*BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA*, December 2013. Vol. **10**(2), 607-612

# Anti-oxidant and Anti-hyperglycemic Properties of Methanolic Extracts of Medicinal Plants

**K.S. Chandrashekharaiah[1]* Menaka N Bolaki[1], Gupta Shivani Sanjay[1], Aanchal Bathija[1], V Krishna Murthy[1], M Narayanaswamy[2] and N Ramachandra Swamy[3]**

[1]Department of Biotechnology, PES Institute of Technology, BSK III Stage, Bangalore-560 085, India.
[2]Department of Molecular Biology, Bangalore University, Bangalore - 560 056, India.
[3]Department of Biochemistry, Bangalore University, Bangalore - 5600 01, India.

DOI: http://dx.doi.org/10.13005/bbra/1171

(Received: 15 August 2013; accepted: 10 October 2013)

**Total phenolic content, DPPH free radical scavenging activity and alpha amylae inhibitory potential was determined for three selected medicinal plants – *Gymnema* sylvestre, *Terminalia arjuna* and *Tinospora cordifolia.* The plant extracts were prepared with methanol. The total phenolic content of methonolic extracts of *Gymnema* sylvestre, *Terminalia arjuna* and *Tinospora cordifolia.* were 6.862, 20.862 and 7.987 mg GAE/g plant material respectively. All the three plants showed anti oxidant activities with their $IC_{50}$ values were 6.862, 20.862 and 7.987 $\mu$g/ml compared to $IC_{50}$ value of the standard L-Ascorbic acid, which was 11.59$\mu$g/ml. The extracts of *Gymnema* sylvestre showed ±- amylase inhibition. Thus the results provided evidence that among the studied plants, *Gymnema sylvestre* potential sources of natural antioxidant and antidiabetic activity.**

**Key words:** Medicinal plants, total phenols, antioxidant activity, amylase inhibitory activity.

*Diabetes mellitus* is one of the leading epidemics of the world. It is a group of metabolic disorders with one common manifestation – hyperglycemia. Management of diabetes is a global problem and there are free radicals that leads to oxidative stress which causes diseases associated with diabetes. Antidiabetic medications include sulphonylureas, biguanides, meglitinides and thiazolidinediones. Generally all these drugs are administered orally with the exception of exenatide, liraglutide, insulin and pramlintide. With the progress of the disease, monotherapy fails to act due to which there is a need for multiple combinations of drugs. Each of these drugs have their own side effects, some of which are nausea, hypoglycemia, weight gain, liver diseases, gastrointestinal disorders, mild anorexia, abdominal discomfort, flatulence and diarrhea. Keeping these side effects in mind, there is a need to switch to drugs which are non toxic, have greater efficacy and are cost effective. There are about 800-1000 medicinal plants that have anti-diabetic potential. The aim of this project was to determine the total phenolic content, antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay and ±-amylase inhibitory activity from *Gymnema* sylvestre, *Terminalia arjuna* and *Tinospora cordifolia.*

* To whom all correspondence should be addressed.
Tel.: +91 80 26721983 x 344; fax: +91 80 26720886.
Email: kschandraks@gmail.com

## MATERIALS AND METHODS

The plant materials *(*Leaves of *Gymnema sylvestre* and *Tinospora cordifolia* and the bark of *Terminalia arjuna*) *were* purchased from University of Agricultural Sciences, Bangalore, India. The Gallic acid and DPPH were purchased from Sigma chemical company, St. Louse, USA. All other

chemicals and reagents used were of analytical grade.

**Preparation of plant extracts**

The leaves and bark were washed with distilled water. The leaves and bark of the plants were processed according to the method of Ranilla *et al* (2010). The cleaned leaves and bark were spread on a paper in cleaned, ventilated laboratory and air dried at 25°C for fifteen days. Dried samples were grounded into a powder using a blender and were stored in polythene bags at 4°C until further use. The powdered samples were subjected for extraction with water, methanol, butanol and ethyl acetate. The powdered samples were taken in a thimble bag made of blotting paper and whatmann filter paper and loaded into a Soxhlet apparatus with water, methanol, butanol and ethyl acetate as solvents (1:20, w/v). The extracts were then concentrated using rotary vacuum dryer at 50°C. The yield obtained was 6.4, 11.5, 7.2, and 6.7 mg/100g in water, methanol, butanol and ethyl acetate respectively. The dried crude extracts were stored at -4°C in air tight bottles until further use.

