

Phytochemical and Antimicrobial Screening of Drumstick Leaves Extracts against Enteric Pathogens

Bansode D. S¹, Chavan M. D²

Department of Microbiology, Walchand College of Arts and Science, Solapur (M.S.), 413006-India

Abstract: The aim of the present study was to determine the antimicrobial activity and phytochemical screening of Drumstick (*Moringa oleifera*) leaves extracts. Four solvents were used for extraction. Those were Ethanol, Methanol, Diethyl ether and Acetone. The solvent extraction was done by using Soxhlet apparatus. Test microorganisms were screened to confirm their viability and identities using standard microbiological methods. The different solvent extracts of Drumstick leaves were tested for antimicrobial activity using the standard agar well diffusion method against nine enteric pathogens, these are *E.coli*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Shigella sonnei*, *Shigella dysenteriae*, *Enterobacter spp.*, *Citrobacter spp.* and *Klebsiella spp.* The Ethanol extract of Drumstick leaves showed highest antimicrobial activity against *Sal.typhi*. The Methanol extract of Drumstick leaves showed highest antimicrobial activity against *Citrobacter spp.* The Diethyl ether extract of Drumstick leaves showed highest antimicrobial activity against *Shigella dysenteriae* while Acetone extract of Drumstick leaves showed highest antimicrobial activity against *E.coli*. The antimicrobial activity of standard antibiotics Ampicillin and Tetracycline were studied in comparison with Drumstick leaves solvent extracts. The MIC values were determined by both agar and broth dilution method. The functional chemical group was determined by Fourier Transform Infrared Spectroscopy (FTIR). The phytochemical analysis of Drumstick leaves solvent extracts showed presence of Alkaloid, Flavonoid, Glycosides, Tanin and Phenolic compounds, Terpenoid etc.

Keywords: Drumstick (*Moringa oleifera*), Phytochemical screening, Enteric pathogens, MIC, FTIR etc.

1. Introduction

Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products. Increased vegetable utilization and consumption are critical to alleviate world-wide incidence of nutritional deficiencies. Drumstick is one of the promising plants which could contribute to increased intake of some essential nutrients and health-promoting phytochemicals. Phytochemicals are present in virtually all of the fruits, vegetables, legumes (beans and peas), and grains we eat, so it is quite easy for most people to include them in their diet. *Moringa oleifera*, or the horseradish tree, is a pan-tropical species that is known by such regional names as benzolive, drumstick tree, kelor, marango, saijhan, and sajna. Drumstick is an aboriginal of Indian subcontinent and has become naturalized in the tropical and subtropical areas around the world. Nearly thirteen species of Drumstick are included in the family Moringaceae. Indians have been using it as a regular component of conventional eatables. Drumstick tree can grow well in the humid tropic or hot dry land with average height that ranges from 5 to 10 m. It can survive in harsh climatic condition including destitute soil without being much affected by drought.

Drumstick is a growing fast, drought-tolerant, and must be cut back several times to make it branch out more. It will readily sprout again and all the valuable products will remain within safe. It seems to thrive in impossible places and never dies. It can be developed easily from seeds or cuttings, compost or manure are not necessary. It can be densely seeded with high yielding. The light shade of the tree is a considerable help to most vegetables. Flowering can be induced through small watering to have a nearly continuous yield. Different parts of this plant contain a sketch of important minerals, and are a good source of protein, various phenolics, vitamins, β – carotene and amino acids.

The Drumstick plant offers a rich and exceptional combination of zeatin, kaempferol, quercetin and many other phytochemicals. It is very significant for its medicinal value. Numerous parts of the plant such as the roots, seed, bark, leaves, fruit, and immature pods, flowers act as cardiac and circulatory drugs, antipyretic, antiulcer, anti-inflammatory, antiepileptic. Other chief medicinal properties of the plant include antispasmodic, diuretic, antihypertensive, cholesterol lowering, hepatoprotective, antioxidant, antidiabetic, antibacterial and antifungal activities. Drumstick parts are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia.

In addition, Drumstick seeds possess water purifying powers by flocculating Gram positive and Gram negative bacterial cells. Drumstick seeds can also be used as a less expensive bio absorbent for the removal of heavy metals. In traditional Indian medicine various parts of the tree are used therapeutically for treatment of venomous bites, ascites and

rheumatism and helps in lowering blood pressure. The root and bark of young trees are considered rubefacient, stomachic carminative, vesicant and abortifacient. The flowers and roots contain an antibiotic that is highly effective in the treatment of Cholera.

