

RTBCE 2014[12<sup>th</sup> August 2014]  
Recent Trends in Biotechnology and Chemical Engineering

## Quality Analysis of Phytocomposition of Branded and Unbranded Honey procured from the Markets of Chennai, India

S. Bhuvaneshwari<sup>1\*</sup>, N. Ashwin Karthick<sup>2</sup>, S. Deepa<sup>2</sup>,  
Hima Aishwarya<sup>2</sup> and N.K. Udaya Prakash<sup>3</sup>

<sup>1\*</sup>Research and Development, MARINA LABS, 40, Anna Nedum Pathai,  
Choolaimedu, Chennai 600094, India

<sup>2</sup>Department of Biotechnology, Jeppiaar Engineering College,  
Semmenjeri, Chennai 600119, India

<sup>3</sup>School of Biological Sciences, Vel Tech Dr. RR & Dr. SR Technical University,  
Avadi, Chennai 600062, India

\*Corres.author: marinalabs@gmail.com

**Abstract:** Honey is a highly valued natural food due to its therapeutic and aesthetic value. The high potency of honey as a medicine is due to its phytoconstituents which serve as chemical repositories. Hence, this study aims at the qualitative analysis of phytoconstituents present in honey, a total of 33 honey samples were purchased from markets of Chennai which includes 25 branded and 8 unbranded honey samples. These samples were analyzed for the presence of phytoconstituents such as Tannins, Phlobatannins, Flavonoids, Saponins, Steroids, Cardiac glycosides and Terpenoids. The results showed the presence of Phlobatannins, Flavonoids, Saponins, Steroids, Cardiac glycosides and Terpenoids in 8, 27, 20, 22, 26 and 21 samples respectively.

**Key words:** Quality, Branded honey, unbranded honey, Phytoconstituents, Chennai.

### Introduction

Honey is defined as “a sweet, sticky yellowish-brown fluid made by bees and other insects from nectar collected from flowers.” Honey is generally obtained from honey combs, built by worker bees<sup>1</sup> which transform nectar from flowers into honey by a process of regurgitation and evaporation. They then store it as a primary food source in waxy honey combs inside the beehive. These honey bees are also cultured commercially for their ability to produce honey that is sold in market as branded products.

From time immemorial honey has been used as a medicine both orally and topically to treat various ailments including gastric disturbances, ulcers, wounds and burns. Ancient Greeks and Egyptians portrayed the medicinal properties of honey in their literatures while Ayurvedha in India and Traditional Chinese Medicine also had Honey as their traditional medicine<sup>2,3</sup>. Honey mixed with warm water is traditionally considered to strengthen circulatory system and also for asthmatics<sup>4</sup>.

The constituents of honey vary regionally. Differences in properties like physicochemical and *B*-trehalose have been reported<sup>5</sup>. The trisaccharides are also found with changing parameters such as pH, enzymes activities, ash of maltotriose, erlose, melezitose, centose 3-*a*5 contents, electrical conductivity and hydroxyl methyl iso-maltosylglucose,1-kestose, isomaltotriose, panose and furfural.

The phytochemical analysis of various branded and unbranded honey samples were conducted in various parts of the world,i.e.,Pakistan<sup>6</sup>, Nigeria<sup>7</sup>, Zimbabwe<sup>8</sup> while in India preliminary phytochemical screening was conducted on Bee-byproducts<sup>9</sup> and on Bee glue (Propolis)<sup>10</sup>. However, with respect to Chennai, there is no report available on the quality of honey and their phytochemical constituents. Hence, the honey samples were obtained from markets of Chennai and qualitative phytochemical analysis was carried out for a total of 33 samples.

## Materials and Methods:

### Collection of samples:

A total of 33 honey samples were procured from the markets of Chennai. Of the 33 samples 25 were branded and 8 were unbranded. Branded honey is the processed honey sold under a brand name, while, unbranded are natural unprocessed honey obtained from the tribal people who collect them directly from the honey combs. The details on the product name of the honey, manufacturer detail and the batch/lot no. is presented in (Table-1).

