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Review Article

RECENT REVIEW ON PLANT MOLECULAR BIOLOGY, PHYTOPHYSIOLOGY, PHYTOCHEMISTRY AND ETHONOPHARMACOLOGY OF *CUSCUTA REFLEXA* ROXB. A WONDERFUL PARASITIC PLANT

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ABSTRACT
Cuscuta reflexa is an evergreen parasitic plant. *Cuscuta* is using traditionally in medicinal system. The plant Cuscuta is used for treatment of disease like fits, melancholy and insanity, headache, disease of spleen, jaundice. Many phytochemicals are found in the plant like cuscutin, luteolin, ducitol, cuscutamin & caffeic acid etc. *Cuscuta reflexa* is one of the richest sources of antioxidant flavonoids. This review article cover the major areas as phytochemistry, medicinal, phytophysiological, genetics and moleculer level study, which will be useful for exploring the hidden aspects on *C. reflexa* in future.
KEY WORDS: Cuscuta reflexa, Parasitic plant, Phytochemistry, Molecular analysis

INTRODUCTION

Amarbel (meaning, immortal vine) is an unusual parasitic vine related to the convolvulaceae (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. Initially the starter plant would have had some roots. Within a few days of germination, the plant, which is touching sensitive, finds a host or dies. 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 and white, having a perfect bell shape and a fleshy calyx, attached directly to the stem nodes.[1]

Cuscuta is traditionally regarded as a rootless, chlorophyll-free parasite completely dependent on a host plant for support and food supply Fig-1. It is generally accepted that water and inorganic nutrients are absorbed through the xylem connections between host and parasite, while organic substances are transported from the phloem of the host to that of the parasite via the phloem connections that appreciable quantities of chlorophyll are present in *C. reflexa* Dodder can be identified by its thin stems appearing leafless, with the leaves reduced to minute scales. From mid-summer to early autumn, the vines can produce small fruit that take the same color as the vine, and are approximately the size of a common pea. It has very low levels of chlorophyll; some species such as *Cuscuta reflexa* can photosynthesize slightly.[2]

Fig-1 *Cuscuta reflexa* with budding and flowering stage, and parasitic nature of plant over another plants

Dodder flowers 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. Dodder seeds sprout at or near the surface of the soil. While dodder germination can occur without a host, it has to reach a green plant quickly; dodder grows toward the smell of nearby plants[1]. If a plant is not reached within 5 to 10 days of germination, the dodder seedlings will after a dodder attaches itself to a plant, it wraps itself around it. If the host contains food beneficial to dodder, the dodder produces haustoria that insert themselves into the vascular system of the host. The original root of the dodder in the soil then dies. The dodder can grow and attach itself to multiple plants. In tropical areas it can grow more or less continuously, and may reach high into the canopy of shrubs and trees; in temperate regions it is an annual plant and is restricted to relatively low vegetation that can be reached by new seedlings each spring. Dodder is parasitic on a very wide variety of plants, including a number of agricultural and horticultural crop species, such as alfalfa, lespedeza, flax, clover, potatoes, chrysanthemum, dahlia, helenium, trumpet vine, ivy and petunias, among others. Dodder ranges in severity based on its species and the species of the host, the time of attack, and whether any viruses are also present in the host plant. By debilitating the host plant, dodder decreases the ability of plants to resist viral diseases, and dodder can also spread plant diseases from one host to another if it is attached to more than one plant.die. Before a host plant is reached, the dodder, as other plants, relies on food reserves in the embryo; the cotyledons, though present, are vestigial.[3] This plant is depurative and purgative and is used in retention of wind and in duration of liver while externally used against itches and skin diseases. Previously, long chain esters of the oleane series and sterol glycosides have been reported from this plant. The methanolic extract and its subsequent ethyl acetate fraction showed significant inhibition against a -glucosidase, while against other [2].

ANTIBACTERIAL, ANTIVIRAL AND ANTIOXIDANT ACTIVITY

The methanol extracts of *Cuscuta reflexa* stem and *Corchorus olitorius* seed showed a broad spectrum of antibacterial activity[4]. In the present study, comparative anti-oxidant activity of alcoholic extracts of *Cuscuta reflexa* was assessed. Anti-oxidant activity of alcoholic extracts of *Cuscuta reflexa* have been analyzed for their free radical scavenging activity by using(1,1-diphenyl-2- picrylhydrazyl) DPPH radical , inhibition of lipid peroxidation induced by FeSO4 in egg yolk , presence of phenolic compound using the Folin-Ciocalteu method and identification of antioxidant compound in bioautographic analysis using DPPH agent. Ascorbic acid was used as a standard. The both extracts neutralized the activities of radicals and inhibited the peroxidation reactions. *Cuscuta reflexa* plant reported to have greater *in vitro* antioxidant activity than other plant. Ethanolic extracts of *Cuscuta reflexa* contained more polyphenol content compared with other species of *Cuscuta*.[5] The in vitro antioxidant of activity of *Cuscuta reflexa* stem has been investigated by estimating degree of non-enzymatic hemoglobin glycosylation majored by the colorimeter at for 40 nm. The ethyl acetate fraction of ethanol extract showed higher activity than other fraction. The antioxidant activity of extract a very closed & identical in magnitude & comparable to that of standard antioxidant compound used.[6] An antiviral substance, showing highly significant virus inhibiting property, has been isolated from the aqueous extract of *Cuscuta reflexa* plants. The biologically active virus inhibitor was purified by fractionation with organic solvents. The antiviral activity of the purified material was increased several folds. It has prevented the infection of several unrelated isometric as well as anisometric viruses in their hypersensitive and systemic hosts. Systemic resistance induced in lower treated as well as upper non treated leaves of host plants, whose lower leaves had been treated with inhibitor, was significantly reversed in presence of Actinomycin D[7].

