



PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF WHOLE PLANT EXTRACT OF *CUSCUTA REFLEXA* GROWING ON DIFFERENT HOST PLANTS

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ABSTRACT

Cuscuta reflexa belongs to the family Convolvulaceae is indigenous medicinal plant and is a well-known folklore remedy for the treatment of liver disorder. Present work deals with the comparative standardization of *Cuscuta reflexa* growing on different host plants i.e. *Alstonia scholaris* and *Ficus virens* by using pharmacognostic and phytochemical investigation of whole plant. The whole plant was extracted with different organic solvents in increasing order of polarity. The results of the preliminary investigation revealed the presence of flavonoids, glycosides & carbohydrates. The methanolic, ethanolic and aqueous extracts of the plant growing on different host plants were investigated by using thin layer chromatography. The aim of the present study was focused on the pharmacognostical and phytochemical properties, which would like to facilitate quick identification and selection of the drug from various adulterates. All the parameters were studied according to the WHO and Pharmacopoeia guidelines to standardize the *Cuscuta reflexa* growing on different host plants.

Key words: *Cuscuta reflexa*, *Ficus virens*, *Alstonia scholaris*, Physico-chemical characterization., TLC.

INTRODUCTION

The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines (Tyler, 2000), particularly in many developed countries, where herbal products have been incorporated into so-called 'alternative', 'complementary', 'holistic' or 'integrative' medical systems. During the latter part of the twentieth century, increasing interest in self-care resulted in an enormous growth in popularity of traditional healing modalities, including the use of herbal remedies. Consumers have reported positive attitudes towards these products because they believe them to be of 'natural' rather than 'synthetic' origin, they believe that such products are more likely to be safe than are drugs, they are considered part of a healthy lifestyle, and they can help to avoid unnecessary contact with conventional 'western' medicine.

Cuscuta reflexa (Giant Dodder) is a genus of about 100-170 species of yellow, orange or red (rarely green) parasitic plants and is commonly known as Akashbela in Hindi (Sharma S *et al.*, 2009). Of the Indian species, *Cuscuta reflexa* has a wide range of distribution extending sometimes up to 6000 ft. or even more above sea level in the hills of South India (Gamble JS, 1921) as well as North India (Singh TCN, 1933). It grows as a large prickly shrub particularly in North-East India (Sikkim, Assam and Nagaland states) (Kumar V *et al.*, 2010). *Cuscuta reflexa* has profound use in Ayurveda and folklore medicine. In India, the plant is traditionally used for various medicinal purposes. The seeds have a bitter bad taste and used as sedative, emmenagogue, diuretic. It is also used in the treatment of disease of the liver and spleen, quartan fever, chronic fevers, griping, purification of blood and cleanse the bowel. The infusion is given in ophthalmia, the decoction in biliousness as a purgative. The stem is used as a purgative. The juice of the plant is inhaled for treating jaundice and warm paste is applied in rheumatism and gout and other affected parts of the body, and the paste of whole plant is applied for relieving

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headache. The crude water extracts of *Cuscuta reflexa* exhibited anti-HIV activity (Mahmood N *et al.*, 1997). The petroleum ether extract of *Cuscuta reflexa* is useful in treatment of androgen induced alopecia by inhibiting the enzyme 5 alpha-reductase (Pandit S *et al.*., 2000; Kokate CK, 1994).

This paper reports the comparative standardization of *Cuscuta reflexa* growing on different host plants i.e. *Alstonia scholaris* and *Ficus virens* procured from the forest department of Ghaziabad, UP, in the month of October. The study includes the determination of physico-chemical constants, preliminary phytochemical screenings of methanolic and aqueous extracts of *Cuscuta reflexa* on different host plants. From the literature, it is clear that there is no comparative work done on this plant till now.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant material of *Cuscuta reflexa* was collected from the host plants viz. *Alstonia scholaris* and *Ficus virens*, grown locally by the forest department of Ghaziabad, UP, in the month of October. Voucher specimen number; NISCAIR/RHMD/consult/2010-11/1591/189 was deposited at the Herbarium of National Institute of Science Communication and Information Resources, New Delhi.

Reagents

All the reagents and chemicals used were of guaranteed reagent quality obtained from Merck, India.

Physico-chemical analysis

Ash Value

Ash content of the plant is generally taken to be the residue after incineration. It represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration. Different Ash values like total ash, water soluble ash and acid insoluble ash were evaluated.

Extractive Value

Extractive value determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. Different extractive values like water soluble and alcohol soluble extractive values were evaluated.

Moisture content

An excess of water in plant materials will encourage microbial growth, the presence of fungi or insects and deterioration following hydrolysis. Limits for water content should therefore be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the

presence of water. Moisture content is determined by Loss on drying method in terms of percent w/w. (Ayurvedic Pharmacopoeia of India, 2005).

