WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 7, Issue 2, 771-782 Research Article ISSN 2278 – 4357

PHARMACOGNOSTIC, PHYSIOCHEMICAL AND PHYTOCHEMICAL STUDIES WITH TLC PROFILING OF *ALLIUM SATIVUM* IN LOCAL REGION OF RAJASTHAN

Veena Sharma*[1], Kanchan Sharma[1], Preeti Sharma[1] and Shatruhan Sharma[2]

[2]Department of Biotechnology, Banasthali University, Jaipur, Rajasthan.

[2]MAI, Jaipur-12, India.

Article Received on
11 Dec 2017,

Revised on 01 Jan. 2018,
Accepted on 21 Jan. 2018,

DOI: 10.20959/wjpps20182-10808

***Corresponding Author**
Veena Sharma
Professor, Department of Biotechnology, Banasthali University, Jaipur, Rajasthan

ABSTRACT

Allium sativum (true garlic) belonging to the Liliaceae family has been widely used for therapeutic and flavoring agent. The presence of sulfur compound such as Allin, Ajoene, Allicin, Diallyl sulphide have great potential to form beneficial activity against various diseases. Our aim of the present study, to perform detailed macroscopic and microscopic analysis which has been carried out by transfer section and powder of *Allium sativum* using MOTIC Digital Microscope. After these evaluations the extraction of *Allium sativum* (bulb) has been carried out by using different extraction methods. The plant extracts were subjected to various phytochemical screening for the identification of bioactive compounds. The identification of phytochemicals was followed by the separation using TLC profiling. The overall aim of the study will contribute to give the important data for identification and separation of appropriate crude drug.

KEYWORDS: *Allium sativum*, Macroscopic and microscopic studies, Phytochemical screening, TLC profiling.

INTRODUCTION

Medicinal plants are very important in traditional medicines and play a key role for the treatment of various diseases. Pharmacognostic and Phytochemical studies include the stepwise processes for the identification and standardization of the plant material. It is the study of physical, chemical, biochemical and biological properties of drug found in nature as well as the search of new drug from natural origin. [1,2]

Phytochemicals are naturally found in the medicinal plants, such as in leaves, vegetables and roots that have protective role against various diseases. Phytochemicals are primary and secondary constituents. Chlorophyll, proteins and sugars are included in essential constituents (primary and secondary) which contain terpenoid, alkaloids and phenolic compounds.[3]

The plants of liliaceae family are known for their essential constituents. Many active components have been investigated in this family. *Allium sativum* known as garlic belonging to the family of liliaceae. Sulfur compounds are reported to be find in highest quantity and are most important constituents of *Allium*. Different parts of garlic such as bulbs, bulblets, flower bulblets, flowers, and leaves are considered as a chief source of medicine.[4]

The main objectives of this study is to evaluate the pharmacognostical, physicochemical phytochemical characteristics and TLC profiling of *Allium sativum*.

MATERIAL AND METHODS

Plant Material: The fresh bulb of experimental plant i.e. *Allium sativum* was collected from the local region of Banasthali Vidyapith, Rajasthan (India). For the study of macro-morphology, fresh garlic bulbs were used and in microscopic, physiochemical, phytochemical characterization dried bulb powder was used and TLC profiling was evaluated after phytochemical screening of bulb powder.

MACROMORPHOLOGY AND MICROSCOPIC STUDIES

Fresh bulb of *Allium sativum* was taken for transfer section (T.S) and microscopic study which was done by various staining methods and after that observable features were captured by Motic digital microscope provided with Motic Image plus 2.0 software at 10X magnification. [5,6,7]

PHYSICOCHEMICAL EVALUATION: Various physiochemical properties such as moisture content, total ash content, foreign organic matter, acid insoluble, water and alcohol extractive values, pH determination and water soluble content were analyzed, followed the WHO guidelines of quality control methods for medicinal plants analysis.[8,9]

FLORESCENCE ANALYSIS: The florescence characters of plant powder were evaluated in daylight as well as in UV light by giving the treatment of various chemical reagents such as nitric acid, sodium hydroxide, picric acid, acetic acid, aniline, hydrochloric acid, and different solvents such as petroleum ether, chloroform, ethyl acetate, methanol etc.[7,8]

PHYTOCHEMICAL INVESTIGATIONS

Preparation of extract- Different extract of *Allium sativum* were prepared by using different extraction techniques and solvent concentration.[7]

I- By Soxhlet

Hydro-ethanol Extract- The collected bulbs of *Allium sativum* were washed well, shade dried and powdered. They were then extracted with 85% hydro-ethanolic solvent using soxhlet apparatus.