PHYTOCHEMICAL AND BIOCHEMICAL STUDIES

Choline kinase is a mitochondrial enzyme in *Cuscuta reflexa.* It can be solubilized from the particles by treatment with 350mm-sodium chloride, or by freezing and thawing. Choline kinase of *C. reflexa* was purified by starting from the crude mitochondrial fraction. A 33-52% recovery of the enzyme, on the basis of the activity in the original homogenate, in 1200-2250-fold enrichment, was affected. 3. The purified preparation of choline kinase had a sigmoid saturation curve with respect to choline, with a Hill number of 2.3, and was inhibited by ADP (competitive in nature and allosteric in binding, with a Hill number of 2.7) and by phosphorylcholine (non-competitive and non-allosteric). The kinetic characteristics of the enzyme were consistent with the K type allosteric model. The enzyme was desensitized, with respect to choline regulation, by prolonged storage in the cold, was activated significantly on warming before assay and was inactivated by high concentrations of sodium chloride.[8]

Carboxymethylcellulase (CMCase) was extracted and purified from an angiosperm parasite *Cuscuta reflexa* free from beta-glucosidase and other enzyme activities. The molecular mass and Stokes' radius of the purified enzyme are 144 kDa and 44 A, respectively. The diffusion coefficient and frictional ratio of the enzyme were 5.15 x 10(-7) cm2/sec and 1.27. The SDS-PAGE revealed homotetrameric nature of the enzyme with a subunit molecular mass of 35 +/- 1 kDa. Titration against DTNB and NBS revealed 19 sulfhydryl groups and 8 tryptophan groups, respectively, per mole of the enzyme. A sharp pH optimum at 5.0 was obtained. Cuscuta CMCase activity is unique amongst plant endoglucanases in being stimulated by Mg2+ and Mn2+ ions and by various thiols. Reaction product analysis, mode of enzyme action and substrate specificity test suggest the endo- nature of the purified CMCase. The enzyme showed K (m) value of 26 +/- 1 mg/ml for carboxymethylcellulose (sodium salt) [9]. A group of scientist reported that neoxanthin, considered an integral component of LHCs, is stoichiometrically replaced by lutein-5, 6-epoxide in the parasitic angiosperm *Cuscuta reflexa*, without compromising the structural integrity of the LHCs. Lutein-5, 6-epoxide differs from neoxanthin in that it is involved in a light-driven deep oxidation cycle similar to the deepoxidation of violaxanthin in the xanthophylls cycle, which is implicated in protection against photodamage. The absence of neoxanthin and its replacement by lutein-5, 6-epoxide changes our understanding of the structure-function relationship in LHCs, has implications for biosynthetic pathways involving neoxanthin (such as the plant hormone abscisic acid), and identifies one of the early steps associated with the evolution of heterotrophy from autotrophy in plants[10].

Two new compounds, 7'-(3',4'-dihydroxyphenyl)-N-[(methoxyphenyl)ethyl]propenamide (4), and 7'-(4'-hydroxy,3'-methoxyphenyl)-N-[(4-butylphenyl)ethyl]propenamide (5) have been isolated from *Cuscuta reflexa* along with five known compounds, 6,7-dimethoxy-2H-1-benzopyran-2-one (1), 3-(3,4-dihydroxyphenyl)-2-propen-1-ethanoate (2), 6,7,8-trimethoxy-2H-1-benzopyran-2-one (3) 3-(4-O-beta-D-glucopyranoside-3,5-dimethoxyphenyl)-2-propen-1-ol(6),2-(3-hydroxy-4-methoxyphenyl)-3,5-dihydroxy-7-O-beta-D-glucopyranoside-4H-1-benzo pyrane-4-one (7), reported for the first time from this species. Structures of these compounds were determined by spectral analysis. These compounds showed strong inhibitory activity against alpha-glucosidase [11].

The parasitic angiosperm *Cuscuta reflexa* has a highly unusual carotenoid composition in that it does not contain neoxanthin, Combined HPLC and mass spectrometric analysis has enabled us to detect in tissues of *C. reflexa* two new types of xanthophylls: lutein-5, 6-epoxide and 9-cis-violaxanthina A team of researchers isolated the LHCIIb complex from thylakoids and analyzed chlorophyll and carotenoid composition. The data show that the 9-cis-violaxanthin is [12] 5-hydroxy-7-methoxy-6-(2,3-epoxy-3-methylbutyl)-flavanone, is isolated from the stems of *Cuscuta reflexa* along with three other known compounds. This new compound has good potential for application especially in the photoactivity of reflexin. It was found to be sensitive to glutathione, forming a fluorescent product that is utilized for sensing nitric oxide (NO). The lowest detection limit of NO analysis was found to be 0.05micro/M present in amounts similar to that of neoxanthin in most plants. On the other hand, lutein-5, 6-epoxide was found to be in substoichiometric quantities, suggesting a peripheral location similar to the loosely-associated all-trans-violaxanthin and also enabling suitable accessibility for the de-epoxidase (VDE). Absorption spectroscopy revealed close similarities of the excited state energies of neoxanthin and 9-cis-violaxanthin in vitro and in intact LHCIIb complex. Resonance Raman analysis clearly indicates a cis conformation of violaxanthin in the complex, confirming the

pigment analysis data and proving that not only does violaxanthin replace neoxanthin as an intrinsic component of LHCIIb in *C. reflexa* but it also adopts the same 9-cis conformation of neoxanthin. These results suggest that the N1 binding site of LHCIIb preferentially binds 9-cis-5, 6-epoxy carotenoids, which has implications for the features of this binding site and its role in the photosystem II antenna assembly and stability.[13]