Extraction

The whole plant of *Cuscuta reflexa* grown on *Alstonia scholaris* and *Ficus virens* were collected and subjected to hot soxhlation as well as cold maceration using petroleum ether (b.p. 40°-60°), methanol, ethanol, ethyl acetate and water as solvents. The different extracts were concentrated using Rota evaporator. Extractive values in different solvents were then determined according to the method reported in literature (The Ayurvedic Pharmacopoeia of India, 2004; Mukherjee PK, 2005)

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Harborne (Harborne JB & Williams CA, 2000). The dried methanolic extract of *Cuscuta reflexa* whole plant was reconstituted in 10 ml of methanol and used for preliminary phytochemical testing for the presence of different chemical groups of compounds.

Qualitative determination using TLC

Thin layer chromatography of methanolic, ethanolic and aqueous extracts of *Cuscuta reflexa* growing on host plants *Alstonia scholaris* and *Ficus virens* were also studied. It is particularly valuable for the qualitative determination of presence of a number of chemical constituents to support the chemical test.

Fluorescence analysis

All the extracts were examined in daylight, short and long UV light. It was carried out according to the method given by Kokoski. (Kokoski J *et al.*, 1958)

RESULTS AND DISCUSSION

Ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 1) of the powdered *Cuscuta reflexa* (whole plant) growing on different host plants revealed a high concentration of total ash, acid insoluble ash, water soluble ash and sulphated ash in *Alstonia scholaris* as compared to *Ficus virens*. Loss on drying of the powdered *Cuscuta reflexa* revealed the presence of 9.43% of moisture in *Alstonia scholaris* and 10.28% of moisture in *Ficus virens*. The foreign matter content of *C. reflexa* whole plant growing on the host plant *Alstonia scholaris* was 1.60% and when growing on the host plant *Ficus virens* it showed the foreign matter content of 1.45% w/w. The results of fluorescence analysis of the powdered whole plant of *Cuscuta reflexa* growing on different host plants are

presented in Table 2 and 3 respectively. From the preliminary phytochemical screening, it has been observed that the ethanolic, methanolic and aqueous extracts of the whole plant contain carbohydrate, amino acids, glycosides and flavonoids (Table 4 and 5). Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The results of hot and cold maceration of petroleum ether (b.p. 40°-60°), methanol, ethanol, ethyl acetate and water extractive values are shown in Table 6 and 7. Thin layer chromatography of methanolic, ethanolic and aqueous

extracts of *Cuscuta reflexa* growing on host plants *Alstonia scholaris* and *Ficus virens* were also studied. The R_f values of ethanolic extracts of *Cuscuta reflexa* growing on *Alstonia scholaris* and *Ficus virens* were 0.41, 0.58, 0.68, 0.79 and 0.88 and R_f values of methanolic extracts of *Cuscuta reflexa* growing on *Alstonia scholaris* and *Ficus virens* were 0.5, 0.53, 0.68, 0.75. These studies help in authentication of the plant since there is no work reported on their comparative pharmacognostical investigation previously.

Table 1. Ash values of the whole plant of *Cuscuta reflexa* growing on *Alstonia scholaris* and *Ficus virens*

Ash values (% w/w)				
<i>Cuscuta reflexa</i> grown on different host plants	Total ash	Acid insoluble ash	Water soluble ash	Sulphated ash
<i>Alstonia scholaris</i>	7.96,	1.49	5.47	7.8
<i>Ficus virens</i>	5.97	1.97	3.48	4.93

Table 2. Fluorescence analysis of powdered *Cuscuta reflexa* whole plant growing on the host plant *Alstonia scholaris*

Sr.No.	Powdered crude drug	Day light	UV (Short) 254 nm	UV (Long) 366 nm
1	As such	Brown	Dark Brown	Brown
2	Drug + Ethanol	Brown	Dark Brown	Brown
3	Drug + Toluene	Brown	Greenish Brown	Greenish Brown
4	Drug + 1N NaOH	Brownish yellow	Greenish Brown	Fluorescent Green
5	Drug + Methanol	Brown	Yellowish Brown	Greenish Brown
6	Drug + Dil Sulphuric acid	Brown	Greenish Brown	Brownish Green
7	Drug + Glacial acetic acid	Brown	Greenish Brown	Greenish Brown
8	Drug + Ferric chloride	Brown	Greenish Brown	Brown
9	Drug + Ammonia	Yellow	Fluorescent Green	Greenish Brown
10	Drug + Nitric acid	Brown	Fluorescent Green	Dark Green
11	Drug + Dilute Iodine	Brown	Light Green	Brownish Green

Table 3. Fluorescence analysis of powdered *Cuscuta reflexa* whole plant growing on the host plant *Ficus virens*

