ISSN: 2278-778X
Research Article
www.ijbio.com

QUALITY CONTROL OF MARKETED CLOVE BUDS - REFERENCE TO THEIR QUALITY AND PURITY AS PER WHO GUIDELINES

Ramlingam P*, Ramakanth Reddy D, Harish Kumar Raju C, Sai Ram Reddy P and Vamshi Krishna PV
Centre for Pharmaceutical Research (CPR), Division of Pharmaceutical Analysis & Quality Assurance, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Chiyyedu, Anantapur -515721, Andhra Pradesh, India.

***Corresponding Author: Dr. Ramlingam P,** Centre for Pharmaceutical Research (CPR), Division of Pharmaceutical Analysis & Quality Assurance, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Chiyyedu, Anantapur -515721, Andhra Pradesh, India.

Received for publication: February 19, 2012; **Accepted**: April 09, 2012.

Abstract: Two marketed clove buds were subjected to crude drug standardization by sampling from retail packages as per WHO recommended guidelines. The marketed clove products viz. brand-A and brand-B were standardized comparatively for various parameters like, powder fineness, foreign matter, ash values, volatile oil content, bitterness value, swelling index, foaming index, heavy metals, Mycological examinations, spectral analysis, qualification of Eugenol by TLC and quantification by HPLC. The results were compared with individual monograph limits specified in Indian Pharmacopoeia. The volatile oil content were estimated by Azeotropic distillation and were found to be 1.5ml and 1.2ml/10gms respectively for brand-A and brand-B against the limit 1.5 to 2.1ml. The UV spectra and ATR-IR spectra of both volatile oils were identical with the finger print of standard Eugenol oil. The foreign matter and ash value for brand-A and brand-B were 1.49%, 5.8% and 3.79%, 6%, respectively and brand-B doesn't complies with the specified monograph limits (NMT 3%) for foreign matter. The Eugenol content in the volatile oil was quantitated by RP-HPLC method and was found to be 93.3.1% and 74.6% respectively for brand-A and brand-B against the standard monograph limit of 85-95%. It was noted that the brand-A was found to be superior quality and whilst brand-B disqualifies in standardization parameters such as Foreign matter, Volatile matter and Eugenol content. World Health Organization has emphasized the need to ensure quality control of medicinal plant products by applying suitable parameters and standards. In order to overcome certain inevitable shortcoming of the Pharmacopoeial monograph and other quality control measures, the present study explores the possible parameters of crude drug standardization for marketed clove buds with special reference to their quality and purity as per WHO guidelines.

Keywords: WHO, Eugenol, Azeotropic distillation, ATR-IR, UV, HPLC.

INTRODUCTION

The World Health Organization (WHO) was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications (Agarwal, 2005). Herbal medicines for human use are required to meet standards which relate to their quality, safety and efficacy. The evaluation of safety and efficacy and their maintenance in practice is dependent upon the existence of adequate methods for quality control of the herbal product. In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine have started getting more apparent, majority of herbal formulations. It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication, which guarantee for purity, safety, potency and efficacy. This duty is discharged by the regulatory authorities by rigidity following various standards of quality prescribed for raw materials and finished products in pharmacopoeias controlling manufacturing formulate through the use of formularies and manufacturing operation through statutory imposed "Good manufacturing practices". All these procedure logically would be apply to all type of medication whether included in modern system of medicine or one of the traditional system such as Ayurvedic system of medicine. Unfortunately the Ayurvedic pharmacopoeias and the formulary have been exempted from the standard attained by present day (Sane, 2002).

Herbal product has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the Ayurvedic formulation is the lack of standard quality control profile. Due to complex nature and inherent variability of the constituents of plant based drugs, it is

difficult to establish quality control parameter and modern analytical technique are expected to help in circumventing this problem. The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is nonavailability of rigid quality control profile for herbal material and their formulations.

Quality controls of synthetic drug offer no problems with very well defined parameters of analysis. In contrast, herbal products represent a number of unique problems when quality aspects are considered. These are because of the nature of the herbal ingredients present therein, which are complex mixtures of different secondary metabolites that can vary considerably depending on environmental and generic factors. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. The task of lying down standards for quality control of herbal crude and their formulation involves biological evaluation for a particular disease area, chemical profiling of the material and lying down specification for the finished product. Therefore, in case of herbal drugs and product, the word "Standardization" should encompass entire field of study from cultivation of medicinal plant to its clinical application (Florey, 1949 and Chaudhari, 1996).

