is no evidence that extra antioxidants stop hair greying, prevent wrinkles or provide a 'fountain of youth'.

MATERIALS AND METHODS

***1) Annona squamosal* (Custard apple)**

Annona squamosa is a small deciduous or semi-evergreen tree in the plant family Annonaceae. It is best known for its fruit, called custard apple, a common name it shares with fruits of several other species in the same genus: *A. cherimola* and *A. squamosa* or sometimes it is called wild-sweetsop, bull's heart, bullock's-heart, or ox-heart.

Synonyms: *Annona lutescens* Saff, *Annona excelsa* Kunth, *Annona laevis* Kunth, *Annona longifolia* Sessé & Moc., *Annona riparia* Kunth.

Scientific Classification

Kingdom	: Plantae
(Unranked)	: Angiosperms
(Unranked)	: Magnoliids
Order	: Magnoliales
Family	: Annonaceae
Genus	: Annona
Species	: A. squamosal.

Charecterstic features

It is a small deciduous or semi-evergreen tree reaching 8 metres (26 ft) to 10 metres (33 ft) tall with an open, irregular crown.

Stems and leaves

The slender leaves are hairless, straight and pointed at the apex (in some varieties wrinkled), 10 centimeters (3.9 in) to 20 centimeters (7.9 in) long and 2 centimeters (0.79 in) to 7 centimeters (2.8 in) wide.

Flowers

The yellow-green flowers are generally in clusters of three or four 2 centimeters (0.79 in) to 3 centimeters (1.2 in) diameter, with three long outer petals and three very small inner ones.

Fruits

The fruits vary in shape, heart-shaped, spherical, oblong or irregular. The size ranges from 7 centimeters (2.8 in) to 12 centimeters (4.7 in), depending on the cultivar. When ripe, the fruit is brown or yellowish, with red highlights and a varying degree of reticulation, depending again on the variety. The flesh varies from juicy and very aromatic to hard with a repulsive taste. The flavor is sweet and pleasant, akin to the taste of 'traditional' custard.

2) Cassia fistula

Cassia fistula prefers deciduous forests, subtropical and tropical regions. They also found in moist forest, woodlands, and mountain habitats. Cassia fistula is widely grown as an ornamental plant. It blooms in late

spring. Growth for this tree is best in full sun on well-drained soil.

Synonyms

Common Name	–	Golden shower tree
Local Name	–	Amaltas
Other Names	–	Purging Cassia, Golden Chain Tree, Indian Laburnum

Scientific Classification

Kingdom	–	Plantae
Division	–	Magnoliophyta
Class	–	Magnoliopsida
Order	–	Fabales
Family	–	Fabaceae
Genus	–	Cassia
Species	–	C. fistula L.

Characteristic features

Leaves: The leaves are smooth, ovate shape, hairy below, alternate, pinnate, and deciduous, with 3-8 pairs of leaflets. The leaf can range from 15 – 60 cm long, with each leaflet ranging from 7 – 15 cm long, and 2-7 cm broad. The leaves will fall periodically, only to be replaced with new foliage. Leaves are absent during flowering time. Leaves usually drop in April as a prelude to flowering which occurs from May to early July. The leaves on this tree are green year round, and remain green until they fall off and are replaced.

Bark: The bark of the young tree is a grey, smooth to slightly ridged and slender, and changes to a darker grey-brown when mature. Stems or young twigs gets spared and forms densely hairy.

Fruit: Fruit is legume, pendulous, cylindrical, and brown in color, 20 to 60 cm long, 1 to 2.5 cm broad, with a pungent odor and containing several seeds. Seeds lenticular, light brown, lustrous. Flower buds are green when immature, and mature into brown to purple-black pods.

Seeds: The pods contain approximately 30 -100 large hard flat, round seeds. Seeds lenticular, lustrous, and light brown in color.

***3) Illicium verum* (Star anise)**

Morphological study

Illicium verum is a medium-sized evergreen tree native to northeast Vietnam and southwest China. A spice commonly called star anise, star anise seed, Chinese star anise or badiam that closely resembles the star-shaped pericarp of the fruit of Illicium verum which are harvested just before ripening.

Standardized common name (English): Star anise.
Ayurvedic name: Takkola.

