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Pharamcological evalution of antioxidant & antidiabetic activity of *decalepis hamiltonii* leaves extract

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Abstract
The plants are the key source of medicine in Ayurveda for treatment and prevention of diseases and maintenance of healthy life. The plants are used in medicine since antiquity. Much of the medicinal plants are documented in the Ancient Ayurvedic classics and these plants are still used successfully to treat different ailments. One of these plants which is used to treat various disease is *Decalepis Hamiltonii* leaves. WHO notes that of 119 plantderived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures? Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. In the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. About 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. It was an integral part of the development of modern civilization. Standardization" of herbal drugs is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection, their extraction, purification process and rationalizing the combination in case of polyherbal drugs. Standardization means adjusting the herbal drugs preparation to a define content of a constituent or a group of substances with known therapeutically activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparation.Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration.

Keywords: Antioxidant, *decalepis hamiltonii*, hypoglycemic, DPPH scavenging

Introduction
Herbal medicine, sometimes referred to as Herbalism or Botanical Medicine, is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities. Herbal plants contain therapeutically active chemical substances that act upon the body. Herbal medicines widely used in health-care in both developed and developing countries are complex chemical mixtures prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetic, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens. WHO notes that of 119 plantderived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures? Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. In the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. About 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material.

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Types of Herbal Medicine

Traditional Chinese Herbalism which is part of Traditional oriental Medicine, Ayurvedic Herbalism which is derived from Ayurvada and western Herbalism which is originally came from Greece and Rome to Europe and then spread to North and South America. Chinese and Ayurvedic Herbalism have developed into highly sophisticated systems of diagnosis and treatment over the centuries. Western Herbalism is today primarily a system of folk medicine.

Advantages of Herbal Medicine

Herbal medicine have long history of use and better patient tolerance as well as acceptance. Medicinal plants have a renewable source, which is our only hope for sustainable supplies of cheaper medicines for the world growing population. Availability of medicinal plants is not a problem especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity. The cultivation and processing of medicinal herbs and herbal products is environmental friendly Cost-effectiveness-prescription drugs cost much more money than herbal medicines.

Standardization of Herbal Drugs

"Standardization" of herbal drugs is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection, their extraction, purification process and rationalizing the combination in case of polyherbal drugs. Standardization means adjusting the herbal drugs preparation to a define content of a constituent or a group of substances with known therapeutically activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparation."Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration.

Importance of standardization

Quality control standards are very vital in developing the herbal formulations,

- To ensure batch to batch uniformity in contents.
- Confirmation of correct amount of dosage or extract per dosage unit.
- Positive control to indicate possible loss or degradation during manufacturing.

Material and Method

Plant Material

Leaves of plant *Decalepis Hamiltonii* was purchased from India Mart. The leaves were washed and excess of water was drained off and dried on filter paper. Shade dried plant material was crushed in electrical mix grinder to a fine powder, and it was further used for the studies.

Preparation of plant extract

The leaves samples were washed thoroughly under running tap water to remove soil particles and finally washed with sterile distilled water. These samples were shade dried and ground in to fine powder. The powdered materials were stored in air tight polythene bags until use. 25 gm of powdered material was extracted by Soxhlet apparatus with 100 ml of methanol. The extract was then filtered through Whatman no 41 filter paper and the filtrate was concentrated through evaporation using rotary evaporator at 500 C. One gram of the extract diluted with 100 ml of methanol and this solution was employed in phytochemical analysis.

Phytochemical Screening

The methanolic extracts of leaves of *Decalepis Hamiltonii* were subjected to preliminary phytochemical tests to detect the presence alkaloids, flavanoids, phenols, saponins, steroids, glycosides, tannins and terpenoids using standard techniques.

Test for identification of Alkaloids

About 3 ml of methanolic extract was taken in a test tube and 5 ml of 1% dilute HCl was added, stirred and kept on a water bath for 20 minutes. The solution obtained was cooled and filtered. 2-3 drops of Mayer's reagent was added to the filtrate. A cream coloured precipitate indicated the presence of alkaloids.

