ISSN : 0973-6204

PHARMBIT

INTERNATIONAL JOURNAL
INDEXING IN "CHEMICAL ABSTRACT"
Vol. XXVII. No. 2, Jul - Dec, 2013

The Official publication of
PHARMACEUTICAL SOCIETY
DEPARTMENT OF PHARMACEUTICAL SCIENCES
BIRLA INSTITUTE OF TECHNOLOGY
MESRA, RANCHI, INDIA
www.bitmesra.ac.in

Contents

PHARMBIT, Jul – Dec 2013; XXVIII (2)

Study of the Test of Inhibitory Effect of *Artemisia vulgaris* towards Different Groups of Bacteria Causing Nosocomial Infections

Lipy Adhikari[1], Pooja Manandhar[2]*, Smriti Gurung[1]

[1]Department of Environmental Science and Engineering, Kathmandu University, Nepal

[2]Department of Natural Science, Kathmandu University, Nepal

Abstract

The study focuses on determining the inhibitory effect of *Artemisia vulgaris* towards different groups of bacteria causing nosocomial infections. In the study, the incidence and abundance of three most common groups of these bacteria (*Staphylococcus* spp*, Enterococcus* spp *and Pseudomonas* spp*)* were determined which are gaining increased resistance to different antibiotics used in treating them. Organisms were isolated from four different hospitals chosen on the basis of their location and mobility of people. The plate exposure method was used to isolate the microorganisms from Post Operative ward, Intensive Care Unit and Operation Theater. The isolated bacteria were then treated by the methanol extract of *Artemisia vulgaris* to determine its inhibitory effect towards the isolated organisms. Gram-staining along with various biochemical tests were undertaken for the identification of the bacterial groups. Among the three groups of target bacteria, only two i.e. *Staphylococcus* spp *and Enterococcus* spp were isolated. *Staphylococcus* spp which was isolated from all four hospitals was found to be more common as compared to *Enterococcus* spp which was isolated from only two hospitals. *Pseudomonas* spp was absent from all four hospitals. Methanol extracts at different concentrations were found to be effective against both *Staphylococcus* spp and *Enterocccus* spp. *Artemisia* extract was effective in treating *Enterococcus* spp from 5% concentration with moderate to encouraging inhibition and with strong inhibitory effect at 40%, 60% and 100% concentration. Similarly, *Staphylococcus* spp showed inhibition from 10% concentration where the effect was encouraging at 10% and very strong at 60% and 100% concentration.

Keywords: Nosocomial, *Artemisia vulgaris,* methanol extract, antibiotics

Introduction

Nosocomial refers to hospital; and those infections that are acquired in hospitals or a long term care facility are termed as *Nosocomial Infections.* Studies document that these infections are major causes of morbidity and mortality[1]. They are a significant problem throughout the world and studies have revealed that the rates of such infections are on the rise[2]. Rates of nosocomial infections are markedly higher in many developing countries, especially for infections that are largely preventable (e.g., those following surgical procedures such as cesarean section). This is attributed to the lack of supervision, poor infection prevention practices, and inappropriate use of

limited resources and overcrowding of hospitals[3]. Another important factor associated with increase in nosocomial infections is the development of antibiotic resistance by a number of microorganisms[4].

A large number of microbes/bacteria have been known to be associated/to cause nosocomial infections. Studies have reported that gram-positive bacteria are the commonest cause of nosocomial infections with *Staphylococcus aureus* being the predominant pathogen[4,5]. The pathogen is responsible for causing around 2 million of the total annual nosocomial infections in world[5].There is a rising concern since *Staphylococci* are gaining resistance against antibiotics used to treat infections like Pneumonia[6]. A study conducted from tertiary care hospitals from western Nepal alone has resulted in the isolation of 162 *S. aureus* strains[7]. Another common nosocomial microbe is *Enteroccus* spp. It has been documented that vancomycin-sensitive enterococcal infections (VRE) are most commonly isolated from highly compromised patients, such as those in the ICU[8]. This particular group has been shown to possess resistance against a broad range of antibiotics such as vancomycin[9]; vancomycin, teicoplanin, penicillin, gentamicin[10]; macrolides fluoroquinolones, tetracyclines, and carbapenems[11]. Similarly, *Pseudomonas* spp. is of serious concern in hospitals contributing to wound related morbidity and mortality worldwide[12]. Its ability to grow well even in the absence of special nutrition, proliferation at room temperature and resistance to antibiotics pose difficulties in treating *Pseudomonas* spp. infections[11].

Natural products against pathogens: Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities[13]. Antimicrobials of plant origin have enormous therapeutic potential[14]. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains[14].They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobial[15].It has been reported that traditional medicine has provided western medicine with more than 40% of all pharmaceuticals [16,17]. Ethanolic as well as methanolic extracts of *Artemisia* spp is known to possess inhibitory effect on bacteria such as *Staphylococcus* at various concentrations[18]. *Artemisia* (*Asteraceae. L).* The active component found in this plant is Artemisinin which is a highly oxygenated sesquiterpene, containing a unique 1,2,4-trioxane ring structure[19]. It has been known to possess antiviral activity against flaviviruses[20] and also used to treat malaria[19, 20]. It is used as the leading treatment for malaria in Africa[21]. Studies have revealed that Artemisinin, sesquiterpene lactone with an unusual peroxide bridge, is the key moiety in the eradication of *Plasmodium falciparum* and the elimination of breast cancer cells[22]. More recently, they have also shown potent and broad anticancer properties in cell lines and animal and their efficacy also extends to phylogenetically unrelated parasitic infections such as schistosomiasis [23].

Materials and Methods

Site Selection: Four Hospitals were chosen for the isolation and identification of different groups of bacteria. Hospitals were chosen on the basis of mobility of people

Research Design: Samples were collected from four different hospitals from three different units namely, Post Operative ward, Intensive Care Unit and Operation Theater.

Part I: Isolation, Enumeration and Identification of Three Groups of Bacteria:

1. Preparation of media: Specific media was prepared for the identification of three different groups of bacteria [24]
2. Sample Collection/Isolation of microorganisms: Plate Exposure Method was used. A total of nine petri-plates were exposed in each unit i.e. POW, OT and ICU with 3 replicates, brought to the lab, incubated at 37 degree C for twenty four hours for the growth of the organism.
3. Enumeration of Colonies: Study and counting of the colonies showing characteristics distinct to the target organism was done. Enumeration was done by taking an average count of three plates.
4. Sub-Culture on Nutrient Agar: The colonies with distinct characteristics were sub-cultured on nutrient agar to obtain pure culture.
5. Gram-Staining: Gram staining was performed for all the pure cultures.
6. Biochemical tests: Based on gram stain results, further biochemical tests were performed.
   i. Catalase/Oxidase test ii. IMVIC test

Table 1: Biochemical tests done and the respective medium used

Test	Medium Used
Catalase	3%H_2O_2
Oxidase	Oxidase disk
Methyl Red	MR/VP broth
Voges-Proskauer	MR/VP broth
Indole	SIM
Citrate	Simmon Citrate Agar
TSIA	Triple Sugar Iron Agar
Litmus milk reduction	Litmus milk

7. Growth of identified organisms in selective media at specific temperature: Organism identified as *Staphylococcs* spp from biochemical tests were grown in Mannitol Salt Agar at 42 °C and as *Enterococcus* spp was grown in Bile Esculin Azide Agar at 44 °C.
8. Re-Confirmation: The biochemical tests were repeated for all the identified organisms

Part II: Methanol Extraction of *Artemisia vulgaris:* *Artemisia vulgaris* was collected, shade dried, grinded into powdered form and soaked in methanol for fifteen days. Approximately two kgs

of *Artemisia vulgaris* was soaked in seven and half liters of methanol. Water-bath was used to generate the extract. Different concentration (5%, 10%, 20%, 40%, 60% and 100%) of *Artemisia* extract was prepared using Dimethyl Sulfoxide (DMSO) solution **I**

Part III: Inhibitory test: Borer method was adopted where 50 µl of extract of different concentrations were taken to fill in the small holes made on Muller Hinton Agar. Plates were then incubated at 37 °C for 18 hours. Diameter of inhibition zone was measured using a scale. Experiment was performed in triplicate and the average diameter of inhibition zone was measured for each sample.

Results and Discussions

A total of 5 types of targeted colonies were seen from 106 samples collected from 4 hospitals. *Staphylococcus* spp was the most common bacteria and it was present in all four hospitals. *Enterococcus* spp was isolated from only two hospitals and found to be less common as compared to *Staphylococcus* spp *Pseudomonas* spp was absent in all four hospitals. Therefore, among the three groups of bacteria causing nosocomial infections in Post Operative Ward, Operation Theater and Intensive Care Unit, *Staphylococcus* spp was found to be most common. Among the two species of Staphylococcus, *S.aureus* was more evident as compared to *S epidermidis.* Studies have reported that gram-positive bacteria are the commonest cause of nosocomial infections with *Staphylococcus aureus* being the predominant pathogen[4,5].

A study conducted from tertiary care hospitals from western Nepal also has revealed the presence of *S. aureus*[7]. According to WHO 2002, health care settings are an environment where both infected persons and persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of nosocomial infections for patients and staff. Nosocomial infections are increased also because of crowding of people, frequent impaired immunity (age, illness, and treatments), new microorganisms and increasing bacterial resistance to antibiotics[1]. Presence of microorganisms in these important wards of all the hospitals suggests that the present practices for the control of microorganisms are not enough. More effort must be made to bring into practice of such methods which will help to check the transmission of pathogens. More than 50% of injections in developing countries are unsafe and many injections are unnecessary which results in an increased incidence of nosocomial infections[16]. The presence of bacteria in the studied hospitals could be attributed to the unsafe practices overcrowding, increased use of antibiotics and inefficient sanitation measures undertaken in the hospital premises. However, intense and extensive research is needed before a concrete conclusion is reached. Inhibitory test using *Artemisia vulgaris* extract on *Staphylococcus* spp and *Enterococcus* spp revealed that the extract was able to inhibit their growth

Table No. 2: Standard used for antimicrobial agent: "Department of Plant Resources", Nepal

Diameter in mm	Interpretation
6	+ weak
10	++ moderate
14	+++ encouraging
18 or above	++++ strong

Table No 3: Inhibitory Effect of *Artemisia vulgaris* on *Staphylococcus* spp

Organism	Micro-pippette	Media used	Concentration	Inhibition
Staphylococcus spp	50ul	Muller Hinton Agar	5%	Negligible
			10%	14mm
			20%	16mm
			40%	17mm
			60%	19mm
			100%	24mm

Table No 4: Inhibitory effect of *Artemisia vulgaris* on *Enterococcus* spp

Organism	Micro-pippette	Media used	Concentration	Inhibition
Enterococcus spp	50ul	Muller Hinton Agar	5%	13mm
			10%	15mm
			20%	17mm
			40%	19mm
			60%	20mm
			100%	25mm

With the increase in concentration of *Artemisia vulgaris*, there was an increase in the zone of inhibition for both the organisms as shows in the tables 3 and 4.

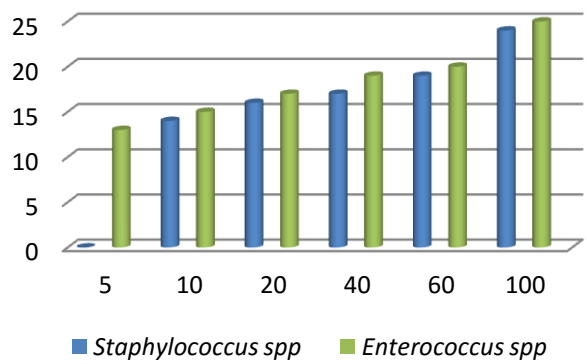


Figure 1: Comparison of Inhibition zone of *Staphylococcus* spp *and Enterococcus* spp

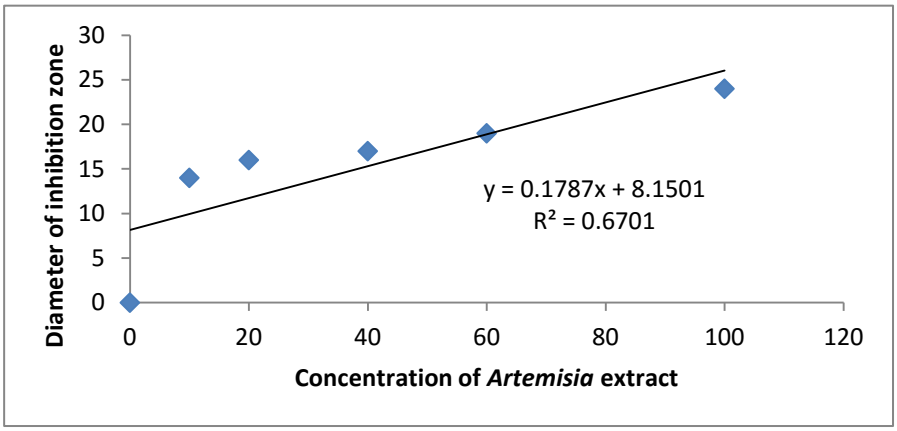


Figure 2: Diameter of inhibition zone VS Concentration of Artemisia extract for *Staphylococcus* spp and *Enterococcus* spp.