Scientific classification

Kingdom	-	Plantae
(unranked)	-	Angiosperms
Order	-	Austrobaileyales
Family	-	Schisandraceae
Genus	-	*Illicium*
Species	-	*I. verum*

Characteristic features

Fruits: The fruit is pedunculate, consisting of eight stellately arranged 10 mm long boat-shaped carpel's, fleshy at first, later becoming woody on drying, wrinkled, straight beaked.

Colour: Brown, dehiscent on the upper suture, internally reddish-brown, glossy and containing a single, flat, oval, lustrous, brittle and brownish-yellow seed.

Odour: The odour of the fruit is agreeable, anise-like, and the carpel's taste sweet and aromatic.

Size: The fruit whorl is 2.5-4.5 cm in diameter with individual carpel's of about 9-19 mm length. The seed is 8-9 mm long and 6 mm broad. The endosperm is bulky and embryo disorganized.

Composition

As with all spices, the composition of anise varies considerably with origin and cultivation method. These are typical values for the main constituents.

- Moisture: 9–13%
- Protein: 18%
- Fatty oil: 8–23%
- Essential oil: 2–7%
- Starch: 5%
- N-free extract: 22–28%
- Crude fibre: 12–25%

Collection of plant materials

The leaves of amaltas and seetaful were collected from herbal garden, Near himayath sagar, Moinabad road, and dried fruits of star anise was procured from *"*jonadumbalaya*"* ayurvedic store, Begumbazar, Hyderabad, Telangana, India.

Standardization of raw materials

All the plant materials were processed and powdered after drying and standardized considering following Physicochemical Parameters.

1. Ash value

The ash content of the crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration. Ash value varies with narrow limits in case of the individual drug but varies considerably in case of different drugs.

***a)* Determination of Total Ash**

About 2g of powdered drug was accurately weighed in a silica crucible, which was previously ignited and

weighed. The powdered drug was spread as a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed for constant weight. The percentage of total ash was calculated with reference to the air-dried drug.

b) **Determination of Water-Soluble Ash**
The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of total ash. The difference in weight was considered as the water -soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

c) **Determination of Acid Insoluble Ash**
The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water; the insoluble ash was transferred into pre-weighed silica crucible. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

2. Loss on drying
5 g of the powdered crude drug was accurately weighed in a tarred dish and dried in an oven at 100-105°C. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

3. Solubility value
Solubility values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

a) **Alcohol soluble extractive**
5g of air-dried coarsely powdered drug was macerated with 100 ml 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. It was then filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish dried at 105^0C and weighed. The percentage of ethanol soluble extractive with reference to the air-dried drug was calculated.

b*)* Water soluble extractive
5 g of the air-dried coarse powder of was macerated with 100 ml of Chloroform water (95ml of water + 5ml of Chloroform) in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. It was then filtered rapidly taking precautions against loss of the solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish dried at 105^0C and weighed. The percentage of water-soluble extractive with reference to the air-dried drug was calculated.

Method of preparation of lehya
Composition of lehya for 100 grams

Ingredients	Quantities
Annona squamosa	6gm
Cassia fistula	6gm
Illicium verum	3gm
Jaggery	60gm
Honey	15gm
Ghee	10gm

Procedure
Equal quantities of jaggery and water are measured, mixed well and boiled for 15 minutes to prepare jaggery syrup. The leaves of Annona Squamosa, Cassia Fistula and fruits of Illicium Verum are pulverized to form fine powder. Weighed quantities of Annona Squamosa (6gm), Cassia Fistula (6gm) and Illicium Verum (3gm) fine powders are added to the jaggery syrup slowly. The formed product is stirred continuously till it becomes thick. To this product, ghee is added and stirred on a medium flame for 15 minutes. Honey is added and stirred for 5 minutes. The prepared formulation is removed from the flame, cool it for 10 minutes and transfer into air tight container.

Standardization of lehya by physicochemical parameters
The preparation for standardization included loss on drying, total ash, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value and PH was estimated.

Nitric oxide scavenging activity
Principle
This method is based on the inhibition of nitric oxide radical generated from sodium nitro prusside in buffered saline and measured by grie's reagent. The absorbance of the chromophore is evaluated at 546nm.

Chemicals required
- Sodium nitro prusside, (10Mm, 2mL).
- Phosphate buffered saline (PBS).
- Griess Reagent.
- 1% Sulfanilamide.
- 0.1% Naphthyl ethylene diamine dihhydrochloride.
- Lehya (10, 50, 100, 200, 400, 800, 1000 µg) in DMSO.
- Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg).

