



IDENTIFICATION OF PHYTOCOMPONENTS IN THE METHANOL EXTRACT OF CUSCUTA REFLEXA GROWN ON NERIUM OLEANDER HOST PLANT THROUGH GC-MS ANALYSIS

POOJA SAINI ^{1*}, EKTA MENGHAN¹¹ AND REKHA MITHAL²

¹Department of Biotechnology1, JECRC University, Jaipur, Rajasthan, India

²Department of Chemistry2, JECRC Foundation, Jaipur, Rajasthan, India

ABSTRACT

Medicinal plants are always been a great source for the search of new chemical entities having therapeutic potentials. *Cuscuta reflexa* is a parasitic plant with therapeutic potentials as antiviral, anticonvulsant activities, bradycardia, antisteroidogenic, antispasmodic and hemodynamic activities and also have an important place in Ayurveda. In the present piece of research work, attempts were made to identify different phytochemicals present in the methanol extract of *Cuscuta reflexa* grown on *Nerium oleander* host plant. GC-MS analysis of crude methanol extract was performed to identify different phytochemicals present in the *Cuscuta reflexa*. GC-MS is an advance technique for the identification of different chemical compounds present in the crude mixture of compounds. Pentanoic acid, 5-hydroxy-, 2, 4-di-t-butylphenyl esters, 1-Hexanol, 5-methyl-2-(1-methylethyl), Phthalic acid, isobutyl undecyl ester, Octadecanoic Acid and Celidoniol are compounds with major peaks in the GC-MS analysis. Most of these compounds are compounds with potentiality to cure various diseases. *Cuscuta reflexa* plant can be used for the isolation and characterization of new chemical compounds that can be used as future drugs against various life threatening diseases.

KEYWORDS: *Cuscuta reflexa*, GC-MS Analysis, Phytochemicals, Medicinal plant



POOJA SAINI*

Department of Biotechnology1, JECRC University, Jaipur, Rajasthan, India

Received on: 12-10-2016

Revised and Accepted on : 06-06-2017

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.3.b478-483>

INTRODUCTION

Medicinal plants have always been a great source for the identification of natural products and bio active compounds from plants having therapeutic potential to deal with many life threatening ailments. Medicinal plants have never ending diversity in having different potential bio active compounds. In the ancient time, therapies of Ayurveda were completely relied on the plant products for treatment of diseases in the form of

plant juice, paste or hot extract of plants. But nowadays plant products are not used in the form of plant juice and paste in allopathic treatments and more emphasis are given on isolation and identification of bio active compounds from medicinal plants having ability to cure disorders. *Cuscuta reflexa* is a parasitic plant which grows as weed plant and become a threat for the farmers by its parasitism nature over the crop plant that destroys crop fields.¹

Table 1
Classification of *Cuscuta reflexa*

| | |
|-----------|----------------|
| Kingdom: | Plantae |
| Division: | Angiospermes |
| Class: | Eudicots |
| Order: | Solanales |
| Family: | Convolvulaceae |
| Genus: | <i>Cuscuta</i> |
| Family: | <i>Reflexa</i> |



Figure 1
***Cuscuta reflexa* grown on *Nerium oleander* host plant**

Although this plant becomes a trouble for the crop cultivars but still *Cuscuta reflexa* have many medicinal activities and called as miracle medicinal plant in the ethno botany. *Cuscuta reflexa* possesses antibacterial, antiviral, anti-inflammatory, anti-diabetic, antifungal, antitumor, hepatoprotective, antifungal activities.² Major secondary metabolites present in the plant includes alkaloids, steroids, phenols, polyphenols, flavonoids etc. *Cuscuta reflexa* is a member of *Cuscutaceae* family and also considered as belonging to morning glory family, *Convolvulaceae*, on the basis of angiosperm phylogeny (Table No.1).³ *C. reflexa* also known as dodder plant, and frequently called as amarbel or akashabela, devil's hair, love vine, witch's hair etc. This plant exhibits very low level of chlorophyll content and photosynthesis activity and grows as homoparasite. *Cuscuta* plant doesn't contain roots and it spends its complete life span over the body of host plant without touching ground surface.⁴ *Cuscuta reflexa* grows over the host plant in parasitic manner and makes a haustorial connection with host plant by penetrating the vascular tissue of the host plant and sucks nutrients by haustorial connection and showed some activities depends on the nature of its host plant. *C. reflexa* also has the ability to choose its appropriate host from many plants on the basis of volatile compounds released from the plants in the process of transpiration. *C. reflexa* is used as