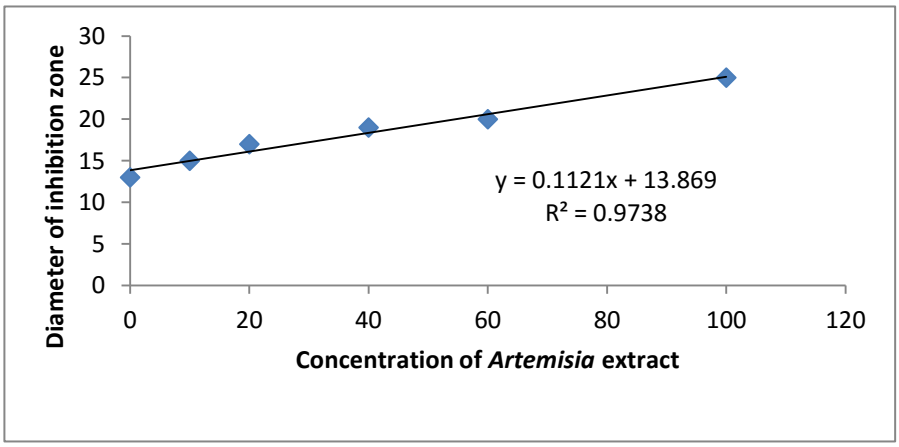


Figure 3: Diameter of inhibition zone VS Concentration of Artemisia extract for *Staphylococcus* spp

Concentrations of *Artemisia* extract positively inhibited the two types of bacterial colony. Although the extract significantly inhibited *Staphylococcus* spp with R^2= 0.67) and *Enterococcus* spp with R^2 = 0.97) of the bacterial colony, t-test showed that the inhibitory effect of the extract was not statistically significant in two strains of bacteria.

The inhibitory test done on *Staphylococcus* spp *and Enterococcus* spp showed that both the organisms were inhibited by methanol extract of *Artemisia vulgaris*. In case of *Enterococcus* spp, inhibition was seen from 5% concentration itself with an inhibition zone of diameter of 13 mm which can be interpreted as moderate to encouraging effect. It can be concluded that *Artemisia vulgaris* has encouraging to strong effect at 10 and 20% concentration and very strong effect at 40% 60% and 100% concentration. However, for *Staphylococcus* spp, the inhibition effect was seen

only from 10% concentration in which the zone of inhibition was 14 mm. This indicates that, at 10% concentration, its inhibitory effect is encouraging. Similarly, at 20 %, 40%, 60% and 100% concentration, the effect was very strong. The result of this study is in accordance with the previous finding where methanolic extracts of various species of *Artemisia* has showed inhibitory action against *Staphylococcus* at higher concentration (10 mg/ mL)[18]. The inhibitory tests performed showed that methanol extract of *Artemisia vulgaris* was effective in treating both *Staphylococcus* spp and *Enterococcus* spp. Methanol extract of *Artemisia vulgaris* was found to have considerable inhibitory effects towards both the organisms. With further necessary studies, it could be used as a better alternative to the existing antibiotics. The plant has the potential to be used as an antimicrobial agent.

Acknowledgement

I would like to express my heartfelt gratitude towards Kathmandu University and Department of Environmental Science and Engineering for providing me with the opportunity of conducting the project from which this paper has been extracted. I extend my sincere thanks to the management team and staff members of all the hospitals for allowing me to take the samples from their hospitals. My special thanks go to my supervisor Ms. Pooja Manandhar and my co-supervisor Ms Smriti Gurung for guiding me during the entire project.

References:

1. Ducel G. Les nouveaux risques infectieux. Futuribles 1995; 203:5-32.
2. Alvarado CJ. The Science of Hand Hygiene: A Self-Study Monograph. University of Wisconsin Medical School and Sci-Health Communications; March, 2000.
3. World Health Organization. Prevention of Hospital –acquired infections, a practical guide. 2nd edition. WHO; 2002.
4. Inweregbu K, Dave J. Pittard, A. Continuing Education in Anaesthesia, Critical Care & Pain. British Journal of Anaesthesia.2005;5(1):14-17
5. Shrestha B, Pokhrel BM, Tribhuban, MM. (2009). Phenotypic characterization of nosocomial isolates of Staphylococcus aureus with reference to MRSA. The Journal of Infections in Developing Countries (impact factor: 1.19).2009;3(7): 554--60
6. Weinstein RA. Nosocomial infection update. Emerg Infect Dis. 1998; (4): 416-420.
7. Tiwari, HK., Das, AK., Sapkota, D., Sivarajan, K., and Pahwa,VK.(2009). Methicillin resistant Staphylococcus aureus: prevalence and antibiogram in a tertiary care hospital in western Nepal. J infect Dev Ctries.2009; 3(9):681-684
8. Centers for Disease Control and Prevention. Recommendations for preventing the spread of vancomycin resistance: recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC) MMWR. 1995; Morb. Mortal Wkly (44):1-13

9. Jones RN, Pfaller MA. Bacterial resistance: a worldwide problem. Diagn Microbiol Infect Dis. 1998; (31):379-388
10. Archibald L, Phillips L, Monnet D. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis. 1997; (24): 211-215
11. Pfaller MA, Jones RN, Doern GV. Survey of blood stream infections attributable to Gram-positive cocci: frequency of occurrence and antimicrobial susceptibility of isolates collected in 1997 in the United States, Canada, and Latin America from the SENTRY. Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis. 1999; (33): 283-297
12. Mayhall CG. Nosocomial burn wound infection. Mayhall GC Ed. Hospital epidemiology and infection control. William and Wilkins Co., Baltimore, MD, USA. 1996; 225-236.
13. Hamburger M, Hostleman K. Bioactivity in plants: the link between phytchemistry and medicine. Phytochemistry. 1991; (3): 3864-3874.
14. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 1998; (60):1-8.
15. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin In: Janick, J. (Ed.), Perspectives on New crops and New Uses. ASHS Press: Alexandr, VA. 1999; 457-462. Available at: http://www.hort.purdue.edu/newcrop/proceedings1999/pdf/v4-457.pdf, Accessed at: 31-01-2013.
16. Samuelsson G. Drugs of Natural Origin: A Textbook of Pharmacognosy. Swedish \ Pharmaceutical Society, Swedish Pharmaceutical Press, Stockholm. 1999; 17
17. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol. 2000; 10 (70), 343-349.
18. Ramezani M, Fazli-Bazzaz BSF, Saghafi-Khadem AD. Antimicrobial activity of four Artemisia species of Iran. Fitoterapia. 2004; (75): 201-203.
19. Brown GD. The Biosynthesis of Artemisinin (Qinghaosu) and Phytochemistry of Artemisia annoua L. (Qinghao).Molecules. 2010; (15):7603-7698.
20. Romero MRT, Serrano MA, Vallejo M, Efferth T, Alvarez M, Marin JJG. (2006). Antiviral Effect of Artemisinin from Artemisia annua against a Model Member of the Flaviviridae Family, the Bovine Viral Diarrhoea Virus (BVDV). Planta Med. 2006 Oct; 72(13):1169-74. Epub 2006 Aug 10
21. Dalrymple G. Artemisia, Agriculture and Malaria in Africa: The Interplay of Tradition, Science and Public Policy. 2006.
22. Ferrira J. Artemisia annua L: The hope against malaria and cancer. USDAARS, AFSRC. 2004; (1224) 25813.
23. Krishna S, Bustamante L, Haynes RK, Staines HM. Artemisinins: their growing importance in medicine. Trends Pharmacol. 2008; 29(10): 520–527.
24. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2000.

PHARMBIT, Jul – Dec 2013; XXVIII (2)

Detection of Microhemagglutinate during Blood Grouping to Minimize the Risk of Mismatched Blood Transfusion

Mukul Chandra Gope

Department of Physiology, Rajendra Institute of Medical Sciences, Ranchi – 834009

Abstract

The method of interpretation of a blood group in practice, widely in the laboratories, is to rely on 'naked eye observation' for the formation of clumps, i.e. observation of Macroclumps (Macrohemagglutinates). In the present study, 137 medical students were blood-grouped to find out the incidence of Microclumping. Samples of around 2% cases were demonstrated microclumping under microscope, initially which were wrongly interpreted as negative (absent clumps) on naked eye observation (for macroclumps).

Key words: Blood grouping, Microscope, Microhemagglutinate, Macrohemagglutinate, donated blood, mismatched transfusion

Introduction

Human blood is an essential element of human life with no substitute. The use of whole blood is now a well-accepted and well-used measure in many major surgeries and emergency care of trauma patients. Blood transfusion has been responsible for saving millions of lives each year around the world. Yet the quantity and quality of blood pool available for transfusions is still a major concern across the globe, especially in the developing countries. According to the World Health Organization South Eastern Asia's estimated blood requirement are about 15 million units per year. India has 2,433 blood banks that require 9 million units of blood annually (Prathama, 2009).

When such large numbers are involved, it is inevitable that problems will occur. These may arise during the actual transfusion or during the pre and post transfusion periods (Wood et al., 2009). Difficulties can arise because of:

- Nonhemolytic febrile reactions: These result from a donor white cell antigen/antibody reaction, the most common complication.
- Fluid overload: This should be rare but may occur in the elderly.
- Benign allergic reactions (rash, pruritis, urticaria) or true anaphylaxis (rare).
- ABO blood group antigen incompatibility, an immune reaction from naturally occurring red cell antibodies causing intravascular hemolysis.
- Transfusion-related lung injury (TRALI): Acute noncardiac pulmonary edema.
- Infection from bacterial, parasitic, or viral contamination.

- Posttransfusion purpura (PTP): Severe thrombocytopenia developing 5–10 days posttransfusion.

Importance of microscopy in Blood Grouping: Red blood cells show different size of agglutinate on mixing with corresponding antisera during blood grouping, depending on the representation of the amount of the surface antigen. Person exhibiting lesser amount of surface antigen on erythrocytes, tend to form microheamagglutinates. Only with the use of microscope such microheamagglutinate can be conformed. Naked eye observation certainly fails to detect such microheamagglutinate.

Rationale of present work: As a common practice, blood groping is done in the loboratories and blood banks, relying solely on naked eye observations i.e. for the macroclumps. This has drawback as to miss the microclumps. Present study is intended to find out the percentage of individuals those exhibit microclumping, which will othrwise be wrongly labelled with a different blood group, with the method in use, in practice.

For the determination of blood group, antisera (A, B or Rh) are mixed with diluted blood (1:16 ratio of blood with normal/isotonic saline). The hemagglutination that occurs after such mixing, can be of four grades (Table 1)

Table 1: Grades of hemagglutination

Grade	Nature of clump/clumps	Observation
Grade 1	Large, single clump	Visible by naked eye
Grade 2	Medium sized clumps	Visible by naked eye
Grade 3	Small sized clumps	Visible by naked eye
Grade 4	Microscopic clumps	Visible by microscope only

So, it is necessary to observe all the samples of the blood groups which do not show macroclumps under microscope to detect Grade 4 type of hemagglutination i.e., microhemagglutination, otherwise Grade 4 will be wrongly interpreted as "no-hemagglutination" on naked eye observation.

Material and Method

137 medical students (RIMS, Ranchi) in 3 groups have been included in the present study to find out the incidence of microhemagglutination. After taking all aseptic precautions, blood drop was obtained by pricking the ring finger of less dominant hand (right hand ring finger for left hander person and vice versa). The drop was mixed with normal saline in 1:16 ratio (i.e. 1 drop of blood was mixed with 16 drops of normal saline). The mixture was thoroughly prepared with help of a wooden stick (tooth-pick).

Three glass slides were taken. One drop of Antisera A, B and Rh (D) were taken at one end of the slides and at the other end of the slides, one drop of the diluted blood was taken as "control". One drop of diluted blood was added to each of the three anti-sera, and mixed properly with separate wooden sticks. At this point, time is noted to calculate the "time interval" after which visible clumps appears on naked eye observation. All the slides demonstrating "no hemagglutination" on naked eye observation were examined under digital microscope (Leica) to rule out micro-hemagglutination.

Results

Table 2: Time interval (min) after which clumps have been observed

Time interval (min)	No of samples
< 2	110
2 to 5	25
5 to 10	2

Table 3: Blood groups with macro and micro clumping

Blood groups		Macro clumps	Microscopic clumps	Total	Percentage
A		26	NIL	26	18.98
B		55	NIL	55	40.15
AB	A	06	NIL	6	04.38
	B	05	1	6	
O		NIL	NIL	50	36.49
		92	01	137	100

Antisera D	Macro clumps	Microscopic clumps	Total	Percentage
Rh +ve	129	2	131	95.62

Table 3 shows the number of macro and micro clumps, observed in the study group. Blood Group A and B have shown macroclumps (Figure 1a), which were visible by naked eye. One case of Blood Group AB was initially labeled as blood Group A, on naked eye observation. On microscopic observation, microclumps were observed with antisera B and therefore finally labeled as Blood Group AB (Figure 1b).

Similarly, in two of the samples, naked eye observation was unable to demonstrate clumps with anti D, hence initially labeled as A –ve and O –ve. Therefore, finally both of the samples were labeled as A +ve and O +ve respectively. The pattern of microclumping observed under microscope was with an approximate clumps size of 15-20 µm.