Procedure
The nitric oxide scavenging activity of Lehya was determined according to the method. Aqueous solution of sodium nitro prusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with

oxygen to produce nitrate ions and which was measured calorimetrically. 3mL of reaction mixture containing 2mL of sodium nitro prusside (10mM) in phosphate buffer saline (PBS) and 1mL of various concentrations of the Lehya were incubated at 37^0C for 4 hours. Control without test compound was kept in an identical manner. After incubation 0.5mL of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds. Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg/ml) was used as standard. The percentage nitric oxide inhibition was calculated from the following formula.[24]

$$\% \text{ Nitric Oxide Inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

Determination of free radical scavenging activity by DPPH method

Principle

This is one of the widely used methods for screening of anti-oxidant activity of plant drugs. DPPH assay method is based on the reduction of absorbance of ethanol solution of DPPH by free radical scavenger.

Chemicals required

- 1,1-diphenyl-2-picryl hydrazide (DPPH)
- Dimethyl sulphoxide (DMSO)
- Lehya (10, 50, 100, 200, 400, 800, 1000 µg) in DMSO
- Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg)
- Ethanol

Procedure

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25mg of DPPH (150µM) was prepared in 100mL of ethanol. To the 0.2 mL of Lehya of different concentrations, 3.8 ml of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg/ml) was used as standard. The percentage DPPH inhibition was calculated from the following formula.[25]

$$\textbf{\% Decolorization} = [1-(\text{ABS}_{\text{sample}}/\text{ABS}_{\text{control}}] \times 100$$

Determination of free radical scavenging activity by Hydroxyl radical method

Principle

This is one of the widely used methods for screening of anti-oxidant activity of plant drugs. Hydroxyl radical method is based on the reduction of absorbance of ethanol solution of Nash reagent by free radical scavenger.

Chemicals required

- EDTA, Phosphate buffer, TCA, Ascorbic acid, Gallic acid
- ***Nash reagent*** (Ammonium acetate, Glacial acetic acid, Acetyl acetone)
- Extract (10, 50, 100, 200, 400, 800, 1000 µg) in DMSO
- Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg)
- Ethanol

Procedure

Hydroxyl radical scavenging activity involves the reaction mixture that contain 1.0 mL of different concentration of extracts (2–10 mg/mL), 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1.0 mL of DMSO (0.85% in 0.1 mol/L phosphate buffer pH 7.4) and 0.5 mL of 0.22% ascorbic acid. The tubes were capped tightly and heated in a water bath at 80–90 °C for 15 min, the reaction was terminated by adding 1.0 mL of ice-cold TCA (17.5%). To the above reaction mixture, 3.0 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2.0 mL of acetyl acetone were mixed and distilled water was added to a total volume of 1 L) was added and incubated at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards. The percentage of inhibition was determined by comparing test with standard.[26]

$$\% \text{ free radical Inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

RESULTS AND DISCUSSION

Standardization of plant material

Physico chemical parameters of crude drugs

S. No.	Parameters	Values(w/w) Annona squamosa	Values(w/w) Cassia fistula	Values(w/w) Illicium verum
1.	Total ash	4.7%	5.8%	8.4%
2.	Acid insoluble ash	1.6%	0.9%	2.7%
3.	Water soluble ash	2.4%	0.5%	4.3%
4.	Loss on drying	10.8%	10.2%	2.8%
5.	Alcohol soluble extractive	20.2%	18.2%	7.6%
6.	water soluble extractive	25.3%	23.1%	9.1%

The ash values are within the limits mentioned in the herbal pharmacopoeia. Annona squamosa showed maximum percentage of both alcohol soluble and water soluble constituents.