**Determination of Total phenolic content**

Total phenolics were estimated by FC method (Spanos *et al.,* 1990). A dilute extract of each plant extract (1.0 ml) or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (2.5 ml, 1:10 diluted with distilled water) and aqueous Na2CO3 (2 ml, 2%). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 720 nm. The standard Gallic acid curve in each solvent was prepared (80 µg/ml) and total phenol values were expressed in terms of Gallic acid equivalent (mg/ g of plant material). Total content of phenolic compound was calculated by the equation:

C= (c*m)/V

where, C= total content of phenolic compound in Gallic acid equivalent, c= concentration of Gallic acid established from the standard curve (µg/ml), m=weight of crude plant extract, V=volume of plant extract.

**Determination of Antioxidant activity**

Antioxidant activity was determined by the DPPH free radical scavenging activity according to the method of Patel *et al (2011*)). Ascorbic acid was used as a reference Standard. 50µl of dilute solutions of different concentrations of the extracts were taken and 100µl of each solvent was added followed by 150µl of DPPH and incubated at room temperature on a Rotary Shaker for 15mins. 3ml of each solvent was added to each of the test tubes and the absorbance was measured at 520nm with respective solvent as blank. Control sample was prepared containing the same volume without any extract and reference was taken as ascorbic acid .Percentage scavenging of the DPPH free radical was measured by using the following equation:

$$\text{Scavenging activity} = \frac{(\text{Absorbance of control} - \text{absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

**Determination of alpha amylase inhibitory activity**

α-Amylase inhibitor activity was determined according to the method of Bernfeld (1955). Alpha amylase was produced from human saliva. 1 ml of enzyme and 1ml of the diluted plant extract were taken in test tubes and incubated at room temperature for 10 minutes. After incubation 1ml of starch was added as substrate and incubated for 10 minutes. The reaction was arrested using 1 ml of DNS reagent (of 3,5-dinitrosalicylic acid) followed by keeping the test tubes in boiling water bath for 10 min. The residual α-Amylase activity was determined by measuring the absorbance at 540 nm.

A standard calibration curve was prepared for the maltose taking 1ml of 360-1800 µg/ml dilutions of maltose. The percentage (w/v) of maltose in the reaction wells was calculated from the corrected absorbance of each test and using the equation of the calibration curve. Control incubations, representing 100% enzyme activity were conducted in the same manner replacing the plant extract with distilled water. The percentage of α-amylase inhibition was calculated by the following equations:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control (enzyme)} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}}$$

**Total Phenolic Contents**

Polyphenols have been said to be important phytochemicals with significant antioxidant capacities and other important

medicinal characteristics. Total phenolic content was determined by the FC method and the calibration curve developed using Gallic acid. A regression equation was obtained from the standard curve and the amount of Gallic acid in the plant samples was calculated from the regression equation:

$$y = 0.004\ x,\ R^2 = 0.995$$

The results were determined in terms of mg Gallic Acid Equivalent per gram of plant material. (Fig 1-a) shows the comparison of the total phenolics of the 3 extracts. Among the three plants tested for their phenolic content, *Terminalia arjuna* showed the highest phenol content (20.862 mg GAE/g plant material), whereas *Gymnema sylvestere* showed the lowest phenol content (~7 mg GAE/g plant material). Similar results were seen by (Shahriar *et al*., 2012) where the methanolic extract of *T.arjuna* showed the highest phenol content when compared with other extracts.

