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Comparative antioxidant activity of *Cuscuta reflexa* and *Cassytha filiformis*

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ABSTRACT

In the present study,comparative anti-oxidant activity of alcoholic extracts of *Cuscuta reflexa* and *Cassytha filiformis* was assessed. Anti-oxidant activity of alcoholic extracts of *Cuscuta reflexa* and *Cassytha filiformis* have been analysed 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 bio-autographic 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 *Cassytha filiformis* which were expressed as IC50. Ethanolic extracts of *Cuscuta reflexa* contained more polyphenols content compared with ethanolic extracts of *Cassytha filiformis.*

Key words: *Cuscuta reflexa, Cassytha filiformis* **,** antioxidant activity, DPPH assay, Ascorbic Acid

INTRODUCTION

The plant *Cuscuta reflexa* Roxb. Coron is a perennial herb of Convolvulaceae family, commonly known as Akashbela in Hindi. The plant is distributed throughout India, Ceylon and Malaya. The *Cuscuta reflexa* has been investigated for antispasmodic, heamodynamic, bradycardia[1], antisteroidogenic[2], antihypertensive, muscle relaxant, cardiotonic [3], psychopharmacological [4], antiviral and anticonvulsant[5] activities. Many chemical constituents have been isolated from *Cuscuta reflexa* such as, cuscutin, amarbelin, beta-sterol, stigmasterol, kaempferol, dulcitol, myricetin, qurecetin, coumarin and oleanolic acid[6]. *Cassytha filiformis* Linn., is perennial, parasitic, herbaceous and leafless plant belonging to family Lauraceae. This plant is distributed throughout India and is used for medicinal purpose in China, Indochina, Madagascar and South Africa. *Cassytha filiformis* is used as antiplatelet agent, vesorelaxant[7], alpha-adrenoreceptar antagonist[8] and antitrypnosomal agent[9]. Some of the isolated compound from this plant are aporphine alkaloid, oxo-aporphine alkaloid, cassyformine, filiformine, cathaformine, lignan, actinodophine, and octenine[10]. In ayurveda, *Cassytha filiformis* is used as substitute for *Cuscuta reflexa*. Literature review reveals that *Cuscuta reflexa* used as antioxidant agent[11]. Since the comparative antioxidant activity of these plants has not been scientifically investigated, the present study was designed to evaluate the antioxidant activity of *Cuscuta reflexa* and *Cassytha filiformis* .

MATERIAL AND METHODS

Plant material

The aerial part of *Cuscuta reflexa* was collected from the field of Charthaval, U. P. and identify by Mr. Mohan Kumar, Dept of Botany, R.K.P.G. College, Shamli, U.P. India. the field of Tirupati, Andhra Predesh and authenticated by Dr. K. Madhva Chetty, Dept of Botany. Vankateswara University, Tirupati, A.P. India.

Anti-oxidant activity

Comparative Anti-oxidant effect of alcoholic extracts of both plants *Cuscuta reflexa* and *Cassytha filiformis* were evaluated and compound responsible for activity were identified by direct Bioautographic analysis performed over precoated Thin Layer chromatographic plates by their respective R_f values.

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Total phenolic content

100mg of extracts were dissolved 100 ml of respective solvent. 10ml from this solution and again made up to 100ml with same solvent. 1, 2, 3, 4, 5 ml of this solution was took in a series of 10ml volumetric flask and 0.6ml of 10% folin-ciocalteu reagent and 2ml of 20% sodium carbonate solution in water was added. Solution was shaken vigorously and allowed to stand for 15min. Absorbance was measured at 650nm against reagent blank. Gallic acid was used as standard.[12]

The DPPH assay:

The method used by Takao *et al* was adopted. 4mg of DPPH was dissolved in methanol (50ml) to obtained a dilution of 80µg/ml. Serial dilution were made with stock solution (10mg/ml)of the plant extracts to obtain concentration of 1-500mg/ml. Diluted solutions (2ml each) were mixed with DPPH (2ml) and allowed to stand for 30min for any reaction to occur. The UV absorbance was recorded at 517nm. The experiment was performed in duplicate and the average absorption was noted for each concentration.[13]

Lipid peroxidation assay

A modified thiobarbituric acidreactive species (TBARS) assay[14] was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media [15]. Lipid peroxidation was induced by FeSO4. Malondialdehyde (MDA), produced by the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm which was measured. Percentage inhibition of lipid peroxidation by different concentrations of the extract was calculated.

