IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(P): 2347-4580; ISSN(E): 2321-8851
Vol. 5, Issue 1, Jan 2017, 121-128

ENDOPHYTIC FUNGI WITH ANTI-DIABETIC ACTIVITIES ISOLATED FROM AMLA FRUITS

KULDEEP SINGH[1], KAVITA SINGH[2] & CBS DANGI[3]

[1]Research Scholar, Department of Biotechnology, RKDF University, Bhopal, Madhya Pradesh, India

[2]Assistant Professor, Department of Biotechnology, RKDF University, Bhopal, Madhya Pradesh, India

[3]Professor & Dean, Faculty of Life Science, RKDF University, Bhopal, Madhya Pradesh, India

ABSTRACT

Many of the endophyte have been show potential *de-novo* synthesis of different bioactive metabolites that are used directly or indirectly as therapeutic agent and several ailments. Diabetes Mellitus is a metabolic disorder caused due to defect in insulin secretion, insulin action or both. Diabetes mellitus is causing serious health problem and due cause of some inheritance or changed lifestyle and food habit. Mycelia extract of *Penicillum, Streptomyces, Rhizopus, Cladosporium, Nigrospora oryze* and *Alternaria* species isolates were reported best inhibition ranging 15 to 38% α-amylase and sucrose. Isolated endophytes have good potency in anti-diabetic ailment.

KEYWORDS: Endophyte, Diabetes, Amla, Glucose Level, Haemoglobin

INTRODUCTION

Medicinal plants are used by humans and animals from very early age. This is occurring in nature randomly. From the development of humans are using different medicinal plants for a number of ailment. Amla are one of them, which are used in remedy of different disease. Different parts of these plants are used for different disease. There are no side effects reported till date for direct use of any medicinal plant part. Microorganisms are also hosted in these plants for his life cycle. These microorganisms sometimes help these plants for protecting from other agents. Mutualism of plant and microorganism are increases the potency of secondary metabolite what they produce. An endosymbiosis of bacterium or fungus with plant parts of its life cycle without causing any disease. Endophytes ever found in almost plants but not all plant endophytes relationships are well studied (Puri *et al.*, 2015; Clay, 2002; Carroll, 1986; Stone *et al.*, 1999; Bacon and White, 2000; Suryanrayanan, 2013). Amla (Indian gooseberry) is a member of Euphrbiceae family and also known as *Phyllanthus emblica* Linn. or *Emblica officinalis* Gaertn. This is an important plant in Ayurveda, Siddha, Unani and naturopathy medicine system (Maurya and Srivastava, 2011). Amla (Indian gooseberry) is a member of Euphrbiceae family and also known as *Phyllanthus emblica* Linn. or *Emblica officinalis* Gaertn. This is an important plant in Ayurveda, Siddha, Unani and naturopathy medicine system (Maurya and Srivastava, 2011). A study of Gangwar reported in year 2015 that 36 endophytic actinomycetes were isolated from roots, stems and leaves of *Emblica officinalis* Gaertn (Gooseberry) and identified as 17 isolates of *Streptomyces* sp., 17 isolates of *Micromonospora* sp. and 02 isolates of *Microbispora* sp. with distribution of endophytic actinomycetes in roots, stem and leaves was 50%, 25% and 25% respectively. To screen the endophyes of medicinal plants and their potency against diabetes were perform in this study.

MATERIALS AND METHODS

The healthy fruits were surface sterilized by modified method of Strobel *et al.*, 1996. The endophytic fungi grown were isolated and maintained on PDA slants. Isolated fungi were identified in order to morphological characteristics viz. colony growth, presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production etc. in reference to Barnett, 1992 by lactophenol and other stains under microscope with 40X resolution. Qualitative and Quantitative characterization of Amylase, Protease, Cellulose and Lipase were performed. Collected compound were access for anti-diabetic activities. α-amylase inhibitory activity, Assay of sucrase inhibition activity and Glucose diffusion method were performed for *in-vitro* antidiabetic properties assessment. *In-vivo* anti-diabetic activities were also performed with animal model and serum glucose level and Blood function test according to standardized laboratory method.

