Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

The isolation, Characterization and Preclinical Studies of Metal Complex of *Thespesia populnea* for the Potential Peroxisome Proliferator-activated Receptors-γ Agonist Activity

Mohini Ashok Phanse, Manohar Janardhan Patil[1], Konde Abbulu[2]

Department of Pharmacognosy, Modern College of Pharmacy, Nigdi, [1]Department of Pharmacognosy, Maratha Mitramandal College of Pharmacy, Kalewadi, Pune, Maharashtra, [2]Department of Pharmaceutics, Mallareddy Institute of Pharmaceutical Sciences, Hyderabad, Telangana, India

ABSTRACT

Background: Diabetes mellitus is an international public health problem since ancient days. The condition is predominantly more severe in developing countries like India where, life is more sedentary due to the even changing lifestyles in this fast-paced global scenario. *Thespesia populnea* is widely used in the ayurvedic system of medicine for treatment of diabetes mellitus in India for years. The aim of this work is to explore the anti-diabetic activity of the isolated compound. **Materials and Methods:** The sesquiterpene isolated from hexane fraction of bark of *T. populnea* modified synthetically then identified by using analytical techniques such as electron paramagnetic resonance spectra for confirmation and the anti-diabetic activity was evaluated by anti-hyperglycemic, hypoglycemic potential. **Result:** In the present work, we have studied the anti-hyperglycemic and hypoglycemic activity of the vanadium complex in glucose loaded and normal animals were shown significantly decreased in plasma blood glucose level. The results derived from preclinical studies confirm the potential of new sesquiterpene. **Conclusion:** The findings could provide evidence regarding the anti-diabetic potential of *T. populnea* by lowering blood glucose level.

Key words: Antihyperglycemic and hypoglycemic activity, diabetes mellitus, *Thespesia populnea*

SUMMARY

- *Thespesia populnea* is widely used in the ayurvedic system of medicine for treatment of diabetes in India. Present study aimed to explore the anti diabetic potential of isolated compound. Isolation of sesquiterpene from hexane fraction of bark of *Thespesia populnea* and modified synthetically then authenticated by using analytical techniques such as electron paramagnetic resonance spectra for confirmation. The modified complex was further assessed for its anti diabetic property in glucose loaded rats. Vanadium complex demonstrated significant reduction in plasma blood glucose level in glucose loaded animals. The results derived from preclinical studies confirm the potential of new sesquiterpene. The present findings conclude that anti diabetic potential of *Thespesia populnea* could be due to lowering blood glucose level by acting on PPAR-γ receptor.

Correspondence:

Asst. Prof. Mohini Ashok Phanse,
Department of Pharmacognosy, Modern College of Pharmacy, Nigdi, Pune, Maharashtra and PhD research scholar JNTUH, Hyderabad, India.
E-mail: phanse_mohini@yahoo.co.in

DOI : 10.4103/0973-1296.168984

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by insulin resistance, hyperglycemia and associated with impaired lipid profile.[1] Considering the high incidence of diabetes mellitus, between 2010 and 2030, there will be a 69% raise in numbers of adults through diabetes in developing country and a 20% raise in developed countries. As a result, there is an increase in demand for a novel drug with a lesser side effect.[2] The main aim of organic and medicinal chemistry is to design, synthesize and produce molecules which act as important human curative agents. The compounds obtain from synthesis or plant source containing heterocyclic ring are of great significance receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry.[3]

In 1982, thiazolidinediones were intensively studied for their anti-hyperglycemic activity.[3] Thiazolidinediones are known to be insulin sensitizers and have been clinically used as anti-diabetic agents. The maleate of rosiglitazone, a medicine of the thiazolidinediones class, showed considerable clinical efficacy against diabetes mellitus.[1] Peroxisome proliferator-activated receptors (PPARs) include three isoforms: α, β/δ, and γ. PPARγ-is the most abundant isoform in adipose tissue, macrophages, monocytes, intestinal cells, skeletal muscle, and endothelium. It plays an important role in the regulation of insulin sensitivity, lipid metabolism, adipogenesis and glucose homeostasis.[4] The target of the thiazolidinediones has been identified as the PPARγ and the glucose-lowering activities of the thiazolidinediones were shown to be closely related to their PPARγ agonistic activity.[5] Still the herbal supplementation and other substitute medicine have gradually increased to use for management of the diabetic disorder.