The IC_{50} value, which is the concentration of test material that reduces 50% of the free radical concentration of the test material, was calculated as mg/ml using this formula.

$$\mathbf{A_{517}\ (Control) - A_{517}\ (Sample) / A_{517}\ (Control)\ _{\times}\ 100}$$

Identification of antioxidant compounds

Anti-oxidant compound were identified by Direct Bioautographic analysis.[16] The alcoholic extracts of *Cuscuta reflexa* and *Cassytha filiformis* were dissolved in respective solvent and chromatographed on precoated silica gel 60 F_{254} plates. The samples were loaded on plates as bands. The plates were developed in selected solvent systems. The plates were dried in air flow for 3hrs then sprayed with 0.008% solution of DPPH in methanol using TLC

sprayer. Plates were placed in dark for 20min. for any reaction to be happened. Anti-oxidant compounds were identified as white spots on dark background. The R_f value of these spots was calculated.

RESULTS:

The preliminary phytochemical analysis revealed the presence of steroids, saponins, triterpenes and flavonoids in *Cuscuta reflexa* and steroid, triterpene, flavonoids and alkaloids in *Cassytha filiformis* in petroleum ether, chloroform, ethanol and aqueous extracts.

Comparative Evaluation of Antioxidant effect

Anti-oxidant effect for alcoholic extract of *Cuscuta reflexa* and *Cassytha filiformis* was evaluated on the basis of its ability to inhibit free radical (DPPH. Reduction in absorbance by different concentration of test sample and ascorbic acid was recorded, result are compiled in (**Table 1) Fig 1 .**The IC_{50} value of standard ascorbic acid was 2.04mg/ml. In *Cuscuta reflexa* the IC_{50} value was obtained 6.89mg/ml where as in *Cassytha filiformis* it was 11.02mg/ml in DPPH assay. Lipid peroxidation assay results are compiled in(**Table 2) Fig 2** in that the IC_{50} value of ethanolic extract of *Cuscuta reflexa* was found to be greater than the ethanolic extract of *Cassytha filiformis*. the IC_{50} value was obtained 37mg/ml in *Cuscuta reflexa* where as in *Cassytha filiformis* it was 46.5mg/ml

Table: 1 % inhibition of Scavenging effect of DPPH free radical.

Conc.(ig/ml)	std	CREE	CFEE
0	0	0	0
1	48.74	38.8	35.06
5	57.23	42.83	41.54
10	67.77	56.87	47.8
50	80.56	71.05	62.27
100	92.94	86.39	80.7
500	97.84	95.53	93.16

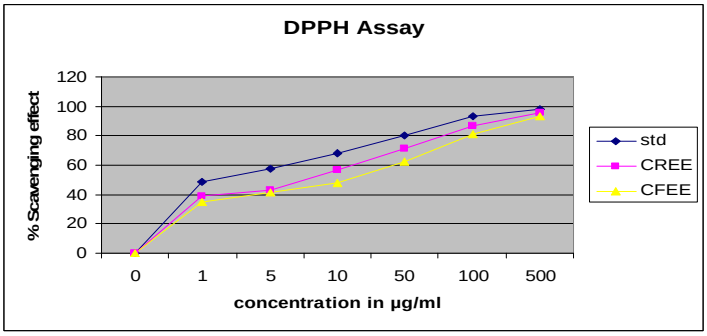

Figure:1 Free radical scavenging effect of standard and test samples.

