Research Article

Kinetic Study of Lantana camara Linn. Secondary Bioactive Molecule Tripene

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Abstract

Allelochemicals are present in almost all plants that have very intricate configuration of bioactive molecules that has an effect on a living organism, tissue or cell. The impending of *Lantana camara* tripene have been isolated and the influence of various solvent on tripene extraction rate, effect of tripene and substrate concentration, pH, temperature, time on reaction rate, behavioural study of silver nanoparticles depends the value of *Lantana camara*. The observed Vmax and Km value for Diethyl ether, Acetone, Methanol and n- Hexane was 5, 4.6 ,4.2 , 6 and 0.8, 1.2, 1.3 1.4 respectively. Optimum pH was 7 for acetone and methanol in phosphate buffer while 4.6 in citrate buffer. Optimum temperature was 5^0C for n-Hexane and Methanol, diethyl ether 30^0C and Acetone 20^0C respectively. Optimum time was observed 2 hrs while lantana leaves silver nanoparticles optimum time 30 min, optimum pH 6.8, Optimum Temperature 40^0C having wavelength 440 nm broaden peak 420 nm to 470 nm appearances of nanoparticles. Change in colour from colourless to brown confirms reduction of Ag^+ ion. Kinetic of *Lantana camara* leaves explored pharmacological properties having industrial applications.

Keywords: *Lantana camara*, Extract, Kinetic Study, Tripene, AgNP's

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Introduction

Phytochemistry is in the stringent sense of the term regarding evaluation of active compounds or metabolites occur in floras. India has a rich tradition of plant based knowledge in health care. The drug yielding plants were screened from the beginning of 19th century for the analysis of secondary metabolites such as alkaloids, saponins, flavonoids, tannin, steroid etc. Certain imperative rudimentary medications used in Ayurveda were chemically analyzed and the constituents were reported from alcoholic extracts of plants [1]**.** Isolation and structural elucidation of natural products was deliberated by various chromatographic and spectroscopic techniques. For natural products future prospects in the development of effective therapeutic compounds as well as elaborately biological pharmacological activities, a new triterpene, Cycloeuphordenol and macro cyclic diterpene esters were isolated from the latex of Euphorbia tirucalli [2]**.** In the present study lantana were selected since *Lantana camara* (Verbenaceae) is an evergreen flowering ornamental weed occurred in arid area and has significantly used as folk medicine for the treatment of chicken pox, measles, asthma, ulcers, swellings, eczema and high blood pressure [2]**.** Bioactive metabolites isolated from *Lantana camara* leaves namely triterpenoids possess, anti-inflammatory and antipyretic activity [3], anti-filarial activity [4, 5, 6], antitumor activity [7] antithrombin activity [8]. Anticancer and anti-proliferative activity [9, 10], Antibacterial activity[11], Haemolytic activity [12], Antifungal activity [9, 13], Antimicrobial activity [14, 15] , Anti mutagenic activity, Antioxidant activity, Wound healing activity, Antimotility activity, Antiurolithiatic activity, Mosquito controlling activity, Anti fertility activity/Embryo toxicity [16, 17, 18]. Due to rich in alliochemicals enzymatic delignification studied along with sensitivity analysis to observe kinetics constant. [19]. Sensitivity parameters inform more cost effective development of species distribution models for lantana for management of invasive species [20, 21]. Uncertainties related to the model presented many challenges [22] and had serious implications for the accuracy of the bioclimatic models species distribution models (SDMs) or ecological niche models (ENMs) output [23, 24, 25, 26].Thus allowing it to expand its potential range, Parameter uncertainty, to quantify the response of lantana bioactive molecule/tripene for perceiving changes in behavior in various concentration of it on reaction rate, extraction rate, substrate, time, temperature, pH were presented in the paper. The

main aim was to identify the parameters that were functionally important and thus provide a better understanding of bioactivity that have a larger impact on lantana distribution. Hence nanoparticle of the tripene also explored to kinetic parameter such as ion concentration, time, wavelength, temperature and pH.