Cite this article as: Phanse MA, Patil MJ, Abbulu K. The isolation, characterization and preclinical studies of metal complex of *Thespesia populnea* for the potential peroxisome proliferator-activated receptors-γ agonist activity. Phcog Mag 2015;11:S434-8.

Thespesia populnea of Malvaceae family is widely employed in the management of diseases. Various experimental findings showed that *T. populnea* bring wide spectrum of activities such as anti-bacterial activity, anti-hepatotoxic activity, anti-steroidogenic activity, anti-implantation activity, cytotoxicity and superoxide anion generation, anti-nociceptive and anti-inflammatory, anti-psoriatic activity, wound healing activity, dermatitis, anti-oxidant activity, Alzheimer's disease, anti-diabetic activity, synergistic activity, immunomodulatory activity, anti-inflammatory, α-amylase inhibitory activity, anti-ulcer activity, anti-oxidant and anti-inflammatory, memory-enhancing activity.[6-25]

Vanadium is a trace mineral that is nearby present in various foods and may be essential in minute amounts in the body. High dose of vanadium has been experienced as an aid to controlling blood sugar level in the crowd with diabetes. It exists in oxidation states of − I, 0, +II, +III, +IV, and + V; the latter two are stable solution structures at physiological pH: Vanadyl (+IV) and vanadate (+V). The oral administrations of inorganic vanadium (IV, V) salts have revealed anti-diabetic activity. Vanadium compounds come first in an insulin-mimetic approach both *in vitro* and *in vivo* has been sound recognized. Both inorganic and organic vanadium compounds have been revealed to lower plasma glucose levels, raise peripheral glucose uptake, progress insulin sensitivity, decline plasma lipid levels and return to normal liver enzyme activities in an array of animal models of both type I and type II diabetes.[26] Vanadyl sulfate is one of the component vanadium's colorful forms and it is sometimes called a vanadium salt. Vanadyl sulfate is the form of preference for the dealing. Vanadyl sulfate management of diabetes is correlated to their anti-hyperglycemic, hyperinsulinemic and antihyperlipidemic effects.[27]

The objective of this study was to ameliorate the effect of *T. populnea* and its modified compound against diabetes mellitus. The findings could provide evidence to support the use of *T. populnea* as an anti-hyperglycemic and hypoglycemic plant. The modified compound characterized by electron paramagnetic resonance (EPR) spectra at low concentration also showed the anti-hyperglycemic and hypoglycemic effect.

MATERIALS AND METHODS

Drug and chemicals

The Glimepiride were purchased from Aventis Pharma Ltd., (Batch No: 20120701). Vanadyl sulfate were purchased from Loba Chemie Lab Reagent Pvt. Ltd., Biochemical estimation of plasma glucose was done by glucose oxidase/peroxidase (GOD/POD) method respectively using standard diagnostic kits from Biolabs India Ltd., India.

Plant material

The dry bark of *T. populnea* was collected from Hilly area of Dehu 15 km from Pune, Maharashtra, India. The plant material were authenticated by Botanical Survey of India with voucher specimen is conserved under reference number BSI/WRC/Herbarium/2011.

Preparation of hexane extract, isolation, and modification of isolated compound

The air dried bark and finely powdered bark was extracted by routine process with 90% hexane, then isolation of the sesquiterpene by using column chromatography with elution of chloroform: Methanol (8:2) solvent system and characterized. This isolated compound complexed with vanadium in alkaline condition with methanolic solutions vanadium sulfate and the respective ligand in 1:2 metal: Ligand. The reaction mixtures were maintained in alkaline condition and stirred 6 h at room temperature. The precipitating metal complexes were collected by filtration, washed with cold methanol.

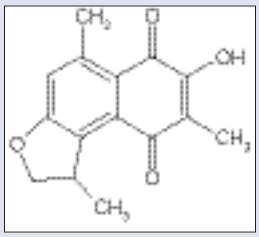

Figure 1: The structures of the compound identified in *Thespesia populnea*

Characterization by electron paramagnetic resonance spectra

The EPR spectra of the vanadium complex provided information on metal ion environment and the electron delocalization. The X-band EPR spectra of vanadium complex, recorded in DMSO glass at 77 K.

