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PHARMACOGNOSTIC STUDY AND ANTI - OXIDANT ACTIVITY OF *CUSCUTA REFLEXA*

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ABSTRACT

Cuscuta reflexa is a holo parasitic plant. It is totally depend on its host plant. Present study deals with the pharmacological action (anti- oxidant activity) of *Cuscuta reflexa* in comparision with its host plant i.e. *Vitex negundo*. *Cuscuta reflex* proved to have different pharmacological actions dependimg on the chemical composition of the host plant. *Vitex negundo* mainly contains flavonoids which having different pharmacological activities such as anti- arthritic, anti-inflammatory, analgesic, anti- oxidant, etc. Therefore present study deals with the anti- oxidant activity of *Cuscuta reflexa* due to presence of flavonoids which may transfer from the host plant i.e. *Vitex negundo.* Estimation of total phenolics and flavonoids reveals that phenolic type of compounds may transfer from host to parasite and this can be better correlated with an anti- oxidant activity of both the plants.

Keywords: *Cuscuta reflexa, Vitex negundo,* Phenolic and flavonoid compounds, Anti-oxidant activity.

INTRODUCTION

CUSCUTA REFLEXA

Amar bel is an unusual parasitic vine related to the Morning glory family. It grows in a prolific manner over host plants (or other support) with inter-twined stems, giving it a common name of Devils Hair. The plant is leafless and rootless. After establishing itself on a host body, it draws nutrition from the host as a stem parasite and the roots wither away. The twining stem develops Haustoria which are root like and penetrate the host stem to draw water and nourishment. The flowers are small, white, having a perfect bell shape.

Cuscuta (Dodder) is a genus of about 100-170 species of yellow, orange or red (rarely green) parasitic plants. The family is Cuscutaceae [1].

VITEX NEGUNDO

Vitex negundo is from Verbenaceae family and is a well-known aromatic shrub. The plant has long spires of pale lilac or rose-colored flowers and small gray-brown, hard fruits, which is the part used medicinally.

Vitex usually grows from three to nine feet tall, but under cultivation can develop to 20 feet tall. The bark is white-felted; the opposite leaves compound with 5-7 leaflets. The fruit is a small hard reddish-black drupe with a persistent calyx [2].

CHARECTERIZATION OF PLANT

CUSCUTA REFLEXA [3]

Botanical name: *Cuscuta reflexa*
Family: Convolvulaceae
Synonyms: Giant Dodder
Hindi: Amar bel, Akashbel
Tamil: Kodiyagundal
Bengali: Swarna lata
Telugu: Sitamma pogunalu

TAXONOMY [4]

Kingdom: Planate

- **Division :** Magnoliophyta
- **Class :** Magnoliopsida
- **Order :** Solanales
- **Genus :** Cuscuta L.
- **Species :** Cuscuta reflexa Roxb – Giant dobb

Habit

Stems yellow or yellowish green, stout, 2-3 mm in diam., with brown spots. Calyx copular; sepals 5, broadly ovate, equal, 2-2.5 mm, with a few tubercles an axially, apex rotund. Corolla white or creamy, fragrant, tubular, 5-9 mm; lobes early Parasitic on Desmodium spp, Rubus spp and Viburnum spp at 1700 -2900 meters in Kashmir. It is also found on Zizyphus jujube and Vitex negundo and has been known to kill these plants.

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DESCRIPTION

MACROSCOPICAL CHARACTERS

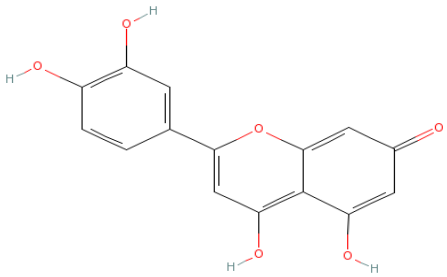
Cuscuta (Dodder) is a genus of about 100-170 species of yellow, orange or red (rarely green) parasitic plants.Dodder flower range in color from white to pink to yellow to cream. Some flower in the early summer, others later, depending on the species. The seeds are minute and produced in large quantities. They have a hard coating, and can survive in the soil for 5–10 years or more.

CHEMICAL CONSTITUENTS

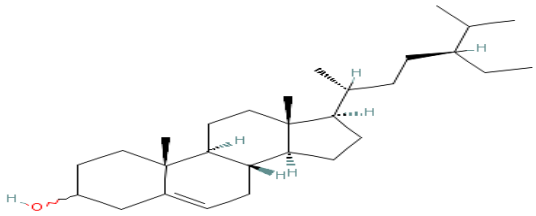
Cuscutalin, cuscutin, luteolin, beta-sitosterol and bergenin (stem); amarbelin (seed); kaempferol (stem and seed)

Structure of Active Ingredients

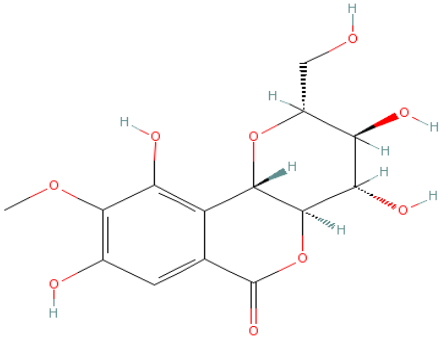
Luteolin



Beta-Sitosterol



Bergenin



USES [5]

Anthelmintic, Carminative, Purgative

- The seeds are alterative, Anthelmintic and carminative
- The stems are used in the treatment of bilious disorders.
- The whole plant is purgative.
- The plant is employed in Ayurvedic medicine to treat difficulty in urinating, jaundice, muscle pain and coughs. The juice of the plant, mixed with the juice of Saccharum officinarum, is used in the treatment of jaundice.