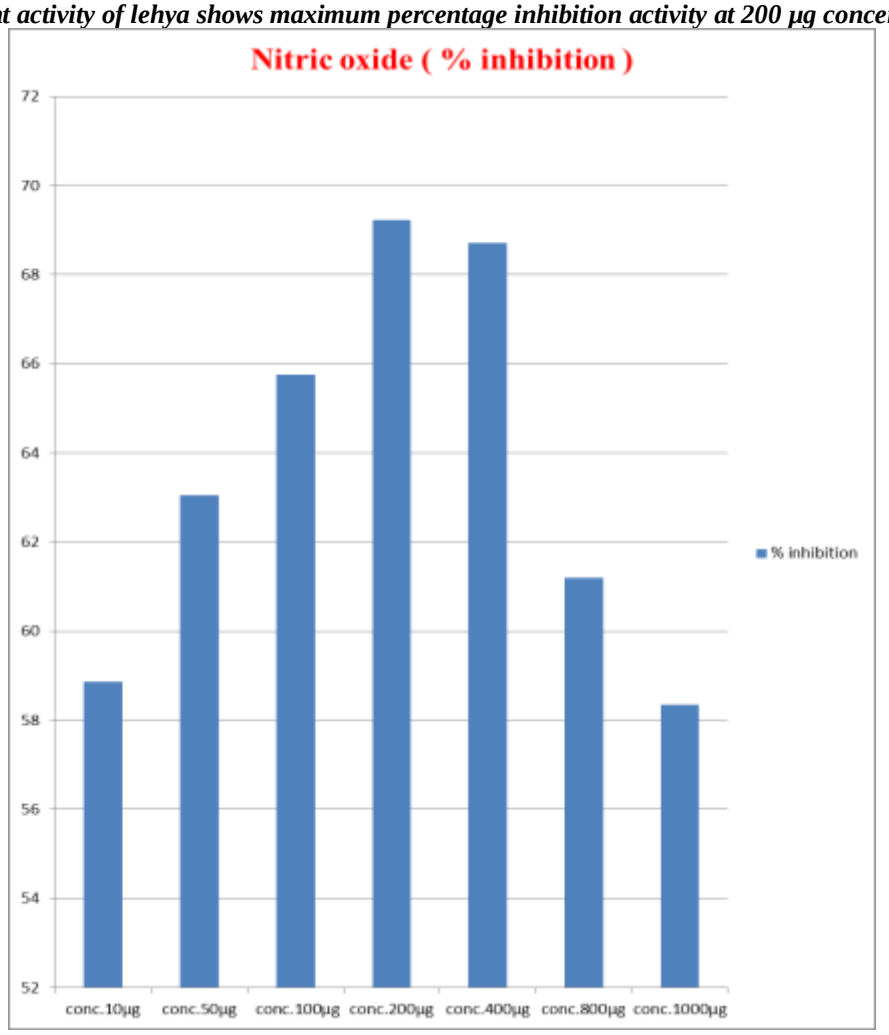
Standardization of lehya

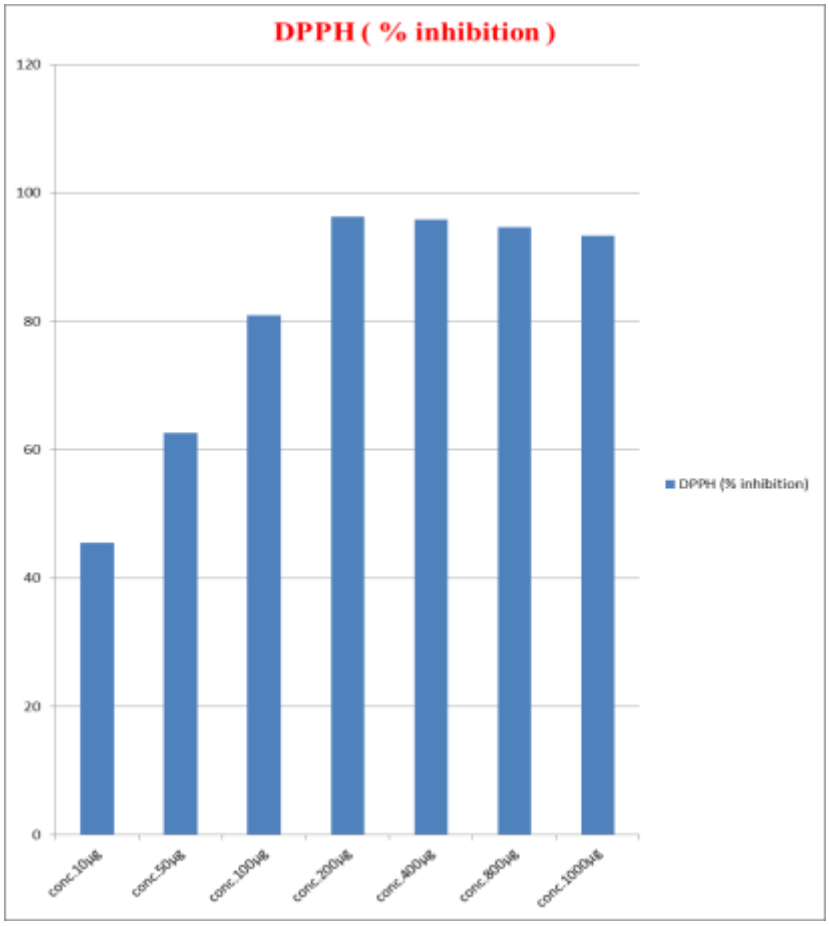
S. No.	Physico-chemical parameter	Standard value	Practical value
1	Description	A blackish brown, semisolid paste with spicy pleasant odour and bitter astringent taste.	A blackish brown, semisolid paste with spicy pleasant odour and bitter astringent taste.
2	Loss on drying	Not more than 28%	23
3	Total ash	Not more than 2%	1.2
4	Acid-insoluble ash	Not more than 1%	0.6
5	Alcohol soluble extractive value	Not more than 19%	15
6	Water soluble extractive value	Not more than 46%	42
7	PH (1%)	Between 4.70-5.00	5