Experimental animals

Male Wistar rats weighing approximately 140–160 g were used. They were housed in an air-conditioned room (approximately 22°C ± 2°C and during the entire experiments). The animals were maintained with pelleted food while tap water was available *ad libitum.* All the animals were monitored carefully and maintained in accordance with the ethical recommendations of animal experimentation protocol (MCP/IAEC/46/2011).

Determination of LD_{50} values for vanadium complex

Albino mice of both sex and of 25–30 g weights were divided into six groups, each containing six animals for the purpose of determining the LD_{50} value of a vanadium metal complex. Each group was caged separately. Seven different doses of 1.75, 5.5, 17.5, 55, 175, 550, and 2000 mg/kg body weight were employed for each test drug. Each animal in every group was administered with a predetermined dose orally. After 24 h, the numbers of dead animals in groups were recorded. The data were tabulated. The toxicological effect was assessed on the basis of mortality, which was expressed as an LD_{50} value. In the groups with survived animals and in the groups with dead animals, the obtained percentages were corrected using the following formula:

- Correction formula for 0% dead group ¼ 100 (0.25/n)
- Correction formula for 100% dead group = 100 [n − 0.25/n].

Where, n represents the number of animals in the group.

After correction, the percentages were converted into probit. The values thus obtained were plotted against log dose. The LD_{50} value was determined.[28,29]

Anti-hyperglycemic study in normal rats

- The Wistar albino rats weighing 140–160 g of either sex were used. The overnight fasted animals were divided into groups (n = 6). The animals were received 10% glucose solution (2 g/kg, p.o.) 30 min after drug administration
- The animals of Group I, II, III, IV, V, VI received (control) 1% gum acacia (1 ml/kg, p.o.), glimepride (0.09 mg/kg, p.o.), ligand vanadium (LVa) complex (5 mg/kg, p.o.), LVa complex (10 mg/kg, p.o, LVa complex (20 mg/kg, p.o.), vanadyl sulfate (0.2 mM/kg, p.o.), respectively.

Biochemical analysis

At the 0th, 30th, 90th, and 150th min (fixed interval of times) after the glucose load, blood was collected by puncturing retro-orbital plexus by

using fine glass capillary, in tubes containing an anticoagulant. Plasma was separated by centrifugation. Plasma glucose levels were determined with a standard diagnostic kit by GOD/POD method [Figure 2].[30,31]

Hypoglycemic study in normal rats

- The Wistar albino rats weighing 140–180 g were used. The overnight fasted animals were divided into groups ($n = 6$)
- The animals of Group I, II, III, IV, V, and VI received 1% gum acacia (1 ml/kg, p.o.), glimepiride (0.09 mg/kg, p.o.), received LVa complex (5 mg/kg, p.o.), LVa complex (10 mg/kg, p.o.), LVa complex (20 mg/kg, p.o.), vanadium sulfate (0.2 mM/kg, p.o.).

Biochemical analysis

At the 0th, 60th, 120th, and 180th min after drug administration blood was collected by puncturing retro-orbital plexus by using fine glass capillary, in eppendorf tubes containing anti-coagulant. Plasma was separated by centrifugation. Plasma glucose levels were determined with a standard diagnostic kit by GOD/POD Method. [30,31]

RESULTS

Plant material

The extraction methods by means of organic solvents are normally used for the pharmacological study. However, the extraction methods might not reflect the real characteristic of traditional Indian medicine; because most traditional Indian medicine remedies are prepared in the form of infusion, decoctions, and tinctures. Boiling water extraction and organic solvent extractions result in different phytochemical profile in the extracts. The present study used the solvent (hexane) extraction process to investigate the real active components that exist in the clinical active extract the plant constituent was isolated by routine process [Figure 1] and incorporated with vanadium sulfate which was thought for the *T. populnea* profound pharmacological activity.

Characterization by electron paramagnetic resonance spectra

The four well-resolved metal hyperfines in the low field region are indicative of monomeric Vanadium complex gives conformation about the complex.

Determination of LD_{50} values for vanadium complex

The LD_{50} for the vanadium complex was found to be 100 mg/kg during a 24 h period were hardly perceptible.