Plant material and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary. WHO has emphasized the need to ensure quality control of medicinal plant products by using modern technique and by applying suitable parameters and standards. In order to overcome certain inevitable shortcoming of the Pharmacopoeial monograph and other quality control measures must be explored. Focus on literature Now-a-days, very few works were published on standardization of herbal drugs and their formulations were attractive area of health research and becoming continuing interest (Chakravarthy, 1993, and Bhanu, 2003). Herein we report the quality control of marketed clove buds with special reference to their quality and purity as per WHO guidelines.

Clove *(Eugenia caryophyllus)* is the aromatic dried flower bud belonging to the family *Myrtaceae*. The clove tree is evergreen that grows to a height ranging from 8-12m having large square leaves and sanguine flowers in numerous groups of terminal clusters. The flower buds first of a pale colour and gradually becomes green, after which they develop into bright red, they are ready for collecting. Cloves are harvested when 1.5 to 2cm long and consists of long calyx, terminating in 4 spreading sepals and unopened petals which form as small ball in the centre.

Therapeutically, it is an antiseptic and effective against *Streptococcus* and *staphylococcus species* and reported to inhibit prostaglandins (Srivastava, 1991 and K.Chaieb, 2007). It was also reported that an infusion of cloves is used as carminative and also to be effective in nausea, vomiting, flatulence and dislepsia (Chaieb, 2007). Now, it is commercially as clove oil for toothaches to produce local anaesthetic effect and antiinfective activity (Lee, K.G and L. Shibamoto, 2001). Various research published for the therapeutic potential of cloves are antioxidant (Miyazawa, M and M. Hisama, 2003), antimicrobial (Mytle, 2006), antimutagnic, antiparasitic (Briozzo J, 1989), nutritional healing (Phyllis B, 2000) glaucoma (Ritch, 2000), ovicidal and adulticidal activity (Yang, 2003).

Herein we report the standardization of two marketed clove buds hereafter named as Brand – A and Brand – B and comparative results with the monograph limits. The brand names and batch numbers of the crude drug under the study is reserved to the author.

EXPERIMENTAL

Clove buds samples Brand- A and B were sampled from retail, Anantapur, Andhra Pradesh, India. Standard eugenol was obtained from Qualigens. Reagents and solvents were of AR grade from Qualigens and glasswares were of borosilicate - class A type and calibrated before use. Systronics UV- Visible spectrometer 2202 was used for obtaining UV spectrum. Brukers FT – IR spectrometer – Alpha with ATR technique was employed for IR Spectra. Agilent LC 1200 with PDA detection and Agilent C18 (150 mm, 4.6 mm, 5 micron) were employed in quantification procedure.

Sampling of clove buds:

About 250 g of two marketed cloves viz. Brand-A and Brand-B were sampled from the pool sample prepared by mixing the contents of the selected brands of same batch.

Standardization of Clove buds:

Morphological characteristics of clove: 50 clove buds each buds were randomly subjected to various morphological evaluation tests like odor, taste, appearance, shape of ovule, length and diameter of bud. The observed results were compared with standards specified and the results were shown table 1.

TS and powder microscopy: Among samples 50 clove buds were selected and soaked in water for 12 hrs and the wet clove buds subjected transverse section microscopy under magnification value of 400.

Phluroglucinol and conc. HCl were used for identifying lignified tissues. Microscopically, all the required characteristics like Ovary with ovarian wall, Parenchymatous dissepiments, ovule, starch, prisms of calcium oxalate, stone cells, Epidermis with stomata, Pollen grains, Lignified fibres of parenchyma, Oil glands, Aerenchyma etc were observed and recorded and interpreted for adulterants and mother clove. Around 10g of clove buds were fine powdered and lignified and were subjected to microscopy evaluation. The observed results were shown in table 1.

Foreign matter: 250g of clove buds were weighed and spread as a thin layer and sorted the foreign matter into groups by visual inspection. Sifted the remainder of the sample through a No. 250 sieve and the dust was regarded as mineral admixture. The separated foreign matter was weighed its content was determined and reported for individual brands in table 2.

Volatile oil content: 100g samples of both brands were transferred in to round bottom flask of Clavenges apparatus and added water sufficient to soak conditions and refluxed until the level of volatile oil remains constant at graduated collecting tube of the apparatus. The reading was noted down and volatile oil content was calculated in ml/10g and was compared with monograph limits. The result was shown in table 2.