Cuscuta represent a compatible host-parasite combination. Cuscuta produces a haustorium that penetrates the host tissue. In early stages of development the searching hyphae on the tip of the haustorial cone are connected to the host tissue by interspecific plasmodesmata. Ten days after infection, translocation of the fluorescent dyes, Texas Red (TR) and 5, 6-carboxyfluorescein (CF), demonstrates the existence of a continuous connection between xylem and phloem of the host and parasite. *Cuscuta* becomes the dominant sink in this host-parasite system. Transgenic Arabidopsis plants expressing genes encoding the green fluorescent protein (GFP; 27 kDa) or a GFP-ubiquitin fusion (36 kDa), respectively, under the companion cell (CC)-specific AtSUC2 promoter were used to monitor the transfer of these proteins from the host sieve elements to those of *Cuscuta*. Although GFP is transferred unimpedly to the parasite, the GFP-ubiquitin fusion could not be detected in Cuscuta. A translocation of the GFP-ubiquitin fusion protein was found to be restricted to the phloem of the host, although a functional simplistic pathway exists between the host and parasite, as demonstrated by the transport of CF. These results indicate a peripheral size exclusion limit (SEL) between 27 and 36 kDa for the simplistic connections between host and *Cuscuta* sieve elements.[14]

The reversed-phase HPLC analysis of a methanol extract of the aerial parts of *Cuscuta reflexa* afforded a non-separable mixture (55: 45) of two novel tetrahydrofuran derivatives, named swarnalin (1) and cis-swarnalin (2), and a known coumarin, 5, 6,7-trimethoxycoumarin (3). The structures of the compounds were elucidated unequivocally by UV, HRFABMS and a series of 1D and 2D NMR analyses. The mixture of 1 and 2 showed significant free radical scavenging activity in the DPPH assay and the RC50 value was found to be 3.80 x 10(-4) mg mL(-1) for the mixture, compared to 2.88 x 10(-5) mg mL(-1) for the positivecontrol, quercetin[15].

The regulatory step in ABA synthesis is the cleavage reaction of a 9-cis-epoxy-carotenoid catalyzed by the 9-cis-epoxy-carotenoid dioxygenases (NCEDs). The parasitic angiosperm *Cuscuta reflexa* lacks neoxanthin, one of the common precursors of ABA in all higher plants. Thus, is *C. reflexa* capable of synthesizing ABA, or does it acquire ABA from its host plants? Stem tips of *C. reflexa* were cultured in vitro and found to accumulate ABA in the absence of host plants. This demonstrates that this parasitic plant is capable of synthesizing ABA. Dehydration of detached stem tips caused a big rise in ABA content. During dehydration, 18O was incorporated into ABA from 18O2, indicating that ABA was synthesized de novo in *C. reflexa.* Two NCED genes, CrNCED1 and CrNCED2, were cloned from *C. reflexa*. Expression of CrNCEDs was up-regulated significantly by dehydration. In vitro enzyme assays with recombinant CrNCED1 protein showed that the protein is able to cleave both 9-cis-violaxanthin and 9'-cis-neoxanthin to give xanthoxin. Thus, despite the absence of neoxanthin in *C. reflexa*, the biochemical activity of CrNCED1 is similar to that of NCEDs from other higher plants. These results provide evidence for conservation of the ABA biosynthesis pathway among members of the plant kingdom.[16]

The plant parasite *Cuscuta reflexa* induces various responses in compatible and incompatible host plants. The visual reactions of both types of host plants including obvious morphological changes require the recognition of *Cuscuta sp.* A consequently initiated signaling cascade is triggered which leads to a tolerance of the infection or, in the case of some incompatible host plants, to resistance. Calcium (Ca (2+)) release is the major second messenger during signal transduction. Therefore, researchers studied Ca (2+) spiking in tomato and tobacco during infection with *C. reflexa*. In recently published study Ca (2+) signals were monitored as bioluminescence in aequorin-expressing tomato plants after the onset of *C. reflexa* infestation. Signals at the attachment sites were observed from 30 to 48 h after infection. In an assay with leaf disks of aequorin-expressing tomato which were treated with different *C. reflexa* plant extracts it turned out that the substance that induced Ca (2+) release in the host plant was closely linked to the parasite's haustoria.[17 18]

Plant infestation with parasitic weeds like *Cuscuta reflexa* induces morphological as well as biochemical changes in the host and the parasite. These modifications could be caused by a change in protein or gene activity. Using a comparative macroarray approach *Cuscuta* genes specifically up regulated at the host attachment site were identified. One of the infestation specific *Cuscuta* genes encodes a cysteine protease. The protein and its intrinsic inhibitory peptide were heterologously expressed, purified and biochemically characterized. The haustoria specific enzyme was named cuscutain in accordance with similar proteins from other plants, e.g. papaya. The role of cuscutain and its inhibitor during the host parasite interaction was studied by external application of an inhibitor suspension, which induced a significant reduction of successful infection events. The study provides new information about molecular events during the parasitic plant--host interaction. Inhibition of cuscutain cysteine proteinase could provide means for antagonizing parasitic plants.[19]

GENETICS AND MOLECULER BIOLOGY

Genomic and molecular biological aspects of c. reflexa showed a powerful tool to enhance the biotechnological studies on other phytomedicinal sources. This includes-

The tissue-specific differences in the 5-methylcytosine (m5C) content in total DNA of the parasite plant *Cuscuta reflexa* have been found: DNA from apical parts of the plant is less methylated (m5C = 4,2 mol %) as compared to the DNA from haustoria and posthaustorial regions (m5C = 5,4 mol %). The base compositions of total DNA preparations from *C. reflexa* grown on various hosts are similar. The m5C amount in stem DNA of the alfalfa plant infected with *C. reflexa* is by approximately 25% higher than that in the non-infected plant DNA. The GC content in alfalfa DNA does not change as a result of infection. Thus, the parasite induces the hypermethylation of DNA in the host plant. It is assumed that the changes in DNA methylation induced by the parasite plant may play a regulatory role and may cause changes in transcription and replication of host DNA[20].