RESULTS

Table 1: Morphological Characterization of Isolated Fungi

Isolate Code	Endophyte (Morphological Basis)	Colour of Mycelia	Pigmentation	Spore Arrangement
E01	*Cladosporiumsp.*	Olive brown	Dark brown	Un-branched
E02	*Nigrosporaoryzae* sp.	White	Black	Un-branched
E03	*Phomopsis* sp.	White	Brown	Spherical
E04	*Streptomyces* sp.	White	Whitish green	Branched filamentous
E05	*Penicillum* sp.	Green	Colorless	Highly branched

Table 2: Qualitative Enzyme Activities Characterization of Isolated Fungi

Name of Endophyte (Morphological basis)	Amylase	Protease	Cellulase	Lipase
Cladosporiumsp.	-	+	+	+
Nigrosporaoryze sp.	-	-	-	+
Phomopsis sp.	-	-	-	-
Streptomyces sp.	+	+	+	-
Penicillum sp.	+	+	+	-

Table 3: Quantitative Enzyme Activities Characterization of Isolated Fungi

Endophyte (Morphological Basis)	Amylase (U/ml)	Protease(U/ml)	Cellulase(U/ml)	Lipase(U/ml)
Cladosporiumsp.	0.00	1.22	0.25	0.16
Nigrosporaoryze sp.	0.00	0.00	0.00	0.25
Phomopsis sp.	0.00	0.00	0.00	0.00
Streptomyces sp.	2.31	1.63	0.45	0.00
Penicillum sp.	2.60	1.45	1.32	0.00

Table 4: In-Vitro Antidiabetic Activities Viz. α-Amylase (%) Sucrase (%)

Name of Endophyte (Morphological Basis)	α-Amylase (%)			Sucrase (%)		
	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg
Cladosporiumsp.	06.0	13.0	22.4	08.7	14.5	27.2
Nigrosporaoryze sp.	05.5	09.6	14.0	07.6	11.5	18.4
Phomopsis sp.	00.0	01.0	01.5	00.0	01.0	02.9
Streptomyces sp.	09.8	17.4	29.3	07.2	15.2	21.5
Penicillum sp.	11.5	23.6	37.6	12.0	25.6	36.2

Table 5: In-Vitro Glucose Diffusion Test

Name of Endophyte (Morphological Basis)	Glucose Diffusion Test		
	50 µg	100 µg	200 µg
Cladosporiumsp.	-	-	+
***Nigrosporaoryze* sp.**	-	-	+
***Phomopsis* sp.**	-	-	-
***Streptomyces* sp.**	+	+	++
***Penicillum* sp.**	+	++	+++

Table 6: In-Vivo Serum Glucose Levels Determination of Diabetic Induced Rats with Treatments

Groups		Serum Glucose Level (Fasting) mg/dL		
		0 Day	30 Days	60 Days
Control		104±5.6	105±6.2	102±5.9
Diabetic control		242±22.5	240±25.8	243±26.5
Diabetic with Glibenclaimide(30mg/kg)		240±20.9	106±7.2	102±8.9
Diabetic with mycelium extract				
E01	**250 mg/kg**	241±19.4	160±12.8	150±11.6
	500 mg/kg	239±21.3	128±10.2	120±10.5
E02	**250 mg/kg**	244±20.8	148±10.6	145±12.1
	500 mg/kg	235±19.8	121±09.2	117±11.6
E03	**250 mg/kg**	239±20.7	152±12.4	148±10.6
	500 mg/kg	229±20.3	124±10.3	118±10.9
E04	**250 mg/kg**	238±19.5	147±11.8	139±12.4
	500 mg/kg.	231±18.9	118±11.4	109±12.7
E05	**250 mg/kg**	241±20.1	162±12.9	151±11.9
	500 mg/kg	239±21.8	131±13.1	121±13.5

Table 7: In-Vivo Haemoglobin Levels Determination of Diabetic Induced Rats with Treatments