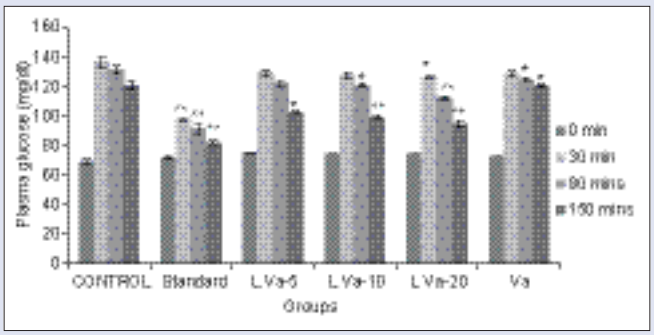

Figure 2: Effect of vanadium complex on plasma glucose in glucose loaded rats (anti-hyperglycemic effect). Values are expressed as mean ± standard error of the mean ($n = 6$), ANOVA followed by Dunnett test (*$P < 0.05$, **$P < 0.01$ when compared with control)

Anti-hyperglycemic study in normal rats

During the anti-hyperglycemic study, treatment with vanadium complex showed a significant decrease in plasma glucose level at the dose of LVa-10 and LVa-20 ANOVA followed by Dunnett test. *$P < 0.05$, **$P < 0.01$ when compared with control group. Group treated with Vanadium complex shows dose-dependent anti-hyperglycemic activity [Figure 2].

Hypoglycemic study in normal rats

In the hypoglycemic studies, Group treated with Vanadium complex at the dose of LVa-10 and LVa-20 showed a significant decrease in plasma glucose level when compared with the control group [Figure 3].

DISCUSSION

The dose of a chemical that has been calculated to cause death in 50%, of a defined experimental animal is $LD_{50.}$ The effects of vanadium complex in p.o doses ranging between 10 mg/kg and 20 mg/kg.

As expected, after starting the glucose tolerance test (anti-hyperglycemic study) in normal rats overloaded with glucose, the plasma glucose concentration was significantly increased when compared with the zero time of this group. Treatment with Vanadium complex showed a significant decrease in plasma glucose level when compared with control group. Pharmacological uses of vanadium consist of lower of triglycerides cholesterol, and glucose levels, diuretic, anti-carcinogenic effect, contraction of blood vessels, enhancement of oxygen affinity of hemoglobin and myoglobin.[32-34]

The anti-hyperglycemic activity of vanadium complex in glucose loaded animals is likely to be mediated by an increase in insulin release from pancreatic β-cells or may be due to increased insulin sensitivity. It is also possible that such effects may also be the result of inhibition of the intestinal absorption of glucose or by stimulating glucose uptake and use in the peripheral tissues or by increasing insulin from pancreatic β-cells.

In the subsequent hypoglycemic studies, it was observed that vanadium complex proved the significant and dose-dependent hypoglycemic effect in normoglycemic animals. This study possesses that Vanadium complex show significant glucose lowering effect in normal rats. The plants showed hypoglycemic activity through the stimulation of insulin release.[35]

CONCLUSION

From the above result it was concluded that these effects may also be the due to of inhibition of the intestinal absorption of glucose or by stimulating glucose uptake and use in the peripheral tissues or by increasing insulin from pancreatic β-cells by acting on PPAR-γ receptors. These results signify innovatively incorporated ligand benefits beyond their nutritional properties. Group treated with

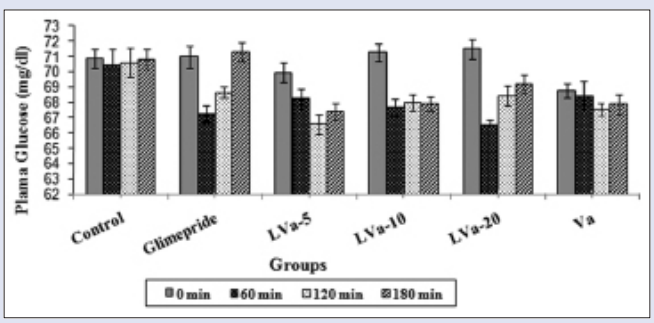

Figure 3: Effect of vanadium complex on plasma glucose in normal rats (hypoglycemic effect). Values are expressed as mean ± standard error of the mean ($n = 6$), when compared with control

Vanadium complex shows dose dependent anti-hyperglycemic and hypoglycemic effect. Thus ligand is considered for developing into a potent anti-diabetic moiety.