A team of researchers cloned and sequenced an area of about 6 kb of the plastid DNA (ptDNA) from the holoparasitic plant *Cuscuta reflexa.* This region contains (in the following order) genes for the cytochrome b6 f-complex subunit V (petG), tRNA(Val) (trnV), tRNA(Met) (trnM), the epsilon- and beta-subunit of the chloroplast ATP-synthase (atpE and atpB) and the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; rbcL). In addition identified other photosynthesis- psbC, and psbD) in *C.*

reflexa by heterologous hybridization. The gene arrangement of the sequenced area is, except for the petG gene, the same as in ptDNAs of other higher plants (e.g. *Nicotiana tabacum)*. Sequence homologies between the *Cuscuta* genes and corresponding genes from higher plants are in the range of 90%. The only significant difference is that the rbcL gene of *C. reflexa* encodes a polypeptide which is 18-23 amino acids longer than in other higher plants. This is remarkable since *C. reflexa* has lost its ability to grow photoautotrophically. The transcript level of the rbcL gene, however, is strongly reduced as compared to tobacco. These findings are compatible with results from Western blotting analysis [21].

Some researchers determined the nucleotide sequence of a 5.3-kb region of the plastid DNA (ptDNA) from the heterotrophic holoparasitic plant *Cuscuta reflexa*. The cloned area contains genes for the D1-protein (32-kDa protein; psbA), tRNA (His) (trnH), ORF 740 (homologous to ORF 2280 from *Nicotiana tabacum*), ORF 77 (homologous to ORF 70), tRNA (Leu) (trnL) and a hypothetical ORF 55 which has no homology to any known gene among higher plants. This 5.3-kb area is collinear with a 12.4-kb region of tobacco ptDNA and has therefore undergone several deletions totaling 7.1 kb. Most of the missing nucleotides belong to one large deletion in the ptDNA of *C. reflexa* of approximately 6.5 kb. This deletion involves two ribosomal protein genes, rpl2 and rpl23, as well as the transfer RNA for Isoleucin (trnI) and a region encoding 1540 amino-acid residues of an ORF 2280 homologue, as compared to tobacco chloroplast DNA. This is remarkable since the remaining genes, especially the psbA gene, are highly conserved in *C. reflexa.* The phylogenetic position of, and the evolutionary change of ptDNA from, *Cuscuta* are discussed [22].

A group of scientist cloned and sequenced an area of about 9.0 kb of the plastid DNA (ptDNA) from the holoparasitic flowering plant *Cuscuta reflexa* to investigate the evolutionary response of plastid genes to a reduced selective pressure. The region contains genes for the 16S rRNA, a subunit of a plastid NAD(P)H dehydrogenase (ndhB), three transfer RNAs (trnA, trnI, trnV) as well as the gene coding for the ribosomal protein S7 (rps7). While the other genes are strongly conserved in *C. reflexa*, the ndhB gene is a pseudogene due to many frameshift mutations. In addition used heterologous gene probes to identify the other ndh genes encoded by the plastid genome in higher plants. No hybridization signals could be obtained, suggesting that these genes are either lost or strongly altered in the ptDNA of C. reflexa. Together with evidence of deleted genes in the ptDNA of C. reflexa, the plastid genome can be grouped into four classes reflecting a different evolutionary rate in each case. The phylogenetic position of Cuscuta and the significance of ndh genes in the plastid genome of higher plants are discussed [23]. Similar type of studies was done to complete cDNA encoding a novel hybrid Pro-rich protein (HyPRP) that was identified by differentially screening 3 x 10(4) recombinant plaques of a *Cuscuta reflexa* cytokinin-induced haustorial cDNA library constructed in lambda gt10. The nucleotide (nt) sequence consists of: (i) a 424-bp 5'-non coding region having five start codons (ATGs) and three upstream open reading frames (uORFs); (ii) an ORF of 987 bp with coding potential for a 329-amino-acid (aa) protein of M(r) 35,203 with a hydrophobic N-terminal region including a stretch of nine consecutive Phe followed by a Pro-rich sequence and a Cys-rich hydrophobic C terminus; and (iii) a 178-bp 3'-UTR (untranslated region). Northern analysis revealed an approx. 1.8-kb mRNA of this gene expressed in the subapical region of the C. reflexa vine which exhibited maximum sensitivity to cytokinin in haustorial induction [24].

A cDNA clone isolated by differentially screening a cytokinin-induced haustorial cDNA library of *Cuscuta reflexa* was sequenced and identified as the gene coding for cytochrome b5, based on the similarity of the deduced amino-acid sequence with that of the cauliflower (60% identity) and tobacco (78% identity) proteins. The 5'-UTR is unusually long (720 bp) and contains 14 potential start codons (ATG) and 10 short ORFs.[25]

Cuscuta reflexa reveals a complete loss of the cis-spliced intron of the rps12 gene in addition to a drastic size reduction of the ndhB pseudogene. It is demonstrated by RT-PCR analysis that the entire gene cluster is transcribed in the form of a multicistronic transcript which also includes the sequences encoded by the ndhB pseudogene. A cDNA containing the correctly transpliced exon 1 of the rps12 transcript can also be amplified. This shows that trans-splicing of the rps12 transcript persists in the plastids of the holoparasite despite the loss of the cis-spliced intron and the loss of many other gene functions. The rps12 and rps7 genes, therefore, still appear to code for functional ribosomal proteins CS12 and CS7, respectively. The conservation of apparently intact ribosomal-protein genes from which correctly processed transcripts are produced is taken as evidence that the translational apparatus of the plastids is still functional and necessary for the expression of the genes remaining in the reduced plastome of a parasitic plant.[26]