Thin layer chromatography (TLC): Commercially available silicagel GF254 precoated aluminum plate and the mobile phase of n-hexane and ethyl acetate (60:450 v/v) were employed in TLC of Clove oil. 10 microliters of extracted clove oils of brand -A, brand –B and Standard Eugenol were spotted at the bottom of the TLC plate in triplicate. The plates were developed in the preconditioned developing tank. The plates were taken out of the chamber after satisfactory development and visualised using 0.7% ferric chloride reagent for eugenol and iodine chamber for other constituents. Retention times were calculated for the purple colored eugenol spot and are reported in table 2.

Total ash: 4g of the ground air-dried material was accurately weighed, placed in a previously ignited, tarred silica crucible and ignited by gradually increasing the heat to 600°C until the formation of white ash, indicating the absence of carbon. Then it is cooled in a desiccator and weighed. The content of total ash in mg per g of air dried material was calculated and reported in table 2.

Water extractable matter: 4.0 g of coarsely powdered air-dried clove was accurately weighed, in a glass-stoppered conical flask and macerated with 100 ml of the water for 6 hours with frequent shaking. Then it was allowed to stand for 18 hours and filtered rapidly without loss any solvent. 50 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath at 105°C for 6 hours and cooled in a desiccator for 30 minutes. The extract was weighed without delay and content of extractable matter was calculated in g per 100 g of air-dried material and expressed as %.

Acid-insoluble ash: To the total ash, 25 ml of hydrochloric acid was added and boiled gently for 5 minutes. The insoluble matter was collected on an ashless filter paper and it was washed with hot water until the filtrate is neutral. It was then transferred to the original crucible and dried on a hotplate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes, then weighed without delay. The content of acid-insoluble ash in mg per g of air-dried material is calculated and reported in table 2

Water-soluble ash: To the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper. It was then washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C.The weight of this residue was subtracted in mg from the weight of total ash. The result was shown in table 2

Swelling index: 10 g of clove buds were soaked in 25ml of water in a 0.2 ml graduated measuring cylinder and volume occupied by clove buds was noted. Then the measuring cylinder was kept aside for 24 hrs with constant shaking for every 1 hr. The change in volume occupied by clove buds was measured including for sticky mucilage and swelling index was calculated for 1g of material. The determination was performed in triplicate and mean value is shown in table 2

Ultraviolet and Infrared spectroscopy: Eugenol was separated by band application preparative TLC using 1mm thickness of silicagel G as stationary phase and n-hexane and ethyl acetate in the ratio of 60:40 v/v. The band was identified under UV light and powder was extracted with methanol. UV spectrum of isolated eugenol of both brand - A and brand - B were recorded and compared with the maxima and valley of standard eugenol by finger print analysis. IR spectra for samples and standard were recorded by ATR-IR spectroscopy technique. The obtained sample spectrum for brand- A and brand-B were superimposed on standard eugenol spectrum for finger print analysis. The spectra were shown in figure 1.

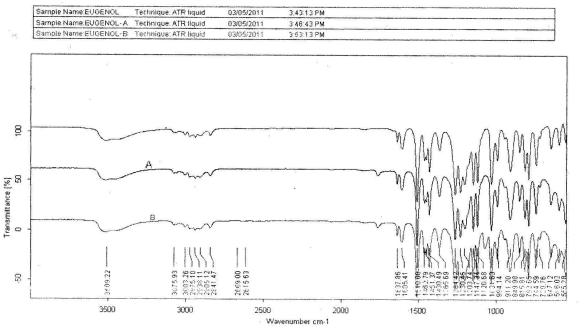

Figure.1: ATR-IR spectra of Standard Eugenol, Brand A and Brand B (Finger print).

High Performance Liquid Chromatography: The quantification of eugenol in High performance liquid chromatography was performed on Agilent 1200 LC series with Gradient EZ Chrom Elite software. The chromatographic conditions were optimized at the detection wavelength of 254 nm, flow rate at 1ml/min for the mobile phase Acetonitrile and buffer pH 3 at the ratio of 85:15 v/v. The method was selected based on the validation report for system precision, method precision, linearity, accuracy and robustness. The validation summary was reported in Table 3. The content of Eugenol in extractive volatile from brand-A and brand-B were quantified against the standard eugenol of 10 µg/ ml, under same chromatographic conditions. The eugenol in sample was identified by comparing the retention time of sample peak with standard peak. The peak purity was checked before quantification by PDA detection. The eugenol content in sample was calculated by direct comparison method. The determination was carried in replicate of five and the mean value was reported in table 1. Chromatograms were shown in Figure 2, 3 and 4.