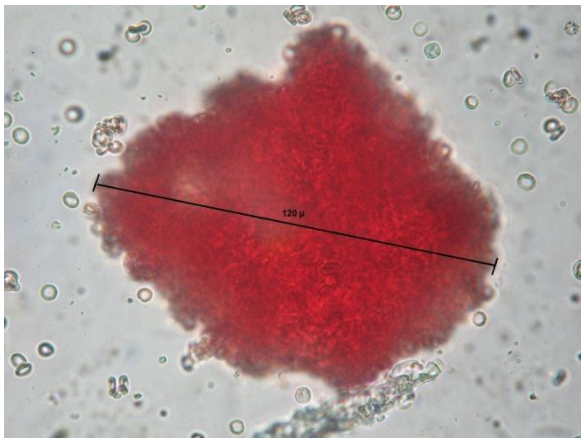


Figure 1a: Macro hemagglutination with approx diameter of 120 µ

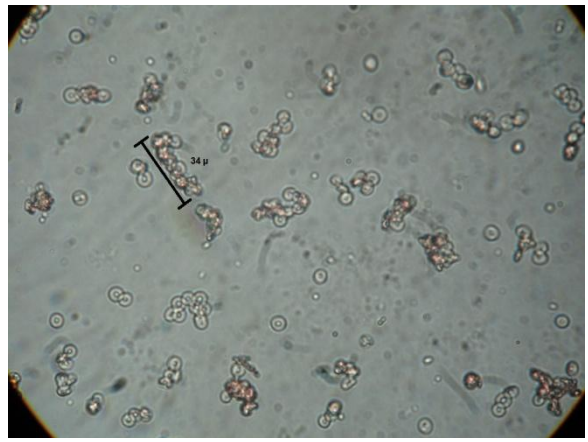


Figure 1b: Microhemagglutination (Antisera B) with approx diameter of 34 µ of the largest hemagglutination

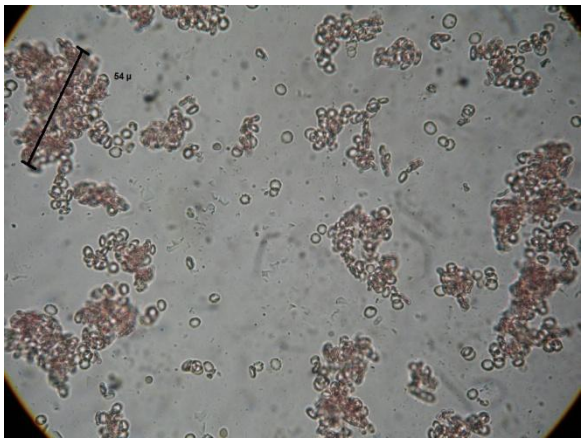


Figure 1c: Microhemagglutination (Antisera D: Rh) with approx diameter of 54 µ of the largest hemagglutination

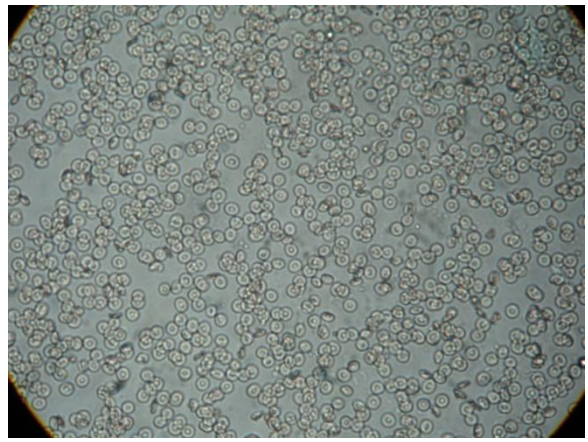


Figure 1d: Undiluted or under-diluted blood tends to form rouleaux, which often are misinterpreted as clump +ve on naked eye observations.

Unfortunately, in many blood banks and test laboratories, undiluted or under-diluted blood (instead of standard 1:16 ratio, they use 1:8 or lower ratio of blood and normal saline and sometimes even undiluted blood also) samples are used for blood grouping. The possible explanation behind this ill practice is the intention of obtaining larger clumps for easy naked eye observation. But this does not help as intended to, since the undiluted or under-diluted blood tends to form rouleaux, on mixing with the antisera (Figure 1d). This creates confusion in clump-negative samples since, the mixture appear hazy on naked eye observations, and often wrongly labeled positive (false positive) for clumps. In Figure 1b the clumps cannot be seen by naked eye

observation, since smallest feature visible to naked eye is 40 micron as per Intel®. Figure 1c, the small clumps, which are visible to naked eye observation with difficulty, provided:

- Acuity - the person has 6/6 acuity of vision;
- Light source - bright day light;
- Background - observed against white background.

Discussion

It has been widely in practice to rely on naked eye observation of the macroclumps formation for the determination of the blood groups. In fact this is a serious mistake, since one cannot see the microclumps by naked eyes. Therefore some of the samples having microclumps are missed out and hence, are interpreted as "negative" (i.e. A -ve, B -ve or O -ve etc). Use of microscope is mandatory (Guyton & C.L. Ghai) while doing blood grouping. The reason of microclumps formation can be understood by Figure 2a and 2b.

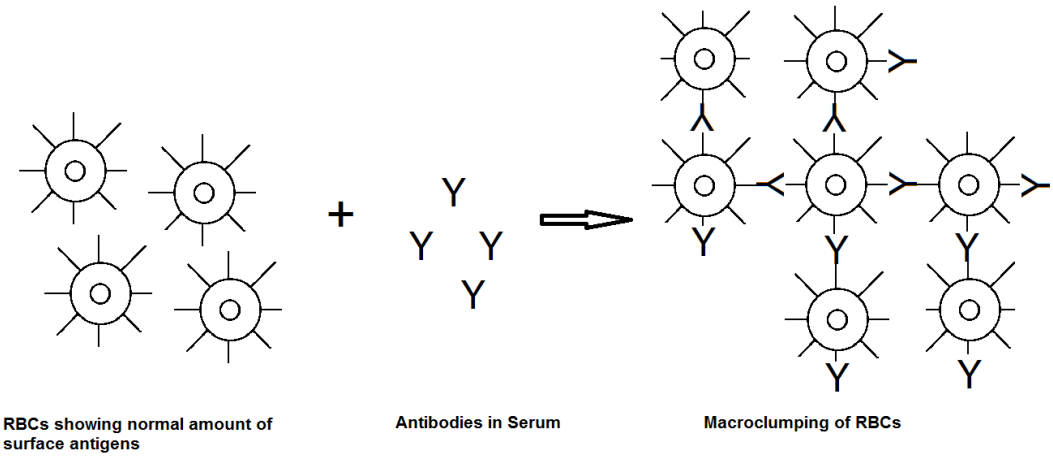


Figure 2a: Formation of large/macro clumps due to normal amount of surface antigens

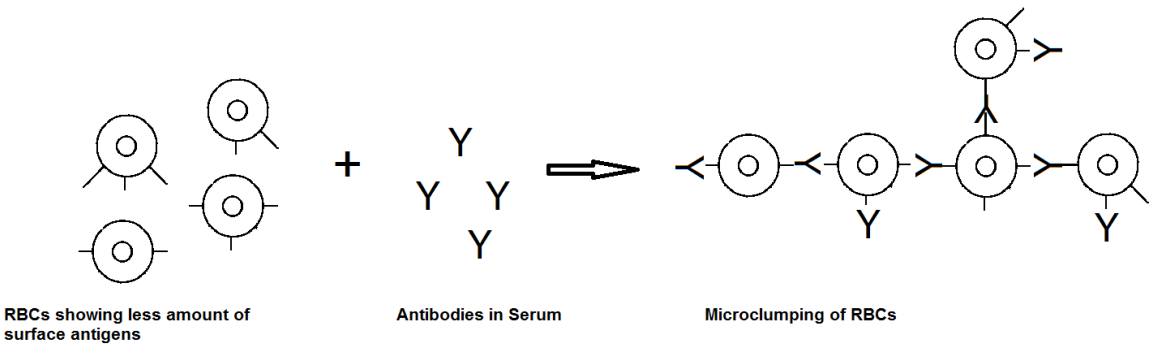


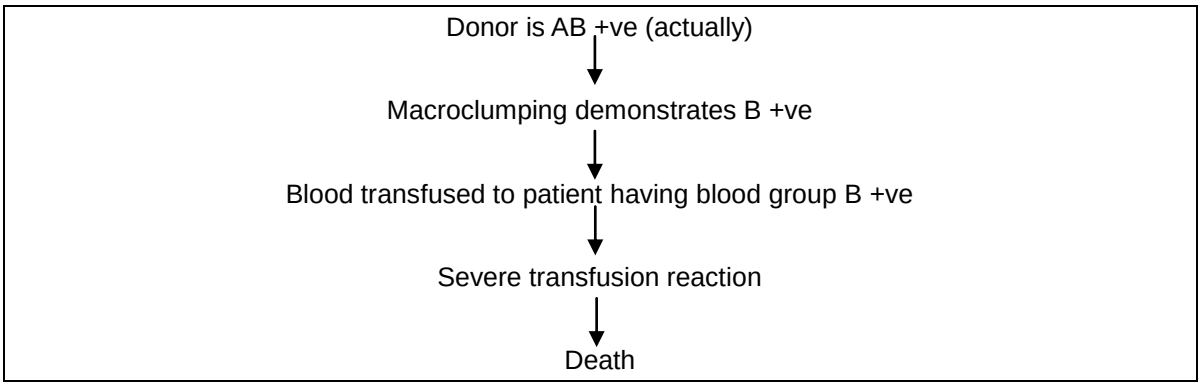
Figure 2b: Formation of micro clumps due to less amount of surface antigens

So, it can be concluded that the representation of lesser amount of surface antigen (possibly genetically determined) on the cell surface of RBCs, can be a cause of formation of microclumps. In the present study group, 3 such samples of microclumping were observed (out of 137 total samples) which equals 2.19% of the total. If we consider a bigger population, such cases will be evident in large number of people.

The Risk

The risk of labeling a “positive” blood group of Rh type as “Negative” is observed only when, the person concerned, ‘donates' blood (as a “Negative” donor) to a “negative blood group”, patient.

While observing many of the cases of mismatched blood transfusion, the cases of microclump misinterpretation and mismatched transfusion are difficult to prove, even in the court. The reason of this is, the donor’s identity is not always available from the blood bank. If such person’s blood exhibits microclumping, it will be wrongly stored as “Negative” group blood and will be provided to a patient having the same negative blood group. On development of ‘transfusion reaction’; it will be difficult to find out the cause, since the doctor will observe the “donated blood” and the “patient” both, as having the same, i.e. of negative blood group. If such a patient dies even then also, many of the other causes of death will be interpreted such as cardio-respiratory failure, drug overdose and interaction etc. So, the exact causes of death will remain elusive. If the donor is found out and, the blood grouping is arranged, then only it will be possible to prove by demonstrating microclumps in the donors blood under microscope, and actual cause of death as 'mismatched transfusion'.



Conclusion

As the study demonstrates the importance of the use of the microscope for blood grouping, it also insists in, for rigorous check and double cheek of "donated blood samples" to avoid the exclusive cases of mismatched transfusion, which are always wrongly interpreted as drug interaction/ adverse reaction or overdose.

Acknowledgement

The author is thankful to 1st year (2K11 batch) students of Rajendra Institute of Medical Sciences, Ranchi for participating in this experiment and for helping in conducting the experiment. Author further likes to thank Dr. Manik Ghosh, Assistant Professor, Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra for his help in digital microscopic observations and photography.

Bibliography

1. P.R. Wood, P.F. Mahoney, J.P. Cooper 'Problems With Blood Transfusion' in Trauma and Orthopedic Surgery in Clinical Practice, 11th edition, 2009. Springer London.
2. C.L. Ghai, Practical Physiology, 7th edition, 2007, Page 105, J.P. Brothers Medical Publishers Pvt. Limited., New Delhi
3. G.K. Paul, Text Book of Practical Physiology, 2nd edition, 2005 , Page 97, Orient Longman, Hyderabad.
4. P. Nayak, Students Handbook of Practical Physiology, Page 58-59, 1st edition, 2006, Mehta Publication , New Delhi.
5. Pinki Chattopadhyay , Practical Physiology, 1st edition, 2011, Page 24, New Central Book Agency, Kolkata.
6. L. Prakasam Reddy, Practical Physiology, 2005, Paras Publishing, Hyderabad.
7. Gyton & Hall, Text Book of Medical Physiology, Page No. 834, 12th edition, 2011, Saunders, Philadelphia.
8. http://www.medwonders.com/medblogs/general/blood-banks-and-india-358.htm
9. http://www.intel.com

Comparative Study on Heavy Metals Concentration in Vegetables reported from different parts of India

Ratna Ghosh, Reshma Xalxo

Department of Home Science (Division of Nutrition), Ranchi University, Ranchi – 834001

Abstract

A comparative study was carried out with the levels of heavy metals present in vegetables reported from different parts (East, West, North and South) of India. This comparative study was carried out to assess the exposure of heavy metals to inhabitant of Ranchi by heavy metals consumption (Arsenic, Cadmium, Chromium, Cobalt, Copper, Nickel and Lead) through the intake of locally available vegetables sold in the various market sites. In our study, the results showed the mean concentrations of Cd, Ni, Pb in vegetables were above the PFA recommended level but in case of Copper, Cobalt, Chromium and Nickel the heavy metal concentration range were found to be much higher in all other sites in comparison to present study. In our study the concentration of Lead content varies from 0.3 to 13.7 mg/kg. The mean Lead concentration in vegetables was higher than the values reported (1.0 to 1.4 mg/kg) in Varanasi, UP but lower than the values reported in Anand, Gujarat; Titagarh WB and Bangalore Karnataka.

Key words: Heavy metal, Comparative study, Varanasi, Titagarh, Bangalore, Anand, Ranchi

Introduction

Vegetables are essential staple food of human's daily diet; contain significant amounts of essential vitamin, nutrients rich in health benefiting phyto-nutrients, minerals and anti-oxidants. Liberal intakes of vegetables in the diet lead to maintain a healthy life which may prevent all diseases and keep an individual energetic and active throughout the life. They are beneficial for important biochemical and physiological process and are necessary for maintaining sound health throughout life (Table 1). In present study we have taken thirteen vegetables from different groups for heavy metal analysis.

Table 1: Nutritional importance of vegetables

Common Name	Botanical Name	Importance
Beet	*Beta vulgaris*	Contain Betacyanin protects heart from potential heart attacks and stroke and helps reduce blood pressure, Prevents plaque formation and reduces bad cholesterol, Great for pregnant mums, Treats osteoporosis & anaemia, Boosts brain power and may treat dementia.