MATERIAL AND METHOD

Materials

Methanol, Acetone, Diethyl ether, n-Hexane**,** Sucrose, Potassium Hydrogen Phthalate, Acetic Acid, Sodium Acetate, Sucrose, Monobasic sodium phosphate, Dibasic Sodium Phosphate, Citric acid, Glycine, Sodium Hydroxide, Borax Acetone and Methanol etc. Glass distilled water used throughout the experimental work.

Plant material

A *Lantana Camara* healthy fresh leaves were collected from campus VPM's B.N.Bandodkar College of Science, Thane in September 2015. Leaves were washed and dried at room temperature under shade. The dried leaves are pulverized using mortar and pestle. Powder was stored in polythene bag at room temperature for further use.

Method

Extraction and isolation of tripene/triterpene

Lantana camara leaves powder (100 gm) was treated with increase in volume of 400 cm^3 methanol and serially refluxed for 18 hrs. Solvent was removed under vacuum (13-14 mm/Hg and distillation temperature up to 58^oC) to observed dark brown residue that was suspended distilled water followed by filtration through Whatman paper No.1 the residue was dissolve in a methanol : water (1**:**7) mixture and extracted with ethyl acetate (2 X 25 cm^3) and with n-butanol (2 X 25 cm^3) The ethyl acetate layer was concentrated under reduced pressure to get crude tripene that was monitored timely with TLC mobile phase $CHCl_3$–CH_3OH (9.8:0.2). Crude tripene was tested by treating active fraction with chloroform (50 µl) followed by 2-3 drops of concentrated H_2SO_4. Warm reaction mixture shown change in colour. It was also confirmed by Salkowaski test and Libermann burchard test was carried out for active fraction from column chromatography was used for its kinetic study.

Influence of solvent on extraction rate of tripene

Tripene (50 µl) was placed in different 100 cm^3 of round bottom flasks. Various solvents (Ethanol, Methanol, Acetone, Chloroform, n-Hexane, Diethyl ether) ranging from 10-100 % was added in separate round bottom flask and refluxed for 0.5- 2.5 hrs The process was repeated thrice in each time average of it was used to calculate the extraction rate.

Effect of triterpene / tripene concentration on reaction rate

The crude triterpene compound (0.5 g) added to Methanol, Acetone, Diethyl ether, n-Hexane separately. Each solvents ranging from 0.1 cm^3 up to 1.0 cm^3 was added and reaction was monitored for 3 cm^3 and reaction rate was measured from 400-700 nm on UV-1800 Spectrophotometer.

Effect of substrate concentration of triterpene / tripene

The crude methanol extracts was concentration to dryness with $CH_2Cl_2.H_2O$. The major active compound/fraction obtained from column was used for substrate study. Four solvents such as Acetone, Methanol, Diethyl ether, n-Hexane with crude compound of triterpene/tripene 0.5 mg was measured 400 nm -700 nm by using various concentration of sucrose without sucrose is acts as control.

Effect of temperature and time on triterpene / tripene

Effect of Temperature on Extraction Rate: According to above experimental results optimum solvent was used to observe optimum temperature. Crude tripene (50 µl) was placed in four different round bottom flask the selected volume of solvents was added. Reaction was refluxed from 30^0C- 100^0C for respective round bottom flask for 2 hrs.

Effect of time extraction rate: Selected solvent optimum extraction temperature was used for extraction time which is depends on extraction rate.

Effect of pH on triterpene / tripene

Crude sample explored at various pH ranging from Acetate Buffer (3.6 to 5.6), Phosphate Buffer (5.8 to 8), Citrate-Phosphate Buffer (3.0 to 6.2), Glycine-NaOH Buffer (8.6 to 10.6) kept in temperature and optimum pH was determine effect. Measured pH at 400 nm -700 nm

Synthesis of silver nanoparticles of tripene [27, 28]

Aqueous extract of Lantana camara

Lantana camara (50 gm) macerated for 3 days in one litter of deionized water at room temperature .The aqueous extract was filtered using filter paper. The filtered extract was stored at room temperature for further use.

Preparation of silver nanoparticles

Aqueous solution of silver nitrate (1 mM) was used for synthesis of nano particles. 5 ml of aqueous extract of *Lantana camara* was added to 95 ml of aqueous solution of 1 mM $AgNO_3$ and heated with stirrer at 80 °C for 60 min. The formation of brown colour was indicated synthesis of silver nanoparticles.