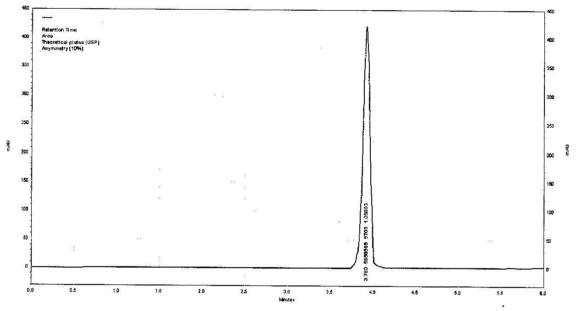

Figure.2: Chromatogram of Standard Eugnol on C18 column

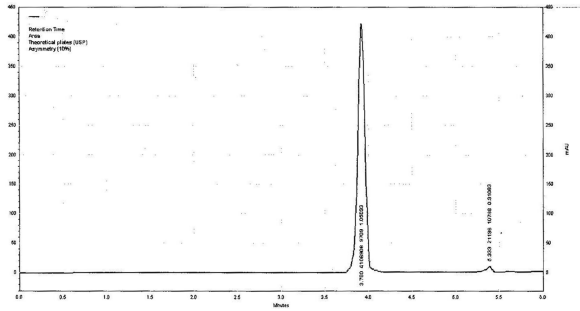

Figure.3: Chromatogram of Eugnol from Brand A on C18

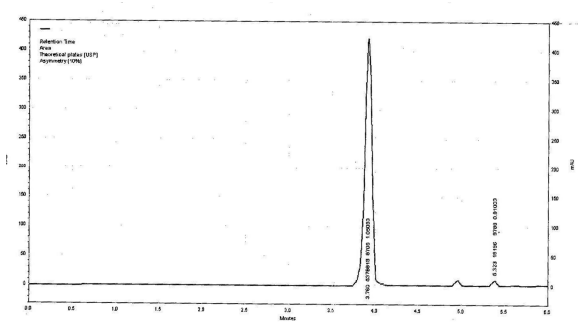

Figure.4: Chromatogram of Eugnol from Brand B on C18 column

Mycological examination: The procedure was adopted as reported by Hiroshi Hitokoto et al (Hiroshi Hitokoto, 1978). 10 g samples of each were taken by a sterile spatula and transferred directly into sterilized flasks containing 90 ml of pre autoclaved potato dextrose agar medium. Before the agar hardened, 1ml portions of each sample were poured and spread on the surface of media in petri dishes. The dishes were incubated at 25°C until the colonies of fungi could be counted and identified. The results were shown on table. 2

Limit test for arsenic and lead: Contamination of clove buds with arsenic and lead can be attributed to many causes including environmental pollution and traces of pesticides. The contents of arsenic and lead were established semiquantitatively by limit test in which colour intensity produced in test was compared with standard. The procedure was adopted as specified in monograph (Ministry of Health, Govt.of India, 2007).

Moisture content: Water content was estimated by azeotropic method. Weighed accurately, 75 g of the clove buds of test brand in aluminum foil and transfer into RB flask and added 100 ml toluene. Added few pieces of porous porcelain and heated the flask gently for 15 minutes. Distil rate was maintained at 2 drops per second until most of the water has distilled over, and then increased to 4 drops per second. As soon as the water has been completely distilled, rinse the inside of the condenser tube with toluene and distillation was continued for 5 more minutes. Then allowed the receiving tube to cool to room temperature and dislodged droplets of water adhering to the walls of the receiving tube by tapping the tube. The water and toluene layers were kept aside to separate and the volume of water was noted down (second distillation). The content of water was calculated as a percentage using the following formula and shown in table 2.

$$\frac{100(n_1 - n)}{w}$$

Where; w = the weight in g of the material being examined;
n = the number of ml of water obtained in the first distillation;
n_1 = the total number of ml of water obtained in both distillations.

RESULTS AND DISCUSSION

In the present study, two marketed clove buds respectively, brand-A and brand-B were standardized comparatively for various quality control parameters like, powder fineness, foreign matter, ash values, volatile oil content, bitterness value, swelling index, foaming index, heavy metals, Mycological examinations, spectral analysis, qualification of Eugenol by TLC and quantification by HPLC. The present study was carried out based on pharmacognostical, quality control test for powder and extracts and qualification and quantification of principle chemical constituents. The pharmacognostical evaluation revealed that both brand A and brand B complied for parameters, however the brand A demonstrated quality compliance of more than 92% in morphological examination for plump, colour and hypathium whilst brand B showed 82%.