Acknowledgments

Authors are indebted to Director JNTU, Hyderabad and Dr. P. D. Chaudhari Dean, Pune University and Principal, Modern College of Pharmacy, Pune for their constant guidance, motivation and being supportive during this course of investigation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. da Costa Leite LF, Veras Mourão RH, de Lima Mdo C, Galdino SL, Hernandes MZ, de Assis Rocha Neves F, *et al.* Synthesis, biological evaluation and molecular modeling studies of arylidene-thiazolidinediones with potential hypoglycemic and hypolipidemic activities. Eur J Med Chem 2007;42:1263-71.
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
3. Jain VS, Vora DK, Ramaa CS. Thiazolidine-2,4-diones: Progress towards multifarious applications. Bioorg Med Chem 2013;21:1599-620.
4. Xiao B, Yin J, Park M, Liu J, Li JL, Kim EL, *et al.* Design and synthesis of marine fungal phthalide derivatives as PPARγ agonists. Bioorg Med Chem 2012;20:4954-61.
5. Yoriko I, Shuichi M, Takamura M, Hiroaki Y, Kasuya A. Interaction between peroxisome proliferator-activated receptor-γ and its agonists: Docking study of oximes having 5-benzyl-2,4-thiazolidinedione. J Mol Graph Model 2001;19:536-42.
6. Gaind KN, Bapna SC. Anti-bacterial activity of *T. populnea.* Indian J Pharm 1967;29:8-9.
7. Shirwaiikar A, Vasanth Kumar A, Krishnanand BR, Sreenivasan KK. Chemical instigation and anti-hepatotoxic activity of *T. populnea.* Int J Pharmacogn 1995;33:305-10.
8. Ghosh T. Bhattacharya K. Preliminary study on the anti-implantation activity of compounds from the extract of seeds of *T. populnea.* Indian J Pharmacol 2004;36:288-91.
9. Kavimani S, Ilango R, Karpagam S, Suryaprabha K, Jaykar B. Anti-steroidogenic activity of floral extract of *Thespesia populnea* Corr. in mouse ovary. Indian J Exp Biol 1999;37:1241-2.
10. Johnson Inbaraj J, Gandhidasan R, Murugesan R. Cytotoxicity and superoxide anion generation by some naturally occurring quinones. Free Radic Biol Med 1999;26:1072-8.
11. Vasudevan M, Gunnam KK, Parle M. Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. J Ethnopharmacol 2007;109:264-70.
12. Shrivastav S, Sindhu RK, Kumar S, Kumar P. Anti-psoriatic and phytochemical evaluation of *T. populnea* bark extracts Int J Pharm Pharm Sci 2009;1:176-85.
13. Nagappa AN, Cheriyan B. Wound healing activity of the aqueous extract of *Thespesia populnea* fruit. Fitoterapia 2001;72:503-6.
14. Hausen BM, Knight TE, Milbrodt M. *Thespesia populnea* dermatitis. Am J Contact Dermat 1997;8:225-8.
15. Ilavarasan R, Vasudevan M, Anbazhagan S, Venkataraman S. Antioxidant activity of *Thespesia populnea* bark extracts against carbon tetrachloride-induced liver injury in rats. J Ethnopharmacol 2003;87:227-30.
16. Vasudevan M, Parle M. Pharmacological actions of *Thespesia populnea* relevant to Alzheimer's disease. Phytomedicine 2006;13:677-87.
17. Parthasarathy R, Ilavarasan R, Karrunakaran CM. Anti-diabetic activity of *T. populnea* bark and leaf extract against streptozotocin induced diabetic rats. International Journal of Pharm Tech Research 2009;1:1069-72.
18. Kumar AS, Venkateshwaran K, Vanitha J, Saravanan VS, Ganesh M, Vasudevan M *et al.* Synergistic activity of methanolic extract of T. populnea (Malvaceae) flowers with oxytetracycline. Bangladesh J Pharmacol 2009;4:13-6.