As the global scenario is now changing towards the use of non-toxic and eco-friendly products, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases. *Moringa oleifera* (Drumstick) is one such plant which is reported to possess several medicinal properties. The different parts of this plant viz. leaves, stem bark, root bark, flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments and some parts are also eaten as vegetable.

2. Literature Survey

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries [1]. For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties [2]. Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of complementary and alternative medicine is now increasing in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections. [3]- [4].

Drumstick (*Moringa oleifera*) is native to the western and sub Himalayan region, India, Pakistan, Asia Minor, Africa and Arabia [5]. The Drumstick tree is cultivated and used as a vegetable (leaves, pods flowers, roasted seeds), for spice (mainly roots), cooking and cosmetics oil (seeds) and as a medicinal plant (all plant organs) [6]. Important medicinal properties of the plant include antipyretic, antiepileptic, anti-inflammatory, antiulcerative [7] antihypertensive [8], cholesterol lowering [9], and antioxidant [10].

In addition, Drumstick seed possesses water purifying powers [11]-[12]. Drumstick is a highly valued plant, distributed in many countries of the tropics and subtropics. It has impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and a good source of protein, vitamin, β carotene, amino acids and various phenolics [13].

Drumstick is a well documented world renowned plant herb for its extraordinary nutritional and medicinal properties. It is a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder and is used in many countries to treat malnutrition and malaria.

Drumstick leaves contain phytochemical having potent anticancer and hypotensive activity and are considered full of medicinal properties and used in siddha medicine [14]. The whole Drumstick plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, the aqueous extracts of roots and barks were found to be effective in preventing implantation, aqueous extracts of fruits have shown significant anti-inflammatory activity, methanolic extracts of leaves have shown antiulcer activity and ethanolic extracts of seeds exhibited anti-tumour activity [15].

Drumstick is used as drug by many ayurvedic practitioners for the treatment of asthma and evaluate the anthelmintic activity of methanolic extract of Drumstick in adult Indian earthworms *pheretima posithuma* at different doses [16]. The different parts of this plant such as the leaves, roots, seeds, barks, flowers, and immature pods contain a quantum of crucial phytoconstituents such as tannins, saponins, alkaloids, steroidal aglycones, reducing sugars, terpenoids, and so on, that act as a cardiac and circulatory stimulants, possess antitumour, antipyretic, anticonvulsant, anti-inflammatory, antiulcer, antispasmodic, antidiabetic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, antifungal, abortifacient, antibacterial, [17]-[19], antiretroviral, antispeticemic, antidiarrhoeal, hepatorenal disorders, cardiovascular, gastrointestinal and haematological disorders [20], anxiety, asthma, bronchitis, cough, diarrhoea, conjunctivitis, cephalgia, arthralgia, psoriasis, semen deficiency, helminthiasis, lactation, pregnancy and diabetes [21], and they are being employed for the treatment of different ailments in the indigenous system of medicine [22]-[24].

3. Problem Definition

The continuous spread of multidrug-resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms.

Multi-drug resistant strains are widely distributed in hospitals and are increasingly being isolated from community acquired infections. All this has resulted in severe consequences including increased treatment failure and health care cost. This has urged the microbiologists all over the world to formulate new antimicrobial agents and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents.

Enteric bacteria belongs to the family enterobacteriaceae, which are large group of Gram negative, peritrichously flagellated or non-flagellated straight rods with simple nutritional requirements. They grow best under aerobic condition but also ferment carbohydrates by an anaerobic pathway. Enterobacteria include coliforms like *E. coli* and other Gram-negative enteric flora, slow lactose fermenting bacteria which could be either normal flora or regular pathogens also forms part of the enteric pathogens.

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections. MRSA infections are very difficult to cure because MRSA strains are resistance against almost all clinically available antibiotics. Enteric Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases. The worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and many other β -lactamase producers has become a major therapeutic problem.

Medicinal plants are the boon of nature to cure a number of ailments of human beings. In many parts of the world medicinal plants are used against bacterial, viral and fungal infections. Evaluation of plants bearing efficiency in healing various diseases is growing in recent years. Innumerable biologically active compounds of plants are found to possess antibacterial properties. Practitioners of Ayurveda and Unani system of medicine regularly employ a large number of Indian medicinal plants as antibiotic agents and over the last 40 years, intensive efforts have been made to discover clinically used herbal antibacterial and antifungal drugs.

4. Materials and Methods

4.1 Collection of Plant Material

Healthy disease free, indigenously grown mature leaves of Drumstick was collected from local area of Solapur (M.S.). The identification of plant material was confirmed by a Botanist in the Dept. of Botany, Walchand College of Arts and Science, Solapur (M.S.).