Common Name	Botanical Name	Importance
Potato	*Solanum tuberosum*	The majority of carbohydrates in potatoes are complex carbohydrates; body's main energy sources, Potatoes contain antioxidants carotenoids and anthocyanins, Provide significant amounts of potassium and vitamin C. Potatoes are part of a healthful diet.
Carrot	*Ductus carrotus*	Regulate the blood sugar, Protect from cardiovascular disease Provide necessary amount of vitamin A & antioxidants; prevent cancer, breast cancer.
Radish	*Raphanus sativus*	One of the very low calorie root vegetables, Contains isothiocyanate anti-oxidant compound called sulforaphane have role against prostate, breast, colon and ovarian cancers, Rich sources of vitamin C helps the body scavenge harmful free radicals, prevention from cancers, inflammation and help boost immunity.
Ginger	*Gingiber officinalis*	Have anti inflammatory, carminative, anti flatulent and anti microbial properties. Contain Zingerone gives pungent character to the ginger root, is effective against E.coli induced diarrhea, especially in children.
Cucumber	*Cucumis sativus*	Cucumbers are 95% water, keeping the body hydrated while helping the body eliminate toxins, The silicon and sulfur in cucumbers help to stimulate hair growth, Contain lariciresinol and pinoresinol reduced risk of several types of cancer including breast, ovarian, uterine and prostate cancer. Cures diabetes, reduces cholesterol and controls blood pressure.
Pea	*Pysum sativum*	Rich in health benefiting phyto-nutrients, minerals, vitamins and anti-oxidants. Excellent source of folic acid, Vitamin A, vitamin C, vitamin K and B groups of vitamins. Helps in maintaining health of mucus membranes, skin, eye-sight, protect from lung and oral cavity cancers.
Beans	*Phaseolus vulgaris*	Rich source of dietary fiber, Reduce blood cholesterol levels, Contain antioxidants such as lutein, zea-xanthin and β-carotene help against oxygen-derived free radicals and reactive oxygen species. Contain healthy amounts of minerals like

Common Name	Botanical Name	Importance
		iron, calcium, magnesium, manganese and potassium.
Cabbage	*Brasssica oleracea*	Cabbage is a very good source of vitamin K; helps in keeping healthy bones, Contains powerful antioxidants like thiocyanates, indole-3-carbinol, lutein, zea-xanthin, sulforaphane and isothiocyanates help protect against cancer, Help reduce LDL.
Spinach	*Spinacia oleracea*	Rich source of Vitamin A, vitamin C, vitamin K, vitamins B-complex (pyridoxine, thiamin, riboflavin, folates and niacin) and minerals (potassium, manganese, magnesium, copper and zinc).
Corriender leaves	*Coriandrum sativum*	Reduce LDL while increasing HDL levels. The leaves are also rich in numerous anti-oxidant polyphenolic flavonoids such as quercetin, kaempferol, rhamnetin and epigenin.
Lady finger	*Abelmoschus esculentus*	Rich sorces of fiber and mucilaginous content help in smooth peristalsis of digested food particles and relieve constipation condition. Contain sound amounts of vitamin A, flavonoid anti-oxidants such as β-carotenes, xanthin and lutein are essential for vision. Good source of iron, calcium, manganese and magnesium.
Tomato	*Lycopersicum esculentum*	Excellent sources of vitamin C, antioxidants, dietary fiber, minerals and vitamins. Concentrated in Lycopene protective of cancers and prevents skin damage from ultra-violet rays and offers protection from skin cancer. Contain flavonoid Zea-xanthin helps protect eyes from age-related macular disease.

Contamination of vegetables with toxic heavy metals has become one of the major causes of concern for human kind. Fruits and vegetables may become contaminated with toxic chemicals by several different pathways. Ambient pollutants from the air may be deposited on or absorbed by the plants, or dissolved in rainfall or irrigation waters that contact the plants. Pollutants may also be absorbed through plant roots from contaminated soil and ground water. The addition of pesticides, soil additives, and fertilizers may also result in food contamination[1]. Heavy metals in surface water, ground water and soils can be either from natural or anthropogenic sources[2] which enter into the plants through surface depositions. Ingestion of heavy metals through food can

cause accumulation in organisms, producing serious health hazards such as injury to the kidney, symptoms of chronic toxicity, renal failure and liver damage[3,4]. Ingestion of contaminated vegetables is a potential pathway of human exposure to toxic chemicals.

It is well known that an excess or deficiency of metals can cause harmful effects in human body. This study is aimed to present a data of levels of heavy metals in different types of vegetables available for the consumption to the inhabitants of Ranchi city from various market sites. This data will help to provide the status of heavy metal contamination in vegetables in Ranchi and also to assure the food safety and to protect the customer.

Analysis of Heavy Metals

Concentrations of Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Nickel (Ni) and Lead (Pb) in the filtrate of digested plant samples were estimated by using Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES) (Model Optical 2100DV ICP-OES, Perkin Elmer, USA) with argon laser. The Spectral range was of 160 nm to 900 nm and resolution of 0.009 nm at 200 nm. The instrument was fitted with UV sensitive dual backside – illuminated Charge Coupled Diode array detector. The vegetables selected for present study were Beet, Potato, Carrot, Radish, Ginger, Cucumber, Pea, Beans, Cabbage, Spinach, Corriender leaves, Lady finger and Tomato. Concentrations of heavy metals were calculated on a dry weight basis. All analyses were replicated six times. The analytical procedures, concentration of heavy metals in individual vegetables and data analysis were already reported[5,6].

Results & Discussion

A comparative study was carried out with the levels of heavy metals present in vegetables reported from different states of India. The sites selected for comparative studies were Varanasi, UP (North); Anand, Gujarat (West); Titagarh, WB (East) and Bangalore, Karnataka (South). In comparison to other sites of India, it was found that the concentrations of heavy metals such as, Cadmium, Nickel and Lead were above the PFA permissible limit but the concentration of Cobalt, Chromium and Copper was within the PFA safe limit in all vegetables collected from sites of Ranchi City. Comparative data were represented in Table 2 to 4.

In Ranchi among different vegetables pea showed highest value of Metal Pollution Index (MPI). The range of MPI (Site-1 to Site-10) was observed in between 2.249 to 3.031. Seven vegetables out of thirteen showed higher MPI i.e. more than 2. These were pea, cucumber, tomato, beans, spinach, lady finger and cabbage. Higher MPI suggests that these vegetables may cause more human health risk due to higher accumulation of heavy metals in the edible portion. The results of the ANOVA (Two-Factor without Replication) suggests that in case of vegetables, the P value (1.12E-38) was found to be less than the significance level (0.05), and F (50.513) was

more than F crit (1.843) i.e. there is significant difference between MPI among the vegetables.

In the present comparative study only Varanasi, UP[7] has reported the MPI values for some of the vegetables taken in our study. In Varanasi the MPI was reported 9.74 for radish. In Ranchi the range of MPI was found to be 0.428 to 1.306 for radish. In case of Cabbage the range of MPI was found to be 0.968 to 2.111 in Ranchi and the MPI reported were 11.82 in Varanasi. The MPI reported in Varanasi were 11.54 for Spinach and the range of same was found to be 1.357 to 2.418 in Ranchi. In case of Lady finger the range of MPI was found to be 1.543 to 2.241 in Ranchi and the same was reported 11.35 in Varanasi. In Tomato the range of MPI was shown 1.719 to 2.495 in Ranchi and in Varanasi[7] the MPI was found to be 11.03. Thus MPI values in all vegetables in present study were below the vegetables taken in Varanasi.

In the present study, the concentration of Lead content varies from 0.3 to 13.7 mg/kg. The mean Lead concentration in vegetables was higher than the values reported (1.0 to 1.4 mg/kg) in Varanasi, UP[8] but lower than the values reported in Anand, Gujarat[9]; Titagarh WB[10] and Bangalore Karnataka[2]. The concentration of Cadmium ranges from 0.1 to 2.2 mg/kg which was found to be higher than the values reported (1.4 to 1.9 mg/kg) in Varanasi, UP but lower than the values reported in all other sites. In case of Copper, Cobalt, Chromium and Nickel the heavy metal concentration range were found to be much higher in all other sites in comparison to present study. Thus from this comparative study it can be concluded that the consumption of average amounts of these contaminated vegetables of Ranchi City have low health risk for the consumers in comparison to vegetables from other parts of India.

Table 2: Comparisons of concentration of Heavy Metals (Cadmium & Lead) in vegetables of different states of India

Vegetables	Cadmium (Cd) mg/kg dry weight					Lead (Pb) mg/kg dry weight				
	Anand Gujarat	Varanasi UP	Titagarh WB	Bangalore Karnataka	**Ranchi Jharkhand (Range)**	Anand Gujarat	Varanasi UP	Titagarh WB	Bangalore Karnataka	**Ranchi Jharkhand (Range)**
Beet	5.0	--	--	--	**0.3-0.7**	149.0	--	--	--	**2.5-7.3**
Potato	--	--	--	--	**0.2-0.5**	--	--	--	--	**0.3-2.4**
Carrot	6.0	--	--	5.6	**0.3-0.8**	78.0	--	--	10.2	**0.8-4.6**
Ginger	6.0	--	--	--	**0.2-0.7**	19.0	--	--	--	**1.8-3.8**
Radish	--	--	17.8	6.2	**0.2-0.6**	--	--	57.6	11.0	**0.8-2.6**
Cucumber	10.0	--	--	--	**0.1-1.6**	122.0	--	--	--	**5.4-12.7**
Pea	--	--	--	--	**0.1-0.8**	--	--	--	--	**6.2-13.7**
Beans	7.0	--	--	3.4	**0.2-0.6**	73.0	--	--	8.0	**3.4-8.7**
Cabbage	--	--	--	--	**0.2-0.9**	--	--	--	--	**1.3- 5.2**
Spinach	--	1.9	14.6	6.8	**0.6-2.2**	--	1.4	49.8	15.8	**0.5-5.8**
Corriender leaves	18.0		14.1	--	**0.3-0.9**	152.0	--	31.1	--	**2.7-8.0**
Lady finger	5.0	1.4	--	--	**0.1-0.6**	66.0	1.0	--	--	**3.4-7.8**
Tomato	--	--	--	2.8	**0.2-0.7**	--	--	--	7.2	**2.5-9.7**

UP: Uttar Pradesh; WB: West Bengal

Table 3: Comparisons of concentration of Heavy Metals (Nickel & Copper) in vegetables of different states of India

Vegetables	Nickel (Ni) mg/kg dry weight					Copper (Cu) mg/kg dry weight				
	Anand Gujarat	Varanasi UP	Titagarh WB	Bangalore Karnataka	**Ranchi Jharkhand (Range)**	Anand Gujarat	Varanasi UP	Titagarh WB	Bangalore Karnataka	**Ranchi Jharkhand (Range)**
Beet	501.0	--	--	--	**0.2-0.6**	323.0	--	--	--	**6.7-12.2**
Potato	--	--	--	--	**0.1-0.6**	--	--	--	--	**2.2-8.9**
Carrot	345.0	--	--	7.8	**0.4-0.8**	573.0	--	--	--	**4.9-5.6**
Ginger	392.0	--	--	--	**0.2-0.5**	502.0	--	--	--	**3.9-8.9**
Radish	--	--	62.7	10.6	**0.2-0.7**	--	--	28.1	--	**4.1-9.1**
Cucumber	2151.0	--	--	--	**1.1-2.9**	592.0	--	--	--	**9.2-15.6**
Pea	--	--	--	--	**3.7-5.8**	--	--	--	--	**8.5-12.3**
Beans	2032.0	--	--	7.8	**3.8-5.8**	838.0	--	--	--	**10.4-17.1**
Cabbage	--	--	--	--	**0.6-3.8**	--	--	--	--	**7.7-14.5**
Spinach	--	--	69.2	9.8	**0.5-0.9**	--	27.6	34.5	--	**10.6-17.7**
Corriender leaves	474.0	--	51.3	--	**0.3-0.6**	1156.0	--	25.1	--	**7.9-12.8**
Lady finger	3034.0	--	--	--	**0.3-2.4**	165.0	18.0	--	--	**9.5-16.1**
Tomato	--	--	--	6.0	**0.3-1.3**	--	--	--	--	**11.5-22.3**

UP: Uttar Pradesh; WB: West Bengal

Table 4: Comparisons of concentration of Heavy Metals (Cobalt & Chromium) in vegetables of different states of India

Vegetables	Cobalt (Co) mg/kg dry weight					Chromium (Cr) mg/kg dry weight				
	Anand Gujarat	Varanasi UP	Titagarh WB	Bangalore Karnataka	**Ranchi Jharkhand (Range)**	Anand Gujarat	Varanasi UP	Titagarh WB	Bangalore Karnataka	**Ranchi Jharkhand (Range)**
Beet	20.0	--	--	--	**0.4-1.6**	--	--	--	--	**0.7-7.2**
Potato	--	--	--	--	**0.2-1.0**	--	--	--	--	**0.6-4.1**
Carrot	11.0	--	--	--	**0.2-0.6**	--	--	--	21.0	**0.8-4.8**
Ginger	15.0	--	--	--	**0.3-0.7**	--	--	--	--	**1.5-4.3**
Radish	--	--	--	--	**0.3-0.6**	--	--	78.0	22.0	**0.1-4.1**
Cucumber	30.0	--	--	--	**0.2-0.8**	--	--	--	--	**1.0-4.7**
Pea	--	--	--	--	**0.5-1.1**	--	--	--	--	**2.2-6.5**
Beans	11.0	--	--	--	**0.4-0.7**	--	--	--	20.0	**0.9-4.0**
Cabbage	--	--	--	--	**0.7-1.4**	--	--	--	--	**0.1-3.4**
Spinach	--	--	--	--	**0.6-1.6**	--	--	96.3	25.0	**2.0-4.5**
Corriender leaves	41.0	--	--	--	**0.4-0.9**	--	--	48.2	--	**0.7-3.7**
Lady finger	9.0	--	--	--	**0.3-0.7**	--	--	--	--	**1.6-4.1**
Tomato	--	--	--	--	**0.6-1.0**	--	--	--	20.0	**2.8-7.8**

UP: Uttar Pradesh; WB: West Bengal

References

1. USEPA (US Environmental Protection Agency), Exposure Factors Handbook, Office of Research and Development. National Center for Environmental Assessment. US Environmental Protection Agency. Washington, DC. http://www.epa.gov/ncea/pdfs/efh/front.pdf (1997).
2. Varalakshmi LR, Ganeshamurthy AN. Heavy metal contamination of water bodies, soils and vegetables in peri urban areas of Bangalore city of India. 2010 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1 – 6 August 2010, Brisbane, Australia. Published on DVD.
3. Abou-Arab AAK, Soliman KM, El Tantawy ME, Ismail BR and Naguid K. Food Chemistry1999; 67: 357-363.
4. Sathawara NG, Parikh DJ, Agarwal YK. Essential heavy metals in environmental samples from western India. Bulletin of Environmental Contamination and Toxicology 2004; 73: 756–761.
5. Ghosh R, Xalxo R, Gope MC, Mishra S, Kumari B, Ghosh M. Estimation of Heavy Metals in locally available vegetables collected from road side market sites (1-4) of different areas of Ranchi City. PHARMBIT XXIII and XXIV: 68-73 (2011).
6. Ghosh R, Xalxo R, Ghosh M. Estimation of heavy metal in vegetables from different market sites of tribal based Ranchi city through ICP-OES and to assess health risk. Current World Environment 2013, 8(3): 435-444.
7. Singh A, Sharma RK, Agrawal M, Marshall FM. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. Food and Chemical Toxicology 2010; 48: 611-619.
8. Sharma RK, Agrawal M, Marshall FM. Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food and Chemical Toxicology 2009; 47: 583–591.
9. Kumar N, Soni H, Kumar R. Characterization of Heavy Metals in Vegetables Using Inductive Coupled Plasma Analyzer (ICPA). Journal of Applied Sciences and Environmental Management 2007; 11(3): 75 – 79.
10. Gupta N, Khan DK, Santra SC. An Assessment of Heavy Metal Contamination in Vegetables Grown in Wastewater-Irrigated Areas of Titagarh, West Bengal, India. The Bulletin of Environmental Contamination and Toxicology 2008; 80: 115–118.