muscle relaxant, as blood purifier, for the treatment of cough, urination disorder, its seeds have anthelmintic and carminative properties and used in the treatment of bilious disorder.⁵ Warm paste of the plant is used for the treatment of rheumatism and to treat headache paste of whole plant is used, juice of *C. reflexa* is also worthy in the treatment of jaundice.⁶ *Cuscuta reflexa* also have the ability to cure frequent urination, flatulence, dry eyes, body pains, itchy skin, constipation, white discharge from vagina, lower back pain, blurred vision, ringing in the ears and also used as hair growth promoters.⁷ In the present work, *Cuscuta reflexa* has been studied for the presence of different bio active compounds. *C. reflexa* grown on *Nerium oleander* host plant were studied by Gas chromatography mass spectroscopy. GC-MS is an advance technique for the identification of different chemical compounds present in the crude mixture of compounds.⁸

MATERIALS AND METHODS

Plant Sample collection and Authentication

Sample plant *Cuscuta reflexa* grown on *Nerium oleander* host plant was collected from the local area of C-scheme, Jaipur, Rajasthan, India. This sample were authenticated and was given identification number and

submitted in Ethno-medicinal Herbarium, Centre with potentials of Excellence funded by DST, JECRC University, Jaipur, India. Further, voucher specimens of *Cuscuta reflexa* was deposited at herbarium of University of Rajasthan, Jaipur, Rajasthan, India and was verified by senior taxonomist of department and provided with accession no. **RUBL211577**.

Processing of plant material

Plant material was collected from the field and screened for the presence of any foreign material. Collected plant sample was shed dried and then whole plant was pulverised.

Extraction of plant sample

Methanol extract of dried whole plant material was prepared by soxhlet extraction technique with pulverised plant material (100g). Diluted plant extract collected was concentrated using rotary evaporator to get dried crude plant extract. The concentrated plant extract was further used for experimental procedures.^{9, 10}

GC-MS analysis of plant extract

The plant extract was subjected to GC MS analysis to identify the various bioactive compounds present. The sample was analyzed in Shimadzu GCMS QP2010 Plus, from Delhi University (DU) India. An aliquot of 2µl of methanol extract was injected into the column, injector temp. 260°C with a split ratio of 10:0, Oven temperature program initial temp is 100°C. 250°C for 5 min, ramp 30 minute to 280°C, hold 69.98 min, ACQ Mode Scan: 40m/z to 600m/z, Column flow is 1.21mL/min and total flow is 16.3ml/min. Flow control with linear velocity which 40.9cm/sec.

Identification of bioactive compounds

Identification of compounds present in the plant extract was performed using National Institute Standard and Technology (NIST) database. Interpretation of mass spectrum GC-MS was done using NIST library having more than 62000 patterns. Spectrum of unknown compounds was compared with the spectrum of known components stored in NIST library. Then the name, molecular weight and structure of the compounds of test sample were determined.

RESULTS

Table 2
Characteristics of *Cuscuta reflexa* grown on *Nerium oleander* Host plant Methanol extract

| Characteristic | Appearance |
|----------------------------|------------------|
| Color of the crude extract | Dark brown color |
| Weight of crude extract | 86.91g |
| % Extract | 11.58% |

$$\% \text{ Extract} = \frac{\text{weight of crude extract}}{\text{weight of dried plant sample}} \times 100$$

Table
3- List of identified components from *Cuscuta reflexa* methanol extract

| Peak | Retention Time | Compound Name | Structure |
|------|----------------|---|-----------|
| 1 | 5.785 | Undecane | C13H28 |
| 2 | 8.545 | Pentadecane | C15H32 |
| 3 | 10.133 | Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters | C14H30O3S |
| 4 | 13.034 | 1-Hexanol, 5-methyl-2-(1-methylethyl) | C9H28O4 |
| 5 | 14.875 | Phthalic acid, isobutyl undecyl ester | C10H22O |
| 6 | 17.027 | Octadecanoic Acid | C23H36O4 |
| 7 | 21.500 | Celidoniol | C21H44O3S |