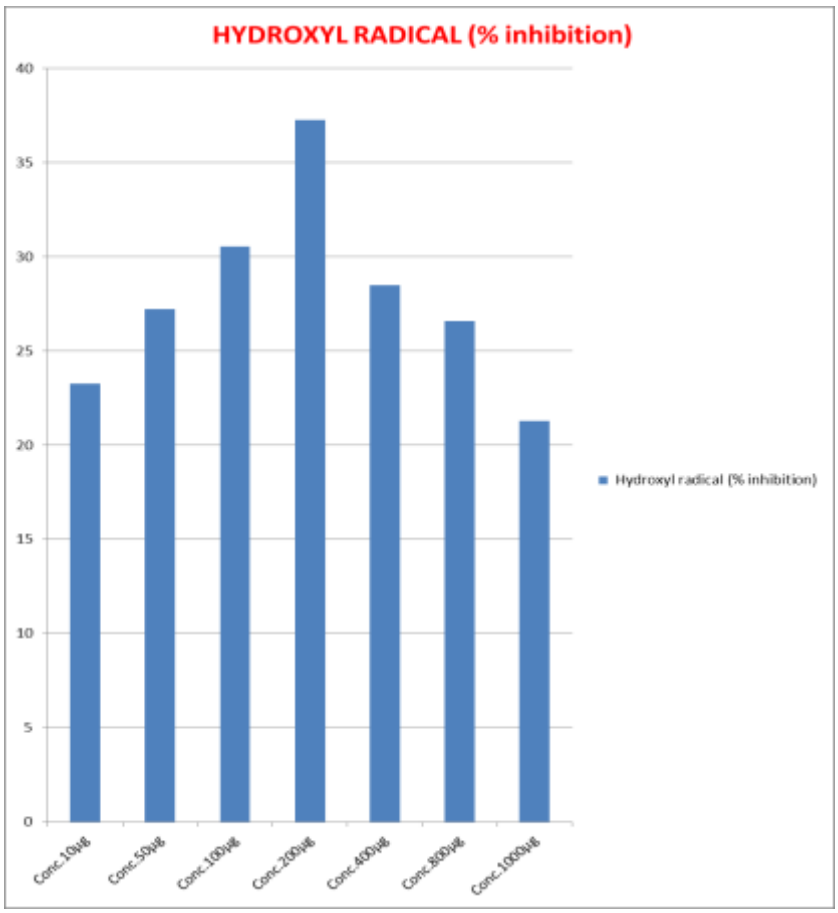
Invitro antioxidant Activity of Lehya

Tablet	Conc. 10µg	Conc. 50µg	Conc. 100µg	Conc. 200µg	Conc. 400µg	Conc. 800µg	Conc. 1000µg
Nitric oxide (%inhibition)	58.87	63.05	65.76	69.22	68.71	61.20	58.34
DPPH (%inhibition)	45.50	62.63	80.98	96.34	95.84	94.63	93.31
Hydroxyl radical (%inhibition)	23.26	27.23	30.54	37.26	28.48	26.59	21.27