Plastids of *Cuscuta reflexa* possess thylakoids and contain both chlorophyll a and b in a ratio similar to that of stem tissue of the systematically closely related but 'normal' green *Ipomoea tricolor*. Light-driven electron transport, as measured by oxygen evolution and indicated by analysis of chlorophyll fluorescence, was present in all chlorophyll-containing species. The photosystem II efficiency was low and ranged from 0.511 to 0.687. The plastid rbcL gene present in all other tested species Low amounts of the large subunit of Rubisco was detected immunologically in all other *Cuscuta species*. Apparently, the genus *Cuscuta* species with different degrees of plastid functionality, ranging from intact chloroplasts, via plastids with impaired protein production and gene expression to plastids with reduced plastome gene content.[27] Transgenic tobacco plants expressing green fluorescent protein (GFP) under the control of the companion cell-specific promoter, AtSUC2, were parasitized by the holoparasite *Cuscuta reflexa* (dodder). GFP, moving in the translocation stream of the host, was transferred to the Cuscuta phloem via the absorbing hyphae of the parasite. An identical pattern of transfer was observed for the phloem-mobile probe, carboxyfluorescein. Following uptake by the parasite, GFP was translocated sand unloaded from the *Cuscuta* phloem in meristematic sink tissues. Contrary to published data, these observations suggest the presence of a functional symplastic pathway between *Cuscuta* and its hosts, and demonstrate a considerable capacity for macromolecular exchange between plant species[28].*Cuscuta* have been found to increase at a similar stage of infection. In contrast to the different behavior with respect to infection, IAA induced both LeAqp2 and TRAMP expression. The observed pattern of LeAqp2 expression during the interaction at a stage where cell elongation occurs together with the water-channel activity in the heterologous expression system suggests a function for LeAqp2 during the tomato-*Cuscuta* interaction[29].

The holoparasitic plant genus *Cuscuta* a range of species whose plastid genomes have different degrees of reductions

in their coding capacity. In this study, , *Cuscuta reflexa, and sp.* that possess substantial physiological differences, were analyzed with respect to the sequence and promoter structure of the rrn16 gene coding for the ribosomal 16S rRNA. Whereas the coding region of this gene is highly conserved among all four *Cuscuta species*, significant differences were observed in the non-coding region 5' of rrn16 with respect to both the length of the intergenic region between rrn16 and trnV and the promoters used to initiate transcription of the rrn16 gene. In the green species *C. reflexa*, rrn16 transcription starts from a functional plastid-encoded RNA polymerase (PEP) promoter [30].

The chlorophyll containing holoparasitic species *Cuscuta reflexa* contains only traces of chlorophyll, were compared with respect to their plastid coding capacity and plastid gene expression at the level of RNA. *C. reflexa* has retained an almost complete plastid genome. It has retained photosynthesis-related genes. Hybridization with radioactive 3'-labelled RNA revealed that in all these species only a small 'parasite-specific' portion of the plastid genome consisting of mainly rRNAs and t RNAs is represented at the level of steady-state RNA. Run-on transcription assays revealed that in plastids of *C. reflexa* the entire genome is transcribed. Hence, the subset of RNA species required for a parasitic lifestyle is preferentially stabilized in *Cuscuta* plastids.[31] Some species of the holoparasitic flowering plant genus *C. reflexa* have retained a plastid genome that encodes photosynthesis-related gene products as well as the plastid-encoded RNA polymerase (PEP. In order to ensure expression of the photosynthesis-related genes in the absence of PEP, a number of adaptations within the plastid genome were required that enable gene transcription mediated exclusively by the nuclear-encoded plastid RNA polymerase (NEP). In this study scientists analyzed promoter sequence conservation and transcription start sites of a typical PEP gene of non-parasitic plants, rbcL, which codes for the large subunit of ribulose bisphosphate carboxylase/oxygenase show that despite high sequence conservation of the coding region of rbcL Primer-extension analyses enabled the identification of transcripts initiated at NEP promoter motifs in *Cuscuta sp.* that are not detectable in the 5' non-coding region of *C. reflexa* [32].

The newly sequenced plastid genomes of *C. reflexa* reveal that the chromosome structures are generally very similar to that of non-parasitic plants, although a number of species-specific insertions, deletions (indels) and sequence inversions were identified observed a gradual adaptation of the plastid genome to the different degrees of parasitism the first documented loss of the gene for a putative splicing factor, MatK, from the plastid genome and (c) a significant reduction of RNA editing. Overall, the comparative genomic analysis of plastid DNA from parasitic plants indicates a bias towards a simplification of the plastid gene expression machinery as a consequence of an increasing dependency on the host plant. A tentative assignment of the successive events in the adaptation of the plastid genomes to parasitism can be inferred from the current data set. This includes (a) a loss of non-coding regions in photosynthetic *Cuscuta species* that has resulted in a condensation of the plastid genome, (b) the simplification of plastid gene expression in species with largely impaired photosynthetic capacity and (c) the deletion of a significant part of the genetic information, including the information for the photosynthetic apparatus, in non-photosynthetic parasitic plants.[33]

Complete DNA sequences of the plastid genomes of two parasitic flowering plants species, which was believed to have been lost during evolution before the emergence of angiosperms. In addition, the existence of silent editing in plant chloroplasts has been confirmed, and some probable reasons for its presence are pointed out herein.[34] The parasitic plant species *Cuscuta reflexa* have independently developed parasitism, the former parasitizing on shoots and the latter attaching to roots. Regardless of these differences, the two species use similar organs, termed haustoria, to attach to the host plant. The morphological similarity can be extended to the molecular level.[35]

MEDICINAL, CLINICAL AND ETHANOPHARMACOLOGICAL STUDIES

Medicinal plants have played an important role in treating and preventing a variety of diseases throughout the world., India still depend on medicinal plants and most of them have a general knowledge of medicinal plants which are used for treating a variety of ailments. The medicinal plants used in folk medicine to treat diabetes mellitus.