**Table 1: Product Details of honey samples**

S.No	Product name	Manufacturer details	Specifications
1	Unbranded Local vendor		
2	Amrutham Honey(Agmark grade)	Packed by Nature food products K.K Nagar, Chennai-600078. Analyzed by A.O© SAGL, Chennai.	Lot No:04 Packed on:07/2013
3	Agmark Kashmir Honey	Processed and Marketed by: Natural ShameethaBeeo.Dhalaraipuram, Viruthunagar-628188. Register under K & V.I.C (Govt of India) <a href="mailto:naturalhoneyform@yahoo.com">naturalhoneyform@yahoo.com</a>	Lot No:674 Packed on:09/2013
4	Natural Honey J.J Agmark	Packaged by Johnson Honey. BondongriMalad(F), Mumbai-400097	Lot No:38 FSS Licence No:11513007002404 St. No:WR/HONEY04/13/ CA No:A/1 NO-003729 Seal No:25551 Packed on:08/13
5	KodaikanalAgmark Honey	Marketed by: Sri Vignesh Cottage Industry Chennai-600015 Graded by:SAGL, Chennai-600078	Lot No:5 St No:SR/H/04/12 Packed on:08/13
6	Unbranded Local vendor		
7	Unbranded VSR Honey		
8	Dabur Honey	Manufactured in India by: Dabur India Ltd. Solan, Himachal Pradesh-173200	Lot No: BDO 365 Packed on:07/13
9	SGS Honey Rex	SGS Cottage Industries Chennai-600052	
10	Unbranded Local Vendor		

11	100% NaturaAgmark Honey	New Indian Apiary Industry Marthandam, KanyaKumari District-629165	Lot No:380/2013 Packed on:09/2013 Agmark CA No:21333 Govt of India
12	Swastik Honey	D.S.T.(Devi Swarna) Trade mark Chennai-600079	
13	Unbranded Local Vendor		
14	100% Natural Marthandam Co- operative Agmark Honey	Co-operative Society Ltd Marthandam, KanyaKumari District-629165 MBKCS Ltd No: 2050 Analyzed by SAGL MTM	Lot No:302/2013 Packed on:06/2013 Agmark CA No:E-2483 Govt of India St No:SRM/H/07/2012 Grade: A B 418702
15	AgmarkAnnai Honey	Repacked and Marketed by:M/S Muruhan Co. Thirumullaiveli, Chennai-600062 <a href="http://www.annaifoodproducts.com">www.annaifoodproducts.com</a> ISO 9001:2008	Packed on:12/2012
16	KhadiAlocory Natural Kashmir Honey	Processed and Packed by: Ashwin Khadi Gramo dyog Samiti Certified by Khadi& Village Industries Commission (Govt of India) Khasara No:197-09 Modinagar Uttar pradesh-201204 <a href="mailto:ashwin_khadi_herbal@yahoo.com">ashwin_khadi_herbal@yahoo.com</a>	Lic No:1271305200000 Packed on:09/2013
17	Devi's Honey Taste		
18	Jay Honey	Packed by:J G S Cottage Industries Pallikaranai, Chennai-600100	S.No:06 Packed on:06/2013
19	New gem Agmark Honey	Natural Honey Zone, Kuzhithurai-629163	Lot No:217/2013 Packed on:05/2013 C.A No:A/2-4664 Govt.of India
20	Unbranded S.K Swastik Honey		
21	Lion Honey	Manufactured & Packed by Lion Dates Imper Pvt. Ltd. Trichy-620002 <a href="http://www.liondates.com">www.liondates.com</a> ISO 9001:2008	Batch No:H105 Packed on:08/2013
22	Coorg Honey	Product of: The Coorg Honey & Wax Product Co-operation Marketing Society Ltd. Govt of India grading station: Madiker Kodagu, Karnataka.	Lot No:21 C.A No:5 2460 Govt of India St No:SR/C/H/01/12B 151119
23	Royal Agmark honey grade Natural Multi floral Honey	Packed by Royal Food Products Chepauk, Chennai-600005 Inspected by State Agmark Grading Laboratory Chennai	Packed on:09/2013 Grade A C.A No: A0 28217 Govt of India
24	Marthandam Star Agmark Honey	Manufactured by: Marthandam Star Honey Traders Munday market, Kanyakumari-629808	Lot No:172/ 2013 Packed on: 07/2013 Grade A C.A No: A/1-0028 Govt of India
25	Hills KodaikanalAgmark Honey	Packed by Sri Balamurugan Cottage Industries, Saidapet, Chennai-600015	Lot No:02 Packed on:06/2013 C.A No:A/1-00936 Govt of India