To determine the optimum factors for synthesized silver nanoparticles

The experiments were carried out in different following conditions for silver ion concentration. Various concentration of silver ion concentration ranging from 0.25, 0.5, 1, 2 and 3 mM was used to achieve optimum concentration of silver ion.

Optimum pH and temperature

pH range from 4, 4.8, 6.8 and 9 and temperature measured from 20°C**,** 40°C, 60°C and 80°C to observed the silver nanoparticles.

Optimum time

The silver ion concentration was measured from 10’ to 80’ and pH of the reaction was adjusted by using NaOH and HCL. The effect of these parameters on the synthesis of silver nanoparticles was monitored by UV-Vis-1800 spectrophotometer.

Lantana camara extract and silver nitrate ratio

The various volume of *Lantana camara* extract 0.1:0.1 ml to 0.5:0.5 ml (V/V) and also *Lantana camara* extract to silver nitrate ratio from 10:90, 5:95, 2.5:97.5, 1:99 and 0.5:99.5 were used to find out extraction for the synthesis of silver nanoparticles.

Characterization of nanoparticles by UV- Visible spectroscopy

The silver nanoparticles of *Lantana camara* leaves extract was studied by the UV-Visible spectroscopy in the wavelength range of 200 to 800 nm. The samples were prepared by the dilution in small aliquots, left from 30 min to 5 hours and monitored through the above wavelength range by UV-1800 Shimadzu double-beam spectrophotometer.

Result and Discussion

Natural products are rich in therapeutic application. Its bioactivity due to molecules has an effect on a living organism, tissue or cell. Most of the active bioactive molecules are organic compounds which are generally non polar and consisting of covalent bonds. Bioactivity Organic molecule is depends on solubility, polarity, extractions of a

specific bioactive compound in the nature and extraction medium. It's very difficult to identify a using uniform process to extract and isolate bioactive compounds from different plants. A series of different physical as well as chemical processes are involved for isolation of bioactive compounds. The process begins with the extraction, isolation and purification by maintaining optimum condition. In the present research work alcoholic solvent extract of *Lantana camara* leaves were prepared to extract bioactive molecule / Tripene.

Extraction of *Lantana camara* Linn leaves tripene was carried out by alcoholic reflux followed by column chromatography using different solvents. Organic solvents contributed exceptionally low yield showing very little difference in physicochemical properties of pentacyclic triterpene. It revealed that interferon of triumphing a pure form of triterpenoids / tripene. Ethyl acetate does not able to extract Tripene completely while from the methanol–water mixture extracted reproducible yield.

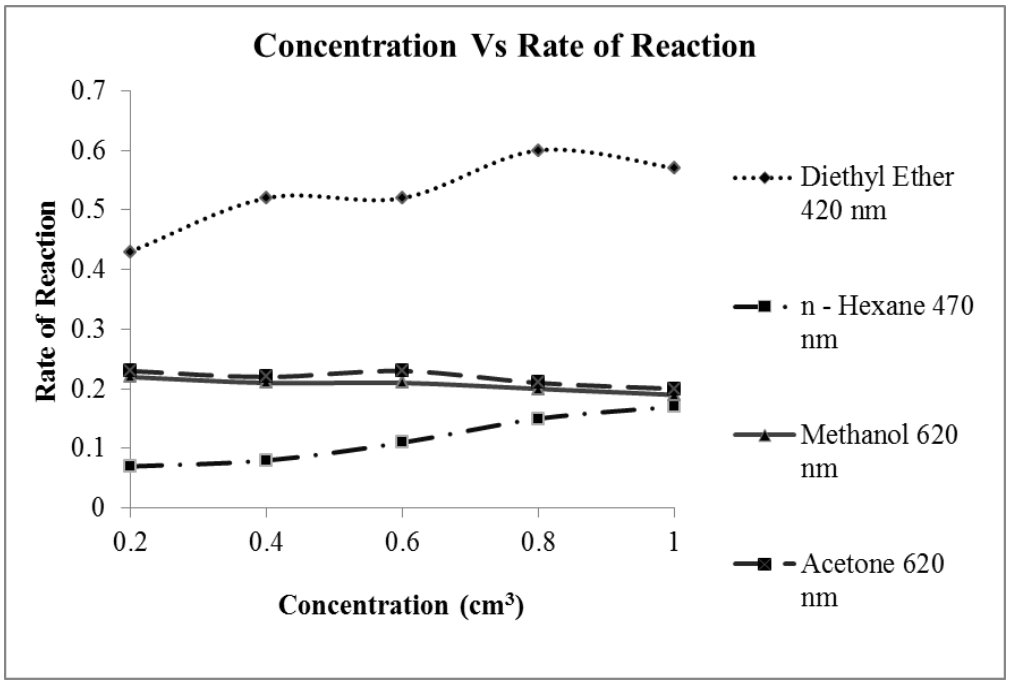
The ethyl acetate layer was concentrated under reduced pressure; the crude tripenes is 0.95 gm from 100 gm of dried leaves powder which was loaded on silica gel column. Proper organic solvents were used for fractional crystallization of crude tripene (0.95 gm) from lantana leaves powder procured 0.24 mg of pure Tripene. When the crude compound loaded on gel chromatogram procure 13 mgs Tripene having greyish elutant. Physical behaviour is same as of in solvent system but during processing for leaves, change in physical property of tripene was observed in different organic solvents.