4.2 Test Pathogens

Nine strains of Enteric pathogenic bacterial cultures were used in this study. These are *E.coli*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Shigella dysenteriae*, *Shigella sonnei*, *Enterobacter spp.*, *Citrobacter spp.* and *Klebsiella spp.* The pure pathogenic bacterial strains were collected from Dept. of Microbiology, V.M. Govt. Medical College, Solapur (M.S.). The collected pure pathogenic bacterial strains were isolated from hospitalized patients at Govt. Civil Hospital, Solapur (M.S.) The cultures were maintained on nutrient agar slants at 4°C and subcultured for 24hr. before use.

4.3 Preparation of Solvent Extracts

Thoroughly washed mature leaves were shade dried and then powdered with the help of electric blender. Twenty five grams of the powder was filled in the thimble and extracted successively with Ethanol, Methanol, Diethyl ether and Acetone using a Soxhlet extractor for 48hr. All the extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. All the extracts were subjected to antibacterial activity assay and phytochemical analysis.

4.4 Antibacterial Activity Assay

Antimicrobial activity of the Drumstick leaves solvent extracts was determined by agar well diffusion method on Muller- Hinton agar medium [25]. Wells were made on Muller- Hinton agar plates using cork borer and inoculum containing 10⁶ CFU/ml of pathogenic bacteria were spread on the solid agar plate with the help of sterile glass rod. Plant extracts were dissolved in 100% dimethylsulphoxide (DMSO) for antimicrobial study. Then 100ul of solvent extract was placed in the cups made in inoculated plates. All the plates were incubated for 24hr. at 37°C. and after incubation period zone of inhibition was measured in mm. Antimicrobial activity of Standard antibiotics Ampicillin and Tetracycline were observed in comparison with Drumstick leaves solvent extracts.

4.5 Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined by both agar and broth dilution methods [26]. For broth dilution tests, 0.1ml of standardized suspension of bacteria (10⁶ CFU/ml) was added to each tube containing different concentrations of solvent extracts (05-50ul/ml) and incubated for 24hr at 37°C. In agar plating method dilutions having 05-50ul of solvent extracts was placed in the cups on the inoculated plates and incubated as mentioned above.

4.6 Phytochemical Analysis

Qualitative Phytochemical Analysis

The Drumstick leaves solvent extracts was tested for the presence of bioactive compounds by using standard method [27].

4.7 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to identify the characteristic functional group in the crude Drumstick leaves powder. A small quantity (5mg) of the powder was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min. to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 4000 to 400cm⁻¹ for 16 times to increase the signal to noise ratio.

5. Results and Discussion

In the present study significant antibacterial activity is observed by all solvent extracts of Drumstick leaves. The results of antimicrobial activity of Drumstick leaves solvent extracts were represented in table 1. The Ethanol extract of Drumstick leaves showed highest antimicrobial activity against *Sal.typhi*. The Methanol extract of Drumstick leaves showed highest antimicrobial activity against *Citrobacter spp.* The Diethyl ether extract of Drumstick leaves showed highest antimicrobial activity against *Shigella dysenteriae* while Acetone extract showed highest antimicrobial activity against *E.coli*. The antimicrobial activity of standard antibiotic Ampicillin were found to be maximum against

Citrobactor spp. while standard antibiotic Tetracycline showed highest antibacterial activity against *Sal.para.A*.

Minimum inhibitory concentration (MIC) of the different Drumstick leaves solvent extracts varied against different test pathogens. The MIC of Drumstick leaves solvent extracts required for test pathogens were represented in table 2. Lowest MIC of 5ul was observed against *Shigella dysenteriae* by Ethanol extract of Drumstick leaves while highest MIC of 50ul was observed against *Salmonella paratyphi B*. Lowest MIC of 10ul was observed against *Citrobactor spp.* by Methanol extract of Drumstick leaves while highest MIC of 45ul was observed against *Enterobactor spp.* Lowest MIC of 5ul was observed against *Sal.typhi* by Diethyl ether extract of Drumstick leaves while highest MIC of 45ul was observed against *E.coli*. Lowest MIC of 10ul was observed against *Sal.para.B*. by Acetone

extract of Drumstick leaves while highest MIC of 45ul was observed against *Sal.typhi*.

The aim of FTIR analysis is to determine the existence of functional group that exists on isolate. The IR spectrum of the crude powder of Drumstick leaves in the form of Kbr pallet is shown in fig 1.