26	R.R Honey Agmark Honey	R.R Honey enterprises Kilpauk, Chennai-600010	Lot No:12 Packed on: 10/2013 Grade A C.A No:AP20584 Govt of India St No:SR/H/04/13 SL.C 577388
27	Reliance Healthy Life		Lot No:RI-045 Packed on:04/2014
28	Unbranded Khadikraft		
29	SKM Honey	Manufactured by SKM Siddha and ayurvedha Company(India) Ltd., Madakurichi, Erode- 638104 ISO 9001:2008 GMF Certified Company <a href="http://www.skmsiddha.org">www.skmsiddha.org</a>	Batch No:H0A13050 MfgLic No:739 Mfg dt:10/2013
30	Unbranded Local Vendor		
31	Sagar Forest Hill Nilgiri Honey	SagarNilgiri Oil Distillery Ooty, Nilgiris Ph:(0423)2440772	Code 152
32	Heritage Agmark Honey	Marketed by Heritage Foods India Ltd Hyderabad-500082 Processed and Packed by Prakruthi Health products, Banglore- 562149 Tested at Pristine Laboratory	Batch No:004 Packed on: 04/2013 C.A No: B162213 Govt of India Grade A
33	Heavena Agmark Honey	Marketed by M/S Heavenly Fuel Pvt Ltd	

### Phytochemical Analysis:

Phytochemical analysis was carried out similar to that of leaf<sup>11-14</sup>. The honey samples were used directly rather than extracting using a solvent.

#### Test for tannins

About 0.5 g of honey was dissolved in 10 ml of water in a test tube and used. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

#### Test for phlobatannins

One ml of honey was boiled with 1ml of 1% Hydrochloric (HCl) acid in a test tube. If the sample carries phlobatannins, a deposition of a red precipitate will occur; this indicates the presence of phlobatannins.

#### Test for saponins

About 1 g of each honey is boiled together with 10ml of distilled water to which 5 ml of distilled water was added in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

#### Test for flavonoids

A few drops of 1% Liquor Ammonia are added to each honey in a test tube. A yellow coloration is observed if flavonoid compounds are present.

#### Test for terpenoids

Around 5 ml of honey was mixed with 2 ml of Chloroform in a test tube and 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added. An interface with a reddish brown colouration is formed if terpenoid is present.

### Test for cardiac glycosides

Five ml of Honey was mixed with 2 ml of glacial acetic acid containing 1 drop of ferric chloride. The above mixture was carefully added to the 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. Appearance of a brown ring indicates the presence of the cardiac glycoside.

### Test for steroids

Two grams of Honey was taken and 10ml of chloroform was added. To the above 2 ml acetic anhydride and few drops of concentrated H<sub>2</sub>SO<sub>4</sub> was added. Appearance of blue green ring indicates the presence of steroids.

### Results and Discussion:

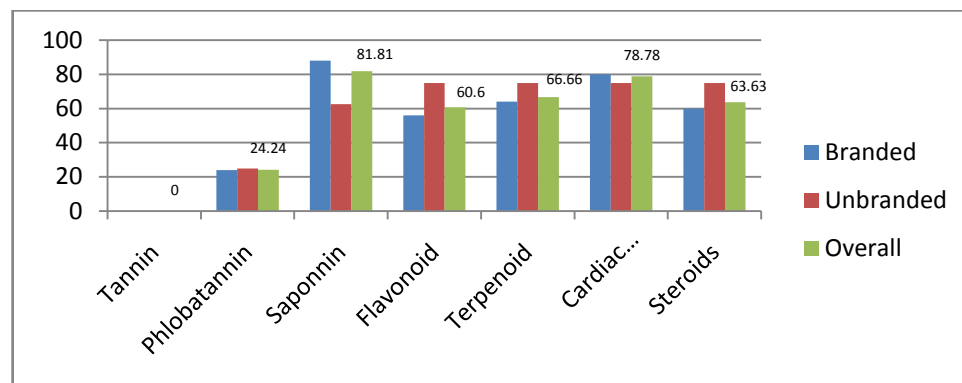
The study confirmed the presence of phytochemicals such as Phlobatannins, Saponins, Flavonoids, Terpenoids, Cardiac glycosides and Steroids in honey. Of the 33 samples analyzed for the presence of phytochemicals 8 showed the presence of phlobatannins, 27 resulted positive for Saponins, 20 for flavonoid, 22 for terpenoids, 26 for Cardiac glycosides and 21 for Steroids while none showed the presence of tannins. The presence or absence of phytocompounds in branded and unbranded honey samples procured from the markets of Chennai, India is provided in (Table- 2). The percent occurrence of different phytochemicals in branded and unbranded honey samples is represented graphically in (Figure- 1).