In this study scientists compared the in vitro antiproliferative activity of extracts from medicinal plants toward human tumor cell lines, including humanery thromyeloid K562, B-lymphoid Raji, T-lymphoidJurkat, erythroleukemic HEL cell lines. Extracts from *Cuscuta reflexa* was the most active in inhibiting in vitro cell proliferation, due to their hepatoprotective, antioxidant, antifungal, antimicrobial and anti-inflammatory medicinal activities. Gas chromatography/mass spectrometry analyses allowed to identify pyrogallol as the common compounds present both in unfractionated and n-butanol fraction of plant extracts. Antiproliferative effects of pyrogallol were therefore determined on human tumor cell lines thus identifying pyrogallol as an active component of *Cuscuta reflexa* extracts[36]. The effect of methanolic extract (ME) of *Cuscuta reflexa* stem Roxb on the onset of reproductive maturity and the ovarian steroidogenesis was studied by means of biochemical techniques. ME of *Cuscuta reflexa* stem treatment cause a remarkable delay in sexual maturation as evidenced by the age at vaginal opening and appearance of first estrus (cornified smear). The same treatment also results in a significant diminution of Delta (5)-3beta-hydroxysteroid dehydrogenase (HSD) and glucose-6-phosphate dehydrogenase (G-6-PD) activity along with a reduction in the weight of ovary, uterus and pituitary. On the basis of above data, it is assumed that the probable cause of delayed maturation in ME of *Cuscuta reflexa stem* treated mice is due to the suppressed ovarian steroidogenesis.[37]

The methanolic extract of *Cuscuta reflexa* stem showed marked protection against convulsion induced by chemoconvulsive agents in mice. The catecholamines contained were significantly increased in the processed extract treated mice. The amount of GABA, which is most likely to be involved in seizure activity, was increased significantly in mice brain after a six week treatment. Results of the present study revealed that both the processed extract.[38]

The petroleum ether extract of *Cuscuta reflexa* Roxb. stem (PECR) was evaluated for its psychopharmacological activities in several experimental models using Swiss albino mice. The PECR was found to cause significant reduction in spontaneous activity and exploratory behavioral profiles. It also showed reduction in muscle relaxant activity by rotarod, 30 degrees inclined screen tests and showed significant analgesic properties as well as potentiated remarkably the

pentobarbitone sodium, diazepam and meprobamate--induced sleeping time. All these results were compared with respective controls for the evaluation of significance. The presence of steroids in the PECR might be responsible for psychopharmacological activities showed a significant anticonvulsive property by altering the level of catecholamines and brain amino acids in mice.[39] Methanolic extract (ME) of *C. reflexa* stem arrested the normal oestrus cycle of adult female mouse and significantly decreased the weight of ovaries and uterus. The cholesterol and ascorbic acid contents in ovaries were significantly increased in the treated mice. Two key enzymes, delta5-3beta-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase, were decreased significantly in ME of C. *reflexa* stem after 17 days of treatment. High level of substrates and low level of enzymes indicate the inhibition of steroidogenesis in treated mice and may be due to the presence of flavonoids.[40]

Cuscuta reflexa and sp. are traditionally acclaimed herbs for their hair growth-promoting potential. In the present study, it was envisaged to prepare herbal formulations containing petroleum ether extracts of the three herbs in varying ratio and evaluating the formulations for the hair growth-promoting activity. The formulations as well as minoxidil (2%) solution (positive control) were applied topically on shaved skin of rats, and the time required for initiation and completion of hair growth cycle was recorded. Hair growth initiation time was markedly reduced to one third on treatment with the prepared formulation compared with control animals. The time required for complete hair growth was also reduced by 32%. Quantitative analysis of hair growth cycle after treatment with formulations and minoxidil (2%) exhibited greater number of hair follicles in anagenic phase compared with growth-promoting capabilities of the plants. The prepared formulation also holds potential for treatment of alopecia.[41] *Cuscuta reflexa* Roxb is evaluated for hair growth activity in androgen-induced alopecia. Petroleum ether extract of *C. reflexa* was studied for its hair growth-promoting activity. Alopecia was induced in albino mice by testosterone administration for 20 days. Its inhibition by simultaneous administration of extract was evaluated using follicular density, anagen/telogen ratio, and microscopic observation of skin sections. To investigate the mechanism of observed activity, in vitro experiments were performed to study the effect of extract and its major component on activity of 5alpha-reductase enzyme. Petroleum ether extract of *C. reflexa* exhibited promising hair growth-promoting activity as reflected from follicular density, anagen/telogen ratio, and skin sections. Inhibition of 5alpha-reductase activity by extract and isolate suggest that the extract reversed androgen-induced alopecia by inhibiting conversion of testosterone to dihydrotestosterone. The petroleum ether extract of *C. reflexa* and its isolate is useful in treatment of androgen-induced alopecia by inhibiting the enzyme 5alpha-reductase.[42] *Cuscuta reflexa* (whole plant) are used in folk medicine of Bangladesh to control blood sugar in patients suffering from diabetes mellitus. The hypoglycemic effects of methanol and chloroform extracts of whole plants of *Cuscuta reflexa* were investigated in oral glucose tolerance tests in Long Evans rats and Swiss, when tested at doses of 100 and 250 mg/kg body weight did not demonstrate any oral hypoglycemic effect when tested in glucose-loaded mice.[43]

The antipyretic activity of aqueous and ethanol extracts from *Cuscuta reflexa* Roxb. (Cuscutaceae) was evaluated using Brewer's yeast induced pyrexia in rats. Both the extracts at 200 and 400 mg/kg body weight dose significantly ($p < 0.05$) reduced the increased rectal temperature. The extracts started reducing the elevated rectal temperature after 3 h of treatment in a dose related manner. At the dose of 400 mg/kg body weight the aqueous and ethanol extract reduced 79 % and 83.8 % respectively of the elevated rectal temperature as compared to reference drug paracetamol (96.5 %) after 6 h of treatment. It was therefore concluded that both the extracts of *C. reflexa* demonstrated antipyretic activity, the ethanol extract was found to be slightly potent than the aqueous extract.[44]