The absorption at 3347cm^{-1} is due to N-H stretching vibration. The bond at 2918cm^{-1} is due to C-H stretching. The absorption at 2849cm^{-1} is due to C-H stretching vibration. The absorption at 1651cm^{-1} is due to C=C stretching. The absorption at 1416cm^{-1} is due to active α -methylene group. The bond at 2918cm^{-1} is due to bending vibration of C-H bonds of methyl group. The results of phytochemical analysis of various solvent extracts of Drumstick leaves were represented in table 3. The phytochemical analysis showed presence of Alkaloid, Flavonoid, Glycosides, Phenolic compounds, Terpenoid etc.

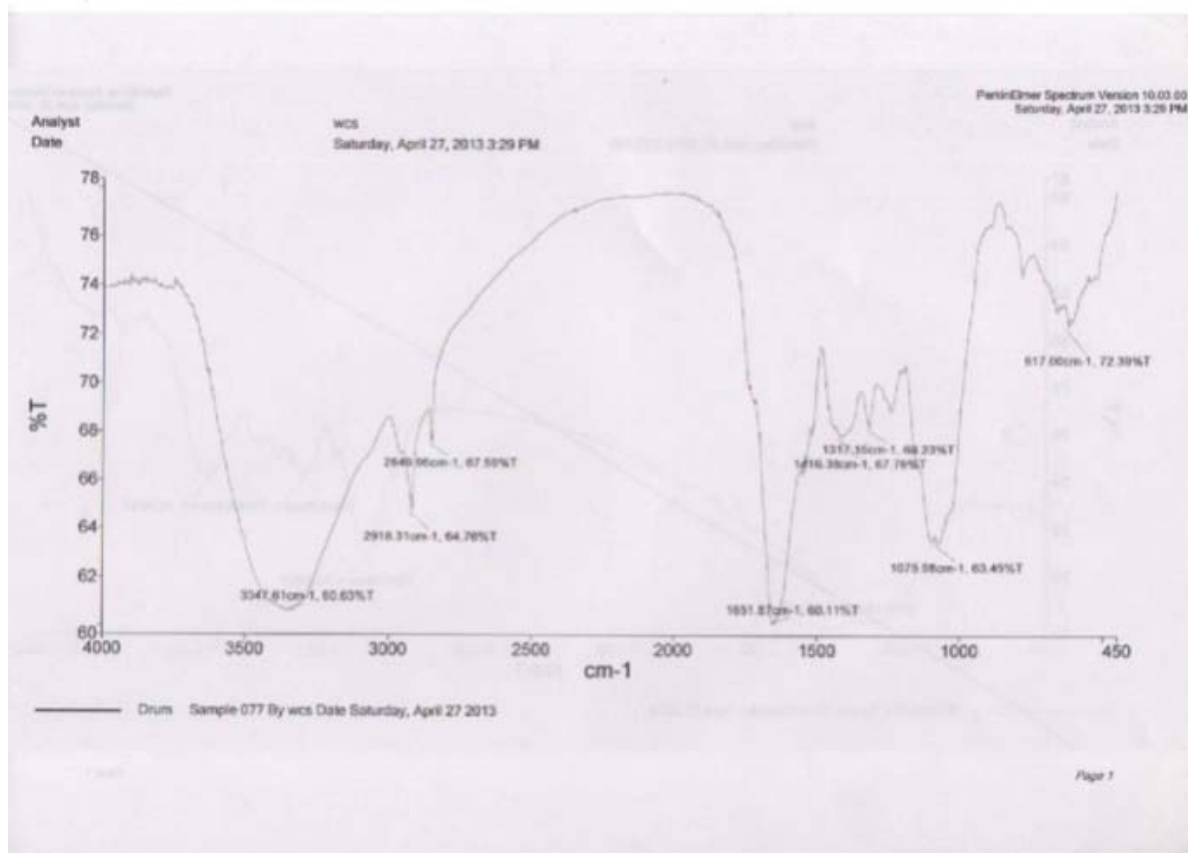


Figure 1: Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Drumstick Leaves.

Table 1: Antibacterial Activity of Different Solvent Extracts of Drumstick Leaves Extracts.