**Table 2. Phytochemical compounds recorded from honey samples procured from markets of Chennai, India**

Branded Honey samples							
Sample No.	Tannin	Phlobatannin	Saponin	Flavonoid	Terpenoid	Cardiac glycoside	Steroids
2	-	-	+	+	+	+	+
3	-	-	+	+	+	+	+
4	-	-	+	-	-	-	+
5	-	+	-	+	+	+	+
8	-	-	+	+	+	+	+
9	-	+	+	+	+	+	-
11	-	+	+	-	+	+	-
12	-	-	-	+	-	+	-
14	-	-	+	-	+	+	-
15	-	-	+	-	+	-	+
16	-	-	+	+	+	-	+
17	-	-	-	+	-	+	-
18	-	-	+	-	+	+	+
19	-	-	+	-	+	+	+
21	-	-	+	+	-	+	+
22	-	-	+	+	+	+	+
23	-	+	+	+	-	+	+
24	-	-	+	-	-	+	-
25	-	-	+	-	+	+	+
26	-	-	+	+	+	+	+
27	-	+	+	-	+	+	-
29	-	-	+	+	-	-	-
31	-	-	+	+	-	-	-
32	-	+	+	-	+	+	-
33	-	-	+	-	-	+	+
Unbranded Honey Samples							
1	-	-	+	+	+	+	+
6	-	+	-	-	-	-	+
7	-	-	+	+	+	+	-
10	-	+	+	-	+	-	+
13	-	-	+	+	+	+	+

20	-	-	-	+	+	+	+
28	-	-	+	+	+	+	+
30	-	-	-	+	-	+	-

**Figure 1: Percent occurrence of phytochemical composition in honey samples procured from markets of Chennai, India**



While the naturally occurring, water-soluble phenolic compounds tannins were absent, condensed tannins of higher molecular weight commonly described as Phlobatannins<sup>15</sup> occurred in 24.24% of the samples that were analyzed. They are formed either due to aging of tissues<sup>16</sup> or due to enzymatic action on dead cells<sup>17</sup> which may have showed their presence when honey bees collect.

Saponins were the highest occurring phytochemical in honey being present in 81.81% of the samples. Foaming is a characteristic feature of saponin whose natural role in plants is thought to be protection against attack by pathogens and pets<sup>18,19</sup>. These molecules also have considerable commercial value and are processed as drugs and medicines, foaming agents, sweeteners, taste modifiers and cosmetics<sup>20</sup>.

Flavonoids and phenolics are the most important groups of secondary metabolites and bioactive compounds in plants<sup>21</sup>. In the present investigation, 60.6% of the samples showed the presence of flavonoids. This confirms that honey can be used as anti-inflammatory and for analgesic activities as flavonoids are responsible for these properties<sup>22</sup>. Flavonoid has been reported as potential anti-ulcer and anti-inflammatory<sup>23</sup> anti-cancer and anti-oxidant<sup>24</sup> and anti-diabetic chemical<sup>25</sup>.

The modified or oxidized terpene is called as terpenoid. Terpenoids which contributes to the scent of most of the plants was present in 66.66% of the samples investigated. Anti-microbial activity of terpenoids has been studied several times<sup>26-28</sup> in the past. Hence, honey can be used as a potential anti-microbial agent.

More than 75 % of the samples resulted positive for cardiac glycosides. In recent time's cardiac glycosides overcame its obstructions of toxicity and may be used for treatment of cancers such as Breast cancer, Colon cancer, Bladder cancer and Lung cancer<sup>29-32</sup>. The results suggest that consumption of honey may reduce the risk of cancer due to the presence of cardiac glycosides in them. Steroid which occurred in 63.63% of the samples are one of the most widely used groups of drugs with or without indication in anesthetic practice<sup>33</sup>. Steroids are reported as controlling agent for topical diseases as eczema etc.

It was found that other than Saponins, the unbranded honey is found to possess most of the secondary metabolites at higher percentage when compared to branded ones. The authors feel that the unbranded honey samples are rich in quality in relation to the presence of phyto constituents when compared to branded ones. Since only a few unbranded honey samples were analyzed, further analysis may support the statement. This study may serve as a reference for further work in honey as an anti-cancer, anti-inflammatory, anti-oxidant agent and exploiting many other medicinal properties of honey. The present study proves that honey samples are found to possess rich secondary metabolites, which without doubt can be used as a potential medicinal agent in traditional system of medicine.