II- By Maceration

Hydro-ethanol Extract- In the method of maceration, 100 gram garlic powder was dissolved in 85% hydro-ethanol and kept for 48 hours in dark.

Aqueous Extract- In this method 100 gram garlic powder was dissolved in 400ml distilled water and kept for 48 hours in dark. Few drops of chloroform was also added to avoid any contamination.

Phytochemical tests: - Various qualitative chemical tests of *Allium sativum* extract was also carried out to detect the presence or absence of secondary metabolites. These phytochemicals has various medicinal properties which are able to fight against many serious diseases.

Table 1: Showing Preliminary Phytochemical Tests.

ALKALOID	TANNINS	CARDIACGLYCOSIDE	STERIODS	FLAVONOIDS
1- MAYER'S TEST 1 ml extract + few drops Mayer's reagent (Potassium mercuric iodine solution) – **cream color precipitate** **2- DRAGENDORFF'S TEST** Few drops extract + 1-2 ml of dragendorff's reagent - **Red color precipitate**	**1- BRAYMER'S TEST** 2ml extract + 2ml H_2O + 2-3 drops $FeCl_3$ (5%) – **green precipitate** **2- VANILLIN HYDROCHLORIDE TEST** 1ml extract + 1ml of vanillin hydrochloride solution -**purple red color**	**1- SALKOWSKI TEST** 2ml extract + 2ml $CHCl_3$ + 2ml $H2SO_4$ (conc.) - **Reddish brown ring** **2- KELLER-KILLIANI TEST** 1mlextract + 4 ml glacial acetic acid + few drops of ferric chloride and conc sulfuric acid (2 ml) - **brown ring**	**1- LIEBERMANN'S TEST** 2ml extract + 2ml $CHCl_3$ + 2ml CH3COOH - **Violet to Blue to Green color**	**1- ALKALINE REAGENT TEST** 1ml extract + few drops NaOH solution -intense yellow color, which turns to colorless on addition of few drops of diluted acid **2- LEAD ACETATE TEST** 1ml extract + 1ml $Pb(OAc)_4$ (10%) - **Yellow coloration**

TERPINOIDS	PROTEINS	REDUCING SUGAR	SAPONINS	CARBOHYDRATES
SALKOWSKI TEST 1ml extract + 2 ml of chloroform and 1 ml H_2So_4 - **reddish brown color**	**NINHYDRIN TEST** 1ml extract + 1 ml of 0.2 % ninhydrin solution. **- violet color**	**FEHLING'S TEST** Filtrate (1 ml) + 1ml each of Fehling solution A & Fehling solution B + boiled **- a colored product, indicates the presence of sugar.**	**FOAM TEST** 0.5ml extract + 5ml H_2O + shake **-foam produced persist for 10 min**	**MOLISH TEST** 2ml extract + 10ml H_2O + 2 drops Ethanolic α-naphthol (20%) +2ml H_2SO4 (conc.) **-Reddish violet ring at the junction**

TLC PROFILING

TLC profiling was carried out to confirm the presence of bioactive compounds in the extract. On the basis of phytochemical investigations hydro-ethanol extract of *Allium sativum* was selected to perform TLC profiling for bioactive compound identification. Silica plates were used as a stationary phase. For the preparation of TLC plate silica gel slurry are used then plates were air dried and kept in hot air oven at 80^0C for 5 minutes. For the mobile phase different solvents on the basis of their polarity were used. These were I-Pet ether:Ethyl acetate:Methanol, and II- Acetone:Ethyle acetate:Methanol.

Table 2: Showing the Solvent Ratio of Mobile Phase.