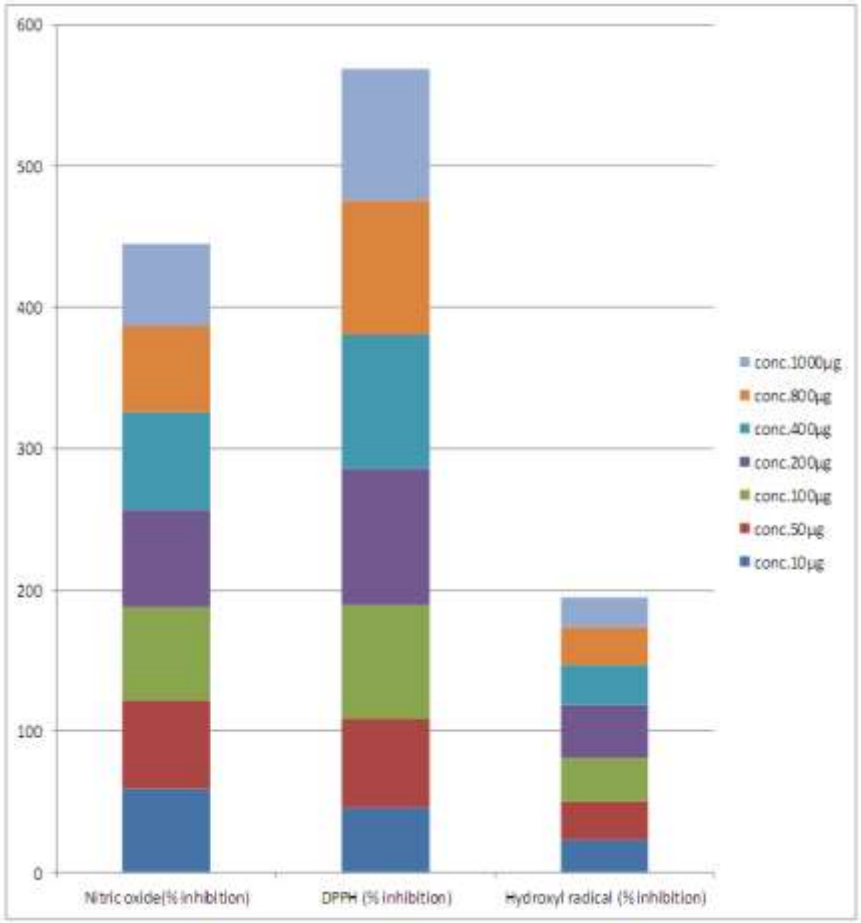
Invitro antioxidant activity of lehya shows maximum percentage inhibition activity at 200 µg concentration.




DPPH (% inhibition)
120
100
80
60
40
20
0
conc.10µg
conc.50µg
conc.100µg
conc.200µg
conc.400µg
conc.800µg
conc.1000µg
DPPH (% inhibition)


HYDROXYL RADICAL (% inhibition)
40
35
30
25
20
15
10
5
0
Conc.10µg
Conc.50µg
Conc.100µg
Conc.200µg
Conc.400µg
Conc.800µg
Conc.1000µg
Hydroxyl radical (% inhibition)

Cumulative Graph of Nitric Oxide, DPPH Scavenging & Hydroxyl Scavenging



SUMMARY

Polyherbal formulations are products from medicinal plants and are considered as safe therapeutic agents besides they are easily available at affordable prices. Polyherbal formulations are available in market are for various ailments. The polyherbal formulation in the form of Lehya meant for antioxidant activity is prepared by using suitable plants reported to possess antioxidant properties such as leaves of Annona squamosa, leaves of Cassia fistula and fruits of Illicium verum. The raw materials were standardized as per Indian herbal pharmacopoeia methods, like Total ash, Acid insoluble ash, and water soluble ash, Loss on drying, Alcohol soluble extractive, and water soluble extractive. The three plants conform to official pharmacopoeia, after standardization polyherbal formulation in the form of lehya was prepared and standardized. The novelty in the formulation is that in this combination lehya is not available in market. Finally the percentage inhibition of free radicals by in-vitro methods such as DPPH and Nitric oxide methods were carried out. Hence it is proved that the active principles in three plants are responsible for the antioxidant properties.

CONCLUSION

The formulated poly herbal lehya was proved to show pronounced in-vitro antioxidant activity and its percentage inhibition was at 200µg concentration was found to be 95.42 of DPPH inhibition, and 69.49 of Nitric oxide. Further after proving its safety and efficacy of lehya in animal models, we can proceed for bulk formulation.

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