Test for identification of Flavonoids

About 3 ml of methanolic extract was taken in a test tube, 1ml of 10% sodium hydroxide was added. A yellow colouration of the solution indicated the presence of flavonoids.

Test for identification of Saponins

About 2 ml of methanolic extract was taken in a test tube was shaken 2 minutes. Fronthing which persisted was taken as evidence for the presence of saponins.

Test for identification of Steroids

About 1 ml of methanolic extract was taken in a test tube and 2 ml of concentrated sulphuric acid was added by the sides of the test tube and red colour at lower layer indicates the presence of steroids.

Test for identification of Tannins

About 1 ml of the methanolic extract was taken in to test tube and 1 ml of freshly prepared 10% ferric Chloride solution was added. Appearance of blue color indicates the presence of tannins.

Test for identification of Terpenoids

About 1 ml of the methanolic extract was mixed with 2 ml of chloroform and 2ml concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of Terpenoids.

Test for identification of Glycosides

3 ml of the methanolic extract was taken in to the test tube and 2 ml of chloroform was added. Sulfuric acid was added careful to form a lower layer. Appearance of reddish brown colour indicates the presence of glycosides.

Test for identification of Phenols

5 ml of methanolic extract was taken in a test tube and 2 drops of ferric chloride solution was added. Formation of green coloured precipitate indicated the presence of phenols.

Animals

The study employed adult Wistar rats (150-200 g) of both sexes. The animals were kept in cages and housed under the same 12:12 h light cycle, with free access to water.

Determination of Antioxidant Activity

DPPH Scavenging Activity (DPPH)

The antiradical power of substances was measured by the decrease of absorption of DPPH (1, 1-Diphenyl-2-picrylhydrazyl). To 950 µl of a methanol solution of DPPH (0.1 mM) were added to 50 µl of the plant leaves extract. After 30 min, the absorbance of the mixture was measured at 517 nm. The ability to scavenge DPPH radical was calculated using the following formula.

$$\%\ Inhibition\ of\ DPPH = \frac{A_c - A_s}{A_c} X\ 100$$

Ac = Absorbance of control
As = Absorbance of sample

Antidiabetic Activity

Hypoglycemic activity in normal rats

Fasted overnight, healthy wistar albino rats weighing 150-200 g were divided into four groups of six rats each.

- A normal control received 0.3% Carboxy methyl cellulose orally in Group 1.
- Glibenclamide (7 mg/kg bwt) was administered orally to normal rats in Group 2.
- Normal rats were given an orally methanolic extract of *Decalepis Hamiltonii* leaves (200 mg/kg bwt) dissolved in 0.3% Carboxy methyl cellulose in Group 3.
- Normal rats were given an orally methanolic extract of *Decalepis Hamiltonii* (400 mg/kg bwt) dissolved in 0.3% Carboxy methyl cellulose in Group 4.
- Blood samples were collected before and after treatment at 1, 2, and 4 hours, and the glucose level was determined using a commercial kit.

Statistical Analysis

The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by the student's test. The values are mean±SD for six rats in each group. Statistical significance was determined at $p<0.05$.

Result & Discussion

Phytochemical screening

The present study contributes valuable information of phytochemical analysis and antioxidant and antidiabetic activity in *Decalepis Hamiltonii*. The results revealed the presence of the phytochemicals like Alkaloids, Flavonoids, Phenols, Steroids, Tannins, Terpenoids, Saponins and Glycosides in the leaves extract of *Decalepis Hamiltonii* (Table-1).

Table 1: Phytochemical analysis of methanolic extracts of leaves from *Decalepis Hamiltonii*.

S.NO	Test for Phytochemicals	Test results
1	Alkaloids	+
2	Flavonoids	+
3	Steroids	+
4	Tannins	+
5	Terpenoids	+
6	Saponins	+
7	Glycosides	+
8	Phenols	+

+ ve Presence of the compound.- ve Absence of the compound.

Acute Toxicity Test

There was no mortality in mice after taking the methanolic extract orally, even at dosages as high as 5 000 mg/kg, indicating that the oral LD50 was more than 5 000 mg/kg.