SN	MOBILE PHASE	RATIO
1	ETHYLE ACETATE: METHANOL	7:2, 6.5:3,6:3 5:4
3	PET ETHER: ETHYLE ACETATE	8:2 ,7:3
4	PET ETHER: ETHYLE ACETATE	7.5:2.5

Glass capillaries were used for loading sample on to the silica plate. Spot was above 2 cm from bottom of plates then the plates were placed vertically in the glass tank covered by glass plate. For the detection of particular compound present in the TLC plate, different reagents and iodine chamber were used. The movement of compound and its distance was calculated by retention factor Rf.[10]

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

RESULTS

Macromorphological and Microscopic studies

All true garlic's are perennial herb. Marco-morphologically garlic has erected pink or purple flowering stem with 2-3 feet height. The medicinal part of the plant is bulb which has diameter about 4-6 cm and containing 8-20 cloves. One whitish papery membranous and

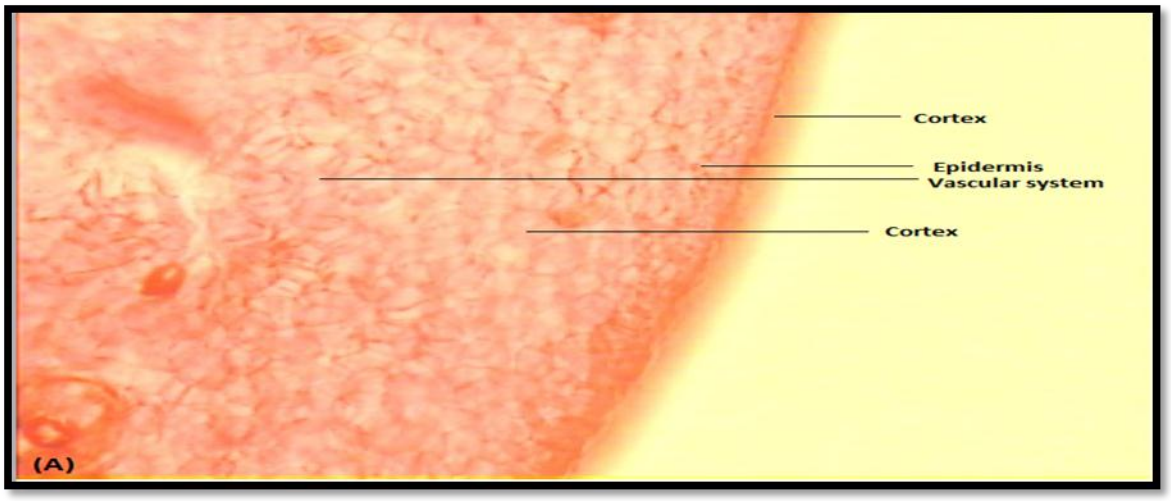
brittle scales (2-3) are present around the cloves attached to one woody stem. Cloves are irregular ovoid with dorsal convex surface, long range 2-3 cm and width 0.4- 0.7 mm. Powdered form of *Allium sativum was* yellow in color with characteristic smell and taste as shown in table 3.

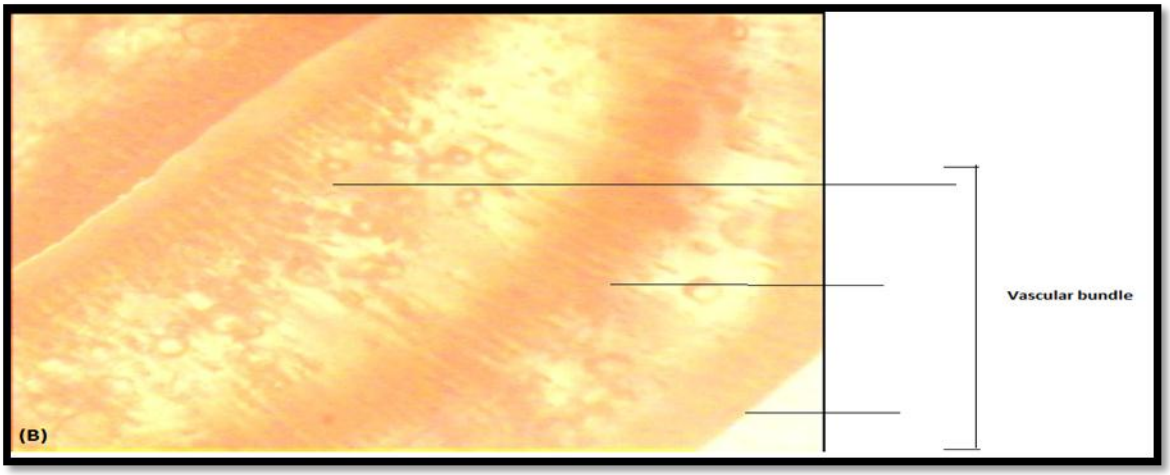
Table 3: Macromorphological Characters of *Allium Sativum*.