Ayurveda: Combating Viral Diseases since Ages

Preeti Awasthi, Siva G Mavuduru, Manik Ghosh*

Birla Institute of Technology, Mesra, Ranchi - 835 215

Abstract

Ayurveda is one of the oldest systems of medicine originated in India and written in Sanskrit language. Plants from Ayurveda showed significant activity against various viral symptoms, though virus and viral diseases were not known at that time. Hence in this article we made an attempt to prove Ayurveda had once combated and still combating against symptoms of viral infections and hence viruses. In modern literatures the antiviral activities of plants (already present in Ayurveda) like *Dracaena cinnabari, Cicer arietinum, Achyranthes aspera, Melia azedarach, Piper longum, Acacia nilotica, Ocimum sanctum, Euphorbia hirta, Alpinia galanga, Cardiospermum halicacabum* and *Carica papaya* were reported. In the present review a comparison was made of the symptoms of viral diseases with therapeutic uses (by translating from Sanskrit to English) of the above plants reported in Ayurvedic Pharmacopoeia of India. There is good co-relation between the symptoms of viral infections and therapeutic uses of Ayurvedic plants, which proves Ayurveda once combated well against symptoms of viral infections and hence viruses. We have made an attempt to show Ayurvedic drugs were successful in combating various viral infections.

Key words: Ayurveda, Viruses, Ayurvedic Pharmacopoeia of India, Symptoms.

Introduction

The term Ayurveda is derived from two words, '*Ayur*' means 'healthy life' and '*veda*' means science.[1] Charaka-Samhita, which is considered to be the oldest book in Ayurveda, along with Sushruta-Samhita and Samhitas of Vagbhataand are considered as *Brihat-trayee.*[2, 3] Ayurveda is divided into eight branches which include internal medicine, treatment of head and neck disease, surgery, pediatrics, toxicology, purification of genetic organs, health and longevity and psychiatry.[4]

In Ayurveda, *Prakruthi* is the body's constitution and according to it, the universe and its subjects are made up of five elements namely earth, air, water, fire and ether. Ayurveda says these five elements with their unique combination make three *doshas* namely *vata, pitta, kapha*. Air and etheral elements are associated with *vata,* fire and water with *pitta*, water and earth with *kapha*. Based on imbalance of these *doshas* the person may develop diseases. When these are in appropriate levels then it is called healthy condition, and if there is any difference in the balance it leads to *vikruthi*, which is ill health. This may leads to *vata dosha*, *pita dosha* and/or *kapha dosha.*[5,6]

Viruses are obligate intracellular parasites, having no internal cellular structure and metabolism. Inside the living organisms they behave as living cells by undergoing replication but outside they remain as inert chemical structures.[7] Various Ayurvedic plants are used for treating symptoms caused by different viral infections. By comparing the therapeutic uses of Ayurvedic plants with symptoms of viral infections, we made an attempt to prove Ayurveda, in the past itself, was successful in combating viral infections.

Plants that can be used as antivirals

Herpes Simplex Virus (HSV) infection is characterized by ulcers, blisters, fever, genital lesions causing burning sensation and enlargement of lymph nodes in the neck.[8, 9] The activity of *Dracaena cinnabari* against HSV was reported by Mothana RA *et al.* They reported methnaolic extract of *Dracaena cinnabari* have activity against HSV.[10] The same plant is referred as *Lohitaniryāsa* in Ayurveda, reported for symptoms like *Vrana* (ulcer) *Raktārśa* (bleeding piles), *Raktapitta* (bleeding disorder), *Rakta-Pradara* (menorrhagia), *Raktasrāva* (bleeding disorder).[11] *Cicer arietinum* had been reported for HSV by Asuman Kan *et al.* who reported that methanolic extract of seeds obtained from *Cicer arietinum* was having activity against HSV.[12] This plant is named as *Cānaka* in Ayurveda has been used for similar symptoms of HSV infection like *Annadravaśūla* (gastric ulcer), *Jwara* (fever) and *Dāha* (burning sensation).[13] Hemantha Mukherjee *et al.* established the antiviral activity of *Achyranthes aspera* against HSV. The methanolic extract (ME) and oleanolic acid (OA) showed significant antiviral activity against HSV. The activity was assessed by Minimum Inhibitory Test (MIT) and Plague Reduction Assay where positive control was Acyclovir and negative control was DMSO (Dimethyl sulfoxide). ME and OA inhibit both HSV-1 and HSV-2 with an EC_{50} of 64.4 µg/ml and 6.8 µg/ml for HSV-1F and 72.8 and 7.8 µg/ml for HSV-2G.[14] In Ayurveda, *Achyranthes aspera* which is referred as *Apāmārga,* reported for *Sula* (pain), *Udara roga* (diseases of abdomen), *Apaci* (cervical adenitis) the same symptoms of HSV infection.[15] So, the above mentioned plants of Ayurvedic origin have anti HSV activities.

Kuniaki applied a patent on an agent obtained from the water or organic solvent extract of *Melia azedarach* containing anti-influenza activity.[16] Symptoms include fever, running nose and breathing problem.[17] The same plant called as *Mahanimba* is used in Ayurveda for treating *Jwara* (fever) and *Śvāsa* (respiratory distress and breathing difficulty). By this it can be estimated *Melia azedarach* once used for treating influenza infections.[18]

Piper longum had been reported to have activity against Hepatitis B Virus (HBV) infection. The common symptoms of HBV include appetite loss, fatigue, low fever, muscle and joint aches, nausea and vomiting.[19] *P. longum* known as *Pippalīmūla* in Ayurveda is reported to have activity against similar symptoms like *Udararoga* (diseases of abdomen) *Gulma* (abdominal lump) *Krmiroga* (worm infestation).[20] Its activity against HBV had been proved by Zhi-Yong Jiang *et al.*. They found that piperine is able to inhibit HBV and inhibited the secretion of Hepatitis B surface

antigen and HBV E antigen. The ethanolic extract was obtained, from where different compounds were isolated among which erythro-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2E-nonenyl]-piperidine, threo-1-[1-oxo-9 (3,4-methylenedioxyphenyl) 8,9-dihydroxy-2E-nonenyl]-piperidine, piperine, guineesine found to have anti HBV activity.[21]

Human Immunodeficiency Virus (HIV) clinical manifestations include diarrhea, red rash that does not itch, fever and flu like infections.[22] The aqueous extract from pods of *Acacia nilotica* had been reported by Khan *et al.,* having activity against HIV by inhibiting reverse transcriptase enzyme.[23] The same plant called as ***Babbūla*** has been used in Ayurveda for *Kustha* (Leprosy / diseases of skin), *Atisara* (Diarrhea), ***Kāsa*** (Cough) and *Krmiroga* (worm infestation), symptoms of HIV infection.[24] *Ocimum sanctum* activity against HIV was reported by Anuya A Rege *et al.* who stated aqueous extract of *Ocimum sactum* can inhibit reverse transcriptase enzyme of HIV.[25] Even in Ayurveda this plant called *Tulasi* has been reported for *Krmiroga* (worm infestation), *Kustha* (Leprosy / diseases of skin), ***Kāsa*** (cough), ***Śvāsa*** (Asthma) and ***Pratiśyāya*** (coryza), similar to symptoms mentioned above.[26] *Euphorbia hirta* has been reported its activity against HIV1 and HIV2. The plant which is referred as *Dugdhik* in Ayurveda reported for treatment of symptoms like *Krmiroga* (worm infestation), ***Kāsa*** (cough), *Kustha* (Leprosy/diseases of skin), *Mutrakrichhra* (dysuria), *Puyameha* (urinary infection), *Sula* (pain / colic), *Tamakasvasa* (bronchial asthma) in Ayurveda which proves this had been used for symptoms of HIV.[27] *Euphorbia hirta* anti-HIV activity was reported by Gyuris Agnes *et al*. They found 50% methanolic extract of *Euphorbia hirta* aerial parts had activity on MT4 human T lymphocyte cell line. The aqueous extract was having dose dependent reverse transcriptase inhibition activity on HIV 1 and HIV 2.[28]

Alpinia galanga is a plant whose activity was reported against HIV which has flu like symptoms, swollen lymph glands and ulcers in the mouth. *A. galanga* in Ayurveda referred as *Kulanjana* has reported activity in Ayurveda against similar symptoms like *Pratisyaya* (coryza) *Svasa* (Asthma) *Hikka* (hiccups) *Sopha* (oedema) *Udara roga* (diseases of abdomen).[29] The anti-HIV activity was reported by Ying Ye and Baoan Li. The methanolic extract was found to contain 1'S-1'-Acetoxychavicol acetate which found to inhibit reverse transport, an essential factor for HIV replication.[30] In Ayurveda, *Cardiospermum halicacabum* referred as *Karnasphota* has reported activity against *Jwara* (fever) *Sopha* (oedema) *Pandu* (anemia) *Sula* (pain).[31] The similar symptoms are also present for HIV infection like fever, swollen lymph glands which states *Cardiospermum halicacabum* has activity against HIV symptoms. Kasi Murugan *et al.* has reported methanolic extract and petroleum ether extract are able to inhibit reverse transcriptase of HIV.[32] This gives a proof that above mentioned plants are successful in treating the various symptoms of HIV infection in the past itself.

Carica papaya had reported to contain activity against dengue which is mainly characterized by Dengue Hemorrhagic fever.[33] In Ayurveda *Carica papaya* is called *Erandkarkati* has been reported for activity against *Krmiroga* (worm infestation), ***Kāsa*** (cough), *Raktavikara* (disorders of

blood).[34] This gives a justification that *Carica papaya* can be used for symptoms of Dengue. Similar results were found by Nisar Ahmad *et al*. There was a disturbance in blood cell levels and reduction of platelet count from 176 x 10^3/µL to 55 x 10/µL, white blood cells from 8.10 x 10^3 /µL to 3.7 x 10^3/µL , and neutrophils count from 84.0% to 46.0%. After administrating the aqueous extract from leaves of C*arica papaya* the values raised from 55 x 10^3/µL to 168 x 10^3/µL, WBC from 3.7 x 10^3/µL to 7.7 x 10^3/µL and neutrophils count from 46% to 78.3%, which is showing *Carica papaya* has effect on Dengue Haemorrhagic fever.[35]

Conclusion

Viral diseases are always a threat for human health and there is a need of development of efficient antiviral drugs. Ayurvedic plants have proved to be valuable sources as antiviral agents in treatment of many viral diseases such as Dengue, Human Influenza Virus, Influenza virus, Herpes Simplex Virus, Hepatitis viruses etc . By comparing the modern literature, symptoms of various diseases with Ayurvedic uses in Sanskrit, we can say Ayurveda, from olden times and still used for treating various viral diseases and the problem is conversion of Sanskrit into modern languages.

References

1. S Mondal. Science of Exercise: Ancient Indian Origin. J Assoc Physicians India. 2013; 61:40-42.
2. K Das, B Kumar. Ancient and Pharmacological Review on *Centella asiatica* (M*andukparni*): A Potential Herbal Panacea. Int J Res Rev Pharm Appl Sci. 2012; 2:1062-1072.
3. M Paliwal, PS Byadgi. Sushruta: A Great Surgeon and Visionary of Ayurveda. Int J Res Ayurveda Pharm. 2012; 3(1):43-46.
4. http://www.medindia.net/ayurveda/ayurveda-branches.asp [Accessed on 2013 Nov, 23].
5. Premal Patel. Ancient Remedies for a Modern You: An Introduction to Ayurveda. Banyan Botanicals Ayurvedic Herbs. http://www.banyanbotanicals.com/wp-content/uploads/2011/08/Banyan-Ayurveda-Ebook-2_22_13.pdf [Accessed on 2013 Nov, 24].
6. Rama Jayasundar. Ayurveda: A Distinctive Approach to Health and Disease. Curr Sci. 2010; 8(7):908-914.
7. Prescott, Harley, Klein. The Viruses: Introduction and General Characteristics. In: Microbiology. The McGraw-Hill Companies; 2002. 362-378
8. http://www.webmd.com/genital-herpes/guide/common-symptoms [Accessed on 2013 Nov,24].
9. Available from: http://www.drugs.com/cg/oral-herpes-simplex-virus-infections.html [Accessed on 2013 Nov, 24].
10. M Ramzi, M Renate, R Christiane, U Lindequist. Phytochemical Screening and Antiviral Activity of Some Medicinal Plants from the Island Soqotra. Phytother Res. 2006; 20:298-302.
11. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 6, Government of India, Ministry of

Health and Family Welfare, Department of Ayush, 104-105.

12. A Kan, B Özçelik, M Kartal. In Vitro Antiviral Activities under Cytotoxic Doses against Herpes Simples Type-1 and Parainfluensa-3 viruses of *Cicer arietinum* L. (Chickpea). Afr J Pharm Pharmacol. 2009; 3(12):627-631.
13. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 6, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 29-31.
14. M Hemanta, O Durbadal, B Paromita, HS Chandel, Bhattacharyya S, TK Chatterjee, PK Mukherjee, S Chakraborti, D Chattopadhyay. Anti-Herpes Virus Activities of *Achyranthes aspera*: An Indian Ethnomedicine, and its Triterpene Acid. Microbiol Res. 2013; 168(4):238-244.
15. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 2, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 7-9.
16. N Kuniaki. Agent Containing *Meliaceae* Plant extract for Preventing and Treating Influenza. Japan Patent 2007254319, A, October 04, 2007.
17. http://www.webmd.com/cold-and-flu/flu-guide/adult-flu-symptoms [Accessed on 2013 Nov,25].
18. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 6, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 79-80.
19. http://www.nytimes.com/health/guides/disease/hepatitis-b/ [Accessed on 2013 Nov, 29].
20. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 2, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 133-134.
21. ZY Jiang, W Liu, X Zhang, J Luo, Y Ma, J Chen. Anti-HBV Active Constituents from *Piper longum*. Bioorg Med Chem Lett. 2013; 23(7):2123-2127.
22. http://aids.gov/hiv-aids-basics/hiv-aids-101/signs-and-symptoms/ [Accessed on 2013 Dec, 2].
23. D Gupta, A Goel, AK Bhatia. Studies on Antiviral Properties of *Acacia nilotica*. J Environ Res Dev. 2010; 5(1):150-151.
24. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 1, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 41-42.
25. A A Rege, RY Ambaye, RA Deshmukh. In-Vitro Testing of Anti-HIV Activity of Some Medicinal Plants. Indian J Nat Prod Resources. 2010; 1(2):193-199.
26. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 2, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 165-167.
27. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 6, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 23-25.
28. Á Gyuris, L Szlávik, J Minárovits, A Vasas, J Molnár, J Hohmann. Antiviral Activities of Extracts of *Euphorbia hirta L*. against HIV-1, HIV-2 and Sivmac251. In Vivo 2009; 23(3):429-432.
29. The Ayurvedic Pharmacopoeia of India, Part 1, Volume 2, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 90-92.
30. Y Ye, B Li. 19S-19-Acetoxychavicol Acetate Isolated from *Alpinia galanga* Inhibits Human

Immunodeficiency Virus Type 1 Replication by Blocking REV Transport. J Gen Virol. 2006; 87:2047-2053.

31. The Ayurvedic Pharmacopoeia of India, Part 1, Volume V, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 67-70.
32. K Murugan, RV Prabu, S Sangeetha, SA Sohaibani. Antiviral Activity of *Cardiospermum halicacabum L.* Extract against Co-Infecting Agents HIV and HBV. J Herbs Spices & Med Plants. 2011; 17(4):403-418.
33. http://www.denguevirusnet.com/signs-a-symptoms.html [Accessed on 2013 Dec, 10].
34. The Ayurvedic Pharmacopoeia of India, Part 1, Volume VI, Government of India, Ministry of Health and Family Welfare, Department of Ayush, 47-48.
35. N Ahmad, H Fazal, M Ayaz, BH Abbasi, I Mohammad, L Fazal. Dengue Fever Treatment with *Carica papaya* Leaves Extracts. Asian Pacific J Trop Biomedicine. 2011; 1(4):330-333.

HPLC Method Development for Qualitative Analysis of different components of Soft Resin obtained from different strains of Lac Insect *Kerria lacca* (Kerr)

Shailesh Kumar Singh[1]*, A. S Vidyarthi[1], K Mukhopadhyay[1], R. Ramani[2], Barij Nayan Sinha[1]

[1]*Birla Institute of Technology, Mesra, Ranchi, Jharkhand*

[2]*Indian Institute of Natural Rasins and Gums, Namkum, Ranchi, Jharkhand*

Abstract

Soft resin is the important constituent of lac obtained from insect *Kerria lacca* (Kerr) For the purpose of component analysis qualitatively; a HPLC method has been developed. As the previos work suggests the presence of four monesters in it, the HPLC chromatogram shows four major peaks. Further a qualitative estimation has been done for lac samples obtained from 20 different strains of lac insect.

Key words: Lac, Soft Resin, Hard Resin, HPLC

Introduction

Lac, a versatile resin, secreted by a tiny insect, *Kerria lacca* (Kerr) is the only commercial resin of animal origin and has considerable socioeconomic significance for India. Lac insect belongs to super family Coccoidea which includes all scale insects. Scale insect is a common name for about 2000 insect species found all over the world. Scale insects range from almost microscopic size to more than 2.5 cm. These insects attach themselves in great numbers to plants. The mouth part of these insects is piercing and sucking type. They can be very destructive to tree-stunting or killing twigs and branches by draining the sap. There are six genera of lac insects, out of which only five secrete lac, and only one, i.e. *Laccifer* secretes recoverable or commercial lac. The commonest and most widely occurring species of lac insect in India is *Laccifer lacca* (Kerr) which produces the bulk of commercial lac.

Since the lac insects thrive and feed on certain species of the tropical trees, it is found distributed in South-East Asian countries. Lac is currently produced in India, Myanmar, Thailand, Malaya, Lao and Yuan province of China. India and Thailand are main areas in the world, while India has prime position in relation to lac production. Lac cultivation is introduced into Thailand from India.

Over 90% of Indian lac produced comes from the states of Bihar, Jharkhand, West Bengal, Madhya Pradesh, Chattisgarh, Eastern Maharashtra and northern Orissa. Some pockets of lac cultivation also exist in Andhra Pradesh, Punjab, Rajasthan, Mysore, Gujarat, and Mirzapur and Sonebhdra districts of Uttar Pradesh.

Broken branches are sold as stick lac and, after grounding and washing with water to

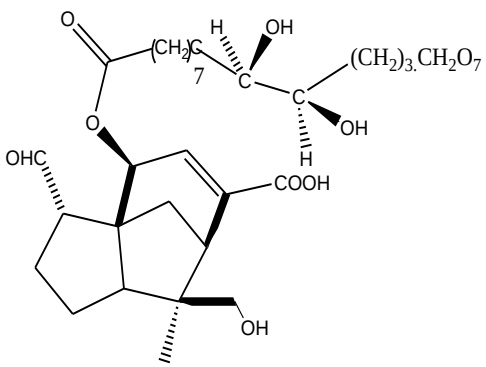
eliminate wood and red pigments (lac dye), seed lac is obtained. Purification of seed lac gives the more homogeneous product known as shellac. Its use in Europe began towards the end of the 16th century mainly as a varnish (mostly known as ‘‘French polish’’) for wooden objects, musical instruments and gilding, as a protective for vinyl disks and mural paintings, as an insulating material for earlier radios and other electrical tools and as an adhesive in the restoration of pottery.

The chemistry of this material has been under investigation, for over a century and a half Stick-lac, the composition of which is dependent to some extent, on the nature of the host tree on which the insect feeds, consists of wax (6-7%), colouring matter (4-8%), “resin” (70-80%), insect debris, moisture and other extraneous matter.

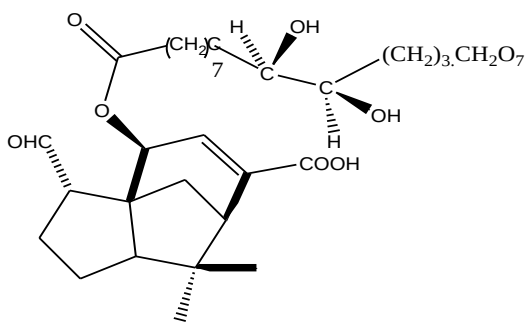
The resin constituting the backbone of shellac is a complex mixture made of mono- and polyesters of hydroxy aliphatic and sesquiterpenoid acids, which can be separated into two fractions: the soft resin, soluble in ether, mainly consisting of monoesters, constituting about 30% of the total resin, and the hard resin, ether insoluble, which has quite complex polyester composition, and takes into account 70% of the total resin. The main shellac ester components are jalaric and laccijalaric acids, 9,10,16- trihydroxyhexadecanoic (aleuritic acid) and 6-hydroxytetradecanoic acids (butolic acid) and its chemical composition seems almost constant, although the amount of some components.

The primary work on the investigation of structure of the components of soft resin was carried out by Sukh Dev et al (1973). The objective of their work was to see if soft resin contains interesters of substantially lower molecular weight than those found in hard resin. By combination of fractional solvent precipitation and chromatography, they isolated all four constituents in essentially pure form. The established the structures using chemical methods and those structures were further supported by their PMR and borne out by partial synthesis.

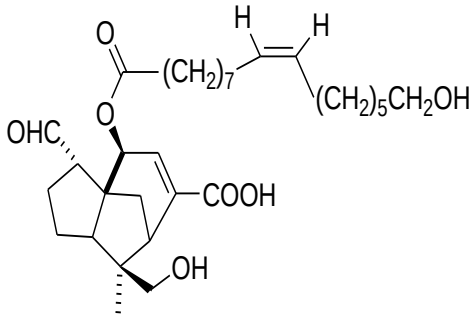
The structures of all constituents of soft resin so far reported are as follows:



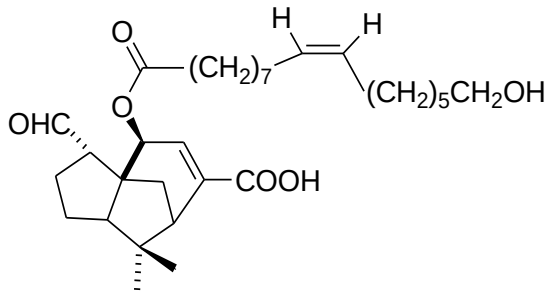
Jalaric acid ester - I



Laccijalaric acid ester - I



Jalaric acid ester - II



Laccijalaric acid ester - II

Various analytical techniques such as chemical analysis and chromatographic and spectrometric methods have been applied to semiquantitative determinations of shellac resins. Khurana et al. and Upadhye et al. systematically reported a procedure for the purification, identification, and semiquantitative determination of the acidic components of a shellac resin. Chauhan et al. analyzed the alkaline hydrolysis products of shellac after converting them into their methyl ester trimethylsilyl ethers followed by gas chromatography (GC).

However, all these methods involve cumbersome pretreatments such as chemical decomposition, derivatization, and/or solvent extraction were inevitably utilized to separate the resin constituents prior to the final analytical measurements. Moreover all the analytical techniques have been used with the objective to determine all the complex constituents of shellac as such or its products like art works or paintings. The discriminative analysis of composition of different constituents of soft resin obtained from different strains of lac insect as such is difficult with the reported methods.

So, objective of this study is to develop a more suitable HPLC method for qualitative analysis of different constituents of soft resin and then perform comparative analysis of them in soft resin samples obtained from different strains of lac insect *Kerria lacca* (Kerr).

Materials and Methods

Materials

Sticklac samples were obtained from Indian institute of Natural Resins and Gums, Namkum, Ranchi, India. HPLC grade acetonitrile and methanol were purchased from sigma Aldrich. Water for HPLC was Milli Q obtained from Millipore filteration system. All other reagents used were of analytical grade.

The chromatography equipment consists of WATERS 510 HPLC with binary pump system, online degasser and single wavelength UV detector. The column used for chromatography is Bondapak C-18 column (4.6 X 250).

Isolation of Soft resin from Sticklac

The method finally worked out for the isolation of "hard resin" is an adaptation of the method of Tschirch and Ludy' with the important modifications of using 78% aqueous ethanol, rather than absolute ethanol, for dewaxing and the precipitation of "hard resin" from ethanolic solution by ether, rather than by water. Seedlac (3 g; 200 mesh) was stirred with distilled water (100 m1) for 15 min, the aqueous part decanted and the process of washing repeated (another 4 times). The washed lac powder was air-dried at room temperature and the resulting material thoroughly dispersed in 78 % Ethanol (30 ml) with mechanical stirring (30 min). The insoluble material on extraction with hot benzene (20 ml x 5) and usual work up gave light orange shellac wax. The total Ethanol extract was concentrated under reduced press (water bath) to 1/5th its volume. The concentrate was cooled and with vigorous stirring slowly diluted with ether (50 ml.). The precipitate was collected by straining through muslin cloth and the product was titurated with ether (4O ml x 3) to furnish an insoluble material, which was soxhleted (12 hr) with dry ether to finally yield the insoluble portion as the "hard resin".

The various ether extracts and washings, mentioned above, were combined and the solvent flashed off. The residue was taken up in chloroform, filtered and the Chloroform, solution extracted with sodium carbonate aq (15 %; 25 ml x 6). The alkaline extract was acidified (phosphoric acid aq 1:1) and the product taken up in Ethyl acetate (25 ml x 5). The combined ethylacetate extracts were washed with brine and worked up to give "soft resin" as a dark red sticky mass.

Estimation of variation with respect to soft resin content, carried out for 30 lac insect lines following the same scheme.

Thin Layer Chromatographic studies of soft resin

TLC of soft resin solution in methanol was carried out using the solvent system; Hexane: Ethyl acetate: glacial acetic acid::20: 80:3. TLC of soft resin showed that four spots are predominating and out of which two are predominating (R_f 0.40 and 0.15).

UV Spectrophotometry Studies

The UV absorption of soft resin was estimated taking methanol as blank and the sample was scanned between the UV regions of 190 to 400. The maximum absorbance was found at 220 nm.

HPLC Method Development

A HPLC protocol has been standardized for the component analysis of soft resin of different strains of lac insect. Several methods have been tried with isocratic flow of solvent system and the best suited protocol for separation of different fractions of soft resin, the solvent system containing Methanol: Water : Acetonitrile (9:0.5:0.5) provided optimum separation (Flow rate- 0.45 ml/min,

C-18 column). The components of soft resin were not resolved properly using isocratic solvent system; the reason may be the very close polarity of acids found in the resins. The method has been again modified and gradient mobile (water: acetonitrile) phase elution has been adopted.

The sample for HPLC is prepared by dissolving 20 mg of soft resin in 10 ml of HPLC grade methanol and then filtering it through 0.45 micrometer syringe filter. The following gradient system has been used:

Time (min)	Flow rate (ml/min)	Water (%)	Acetonitrile (%)	Wavelength (nm)
0.00	1.00	80	20	220
3.00	1.00	80	20	220
15.00	1.00	10	90	220
24.00	1.00	10	90	220
25.00	1.00	80	20	220
30.00	1.00	80	20	220

Result and Discussion

The amount of soft resin is estimated for 20 samples of seedlac obtained from different strains of lac insect *Kerria lacca* (Kerr). The percentage is calculated for each sample from the initial amount of seedlac taken. A comparative table for percentage of hard resin and soft resin is prepared.

Table: 1. Estimation of resins from seedlac obtained from 20 different strains of lac insect

S.N.	CODE	Sticklac (gm)	Seedlac (gm)	Initial weight (gm)	Amount of total Resin (gm)	Amount of Hard Resin (gm)	% of Hard Resin	Amount of Soft Resin	% of Soft Resin
1	LIK0031	14.75	11.22	3.00	2.45	2.12	86.5	0.20	8.16
2	LIK0036	23.57	16.52	3.00	2.29	2.00	87.3	0.17	7.4
3	LIK0038	18.82	12.08	3.00	2.34	2.00	85.4	0.18	7.6
4	LIK039	43.95	32.62	3.00	2.52	1.72	68.5	0.60	23.8
5	LIK0040	21.30	17.00	3.00	2.12	1.63	76.8	0.37	17.4
6	LIK0042	26.20	22.96	3.00	2.30	1.60	69.5	0.51	22.1
7	LIK0065	71.45	62.40	3.00	2.60	2.02	77.6	0.31	11.9
8	LIK003	25.04	19.60	3.00	2.20	1.56	70.9	0.42	19
9	LIK002	4.87	3.86	3.86	2.90	2.23	76.8	0.62	21.3

S.N.	CODE	Sticklac (gm)	Seedlac (gm)	Initial weight (gm)	Amount of total Resin (gm)	Amount of Hard Resin (gm)	% of Hard Resin	Amount of Soft Resin	% of Soft Resin
10	LIK059	2.66	1.90	1.90	1.34	0.97	72.3	0.19	14.1
11	LIK0031	14.75	11.22	3.00	2.45	2.12	86.5	0.20	8.16
12	LIK008	2.57	1.48	1.45	1.18	0.88	74.5	0.18	15.2
13	LIK025	1.34	0.84	0.80	0. 62	0.46	74.1	0.11	17.7
14	LIK056	0.71	0.49	0.45	0.35	0.21	60	0.07	20.0
15	LIK020	5.07	3.39	3.00	2.52	1.83	72.6	0.44	17.4
16	LIK0023	1.75	0.94	0.90	0.72	0.46	63.8	0.13	18
17	LIK046	1.32	0.84	0.80	0.44	0.25	56.18	0.09	20.4
18	LIK004	4.25	2.73	2.70	2.10	1.48	70.4	0.41	19.5
19	LIK021	2.85	1.10	1.00	0.70	0.41	58.57	0.10	14.2
20	LIK0014	1.54	0.84	0.80	0.61	0.37	60.6	0.09	14.75

The soft resin samples were then run in both isocratic and gradient system of mobile phase for obtaining a HPLC chromatogram representing the each monoester at different retention times.

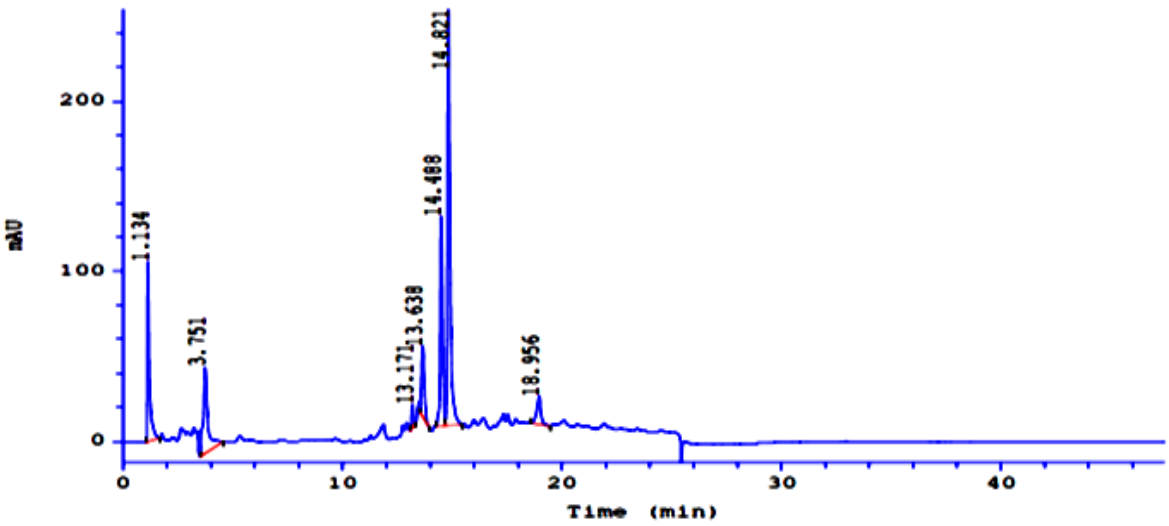


Fig: 1. HPLC chromatogram of soft resin using isocratic mobile phase

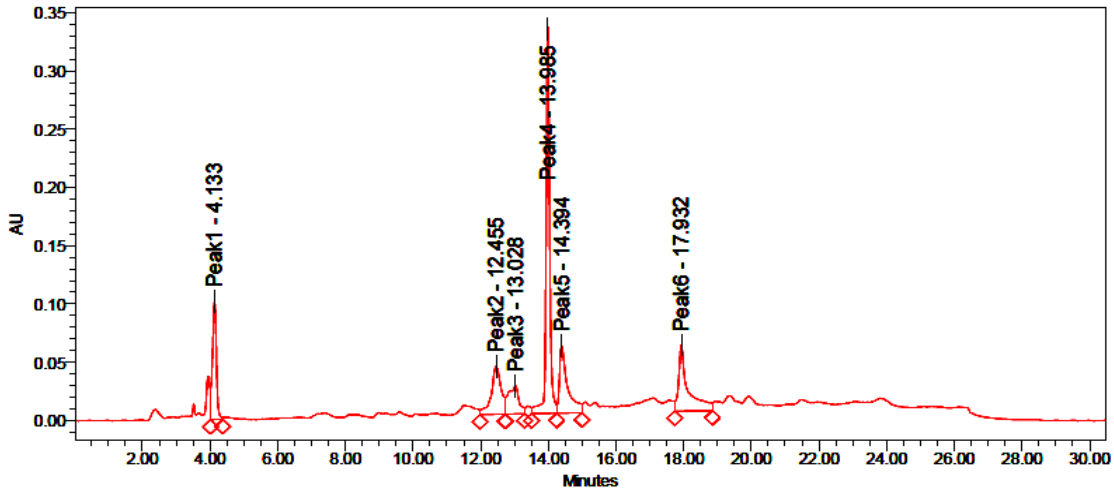


Fig: 2. HPLC chromatogram of soft resin using gradient mobile phase obtained from LIK0031

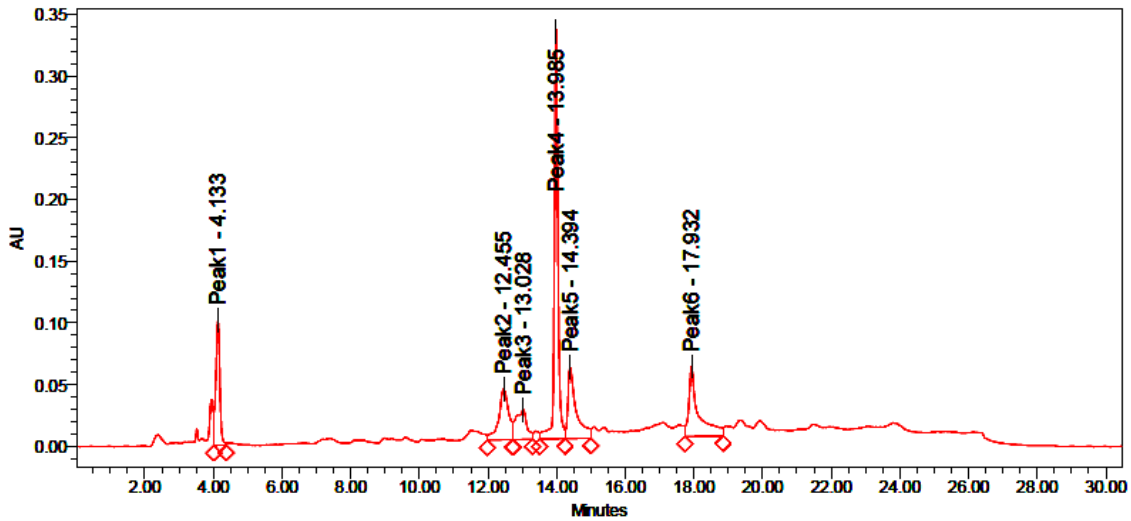


Fig: 3. HPLC chromatogram of soft resin using gradient mobile phase obtained from LIK0039

The HPLC chromatograms showing major peak at retention times 4.074 min, 12.91 min, 13.90 min, 14.17 and 17.91 for majority of samples. The qualitative estimation on the basis of absence or presence of peaks at these particular retention times has been done for all 20 samples. The absence or presence of peak at a particular retention time is shown by denoting by the symbols 0 or 1 respectively.

Table: 2. Comparative analysis for different components of soft resin using HPLC

S.N.	Lac Strain	RT- 4.074	RT- 12.916	RT-13.90	RT-14.17	RT-17.814
1	LIK0003	1	1	1	1	1
2	LIK0021	1	0	1	1	1
3	LIK0031	1	0	1	1	1
4	LIK0036	1	0	1	1	1
5	LIK0038	1	1	1	1	1
6	LIK0039	1	1	1	1	1
7	LIK0042	1	0	1	1	1
8	LIK0055	1	1	1	1	1
9	LIK0040	1	1	1	1	1
10	LIK0002	1	1	1	1	1
11	LIK0023	1	0	1	1	1
12	LIK0059	1	0	1	1	1
13	LIK0014	1	1	1	1	1
14	LIK0065	1	1	1	1	1
15	LIK0025	1	1	1	1	1
16	LIK0056	1	0	1	1	1
17	LIK0004	1	0	1	1	1
18	LIK0008	1	1	1	1	1
19	LIK0046	1	1	1	1	1
20	LIK0020	1	1	1	1	1

The four peaks are present in almost all the samples indicating the presence of four monoesters in soft resin obtained from each sample.

Conclusion

The soft resin containing four monoesters is having relatively higher UV absorbance at wavelengths more than 210 nm than the polymeric esters present in hard resin which show negligible UV absorbance at wavelengths above 210 nm. The monomeric nature of esters enables to obtain reproducible chromatograms for each sample of soft resin which helps in giving a better picture about the different components. The unavailability of standards of these monomeric esters is the only limitation for estimating quantitatively each component.

Acknowledgement

The authors are thankful to Dr. K. K. Sharma and Lac production division of Indian institute of Natural Gums and Resins for providing lac samples obtained from different strains of lac insect.

References

1. Xia, W., Zhang, L. J., Ming, Y. F., Juan, X. J. 2006; Present research on the composition and application of lac. Forestry Studies in China. 8, 64-70.
2. Sharma, S. K., Shukla, S. K., Vaid, D. N. 1983; Shellac-Structure, Characteristics & Modification.Defense Science Journal. 33, 261-271
3. Khurana, R. G., Singh, A. N., Upadhye, A. B., Mhaskar, V. V., and Sukhdev. 1970; Chemistry of lac resin-iv- pure lac resin-l: isolation and quantitative determination of constituent acids.Tetrahedron. 26, 4177- 418.
4. Singh, A. N., Upadhye, A. B., Mhaskar, V. V., and Sukhdev. 1973; chemistry of lac resin-vi “components of soft resin”. Tetrahedron. 30, 867 - 874.
5. Schaeffer, B.B., Gardner, H.W. 1938. Nature and constitution of Shellac. Industrial and Engineering Chemistry. 30, 333-35.
6. Sao, K. P., Pandey, S. K., and Baboo, B. 2008; Formaldehyde-free sticklac and arhar stick composite board. Journal of Scientific and Industrial Research. 67, 314-18.

Is India a dumping ground for drugs and Indian population guinea pigs?

Suresh R. Saravdekar

Ex- Assistant Director, Ministry of Medical Education & Drugs, State of Maharashtra

Introduction

Indian pharmaceutical industry currently ranks third in terms of volume of production (10% of global production share). Globally, India ranks at 1st in exports of pharmaceuticals by volume. It provides about 40% of the generic and over-the-counter drugs to U.S. and other developed countries. Now, India is called **as "Pharmacy of the world".**

However , this growth of Indian Pharmaceutical industry is solely, based on economics of business and return on investment by all possible means , even putting the safety and health of common man at risk. Why I am saying so? Let's have a close look at the scenario of how drugs and medicines are made available and used in India. Let's ask some basic questions and try to find ourselves answers.

1. Which medicines are required in India?

The needs of medicines of any country depend on the diseases prevalent in the country. In India, our major population is mainly affected by infectious and communicable diseases like Tuberculosis, Malaria, HIV AIDS, Pneumonia, Typhoid, Diarrhea, Hepatitis, Leptospirosis, Dengue, Chicken-Gunea, Anemia etc. So, Indian population needs medicines primarily to treat these diseases. The threat of non-communicable diseases like diabetes is emerging in a big way to increase disease burden.

2. How many medicines India need?

Based on the disease profile in India, the medicine experts at National level have prepared a list of essential drugs called "National List of Essential Medicines "(NLEM). This list contains 348 medicines. If all necessary formulations of these drugs summed up, still the total number of medicines required in India will not be more than 1000 formulations of these 348 essential medicines.

3. As against above requirement, how many medicines are licensed to market in India?

Presently, approximately 90,000 different formulations of medicines have been licensed and are being marketed in India as against requirement of 1000 formulations.

4. Which are the topmost selling medicines in Indian market?

Following is the list of top 26 selling brands of medicines in India (2013-14, IMS data-) arranged as highest selling product at the top of list-

1. COREX
2. PHENSEDYL
3. HUMAN MIXTARD30/70
4. VOVERAN
5. REVITAL
6. AUGMENTIN
7. MONOCEF
8. VOLINI
9. DEXORANGE
10. LIV-52
11. GLYCOMET-GP
12. CLAVAM
13. BETADINE
14. BECOSULES
15. GALVUS MET
16. TAXIM
17. ZIFI
18. THYRONORM
19. ACILOC
20. ZINETAC
21. TAXIM-O
22. ASTHALIN
23. ZINCOVIT
24. LANTUS
25. CALPOL
26. SHELCAL

With current rate of prescriptions written for these drugs, approximately Rs. 4600 Cr. are spent annually on the purchase of these top 26 brands of medicines.

5. How many of these top selling 26 brands of medicines are non-essential?

Look at those marked in red. Looking at the disease profile of India, the medicines marked in red, are definitely not relevant and hence not essential. With the current rate of prescriptions of these brands, approx Rs.2300 crores are spent annually on these 13 non-essential medicines (50% of the top 26 brands). Most of these medicines are sold on OTC and without prescription and denotes extent of self-medication in India. Though, the medicines Volini (diclofenac) & Shelcal (Calcium) are essential but their corresponding sales figures points merely at their overutilization & over consumption.

If we compare the expenditure on non-essential medicines with the annual health budget of some of the states (Shown in block below), there is sheer wastage of money while the needs are unattended.

Annual sale of few non-essential drugs

	Amt in Rs. Cr
Corex & Phynsydyl (288+274):	**562**
Revital:	**221**
Dexorange:	**230**
Liv 52:	**200**
Acoloc & Zentac (146 +146):	**292**
Becasules & Zincovit (166+142):	**308**
Total:	**1811**

National Annual State Health Budget of few states in India

	Amt in Rs. Cr
Manipur	**981**
Sikkim	**1767**
Mizoram	**2400**

6. What is the Focus of Research activities of World Pharmaceutical Industry?

- ✓ All Inventions are targeted only towards life style diseases prevalent in developed countries - i.e. mainly, diseases like Diabetes, Hypertension, Anxiety etc & Autoimmune diseases
- ✓ All Inventions are mainly targeted towards

  A**)** "Me Too Drugs" For example Pantoprazole, Lansoprozole, Rabeprazole, Esomaprozole, which are me too derivatives of omeprazole and, Paroxetine, Reboxetine, Duloxetin which are me too derivatives of Fluoxetin and

  B) Towards irrational Fixed Dose Combinations (FDCs) – For example

Diyclomine +Paracetamol
Diyclomine +Paracetamol+ Diclofenac
Diyclomine +Paracetamol+ Domperodol
Dicyclomine+ Paracetamol+ Clinidium+ dimethocone
Dicyclomine+ Chlordizepoxide+ Clinidium
Dicyclomine +Mafenamic acid+ Dimethylpolysiloxane
Dicyclomine +Nimesulide
Ranitidine + Ondansetron
Ranitidine + Domperidone

- ✓ Hardly any new drug invented to target diseases Prevalent in developing countries like India, e.g. - During last 5 decades, hardly any drug is invented to treat Tuberculosis, Malaria, Dengue, Leprosy, Hepatitis, Ameobiasis, etc.

It is not surprising then, that still no effective drugs are available for – Kala Azar, leptospirosis, filariasis and many diseases prevalent in these countries.

7. What is it that invented in India? Which are the medicines invented for diseases prevalent in India?

To encourage innovation Indian Government has provided 150 t0 200 % tax subsidy on R & D expenses, However not a single new drug invented by Indian pharmaceutical industry during last 60 years. Throughout these years, Indian manufacturers remained as mere generic manufacturers of the drugs required in the developed countries.

8. What is it that licensed in India? What is the licensing procedure adopted by our Central Drugs Standards Control Organisation (CDSCO)?

As per the **Findings of the Parliamentary Committee Report - May 2012, presented to the Rajya Sabha on 8th May'2012,** approximately, 1,600 applications were received annually by CDSCO, during 2005-2009. These applications included-

- NDA- New Drugs to be introduced for the first time in the country,

- Modified or new claims of old approved drugs , namely , new indications , new dosage forms, etc., and
- New Fixed-Dose Combinations (FDCs) of two or more drugs.

The committee found serious issues with the way approvals for foreign drugs are given and the clinical trials are being carried out.

Here are some of the startling findings:

- Of the 42 drugs picked up randomly for scrutiny by the panel, the health ministry could not provide any documents on three drugs - pefloxacin, lomefloxacin and sparfloxacin. *Reason:* their files were untraceable. The documents related to sponsors, clinical trials, overseas regulatory status, names of experts consulted and post-marketing safety reports are not traceable. "All these drugs had been approved on different dates and different years creating doubt if disappearance was accidental," says the report, adding that all these were "controversial" drugs. One was never marketed in the US, Canada, Britain, Australia and other countries, while the other two were discontinued. All three drugs are still sold in India.
- Of the 39 drugs on which information was available, the panel found that in the case of 18 drugs, adequate clinical trials had not been conducted - many of the drugs had been tested on fewer patients and in fewer hospitals than what is legally mandated.
- There are 13 drugs which were not sold in much of the developed world, and the report said none of these drugs "have any special or specific relevance to the medical needs of India".
- In the case of 25 drugs, the opinion of medically qualified experts was not obtained before approval.
- A total of 31 new drugs, by the health ministry's own admission, were approved between January 2008 and October 2010 without conducting clinical trials on Indian patients. The ministry says that the authorities have the power to approve drugs without clinical trials in the public interest. "No explanation," the report says, "is available as to what constitutes public interest."
- Every month, on average, the authorities say, one drug is approved in India without trials.
- A review of expert opinion on various drugs showed that an "overwhelming majority are recommendations based on personal perception without giving any hard scientific evidence or data". More shockingly, the panel found adequate evidence to conclude that "many opinions were actually written by the invisible hands of drug manufacturers and experts merely obliged by putting their signatures".
- The panel believes that there is "sufficient evidence to conclude that there is collusive nexus" between drug makers, authorities and some medical experts.
- This is not all. The panel expresses concern over the continued sale of potentially

harmful drugs in India, years after such products were banned or withdrawn in developed countries and the prevalence of "sub-standard" drugs is 7-8% of total sales in the Indian market.

The present drug situation in India is paradoxical. As per WHO findings, 80% Indian population is paying for health care out of pocket (OPP), still around 50% Indian population does not have access to even essential medicines. World Bank study has concluded that - ***"In India, Out-of-pocket medical costs alone may push 2.2% of the population below the poverty line in each year"***

The above facts & findings raise one shocking and alarming question in our minds -

Is India a dumping ground for drugs and Indian population guinea pigs?

We need answer to this agonizing question from our policy makers, legislators, regulators and the pharmaceutical industry.

References

1. AIOCD Pharma Data on Pharmaceutical Market
2. National List of Essential Medicines
3. Department-Related Parliamentary Standing Committee On Health And Family Welfare - *Fifty-Ninth Report On The Functioning Of The Central Drugs Standard Control Organisation (Cdsco) (Presented To The Rajya Sabha On 8th May, 2012)*

Instructions to Authors

PHARMBIT is an official scientific journal and biannual publication of Pharmaceutical Society of Department of Pharmaceutical Sciences, Birla Institute of Technology, Ranchi. The journal is devoted to publish review and research articles in pharmacy and the related disciplines of pharmaceutical education. PHARMBIT is abstracted in Chemical Abstract, USA, Index Copernicus, and Natural Science Database, USA since 2008; making our publications International.

Manuscripts will be subjected to peer review process to determine their suitability for publication provided they fulfill the requirements of the journal. After reviewer's comments the revised manuscript should be submitted by e-mail or in CD prepared in MS Word.

Submission of a manuscript to PHARMBIT for publication implies that the same has not been either published or under consideration for publication in another journal. The author should confirm during submission of manuscript.

PREPARATION OF MANUSCRIPTS: RESEARCH PAPERS

Manuscripts should be concisely written and conform to the following general requirements: Manuscripts should be typewritten in 1.5 space in A4 sized sheets, only on one side, with a 1.0 inch margin on both sides. Research Papers, should not exceed 8-10 pages, Review Articles, 12-15 pages and Short Communications, 4-5 pages. Pages should be numbered consecutively, starting with the title page and the matter arranged in the following order: Title, Name and Address, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion or Results and Discussion, Acknowledgements and References. All other section titles should be in capital letters while subtitles in each section shall be in bold face lower case.

TITLE PAGE - Title page should contain title of the paper in bold face, title case (font size 14), names of the authors in normal face, upper case (font size 12) followed by the address(es) in normal face lower case. The author to whom all correspondence be addressed should be denoted by an asterisk mark.

ABSTRACT - Start on a new page after the title page and should be typed in single-space to distinguish it from the Introduction. Abstracts should briefly reflect all aspects of the study, as most databases list mainly abstracts. Short Communications as well as Review Articles should have an Abstract.

KEYWORDS – 4 to 5 Keywords related to topic

INTRODUCTION - Start immediately after the Abstract, as the next paragraph, but should be typed in double-space. The Introduction should lead the reader to the importance of the study; tie-up published literature with the aims of the study and clearly states the rationale behind the investigation.

MATERIALS AND METHODS - Start as a continuation to introduction on the same page. All important materials used along with their source shall be mentioned.

RESULTS - All findings presented in tabular or graphical form shall be described in this section. The data should be statistically analyzed and the level of significance stated. Results section shall start after materials and methods section on the same page.

DISCUSSION - This section should follow results, deal with the interpretation of results, convey how they help increase current understanding of the problem and should be logical. Results and discussion of results can also be combined under one section, Results and Discussion.

ACKNOWLEDGEMENTS - Should be given after the text and not in the form of foot-notes.

REFERENCES - References should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order). Identify references in text, tables, and legends by Arabic numerals in superscript.

ARTICLES IN JOURNALS

- KV Devi, RS Pai. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci. 2006; 68:1-6.

BOOKS AND OTHER MONOGRAPHS

- Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
- Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.
- Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2 ed. New York: Raven Press; 1995. p. 465-78.

ILLUSTRATIONS

Tables and Figures - They should be inserted within the text. Tables should not be very large that they run more than one A4 sized page. Tables should be numbered consecutively in Arabic

numerals and bear a brief title in lower case bold face letters above the table. Figures should be numbered consecutively in Arabic numerals and bear a brief title in lower case bold face letters below the figure.

PREPARATION OF MANUSCRIPTS: REVIEW ARTICLES

If should be about 15 pages long, contain up-to-date information, comprehensively cover relevant literature and preferably be written by scientists who have in-depth knowledge on the topic. All format requirements are same as those applicable to full papers. Review articles need not be divided into sections such as materials and Methods and results and Discussion, but should definitely have an abstract and introduction, if necessary.

PREPARATION OF MANUSCRIPTS: SHORT COMMUNICATIONS

The journal publishes exciting findings, preliminary data or studies that did not yield enough information to make a full paper as short communications. These have the same format requirements as full papers but are only up to 5 pages in length. Short Communications should not have subtitles such as Introduction, Materials and Methods, Results and Discussion - all these have to be merged into the running text. Short Communications preferably should have only 1-2 illustrations.

Submission: Authors are required to submit their manuscript by post or by e-mail (pharmbit@outlook.com).

Note: The Editor does not claim any responsibility, liability for statements made and opinion expressed by authors.

Dr. R. N. Gupta
Chief Editor, PHARMBIT
Scientific Journal of Pharmaceutical Society
Department of Pharmaceutical Sciences & Technology
BIRLA INSTITUTE OF TECHNOLOGY
MESRA, RANCHI-835215
Website: www.bitmesra.ac.in
Fax: 0651-2275290; Phone: 0651-2275444 (Ext.: 4423)
E-mail: pharmbit@bitmesra.ac.in

PHARMBIT

ISSN: 0973-6204

Scientific Journal of Pharmaceutical Society

Indexing in "Chemical Abstract & Natural Science Database"

Contents

Published by:

Pharmaceutical Society

Department of Pharmaceutical Sciences & Technology

BIRLA INSTITUTE OF TECHNOLOGY

Mesra, Ranchi, Jharkhand (INDIA)

pharmbit@bitmesra.ac.in