Table: 2 % inhibition of Scavenging effect of Lipid peroxidation assay

Conc.(ig/ml)	std	CREE	CFEE
0	0	0	0
1	25.9	8.86	3.18
5	35.34	16.81	6.7
10	44.65	33.86	21.93
50	64.31	60.01	54.31
100	75.68	68.18	61.7
500	85.56	79.2	70.68

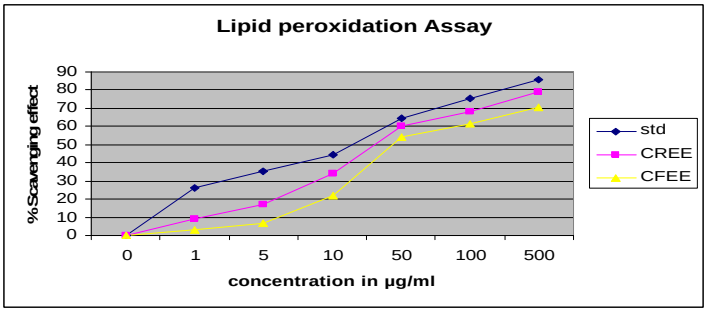

Figure:2 Lipid peroxidation effect of standard and test samples

Total polyphenol content:

Total Phenolics were estimated by folin-ciocalteu reagent by using Gallic acid as standard. Amount of phenolics were determined by using interpolating the absorbance on standard curve. Total polyphenol content was found to be 3.25% in alcoholic extract of *Cuscuta reflexa* . In alcoholic extract of *Cassytha filiformis* the total polyphenol content was found to be 1.15%, results are compiled in **Fig 3**

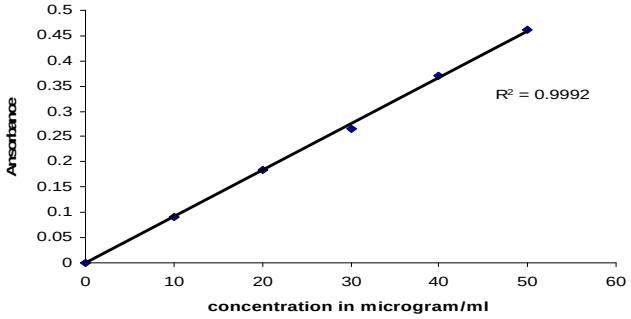

Figure:3 Standard curve for Gallic acid

Bioautographic analysis for identification of antioxidant constituents

In bioautographic analysis, alcoholic extract of *Cuscuta reflexa* eluted with the mobile phase toluene: ethyl acetate (5:7) whereas the *Cassytha filiformis* eluted with the mobile phase chloroform: methanol (8:2). After the sprayed with DPPH, 4 spots 0.15, 0.18, 0.32, 0.35 were obtained in *Cuscuta reflexa* whereas, in *Cassytha filiformis* only two spots 0.024,0.38 were found, result are compiled in **Fig: 4**

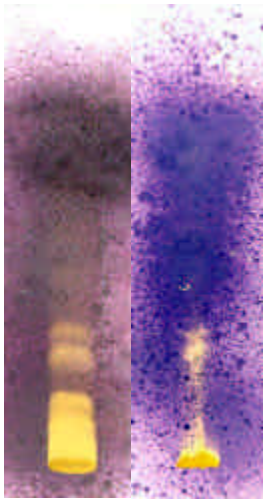

Figure:4 Bioautographic analysis for identification of antioxidant constituent

DISCUSSION

The present study attempts comparative evaluation of stem part of *Cuscuta reflexa* and *Cassytha filiformis* on anti-oxidant effect of alcoholic extracts of both plant. Anti-oxidants are known to protect the body against free radical mediated toxicity. The antioxidant activities of the medicinal plants of India were measured in different systems of assay, e.g. DPPH assay, superoxide radical-scavenging assay, hydroxyl radical-scavenging assay and lipid peroxidation assay. Antioxidant compound reduced the oxidation of compound and oxidized itself at their place. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms.

DPPH radicals are widely used in evaluation of antioxidant activity. When DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm. Results showed that, the scavenging activity against DPPH radicals of alcoholic extracts of both plants Cuscuta reflexa and *Cassytha filiformis* was found, while ascorbic acid was used as standard. All three samples exhibit dose-dependent anti-DPPH radical activity. IC50 values were calculated from these regression equations. IC50 value is inversely related to the activity.

However, considering all the results, it can be concluded both *Cuscuta reflexa* and *Cassytha filiformis* possess antioxidant activity, in which *Cuscuta reflexa* being more effective than the *Cassytha filiformis* in scavenging free radicals and superoxide radical .Among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules, *Cuscuta reflexa* is a good scavenger of superoxide radical and

DPPH radical. *Cuscuta reflexa* also has the highest total phenol content. This property of *Cuscuta reflexa* could possibly be related to its higher polyphenol content.