M/O	Zone of Inhibition (mm)					
	Ethanol	Methanol	D.E.	Acetone	Ampi	Tetra
<i>E.coli</i>	5	4	3	6	13	14
<i>Sal.typhi</i>	7	4	6	4	14	11
<i>Sal.para A</i>	4	3	5	2	10	15
<i>Sal.para B</i>	3	5	3	5	11	12
<i>Shigella sonnei</i>	4	3	2	2	06	12
<i>Shigella dysenteriae</i>	6	4	7	5	12	11
<i>Enterobactor spp.</i>	5	3	3	4	12	14
<i>Citrobactor spp.</i>	4	6	5	4	15	13
<i>Klebsiella spp.</i>	6	4	5	3	09	11

(Ampi- Ampicillin, Tetra- Tetracycline)

Table 2: MIC of Different Solvent Extracts of Drumstick.

M/O	Minimum Inhibitory Concentration (ul/ml)			
	Ethanol	Methanol	D.E.	Acetone
<i>E.coli</i>	20	20	45	35
<i>Sal.typhi</i>	10	20	05	45
<i>Sal.para A</i>	40	40	20	40
<i>Sal.para B</i>	50	15	40	10
<i>Shigella sonnei</i>	45	40	40	50
<i>Shigella dysenteriae</i>	05	30	10	20
<i>Enterobactor spp.</i>	25	45	30	25
<i>Citrobactor spp.</i>	30	10	15	35
<i>Klebsiella spp.</i>	15	35	15	40

(D.E. - Diethyl ether)

Table 3: Phytochemical Analysis of Drumstick Leaves Solvent Extracts.

Test	Ethanol	Methanol	D.E	Acetone
Alkaloid	+	+	+	+
Flavonoids	+	+	+	+
Tanin	-	-	-	-
Saponins	-	-	-	-
Glycosides	+	+	-	-
Carbohydrate	-	-	+	+
Phytosterol	-	-	-	-
Phenolic Compounds	+	+	+	+
Terpenoid	+	+	-	-

(+=Present, -=Absent)

The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive compounds and these various solvent extracts of Drumstick leaves can be used as potential source of drugs in the treatment or control of intestinal disorders.

Various medicinal plants are valuable and radially available resources for primary health care and complementary healthcare system. Undoubtedly medicinal plants containing substances of medicinal value that has yet to be discovered, though large numbers of plants are constantly being screened for their antimicrobial activity. These plants may prove to be a rich source of compounds with possible antimicrobial activities. Due to several intricacies of modern or the orthodox antibiotics, there has been a significant shift towards alternative therapy and herbal remedies. Antibiotic screening on natural products obtained from Drumstick used in the Complementary and Alternative Medicine is a major thrust of research and development. Therefore, in a bid to discover new antimicrobials that would be effective against enteric pathogens, phytochemical screening and investigations into the antibacterial profile of the various solvent extracts of Drumstick leaves were carried out.

6. Conclusion

The present study suggested that, the various solvent extracts of Drumstick leaves have a great potential as antimicrobial agents against selected enteric pathogens and they can be used as an alternative medicine in the treatment or control of enteric bacterial infections. The antimicrobial activity and MIC assays showed promising evidence for the antimicrobial activity of Drumstick leaves solvent extracts against selected enteric pathogens. Phytochemical analysis showed presence of antimicrobial substances in the studied extracts. The results revealed the presence of medicinally important constituents in these solvent extracts. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, the Drumstick leaves solvent extracts could be seen as a good source for useful drugs.

7. Acknowledgement

The authors are grateful to Head, Dept.of Microbiology, Dr.V.M.Government Medical College, Solapur (M.S.) for provision of pure cultures of Enteric Pathogens.

8. Future Scope

This *in vitro* study demonstrated that naturally available medicines can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants as a medicine suggests that they represent an economic and safe alternative to treat infectious diseases. This study exposed that traditional medicines are still used by peoples and it is established the value of a great number of plants used in tribal medicine. Thus, Drumstick tree offers very interesting opportunities as food supplement, nutrition, vegetable, oil, water treatment, green manure, foliar spray, natural fertilizer, livestock feed, fodder, medicine, cosmetic and care products.

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Author Profile

Bansode D.S. (Research student): Completed M.Sc. in Microbiology from S.R.T.M.U. Nanded in 2009. Worked as F.D.A. (Maharashtra Government) Approved Microbiologist in Quality Control Department of Pharmaceutical MNC. Pursuing Ph.D.

Chavan M.D. (Research guide): Working as Head, Dept. of Microbiology in Walchand College of Arts and Science. Having 29 years teaching experience. Presented several papers in National and International conferences. Published many papers in different National & International journals. Worked on different academic committees in university. Received

- 1) Best and Ideal teacher award from Lions Club.
- 2) Paryavaran Mitra Puraskar from Govt. of Maharashtra.
- 3) Appreciation award from Rotary club
- 4) Indira Gandhi Sadbhawna Award