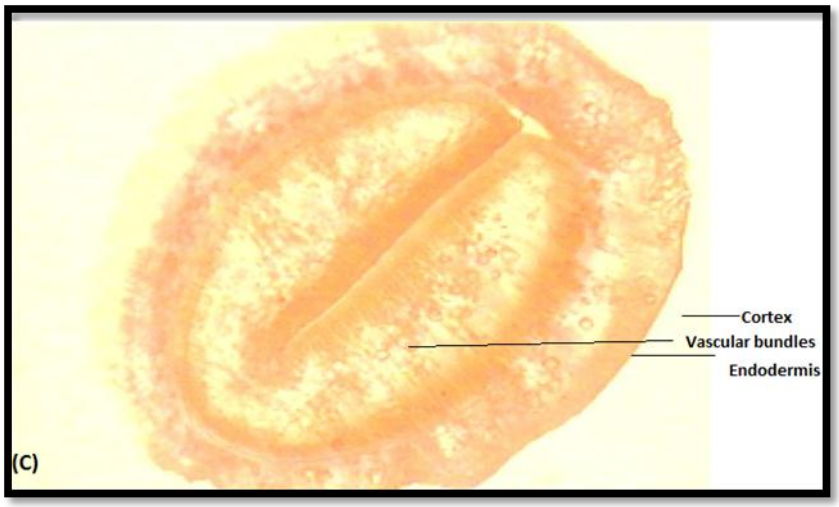
Sr.no	MACROMORPHOLOGICAL CHARACTERS	OBSERVED CHARACTERS
1.	Color	Light yellow, off white
2.	Odor & taste	Characteristics pungent
3.	Size ▪ Length ▪ Width	 2-3 cm 0.4-0.7 mm
4.	Shape	Ovoid

The observable characteristics of T.S section of garlic bulb contains cuticle, epidermis, cortex, endodermis and bicollateral vascular bundles arranged in a ring and scattered below endodermis. The parechymatous cells are found in cortex region (fig A, B, C).

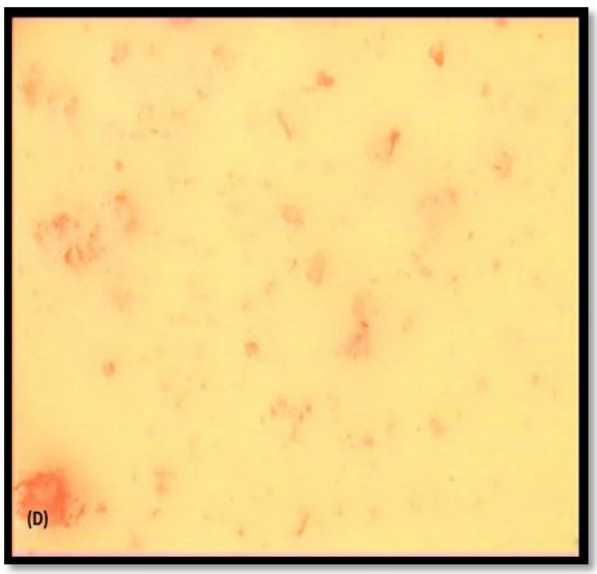
Microscopic evaluations of powder of *Allium sativum* showed the presence of xylem vessels, Lignified fibers, trichomes and calcium oxalate crystals, fragments of cortex cells and vascular bundles (fig D, E, F).