In the comparative antioxidant activity, the results showed that the ethanolic extract of *Cuscuta reflexa* has greater anti-oxidant activity than the ethanolic extract of *Cassytha filiformis*.

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REFERENCES

1. Hassan Gilani A.U. and Aftab K, Pharmacological action of *Cuscuta reflexa*. *Int. J. Pharmacog.*, (1992) **30**, 296
2. Gupta M., Mazumdar U.K, Pal D.K and Bhattacharya S, Anti-Steroidogenic activity of methanolic extract of *Cuscuta reflexa* roxb. stem & *Corchorus olitorius* Linn. seed in mouse ovary, *Indian Journal of Experimental Biology* 2003; 41 (6): 641.
3. Singh G. S and Garg K. N, Some pharmacological studies on *Cuscuta reflexa* plant, *Indian Journal of Pharmacognosy* 1973; **5**(2): 344–345
4. Pal D., Panda C., Sinhababu S., Dutta A. and Bhattacharya S., Evaluation of Psychopharmacological effect of petroleum ether extract of *Cuscuta reflexa* Roxb. Stem in mice, *Acta Pol Pharma* 2003; 60(6): 481–486
5. Gupta M., Mazumdar U.K., Bhattacharya S. and Chakrabarty S.,Studies on brain biogenic amines in methanolic extract of *Cuscuta reflexa* Roxb. & *Corchorus olitorius* Linn. seed treated mice, *Acta Pol Pharma* 2003; 60(3): 207–210
6. Mohammad Ali V., Studies in the chemical constituents of *Bombax ceiba* and *Cuscuta reflexa*, University of Karachi/H.E.J Research Institute of Chemistry. (Thesis) 2004; 157
7. Wu, Yang-Chang, Chang Fang-Rong, Chao Ya-Chief and Teng Che-Ming, Antiplatelet and vasorelaxing action of aporphinoids from *Cassytha filiformis*., *Phytochemical Research*, 1998; 12 (SI): 539–541
8. Hoet S., Stévigny C., Block S., Opperdoes F., Colson P. and Baldeyrou B., Alkaloid from *Cassytha filiformis* & related aporphines: Antitrypnosomal activity, cytotoxicity & interaction with DNA & topoisomerases. *Planta medica*, 2004;70:407–413
9. Chang C. W, Ko F. N, Su M. J, Wu Y. C and Teng C. M, Pharmacologic alevaluation of ocoteine, isolated compound from *Cassytha filiformis* in rat thoracic aorta. *Japanese Journal of Pharmcology* 1997;73(3): 207
10. Chang Fang-Rong, Chao Ya-Chief, Teng Che-Ming and Wu Chang Yang, Chemical constituents from *Cassytha filiformis*. *Journal of Natural Product*, 1998; 61(7): 863–866
11. Patil Amol, Patil Vikas, Chaudhari Kundan, Patil Vijay, Chaudhari Rajesh *In vitro* free radicals scavenging activity of stems of *Cuscuta reflexa* ,*Journal of Pharmacy Research* ,2009 ;2(1): 58-61
12. Singleton V. L. , Joseph A. Colorimetry of total phenolics with phosphormolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 1965;16(3):144-158.
13. Békro J.A.M, Konan K.M,. Djié Bi M.G *et al* Phytocompounds of the Extracts of Four Medicinal Plants of Côte D'ivoire and Assessment of their Potential Antioxidantby Thin Layer Chromatography. *European Journal of Scientific Research*..2008; 24(2): 219-228
14. Ohkowa, M., Ohisi, N., & Yagi, K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry*, 1979;95; 351–358.
15. Ruberto, G., Baratta, M. T., Deans, S. G., & Dorman, H. J. D. Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. Planta Medica, 2000; 66: 687–693.
16. Lihua Gu, Tao Wu, Zheygta Wang. TLC-bioautography guided isolation of antioxidant compounds from fruits of *Perilla fruatescens*. *Food Science and technology*. 2009; 42 :1131-36

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