The same quantity of lantana leaves powder when treated with aqua reflux process followed the same process of solvent extraction; but there was no separation of layers. Colour of the layer was dark brownish liquid. For the separation of layers excess of ethyl acetate was added but unable to separate the layers though the process was repeated in different season. It indicates that aqueous reflux method followed by vacuum distillation does produced results as compared to organic solvent reflux method. HPLC techniques are used to sort the nature of tripene in each solvent during process after ethyl acetate (crude). Warm reactions shows red coloration indicates presence of tripene. λ max was measured at various concentrations of Tripene exhibited different UV maxima for each range of concentration such as 0.4 cm^3 at λmax 470 nm.

The initial concentration produced significant depth knowledge of molecule. All mass transfer resistances of all molecules between the aqueous and dense phases of tripene Consequently, change in behavioural pattern crude tripene concentration procure on reaction time and rate of reaction influence by diethyl ether at 420 nm when 0.8 cm^3 concentration was used without substrate while the 0.6 cm^3 of sucrose substrate shows maximum rate of reaction of diethyl ether at 420 nm (**Figure 1a**). When reaction was catalysed with sucrose along with ratio of solvents, it is very difficult passes free radical in chain process. This reaction produce intermediate complex having possibility is conversion towards the catalyst and substrate where the rate of reaction is greater than that of under goes starting reaction.

Sucrose was used as a substrate throughout the experiment to observed maximum reproducible results. According to Arrhenius concept of chemical reaction involves reactant and activated complex. If intermediate complex is less stable species it produces direct product. Vmax and Km was differing with different solvent and ratio as well characteristically (**Table 1**, Figure 1b). Optimum temperature for Acetone was at 20^0C solvent at 620 nm while methanol and Diethyl ethers solvent was 5^0C and 30 ^{0}C at 660. n- Hexane does differ much at 620 nm and at 660 nm (**Figure 2**) and other buffers such as Acetate buffer (3.6 -5.6), Glycine- NaOH buffer (8.6- 10.6), Phosphate buffer, Citrate buffer optimum pH 4.6 at 400 nm of acetone solvent (Figure 2).

These elevated temperatures from 05^oC to 30^oC for different solvents result in improved extraction efficiencies since desorption of analytes from active sites in the matrix was increased. Additionally, solvents have higher capacity to solubilize analytes at higher temperatures, while surface tension and solvent viscosity decrease with temperature, which mayl improve sample wetting and matrix penetration, respectively. Alteration of temperatures on extraction percentage of tripene showed change in percentage of yield. The yield of the lantana leaves crude tripene was gradually enhanced with increase in temperature until 80°C, probably due to increased diffusivity of solvent into the internal parts of the matrix under elevated temperatures. Nevertheless, simultaneously with increased tripene extract ability, increased amount of matrix components would be co-isolated at higher temperature. Therefore, 80°C is optimal temperature for the extraction process though it showed 5^oC to 30^oC in solvents.