**Table 1.** Total phenolic (expressed as gallic acid equivalents) content from the extracts of *Gymnema sylvestere, Terminalia arjuna* and *Tinospora cordifolia*

| Plant Species | Phenolic content ( GAE mg/g plant material) |
|---|---|
| *Gymnema sylvestere* | 6.862 |
| *Terminalia arjuna* | 20.862 |
| *Tinospora cordifolia* | 7.987 |

**Table 2.** Antioxidant activity of extracts of *Gymnema sylvestere, Terminalia arjuna* and *Tinospora cordifolia* and L-Ascorbic acid at different concentrations and their $IC_{50}$ Values.

| Plant Species | Phenolic content ( GAE mg/g plant material) |
|---|---|
| *Gymnema sylvestere*- $IC_{50}$ (µg/ml) | 6.862 |
| *Terminalia arjuna*- $IC_{50}$ (µg/ml) | 20.862 |
| *Tinospora cordifolia* - $IC_{50}$ (µg/ml) | 7.987 |
| *L-Ascorbic acid*- $IC_{50}$ (µg/ml) | 11.59 |

**Antioxidant capacity**

Natural antioxidants present in plants are responsible for inhibiting or preventing the injurious effects of oxidative stress caused by free radicals in the body. Polyphenols present in plants have said to be efficient free radical scavengers. (Khalaf *et al.,* 2008). Thus after determining the total phenol content of the plants, the antioxidant activities of the plants were estimated for their free radical scavenging activity (Patel *et al*.,2011).

The assay was performed at different concentrations for the standard i.e., L ascorbic acid and the methanolic crude extract of the three plants. Scavenging activity was calculated and the graphs of scavenging activity (%) against concentrations were plotted in each case. In the present study, the extracts of all the three plants were found to be effective scavengers against DPPH radical. The $IC_{50}$ values were calculated and compared with L- ascorbic acid (Table 2, Fig. 1)). The $IC_{50}$ values were in the range of 16-34 µg/ml and the extract of *Gymnema sylvestere* showed $IC_{50}$ of 16.8µg/ml which indicated highest DPPH radical scavenging activity, while the weakest scavenger was the extract of *Tinospora cordifolia* (33.4 µg/mL). Figure 2 shows the total phenol content and antioxidant capacities of the plants. The extracts do not show any significant correlation between the antioxidant capacity and phenol content. *Terminalia arjuna* has the highest phenol content with an $IC_{50}$ of 18 µg/ml and *Gymnema sylvestere* has the lowest phenol content i.e. 6.862 mg GAE/g plant material with an $IC_{50}$ of 16.8 µg/ml. *Tinospora cordifolia* has a phenol content of 7.987 mg GAE/g plant material , but its $IC_{50}$ value is 33.04. Although many studies of (Shan *et al*.,2005; Wu *et al*., 2006; Wong *et al*., 2006; Yang *et al*.,2002) have shown correlation between the total phenol content and antioxidant activity, bit in our studies there is no correlation and this was in accordance with the studies of Bajpai *et al* (2005) and Sengul *et al* (2009).

No correlation between antioxidant capacity and total phenol content could be due to many factors like the antioxidant capacity was not entirely due to the phenol contents, but could be due to presence of other phytochemicals also. Also Folin Ciocalteau method is not a completeindicator