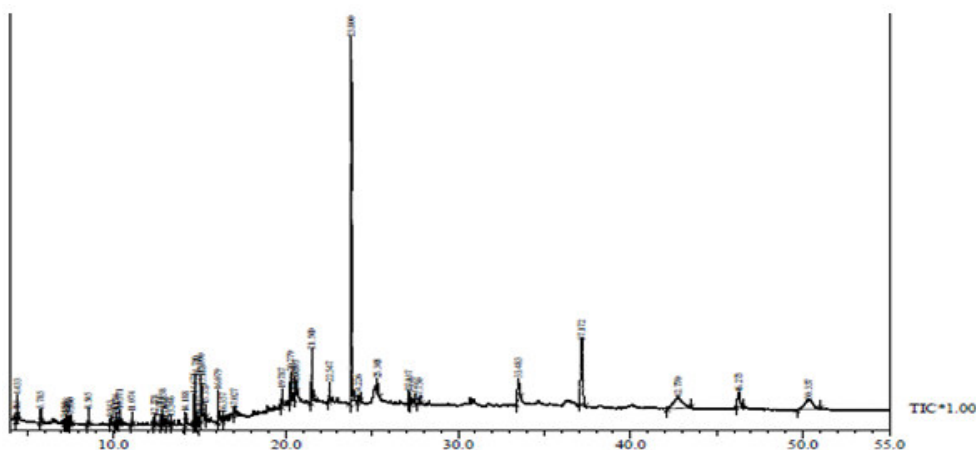
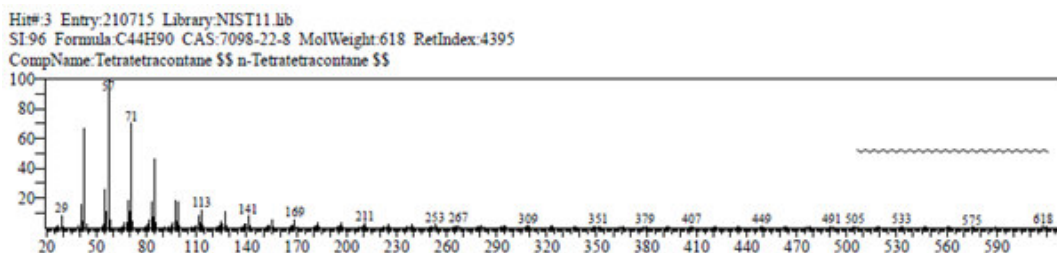
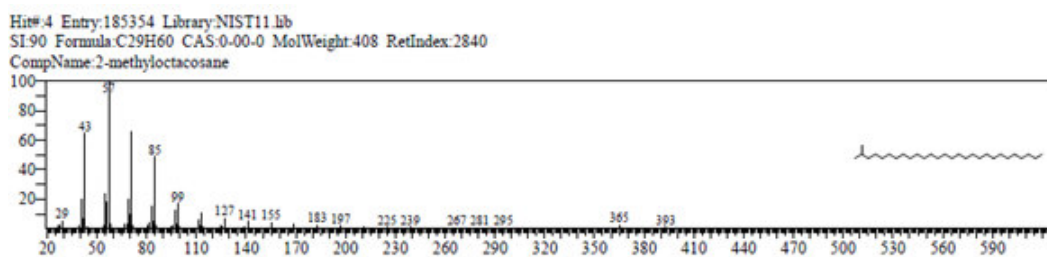
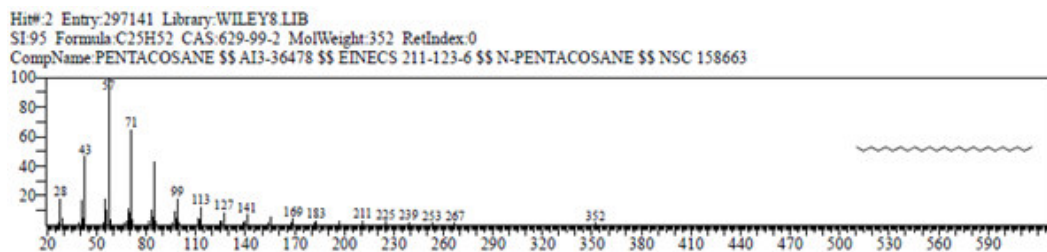
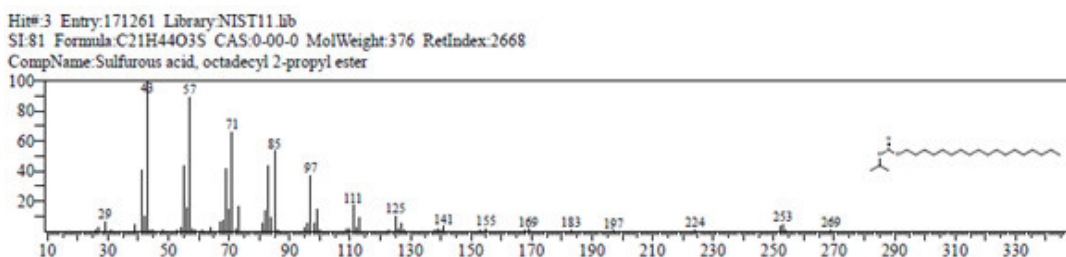
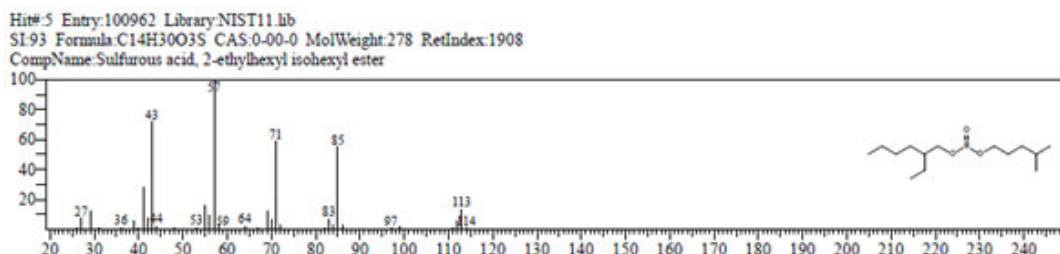
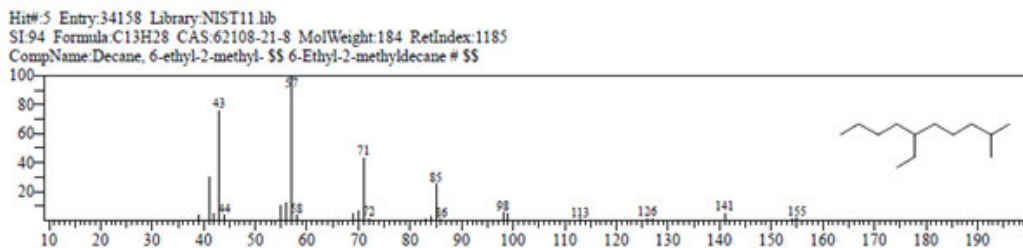