Figures: (A),(B) and (C) T.S of *Allium sativum*.



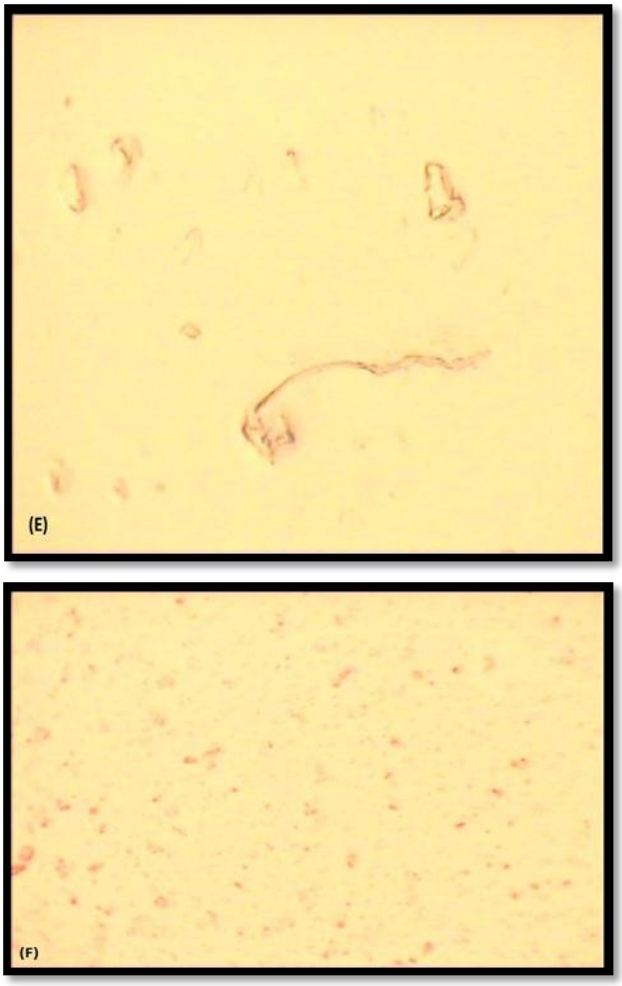


Figure: Microscopic studies of powder of *Allium sativum* (D) Xylem vessels, (E) lignified fibers and (F) trichomes and calcium oxalate crystals.

PHYSICOCHEMICAL EVALUATION

Physiochemical analysis of *Allium sativum* has done from bulb powder which is depicted in table no 4.

Table 4: Results are in the mean ± S.D. and has carried out in triplicate (*n*=3).

Sr. no	PARAMETERS STUDIES	Values (%) w/v
1	Foreign matter analysis	0.04 ± 0.005
2	Moisture content	0.93± 0.001
3	Water soluble extractive value	31.58± 0.02
4	Alcohol soluble extractive value	8.26± 0.04
5	pH	6.9± 0.1
6	Total ash content	1.68± 0.01
7	Acid soluble ash content	0.36± 0.01
8	Water soluble ash content	0.52±0.02

FLORESCENCE ANALYSIS: Light with short wavelength is more active to produce fluorescence and that's why UV light produces fluorescence in the presence of many chemicals that fluoresce cannot visualized in day light. This phenomenon formed by many phyto-constituents present in the plant material.

Table 5: Showing the Fluorescence Analysis Exhibited by Phytochemicals.

Sr.no.	Reagents	Ordinary light	260 nm (short UV)	366 nm (long UV)
1.	Conc. HCL	Light blue	Pale yellow	Greenish yellow
2.	Conc. HNO_3	Pale yellow	Light green	Dark green
3.	Conc. H_2SO_4	Dark yellow	Brown	Dark brown
4.	Acetic acid	White	No color	No color
5.	Aniline	Red	Brown	Dark brown
6.	Picric acid	Pale yellow	Yellow	Greenish yellow
7.	Dil. Ammonium solution	No color	Cream	White
8.	5% ferric chloride	Light yellow	Green	Greenish yellow
9.	10% NaOH	Cream	Pale yellow	Yellow
10.	Pet ether	Pale yellow	Yellow	Greenish yellow
11.	Chloroform	No color	Cream	Pale yellow
12.	Ethyle acetate	No color	Pale yellow	Pale yellow
13.	Methanol	Light blue	Bluish green	Green
14.	water	No color	Pale yellow	Yellow

Table 6: Average Yield of Plant Extract.