## Conclusion:

The study was conducted to know the quality of branded and unbranded honey samples procured from the markets of Chennai for the presence of phytochemicals like Tannin, phlobatannin, flavonoid, steroids, cardiac glycosides, saponins and terpenoids. The study proves that the samples are rich in secondary metabolites. Though, the occurrence of phytochemicals in both branded and unbranded honey remained almost same, unprocessed natural unbranded honey is preferred for consumption than branded ones.

## References:

1. Standifer LN. "Honey Bee Nutrition and Supplemental Feeding", Beekeeping in the United States.
2. Pecanac, M., Janjic, Z., Komarcevic, A., Pajic, M., Dobanovacki, D., Miskovic, S.S., "Burns treatment in ancient times". *Med Pregl*, 2013, 66, 5-6, 263-267.
3. Altman, Nathaniel, "The Honey Prescription: The Amazing Power of Honey as Medicine" Inner Traditions / Bear & Company. March 2010, 60-62.
4. Pand, K.N., "Simple Yoga for Good Health" India: Star Publications 2008.
5. Terrab, A., A.G. Gonzalez, M.J. Diez and J.H. Francisco, "Characterization of Moroccan unifloral honeys using multivariate analysis". *J. Eur Food Res and Technol.*, 2013, 218, 88-95.
6. KhaliqurRahman, ArshadHussain, ShafqatUllah and ImdadUllah Muhammad Zai, "Phytochemical Analysis and Chemical Composition of Different Branded and Unbranded Honey Samples", *International Journal of Microbiological Research*, 2013, 4, 2, 132-137.
7. Nwankwo, C.M., Ezekoye, C.C., Igbokwe, S.O., "Phytochemical screening and Anti-microbial activity of Apiary Honey produced by Honey Bee (*Apis mellifera*) on clinical strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*" *African Journal of Biotechnology*, June 2014, 13, 23, 2367-2372.
8. P. Dzomba, N. Ngoroyemoto, L. Mutandwa and D. Shasha, "Phytochemical Screening and Biological Activities of *Hypotriginasquamuligera* Raw Honey", *International Journal of Biochemistry Research & Review*, 2012, 2, 3, 98-105.
9. RajinderKaur, PreetiKalia, Neelima R. Kumar and KusumHarjai, "Preliminary studies on different extracts of some honey bee products", *Journal of Applied and Natural Science*, 2013, 5, 2, 420-422.
10. PreetiKalia, Neelima R Kumar and KusumHarjai, "Phytochemical screening and antibacterial activity of different extracts of Propolis" *Int. J. of Pharmaceutical and Biological Research*, Jan 2013, 3, 6, 212-222.
11. Udayaprakash NK, Selvi CR, Sasikala V, Dhanalakshmi S, Bhuvaneshwari S. "Phytochemistry and bioefficacy of a weed, *Dodonaea viscosa*". *Int J Pharm Pharm Sci*. 2012;4, 5, 509-512.
12. Udayaprakash NK, Jahnavi B, Abhinaya K, Rajalin AG, Sekarbabu H, "Phytochemical analysis of common weeds of Northern districts in Tamil Nadu". *Int J Applied Biol.*, 2011, 2, 25-28.
13. N. K. UdayaPrakash, S. Bhuvaneshwari, A. Balamurugan, B. Radhika, R. Bhagya, N. SriPriya, L. Prameela, S. Sarojini, R. Vigneshwari, M. Chandran and S. Arokiyaraj, "Studies on Phytochemistry of 100 Plants in Chennai, India", *British Journal of Pharmaceutical Research*, 2013, 3, 3, 407-419.
14. Evans WC. Trease and Evan's Pharmacognosy, 14th ed. London:WB Saunders, 1996.
15. Foo, L.Y., Karchesy, J.J., "Chemical nature of phlobaphenes", In: Hemingway RW, Karchesy JJ, editors. *The Chemistry and Significance of Condensed Tannins*, Plenum Press; 1989.
16. Porter, L.J., "Structure and chemical properties of the condensed tannins" In: Hemingway RW, Laks PE, editors. *Plant polyphenols: synthesis, properties, significance*. Plenum, New York, 1992.
17. Scalbert A., "Tannins in woods and their contribution to microbial decay prevention", In: Hemingway RW, Laks PE, editors. *Plant polyphenols: synthesis, properties, Significance*. Plenum, New York, 1992.
18. Price, K.R., Johnson, I.T and Fenwick, G.R. "The chemistry and biological significance of saponins in food and feeding stuffs", *Crit Rev Food Sci Nutr.*, 1987, 26, 27-133.
19. Morrissey, J.P. and A.E. Osbourn. "Fungal resistance to plant antibiotics as a mechanism of pathogenesis", *Microbiol Mol Biol Rev.*, 1999, 63, 708-724.
20. Hostettmann, K.A. and Marston, A. "Saponins-Chemistry and pharmacology of natural products", Cambridge University Press, 1995.
21. Kim DO, Jeond SW, Lee C. "Antioxidant capacity of phenolic phytochemicals from various cultivars of plums", *Food Chem*. 2003, 81, 321-326.
22. Chakraborty, A., Devi, R.K.B., Rita, S., Sharatchandra, K and Singh, T.I., "Preliminary studies on anti-inflammatory and analgesic activities of *Spilanthes acmellain* experimental animal models", *Indian. J. P. Pharmacol.*, 2009, 36, 3, 148 - 150.