To determine anti-inflammatory and anti-cancer activities of *Cuscuta reflexa* in cell lines (in vitro).Anti-inflammatory activity of the water extract was analyzed in vitro using lipopolysaccharide (LPS) induced inflammatory reactions in murine macrophage cell line RAW264.7. The expression of COX-2 and TNF-α genes involved in inflammation was analysed by SQ RT-PCR. EMSA was conducted to analyze the influence of the extract on NF-κB signaling. Anti-cancer activity was analyzed on Hep3B cells by MTT assay, DAPI staining, annexin V staining and SQ-RT PCR analysis of BAX, Bcl-2, p 53 and surviving. The extract down regulated LPS induced over expression of TNF-α and COX-2 in RAW264.7 cells; blocked NF-κ B binding to its motifs and induced apoptosis in Hep3B cells as evidenced from MTT, DAPI staining and annexin V staining assays. The extract up regulated pro-apoptotic factors BAX and p53, and down regulated anti-apoptotic factors Bcl-2 and surviving. The study showed that *Cuscuta reflexa* inhibits LPS induced inflammatory responses in RAW264.7 cells through interplay of TNF-α, COX-2 and NF-κ B signaling. It induced apoptosis in Hep3B cells through the up regulation of p53, BAX and down regulation of Bcl-2 and surviving.[45]

There was great agreement among informants regarding phytotherapeutic uses of medicinal plants with factor of informants' consensus (F(IC)) value ranging from 0.84 to 1, with an average value of 0.94.Study reveals that there is great agreement among informants for the usages of *Cuscuta reflexa* Roxb & some other plants. These species may be used for the development of new, cheep, effective, and eco-friendly herbal formulations for veterinary healthcare management. Further investigation of these herbal formulations for veterinary healthcare management will require safety and efficacy testing. There is an urgent need to formulate suitable conservation strategies for wildly growing phytotherapeutics to overcome their depletion from natural resources and to make these practices more eco-friendly.[46]

Cuscuta reflexa Roxb.is a golden yellow, leafless, perennial, parasitic herb of the family Convolvulaceae. *C.reflexa* has been investigated for antispasmodic, hemodynamic, anticonvulsant, antisteroidogenic, antihypertensive, muscle relaxant, cardiotonic, antiviral, antibacterial, antioxidant, cholinergic, diuretic and hair growth activities. Many chemical constituents have been isolated from C. reflexa such as cuscutin, amarbelin, β-sitosterol, stigma sterol, kaempferol, dulcitol, myricetin, quercetin, coumarin and oleanolic acid. This review presents a detailed survey of the literature on pharmacognosy, phytochemistry and traditional and biological medicinal uses of *C. reflexa*[47]. *Cuscuta reflexa* plants used traditionally in treating diabetes mellitus suggests that eleven plant species make claims of new reports on antidiabetic efficacy. Some researchers reported that plant has an antidiabetic effect on rodent models but none have sufficient clinical evidence of effectiveness. The wide variety of medicinal plants that are used to treat diabetes mellitus in this area supports the importance of plants in the primary

healthcare system of the rural people of Lohit district of Arunachal Pradesh. The finding of new plant uses in the current study reveals the importance of the documentation of such ethnobotanical knowledge [48].

Methanol extract of *Cuscuta reflexa* Roxb. Stem (MECR) contain flavonoids (0.2%) (MECO) was found to contain steroids and cardenolide glycosides. Effects of multiple weekly dose of MECR (25, 50, 75 mg/kg, i.p.) and MECO (15, 20, 25 mg/kg, i.p.) on liver and kidney functions and hematological parameters in mice were studied. No significant alteration of RBC` count and hemoglobin content was observed in all dose level of treatment in MECR and MECO treated mice whereas significant increase of clotting time was seen in moderate and high doses in both case. MECR and MECO both caused significant increase in WBC count only in high dose level of treatment. Both the extracts in medium and high dose level increased SGOT, SGPT, NPN and plasma cholesterol significantly. Serum alkaline phosphatase and total bilirubin were also increased by both moderate and high dose level of treatments in MECR and MECO treated mice respectively. Low dose of both the extract did not exhibit any significant change of creatinine and serum protein level. But high dose level of MECR and MECO significantly increased creatinine level. Increase in plasma cholesterol may be due to decrease in cholesterol catabolism owing to liver dysfunction of due to the intake of MECO itself as it was found to be steroid in nature. Elevated level of SGOT, SGPT and serum alkaline phosphatase activity in moderate and high dose level of weekly treated mice may be due to improper liver function following the treatment. Increased urea, non protein nitrogen and creatinine content in blood have been observed with impaired renal function. The slightly higher toxicity in case of MECO treated mice may be due to the presence of cardenolide glycosides in the stem [49].