Groups		Haemoglobin gm/dL		
		0 Day	30 Days	60 Days
Control		15.80±0.85	15.20±0.80	15.40±0.86
Diabetic control		10.10±1.00	09.52±0.95	07.15±0.98
Diabetic with Glibenclaimide(30mg/kg)		10.25±0.95	12.60±0.88	13.20±0.92
Diabetic with mycelium extract				
E01	**250 mg/kg**	09.95±0.94	10.92±0.91	12.05±1.00
	500 mg/kg	10.05±0.98	11.56±0.94	13.00±1.10
E02	**250 mg/kg**	10.10±1.00	10.95±0.93	12.00±0.98
	500 mg/kg	10.16±0.85	11.02±0.85	12.90±0.99
E03	**250 mg/kg**	09.90±0.97	10.00±0.88	11.15±0.95
	500 mg/kg	10.15±0.86	11.45±0.87	12.55±0.89
E04	**250 mg/kg**	10.00±0.92	10.25±0.91	11.10±0.82
	500 mg/kg.	09.80±0.87	11.10±0.88	12.15±0.95
E05	**250 mg/kg**	10.05±0.93	10.85±0.86	11.90±0.98
	500 mg/kg	09.85±0.99	11.30±0.83	12.70±0.93

Cladosporium sp., *Nigrosporaoryze* sp., *Phomopsis* sp., *Streptomyces* sp. and *Penicillum* sp. endophytes were found in amal fruit. Morphology of endophytes was determined according to their colour of mycelia, pigmentation and spore arrangement as in *Cladosporiumsp*. Olive brown, Dark brown and Un-branched; *Nigrosporaoryzae* sp. White, Black and Un-branched; *Phomopsis* sp. White, Brown and Spherical; *Streptomyces* sp. White, Whitish green and Branched filamentous; *Penicillum* sp. Green, Colorless and highly branched. Qualitative characters of isolated endophytes were shown as Amylase in *Streptomyces* sp. and *Penicillum* sp., Protease and Cellulase in *Cladosporiumsp*., *Streptomyces* sp.