The powder and chemical microscopy of the brands revealed that brand B showed negative results for Stone cells, starch and prism of calcium oxalate crystals. The results were shown in Table 1. The powder fitness for brand A and brand B were 100% and 91% respectively. The foreign matter and ash value for brand-A and brand-B were 1.49%, 5.8% and 3.79%, 6%, respectively and brand-B doesn't complies with the specified monograph limits (NMT 3%) for foreign matter. The volatile oil content were estimated by Azeotropic distillation and were found to be 1.5ml and 1.2ml/10gms respectively for brand-A and brand-B against the limit 1.5 to 2.1ml. The UV spectra of tests and standards were 281nm and also ATR-IR spectra of both volatile oils were identical with the finger print of standard Eugenol oil in between 2000-400 cm^{-1}.

Table 1: Pharmacognostical evaluation of marketed Clove buds (brand A and brand B)

S.No.	Characteristics	Specification	Brand-A	Brand-B
1	Plump	Heavy	95%	84%
2	Colour	Brown	98%	90%
			92%	87%
3	Hypanthium	Subcylindrical	94%	83%
		10-13mm long, 4mm wide, 2mm thick	95%	82%
			92%	84%
4	Corallo	Dome shaped	Complies	Complies
5	Odour and taste	Pungent aroma	Complies	Complies
6	Ovary with ovarian wall	Present	Complies	Complies
7	Parenchymatous dessepiment	Present	Complies	Complies
8	Ovule	Present	Complies	Complies
9	Starch	Present	Complies	Absent
10	Prisms of calcium oxalate	Present	Complies	Absent
11	Stone cells	Present	Complies	Absent
12	Epidermis with stomata	Present	Complies	Complies
13	Pollen grains	Present	Complies	Complies
14	Lignified fibres of parenchyma	Present	Complies	Complies
15	Oil glands	Present	Complies	Complies
16	Aerenchyma	Present	Complies	Complies

% results indicate compliance of specification out of 50 sample buds

The fingerprint examinations of eugenol of test brands against standards were shown in Figure 1. The Eugenol content in the volatile oils were quantified by a validated RP-HPLC method (Table 3) and found to be 93.3.1% and 74.6% respectively for brand-A and brand-B against the standard monograph limit of 85-95%. Chromatograms were shown in Figure 2, Figure 3 and Figure 5. It was noted that the brand-A was found to be superior quality and whilst brand-B disqualifies in standardization parameters such as Foreign matter, Volatile matter and Eugenol content. Limit test for lead and arsenic was passed for both brand A and brand B.

The results were compared with individual monograph limits specified in Indian Pharmacopoeia and were shown in Table 2. RP-HPLC chromatogram showed that an additional constituents for brand A at 5.23 min whilst for brand B two constituents at 4.91 mins and 5.23 mins, respectively. The mycological examination was negative for both brands and it was supported by literature on antimicrobial property of eugenol. Based on the above observation it is concluded that Brand A passes the test for quality and purity whilst Brand B fails in quality with respect to volatile content, % Eugenol, powder fitness, forging matter, powder microscopy for stone cells, micro chemical test for starch and calcium oxalate crystals

Table 2: Standardization results for commercial clove buds (brand-A and brand-B).

S.No.	Parameter	Quality control limits	Brand-A	Brand-B
1	Powder fineness	All particles should pass through sieve no.2000	100%	91%
2	Sampling	250 g	250 g	250 g
3	Foreign matter	NMT 3%	1.49%	3.79%
4	TLC (Rf value)	0.86	0.86	0.85
5	Ash value	5 to 7%	5.8%	6.0%
6	Extractable matter (H2O)			
7	Acid insoluble extract	5-8%	5.2%	4.3%
8	Water insoluble extract			
9	Volatile oil (ml/10gms)	1.5 to2.1 ml.	1.5ml	1.2ml
10	Bitterness	NLT 3000	4000	3000
11	Swelling index	No swelling	No swelling	No swelling
12	Foaming index	No foaming	No foaming	No foaming
13	Arsenic	NMT 40ppm	Passes	Passes
14	Lead	NMT 40ppm	Passes	Passes
15	Mycological examination	Nil	Nil	Nil
16	UV (λ max)	281nm	281nm	281nm
17	FT-IR (ATR)	Fingerprint in between 2000- 400 cm-1	Passes	Passes
18	RP-HPLC Rt % Eugnol No. Components	Rt 3.76 % : 100% 01(eugenol)	Rt 3.76 % 94% 02	Rt = 3.76 %: 75% 03