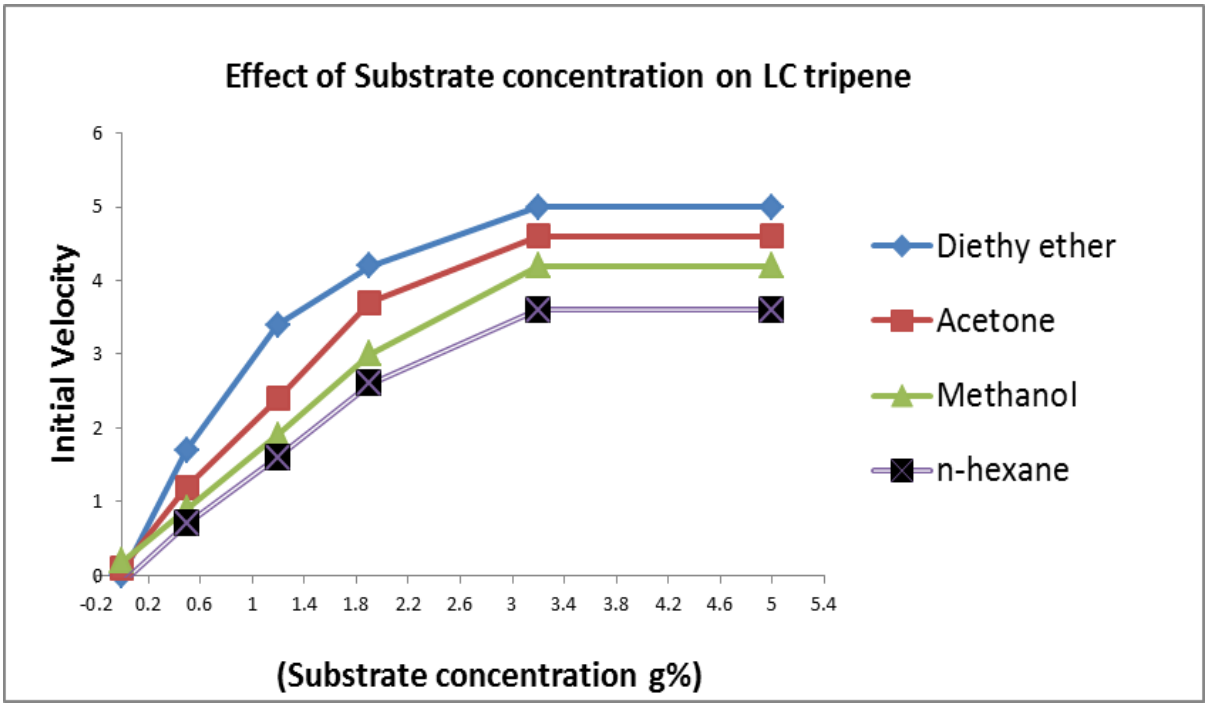


Figure 1 (a) Effect of tripene concentartion on rate of reaction **(b)** Effect of substrate concentartion on tripene bioactivity

Table 1 Vmax and Km of organic solvent

Solvent	Vmax	Km
Diethyl ether	5	0.8
Acetone	4.6	1.2
Methanol	4.2	1.3
n-hexane	3.6	1.4