VITEX NEGUNDO [6]

- **Botanical name:** *Vitex negundo*
- **Family:** Verbenaceae
- **Habitant:** Wasteland up to 2000 meters in the Himalayas.

TAXONOMY

- Kingdom: Plantae
- Order: Lamiales
- Family: Lamiaceae
- Genus: Vitex
- Species: Vitex negundo

CHEMICAL CONSTITUENTS

Casticin, Luteolin,P –hydroxybenzoic acid, D-fructose

USES: Antibacterial, Antitumor, Astringent**,** Expectorant**,** Sedative**,** Tonic.

MATERIALS AND METHODS

Material

The plant of *Cuscuta reflexa* and *Vitex negundo* were collected from the sayaji garden, Vadodara, in month of saptember.shade plants are dried and coarsely powdered by using the pulverized. The powdered drug was then passing through sieves no #40 and used for extraction process.

Method

TOTAL FLAVONOID ESTIMATION [7]

Quercetin solution: The solution was prepared by adding 5 mg of Quercetin in 50 ml of methanol from this solution different concentration (20-100) µg/ml were prepared.

Sample solution: Sample solution was prepared by dissolving 10 mg of the extract in 100 ml of methanol to give (100 µg/ml) solution.

Potassium acetate solution: Potassium acetate solution was prepared by dissolving 9.8 g of potassium acetate in 100 ml of distilled water.

Alluminium nitrate (10% w/w): Alluminium nitrate solution was prepared by dissolving 10 g of Alluminium nitrate in 100 ml distilled water.

Procedure: Take sample solution 0.5ml, add ethanol 1.5ml,alluminium nitrate 0.1ml (10%),than add potassium acetate solution 0.1ml(1M), than add water 2.8 ml. mixed it and kept ambient temperature for 40min.the absorbance is measured at 415nm.total flavonoid was calculated according standard curve established with Quercetin.

TOTAL PHENOLIC ESTIMATION

Total phenolic content of methanol extract was determined using Folin-ciocalteu reagent.in this method the blue colour formed due to the polyphenol present in the extract was measured at 760 nm using UV Spectrophotometer [8].

Chemicals: Folin-ciocalteu reagent, Gallic acid, Sodium carbonate

Reagent preparation

- **Folin-ciocalteu (phenol) reagent:**The reagent was prepared by diluting the 5 mi of the reagent to 25 ml with water to give a stock solution.
- **Sodium carbonate:**It was prepared by dissolving 29 g of sodium carbonate in 100 ml of water.
- **Gallic acid solution:**The solution was prepared by adding 5 mg of Gallic acid in 50 ml of water .from this solution different concentration (20-100) µg/ml were prepared.
- **Sample solution:**Sample solution was prepare by dissolving 10 mg of the extract in 100 ml of methanol to give (100 µg/ml).

Procedure: The extract (0.1ml)was mixed with the Folin-ciocalteu (phenol) reagent (0.2ml),water(2ml) and sodium bicarbonate (15%w/v,1 ml) , absorbance was measured at 760 nm after 2 hr incubation period at 50^0temperature for 10 min. the total phenolic was calculated according standard curve of established with Gallic acid solution.

TOTAL ASH VALUE [9]

- Ash values are used to determined quality and purity of crude drug.
- The object of ash is to remove all traces of organic material interfering in analysis of inorganic element.

TOTAL ASH

2 gm of accurately weighed air dried leaf powder was taken in a crucible and was kept in a muffle furnace for ignition at temperature up to 450 degree. The crucible was then taken out from furnace, cooled and weighed. The total ash was calculated by subtracting the weight of crucible with ash after ignition from the weight of crucible with drug powder before ignition. Percentage of total ash was calculated with reference to air dried drug.

ACID INSOLUBLE ASH

- Ash contains inorganic radicals like phosphate, carbonate and silicates of sodium, potassium, calcium. These variables affect the Total ash value.
- Such variable removed by treating with acid and then acid insoluble ash value is determined

Procedure: The ash obtained in the total ash method was boiled with 25ml of 2N HCl for 5 minutes. Insoluble matter was collected on ash less filter paper and washed with hot water. The material retained on the paper along with the paper was further ignited and weighed. Percentage of acid insoluble ash was calculated with reference to air dried material.