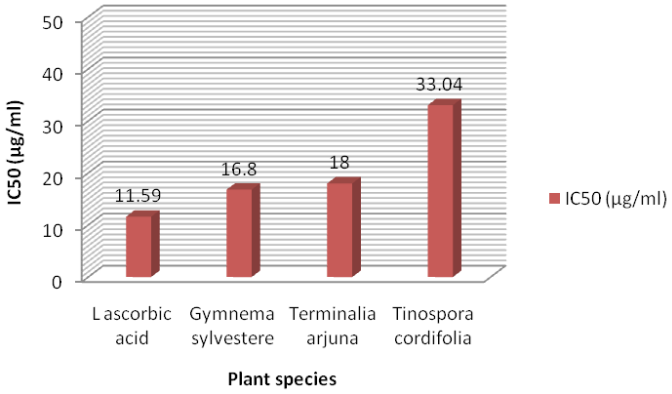


**Fig. 1.** Comparison of $IC_{50}$ values of the plants.

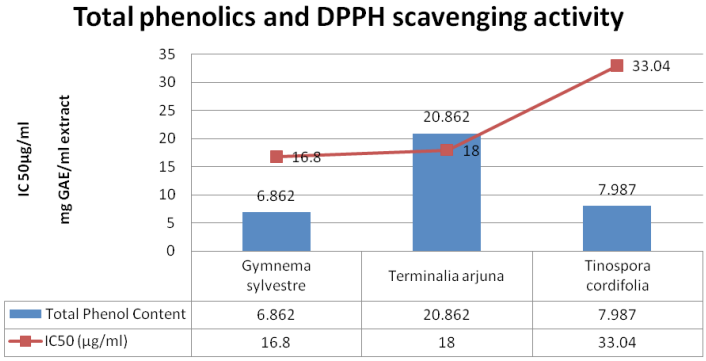


**Fig.2.** DPPH scavenging activity and Total phenol content.

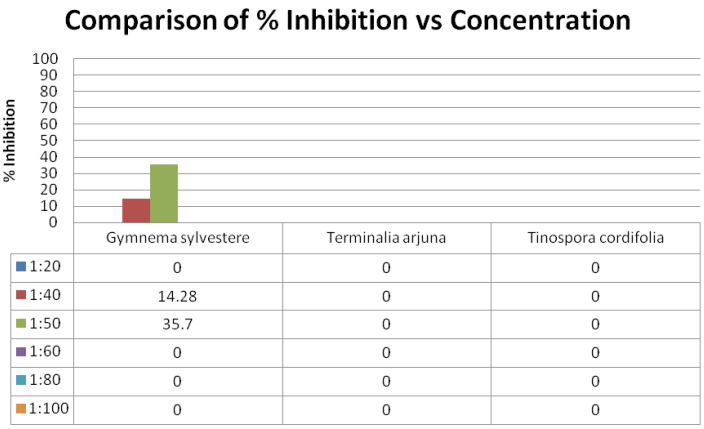


**Fig. 3.** Comparison of Alpha amylase inhibition of the 3 plants.

of the phenol content and thus there may be other phenol compounds present that contribute to the antioxidant capacities.

**Alpha amylase inhibitory activity**

Alpha amylase is one of the key enzymes that play a role in digestion of starch and glycogen and carbohydrate metabolism. Its inhibition is one of the strategies for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity. It is involved in carbohydrate metabolism and thus

inhibiting it would lead to reduced post prandial blood sugar (Paloma *et al* 2012).

The three plant extracts were tested for alpha amylase inhibition. The standard maltose curve was plotted to note the absorbance corresponding to the maltose content. The extracts were then tested for amylase inhibition at different concentrations and the % inhibition was calculated by the amount of maltose present after inhibition of the enzyme and the amount of maltose present in the control i.e. only the enzyme – alpha amylase. Amongst the 3 plants *Terminalia arjuna* and *Tinospora cordifolia* did not show amylase inhibition. *Gymnema sylvestere* showed amylase inhibition (Fig. 3), but the inhibition is not concentration dependant.

## CONCLUSION

Highest total phenolic content was seen in *Terminalia arjuna* (20.862 mg GAE/g of plant material) and lowest was seen in *Gymnema sylvestere*(6.862 mg GAE/g of plant material). All the plants showed good antioxidant activity. Alpha amylase inhibitory activity was observed only in *Gymnema sylvestre*. *Tinospora cordifolia* and *Terminalia arjuna* did not show alpha amylase inhibition. Among the three selected plants, *Gymnema sylvestre* showed alpha amylase inhibition and free radical scavenging capacity and thus is a potent sources for anti-diabetic and antioxidant agents.