and *Penicillum* sp. whereas Lipase in in *Cladosporiumsp.* and *Nigrosporaoryze* sp. Quantity of Amylase recorded in *Streptomyces* sp. 2.31 U/ml and *Penicillum* sp. 2.60 U/ml, Protease in *Cladosporiumsp.* 1.22U/ml, *Streptomyces* sp. 1.63 U/ml and *Penicillum* sp. 1.45 U/ml, Cellulase in *Cladosporiumsp.* 0.25U/ml, *Streptomyces* sp. 0.45 U/ml and *Penicillum* sp. 1.32 U/ml whereas Lipase in in *Cladosporium*sp.0.16 and *Nigrosporaoryze* sp. 0.25.α-amylase (%) activity for *in-vitro* antidiabetic activities was assessed and recorded at 50 µg, 100 µg and 200 µg doses in*Cladosporiumsp.* by 06.0, 13.0 and 22.4; *Nigrosporaoryze* sp. 05.5, 09.6 and 14.0; *Phomopsis* sp. 00.0, 01.0 and 01.5; *Streptomyces* sp. 09.8, 17.4 and 29.3; *Penicillum* sp. 11.5, 23.6 and 37.6. Sucrase(%) was recorded at 50 µg, 100 µg and 200 µg doses in*Cladosporiumsp.* by 08.7, 14.5 and 27.2; *Nigrosporaoryze* sp. 07.6, 11.5 and 18.4; *Phomopsis* sp. 00.0, 01.0 and 02.9; *Streptomyces* sp. 07.2, 15.2 and 21.5; *Penicillum* sp. 12.0, 25.6 and 36.2. Positive Glucose diffusion test was reported at 200 µg in *Cladosporiumsp*. and *Nigrosporaoryze* sp.; at 50 µg, 100 µg and 200 µg *Streptomyces* sp. and *Penicillum* sp. gives positive, fairly positive and strong positive results. Serum glucose (fasting) levels in Control, diabetic control, diabetic treated with glibenclaimide and treated with different mycelia extracts of isolated fungi with 250mg/kg and 500mg/kg concentration were reported in table 6. Levels of serum glucose (mg/dL) in control at 0^{th}, 30^{th} and 60^{th} days were 104±5.6, 105±6.2 and 102±5.9 respectively; diabetic control 242±22.5, 240±25.8 and 243±26.5 at 0^{th}, 30^{th} and 60^{th} day samples respectively; diabetic with glibenclaimide treated samples 240±20.9, 106±7.2 and 102±8.9 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E01 mycelia extract 241±19.4, 160±12.8 and 150±11.6 at 0^{th}, 30^{th} and 60^{th} day correspondingly ; diabetic treated with 500mg/kg E01 mycelia extract 239±21.3, 128±10.2 and 120±10.5 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E02 mycelia extract 244±20.8, 148±10.6 and 145±12.1 at 0^{th}, 30^{th} and 60^{th} day correspondingly ; diabetic treated with 500mg/kg E02 mycelia extract 235±19.8, 121±09.2 and 117±11.6 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E03 mycelia extract 239±20.7, 152±12.4 and 148±10.6 at 0^{th}, 30^{th} and 60^{th} day correspondingly; diabetic treated with 500mg/kg E03 mycelia extract 229±20.3, 124±10.3 and 118±10.9 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E04 mycelia extract 238±19.5, 147±11.8 and 139±12.4 at 0^{th}, 30^{th} and 60^{th} day correspondingly; diabetic treated with 500mg/kg E04 mycelia extract 231±18.9, 118±11.4 and 109±12.7 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E05 mycelia extract 241±20.1, 162±12.9 and 151±11.9 at 0^{th}, 30^{th} and 60^{th} day correspondingly; diabetic treated with 500mg/kg E05 mycelia extract 239±21.8, 131±13.1 and 121±13.5 at 0^{th}, 30^{th} and 60^{th} day respectively. Haemoglobin level in Control, diabetic control, diabetic treated with glibenclaimide and treated with different mycelia extracts of isolated fungi with 250mg/kg and 500mg/kg concentration were reported in table 7. Levels of haemoglobin (gm/dL) in control at 0^{th}, 30^{th} and 60^{th} days were 15.80±0.85, 15.20±0.80 and 15.40±0.86 respectively; diabetic control 10.10±1.00, 09.52±0.95 and 07.15±0.98 at 0^{th}, 30^{th} and 60^{th} day samples respectively; diabetic with glibenclaimide treated samples 10.25±0.95, 12.60±0.88 and 13.20±0.92 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E01 mycelia extract 09.95±0.94, 10.92±0.91 and 12.05±1.00 at 0^{th}, 30^{th} and 60^{th} day correspondingly ; diabetic treated with 500mg/kg E01 mycelia extract 10.05±0.98, 11.56±0.94 and 13.00±1.10 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E02 mycelia extract 10.10±1.00, 10.95±0.93 and 12.00±0.98 at 0^{th}, 30^{th} and 60^{th} day correspondingly ; diabetic treated with 500mg/kg E02 mycelia extract 10.16±0.85, 11.02±0.85 and 12.90±0.99 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E03 mycelia extract 09.90±0.97, 10.00±0.88 and 11.15±0.95 at 0^{th}, 30^{th} and 60^{th} day correspondingly; diabetic treated with 500mg/kg E03 mycelia extract 10.15±0.86, 11.45±0.87 and 12.55±0.89 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E04 mycelia extract 10.00±0.92, 10.25±0.91 and 11.10±0.82 at 0^{th}, 30^{th} and 60^{th} day correspondingly; diabetic treated with 500mg/kg E04 mycelia extract 09.80±0.87, 11.10±0.88 and

12.15±0.95 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg E05 mycelia extract 10.05±0.93, 10.85±0.86 and 11.90±0.98 at 0^{th}, 30^{th} and 60^{th} day correspondingly; diabetic treated with 500mg/kg E05 mycelia extract 09.85±0.99, 11.30±0.83 and 12.70±0.93 at 0^{th}, 30^{th} and 60^{th} day respectively; diabetic treated with 250mg/kg.