WATER SOLUBLE ASH

The ash obtained from total ash was boiled with 25ml water for 5 minutes. All the insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 1 hr. The percentage of water soluble ash was calculated by subtracting weight of insoluble matter from weight of total ash. Difference between weights represents water soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

LOSS ON DRYING

Weigh the 5 g of the powdered drug in to the porcelain dish. Place the porcelain dish in the oven at 110^0 c. dry the sample at constant weight. After drying opens the drying chamber, cool to at room temperature before weighin.

ANTI-OXIDANT ACTIVITY

Antioxidants are chemical compound that can bind to free oxygen radicals preventing these radicals from damaging healthy cells. Free radicals are naturally produced in the body through the normal metabolism of amino acid and fats. These free radicals are unstable molecules that can freely react with and destroy healthy cells. They can bind to and alter the structure of DNA thus leading to mutations an eventually to cancer. Besides cancer, this oxidative stress on the cells can lead to heart, eye and neurogical diseases. Antioxidant activity due to the inhibition of super oxides, but mostly they act through mechanism of inhibition of super oxide [10].

Determination of reducing power assay

The reducing capability was measured by the transformation of ferrous to ferric in the presence of different extract at 700 nm as the reported method.

Table 1. Total Flavonoid Content

Sample	Concentration (µg/ml)	Absorbance (760nm)
Quercetin solution	10	0.064
	20	0.121
	40	0.230
	60	0.301
	80	0.389
	100	0.681
Cuscuta reflexa	100	0.161
Vitex negundo	100	0.173

Table 2. Concentration from Graph

	Concentration(µg/ml)
Cuscuta reflexa	29.37
Vitex negundo	31.51

Table 3. Total Phenolic Content

Sample	Concentration (µg/ml)	Absorbance (760nm)
Gallic acid solution	10	0.291
	20	0.318
	40	0.328
	60	0.433
	80	0.436
	100	0.478
Cuscuta reflexa	100	0.178
Vitex negundo	100	0.153

Table 4. Concentration from Graph

	Concentration(µg/ml)
Cuscuta reflexa	26.23
Vitex negundo	11.95

Table 5. Total Ash Content

PARAMETER	*Cuscuta reflexa*	*vitex negundo*
TOTAL ASH	7.5%	11.0%
WATER SOLUBLE ASH	2.3%	9.6%
ACID INSOLUBLE ASH	4.8%	0.8%

Table 6. Loss On Drying

Plant	%w/w
Cuscuta reflexa	7%
vitex negundo	8%

Table 7. Anti-Oxidant Activity

S No	sample	Concentration (µg/ml)	Absorbance (700 nm)
1	Ascorbic acid	10	0.098
		20	0.141
		40	0.165
		60	0.170
		80	0.187
		100	0.201
2	*Cuscuta reflexa extract*	10	0.106
		25	0.113
		50	0.128
		100	0.144
3	*Vitex negundo extract*	10	0.121
		25	0.125
		50	0.158
		100	0.165

The concentration of drug of increases, the absorbance increases, means it shows the Anti-oxidant activity

Fig.1. *Cuscuta reflexa*

Fig 2. *Cuscuta European* in flower

Fig. 3. Vitex negundo

Fig. 4. Total Flavonoid content

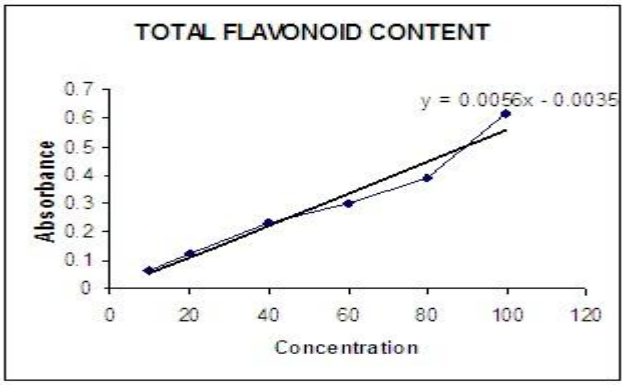
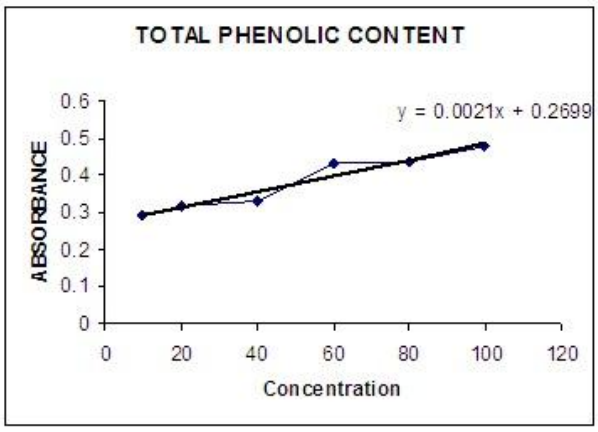


Fig. 5. Total phenolic content



CONCLUSION

Based on practical pharmacological activity it was concluded that, the activity of methanol extract was confirmed against standard ascorbic acid; hence anti-oxidant activity was shown through the reducing power assay.

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