Figure 2
Mass chromatogram of *Cuscuta reflexa* grown on *Nerium*

oleander host plant Methanol Extract
Identified components from *Cuscuta reflexa* methanol extract



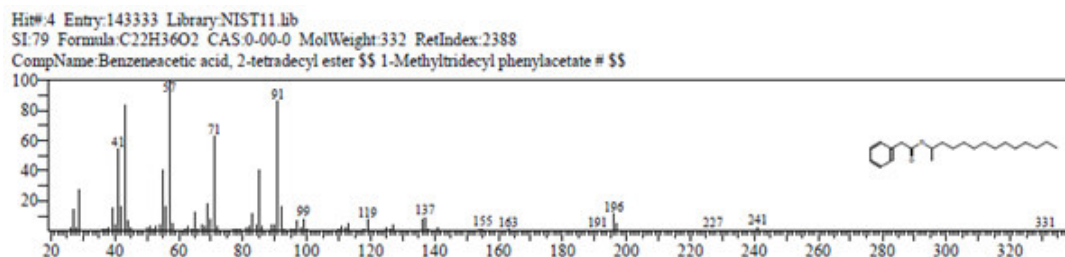


Figure 3
List of identified components from *Cuscuta reflexa* methanol extract are ascertained with WILEY8. LIB. and NIST11. Library