DISCUSSIONS

Morphological characters of E01 isolated fungi were olive brown colour, dark brown pigment and un-branched spore arrangement and shows positive enzyme activities for protease, cellulose and lipase with 1.22, 0.25 and 0.16 U/ml production. Isolated endophytic fungi with these characters were earlier reported as species of *Cladosporium* (Ogorek *et al.,* 2012; Braun *et al.,* 2003). Morphological characters of E02 isolated fungi were white colour of mycelia, black pigmentation and unbranched spore arrangement and shows positive enzyme activities for lipase only with 0.25 U/ml productions. Similar result was reported by Abbas and Mohammad in 2014 and confirms the isolated endophytic fungi with these characters were a species of *Nigrosporaoryze.* Endophytic isolate E03 was reported white colour, brown pigment and spherical spore arrangement morphological characters but not shown any enzymatic activities. Farr *et al.* reported in year 2002 that isolate species having similar characters were a member of *Phomopsis*. Morphological characters of E04 isolated fungi were white colour of mycelia, whitish green pigmentation and branched filamentous spore arrangement and shows positive enzyme activities for amylase, protease and cellulose with 2.31, 1.63 and 0.45 U/ml production. Isolated endophytic fungi with these characters were earlier reported as species of *Streptomyces* (Tadai *et al.,* 2006). Morphological characters of E05 isolated fungi were green colour, colourless pigment and highly branched spore arrangement and shows positive enzyme activities for amylase, protease and cellulose with 2.60, 1.45 and 1.32 U/ml productions. Similar result was reported by Tiwari *et al.,* 2011 and confirms the isolated endophytic fungi with these characters were a species of *Penicillum.* Mycelia extract of *Penicillum, Streptomyces, Rhizopus, Cladosporium, Nigrospora oryze* and *Alternaria* species isolates were reported best inhibition ranging 15 to 38% α-amylase and sucrose. Isolates of *Acremonium, Phomopsis, Curvularia* and *Fusarium* species were showed low potency to inhibit α-amylase and sucrose. Similarly *Penicillum, Streptomyces, Rhizopus, Cladosporium, Nigrospora oryze* and *Alternaria* species chronically gives best results as compare to isolates of *Acremonium, Phomopsis, Curvularia* and *Fusarium* species in glucose diffusion test. Ushasri and anusha worked on *in-vitro* anti-diabetic properties in 2015 and reported that the endophytic extracts have good potency to α-amylase and sucrose inhibition with up to 23.7% inhibition. Wan *et al.,* (2013) also reported that swartia extract resulted best result for reducing diabetes. Blood functions tests (% haemoglobin and White blood cells count) and Body weight studies were performed at 0^{th}, 30^{th} and 60^{th} days for *in-vivo* anti-diabetic activities determinations. Yuan and associates (2014) worked on effect of *Actinida* extract in diabetic induced mice and reported that alpha-glucosidase inhibitory activity test, ethanol extract of roots showed the best inhibitory activity (74.2%, 6 mg/ml). Ushasri and Anusha (2015) previously reported the effect of endophytic extract on allexon induced diabetic mice that showed highest glycosidase inhibition in treated mice. In this study serum glucose level was decrease from 240gm/dL to 102gm/dL in 60 day administration.

CONCLUSIONS

Mycelia extract of Penicillum, Streptomyces, Rhizopus, Cladosporium, Nigrospora oryze and Alternaria species isolates were reported best inhibition ranging 15 to 38% α-amylase and sucrose. Isolated endophytes have good potency in anti-diabetic ailment. This is need to extend identification of novel compound present in these isolates, who gives antidiabetic activities.