SN	Solvents	Color and nature of extract	Average yield (g/100g of plant powder)
1	Hydro-ethanol (Soxhlet)	Dark brown, sticky	20.69 $\pm$ 0.01
2	Aqueous (Meceration)	Reddish brown, sticky	31.58 $\pm$ 0.02
3	Hydro-ethanol (Meceration)	Dark yellow, stick	16.43 $\pm$ 0.07

PHYTOCHEMICAL SCREENING

Phytochemical screening of *Allium sativum* was done successfully. The aqueous, hydroethanol (Macerated) and hydroethanolic (Soxhlet) extract of *Allium sativum* showed positive result of phytochemicals tests. Alkaloids, flavonoids, terpenoids, saponins cardiac glycosides, protein, reducing sugar and carbohydrates are positively present in all extracts with high, moderate and in trace amount. According to this study, hydroethanolic extract of garlic bulb was analyzed and gave better results than others. This can be happened due to better extraction capacity of soxhlet and slightly polar solvent.

Table 7: Showing the Phytochemical Qualitative Analysis.

S. N	PHYTOCHEMICALS	TESTS	EXTRACT BY SOXHLET	EXTRACT BY MECERATION	
			HYDROETHANOL	AQUEOUS	HYDROETHANOL
1	ALKALOIDS	Mayer's test Dragendorff's test	+ +++	++ ++	+ +
2	TANNINS	Braymer's test Vanillin hydrochloride test	– –	– –	– –
3	SAPONINS	Foam test	++	+	+
4	CARDIAC GLYCOSIDE	Salkowski test Keller-killiani test	+++ +++	+ +	++ ++
5	FLAVONOIDS	Alkaline reagent test Lead acetate test	++ ++	+ +	+ +
6	TERPENOIDS	Salkowski test	+++	++	++
7	PROTEINS	Ninhydrin test	++	+	+
8	REDUCING SUGAR	Fehling's test	_	_	_
9	CARBOHYDRATE	Molish test	+++	++	++

+ = LOW AMOUNT, ++ = MODERATE AMOUNT, +++ = HIGH AMOUNT

TLC PROFILING

TLC profiling of hydroethanol extract has been carried out to achieve some chromatographic studies to confirm the presence of active components in *Allium sativum*. TLC profiling shows different spots with different Rf values.

(A)  **(B)** 

Fig: TLC profiling of *Allium sativum* hydroethanol extract. (A) Solvent system 1 [Ethyle acetate+ Methanol (6.5:3, 7:2) in iodine and spray derivatization (B)].

DISCUSSION

According to pharmacological as well as pathological discoveries, the phytochemicals are essential compounds in plants. The Phytochemical was performed various mechanisms in the medicinal plant such as protecting from oxidative stress, prevent the risk of certain deleterious diseases and possesses antibacterial, anti-fungal properties.[11,12,13] It control and regulate hormonal action, and stimulate important enzymes to keep balance in hormonal level e.g estrogen. Saponins are found to interfere with the DNA replication and protect the cell to get cancerous. It also has some anti- adhesion property. *Allium sativum* possesses various phytochemicals confirmed by some screening which showed it's therapeutic and antioxidant activity of scavenging free radical. In the present study we evaluated the pharmacognostical, physicochemical, phytochemical analysis and TLC profiling of *Allium sativum*. The macromorphology and microscopy with fluorescence and physiochemical analysis are the easiest way to determine the plant characteristics such as their structure and chemical nature for identifying the authenticity of desire plant. [14,15]