Sr. No.	Powdered crude drug	Day light	UV (Short) 254 nm	UV (Long) 366 nm
1	As such	Brown	Brown	Brown
2	Drug + Toluene	Brown	Greenish Brown	Greenish Brown
3	Drug + Ethanol	Brown	Dark Brown	Brown
4	Drug + Glacial acetic acid	Brown	Greenish Brown	Greenish Brown
5	Drug + 1N NaOH	Yellowish Green	Light Green	Fluorescent Green
6	Drug + Methanol	Brown	Yellowish Brown	Greenish Brown
7	Drug + Dil H ₂ SO ₄	Brown	Greenish Brown	Brownish Green
8	Drug + FeCl ₃	Brown	Greenish Brown	Brown
9	Drug + Ammonia	Yellow	Fluorescent Green	Greenish Brown
10	Drug + Nitric acid	Brown	Fluorescent Green	Dark Green
11	Drug + Dil Iodine	Brown	Light Green	Brownish Green

Table 4. Results of phytochemical screenings of extracts of *C. reflexa* whole plant growing on the host plant *Alstonia scholaris*

Test name	Direct extraction		
	Methanolic Extract	Ethanolic extract	Aqueous extract
Carbohydrate			
Molisch's test	+	+	+
Barfoed's test	-	-	+

Selivanoff's test	-	-	-
Test for Pentoses	-	+	-
Alkaloids			
Dragendorff's Reagent	-	-	-
Mayer's reagent	-	-	-
Wagner's Reagent	-	-	-
Amino acid			
Ninhydrin test	-	+	+
Millon's test	-	+	-
Test for waxes	-	+	-
Test for steroids & Triterpenoids			
Salkowski test	+	-	-
Test for Starch	-	-	-
Test for flavonoids			
Shinoda test	+	+	+
Alkaline reagent test	+	+	+
Zinc hydrochloride test	+	+	+
Cardiac glycosides			
Raymond's reagent	-	-	-
Picric acid	-	-	-
Anthraquinone Glycosides			
Borntrager's test	+	+	+
KOH	-	-	-
General test	-	-	-

Table 5. Results of phytochemical screenings of dried extracts of *C. reflexa* stem growing on *Ficus virens*

Test name	Direct extraction		
	Methanolic Extract	Ethanollic extract	Aqueous extract
Carbohydrate			
Molisch's test	+	+	-
Barfoed's test	-	-	-
Selivanoff's test	+	-	-
Test for Pentoses	-	-	-
Alkaloids			
Dragendorff's Reagent	-	-	-
Mayer's reagent	-	-	-
Wagner's Reagent	-	-	-
Amino acid			
Ninhydrin test	-	+	+
Millon's test	-	+	-
Test for waxes	-	+	-
Test for steroids & Triterpenoids			
Salkowski test	+	-	-
Test for Starch	-	-	-
Test for flavonoids			
Shinoda test	+	+	+
Alkaline reagent test	+	+	+
Zinc hydrochloride test	+	-	+
Cardiac glycosides			
Raymond's reagent	-	-	-
Picric acid	-	-	-
Anthraquinone Glycosides			
Borntrager's test	+	+	+
KOH	-	-	-
General test	-	-	-

Table 6. Extractive values of *Cuscuta reflexa* whole plant growing on the host plant *Alstonia scholaris* and *Ficus virens* by hot maceration.

EXTRACTIVE VALUE (% w/w)					
Plant	Types of Extract				
<i>Cuscuta reflexa</i> grown on different host plants	Ethyl acetate	Petroleum ether	Ethanollic	Methanolic	Water
<i>Alstonia scholaris</i>	2.0	1.2	21	9.2	26.0
<i>Ficus virens</i>	1.2	1.2	18	14	29.2

Table 7. Extractive values of *Cuscuta reflexa* whole plant growing on the host plant *Alstonia scholaris* and *Ficus virens* by cold maceration.

EXTRACTIVE VALUE (% w/w)					
Plant	Types of Extract				
<i>Cuscuta reflexa</i> grown on different host plants	Ethyl acetate	Petroleum ether	Ethanollic	Methanolic	Water
<i>Alstonia scholaris</i>	2.4	1.6	13.6	13.6	22.8
<i>Ficus virens</i>	5.6	1.6	24.8	12.0	23.2

CONCLUSION

Hence, the current research assists to differentiate the two host plants of *Cuscuta reflexa* based on their standardization and physicochemical parameters. The study showed that the all mentioned parameters vary with the host on which the dodder parasitizes. In conclusion, all the parameters, which are being reported,

could be useful in identification of distinctive features of drugs and also valuable as raw material in manufacturing a stable herbal product. As there is no pharmacognostic work on record of this traditionally much valued drug on these different host plants, the present work was taken up with a view to lay down standards, which could be useful to detect the authenticity of this medicinally useful plant.

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