REFERENCES

1. Bacon CW and White JF. (2000). Microbial endophytes, Marcel Deker Inc., New York. de Bary A. (1866). Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten. Hofmeister's handbook of physiological botany, vol. II, Leipzig, Germany.
2. Barnett HL. (1992) Illustrated Genera of imperfect fungi. Berg. Pub. Co., Minneapolis. pp. 213.
3. Carroll GC. (1986). the biology of endophytism in plants with particular reference to woody perennials. In Fokkema, N. J.; Van den Heuvel, J. *Microbiology of the phyllosphere*. Cambridge: Cambridge University Press. pp. 205-222.
4. Clay K and Schardl C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. The American Naturalist. 160(4): 99 - 127.
5. Farr DF, Castlebury LA and Rossman AY. (2002). Morphological and molecular characterization of Phomopsis vaccinii and additional isolates of Phomopsis from blueberry and cranberry in the eastern United States. Mycologia. 94(3): 494-504.
6. Gangwar M, Kaur N, Saini P and Kalia A. (2015). The diversity, plant growth promoting and anti-microbial activities of endophytic actinomycetes isolated from Emblica officinalis Gaertn. International Journal of Advanced Research. 3(4): 1062-1071.
7. Maurya U and Srivastava S. (2011). Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer: A review, International Journal of Research in Pharmaceutical Chemistry. 1(4): 1152-1159.
8. Mohammad AH and Mohammed NH. (2014). Morphological, molecular and pathological study on Nigrospora oryzae and Nigrospora sphaerica, the leaf spot fungi of date palm Basra Journal for Date Palm Researches. 13 (1-2): 26-38.
9. Ogórek R, Lejman A, Pusz W, Miłuch A and Miodyńska P. (2012). Characteristics and taxonomy of Cladosporium fungi. Mikologia Lekarska. 19(2): 80-85.
10. Puri A, Padda KP and Chanway CP. (2015). Can a diazotrophic endophyte originally isolated from lodgepole pine colonize an agricultural crop (corn) and promote its growth?. Soil Biology and Biochemistry. 89: 210-216.
11. Stone J, Bacon C and White J. (1999). Bacon, C and White, J., Ed. An overview of endophytic microbes: endophytism defined. Chapter in book: Microbial Endophytes (Marcell-Dekker). pp. 29-33.
12. Suryanarayanan TS. (2013). Endophyte research: going beyond isolation and metabolite documentation.

FungalEcology6 (6): 561–568.

13. Taddei A, Rodríguez http://www.sciencedirect.com/science/article/pii/S0944501305000844 - cor1MJ, Márquez-Vilchez E and Castelli C. (2006). Isolation and identification of *Streptomyces* spp. from Venezuelan soils: Morphological and biochemical studies. I. Microbiological Research. 161(3): 222–231.
14. Tiwari KL, Jadhav SK and Kumar A. (2011). Morphological and Molecular Study of Different Penicillium Species Middle-East Journal of Scientific Research. 7(2): 203-210.
15. Ushasri R and Anusha R. (2015). *In vitro* anti-diabetic activity of ethanolic and acetone extracts of endophytic fungi *Syncephalastrum racemosum* isolated from the seaweed *Gracilaria corticata* by alpha-amylase inhibition assay method. Int.J.Curr.Microbiol.App.Sci. 4(1): 254-259.
16. Wan LS, Chen CP, Xiao ZQ, Wang YL, Min QX, Yue Y and Chen J. (2013). In vitro and in vivo anti-diabetic activity of Swertia kouitchensis extract. J Ethnopharmacol. 147(3): 622-30.
17. Yuan X, Hu X, LiuY, SunH, Zhang Z and Cheng D. (2014). *In vitro* and *In vivo* Anti-Diabetic Activity of Extracts from *Actinidia kolomikta.* International Journal of Biology. 6(3).

AUTHOR DETAILS

Dr. CBS Dangi (Professor. & Dean)

Ph.D. in Human Genetics with good experience in molecular cytogenetics and clinical genetics thalassemia. Competent and versatile Clinical Geneticist. Guided dissertations to clinicians, non clinicians, medicos, non medicos. Eleven years of laboratory diagnostic and academic experience in India and overseas. Possesses a sound working knowledge in laboratory techniques. Managerial experience includes career development program, laboratory operations, educational and research institution development in the field of medicos as well as non-medicos area. Provide genetic counseling to medallion and multi factorial genetic disorders.

Kuldeep Singh

Ph.D. student in Department of Biotechnology, RKDF University, Bhopal under supervision of Dr. CBS. Dangi, Dean Faculty of Life Science, RKDF University, Bhopal.

Kavita Singh

Assistant Professor in Department of Biotechnology, RKDF University, Bhopal