Table.3: Validation summary for RP-HPLC method

Validation Parameter	Results	Acceptance Criteria
Linearity	5 – 50 µg/ml with R2 = 0.9991	R2 = 0.99
System Precision	0.987 % (RSD)	2%
Method Precision	1.076 % (RSD)	2%
Intermediate	1.28 % (RSD)	2%
Recovery	98.5 – 101.3%	98-102%
LOD	0.2 µg/ml	--
LOQ	0.7 µg/ml	--

CONCLUSIONS

The present study revealed the quality difference in the marketed crude clove buds and was compared with adequate parameters as per WHO guidelines and this study may be an example for standardizing other crude drugs which are in the market.

REFERENCES

1. Agarwal, A, 2005. Critical issues in Quality Control of Herbal Products. *Pharma Times,* 37(6): 9-11.
2. Briozzo J, Nuzen L, Chirife J and Herzge L, 1989. Antimicrobial activity of clove oil dispersed in concentrated sucrose solution. *J appl bact.* 66: 69-75.
3. Bhanu P. S. Sagar, T. K. and R. Zafer, 2003. Failure and successes of Herbal Medicines, *The Indian Pharmacist,* 3: 17-23.
4. Chaudhari, R. D, 1996. *Herbal drug industry,* Eastern Publisher, New Delhi, 1st Edition: p498- 499.
5. Chaieb, K, T. Zmantar, R. Ksouri, H. Hajlaoui, K. Mahdouani, C. Abdelly and A. Bakhrouf, 2007.
6. Antioxidant properties of essential oil of*Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. *Mycosis.* 50: 403-40.
7. Chakravarthy, B. K, 1993. Standardization of Herbal products. *Indian. J. Nat. Product,* 9: 23- 26.
8. Florey, H. W, Chain, E and Florey, M. E, 1949. *The Antibiotic,* Oxford University Press, New York: 576-628.
9. Hiroshi Hitokoto, Satoshi Morozumi, Tomoaki Wauke, Senzo Sakai and Hiroshi Kurata, 1978. Fungal contamination and mycotoxin detection of powderedherbal drugs, *Applied and environmental microbiology,* 2: 252-256.
10. K.Chaieb, H. Hajlaoui, T. Zmantar, A.B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf, 2007. The chemical composition and biological activity of essential oil, *Eugenia cryophyllata* (*Syzygium aromaticum* L. Myrtaceae): A short review. *Phytother. Res.* 21: 501-506.
11. Lee, K.G and L. Shibamoto. 2001. Inhibition of Quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett. Appl. Microbiol.* 49: 354-360.
12. Ministry of Health, Govt.of India, 2007. *Indian Pharmacopoeia.* Vol.1: 76-77.
13. Miyazawa, M and M. Hisama, 2003. Antimutagenic activity of phenylpropanoides from clove (*Syzygium aromaticum*). *J. Agric. Food Chem.* 51: 6413-6422.
14. Mytle, N., G.L. Anderson, M.P. Doyle and M.A. Smith, 2006. Antimicrobial activity of clove (*Syzgium aromaticum*) oil

in inhibiting *Listeria monocytogenes* on chicken frankfurters. *Food Control.* 17: 102-107.

15. Phyllis B, and B. James, 2000. *Prescription for Nutritional Healing.* 3rd Edn. Avery Publishing Group Inc., USA., ISBN-13: 978-1583330777, 784.
16. Ritch, R., 2000. Potential role for Ginkgo biloba extract in the treatment of glaucoma. *Med. Hypothesis.* 54 (2): 221-235.
17. Sane, R.T, 2002. Standardization, Quality Control and GMP for Herbal drugs.*Indian Drugs.* 39 (3): 184-190.
18. Srivastava, K.C and N. Malhotra. 1991. Acetyl euginol, a component of oil of cloves (*Syzygium aromaticum* L.) inhibits aggregation and alters arachidonic acid metabolism in human blood platelets. *Prostaglandins Leukot. Essential Fatty Acids.* 42: 73-81.
19. Yang, Y.C, S.H. Lee, W.J. Lee, D.H. Choi and Y.J. Ahn, 2003. Ovicidal and adulticidal effects of *Eugenia cryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *J. Agric. Food Chem.* 51: 4884-4888.

Source of support: Nil
Conflict of interest: None Declared