Table No.2 represents the characteristics of plant extract prepared with %extract. Table no.3 and figure no. 3 showed the chemical structure of bio active compounds in the methanolic extract of *Cuscuta reflexa*. Figure no. 2 showed the mass spectrum of investigating plant extract.GC-MS spectrum of methanol extract of *Cuscuta reflexa* grown on *Nerium oleander* host plant confirmed the presence of different bio molecules along with chemical structure of bio active compounds present in the crude plant extract that are responsible for the medicinal properties of sample plant.

DISCUSSION

Cuscuta reflexa, a parasitic weed plant has been studied to identify its pharmacological activities as Jha et al, 2011 stated that hydro alcoholic extract of *Cuscuta reflexa* showed hepatoprotective activity against paracetamol induced hepatic damage in albino rats acting as strong hepatoprotective agent.¹¹ Dandopani et al, 2011 stated that chloroform and ethanol extract of *C. reflexa* illustrated antitumor activity against Ehrlich ascites carcinoma tumour in mice at doses of 400 and

200 mg/kg body weight orally.¹² Methanol extract of *C. reflexa* showed hypoglycaemic activity by momentous inhibition of α -glucosidase enzyme and delayed the absorption time of glucose after meal. α -Glucosidase is a membrane bound enzyme at the epithelium of small intestine.¹³ Methanol extract of *C. reflexa* posse's antioxidant and anti bacterial activity.¹⁴ Ethanolic extract of *Cuscuta* showed decrease in arterial blood pressure and heart rate in anaesthetised rats in dose dependent manner.¹⁵ Alcoholic extract of *C. reflexa* showed positive response in cardiotoxic and inotropic activities on the perfuse frog heart, and it also caused decrease in dog blood pressure.¹⁶ In wistar rats aqueous and alcoholic extract of *C. reflexa* showed diuretic activity.¹⁷ Stems of the plant exhibit spasmolytic action and worked as relaxant in guinea pig and rabbit, aqueous and alcoholic extract also showed acetyl choline like action.¹⁸ Pharmacological activities showed by *Cuscuta reflexa* are due to the presence of various bio actives in the plant. The presence of these potential bio active compounds made this plant medicinally imperative (Table No.4).

Table 4
Pharmacological activities of compounds identifies from GCMS analysis

| Compound Name | Pharmacological Activity | Reference |
|-------------------|---|-----------------------------------|
| Pentadecane | Against Gastric ulcers | Ozkan et al (2016) ¹⁹ |
| Undecane | Antimicrobial Activity | Gibka et al (2009) ²⁰ |
| Phthalic acid | Antiviral Acitivity | Uddain et al (2013) ²¹ |
| Octadecanoic acid | Anti-inflammatory and anti-arthritis Activity | Jones et al (2002) ²² |

CONCLUSION

The present study helps to predict the chemical formula and structure of different natural compounds present in the crude extract of *Cuscuta reflexa* (Table No.3 and Figure No.3). The existence of various bio active compounds in the *Cuscuta* reveals its potent ability as being medicinal plant in the Ayurveda. Further