23. Villar, A., Gasco, M.A, Alcaraz M.J, “Anti-inflammatory and anti-ulcer properties of hypolaetin-8-glucoside, a novel plant flavonoid”, *J Pharm Pharmacol.* 1984; Vol. 36:820-823.
24. Ghasemzadeh, A., Jaafar, H.Z.E., “Anticancer and antioxidant activities of Malaysian young ginger (*Zingiberofficinale*R.) varieties grown under different CO<sub>2</sub> concentration”, *J Med Plant Res.* 2011,5, 3247-3255.
25. Brahmachari, G., “Bio-flavonoids with promising anti-diabetic potentials: A critical survey”, *Opportunity, Challenge Scope Nat Prod Med Chem.*,2011, 187-212.
26. Islam, A.K., Ali, M.A., Sayeed, A., Salam, S.M., Islam, A., Rahman, M., Khan, G.R.,Khatun, S.,“An antimicrobialterpenoid from *Caesalpinia pulcherrima* Swartz.Its characterization, antimicrobial and cytotoxic cactivities”, *Asian J. Plant Sci.*2003, 2,17-24.
27. Morales, G., Sierra, P., Mancilla, A., Paredes, A., Loyola, L.A., Gallardo, O., Borquez J, “Secondary metabolites from four medicinal plantsfrom northern Chile: Antimicrobial activity and bio-toxicity against *Artemiasalina*”,*J. Chil. Chem. Soc.*,2002, 48, 13-18.
28. John, A.J.,Karunakran,V.P., George, V.,“Chemical composition and antibacterial activity of *Neolitsea foliosa* (Nees), Gamble var.*caesia* (Meisner) Gamble”, *J. Essent. Oil Res.* 2007,19, 498-500.
29. Stenkqvist, B., Bengtsson, E., Eriksson, O., Holmquist, J., Nordin, B., and Westman-Naeser, S. “Cardiac glycosides and breast cancer”, *Lancet*,1979, 10, 563.
30. Frese, S., Frese-Schaper, M., Anne-Catherine, A., Miescher, D.,Zumkehr, B., and Schmid, R.A., “Cardiac glycosides initiate Apo2L/TRAILinducedapoptosis in non-small cell lung cancer cells by up-regulation of death receptors 4 and 5”, *Cancer Res.*2006,66, 6867–5874.
31. Davies, R.J., Sandle, G.I., and Thompson, S.M., “Inhibition of the Na<sup>+</sup>,K<sup>+</sup>-ATPase pump during induction of experimental colon cancer”, *Cancer Biochem.Biophys.*,1991,12, 81–94.
32. Espineda, C., Seligson, D.B., Ball, Jr., W., Rao, J., Palotie, A., Horvath,S., Huang, Y., Shi, T., and Rajasekaran, A.K., “Analysis of the Na,KATPasealpha- and beta-subunit expression profiles of bladder cancer using tissue microarrays”, *Cancer*,2003, 97, 1859–1868.
33. Shaikh, S., Verma, H., Yadav, N., Jauhari, M., Bullangowda, J., “Applications of Steroid in Clinical Practice: A Review”, *ISRN Anesthesiology*, 2012, 10.

\*\*\*\*\*