The phytochemical analysis was conduct to identify the presence of phytochemicals in three extracts of *Allium sativum* i.e. hydro-ethanol (soxhlet) aqueous and hydro-ethanol (maceration). The comparative phytochemical analysis of all these three extracts showed the positive results with high, moderate and low amounts of phytochemicals. The hydro-ethanol (soxhlet) extract showed appreciable results leading to the TLC profiling. TLC profiling of hydroethanol extract of *Allium sativum* determined the separation of phytochemicals. The separation of phytochemicals in the TLC plate gives different Rf values with different mobile phase. Difference in the Rf values depend upon the polarity of compound so that the solvent system which analyzed appropriate is used for the further isolation from the plant extract. Rf value is very important factor to decide the appropriate mobile phase for the separation of single compound by column chromatography.[16,17]

CONCLUSION

After these investigations and results obtained in the present study *Allium sativum* possesses bioactive compounds and as a high source of secondary metabolites. *Allium sativum* identified as a great potential of natural antioxidant that inhibit oxidative stress and various diseases. The alkaloids, flavonoids, tannins, terpenoids, saponins, phenol, cardiac glycoside, reducing sugar are all phytochemicals having a great importance to produce various antibiotics for the cure of dangerous diseases. The extraction, identification and separation of

bioactive compounds would be proven as main priority for the further pharmacological studies.

ACKNOWLEDGMENTS

The authors acknowledge the cooperation and support of Department of Bioscience and Biotechnology and Department of Pharmacy, Banasthali vidyapith to finish the experiments of research work.

REFERENCES

1. Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol, 2000; 30: 379-384.
2. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev, 2007; 1: 97-104.
3. Abdul Wadood, Mehreen Ghufran, Syed Babar Jamal, Muhammad Naeem, Ajmal Khan, Rukhsana Ghaffar and Asnad. Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Journal of Biochemistry & Analytical Biochemistry, 2013; 2.
4. Rekha Jadon, Shruti Dixit. Phytochemical extraction and antimicrobial activity of some medicinal plants on different microbial strains. Journal of Medicinal Plants Studies, 2014; 2: 58-63.
5. Kadam PV, Yadav KN, Deoda RS, Navasare VS, Shivatare RS and Patil MJ. Pharmacognostical evaluation of root of *Alipinia galaga willd*. International Journal of Pharmacy, 2012; 2(2): 426–431.
6. Kadam PV, Yadav KN, Patel AN, Navasare VS, Bhilwade SK and Patil MJ: Phytopharmacopoeial specifications of *Garcinia indica* fruit rind. Phcog J, 2012; 4(31): 23-28.
7. Kadam PV, Deoda RS, Shivatare RS, Yadav KN and Patil MJ: Pharmacognostic, phytochemical and physicochemical studies of *Mimusops elengi linn* stem bark (sapotaceae). Scholar Research Library, 2012; 4(2): 607-613.
8. Anonymous, World Health Organization (WHO), Quality control methods for medicinal plant material. Geneva, 1998.
9. Kadam PV, Yadav KN, Narappanawar NS, Shivatare RS, Bhusnur HU and Patil MJ. Development of Quality standards of *Terminalia catappa* leaves. Phcog J, 2011; 3(26): 19-24.

10. Stahl E. Thin layer chromatography: a laboratory handbook. Springer, Berlin, Heidelberg, 2007.
11. Khandelwal KR. Practical pharmacognosy. Nirali Prakashan, Pune, 2002.
12. Snowdon DW, Janckson. Atlas of microscopy of medicinal plants, culinary herbs and spices. CBS Publishers and Distributors, New Delhi, 2005.
13. Anonymous. Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, 1998.
14. Joseph L, George M. Pharmacognostical profiling of Geranium ocellatum leaves. Int J Med Arom Plants, 2011; 1(3):351–354.
15. Babu K, Shankar SG, Rai S. Comparative pharmacognostic studies on the barks of four Ficus species. Turk J Bot, 2010; 34(3): 215-224.
16. Kokate CK, Purohit AP, Gokhale SB. Pathway to screen phytochemical nature of natural drugs. In: Pharmacognosy. Nirali Publication, Pune, 2008.
17. Karthika K, Jamuna S, Paulsamy S. TLC and HPTLC fingerprint profiles of different bioactive compounds from the tuber of Solena amplexicaulis. J Pharmacogn Phytochem, 2014; 3(1): 198–206.