19. Jayakumari S, Rajkumar M, Joanofarc J, Srinivasa Rao G, Sadish Kumar S, Umadevi SK. Hypoglycaemic, anti-diabetic and anti-ulcer screening of *Thespesia populnea* Linn. Nat Prod Sci 2003;9:167-9.
20. Gaikwad SB, Mohan GK. Immunomodulatory activity of methanolic extract of *T. populnea* leaves in Westar albino rats. Asian J Pharm Clin Res 2011;4:99-101.
21. Shah AS, Alagawadi KR. Anti-inflammatory, analgesic and antipyretic properties of *Thespesia populnea* Soland ex. Correa seed extracts and its fractions in animal models. J Ethnopharmacol 2011;137:1504-9.
22. Sangeetha R, Vedasree N. *In Vitro* α-amylase inhibitory activity of the leaves of *Thespesia populnea.* ISRN Pharmacol 2012;2012:515634.
23. Patil PH, Patil JY, Mahale JN, Patel JB, Surana SJ. Evaluation of anti-ulcer activity of the terpenoid fraction from the leaves of *T. populnea* (L) (Malvaceae) in albino rats. Res J Pharm Biol Chem Sci 2010;1:495-513.
24. Sarma DS, Venkata A, Babu S, Krishna KR. Anti-oxidant and anti-inflammatory activities of *T. populnea* Linn. Int J Res Pharm Chem 2011;1:674-6.
25. Vasudevan M, Parle M. Memory-enhancing activity of *T. populnea* in rats. Pharm Biol 2007;45:267-73.
26. Arya GS, Hedaytullah MD, Raghvendra, Yadav A, Sachan K. Treating diabetes mellitus with vanadium salts - a future prospectus: A review. International Journal of Pharmaceutical Sciences Review and Research 2011;8:183-5.
27. Ali W. Anti-hyperglycemic effect of chicory leaves and vanadium consumption on diabetic experimental rats. World J Dairy Food Sci 2012;7:167-73.
28. OECD. Guidelines for Testing of Chemicals 425, Acute Oral Toxicity-Up-and-Down Procedure. OECD publisher: OECD; 2001. p. 1-26.
29. Kesari AN, Gupta RK, Singh SK, Diwakar S, Watal G. Hypoglycemic and antihyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats. J Ethnopharmacol 2006;107:374-9.
30. Bhat ZA, Ansari SH, Mukhtar HM, Naved T, Khan NA, Chashoo IA. Anti-hyperglycemic activity of the alcoholic extracts of *Aralia cachemirica* Decne roota. J Nat Remedies 2005;5:160-4.
31. Mukhtar HM, Ansari SH, Ali M, Bhat ZA, Naved T. Effect of aqueous extract of *Cyamopsis tetragonoloba* Linn. beans on blood glucose level in normal and alloxan-induced diabetic rats. Indian J Exp Biol 2004;42:1212-5.
32. Poucheret P, Verma S, Grynpas MD, McNeill JH. Vanadium and diabetes. Mol Cell Biochem 1998;188:73-80.
33. Rehder D. Structure and function of vanadium compounds in living organisms. Biometals 1992;5:3-12.
34. Thompson KH, Leichter J, McNeill JH. Studies of vanadyl sulfate as a glucose-lowering agent in STZ-diabetic rats. Biochem Biophys Res Commun 1993;197:1549-55.
35. Meliani N, Dib Mel A, Allali H, Tabti B. Hypoglycaemic effect of *Berberis vulgaris* L. in normal and streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed 2011;1:468-71.


Mohini Ashok Phanse


Manohar Janardhan Patil


Konde Abbulu

ABOUT AUTHORS

Mohini Ashok Phanse, Assistant professor, Department of Pharmacognosy, Modern College of Pharmacy, Nigdi, Pune, India. PhD research scholar at JNTU, Hyderabad, Telangana, India.

Manohar Janardhan Patil, Principal, Department of Pharmacognosy, Maratha Mitramandal College of Pharmacy, Kalewadi, Pune, Maharashtra, India.

Konde Abbulu, Principal, Department of Pharmaceutics, Mallareddy Institute of Pharmaceutical Sciences, Hyderabad, Telangana, India.