## REFERENCES


1. Arayne Saeed.M, Sultana Najma, Mirza Agha Zeeshan, Zuberi Hashim .M and Siddiqui Farhan Ahmen, 2007. *In Vitro* Hypoglycemic activity of methanolic extract of some indigenous plants, *Pak. J. Pharm. Sci*. **20**(4), 268-273.
2. Arya Aditya, Abdullah Ameen Mahmood , Haerian Batoul Sadat, Mohd Mustafa Ali, 2012. Screening for Hypoglycemic Activity on The Leaf Extracts of Nine Medicinal Plants: *In-Vivo* Evaluation, *E. Jouranl of Chemistry*, **9**(3), 1196-1205.
3. Bajpai M, Pande A, Tewari SK and Prakash D, 2005. Phenolic contents and antioxidant activity of some food and medicinal plants, *International Journal of Food Sciences and Nutrition,* **56**(4): 287-291.
4. Bernfeld P, 1955. Amylases, ± and ², *Meth. Enzymology*. **1**, 149-158.
5. Chaudhari M and Mengi S, 2006. Evaluation of phytoconstituents of Terminalia arjuna for wound healing activity in rats, *Phytother Res.,* 20(9), 799-805.
6. Devi Sundaresan Rethinam, Narayan Shoba, Vani Ganapathy and Devi Srinivasulu Shyamala Chennam, 2007. Gastroprotective effect of *Terminalia arjuna* bark on diclofenac sodium induced gastric ulcer, *Chemico-Biological Interactions*, **167**(1), 71-83.
7. Dwivedi Shridhar, 2007. *Terminalia arjuna* Wight & Arn.-A useful drug for cardiovascular disorders, *Journal of Ethnopharmacology,* **114**(2), 114-129.
8. Doss A, Pugalenthi M, Rajendrakumaran D and Vadivel V, 2009. In vitro antioxidant properties of certain indigenous medicinal plants from Western Ghats of India. *Asian J.Exp.Biol.Sci*., **1**(3).
9. Ekta Menghani, Arvind Pareek, Roshan Singh Negi and Ojha C K, 2010. AntiDiabetic Potentials of Various Ethano-medicinal Plants of Rajasthan, *Ethanobotanical Leaftes,* 14, 578-583.
10. Gulati Vandana, Harding Ian H and Palombo Enzo A, 2012. Enzyme inhibitory and antioxidant activities of traditional medicinal plants:Potential application in the management of Hyperglycemia, BMC Complementary and Alternative Medicine 2012, 12:77.
11. Beatriz Luna, BCPS and Fengelos Mark N, 2001. Oral Agents in Management of Type 2 Diabetes Mellitus, *Am Fam Physician*, **63**(9), 1747-1757.
12. Khalaf Nooman A, Shakya Ashok K Al-Othman Atif, Zaha El Agbar and Husni Farah, 2008. Antioxidant Activity of Some Common Plants, *Turk J Biol*., **2**, 52-55.
13. Khanna Gopiesh Venkatesan and Kannabiran Krishnan, 2009. Anticancer-cytotoxic activity of saponins isolated from the leaves of *Gymnema sylvestre* and *Eclipta prostrata* on HeLa cells, *International Journal of Green Pharmacy*, **3**(3), 227-229.
14. Khanna V G, Kannabiran K., Rajakumar G, Rahuman A A and Santhoshkumar T, 2011. Biolarvicidal compound gymnemagenol isolated from leaf extract of miracle fruit plant, *Gymnema sylvestre* (Retz) Schult against malaria and filariasis vectors, *Parasitol Res*., **109**(5), 1373-1386.
15. Malik K Jitender, F V Manvi, K R Alagawadi and M Noolvi, 2008. Evaluation of anti-inflammatory activity of *Gymnema sylvestre* leaves extract in rats, *International Journal of Green Pharmacy,* **2**(2), 114-115.