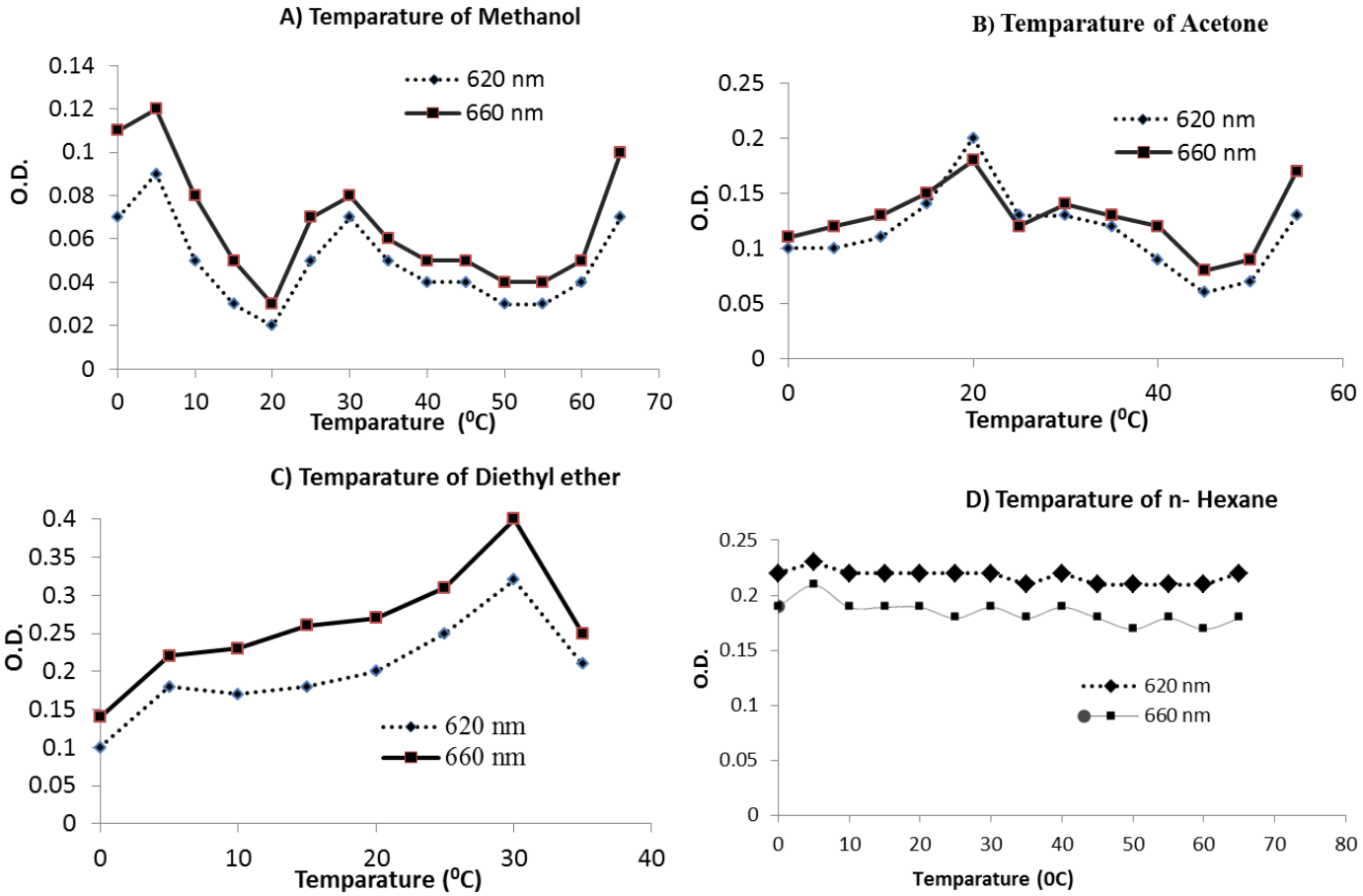


Figure 2 Effect of temperature on tripene in relation to wavelength.

Crude tripene explored at various pH ranging from Acetate Buffer (3.6-5.6), Phosphate Buffer (5.8- 8); Citrate-Phosphate Buffer (3.0 to 6.2) Glycine-NaOH Buffer (8.6 to 10.6) exhibited optima pH 4 ,7 ,4.8 and pH 9 for respective buffers at 620 nm when acetone and methanol used. (**Figure 3**)

Silver nanoparticles of lanatan leaves tripene

Aqueous solution of silver nanoparticles exhibited white in colour while tripene solution yellow in colour. Reduction of silver ion into silver nanoparticles during exposure to the lantana leaves extracts followed the visual changes in colour from yellow to dark brown indicated the reduction of Ag^+ via complexation and formation of AgNP's (**Figure 4**). It is generally recognized that UV–VIS spectroscopy used to examine size- and shape-controlled nanoparticles in aqueous suspensions.

The progress of two new absorbance bands observed at 400 nm, 420 nm and 470 nm. These characteristic colour variations are due to the resonance excitation in the metal nanoparticles confirming the activity of particles size was reduced. Absorbance peak at 440 nm showed in the reaction mixture indicated silver nanoparticles were formed.(**Figure 5)** .(**Figure 5)** . Broadening of peak indicated nanoparticles are poly dispersed.