investigation may lead to isolation, identification and structure elucidation of pure bio active compounds from *Cuscuta reflexa* and screening of its pharmacological activity will be supportive for further drug development.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Dawson JH, Musselman LJ, Wolswinkel P, Dorr I. Biology and control of *Cuscuta*. Rev Weed Sci. 1994; 6(1): 265–317.
2. Costa-Lotufo LV, Khan MTH, Ather A. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. J Ethnopharmacol. 2005; 99(1): 21-30.
3. Story R., Some plants used by the Bushmen in obtaining food and water. Mem. Bot. Survey S. Afr. 1958; 30: 1-115.
4. Dawson JH, Musselman LJ, Wolswinkel P, Dorr I. Biology and control of *Cuscuta*. Rev Weed Sci. 1994; 6: 265–317.
5. Khan S, Mirza KJ, Abdin MZ. Development of RAPD markers for authentication of medicinal plant *Cuscuta reflexa*. EurAsia J BioSci. 2010; 4: 1-7.
6. Siwakoti M, Siwakoti S. Ethnobotanical studies of Satars of Jhapa district, Nepal: A case study of Haldibari VDC. A report submitted to University Grants Commission, Kathmandu, Nepal. 1996.
7. Pandit S, Chauhan NS, Dixit VK. Effect of *Cuscuta reflexa* on androgen induced alopecia. J Cosmet Dermatol. 2008; 7(3): 199-204.
8. Ezhilan BP, Neelamegam R. GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. Pharmacognosy Res. 2012; 4(1): 11-14.
9. Nayan RB, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L.: An ethnomedicinal plant. J Adv Pharm Technol Res. 2011; 2(2): 104–109.
10. Kanthal LK, Dey A, Satyavathi K, Bhojaraju P. GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. Pharmacognosy Res. 2014; 6(1): 58-61.
11. Jha U, Shelke T. Hepatoprotective activity of hydroalcoholic extracts of *Cuscuta reflexa* roxb in paracetamol intoxicated albino rats. IJRAP. 2011; 2(4): 1290-1293.
12. Dandopani C, Sahu KR, Jha KA, Dwivedi J. Evaluation of Antitumor Activity of *Cuscuta reflexa* Roxb (*Cuscutaceae*) Against Ehrlich Ascites Carcinoma in Swiss Albino Mice. Tropical Journal of Pharmaceutical Research. 2011; 10(4): 447-454.
13. Eram A, Ahmed A, Ghulam M, Abdul M, Nighat A, Syed HA. α -Glucosidase Inhibitory Constituents from *Cuscuta reflexa*. Chem Pharm Bull. 2002; 50(1): 112-114.
14. Mahmood N, Piacente S, Burke A, Khan A, Pizza C. Constituents of *Cuscuta reflexa* are anti-HIV agents. Antivir Chem Chemother. 1997; 8: 70-74.
15. Gilani A. U. H., Aftab K. (1992). Pharmacological actions of *Cuscuta reflexa*, International journal of Pharmacogn., 30(4), 296-302.
16. Singh G. S., Garg K. N. (1973). Some pharmacological studies on *Cuscuta reflexa* plant (Aakash bel), Indian journal of Pharmacol., 5(2), 344-345.
17. Sharma S., Hullatti K. K., Prasanna S. M., Kuppast I. J., Sharma P. (2009). Comparative study of *Cuscuta reflexa* and *Cassytha filiformis* for diuretic activity, Pharmacognosy Res.,1(5), 327330.
18. Prasad D. N. (1965). Preliminary pharmacological investigations on *Cuscuta reflexa* Roxb, Indian Journal Med Res., 53, 465-470.
19. Ozkan G, Kamiloglu S, Ozdal T, Boyacioglu D, Capanoglu E. Potential uses of Turkish medicinal plants in the treatment of various diseases. Molecules. 2016; 257(21): 1-32.
20. Gibka J, Kunicka-Styczyńska A, Gliński M. Antimicrobial activity of undecan-3-one, undecan-3-ol and undec-3-yl acetate. Experimental Immunology. 2009; 43(3): 154–157.
21. Uddain SJ, Bettadapura J, Guillon P, Grice D, Mahalingham S, Tiralongo E. In-vitro antiviral activity of a novel phthalic acid ester derivative isolated from the Bangladeshi mangrove fern *Acrostichum aureum*. Journal of antivirals and antiretrovirals. 2013; 5: 139-144.
22. Jones PJ. 7 Functional foods more than just nutrition. Clinical nutrition CMAJ. 2002; 166: 1555-1563.

Reviewers of this article



Dr M Saiyad Musthafa ,M.Sc.,M.Phil.,Ph.D

Assistant Prof, Dept of Zoology, The New College, Royapettiah , Chennai, India

Prof. Dr. M. Ranga Priya, M. Pharm., Ph.D., R.Ph.

Professor , Dept of Pharmaceutics, Sun Institute Of Pharmaceutical Education & Research, Kakupall, Nellore Rural, Nellore, Andhra Pradesh 524346



Prof. Dr. K. Suri Prabha

Asst. Editor , International Journal of Pharma and Bio sciences.



Prof. P. Muthu Prasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript