INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
© VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N)
www.vmsindia.org

TOXICOLOGICAL STANDARDIZATION OF HAEMATINIC HERBAL FORMULATIONS MARKETED IN INDIA BY USING SPECIFIC MEDIAS AND AAS

Anil Chandewar[1], Sanjay Bais[2] and Ravindra Bakal[3]
[1]P.Wadhwani College of Pharmacy, Yavatmal, India--445001
[2]Mauli College Of Pharmacy, Udgir, India-413517
[3]College of Pharmacy, Sakegaon,Bhusawal-India
avchandewar@rediffmail.com

Abstract:
In India, drugs of herbal origin have been used in traditional systems of medicines such as *Unani* and *Ayurveda* since ancient times. W. H. O. limits for Escherichia coli 10^1/gm CFU, Staphylococus.aureus 10^5/gm cfu, and Pseudomonas aeruginosa 10^3 /gm cfu and Salmonella species-nil cfu. WHO mentions maximum permissible limits in raw materials only for arsenic, cadmium, and lead, which amount to 1.0, 0.3, and 10 ppm respectively. The main purpose of the investigation was to document evidence for the users, and practitioners of marketed Haematinic herbal formulations. In the present study Haematinic herbal formulations marketed in Yavatmal India were determined for the presence of microbial and heavy metal content. The investigations were performed by using Specific medias and atomic absorption spectrometry. The present work indicates that there is presence of Heavy Metal contents in Herbal formulations selected for study. It was found that Arsenic content in formulations was below the Permissible limit in all formulations. The Cadmium and lead content in six formulations which were above the permissible limits. Such formulations are injurious to health of patient if consumed regularly. The specific Medias were used to determining the presence of Escherichia coli 4 samples, Staphylococcus aureus 3 samples, and P. aeruginosa 4 samples. The data indicated suggest that there is requirement of in process improvement to provide better quality for consumer health in order to be competitive in international markets. The presence of microbial and heavy metal content above WHO limits indicate that the GMP was not followed during manufacturing of herbal formulations marketed in India.
Keywords:-Herbal formulation, Heavy metals, Micro-organism, AAS, Specific Medias

1.0 Introduction

Herbal medicines are plant derived materials and preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants, inorganic materials or animal origin. Herbal medicine preparations are developed and created drugs by the modern pharmaceutical industry. Nowadays, they are manufactured and sold most widely on the pharmaceutical market for curing diseases and promoting public health worldwide.[1]

Herbal drugs have been used since ancient times as remedies and treatment for a range of diseases. Western pharmaceutical drugs play a major role in modern medicine, but traditional medicine are used by approximately 60% of people in rural areas still make an important contribution in health care.[2]

In India, the unscientific methods of collection, storage, transportation and congenial climatic conditions make the raw materials of herbal drugs prone to fungal infestations. The raw materials are collected using unscientific methods and are commonly exposed to many microbial contaminants. The raw materials are often deteriorated by microorganisms before harvesting, and during handling and storage.[3]

The microbial quality of pharmaceuticals is influenced by the environment and quality of the raw materials used during formulation. Some infectious outbreaks have been associated with the use of heavily contaminated raw materials of natural origin.

1.1 SOURCES OF CONTAMINATIONS IN HERBAL PRODUCTS.

The practices of most ethnic herbal medicine include the use crude or raw herbs that are collected from the wild or from cultivated fields and their prepared or ready-made products.

Toxic contaminants may come from:

- Environments and conditions that the medicinal plants are grown or collected
- The conditions under which they are dried and processed.
- The storage conditions and conditions during transport.
- Unhygienic use of medicines by patients
- The manufacturing processes when the ready-made medicinal products are produced.[4]
- WHO, (1998) mentions maximum permissible limits in raw materials only for arsenic, cadmium, and lead, which amount to 1.0, 0.3, and 10 ppm, respectively. The concentration of heavy metals is one of the criteria that make raw plants admissible to the production of medicines due to the fact that amount

taken increases with the concentration, increased by constant mass of a taken dose.[5]

Herbal medications are claimed and widely believed to be beneficial; however, there have been reports of acute and chronic intoxications resulting from their use. The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies. A common misperception is that medicaments of natural substances cannot be present in toxic concentrations in a variety of herbal preparations and dietary supplements[6]

Arsenic

Arsenic is a highly toxic, naturally occurring grayish- white element used as a poison in pesticides and herbicides. Arsenic is also found as an ingredient in pigments and wood preservatives. Arsenic contained in wolmanized lumber will not release toxic compounds unless burned. Arsenic can be harmful through inhalation, absorption through skin and mucous membranes, skin contact, and ingestion.[7]

Cadmium

Cadmium is a toxic heavy metal, well known for its occupational health risk, and cadmium (as a pollutant of air and water) is an increasing public health concern. Inhalation of cadmium fumes or dust is the primary cause of cadmium exposure[8]

- Most studies have centered on the detection of early signs of kidney dysfunction and lung impairment in the occupational setting, and, in Japan, on the detection and screening for bone disease in general populations exposed to cadmium-contaminated rice. More recently, the possible role of cadmium in human carcinogenesis has also been studied in some detail.[9]

Lead

Lead is a ubiquitous toxicant. Lead poisoning is an insidious disease that can result in developmental delays, behavioral disorders and irreversible brain damage. The major signs and symptoms of lead poisoning are pallor, gingival lead line, gastrointestinal disorder, and anemia, renal and neurological symptoms (peripheral neuropathy, ataxia and memory loss) in adults. Chronic exposure to lead is associated with renal dysfunction whilst, chronic lead toxicity will also lead to sterility in adults.[10]

2.0 About haematinic herbal formulations

A medicine that increases the hemoglobin content of the blood and used to treat iron-deficiency anemia. The herbal formulations selected for study were as follows,

Each tablet contains: Pravalpishti, Agasthibhasma, Andatwakpishti and amalaki (Embellica officinalis) 50mg each, Mandoorbhasma 15mg, Suvarna makshik Bhasma 10mg, Binders and Excipients q.s.

3.0 Experimental work:

3.1 Sample collection

The ten herbal formulation of Haematinic herbal formulation marketed by various herbal manufacturers were collected from the retail medical stores of Yavatmal (Vidarbha region, India). The formulation were given code ie HT1 to HT10

3.2 Materials & methods

Serial dilutions were made and viability assessed using the pour plate method. The plates were incubated at 37oC for 24h. The plate was placed on a colony counter and the number of colony forming units was taken. The microbial content was taken as the mean of duplicate determinations. The media utilized were Nutrient agar, Cetrimide Nutrient agar, Salt Nutrient agar, MacConkey agar[11]

3.3 Pathogen determination

3.3.1 Determination of S. aureus

10 mg of the sample was added into Tryptic soya broth and incubated at 37°C for 24 hours. The sample was then streaked on Vogel-Johnson agar and incubated at 37°C for 24 hours. A single colony on each plate was then resteaked on Mannitol salt agar and incubated at 37°C for 24 hours. After the incubation, the colonial morphology was observed[12]

The results are expressed in Table No.2 and Figure No.1

3.3.2 Determination of Escherichia coli

Suspend 10 gm of the specimen in lactose broth or any other broth, which has no antibacterial effect to make 100ml (may adjust PH at 7). It is called pretreatment material Incubate 100ml of pretreatment material at 30-37°C for 2-5 hrs. Transfer amount of above homogenized pretreatment material containing 1gm or 1ml of the material being examined to 100ml of MacConkey broth and incubate at 43-45c for 18-24hrs Prepare subculture on a plate with MacConkey agar and incubate at 43-45c for 18-24hrs Growth of red generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by.a reddish zone of precipitation, indicates the possible presence of E.coli.[13] The results are expressed in Table No.2 and Figure No.1

3.3.3 Determination of P. aeruginosa

The diluted sample was streaked onto Cetrimide agar plate. After the incubation at 37°C for 24 hours, the green colonies were tested for oxidase reaction and subcultured into Triple sugar iron medium. Growth of bacteria and the reaction results were observed.[14]

The results are expressed in Table No.2 and Figure No.1

3.3.4 Calibration of Equipment

For the studied elements we established the following sensitivity and detection limits, respectively of the used flame atomic absorption Spectrophotometer (AAS) apparatus. As 0.02 and 0.08 ppm, Cd 0.2 and 1.0 ppm, Pb 2 and 10.0 ppm

3.3.5 Extraction of heavy metals from Herbal formulations:

A sample of 10gm of each herbal formulation was taken in a silica crucible and heated to remove the moisture. It was then put in a muffle furnace at 450°C, for 2 hours, to remove the organic material. The ash was digested in 5 ml dilute HCL + 1ml HNO_3, cool, 20 ml distilled water added. Filtered and the filter paper were washed distilled water, in 100ml volumetric flask. It was made to 100ml with distilled water and suitable dilutions were prepared. This filtrate contained the metal-like arsenic, lead cadmium. The Arsenic, cadmium and Lead were determined by Atomic Absorption Sphectrophometer.[15]

4.0 Results

Table1 W.H.O. Limits for microbial contamination

Microorganism	**Finished product Cfu**
Escherichia coli	10^1/gm
Staphylococus.aureus	10^5/gm
Pseudomonas aeruginosa	10^3 /gm
Salmonella species.	Nil

Table 4.1. Determination of Microbial content in herbal formulations

Sr.No	Formulation code	Pseudomonas aeroginosa (10^3 cfu /gm) Mean±SD	Escherichia coli (10cfu/gm) Mean±SD	Staphylococcus aureus (10^5cfu/gm) Mean±SD
1	HT1	11.5 ± 1 x 10^2	-	6.5 ± 1 x 10^4
2	HT2	*13.5 ± 2 x 10^2	3.50 ± 1 X 10	14.00 ± 2 x 10^4
3	HT3	6.5 ± 1 x 10^2	-	6.50 ± 1 x 10^4
4	HT4	6.5 ± 1 x 10^2	-	9.50 ± 2 x 10^4
5	HT5	5.5 ± 1 x 10^2	2.50 ± 1 x 10	7.50 ± 2 x 10^4
6	HT6	6.5 ± 1 x 10^2	-	5.50 ± 1 x 10^4
7	HT7	4.5 ± 2 x 10^2	-	3.50 ± 1 x 10^4
8	HT8	*12.5 ± 1 x 10^2	2.50 ± 1 x 10	12.50 ± 2 x 10^4
9	HT9	5.5± 1 x 10^2	-	15.50 ± 2 x 10^4
10	HT10	13.5 ± 2 x 10^2	1.50 ± 1 x 10	8.5 0 ± 1 x 10^4

* indicates abovbe permissible limit

Table No.2 Comparative determination of Microbial contamination in Haematinic herbal formulation

Table 4.2 Determination of Heavy metal content in herbal formulations, Comparative data of Heavy metals contents in formulation

Formulation code	Heavy metal content mg/kg		
	Arsenic Mean±SD	Cadmium Mean±SD	Lead Mean±SD
HT1	0.33±0.36	*0.45±0.23	*12.00±0.25
HT2	0.25±0.35	BDL	6.33±0.36
HT3	0.54±0.23	0.18±0.64	7.65±0.25
HT4	0.64±0.14	BDL	6.97±0.45
HT5	0.32±0.18	*0.45±0.15	*13.22±0.65
HT6	0.11±0.26	*0.59±0.32	*15.68±0.61
HT7	0.12±0.14	* 0.33 ± 0.29	*13.58±0.45
HT8	0.18±0.25	BDL	5.66±0.58
HT9	0.35±0.24	*0.56±0.27	*13.78±0.68
HT10	0.23±0.15	*0.42±0.36	*15.74±0.24

BDL=Below Detectable Level,* indicate above permissible limit.

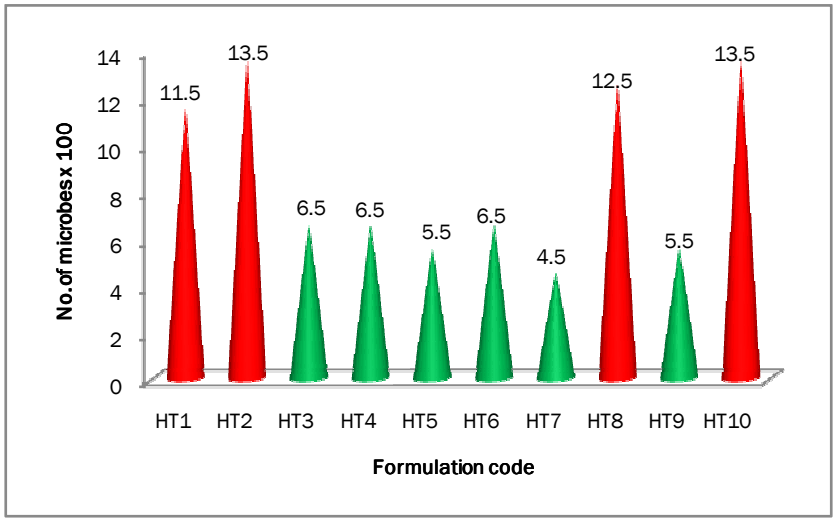

Figure 1: Pseudomonas aeruginosa contamination in Haematinic herbal formulation

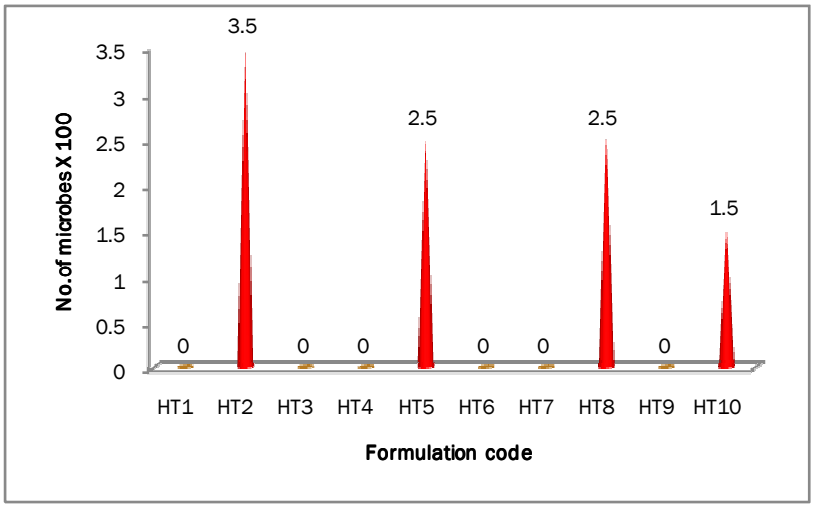

Figure 2: Escherichia Coli contamination in Haematinic herbal formulation

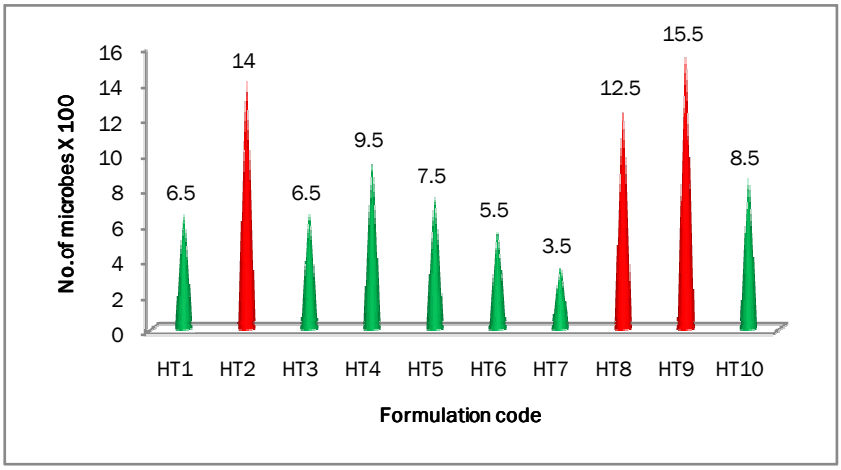

Figure 3: Staphylococcus aureus contamination in Haematinic herbal formulation

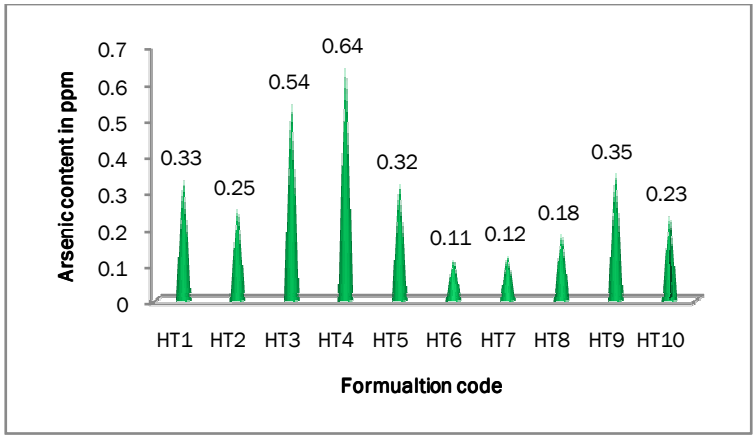

Figure 4: Arsenic content in Herbal formulation HT1 to HT10

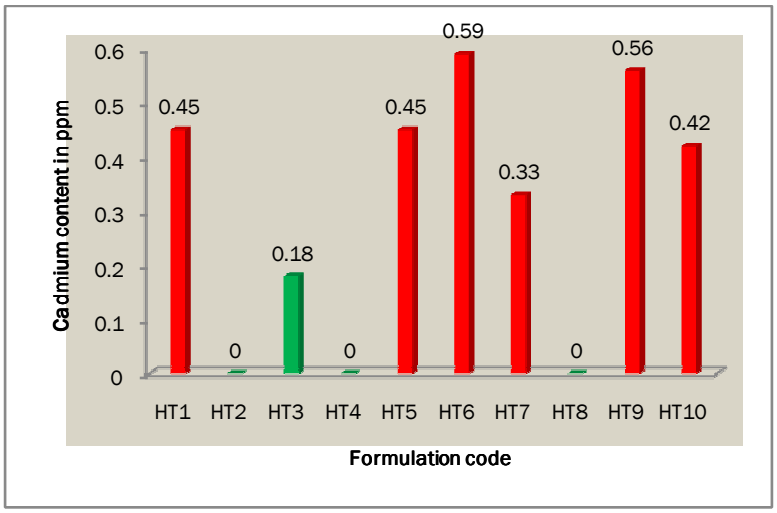

Figure 5: Cadmium content in Herbal formulation HT1 to HT10

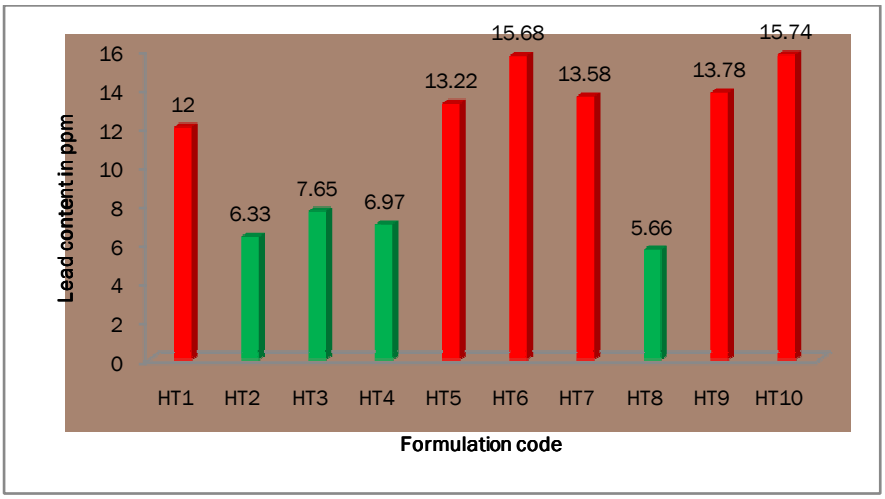

Figure 6 Lead content in Herbal formulation code HT1 to HT10

5.0 Discussion

The present study reports microbial contaminations suggest that in haematinic marketed herbal products widely distributed over the country. It was found that the formulations code HT1, HT 2, HT8 and HT10 were contaminated by Pseudomonas aeruginosa and HT 2, HT 5, HT 8 and HT 10 were contaminated by Escherichia coli whereas the formulations having code no. HT 2, HT 8 and HT 9 were contaminated by Staphylococcus aureus more than the limit prescribed by WHO if such product was consumed by patient there was possibility of infection. Medicinal plants have

been generally used for decades. Consumers can easily acquire pathogenic microorganisms by consuming contaminated products. The results from this study suggest that the production of herbal products is still in critical situation in terms of quality and safety. Very low product quality can be derived from many factors such as cultivation, harvest, manufacturing procedure, transportation, and storage. The good handling must be carried out starting from raw materials to finished products.

It was found that Arsenic content in Herbal formulations was below the Permissible limit in all formulations.. The Cadmium content in H1 (0.45 ppm), H5 (0. 45 ppm), H6(0.33 ppm), H9 (0.56 ppm), H10 (0.42 ppm) which are above the permissible limits. The lead content in H1,(12 ppm),H5 (13.22 ppm),H6 (15.68 ppm),H7 (13.58 ppm) H9 (13.78 ppm),and HT10 (15.74) which are above the permissible limits. Such formulations are injurious to health of patient if consumed regularly.

Conclusion

- People generally use herbal medicine for prolonged period of time to achieve desirable effects. Prolong consumption of such herbal medicine might reduce chronic or subtle health hazards. Thus our findings indicate that the medicinal plant or plant parts used for different diseases must be checked for heavy metals contamination in order to make it safe for human consumption.
- The general belief that herbal preparations are natural and, therefore, inherently safe harmless and without any adverse effects is sometimes unfounded. Toxic effects of herbal preparations have been attributed to several factors including contamination by poisoning through traditional Chinese, Indian and Malaysian medicines have been reported.

References

1. Don Woong Choi et al, Regulation and quality control of herbal drugs in Korea, Toxicology,2002,181-182,.581-586.
2. Z.C.Khanyile, N.Singh, M.Smith, F.O.Shode, S.Mngomezulu and Y.H.Dewir, Camparative Assessment of Bacterial contamination in Commercial Herbal product of Lessertia futescens, American-Eurasian J.Agric & Eniron.sci, 2009,5(4);494-499.
3. N. K. Dubey, Ashok Kumar, Priyanka Singh and Ravindra Shukla Microbial contamination of raw materials: A major reason for the decline of India's share in the global herbal market Current science, September 2008 , 95,(6),25
4. K. Chan, Review: Some aspects of toxic contaminants in herbal medicines Chemosphere, 2003,52, 1361–1371.
5. Xue zian et al, Overview on External Contamination Sources in Traditional Chinese Medicines, Modernization of traditional and Chinese medicine,2008,10 (1) 91–96
6. E. Obi, Dora N. Akunyili, B. Ekpo , Orish E Orisakwe, Heavy metal hazards of Nigerian herbal remedies, Science of the Total Environment,2006,369,35–41
7. http//www.greenfacts.org/en/arsenic.
8. P K Sethi, Dinesh Khandelwal, Nitin Sethi, Cadmium Exposure: Health Hazards of Silver Cottage Industry in Developing Countries, Journal of medical toxicology March,2006, 2, (1) 14-15.
9. www.Cadmium.org Cadmium exposure and human health
10. Current status of lead in India, Released on World Environment Day, 2001.
11. Pulak K.Mukharji,sujay Rai,Sauvik Bhattacharya, Atul Wahile,Bishnu Padasaha,Marker analysis of poly herbal formulation-A Triphala-A well-known Indian Traditional Medicine, Indian Journal of Traditional Knowledge,2008, 7(3) 379-383
12. Adenike Okunlola, Babatunde A. Adewoyin and Oluwatoyin A. Odeku, Evaluation of Pharmaceutical and Microbial Qualities of Some Herbal Medicinal Products in South Western Nigeria Tropical Journal of Pharmaceutical Research, March-2007; 6 (1) 661-670
13. Waterman RF, Sumner ED, Baldwin JN and Warren FW. Survival of Staphylococcus aureus on pharmaceutical oral solid dosage forms. J. Pharm.Sci. 1973; 62,1317 – 1320
14. Van Doorne H. and Claushauis EPM. The quantitative determination of Enterobacteriaceae in pharmaceutical preparations. Int. J. Pharm. 1979; 4: 119 - 125.
15. Sharma A.K, Gaurav S.S, Balkrishna, A rapid and simple scheme for the standardization of polyherbal drugs, Int J Green Pharm,2009,3,134-140.