The result is correlated with [29]**.** Optimum time for formation of particles is 30 min.They exhibited that rate of formation and size of AgNP's was dependent on lantana extract quantity and effective as antibacterial activity with the decrement of particle size against all tested bacterial cultures [30] having antioxidant efficacy in berry AgNP's [31]. Silver nanoparticles (AgNP's) have been synthesized by *Lantana camara* leaf extract through simple green route and evaluated their antibacterial and catalytic activities by [30].

Phytochemical analysis of L. camara fruit extract reveals the aqueous extract contains carbohydrates, glycosides and flavonoids. [32]. The Ag+ reduction was based on these three molecules [33] The larger amount of flavonoids present in aqueous extract may act a major role in Ag+ reduction reaction showing its characteristics temperature 40^0C with optimum pH 6.8 (**Figure 6).**

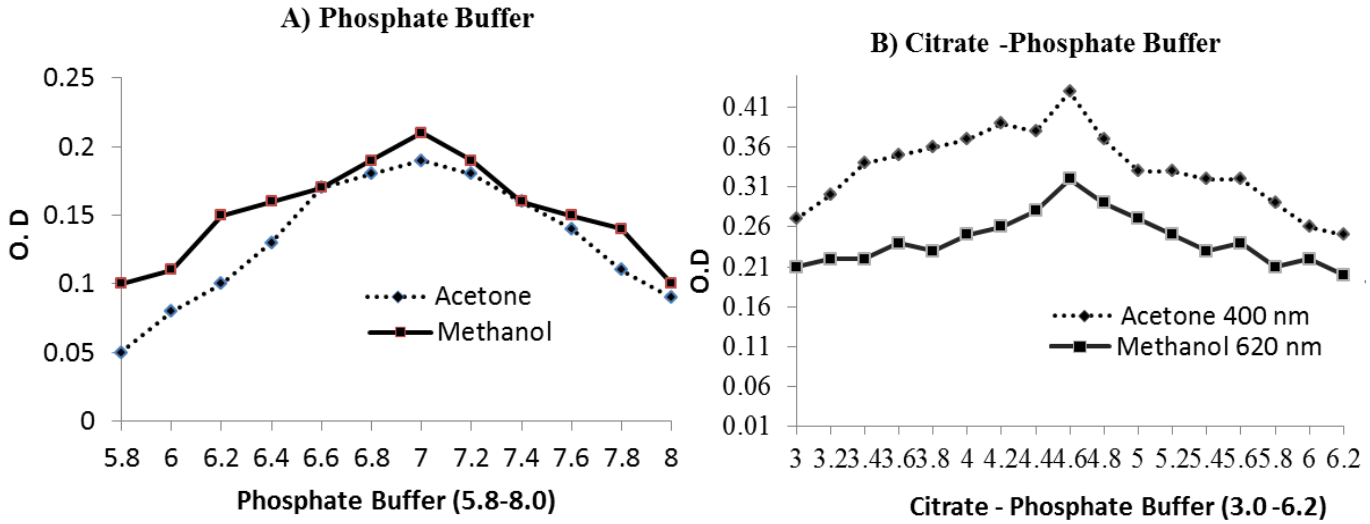


Figure 3 Effect of buffers/ pH range on tripene reaction rate

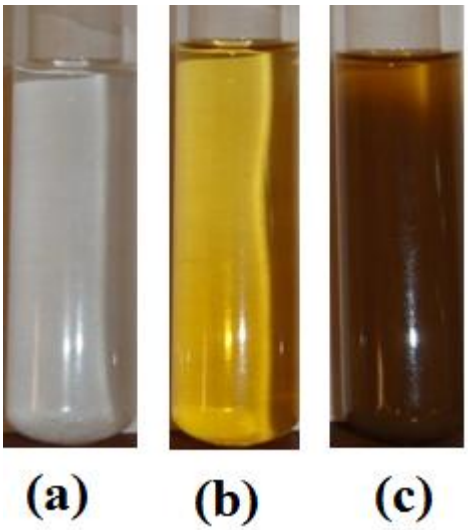


Figure 4 Changes in colour of nano particles (a) 1 mM $AgNO_3$ without *Lantana camara* extract (b) Aqueous leaf extract *Lantana camara* (c) Changing colour from yellowish to dark brown after adding 1 mM $AgNO_3$ and heated at 80 °C for 30 min