Table 2: LD50 and ED50 of *Decalepis Hamiltonii*

Plant Name	LD 50	ED 50
Decalepis Hamiltonii	5000 mg/kg	200mg/kg

Evaluation Antioxidant Activity

DPPH Scavenging Activity (DPPH)

Based on the constituent present, further antioxidant study was carried out for leaves extracts of *Decalepis Hamiltonii* plant. There are various methods to determine antioxidant activities among them. DPPH methods were used to determine antioxidant property of the said plant and The IC50 of the DPPH radical scavenging was 126.6±1.07 µg/ml respectively for the plants leaves.

Table 3: Antioxidant activity of *Decalepis Hamiltonii* leaves extract assessed by DPPH

Activity	DPPH (%)	IC50 (µg/ml)
Decalepis Hamiltonii	81.53±0.62	126.6±1.07

Values are expressed in mean±SEM. Means in each column followed by different letters are significantly different ($p<0.05$). Antioxidant studies have relation with the phenolic content of the plants. In the present study, leaves extract showed more phenolic and flavonoid contents, hence, leaves extract of *Decalepis Hamiltonii* showed higher scavenging property that may be due to the present of hydroxyl groups existing in the phenolic and flavonoid compounds chemical configuration that can provide the essential constituents as a radical scavenger with redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be

used as a basis for rapid screening of antioxidant activity. Plant flavonoids also have antioxidant activity in this methods. Furthermore, flavonoids are also highly effective antioxidant agents for most oxidizing molecules and various other free radicals implicated in various diseases. They suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and protect oxidations. It was also reported that antioxidant activity inversely dependent on IC50 value. The present study also showed the same trend where leaves extract showed higher phenolic and flavonoid content and as well as higher antioxidant activity. DPPH methods gave additional benefits with phenolics and flavonoids for significant results.

Evaluation Antidiabetic Activity

Hypoglycemic activity in normal rats

Table 5 shows the blood glucose levels in euglycemic rats after 0, 1, 2, and 4 hours of administration. Glibenclamide (7 mg/kg) and methanolic extract of *Decalepis Hamiltonii* (200 mg and 400 mg/kg) administration to euglycemic rats was not significant at 1 h, but was significant at 4 h ($p<0.05$) compared to control.

Table 4: Effect of MEDH on blood glucose level in normal rats

S. No.	Group	Blood Glucose level mg/dl (Mean±SD)			
		Fasting	Time (h) after treatment		
			1	2	4
1	Control (0.3%CMC)	71.02±0.48	69.15±0.60	67.86±0.58	70.46±0.85a
2	Glibenclamide (7mg/kg)	75.23±0.67	65.23±0.70	57.35±0.60	53.55±0.84b
3	MEDH (200 mg/kg)	69.16±1.14	66.24±0.52	61.22±0.47	57.23±0.82c
4	MEDH (400 mg/kg)	74.13±1.17	64.12±0.50	56.63±0.57	51.15±0.95b

Values are expressed as mean ±SD. ANOVA followed by Duncan's multiple range tests.

Values not sharing a common superscript differ significantly at $p\leq 0.05$.

Flavonoids have a high nutritional value because they are part of our usual diet, which could be explained by their rapid metabolism, elimination, and relatively low bioavailability. Flavonoids or flavonoid-rich foods can reduce the risk of diabetes by modulating glucose uptake and insulin secretion. Diabetes is a chronic metabolic condition recognized worldwide as an important cause of premature death and disability, especially in the developing.

Conclusion

In future, further investigation might provide an insight to identify and characterize the exact active phytoconstituents responsible for the antioxidant and antidiabetic effect and to elucidate the exact mechanism of action, which is responsible for the observed significant activity with low toxicity and better therapeutic index. Finally result showed the path to the future researchers to find out more therapeutic activities on leaves extracts and to discover novel phytoconstituents for effective treatments. The LD50 of the plant revealed that the plant is safe and/ or non-toxic in rats when doses less than 5000 mg/kg of body weight of animals. All the above data obtained from *Decalepis Hamiltonii* studies indicate that the plant species has both antidiabetic and antioxidant activities which support or justify the reported folkloric and anecdotal use of *Decalepis Hamiltonii* in the treatment and/ or management of diabetes and oxidative in various regions.

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