PLANT PHYSIOLOGY

Protoplasts isolated from *Cuscuta reflexa* exhibited a higher rate of exogenous NADH oxidation as compared to NADPH in the dark. NADPH oxidation was monitored by measuring the rate of oxygen consumption and this oxidase system was sensitive to blue light. Both NADH oxidase and its blue light sensitivity were inhibited by -SH group reacting agents. The corresponding changes occurring in H+-extrusion activity and intracellular ATP levels were also monitored. Stimulation of NADH oxidation under blue light corresponded to increased rate of H+-extrusion andintracellular ATP level, the converse was also true under NADH oxidase inhibitory conditions. These observations suggested a close functional association between blue light-sensitive plasma membrane bound redox activity and H+-ATPase in this tissue. Further, concanavalin A binding of protoplasts resulted in a loss in NADH oxidase activity and its blue light sensitivity suggesting apoplastic location and glycoprotein nature of the blue light sensitive NADH oxidase system in *Cuscuta.*[50]

The growth of dodders, *Cuscuta reflexa*, on the partially incompatible host *Euphorbia pulcherrima* is studied, by bark growths to the formation of the dodder haustoria and prevents dodder from obtaining normal growth. The growth instead becomes extremely branched, coral-like, and dodder lacks the ability to form haustoria. After a period of coral-like growth, long shoots sprout, resembling the normal growth. These long shoots mark an ending phase for dodder, which dies shortly after without having flowered. During the coral-like growth phase, dodder develops transfer cells in the parenchyma cells bordering the vessels of the xylem in the shoot. The transfer cells have not been observed when dodder is grown on the compatible host Pelargonium zonale. A coral-like growth phase has also been observed at the establishing phase when dodder is grown in vitro on agar; later a more normal growth form takes over. In this coral phase, xylem transfer cells are also developed. The fluorochromes carboxyfluorescein and Texas Red were loaded into the host in the phloem and xylem, respectively, and detection of these fluorochromes in the dodder stem indicated that a functional haustorial contact developed for both vascular systems. The results show that *Cuscuta sp*. have the genetic ability to develop xylem transfer cells and use this in response to developmental stress [51].

A suppressive subtractive hybridization technique was used to identify genes, which were induced during the early phases of the interaction between dodder (*Cuscuta reflexa),* a phanerogamic parasite, and its incompatible host plant tomato. One of the identified genes encodes a tomato xyloglucan endotransglycosylase/hydrolase (XTH)--an enzyme involved in cell wall elongation and restructuring. The corresponding LeXTH1 mRNA accumulated 6 h after attachment of the parasite. In contrast, wounding did not influence the expression level. Subsequent to LeXTH1 mRNA accumulation, an increase in XTH activity at the infection sites as well as in adjacent tissues was observed. The effect of IAA on LeXTH1 expression was analyzed because the concentration of this phytohormone is known to increase in the tomato tissue during the interaction with the parasite. LeXTH1 mRNA accumulation was in fact induced by external application of auxin. However, in the auxin-insensitive tomato mutant diageotropica, *Cuscuta* induced LeXTH1-mRNA accumulated with a time course similar to wild type tomato. Thus, *auxin* appears not to be an essential signal for infection-induced LeXTH1 activation. Our data suggest a role for xyloglucan transglycosylation in defence reactions associated with the incompatible tomato- *Cuscuta* interaction[52]. Effect of cadmium on growth, antioxidative enzymes namely catalase, peroxidase, glutathione reductase, level of glutathione and phytochelatin synthesis was investigated in callus and seedlings of *Cuscuta reflexa*. A time, concentration and tissue dependent response of Cd was observed. Cd inhibited the growth of callus and seedlings by 50% at 300 and 500 micromol/L concentrations, respectively. Shorter exposure of low concentration of Cd led to augmentation of antioxidant activity, both in callus and seedlings, while longer exposure and high concentration of Cd led to a concentration dependent decrease in callus. Analysis of phytochelatin (PC) synthesis in callus and seedlings of *C. reflexa* revealed both quantitative and qualitative changes. Cd at low concentrations led ` to synthesis of predominantly PC4, while at higher concentrations, PC3 was the` major form being synthesized. Amelioration of antioxidative systems of C. reflexa` in response to Cd stress might be playing a protective role, alleviating the` damaging effects of ROS, generated during Cd stress. Concomitantly, chelation and` sequestering of toxic Cd ions in this parasite was mediated by synthesis of PC. The response to Cd stress shown by this holoparasitic plant was found to be similar to those of non-parasitic plants (hosts) [53].

OTHER IMPACTS

A toxic effect of alpha-alpha-trehalose in an angiospermic plant, *Cuscuta reflexa (*dodder), is described. This disaccharide and its analogs, 2-aminotrehalose and 4-

aminotrehalose, induced a rapid blackening of the terminal region of the vine which is involved in elongation growth. It is concluded that the toxic effect of trehalose in Cuscuta is because of the very low trehalase activity in the vine. As a result, trehalose accumulates in the vine and interferes with some process closely associated with growth. It is concluded that, if allowed to accumulate within the tissue, trehalose may be potentially toxic or inhibitory to higher plants in general.[54] Trehalose, an alpha-alpha-diglucoside, induced a rapid blackening and death of shoot tips of *Cuscuta reflexa* (dodder) cultured in vitro. The onset of toxic symptom was delayed if any of the several sugars which support the in vitro growth of *Cuscuta* was supplied with trehalose. The rate of trehalose uptake or its accumulation in the tissue was not affected by sugar cofeeding. The symptom of trehalose toxicity was duplicated by 2-deoxyglucose, which has been shown to be a potent inhibitor of cell wall synthesis in yeast. Trehalose interferes with the synthesis of cell wall polysaccharides, the chief component of which was presumed to be cellulose.[55] Dodder or *Cuscuta sp.* are holoparasitic plants subsisting on other dicotyledonous plants. The infection process is initiated by adherence of to the host surface, followed by penetration attempts by hyphae. In the case of a successful infection, these organs connect the parasite's vascular tissue to that of the host.[56]

CONCLUSION

C. reflexa is a wonderful parasitic plant having enormous range of medicinal activity .in this article have assembled almost all information related to different research activity of plant. Same type of review paper has been published on *Tribulus terrestris*, a traditionally important wild medicinal herb of waste lands, which became a popular article for further investigations on particular medicinal herbs.[57] This review will help to researchers & scholars to go deep in this area as plant indicate vast range of phytochemical related to origin so it can be suggested the further work can be done on *C. reflexa* which is collected from different season & agro climatic zone .Definitely it is assume that research will be able to find out more suitable & specific drug plant having particle activity in specific season.

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