16. Manjrekar P N, Jolly C I and Narayanan S 2000. Comparative studies of the immunomodulatory activity of *Tinospora cordifolia* and *Tinospora sinensis*, *Fitoterapia*, **71**(3), 254-257.
17. Middha Sushil Kumar , Talambedu Usha, Tripathi Pranav, Marathe Kirti Yashwant , Jain Tarun , Bhatt Bhargavi , Masurkar Yogini Pradeep and Pande Veena , 2012. An *In vitro* studies on indigenous ayurvedic plants, having hypoglycemic activity, *Asian Pacific Journal of Tropical Disease*, 46-49.
18. Modak Manisha, Dixit Priyanjali, Londhe Jayant, Ghaskadbi Saroj, Thomas Paul A Debasagayam, 2007. Indian Herbs and Herbal Drugs Use for the Treatment of Diabetes, *J Clin Biochem Nutr*., **40**(3), 163-173.
19. Molyneux P, 2003.The use if the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity,Songknakaria *J.Sci.Technol*., **26**(2), 211-219.
20. Moorthi P. Sathiya , M. Deecaraman1 , K. Praveen kumar, N. Kishan vaidyanat, Kalaichelvan P T, 2009. Screening of a- Amylase inhibitory activities from natural sources, *Advanced Biotech*., 33-39.
21. Neelesh Malviya, Sanjay Jain, Sapna Malviya, 2010. Antidiabetic potential of medicinal plants, *Acta Poloniae Pharmaceutica Drug Research*, **67**(2) 113-118.
22. Paloma Michelle de Sales, Paula Monteiro de Souza, Luiz Alberto Simeoni, Pérola de Oliveira Magalhães, and Dâmaris Silveira, 2012. ±-Amylase Inhibitors: A Review of Raw Material and Isolated Compounds from Plant Source, *J Pharm Pharmaceut Sci* **15**(1): 141 – 183.
23. Patel Rajesh M and Patel Natvar J, 2011. In vitro antioxidant activity of coumarin compounds by DPPH , Super oxide and nitric acid free radical scavenging activities. *Journal of Advanced Pharmacy Education and Research,* **1**, 52 -68.
24. Rani Phulan and Khullar Neeraj, 2004. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi, Phytotherapy Research*, **18**(8), 670-673.
25. Ranilla LG, Kwon Y I, Apostolidis E and Shetty K, 2010. Phenolic compounds, antioxidant activity and *in vitro* inhibitor potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America, Bioresource Technology, *101.4676*-4689.
26. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z and Ercisli S, 2009. Total phenol content, antioxidant and antimicrobial activities of some medicinal plants, *Pakistan Journal of Pharmaceutical Sciences*, **22**(1),102–107
27. Shahriar Mohammad, Akhter Savika, Hussain Ismail Md, Haque Aminul Md and Bhuivian Ahmed Mohiuddin, 2012. Evaluation of *in vitro* antioxidant activity of bark extracts of *Terminalia arjuna, Journal of Medicinal Plants Research,* **6**(39), 5286-5298.
28. Shikha Batra, Prakash Badri Nagori and Nikhil Batra, 2011. Medicinal Plants of Rajasthan (India) with Antidiabetic Potential, *International Research Journal of Pharmacy*, 2(3), 1 - 7.
29. Spanos, G A, Wrolstad R E, Heatherbell D A, 1990. Influence of processing and storage on the phenol composition of apple juice, *J. Agric. Food Chem*., **38**, 1572- 1579.
30. Sridhar S B, Sheetal U D, Pai M R S M and Shastri M S, 2005. Preclinical evaluation of the antidiabetic effect of *Eugenia jambolana* seed powder in streptozotocin-diabetic rats, *Brazilian Journal of Medical and Biological Research*, 38, 463-468.
31. Sultana Bushra , Anwar Farooq , and Ashraf MuhammadBushra, 2009. Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts, *Molecules,* 14*,* 2167-2180.
32. Thamilvaani Manaharan, Uma Devi Palanisamy and Cheng Hwee Ming, 2012. Tropical Plant Extracts as Potential Antihyperglycemic Agents, *Molecules***, 17**, 5915-5923.
33. Tiwari Prashant, Kumar Bimlesh, Kaur Mandeep, Kaur Gurpreet, Kaur Harleen, 2011. Phytochemical screening and Extraction: A Review, *International Pharmaceutica Sciencia,* **1**(1), 98-106
34. Tomar G S, Tiwari S K, Chaturvedi G N, 1982. Kalmegh (Andrographis paniculata Nees) and its medicinal status, Nagarjun, 76-78.
35. Waterhouse Andrew, 1999. Folin-Ciocalteau Micro Method for Total Phenol in Wine, Methods in Enzymology, 299,152-178.
36. Vasim Khan, Abul Kalam najmi, Mohd. Akhtar, Mohd. Aqeel, Mohd Mujeeb and Pliiai K K 2012. A pharmacological appraisal of medicinal plants with antidiabetic potential, *J Pharma Bioallied Sci*, **4**(1), 27-42.