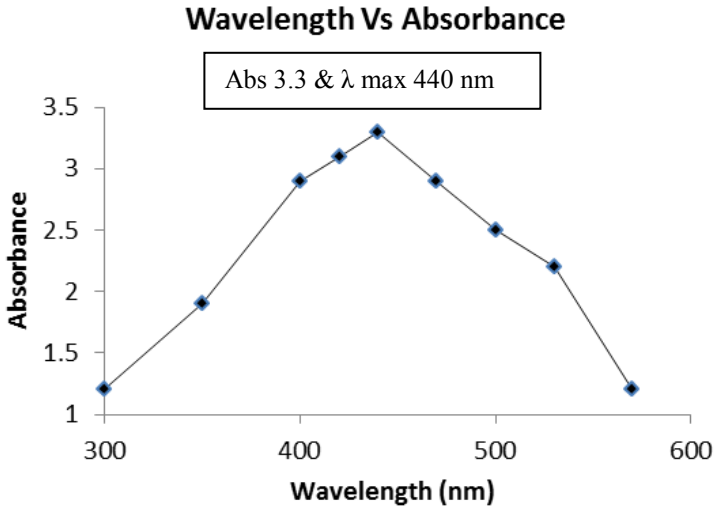


Figure 5 UV absorption reductions of silver ions to silver nanoparticles after heating at 80 °C for 30 min

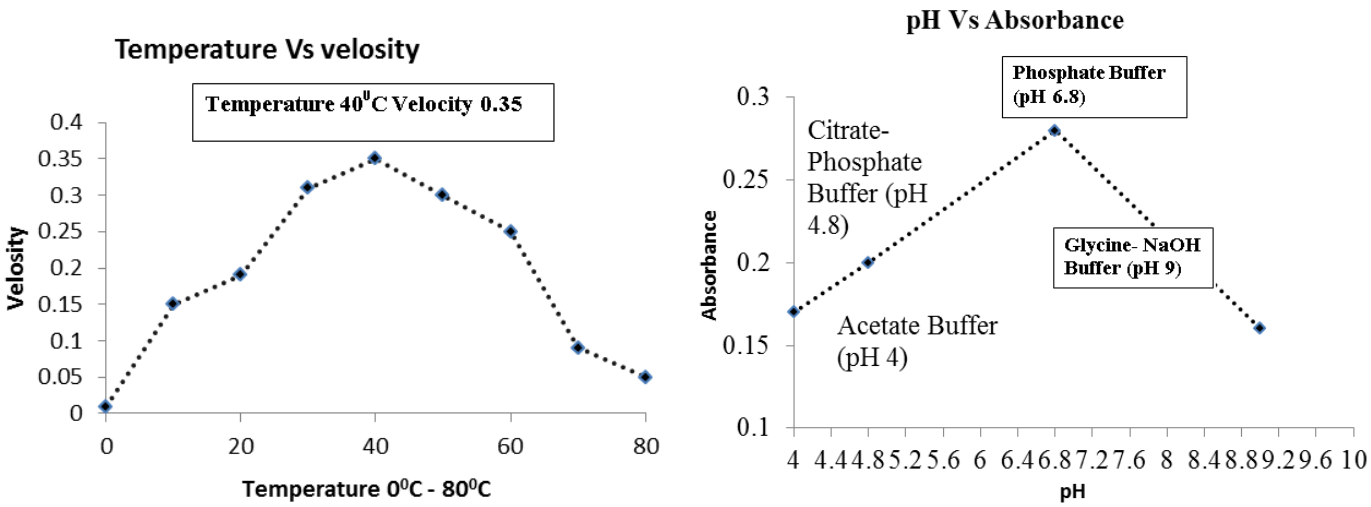


Figure 6 Change in behaviour of lantana silver particles at different temperature and pH

Conclusion

The plant leaves contain a number of medicinally important compounds. Tripene was separated from *Lantana camara to study* chemical nature in kinetics parameters. Study has precise and reproducible evaluation enhanced manifold for its trade in industry, in scientific research as one of the precursor.

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